DIPLOMARBEIT

Titel der Diplomarbeit

„Impact of ABC transporters on secondary fungal metabolites as remedies or toxins“

verfasst von

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angestrebter akademischer Grad

Magister der Pharmazie (Mag.pharm.)

Wien, 2014

Studienkennzahl lt. Studienblatt:	A449
Studienrichtung lt. Studienblatt:	Pharmazie
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Contents

Abstract

ATP-binding cassette (ABC) transporters are righteously gaining ever-growing scientific attention. Initial evidence pointed this protein transporter class to be involved in the uptake of nutrients in lower organisms. However, their presence was later found in a variety of cell types across most distinct organisms. Moreover, it was revealed in subsequent discoveries over the past decades that their roles reach far more than being simple nutrient uptake mediators. In humans, their physiological roles are numerous (e.g. phospholipid transport, chloride channels, lipid metabolism, permeability regulation in different blood-tissue barriers), as well as the pathological disorders that manifest in different tissues and organs as a result of mutations in ABC-transporters that lead to functionality loss, including cystic fibrosis, progressive familiar intrahepatic cholestasis, X-linked sideroblastic anemia and many other illnesses. The relevance of this transporter class in pharmacotherapy has been increasingly recognized over the past decades as they have been shown to be majorly involved in transport of a wide range of drugs, reaching from statins used in treating lipid disorders to different chemotherapeutic agents clinically used to treat malignant conditions. Their expression, especially so overexpression, has been associated with treatment failure in a wide range of malignant forms. Given their wide distribution in the human organism and the broad spectrum of compounds that they can transport across cell membranes and tissue barriers, this transporter class is also highly relevant in drug metabolism, encompassing absorption, distribution, and excretion. Moreover, ABC-transporters add certain value to drug-drug interactions in the context of pharmacokinetics.

Secondary metabolism of bacteria, fungi, algae, and plants has been long known to generate a variety of biologically active compounds. The fungal kingdom, comprised by more than a million different species, is a rich source of remedies and mycotoxins.

However, despite the fact that different forms of fungal extracts have been long used in traditional medicine around the globe, the utilization of pure substances isolated from fungi as remedies commenced in the 20th century. Penicillin antibiotics, a prominent and very widely used class of antibiotics, have emerged from the discovery of penicillin G in the late 1920s, and were followed by other antibiotics, including cephalosporins and fusidic acid, and the antimycotic drug griseofulvin. Immunosuppressive drugs cyclosporine A and mycophenolat-mofetil, which were discovered shortly thereafter, have paved the way into the era of organ transplantation, and are also of major clinical relevance in treating numerous autoimmune disorders. Statins, which are amongst the most commonly used and important drugs nowadays, go back to the discovery of the fungal metabolite compactin, which never lived up to its clinical use; and lovastatin, the first statin introduced to general clinical use in treating blood lipid disorders. Ergot alkaloids are a particularly interesting and large group of related compounds, which are effectively used in treating different conditions, such as Parkinson's disease, postpartum bleeding, hypotonic disorders; but also have a long history in regards to their toxic potential and are starting points in modern developing of strong hallucinogenic drugs. Mycotoxins are secondary fungal metabolites that have been partly associated with adverse health effects. For a great part of mycotoxins, well-established *in vivo* health effects have been observed only in animals. In humans, mycotoxins are mostly ingested through consumption of food commodities, such as cereals and their by-products, dairy products, fruits etc., which are contaminated with fungi and their mycotoxin metabolites. Adverse health effects have been proven and are clearly evident for aflatoxins, particularly for aflatoxin B_1, for which strong evidence has indicated it to be highly hepatotoxic and hepatocarcinogenic. With respect to exposure to other mycotoxins, many uncertainties still remain, as existing evidence is generally sparse and relies predominantly on *in vitro* experiments. Moreover, ambiguous health-related effects

have been reported in many of the conducted experiments making it difficult to establish a clear dose cut-off between negative and positive effects, which is an additional constraint in defining the health perspective related to consumption of fungi-contaminated food products.

In this regard, it is relevant to investigate how ABC-transporters interact with secondary fungal metabolites, both remedies and mycotoxins. In this literature review, studies will be presented in detail to provide an overview of such interactions at different levels. Beginning from genetic features of ABC-transporters in relation to pharmacokinetics of lovastatin and cyclosporine A, through the influence of ABC-transporters on absorption, tissue distribution, and excretion of secondary fungal metabolites; the role of ABC-transporters in protection and determining of toxicity of mycotoxins; importance of these interactions for pharmacokinetic profile of drugs, and many other aspects will be discussed.

The first part provides an overview of ABC-transporters' discovery, structure, mechanism of action, and human ABC-transporters with regards to physiology and pathology. In the second part, secondary fungal metabolites will be presented that are used as remedies, in addition to most important mycotoxins. In the third part, studies that analyze different interactions between ABC-transporters and secondary fungal metabolites are presented. At the end, an overall conclusion will be presented with a summary of what has been found in this review.

Abstrakt

ABC-Transporter bekommen immer mehr wissenschaftliche Bedeutung. Erste Beweise wiesen darauf hin, dass diese Klasse von Transportern an der Aufnahme von Nährstoffen beteiligt sei. Ihre Existenz in unterschiedlichen Zelltypen in verschiedenen Organismen wurde aber später bewiesen. In den letzten Jahrzehnten wurde auch gezeigt, dass sie viele andere Rollen haben auβer einfacher Nährstoffaufnahme. Im menschlichen Körper haben sie viele physiologische Rollen, z.B. Transport von Phospholipiden, Chloridkanäle, Lipidmetabolismus, Regulierung der Permeabilität in unterschiedlichen Blut-Gewebe Barrieren; aber auch viele pathologische Erscheinungen resultieren aus Mutationen in ABC-Transportern, die zu Funktionalitätsverlust führen, wie cystische Fibrose, progressive familiäre intrahepatische Cholestase, X-verbundene sideroblastische Anämie und viele andere Erkrankungen. Die Relevanz dieser Transporterklasse in Pharmakotherapie nahm in den letzten Jahrzehnten an Bedeutung zu aufgrund der Beweise, dass sie stark am Transport vieler Wirkstoffe beteiligt sind, von den lipidsenkenden Statinen bis zu Chemotherapeutika, die klinisch in der Therapie von malignen Erkrankungen eingesetzt werden. Die Exprimierung, vor allem Überexprimierung, ist verbunden mit Therapieversagen in vielen malignen Formen. Aufgrund sehr breiter Verteilung im menschlichen Körper und dem breitem Spektrum von Substanzen, die sie über Zellmembranen und Gewebebarrieren transportieren können, hat diese Transporterklasse groβe Bedeutung auch in Metabolismus von Wirkstoffen, inklusive Absoprtion, Verteilung, und Ausscheidung. Weiterhin tragen sie auch den Interaktionen zwischen Wirkstoffen im Sinne der Pharmakokinetik bei.

Es ist lang bekannt, dass im Sekundärmetabolismus von Bakterien, Pilzen, Algen, und Pflanzen eine Groβzahl von biologisch aktiven Substanzen aufgebaut wird. Die Welt der

Pilzorganismen, die über eine Million verschiedene Arten enthält, ist eine groβe Quelle an Arzneistoffen und Mykotoxinen. Obwohl Pilzextrakte in unterschiedlichsten Formen sehr lange in der traditionellen Medizin weltweit eingesetzt werden, begann die arzneiliche Anwendung von reinen Pilzsubstanzen erst im 20. Jahrhundert. Penicilline, eine sehr bekannte und breit eingesetzte Klasse von Antibiotika, wurden nach der Entdeckung von Penicillin G am Ende der 1920er Jahre entwickelt. Andere Antibiotika wie Cephalosporine und Fusidinsäure, sowie das Antimykotikum Griseofulvin, folgten. Die Zeit der Organtransplantationen begann mit der Entdeckung immunsuppressiver Wirkstoffe Cyclosporin A und Mycophenolatmofetil, die einen groβen klinischen Wert auch in der Therapie von Autoimmunerkrankungen haben. Statine, eine der wichtigsten und häufigst eingesetzten Wirkstoffklassen heutzutage, gehen auf die Entdeckung des Pilzmetaboliten Compactin zurück, der aber nie zur klinischen Anwedung eingeführt wurde, und Lovastatin, das erste Statin eingesetzt in der Therapie von Lipidstörungen. Ergotalkaloide bilden eine besonders interessante und groβe Gruppe von Wirkstoffen, die effektiv in der Therapie von verschiedenen Erkrankungen eingesetzt werden, wie z.B. Morbus Parkinson, postpartum Blutung, Hypotonie. Andererseits haben Ergotalkaloide eine lange Geschichte in Bezug auf ihr toxisches Potential, und sind auch Ausgangssubstanzen in der modernen Entwicklung von starken Halluzinogenen. Mykotoxine sind sekundäre Pilzmetabolite, die teilweise mit negativen Gesundheitseffekten verbunden sind. Für die Mehrheit von Mykotoxinen bestehen feste *in vivo* Beweise in Bezug auf Gesundheitseffekte nur in Tieren. Menschen nehmen Mykotoxine meistens durch die Konsumation von Nahrungsmitteln auf, wie z.B. Getreide und ihre Produkte, Milchprodukte, Obst usw., die mit Pilzen und durch sie hergestellte Mykotoxinen kontaminiert sind. Negative Gesundheitseffekte auf Menschen wurden klar bewiesen und stehen fest für z.B. Aflatoxine, insbesondere Aflatoxin B_1, das eine starke hepatotoxische und

hepatocarcinogene Wirkung hat. Was die Gesundheitswirkungen von anderen Mykotoxinen betrifft, viele Unsicherheiten bestehen noch immer aufgrund allgemein nichtausreichenden Beweisen und der Tatsache, dass diese aus *in vitro* Experimenten stammen. Zusätzlich ist es oft schwierig genaue Dosengrenzen bezüglich Gesundheitseffekte zu setzen, weil in vielen Experimenten zweideutige Ergebnisse erhalten wurden – ein limitierender Faktor für die genaue Bestimmung welche Auswirkung die Konsumierung von pilzkontaminierten Nahrungsmitteln auf die Gesundheit hat.

Es ist daher relevant zu erforschen wie ABC-Transporter mit sekundären Pilzmetaboliten als Arzneistoffe und Mykotoxine interagieren. Studien über solche Interaktionen auf unterschiedlichen Niveaus werden in dieser Literaturarbeit präsentiert. Wie sich die genetische Ausstattung von ABC-Transportern auf die Pharmakokinetik von Lovastatin und Cyclosporin A auswirkt, welchen Einfluss ABC-Transporter auf die Absorption, Gewebsverteilung, und Ausscheidung von sekundären Pilzmetaboliten haben, wie sie vor der toxischen Wirkung der Mykotoxine schützen und deren Grad mitbestimmen, welchen Einfluss diese Wechselwirkungen auf den pharmakokinetischen Profil haben, und viele andere Aspekte werden diskutiert.

Diese Literaturarbeit ist aufgebaut aus drei Hauptteilen. Im ersten Teil werden die Entdeckung der ABC-Transporter, ihre Struktur und Wirkmechanismus, und menschliche ABC-Transporter im Sinne der Physiologie und Pathologie präsentiert. Der zweite Teil gibt einen Überblick über sekundäre Pilzmetabolite, die als Arzneistoffe eingesetzt werden, sowie über die wichtigsten Mykotoxine. Die Wechselwirkungen zwischen den ABC-Transportern und sekundären Pilzmetaboliten analysierenden Studien werden im dritten Teil in Detail präsentiert. Eine allgemeine Schlussfolgerung, die aus den identifizierten Studien hervorgeht, wird am Ende präsentiert.

I Introduction and overview of ABC-transporters

1.1 Historical perspective on the discovery of ABC-transporters

The first scientific concepts on ABC-transporters were set in bacterial models which were used to characterize the basic principles of their structure and function. Thorough research was conducted in the 1970s on transporter systems in bacteria that regulate the uptake of vital nutrients with *Escherichia coli* (*E. coli)* and *Salmonella typhimurium* (*S. typhimurium)* as test models. Results showed that the uptake is regulated by different transporter systems, which can be classified into three groups: i) the phospotransferase systems, ii) secondary transporters, driven by energy from the electrochemical gradient, and iii) primary systems, which are shock-sensitive and use energy derived from the hydrolysis of adenosine triphosphate (ATP). The shock sensitivity of primary transporters results from a loss of a substrate-binding protein, located in the periplasm. The gene sequence of such a protein was discovered for the first time in *S.typhimurium* and published in 1982 – the histidine transporter (1). Four different genes were found within the transport operon – hisJ, hisQ, hisM and hisP (2), the first coding for the substrate-binding protein in the periplasm, while products of the other three are attached to the membrane (1). A transporter from *E. coli* responsible for the transport of maltose and maltodextrin, product of the MalK gene, was found to be extensively homologous to the HisP protein in terms of amino acid sequence (about 35%), implying that periplasmic-binding protein type transporters to a great part most probably evolved from a single ancestor (3). In 1985, it was proposed that hisP and malK share great amino acid homology with another protein, oppD, the membrane component of an oligopeptid transporter, and that they all contain a nucleotide binding site (4). The existence of a nucleotide binding site was already recognized in other ATP and adenosine diphosphate (ADP) binding proteins, such as ATP-synthase, myosin, phosphofructokinase and adenylate

kinase (5). In parallel, it was recognized that ATP binding couples to energy delivery for the transport process (4, 6, 7).

It was suggested in 1986 that such bacterial transport proteins with ATP binding sites all belong to a superfamily, characterized by four main structural domains (1). Being highly similar in both structure and function, these transporters are involved in a great number of cellular processes, such as transport across membranes, division of cells, or elimination of haemolysin from the cell (8).

The discovery of eukaryotic ABC-transporters went along. Initial evidence was obtained in the Chinese hamster ovary cell line, and indicated a correlation between transport proteins located in the cell membrane and the occurrence of a phenotype characterized by drug resistance. Treatment of these cells with colchicine resulted in a concomitant resistance against daunomycin and puromycin. In 1979, the responsible protein was purified – a glycoprotein with a molecular weight of 170 kDa. In addition, it was recognized three years later that cells originally sensitive to drug treatment can acquire drug resistance following genetical transfer of desoxyribonucleic acid (DNA) isolated from colchicine-resistant cells. The underlying multidrug resistance protein 1 (MDR1) gene was successfully cloned in 1985 (9). In 1986, human complementary DNA (cDNA) of the MDR1 gene was obtained from a cell line called KB-C2.5, which was characterized by multidrug resistance after selection with colchicine. In 1989, the complementary DNA (cDNA) of the human MDR1 gene was obtained from healthy tissue (10).

Finally, the term ATP-binding cassette transporters was brought to use in 1990 (11), putting basics for future research on this unique and widespread family of proteins.

To date, a variety of ABC-transporters has been discovered throughout a range of species. For instance, 28 different ABC-transporters have been found in *Saccharomyces*, 51 in *Drosophila*, and a total of 69 in *E. coli* comprise an estimated 5% of its genome (12).

1.2 Structure and mechanism of action

1.2.1 Structure

According to their structure, ABC-transporters can be classified in half- and full-transporters (13).

The prototype ABC full transporter consists of four domains - two pore forming transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs) (13). TMDs are responsible for ligand binding and drive the protein's specificity, while NBDs represent the site of ATP binding and hydrolysis, delivering energy needed for the transport process (12). NBDs have a hydrophilic structure and are located on the cytosolic side of the membrane (1).

In the case of half-transporters, only one of each domain is present, and homodimerization, as in ABCG2, or heterodimerization is needed for proper function. Most evolutionary scientists believe that half-transporters were the first to appear, followed by full transporters which developed through multiplication. Additional domains have been discovered in some ABC-transporters, such as ABCC7, which most likely exert regulatory functions (13).

The TMDs are not necessarily homologous in their sequence or structure across the family of ABC-transporters. Their variability reflects on the very broad range of substrates that are transported (14). Typically, these domains are embedded in the membrane multiple times in form of α-helices, forming the substrate translocation pathway (1). Some α-helices can also serve adjunct functions such as insertion in the membrane bilayer or have a regulatory function (1).

According to the structural organization of the TMDs, ABC-transporters can be generally divided into three groups — ABC exporters and Type I and II ABC importers (14).

ABC exporters have been discovered in sequences of all currently known genoms. Sav1866 from *Staphylococcus aureus* (*S. aureus*) was the first ABC exporter with identified crystal structure, depicted through high-resolution technique (15). This bacterial model was shown to have 6 membrane spanning helices per TMD. Therefore, a total of 12 α-helices are found in a full transporter (14).

ABC importers Type I mediate the uptake of vital nutrients such as amino acids and sugars after their binding and delivery by special proteins (15). Various models have been used to describe the architecture of TMDs in this transporter type; such as the TMD of the molybdate transporter (ModB), the first model to be used; followed by TMDs of the maltose transporter (MalF and MalG) and the methionine transporter (MetI). The number of putative α-helices per TMD is not universal across the group; it ranges from 5 in MetI to 8 in MalF (14).

ABC importers Type II are used to absorb larger molecules, e.g. metal chelates such as heam or Vitamin B_{12}. A Vitamin B_{12} transporter from *E. coli*, called BtuCD was the first model used for structural characterization of this transporter class, followed by the homologue transporter from *Haemophilus influanzae* (*H. influanzae)*, the HIF protein (15). Data retrieved from their structural analysis suggest that each TMD consists of 10 helices, or 20 for a full transporter (14, 15).

The periplasmic binding protein (PBP) is a common subunit in many ABC-transporters. It can be located in the periplasm, as in the case of gram negative bacteria, or linked to the external

side of the membrane by lipid structures in outer membrane and periplasm lacking gram positive bacteria. It has been recognized that PBPs are not only responsible for solute binding and delivery; they also contribute substantially to specificity and affinity of substrate transport through a mechanism still unknown. In addition, the presence of PBPs corresponds absolutely with the inwards-directed nature of substrate transport (1).

The NBDs represent the conserved part of ABC-transporters. Initial findings have suggested that these hydrophilic domains are embedded in the membrane bilayer and reach the extracellular side. However, data from novel findings have proven that they are peripherally attached only on the cytoplasmic side of the membrane (1).

The structure of NBDs is divided into two sub-domains. The catalytic sub-domain contains several typical motifs, including the Walker A motif (also called P loop), Walker B motif (with four hydrophobic residues), H motif, and a Q loop. The α-helical sub-domain has a typical LSGGQ motif, also called ABC signature (14), which stands for the single letter code of the constituting amino acids (15). While the Walker A and B motifs occur also in other proteins that bind nucleotides, other, such as the ABC signature and the H loop are characteristic only for ABC-transporters. It was shown in eukaryotic P-glycoprotein (P-gp) that the two NBDs are equally involved in binding of ATP, as mutational changes in one of the domains results in failure to mediate drug efflux from cells and hydrolyze ATP, indicating that the NBDs are coupled to each other in allosteric manner (12). Therefore, the presence of both NBDs is needed for proper hydrolysis of ATP (1).

The conserved regions of the NBD pair are oriented to each other in a “head-to-tail” manner in a full ABC-transporter. Specifically, the Walker A motif of one sub-domain faces the LSGGQ signature motif of the other. This way, two nucleotide binding sites are formed in the common interface. In the catalytic stage, ATP is pushed into a sandwich between the two

NBDs and the interface site is closed (15). Precise structural characterization of the NBDs in the catalytic state has been hard to achieve, because they form the complex for a very short time in physiological environment to exhibit their hydrolytic activity. Several models have been used in order to obtain more detailed information, such as the hemolysin exporter HlyB or the MJ0796 transporter. It was shown that two ATP molecules are captured in the common interface of the closed NBD dimeric conformation, suggesting that NBDs function in synchronization. These findings have been confirmed in diverse other models, e.g. GlcV, MalK, MutS, and Rad50 (12).

The ways in which the four core domains are attached to each other to form a functional transporter can vary between the diverse ABC-transporters (Figure 1) (1). In the ABC importer BtuCD, single polypeptides form all four domains (14). In a great part of eukaryotic ABC-transporters all domains are located on a single polypeptide chain. The transporter can also have one domain type located on a single polypeptide chain (1). In Sav1886 model from *S. aureus*, two separate polypeptide chains were found, each containing a single sample of the two domain types (14). Finally, an ABC-transporter from *E. coli* called YhiGHI encompasses three polypeptide chains – one chain contains a TMD and a NBD domain, while the other two chains contain only one domain type (1).

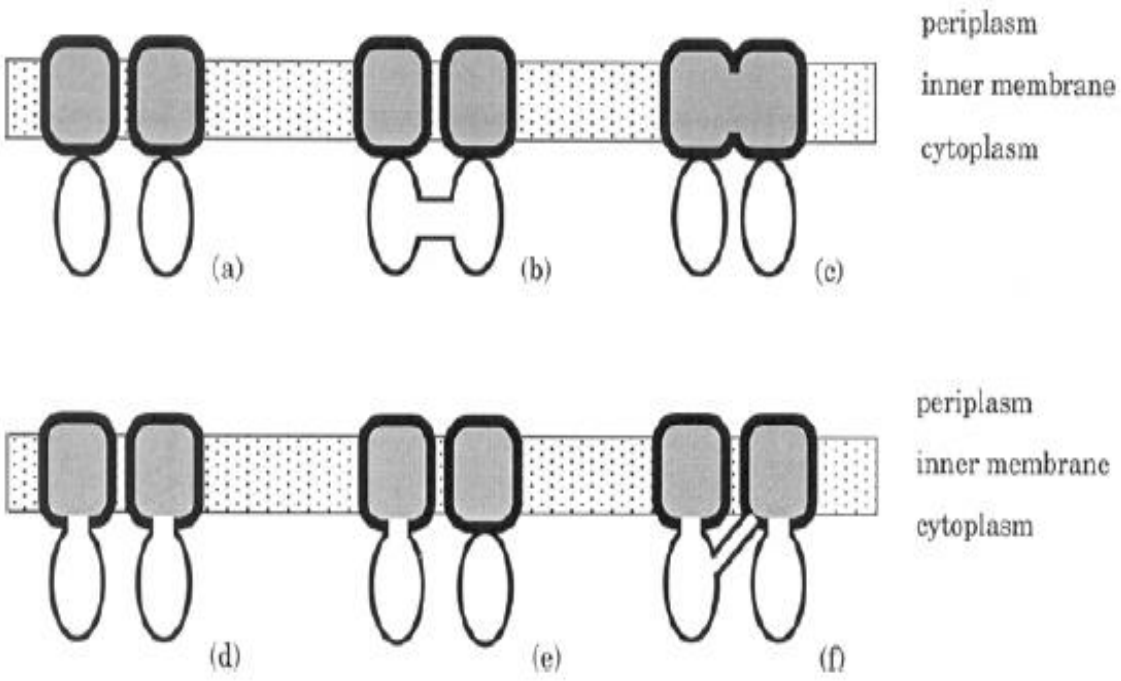


Figure 1: Structure of ABC transporters. There are typically four domains – two TMDS (shown in shaded forms) and two NBDs (shown in white ovals on the intracellular side of the cell membrane). These can be arranged in different ways: (A) each domain is an individual polypeptide chain, NBDs (B) or TMDs (C) are fused together, (D) two separate chains, each containing a TMD and NBD, (E) one chain containing a TMD and NBD, with two other domains on individual chains, (F) one polypeptide chain containing all four domains, a common combination in eukaryotes (1).

Despite the discovery and structural characterization of many sub-domains, successful identification of the complete architecture of an ABC-transporter has been challenging to accomplish. The eukaryotic P-gp is a pioneer in this field - its overall structure has been clarified using techniques like single particle imaging or cryoelectron microscopy, the later providing more details. It was recognized that the TMDs are oriented in a manner which generates a hydrophilic pore larger than the one seen in other ATP binding proteins. An additional characteristic is the tight binding between NBDs and TMDs, as oppose to their separation seen in e.g. Ca^{2+} ATPase (1).

Clarification of the nature of ABC-transporter into greater details requires a multidisciplinary approach, including biophysics, structure analysis, biochemistry and physiological aspects.

Detergents can theoretically be used to isolate ABC-transporters from the lipid structure of the membrane in preparation for protein crystallization. However, they can also induce conformational changes in the isolated protein. It is a particularly challenging task to obtain ordered three-dimensional crystal structures of ABC-transporters, given their remarkably dynamic nature. However, structural characterization is an essential step in enhancing our understanding on the functional properties of ABC-transporters (15).

1.2.2 Mechanism of action

There is still a poor understanding of the exact mechanism of action of ABC-transporters (1). The most thourough characterization to date has been achieved for P-gp. Pharmacological analysis looking into drug affinity has provided the basic model to explain how these compounds are transported by P-gp (12). Similar mechanisms of transport seem to exist between ABC-transporters found in prokaryotes and mammals (1).

The existence of substrate binding sites characterized by different affinity towards the substrate has been suggested. In the case of ABC exporters, the extracellular site normally exerts low affinity, whereas the intracellular site binds substrates with high affinity. The transport begins with substrate binding by TMDs on the cytoplasmic side of the membrane (1). This initial step induces conformational changes in the TMDs, which further results in higher affinity for nucleotide binding in the NBDs. The communication flows along the intracellular loops of TMDs, which are positioned close to Walker A motifs and Q loops of NBDs. Although substrate binding appears to be correlated with higher NBD affinity for ATP, there is still weak evidence for a well-established correlation (12).

In some cases, energy necessary for the conformational changes in the TMDS has been demonstrated to come from the nucleotide binding itself. Binding of two ATP molecules leads to altered conformation of NBDs, pushing them into a closed form. This step has been

suggested to deliver sufficient energy for conformational changes in TMDs. This assumption originates from several models, e.g. from P-gp and ABCC7. The changes are so large that the common architecture of TMDs is fractioned and new interactions generated (12). Altered NBD conformation following the nucleotide hydrolysis is communicated to TMDs. Generally, these conformational changes affect the affinity of substrate binding sites. In case of ABC-transporters which act as exporters, reduced affinity of the intracellular binding site induces substrate transport outside the cell (1).

The hydrolysis of ATP is generated directly through NBD dimer building (12). The functionality of both NBDs is required for the hydrolysis of ATP. Yet, only one subunit hydrolyzes a molecule at a time, i.e. the NBDs do not perform their catalytic function simultaneously (1). The number of hydrolyzed ATP molecules per translocation cycle is often two, as in P-pg. However, energy derived from hydrolysis of a single ATP molecule can be enough for some ABC-transporters to engage into conformational changes, as in the case of ABCC1 and ABCC7. The hydrolysis of ATP shifts the NBD dimer into an unstable conformation. After the elimination of phosphate from the pocket, the conformation is reversed back to the stable form. The remaining ADP binds to NBDs with very low affinity. Analysis data of P-gp suggest that substrate binding sites are sensitive to conformational changes once phosphate has been eliminated, pushing them back into the high affinity substrate binding state (12).

The mechanism of action is not very different in bacterial ABC importers. The initial substrate binding by TMDs occurs on the extracellular side, once it has been captured by the PBP subunit. Substrate uptake into the cell is also regulated by PBPs, which mediate affinity reversal in the substrate binding sites (1).

1.3 Overview of human ABC-transporters - physiology and pathology

To this date, the human genome was found to contain a total of 49 different ABC-transporter genes (18). Multiple examples of pseudogenes have been discovered in the human genome (16). The entire family of ABC-transporters has been divided into seven sub-families (ABCA-ABCG) (16, 17, 18, 19). Phylogenetic analysis was applied to obtain this nomenclature (19). The division into sub-families arises from genetic similarities, organization of the domains, and homology in the sequence of the two main domain types (17).

1.3.1 ABCA Subfamily

The ABCA subfamily consists of two different groups, with 12 different full-transporters identified. ABCA1-4, A7, A12, and A13 form one group, and are products of genes located on multiple chromosomes. The second group, comprising the transporters ABCA5, ABCA6, ABC8-10, is completely encoded by genes located on chromosome 17q24 (17).

The **ABCA1** transporter is most likely located in the cell membrane, containing a large number of sugars in its structure (17). In accordance with its expression, which is stimulated by sterol molecules and nuclear hormone receptors (20), the importance of ABCA1 is principally set in the metabolism of lipid molecules (17, 20). Its physiological role presumably consists of mediating the efflux of cholesterol and phospholipids to lipid poor apolipoproteins, which then form nascent high-density lipoprotein (HDL) (17). Consequently, transporter deficiency is associated with reduced HDL levels, as demonstrated in mice. However, the exact role of ABCA1 in the lipid transport process still remains unclear. Because this transporter has an effect on the levels of lipids contained in the membrane, it is clearly involved in determining membrane's fluidity. This further reflects on the proteins residing in the membrane in terms of their mobility and bonding with membrane lipids. On

molecular level, human ABCA1 seems to require magnesium ions to bind ATP, and has only week hydrolytic activity at baseline. Its hydrolytic activity does not change even after adding its typical lipid ligands. Upon this, ABCA1 has been discussed to have a rather regulatory activity or to require other assisting molecules for its complete function (20).

Several pathological disorders have been associated with mutations found in ABCA1 transporter. Tangier disease, characterized by abnormal transport of cholesterol between peripheral tissues and the liver, is an example (20). Defects in ABCA1 cause a disruption of lipid removal from macrophage cells, leading to early onset of atherosclerotic changes and reduced levels of HDL circulating in the vessels (17). In addition, mutations in ABCA1 have been identified in patients suffering from other lipid disorders, such as familial hypoalphalipoproteinemia, and non-Tangier low HDL levels (20).

ABCA2 is found in brain cells, particularly in oligodendrocytes. Based on its similarity to ABCA1, the ABCA2 transporter is probably involved in the lipid metabolism of nerve and glia cells. The protein can also transport cytotoxic drugs from the cell, as demonstrated in ABCA2-overexpressing ovarian cancer cell line (20).

ABCA3 is primarily expressed in lung tissue, and has recently been associated with the formation of surfactants, which play an important role in lung function in newborns, as well as in adults (20).

The **ABCA4** transporter is expressed only in the eye retina (17), where it has been proposed to transport retinol and phospholipid substrates from the external segment disks of the receptors into the cell (20). The ATPase activity of ABCA4 is stimulated by the aforementioned molecules, as shown in purified transporter. A number of recessive pathological disorders have been recognized to result from mutations in ABCA4. These

include retinitis pigmentosa, caused by fully dysfunctional ABCA4 transporter; as well as Stargardt disease, with partial loss of transporter function; and cone rod dystrophy. Mutations in ABCA4 are additionally associated with increased risk of age-related macular degeneration. It has been discussed that the pathological accumulation of retinoid compounds in epithelial structures posterior to retina is one of the mechanisms occurring at initial stage of the disease (20).

The function of all other transporters of the ABCA subfamily remains unrevealed. The range of their localization throughout the human organism is wide – organs of their localization include the liver, pancreas, testes, muscles, heart, etc. (20).

1.3.2 ABCB Subfamily

The ABCB subfamily can be found only in mammalians. The family is generated by a total of 11 genes that encode four full and seven half-transporters (18). A variety of functions is exerted by transporters of the ABCB subfamily. Besides drug transport, they are involved in transport of iron, and peptides which play a role in presentation of antigen structures. Apart from the plasma membrane, these transporters reside within membranes of different cell vesicles, such as mitochondria and lysosomes (17). Although requiring further clarification, mutational changes and subsequent defects in ABCB transporters have been associated with a number of pathological disorders, ranging from lethal neonatal syndrome to X-linked sideroblastic anemia with ataxia (18).

ABCB1, also known as multidrug resistance protein 1 (MDR1) or P-gp was the very first ABC-transporter recognized in human cells. Initially characterized as a determinant of multidrug resistance in malignant cells, ABCB1 is the best described ABC-transporter today.

Compounds transported by ABCB1 are typically hydrophobic. Many of them are natural cytotoxic products from different groups, such as anthracyclines (doxorubicin) or alkaloids (colchicine, vinblastine) (20). Apart from cancer cells, ABCB1 is located in a variety of normal cells with secretory function, such as in the intestine (epithelial cells), liver (bile canaliculi cells), kidneys (proximal tubular cells), and brain (cells of the endothelium), where it is responsible for the elimination of cytotoxic substances and metabolic products (17). ABCB1 has also been discovered in large numbers in hematopoietic stem cells, where it is assumed to exert the same, protective function. An additional function of this protein is given in the blood-brain barrier. There, ABCB1 seems to be responsible for the transport of compounds that cannot access brain cells through simple processes, like diffusion (20).

The presence of half-transporters **ABCB2** and **ABCB3** is necessary for the formation of major histocompability complex I (MHC I) (20). Hence, they are also referred to as transporters associated with antigen presentation (TAP) 1 and 2 (21). Embedded in the endoplasmic reticulum membrane, they are responsible for translocation of peptide compounds from the cell cytoplasm into the reticulum. The peptides can further be presented as part of the major histocompability complex I (MHC I) (17). This function is exerted once the half-transporters have assembled to form a fully functional heterodimer complex. This complex is assumed to have particularly high specificity for peptides consisting of 9-12 amino acids. Deficiencies in TAP-1 may lead to impaired presentation of antigen structures, and scarce MHC-I molecules and cytotoxic (CD8+) T-cells, as shown in experiments conducted in mice. Functionality of the TAP complex can be disturbed by certain compounds generated in Herpes simplex, and other DNA-viruses. Cancer cell lines and examples of hereditary impaired immune function have also been found that display mutational changes in TAP (20).

ABCB4 is a full transporter. It shows strong homology with ABCB1, and is genetically located on the same chromosome. Despite functional disparities, these two genes developed through duplication. The ABCB4 transporter is primarily expressed in cells along the bile canaliculus in the liver. However, it is also seen in other cells and organs, including the heart and B-lymphocytes. Functionally, this ABC-transporter seems to be involved in the transport of phosphatidylcholin. Mutational changes in the ABCB4 gene lead to progressive familial intrahepatic cholestasis (PFIC) 3. Moreover, they are correlated with intrahepatic cholestasis of pregnancy (20).

ABCB5 is an ubiquitously found full transporter, and its function still remains unclear (20).

ABCB6 and **ABCB7** are similar proteins in terms of structure (half-transporters), expression site (inner membrane of cellular mitochondria), and they both transport metal-containing compounds. ABCB6 and ABCB7 are believed to play a role in the export of an iron complex and heme from the mitochondrium, respectively (17). Both transporters have been shown to exert a complementary function in yeast, which, due to a deficient ABC gene displays impaired cytosolic translocation of a forerunner of the Fe/S complex (20). Mutational changes in both transporters are linked to hereditary diseases. Mutations in the ABCB6 gene can lead to lethal metabolic syndrome (17), and in ABCB7 to X-linked sideroblastic anemia with hypochromic and microcytic red blood cells, and ataxia of the little brain, which typically have an onset already at early life stage (20).

The function of **ABCB8** and **ABCB10**, which are found in mitochondria, and of **ABCB9**, found in lysosomes, still remains unclear (20).

ABCB11 is a full transporter, and can be found mainly in the liver cells along the bile canaliculus, where it is involved in the secretory transport of compounds typically found in the bile. Mutational changes in this ABC-transporter are seen in cases of PFIC 2 (20).

1.3.3 ABCC Subfamily

This family comprises a total of 12 different transporters, which show structural similitude, as they are all full transporters, but vary strongly in terms of their specific functions (17).

ABCC1, also called multidrug resistance-associated protein 1 (MRP1) can be found in cancer cells. It was originally found in NCI-H69, a small cell lung carcinoma cell line. These cells exhibited resistance to multiple drugs not based on P-gp overexpression. The encoding gene is located near to the ABCC6 gene, and it is clear that they were generated by duplication. Like P-pg, ABCC1 is involved in the elimination of a wide spectrum of cytotoxic compounds from the cell, such as doxorubicin, colchicine, vincristine etc. (20). The primary ligands for ABCC1 are organic anions, and they are often transported in conjugated form with glutathione (GSH), glucuronate, and sulfate groups. ABCC1 can also couple the transport of substrates with GSH in unconjugated form (17). A physiological substrate of ABCC1 seems to be leukotriene C4 (LTC4), which plays an important role in neuronal dendrites, and it was demonstrated that their migration can be impaired in ABCC1 deficient mice (20).

ABCC2 (MRP2) is found in apically directed membranes of epithelial cells in the liver, intestine and kidney. There, it is primarily involved in elimination of toxic substances (17). Similar to other ABC-transporters, the main expression site of ABCC2 in the liver are cells localized along the bile canaliculus. Here, it eliminates organic anion compounds from the hepatocytes into the bile. Initially, the nature of substrates for ABCC2 was characterized in the TR-rat, which had impaired transport of charged organic compounds and icterus (20).

Mutational changes in the transporter were later also discovered in humans suffering from the Dubin-Johnson syndrome. This syndrome is characterized by an impaired elimination of conjugated anionic compounds into the bile, resulting in severe conjugated hyperbilirubinemia. The transporter also seems to be associated with multidrug resistance in cancer cells (17).

ABCC3 (MRP3) expression sites are similar to those of ABCC2 (17), with liver being the main organ of expression (20). However, opposite to ABCC2, this transporter is embedded in the basolateral side of cellular membranes. The primary substrates are organic anion compounds, which are often transported in conjugation with glucuronate moiety (17). This transporter is also associated with drug resistance (20).

ABCC4 (MRP4) is found weakly expressed in a variety of cell types (20). Although its physiological role remains unrevealed, the therapeutic relevance of this ABC-transporter lies in its ability to transport nucleoside analogues, an important class of compounds used in e.g. treatment of human immunodeficiency virus (HIV) infections, such as azidothymidine monophosphate (17).

ABCC5 is commonly found in different tissues (17, 20). Its physiological role is still uncertain. However, this transporter has therapeutic relevance similar to the one of ABCC4, as it can mediate the elimination of nucleoside analogues from cells, used in treatment of virus infections and hematopoietic malignancies (17).

ABCC6 is primarily found in the liver and kidney. GSH-conjugated compounds are suggested to be a substrate of ABCC6. Mutational changes in ABCC6 are associated with the recessive hereditary disease pseudoxanthoma elasticum. Skin with calcificated fibers and hemorrhage in

the eyes, along with pathological changes in the cardiovascular system are the main clinical characteristics of this disorder (20). Interestingly, the affected organs and tissues are not the primary organs of ABCC6 expression, implying that the transporter has a rather indirect role in the etymology of the disease (19).

ABCC7 (cystic fibrosis transmembrane conductance regulator - CTFR) is not a typical ABC-transporter – the protein is a cyclic adenosine monophosphate (cAMP)-dependent chloride ion channel (19). Organs with exocrine activity, such as kidneys, pancreas and intestinal system, are principal expression sites of ABCC7. In addition, unlike other ABC-transporters, ABCC7 has a special domain that can be phosphorylated and regulates the protein's function (20). The pathological relevance of ABCC7 is related to cystic fibrosis. Clinically, the disorder primarily affects the exocrine activity in organs with high levels of expression. It is characterized by abnormally high content of electrolytes in the sweat, frequent infections of the broncho-pulmonary system, intestinal abnormalities (meconium ileus) (17), impaired pancreas activity resulting in deficiencies in important nutrients, and defects in vas deferens resulting in infertility in men (19). In terms of prevalence, cystic fibrosis is most commonly seen in populations with Caucasian background, where every 1 in 2500 to 900 individuals is affected. In contrast, those with African and Asian background have much lower prevalence (20).

ABCC8, also called sulfonylurea receptor 1 (SUR1) is another unique ABC-transporter, as its primary function is not the transport of substrates, but regulation of the ATP-sensitive potassium channel. A total of four SUR1 proteins join with the same number of pore-generating Kir6.2 subunits to form the ion channel. A variety of cell types are equipped with such potassium channels, which join their metabolic pathways with electrical impulses. Functionally, SUR1 can exert hydrolysis of ATP molecules in its nucleotide binding pocket,

which increases the likelihood for the potassium channel to open. In opposite, increases in cellular ATP levels result in closing of the potassium channel, as ATP binds to Kir6.2 subunit (22). Beta cells located in the pancreatic isles, which are responsible for insulin synthesis and secretion (23), are an important cell type with such potassium channels. The regulatory unit SUR1 in these pancreatic potassium channels is directly involved in the regulation of insulin secretion. Sulfonylurea compounds are drugs that bind with high affinity to SUR1 and subsequently lead to closing of the potassium channel, resulting in stimulation of insulin secretion. Thus, they are used in patients with non-insulin dependent diabetes mellitus (type II). Mutational changes in SUR1 are associated with abnormalities in regulation of blood insulin and glucose levels. Hyperinsulinism of infancy is a condition with early onset, characterized by defect insulin secretion, resulting in elevated levels of the hormone even in hypoglycemia. This disorder can be dangerous as it can irreversibly harm the brain if not treated. Moreover, mutations causing a defect in activation of SUR1 are associated with neonatal diabetes (22).

ABCC9 (SUR2), a protein similar to ABCC8, is primarily expressed in muscle tissue. Opposite to SUR1, sulfonylurea compounds show only low affinity for SUR2 (20).

The function of **ABCC10**, as well as **ABCC12,** still remains unknown and requires further elucidation (20).

ABCC11 could be pathologically relevant, as it was found that the transporter is overexpressed in leukemic T-cells which are resistant to treatment with nucleoside compounds (20).

1.3.4 ABCD Subfamily

This subfamily comprises four different half-transporters. To become fully active they have to build heterodimer or homodimer complexes (17). It is suggested that transporters of this subfamily are involved in the regulation of cellular lipids, and like ABCA and ABCG transporters, they are oriented on the peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor family (19).

ABCD1 gene encodes a protein expressed in peroxisomes, where it presumably plays a role in the transport of very long chain fatty acids (VLCFAs). Mutational changes in ABCD1, most of which are point mutations, are associated with adrenoleukodystrophy (ALD). The X-linked form (X-ALD), which becomes apparent in late childhood stage, is a recessive hereditary disease with deficiencies in adrenal glands and typical degenerative processes affecting the nervous system. ALD can also have an onset in early infancy, as deletion mutations in ABCD1 together with another gene have been found in newborns with the so called contiguous ABCD1 DXS1357E deletion syndrome. Overall, ALD is characterized by unbranched fatty acids with chains between 24 and 30 carbon atoms in length, which amass in cerebral cholesterol esters and adrenal cortex. A therapeutic strategy for ALD based on the use of erucic and oleic acid in combination (Lorenzo's oil) turned out to be successful in bringing blood VLCFAs concentrations into normal range, but failed to substantially decelerate the overall progression of ALD, most likely due to incapability to reduce the cerebral levels of fatty acids (20).

ABCD2 is very similar to ABCD1, as they overlap strongly in their amino acid sequences (66% homology) and patterns of exons and introns. The transporter is located in peroxisomes, and can be primarily found in brain and cells of the adrenal glands. It has been shown that high levels of ABCD2 expression in individuals suffering from X-ALD can reconstitute the

defect cellular mechanism of peroxisomal beta oxidation in fibroblasts. In addition, fibrates, compounds used therapeutically in patients with lipid disorders, can induce ABCD2 gene in a process that requires peroxisome proliferator activated receptors, which possibly presents a novel strategy in treatment of X-ALD (20).

ABCD3 is also a peroxisome-located transporter. Mutational changes in ABCD3 were discovered in some individuals suffering from Zellweger syndrome. However, significant correlation between ABCD3 and this disease could not be detected so far (20).

ABCD4 is a peroxisomal protein, whose amino acid sequence shows a certain overlap with all other transporters of the family (25-27%) (20).

1.3.5 ABCE and ABCF Subfamily

Genes coding for transporters of ABCE and ABCF subfamily solely encode their NBD domains. Hence, transporters of these subfamilies lack TMDs. Despite this remarkable difference, their NBD domains are typical for ABC-transporters and thus, they belong to the transporter superfamily. All transporters of these two subfamilies are located in the cytosol, and not in cellular membranes (20).

ABCE1, which is the only transporter in the subfamily, can spot oligoadenylate, which is produced in reaction to infections with some viruses (19). The transporter is also capable of inhibiting the interferon-dependent ribonuclease RNase L. Additionally, the transporter also seems to play a role in formation of HIV capsids (20).

The **ABCF** subfamily comprises three different transporters, and their exact function still remains unknown. The ABCF1 gene is located within the human leukocyte antigen 1 (HLA I)

complex, and underlies an activation by tumor necrosis factor α (TNF α). A protein product of the GNC20 gene identified in yeast *Saccharomyces cerevisiae*, which is similar to human ABCF1, is necessary for the phosphorylation of elF2, a factor needed to initiate protein translation. In humans, ABCF1 protein joins with ribosomes, and can be obtained together with elF2 in purified form, implying that its role in humans could be similar (20).

1.3.6 ABCG Subfamily

The ABCG subfamily consists of six half-transporters. All six members have uniquely ordered domains, as the NBDs are localized at the N-terminus, and the TMDs at the C-terminus of the protein (20).

The **ABCG1** gene shares great homology with the "white gene" in *Drosophila*. The fly gene codes for a protein that is part of a molecular complex responsible for the transport of guanine and tryptophan, compounds that serve as forerunners of ocular pigment in *Drosophila*. It is suggested that ABCG1 and ABCG4 genes formed through duplication, as they are the only members of this subfamily that show certain intron similarities. ABCG1 is found in macrophages, where its gene can be induced by cholesterol molecules (20). This protein is increased after acetylated low-density lipoprotein (LDL) treatment, and down regulated following treatment with HDL-3 at both mRNA and protein levels in monocyte-derived macrophages.

This implies that ABCG1 plays a role in eliminating cholesterol from cells. In addition, two different promoter regions in ABCG1 genes were shown to be sensitive to hydroxycholesterol and retinoic acids in macrophage cells (20).

ABCG2 (breast cancer resistance protein - BCRP) is a half-transporter which becomes fully functional in the form of a homodimer. The ABCG2 gene was first discovered in cells that

exhibited resistance to mitoxantrone, but were not characterized by overexpression of ABCB1 or ABCC1. ABCG2 typically occurs in cells resistant to anthracycline class of chemotherapeutics. Additionally, transfection with the ABCG2 gene results in cells resistant to chemotherapeutic agents. ABCG2 transporter is abundantly found in placental trophoblasts, where it is supposed to deliver substances to the fetus or eliminates harmful toxicants. The transporter is also found in intestinal cells. Amplified ABCG2 gene was discovered in mice lacking other genes typically associated with drug resistance (e.g. Abcb1, Abcc1) following treatment with mitoxantrone, topotecan, or doxorubicin, which is indicative of the ABCG2 relevance in the context of cellular drug resistance development in mammals (20).

The **Abcg3** gene is primarily expressed in mouse hematopoietic cells and no equivalent is found in humans (20).

ABCG4 is particularly found in the brain, but specific forms that result from alternative transcription ways have been identified in hematopoietic cells, as well as in the lung. Just like in the case of the closely related ABCG1, oxysterols and retinoid compounds can lead to induction of the ABCG4 gene (20).

ABCG5 and **ABCG8** gene are located adjacently on the same chromosome (2p21). Mutations in these two genes are pathologically relevant in familial sitosterolemia, characterized by an impaired transport of steroid compounds (cholesterol, plant and fish steroid compounds). It is suggested that the two half-transporters unite in a heterodimer complex which mediates the transport of sitosterol, since equally increased levels of sitosterol have been found in patients carrying only one mutated gene. However, Asian and Caucasian individuals affected by this disorder have been shown to carry only one mutated gene (ABCG5 and ABCG8,

respectively), which strongly suggests that individual functions are also attributable to these transporters (20).

II Secondary fungal metabolites – Remedies and toxins

It is presumed that the fungal kingdom comprises more than a million different species inhabiting diverse environments, which contributes to diversity in their primary and secondary metabolism (24).

Unlike ubiquitous compounds such as amino acids, lipids, and carbohydrates, that are products of the primary metabolism and are essential for maintaining cellular function and growth, secondary metabolic pathways generate a wide range of different and complex compounds that are principally not crucial for the cell or the organism. Prominent examples of secondary metabolic products include terpenoid compounds, alkaloids, and phenyl propane derived substances as well as the related phenols (25). So far, the biological significance of the secondary metabolism for the producing fungi is still largely unexplained. However, it is suggested that the generated compounds could have a protective role against other potentially constraining organisms, assuring a safe habitat this way. In addition, potential functions in parasitic and symbiotic interactions are under research (24).

Biological activity is frequently given for products of secondary fungal metabolism, and the compounds mostly have low molecular weight. A secondary fungal metabolite is mainly specific for a fungal taxonomic group. Thorough research on secondary products generated in fungi started in the 1920s. In mould, at least 200 different compounds were characterized through work guided by Harold Raistrick. But, the actual focus on compounds generated in fungal organisms started to increase only after penicillin has been discovered. With growing

pharmaceutical interest in natural fungal and other microbial metabolites, several thousand substances have been found that have potent biological functions in a wide range of cells, reaching from bacteria to human cancer cells. With respect to fungal compounds, at least 750 those discovered during 1993 – 2001 period are biologically active against bacteria, fungi or tumor cells. Secondary fungal metabolites can be characterized based on their biosynthesis and accordingly grouped into e.g. polyketides, which include aflatoxins and lovastatin; non-ribosomal peptide compounds, like cyclosporine; terpenes, such as aristolochenes and trichothecenes; and indole alkaloids, such as ergotamine. An additional classification considers the biological activity and consequent potential use of fungal metabolites, giving rise to pharmaceutically used compounds and mycotoxins (26).

An overview of important secondary fungal metabolites is given below following the later classification.

2.1 Pharmaceutically used secondary fungal metabolites

2.1.1 Antibiotics

2.1.1.1 Penicillin G

Alexander Fleming, doctor at St. Mary's Hospital in London, set basis for the discovery of penicillin in 1929 as he noticed that a Petri plate containing a *S. aureus* culture had a bacteria free zone surrounding an area contaminated with mold. For further elucidation of the phenomenon, he isolated and cultured the spotted mold and observed repeatedly the same inhibitory effect of the mold culture on bacterial growth. Thus, a certain mold compound was suggested to be responsible. The growth inhibitory effect was not observed only for *S. aureus,* but also for other staphylococci, gonococci, meningococci, pneumococci, *Corynebacterium diphtariae* etc. In contrast, *E. coli, Proteus vulgaris, H. influenzae, Pseudomonas pyocyaneus* along with other bacterial strains were completely resistant. Further toxicological experiments

conducted in animals underscored the potential of the compound for therapeutic use. The substance was named penicillin, as the producing mold was identified to belong to the *Penicillium* genus. Given its instability and production difficulties, penicillin provoked only small interest in the decade to follow. The effect of penicillin was described as selectively bacteriostatic by Fleming in several publications. In 1932, under leadership of Clutterback, methods for scaled up cultivating the producing mold were found, and the chemical nature of penicillin was described. In early 1940s, Florey et al. confirmed the therapeutic effectiveness of penicillin, acknowledging its toxicity to be very low in comparison to other antibiotics, and revealed new extraction and purification techniques. These promising findings excited enormous interest in penicillin, and a massive production was started in American pharmaceutical companies in collaboration with research and laboratory centers (27).

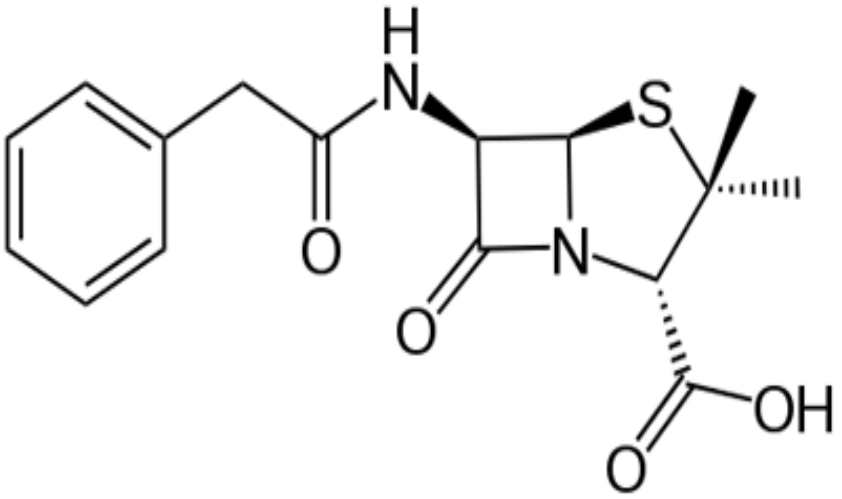


Figure 2: Chemical structure of penicillin G (28).

Penicillin G was the first clinically used antibiotic from the large group of β-lactams. Thorough research has led to identification of 6-aminopenicillan acid as the basic chemical component, opening the possibility to produce semisynthetic, improved penicillin antibiotics. Generally, 6-aminopenicillan acid has low chemical stability, and consists of a thiazolidine and a β-lactam ring, a feature necessary for the antibiotic activity. The antibiotic structure can be broken down if it is exposed to the enzyme β-lactamase, which leads to activity loss, and

amidase, through which 6-aminopenicillan acid is generated and further used for semisynthetic antibiotics (29).

The mold observed by Alexander Fleming was *Penicillium notatum*. A large-scale industrial production of penicillins nowadays is using primarily the fungal strains *Penicillium chrysogenum* and *Acremonium chrysogenum*. Understanding of regulatory mechanisms for biosynthesis of β-lactam antibiotics has been achieved by studying the model fungus *Aspergillus nidulans* (29).

The biosynthesis of penicillins begins with three amino acids – L-α-aminoadipinic acid (L-α-AAA), L-cysteine and L-valine. In molds, L-α-AAA is generated in a specific lysine biosynthesis pathway, and it can also be formed in a lysine catabolic pathway that is yet not characterized. The three amino acids join to form the tri-peptide molecule δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV), a step catalyzed by the enzyme ACV-synthase. The linear ACV chain condensates to form a bicyclic ring, consisting of a β-lactam and a thiazolidine ring. This cyclic molecule shows low antibacterial activity and is called isopenicillin N; the step is catalyzed by the enzyme isopenicillin N-synthase. The last biosynthetic step consists of replacing the hydrophilic L-α-AAA rest by a hydrophobic acyl group, which is performed by the enzyme acyl coenzyme A isopenicillin N-acyltransferase. By adding phenylacetic acid into the fermentation broth, the biosynthesis is directed to produce penicillin G (29).

The antibiotic effect of penicillin G is achieved by docking on penicillin-binding proteins, which are localized in the cell wall of the bacterium, where they play a role in its formation. Specifically, penicillin G disrupts the last step in cell wall formation, causing breaking down of the bacterial cell introduced by autolytic enzymes, e.g. autolysins. In addition, penicillin G potentially interacts with an inhibitor of autolysins (30).

2.1.1.2 Cephalosporin C

In 1945, Guiseppe Brotzu from the Institute of Hygiene in Sardinia, Italy isolated *Cephalosporium acremonium* from a surprisingly pure seawater sample. It was later confirmed that the fungus produces an antibacterial compound, which led to its initial therapeutic use in raw extract form in patients suffering from staphylococci and streptococci infections. Soon thereafter, Florey et al. suggested that it consists of several different antibacterial compounds. At first, cephalosporin P was identified, which was later recognized as a mixture of multiple related compounds. The identification of cephalosporin N followed soon. This substance showed low stability and had a broad spectrum of activity that corresponded with the one demonstrated by Brotzu, and was later on recognized as a new penicillin antibiotic by Abraham et al. who therefore named it penicillin N. Research aimed at structural characterization of the compound led to the discovery of cephalosporin C, a by-product that turned out to be fairly stable as it was quite resistant to β-lactamase. The chemical structure of cephalosporin C was identified in 1961, and although the compound led to development of four generations of semisynthetic cephalosporins, it was never brought to market for clinical use as antibiotic (31). Examples of bacteria that produce cephalosporins have also been found, such as *Streptomyces clavuligenes* producing cephamycin C, and cephabacins from *Lysobacter lactamgenus* (29).

Cephalosporin C is derived from 7-amino-cephalosporinic acid, a structure constituted from a dihydrothiazine and a β-lactam ring. Semisynthetic cephalosporins cannot be produced through fermentation; the starting compound 7-amino-cephalosporinic acid is rather generated by hydrolyzing cephalosporin C. The antibiotic activity of cephalosporin C is almost a hundred-fold lower than the one of penicillin G, with gram-negative and gram-positive bacteria equally affected (29).

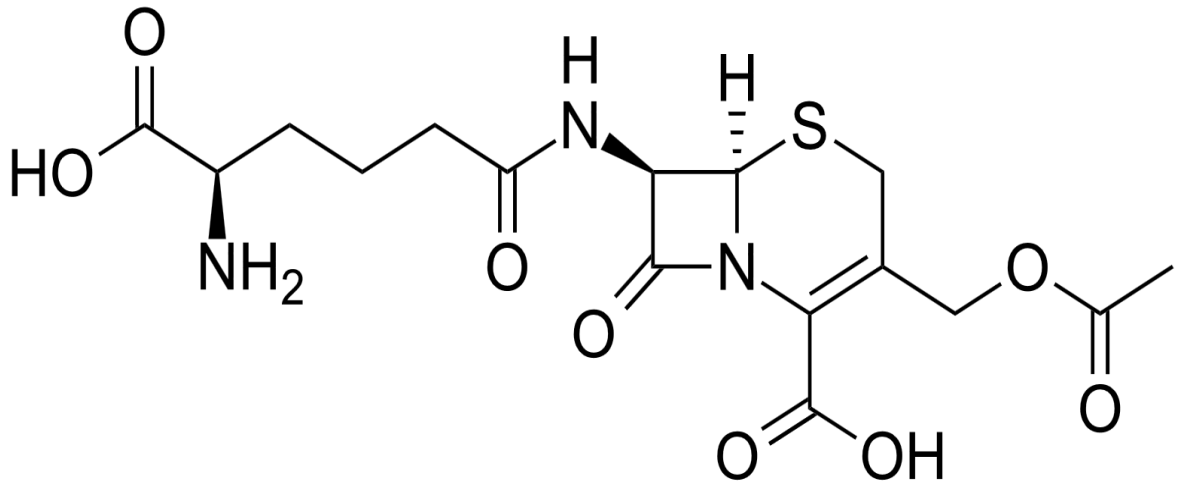


Figure 3: Chemical structure of cephalosporin C (32).

The biosynthesis of cephalosporins starts by generating penicillin N from isopenicillin N through isomerization of L-α-AAA side rest to its D-enantiomer, with isopenicillin N-epimerase catalyzing the reaction. Penicillin N is the starting point in the synthesis of cephem-compounds (cephalosporins and cephamycins). In the next step, penicillin N is converted to desacetoxycephalosporin C (DAOC) by desacetoxycephalosporin C-synthase, which is done by cleaving the thiazolidine ring and forming a dihydrothiazine ring. The 3-methyl rest of DAOC undergoes oxidation and hydroxylation, which creates desacetylcephalosporin C (DAC) with the help of DAOC-synthase/DAC-hydroxylase. In the last step of cephalosporin C biosynthesis, the acetyl rest of acetyl-CoA is transferred to the hydroxyl group of DAC, with acetyl-CoA DAC acetyltransferase catalyzing the reaction (29).

The mechanism of action of cephalosporins is identical to the penicillin one; hence they inhibit the bacterial cell wall synthesis. They are used very often instead of penicillins for

infectious diseases caused by gram-negative bacteria, and are commonly used as prophylactic therapy in chirurgic procedures (29).

The first generation of cephalosporins comprises cefapirin, cefalotin, cefazolin, cefadroxil among others, which are active against gram-positive cocci, *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* and are mostly used to treat skin infections caused by staphylococci. The second generation consists of cefuroxime, cefaclor, cefoxitin and others, and is used against the same bacterial strains as the first generation, but have better characteristics as they show higher resistance to β-lactamase produced in gram-negative bacteria. Cefotaxime, cefoperazone, and ceftazidime are examples of the third generation cephalosporins, and are less effective in treating infections caused by gram-positive cocci than members of the first generation, but are far more effective in combating gram-negative bacteria and are therefore used in patients with meningitis caused by gram-negative aerobic bacteria. The fourth generation with cefepime, cefquinome, and cefpirome along with other members, is resistant to β-lactamase from staphylococci, enterobacteria and *Pseudomonas aeruginosa* (29).

2.1.1.3 Fusidic acid

First reports on fusidic acid date back to early 1960s, when the antibiotic was isolated from a *Fusidium* culture. Its antibacterial activity was confirmed for a wide range of microorganisms, including penicillin-sensitive and penicillin-resistant *S. aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Mycobacterium tuberculosis* etc. Very high half maximal inhibitory concentrations (IC_{50}) were needed for bacterial strains of *S. typhimurium* and *Shigella dysenteriae* and for yeasts *Candida albicans* and *Aspergillus fumigatus*; hence they show only minimal sensitivity to fusidic acid (33).

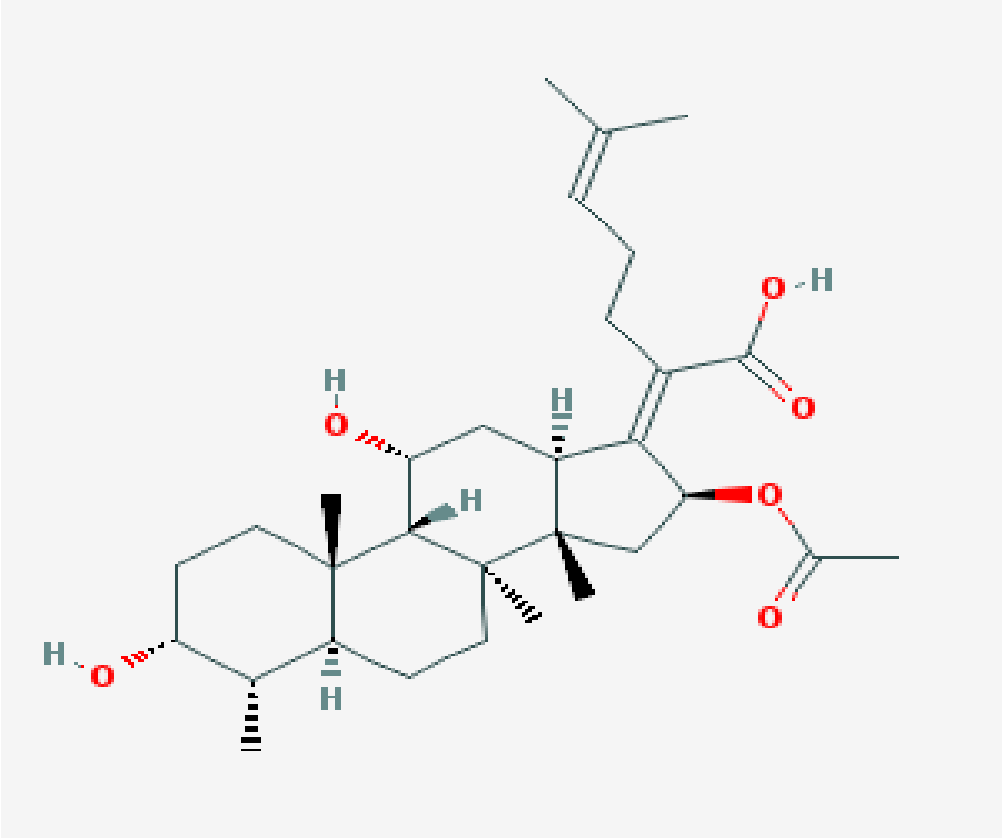


Figure 4: Chemical structure of fusidic acid (34).

Fusidic acid is structurally a steroid antibiotic, carrying a branched side chain in C_{17} (34).

The antibacterial effect of fusidic acid is based on interference with bacterial protein synthesis. Precisely, fusidic acid inhibits the elongation factor G (EF-G), an important component in the protein synthesis needed for the translocation of tRNA together with the evolving peptid chain from the acceptor site to the donor site. Fusidic acid binds to the ribosome-EF-G complex, and inhibits the release of EF-G from the complex, making further peptide synthesis impossible (35).

Clinically, fusidic acid is used in patients suffering from *S. aureus* infections, including methicilin-resistant strains, and in cases of pseudomembranous enterocolitis caused by *Clostridium difficile* (35).

2.1.2 Antimycotics

Griseofulvin

Griseofulvin was discovered in late 1930s, as Oxford et al. isolated and characterized several metabolic products from *Penicillium griseofulvum* (36).

Griseofulvin was the first antimycotic compound used for oral therapy of dermatophytes infections. *In vitro* experiments have proven griseofulvin to be active against *Microsporum, Epidermophyton* and *Trichophyton,* but there is no activity against other fungi or bacteria. The underlying mechanism for its inhibitory effect on dermatophytes is relatively poorly understood. The suggested mechanism starts with accumulation of griseofulvin in immature keratin cells, which is particularly seen in affected tissue compartments. Once new keratin molecules are formed, griseofulvin can bind to keratin precursor cells, making them resistant to fungal infections. The newly formed drug-keratin complex then reaches invaded skin tissue, penetrates into fungal cells and binds to microtubular system involved in cellular mitosis, through which the mitotic process is disrupted. It is suggested that griseofulvin also disrupts the synthesis of nuclear acids (37).

Therapeutically, griseofulvin is applied in patients with infections manifesting on the skin, nails and hair that are caused by e.g. *Tinea cruris, Tinea corporis,* or *Tinea pedis* and its use is associated with only mild side effects (37).

2.1.3 Statins – lipid-lowering drugs

Population studies extensively carried out during the 1950s demonstrated a clear correlation between elevated levels of cholesterol, in particular LDL cholesterol and increased mortality risk in individuals with coronary heart disease (CHD). In opposite, HDL cholesterol was

associated with reduced mortality risk. These findings led to the hypothesis that a reduction of total and especially LDL cholesterol in patients with elevated blood levels could decrease the risk of heart attack and other adverse events. After the National Institutes of Health Coronary Primary Prevention Trial it became undoubtedly clear that a reduction of increased LDL cholesterol through a combination of proper diet and drug treatment would decrease the CHD risk. In the 1950s and 1960s, the exact mechanisms of cholesterol biosynthesis was elucidated, but initial therapeutic strategies were unsuccessful, as triparanol, a biosynthesis inhibitory compound used in the 1960s was associated with adverse effects affecting the eyes and the skin and was soon taken out from use. 3-Hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase is the key component in the biosynthesis of cholesterol, as this enzyme limits the rate of production. In the 1970s, a completely new substance named compactin, isolated from a *Penicillium citrinum* broth was discovered. It was soon recognized that the newly discovered compound has an inhibitory effect on HMG-CoA reductase in various animal models, and lowers total and LDL cholesterol levels in individuals suffering from heterozygous familial hypercholesterolemia. A research group from Merck discovered another compound with the same inhibitory effect in 1978, isolated from *Aspergillus terreus*. They called their newly discovered inhibitor mevinolin, but the name was later changed to lovastatin. Studies on lovastatin involving healthy individuals started in 1980, underlining its effectiveness in reducing LDL cholesterol in healthy participants, with no adverse effects. Trials had to be stopped based on lovastatin's strong similarity to compactin, which had to be taken out of clinical studies most likely due to severe toxic effects in animal models, and new trials were not carried out up until 1984. In 1987, lovastatin was brought into use after being approved by the United States Food and Drug Administration. Several studies carried out thereafter confirmed the effectiveness of statin drugs, advocating their clinical use (38).

Both lovastatin and mevastatin have very closely related structures with HMG, which is a component of HMG-CoA, an important biological substrate molecule in the biosynthesis of

cholesterol based on mevalonic acid. Lovastatin inhibits the enzyme HMG-CoA reductase in a competitive manner, to which it has a 20.000-fold higher affinity than the biological substrate itself. As a prodrug, lovastatin has to be converted into active form in the organism by hydrolyzing its lactone ring, which generates a β-hydroxyacid (39).

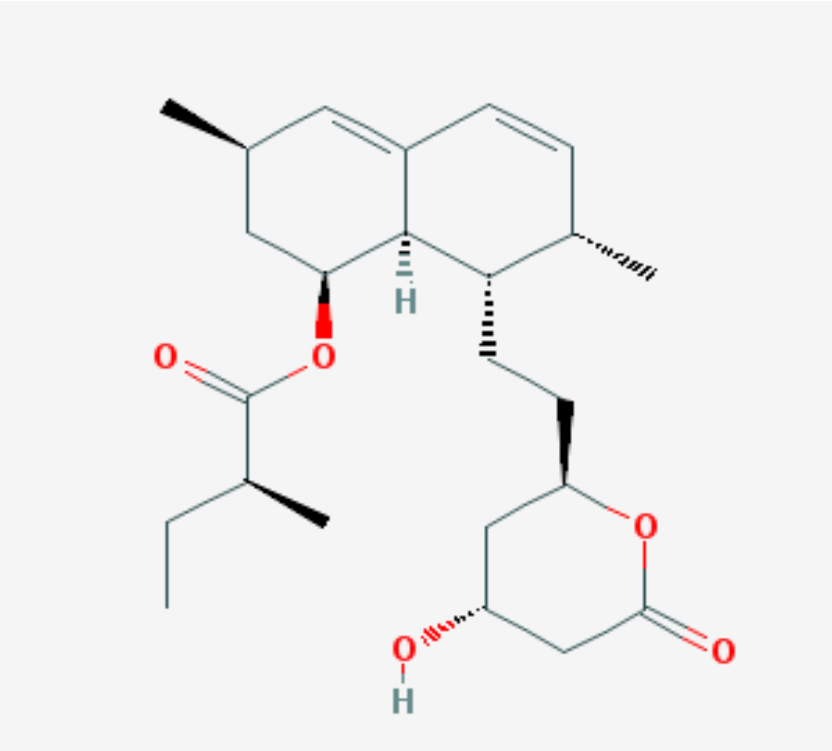


Figure 5: Chemical structure of lovastatin (40).

Lovastatin is clinically used in patients suffering from primary hypercholesterolemia and mixed dyslipidemia to lower their increased levels of total and LDL cholesterol, apo B and triglycerides, in combination with a dietary regime. Its use should prevent the primary onset of CHD and decelerate the progression of atherosclerosis in heart vessels in individuals already affected by CHD (39).

2.1.4 Ergot alkaloids

Claviceps purpurea, a parasitic ascomycetes fungus forms a resting form (sclerotium) called ergot, which is rich in indol alkaloids. In medieval period it was associated with intoxications specifically termed as ergotism characterized by convulsions and gangrene in extremities, which affected large proportions of populations and had a high death toll. The intoxications were caused by consumption of rye contaminated with ergot, which was frequently found across Europe, particularly in Germany and France which had optimal climate for the fungus to grow. At the same time, ergot was widely used by midwives to quicken childbirth and inhibit the following bleeding, and was also used in treatment of headaches with vascular cause, like migraine. Both, intoxications and medical use of ergot are due to its indol alkaloid content, many of which are toxic for nerve cells and can promote constriction of blood vessels. The vasoconstrictive properties are responsible for gangrene in cases of intoxications, as well as for therapeutic use to prevent bleeding and relieve migraine. In order to prevent ergot poisoning, the resting fungal forms are nowadays mechanically removed from cereals (41).

With respect to chemical structure, ergot alkaloids are amide compounds derived from D-lysergic acid, a terpenoid indol acid. They can be found in a variety of fungi, particularly in *Clavicipitaceae*, but also in *Ipomoea violaceae* and *Turbina corymbosa*, plants from the *Convolvulaceae* family. The pharmacological activity of ergot alkaloids, of which at least 50 different have been discovered in ergot by today, depends on the acid they are derived from. Ergot alkaloids derived from lysergic acid are active, as oppose to the inactive isolysergic acid derivates. Lysergic acid-derived ergot alkaloids are further classified into amide-related and peptide-related compounds, and clavines. Basic steps in the biosynthesis of ergot alkaloids include the formation of dimethylallyltryptophan by condensing tryptophan and isopentenyl diphosphate, methylation, oxidative decarboxylation, formation of a ring, and repeated oxidation (41).

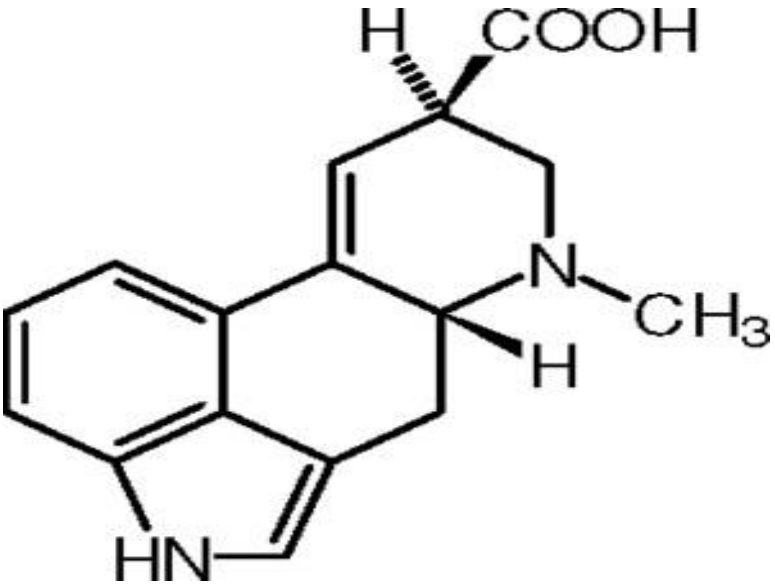


Figure 6: Chemical structure of lysergic acid (42).

The pharmacological activity of ergot alkaloids is based on their tetracyclic ring component, as this structural part is similar to neurotransmitters like noradrenaline, serotonin or dopamine, which enables them to interact with their biological receptors. Ergot alkaloids can be antagonistic or agonistic, but can also be partial-agonistic and antagonistic at the same time, and the way they act is strongly determined by the C_8-rest on their ergoline ring. Peptide-related members are vasoconstrictive and adrenolytic, as they predominantly bind to adrenergic receptors. In opposite, amid-related and clavine members primarily affect the serotonin system and act anti-serotoninergic. Some ergot alkaloids can also bind to dopamine receptors, or even stimulate nerve endings to release dopamine (41).

Ergocryptine, ergotamine, ergometrin, and lysergic acid are prominent examples of naturally occurring ergot alkaloids, which served as starting point in synthesis of semisynthetic compounds. Both groups have potent pharmacological effects, and can thus be medically used in treatment of various disorders, including Parkinson's disease and migraines. Additionally, they are used for gynecological treatment, such as to initiate labour, inhibit postpartum

bleeding, and decrease breast milk production. Moreover, ergot alkaloids like lysergic acid and the derived lysergic acid diethylamide (LSD) have strong hallucinogenic effect even if low doses are administered (41).

2.1.5 Immunosuppressive drugs

2.1.5.1 Cyclosporine A

The discovery and development of cyclosporine A followed an analysis of a soil sample brought back from Norway to Switzerland by a worker of Sandoz pharmaceutical company. A fungus isolated from this sample, called *Tolypocladium inflatum (T. inflatum)* was found to produce lipophilic compounds that were suggested to be cyclopeptides. In diverse test settings the antifungal potency of a mixture of two metabolites called 24-556 was examined, but results were not convincing. However, data showed minimal toxicity in animal models. Additionally, an immunosuppressive potential was observed. This effect was based on inhibition of lymphocyte proliferation, while not affecting other somatic cells. Afterwards, the metabolites were separately characterized and were named cyclosporine A and B. Despite strong homology, cyclosporine A was proven to have convincingly higher immunosuppressive potential. Moreover, a strong anti-inflammatory potential for cyclosporine A was observed in mice suffering from adjuvant arthritis, a disorder analogue to rheumatoid arthritis in humans. Thus, the first development process of cyclosporine A started. Structural characterization of cyclosporine A followed in 1976, when the cyclopeptide assumption was confirmed. A variety of cyclosporines is generated in *T. inflatum*, with analog structures consisting of 11 amino acids, out of which mostly a single amino acid is different across the cyclosporine group. Clinical trials on immunosuppression of cyclosporine A in cases of organ transplantation soon followed, and yielded predominant success, but also revealed side effects, such as reversible renal dysfunction of moderate to severe degree.

Despite side effects associated with its use, cyclosporine A is the drug of choice in patients undergoing organ transplantation (43).

Several underlying mechanisms are thought to play a role in the immunosuppresive activity of cyclosporine A. The mechanism based on inhibition of the calcineurin/NFAT pathway, which is well described, starts with cyclosporine joining into a complex with cyclophillin. The complex binds to calcineurin, a protein located in the cytosol, which belongs to the serine/threonine phosphatase superfamily. Physiologically, calcineurin cleaves the phosphate groups from NFAT1, NFAT2 and NFAT3 proteins, enabling their translocation into the cell nucleus, where they can activate transcription of genes coding for IL-2, IL-4, and CD40L. The cyclosporine A-cylophillin complex disrupts this process by docking to the A subunit of calcineurin, which is its actual catalytic site. This results in reduced expression of cytokines in T-cells and consequently reduces their activation. Additional mechanisms for cyclosporine A immunosuppression might include effects on c-Jun N-terminal kinases (JNK) and p38 pathways (44).

Besides its use in patients undergoing organ transplantation, including kidneys, liver and heart transplants, it is also indicated in autoimmune diseases, including rheumatoid arthritis, and severe cases of psoriasis (45).

2.1.5.2 Mycophenolic acid, Mycophenolat-mofetil

Mycophenolic acid was first isolated in 1896 from a corn broth, which contained the fungus *Penicillium brevicompactum*. However, the compound provoked enough interest only in late 1960s, after recognizing its capability to inhibit cancer cell growth by inhibiting the enzyme

inosine-5'-monophosphat-dehydrogenase (IMPDH), but the discovery was not further persued. In 1982, Allison and Eugui performed yet another research on mycophenolic acid, as they were eager to develop a new compound that would only suppress lymphocytes, but would not be associated with toxic effects or potentially provoke renal dysfunction like cyclosporine A. Their knowledge in purine metabolism of lymphocytes and experience with the use of azathioprin, an inhibitor of purin synthesis, led them to identify the enzyme IMPDH as target molecule, and subsequently mycophenolic acid as a potentially active compound. A clear demonstration of the immunosuppressive activity of mycophenolic acid soon followed by Morris and co-workers. Mycophenolic acid was finally marketed as an immunosuppressive drug after thorough research and work performed by Sollinger et al. from the American Society of Transplant Surgeons (46).

Mycophenolic acid's immunosuppressive effect is based on its selective inhibition of IMPDH, which is blocked reversibly and uncompetitively. Thereby, de novo synthesis of guanosine-type nucleotides is disrupted, affecting strongly both T- and B-lymphocytes, which have to de novo synthesize purine nucleotides in order to proliferate. Consequently, mycophenolic acid is a cytostaticum for both lymphocyte cell types, making them insensitive to stimuli of mitogenic and allospecific type. This inhibition is reversible, as cells can proliferate again if guanosine or deoxyguanosine is provided. In addition, B-lymphocytes can be inhibited in their production of antibodies by mycophenolic acid, and glycoproteins found on lymphocytes and monocytes can lack sugar components needed for adhesion with endothelium due to inhibited glycosylation, thus making them unable to infiltrate the inflammed tissue. Mycophenolic acid is clinically used in organ transplant patients, such as in cases of kidney transplants together with cyclosporine and corticosteroids (47).

2.1.6 Oxytetracycline

Tetracyclines are very effective in treating a variety of infectious diseases. Their emergence goes back to the discovery of Benjamin Minge Duggar in 1948, an American botanic scientist, who investigated soil-inhabiting mold species. A specific mold species was found to have antibacterial activity evident in a range of bacterial strains, including *Bacillus, Streptococcus,* and *Staphylococcus* strains. The compound responsible for such an activity was named aureomycin, the name resembling its colour (aureus - golden) and producer (mykes - fungi) (48).

Oxytetracyline was discovered as the following tetraycycline antibiotic. It is produced in the actinomycete bacteria *Streptomyces rimosus*. Due to its lipohilic nature, the antibiotic can readily penetrate the cell membrane or it enters the cells through porin channels by passive diffusion. Oxytetracyline acts by docking to the 30S subunit of ribosomes in a reversible manner, making amino-acyl tRNA unable to bind on the A ribosomal site. Oxytetracycline is used in treating both gram-positive and gram-negative bacteria associated infections (e.g. *Mycoplasma pneumoniae, Pasteruella pestis, H. influenzae*) (49).

2.2 Overview of most important mycotoxins

2.2.1 Aflatoxins

Aflatoxins are secondary metabolites found in several different *Aspergillus* species, including *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus*. An estimated 20 different aflatoxins have been identified to date, with aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) having the greatest importance as they can occur in mold contaminated food, such as cereals and nut products. Aflatoxins are most abundant in warm climate areas, but can also be found in contaminated food in other areas of the world, partly due to world food trading (50).

The first step in the biosynthesis of aflatoxins, which is thought to involve a variety of 30 different genes, consists of generating norsoloronic acid, a stable compound originating from acetate. The discovery of norsoloronic acid in *Aspergillus parasiticus* was the pillar in elucidating the production of aflatoxins, as it enabled other important biosynthesis intermediates to be identified. The last step in aflatoxin biosynthesis consists of converting O-methylsterigmatocystin and dihydro-O-methylstergimatocystin to AFB_1 and AFG_1, and AFB_2 and AFG_2, respectively (51).

Other aflalatoxins include M_1 (AFM_1), M_2 (AFM_2), GM_1 ($AFGM_1$) and GM_2 ($AFGM_2$), which can be found at smaller scale in *Aspergillus parasiticus*. AFM_1 and AFM_2 were first found in cow milk, but can also be generated in mammalian organisms through conversion of AFB_1 and AFB_2 through hydroxylation in the liver (51).

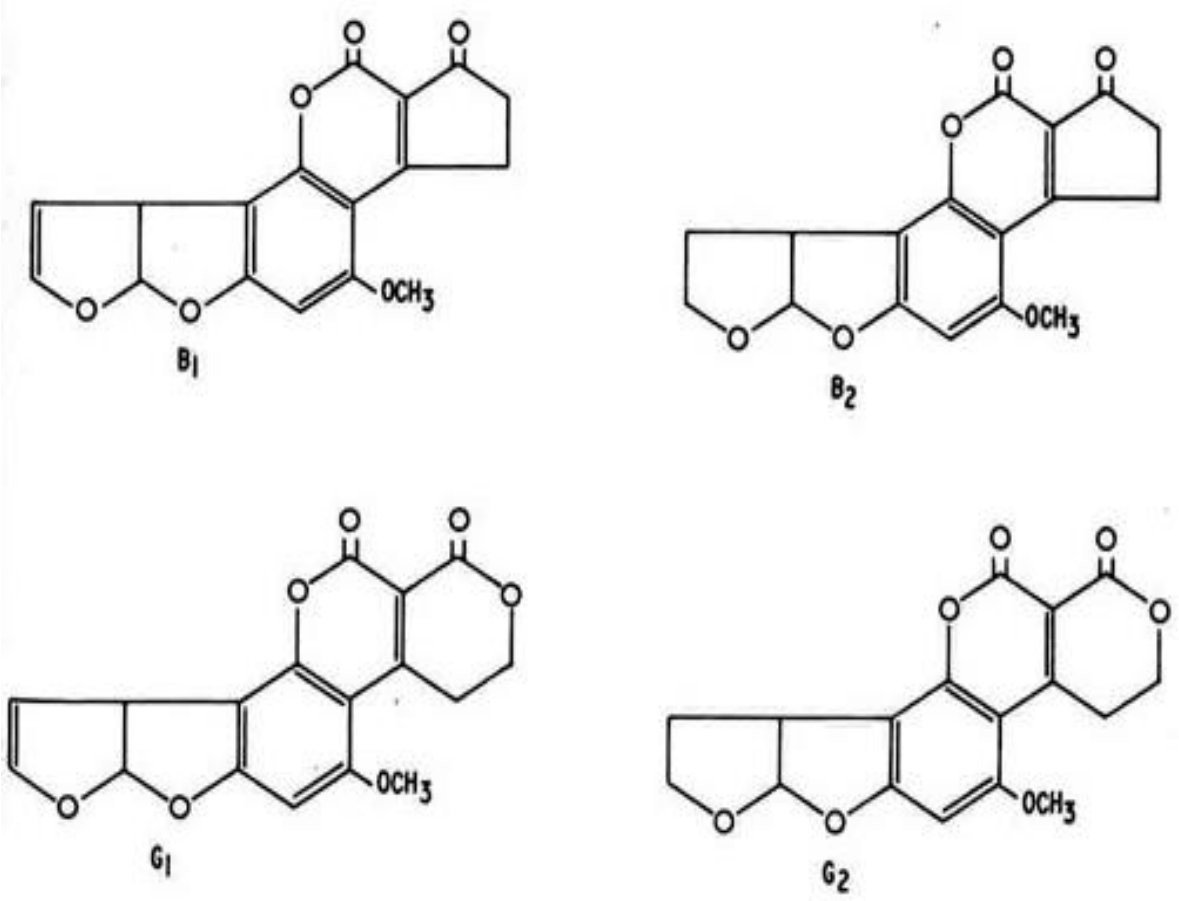


Figure 7: Chemical structure of AFB_1, AFB_2, AFG_1, and AFG_2 (52).

AFB_1 is considered the most important member, as it is the most abundant of all aflatoxins, and has the greatest proven toxic potential. Its toxic activity is initialized by its conversion into endo- and exo-epoxide form, with AFB_1-8,9-exo-epoxide being the main product responsible for DNA binding and subsequent formation of 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB_1. The DNA AFB_1 intercalation product is responsible for the mutagenic character of the mycotoxin. Aflatoxins Q_1 (AFQ_1), AFM_1 and aflatoxin P_1 (AFP_1) are other products of AFB_1 metabolism. However, like AFG_1, AFG_2, and AFB_2, these compounds are less prone to formation of toxic epoxides, a feature that decreases their toxic potential. The aforementioned metabolism of AFB_1 in humans is performed by enzymes of the CYP-family; with CYP3A4 being responsible for its conversion to AFB_1-exo-epoxide and AFQ_1, and CYP1A2 that predominantly generates the endo-epoxide form and AFM_1 (53).

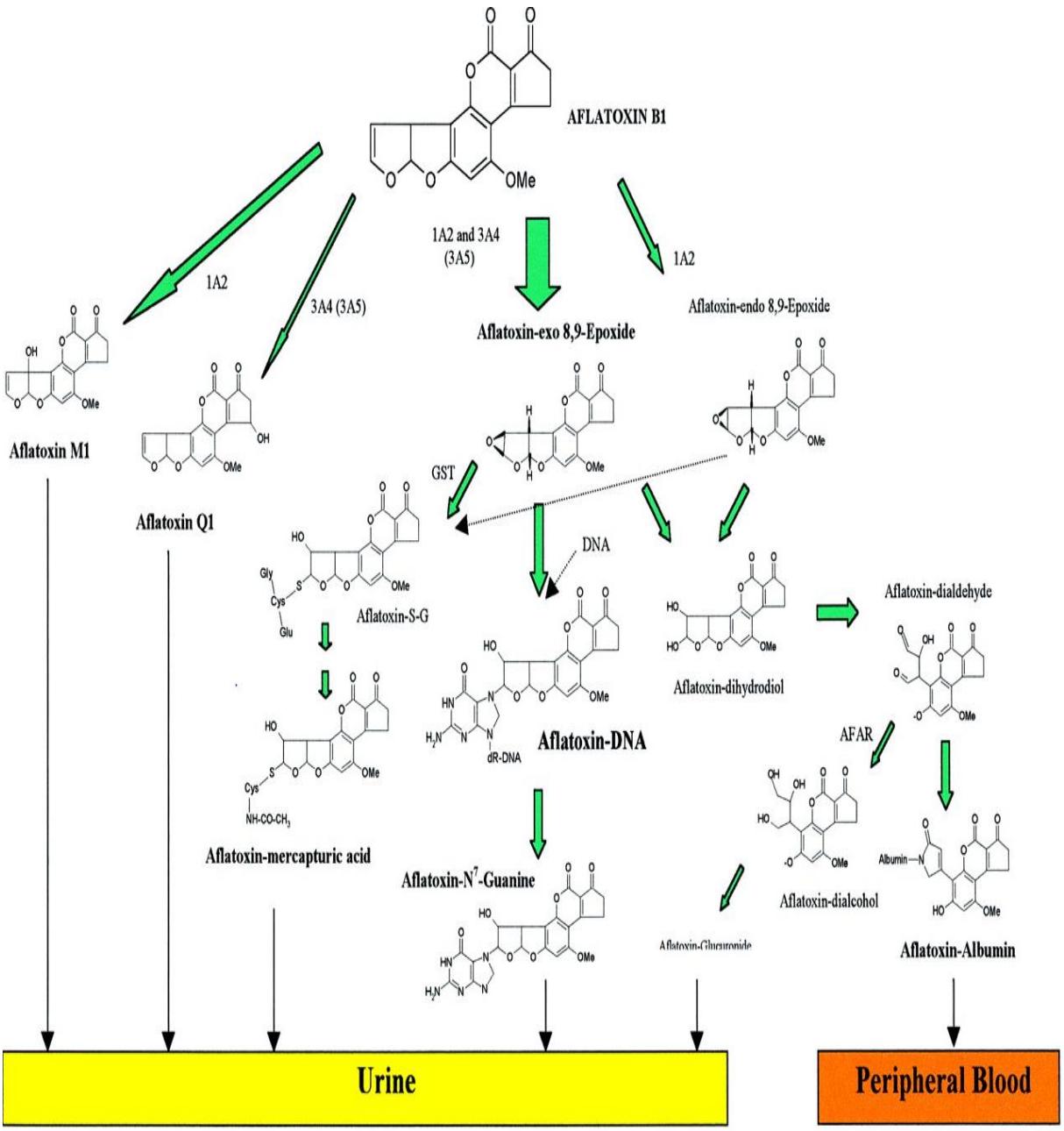


Figure 8: Conversion of AFB_1 in the body resulting in reactive metabolic products and biomarkers. 1A2, 3A4 and 3A5 stand for the respective CYP-enzymes, GST – glutathione-S-transferase, AFAR- aflatoxin-aldehyde-reductase, Aflatoxin-S-G – glutathione conjugated aflatoxin (53).

Conjugation of AFB_1 endo- and exo-epoxides with glutathione in its reduced form is the main metabolic pathway in abolishing the toxic potential, and is performed by glutathione-S-transferase (GST). In addition, the epoxides can be hydrolyzed in a non-enzymatic manner to AFB_1-8,9-dihydro-diol, which is subsequently converted into a dialdehyde phenolate ion, and further reduced to a dialcohol by the enzyme aflatoxin aldehyde reductase (53).

Cases of acute aflatoxin intoxications in humans have been observed, particularly in Africa and Asia, but exposure to aflatoxins is mostly associated with subacute and chronic liver conditions, including cancer, chronic hepatitis, hepatomegaly, icterus, and cirrhosis which become apparent when low dosis of aflatoxins are repeatedly taken. The hepatic carcinogenic properties are most strongly associated with AFB_1, whereas AFM_1 seems to primarily affect the kidneys. Aflatoxins are also thought to play a role in other disorders, such as kwashiorkor, Reye's syndrome and conditions affecting the immune system (50).

2.2.1 Ochratoxins

Ochratoxin A has the greatest importance and abundance from all ochratoxins. This mycotoxin occurs in tropical areas, where it is generated in *Aspergillus* species, like *A. ochraceus*, but can also be found in areas with temperate climate, where *Penicillium verrucosum* is the producing fungus. Ochratoxin A was originally observed in contaminated corn, but its widespread occurence has been reported in a variety of food (54).

Structurally, ochratoxin A is constructed from a dihydro-isocoumarin ring, with a carboxy group in position 12 that connects the ring with a phenylalanine part. Other occuring members are ochratoxins B, C, α and β (54).

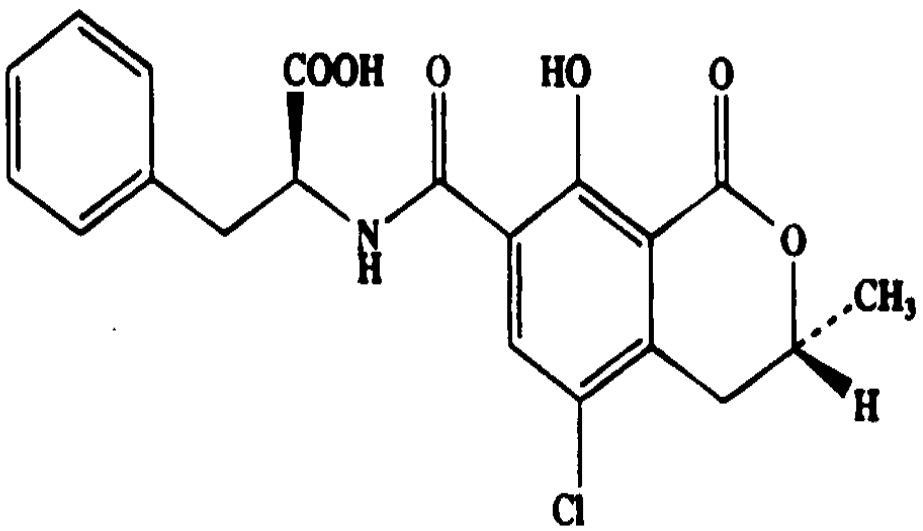


Figure 9: Chemical structure of ochratoxin A (54).

The toxic potential of ochratoxin A becomes apparent primarily in the kidneys, as acute and chronic renal lesions can manifest as a consequence of toxin exposure. Its damaging effect on kidneys has been proven in all mammals. In addition, ochratoxin A was confirmed to have a teratogenic effect in several species, including mice, hamsters and chicken. It also has an effect on the immune function in some mammals. Although the exact underlying mechanism remains unknown, the genotoxic properties of ochratoxin A have been demonstrated in experiments conducted *in vitro* and *in vivo* (54).

2.2.2 Patulin

Penicillium griseofulvum and *Penicillium expansum* were the first identified fungal species to produce patulin. Nowadays it is known that this mycotoxin can be produced across a range of species from different genuses, including *Penicillium* (*P. carneum, P. concentricum, P. clavigerum, P. coprobium, P. dipodomyicola, P. expansum, P. gladioli, P. gladnicola, P. griseofulvum, P. marinum, P. paneum, P. vulpinum and P. sclerotigenum*); *Aspergillus* (*A. clavatus, A. longivesica, and A. giganteus*); *Paecylomyces* (*P. saturatus*); and *Byssochlamys* (*B. nivea*) (55).

Patulin is the most abundantly occurring mycotoxin in apples and food commodities produced from apples, in which *Penicillium expansum* has been recognized to contribute to the greatest proportion of patulin occurrence (55).

Structurally, patulin belongs to the polyketide family, which also comprises other important mycotoxins, such as the already mentioned aflatoxins or ochratoxins (55).

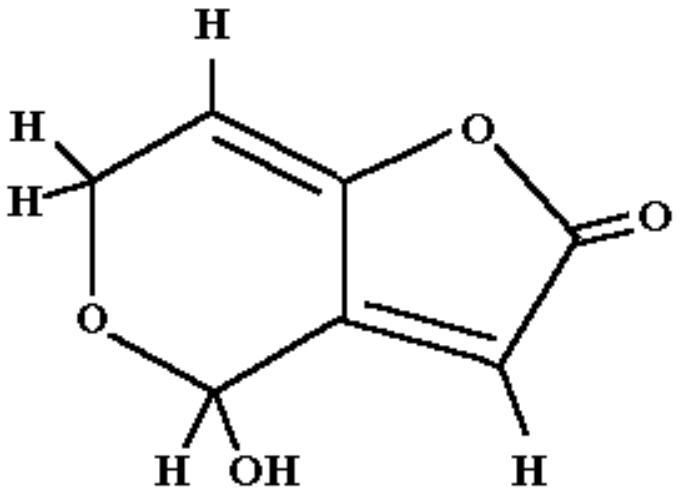


Figure 10: Chemical structure of patulin (56).

The toxical features of this mycotoxin arise from its affinity to bind to sulfhydryl groups, allowing it to interfere with multiple enzymes. In parallel, compounds generated by addition of patulin to cysteine have been proven to have smaller acute toxic, teratogenic, and mutagenic potential than the mycotoxin itself. Acute intoxication with patulin can manifest in agitation, convulsive reactions, dyspnea, congestion in the lungs, edema, ulcera, hyperemia and gastrointestinal distension. In sub-acute intoxication cases, patulin primarily affects the gastrointestinal function, resulting in ulcera, distension and hemorrhage. At higher doses, kidney function can also be affected. In addition, patulin was identified as a genotoxic compound by the World Health Organization. Its embryotoxic and teratogenic potential have been demonstrated in several animal models; and its potent effects on the immune function were confirmed *in vitro* and *in vivo* (55).

2.2.3 Citrinin

Penicillium citrinum was the first fungal species identified to produce citrinin. Subsequently, the production of this mycotoxin was also demonstrated in several *Penicillium* species, including *P. expansum* and *P. verrucosum*; in *Aspergillus terreus* and in *Monascus ruber* (57).

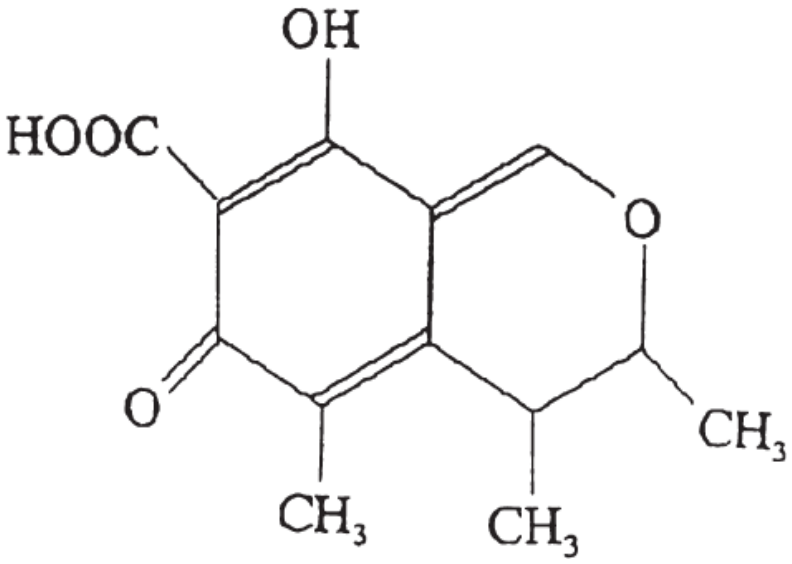


Figure 11: Chemical structure of citrinin (57).

Although citrinin shows a certain degree of antibacterial activity against gram-positive bacteria, it has never lived up to clinical use based on its damaging effects on kidneys. Additonally, toxic potential was also observed in the liver and bone marrow. Despite its early isolation, the exact mechanisms underlying its toxicity remain poorly understood and have predominantly been studied *in vitro*. Citrinin often occurs together with ochratoxin A in feed and food commodities, and their toxic effects on kidneys are associated with a renal disease known as Balkan endemic nephropathy (57).

Properties of citrinin in terms of its toxic, carcinogenic, teratogenic, nephrotoxic, and mutagenic effects require further research and elucidation. Currently, citrinin falls in the Group 3 carcinogenic compounds according to the International Agency for Cancer Research,

as there is only insufficient evidence on its carcinogenic potential, and they are based on animal trials (57).

2.2.4 Trichothecenes

Trichothecenes constitute a major structural group of compounds, which carry a double bound between carbon atoms 9 and 10, whereas their positions C-12 and C-13 are closed into an epoxy ring. Although they are many times called 'Fusarium toxins', these mycotoxins can actually be produced in a number of fungal genera, such as *Trichoderma*, *Verticimonosporium*, *Stachybotrys*, *Cephalosporium*, and *Myrothecium*. Until today, hundreds of different trichothecenes have been discovered, but only a small number is of importance in terms of agriculture. At least 20 different species from the *Fusarium* fungal genus are able to produce trichothecenes, such as *F. sporotrichioides*, *F. moniliforme*, and *F. clumorum*. Based on their chemical structure and presence of a side chain in position C_7, trichothecenes are divided into group A and group B. According to reports, the most common occurring members of the group A are T-2 toxin, HT-2 toxin, monoacetoxy scirpenol, diacetoxyscirpenol, and neosolaniol. Besides deoxynivalenol, the group B also comprises nivalenol, 3-acetoxynivalenol, 15-acetoxynivalenol, and fusarenon X. Macrocyclic trichothecenes produced in e.g. *Stachybotrys arta*, constitute a special group, with members such as satratoxins, verrucarins, and roridins (58).

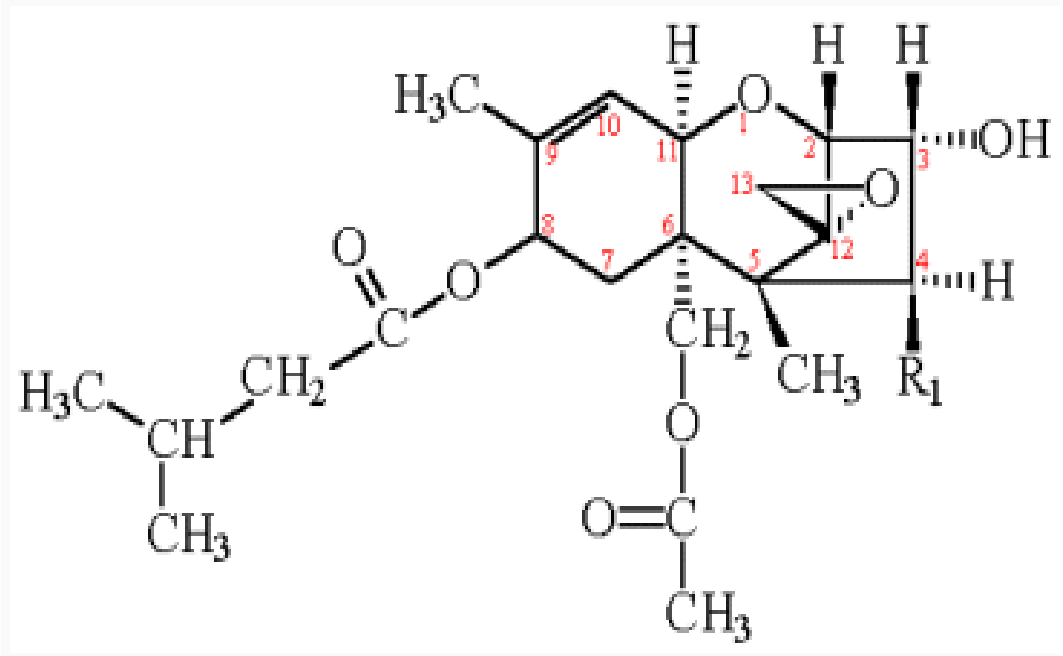


Figure 12: Chemical structure of trichothecenes from group A (58).

With generally a wide range of LD_{50} values, trichothecenes have an acute toxic potential if administered orally or by intraperitoneal route. Macrocyclic mycotoxins have the greatest acute toxic potential, followed by T-2 toxin, but are of smaller overall agricultural importance than deoxynivalenol because they are less abundant. Acute intoxication with trichothecenes results in gastrointestinal problems (e.g. emesis), abortion, anemia, and leukopenia among other symptoms. They are associated with acute cytotoxic and immunosuppressive effects. Their mutagenic or cancerogenic potential has not been demonstrated. However, they can disrupt the synthesis of DNA and proteins (58).

In humans, alimentary toxic aleukia (ALA) is the best described condition caused by trichothecenes intoxication. A large scale epidemic of ALA in Russia dating back to past century is believed to have been partially caused by T-2 toxin. In individuals who are repeatedly exposed to trichothecenes, dermal rashes can be observed, which eventually can develop to necrotic lesions (58).

2.2.4.1 Deoxynivalenol

Deoxynivalenol is a mycotoxin from the trichothecenes group, with *Fusarium graminearum* being the primary producing fungal species. Deoxynivalenol is the best characterized and most abundant mycotoxin found in contaminated grains and derived food commodities. This mycotoxin is commonly also called vomitoxin, as it causes emesis once consumed, based on its interaction with dopamine receptors in the central nervous system (CNS). Vomiting can also be accompanied by symptoms such as acute nausea, diarrhoea, pain in the abdominal area etc. (59).

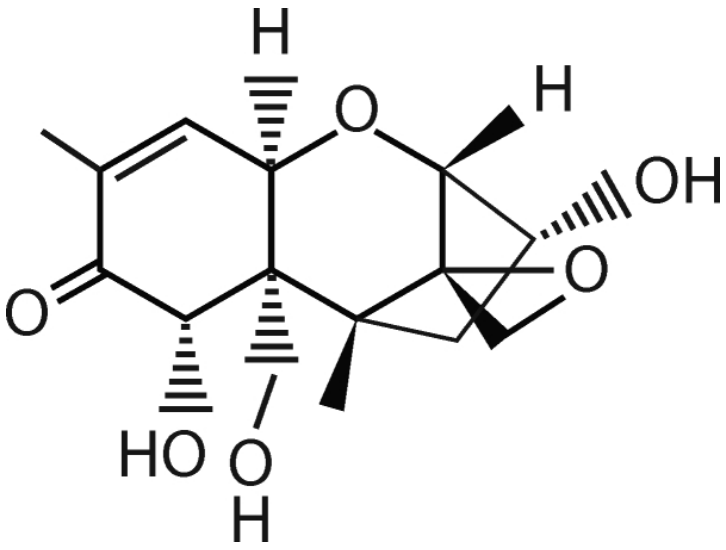


Figure 13: Chemical structure of deoxynivalenol (59).

Many experiments have been carried out in animal models aiming to elucidate the underlying mechanism of action, identify the tissues that might be affected and determine doses for possible adverse effects. However, further research is needed to clarify the nature of deoxynivalenol with respect to its toxic potential on cellular, genetic, immune and other levels. Carcinogenic effects of deoxynivalenol in humans have not been proven in an adequate manner, and this mycotoxin therefore falls into the Group 3 carcinogenics, according to the International Agency for Cancer Research (59).

2.2.5 Fumonisins

This mycotoxin group encompasses a minimum of 15 highly similar polar compounds that often contaminate maize. Their actual identification occurred in the 1980s, despite the fact that they have been associated with effects in animals long before. *Fusarium* species (e.g. *F. moniliforme, F. proliferatum, F. nygamai, F. anthophilum)* are the primary source of this mycotoxin class (60).

Structurally, they consist of a long hydrocarbon chain carrying methyl, amino and hydroxy groups; and typically two esters formed between hydroxyl groups of the main chain and propane-1, 2, 3-tricarboxylic acids. Fumonisin B_1 is the most prominent member of the class (60).

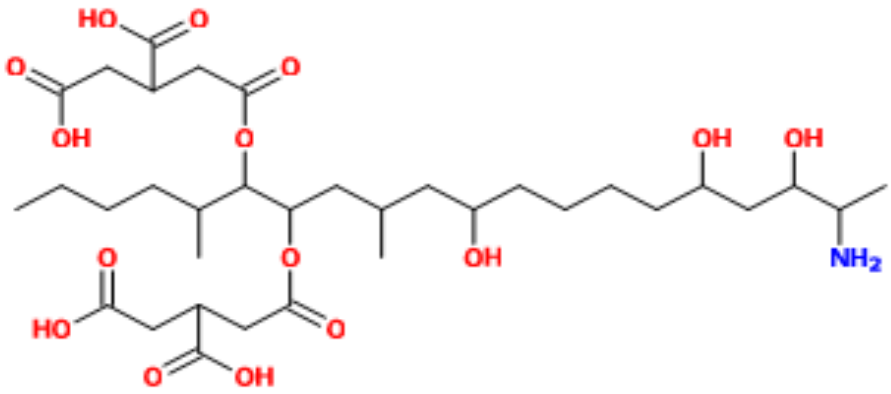


Figure 14: Chemical structure of fumonisin B_1 (60).

The occurrence of fumonisins is frequently accompanied by other mycotoxins, including aflatoxins and deoxynivalenol. Toxic effects of fumonisins are primarily caused by their impact on biosynthesis of sphingolipids, particularly due to the inhibition of ceramide synthetase. In animals, effects such as appetite loss, lethargia, neurotoxicosis with brain edema, hepatic lesions, lung edema, and hydrothorax are associated with consumption of fumonisin-contaminated feed (60).

As for their effects in humans, there seems to be a positive correlation between prevalence of esophageal carcinoma and maize intake in certain parts of the world. However, the exact health-related effects of fumonisins in humans remain poorly elucidated and require further and thorough investigation (60).

2.2.6 Zearalenone

As for a variety of other mycotoxins, *Fusarium* species are also the primary producers of zearalenone, especially *F. graminerum, F. culmorum, F. equiseti* and *F. verticillioides*. Structurally, zearalenone is a lactone of resorcinic acid. With respect to contaminated food commodities, this mycotoxin is predominantly found in maize, although its occurance was also observed in wheat, barley, and sorghum along with other crops. Zearalenone is very effectively absorbed and can be subsequently converted into α- and β-zearalenone through reductive metabolism, as well as conjugated with glucuronide groups through metabolic activity of the liver and small intestine (61).

With regards to its health-related effects, they primarily concern the reproductive system. In various animal species, its influence on estrogen-related processes has been demonstrated. For instance, in female pigs consumption of zearalenone is associated with abortion, stillbirth, underweight of the offspring, vulval changes (e.g. swelling), breast swelling accompanied by vesicular follicles, and ovarial changes characterized by cystic follicles (62).

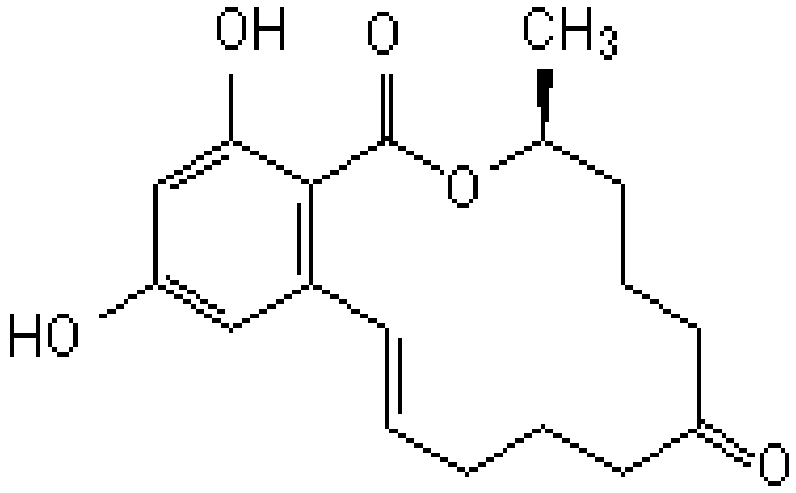


Figure 15: Chemical structure of zearalenone (62).

Metabolic conversion of zearalenone is not entirely protective against these effects, as both α- and β-zearalenone are also associated with estrogenic effects. Early phases of development, such as fetal and neonatal period, are thought to be especially susceptible to zearalenone, based on metabolic underdevelopment and consequently higher exposure (61).

Exact effects of zearalenone on human health are still not entirely known. However, pubertal changes that affected several thousand Puerto Rican youngsters, as well as cases cervical carcinoma was suggested to be based on zearalenone uptake (62).

2.2.7 Cyclohexadepsipeptides beauvericin and enniatin

Beauvericin (BEA) consists of 3 residues N-methyl-L-phenylalanine alternating with D-hydroxy-isovalerianic acid. They are linked with ester and amino bonds forming an 18-membered ring. This lipophilic mycotoxin is the primary bioactive component of the fungi *Beauveria bassiana.* However, a variety of other fungal species have been found to produce beauvericin, including the genus *Paecilomyces* (*P. fumosoroseus, P. tenuipes*), *Polyporus* (*P. sulphureus*), and *Fusarium* (*F. subglutinans, F. verticilloides, F. proliferatum, F. equiseti, F. anthophilum, F. poae, F. dlamini, F. sambucinum* among others). In addition, two analogous compounds have been identified in *B. bassiana* - BEA (A) and the less occuring BEA (B). These compounds differ in their molecular mass (797 Da and 811 Da for (A) and (B),

respectively) and lipophilicity, due to the different number of methyl groups that they carry (Figure 16) (63).

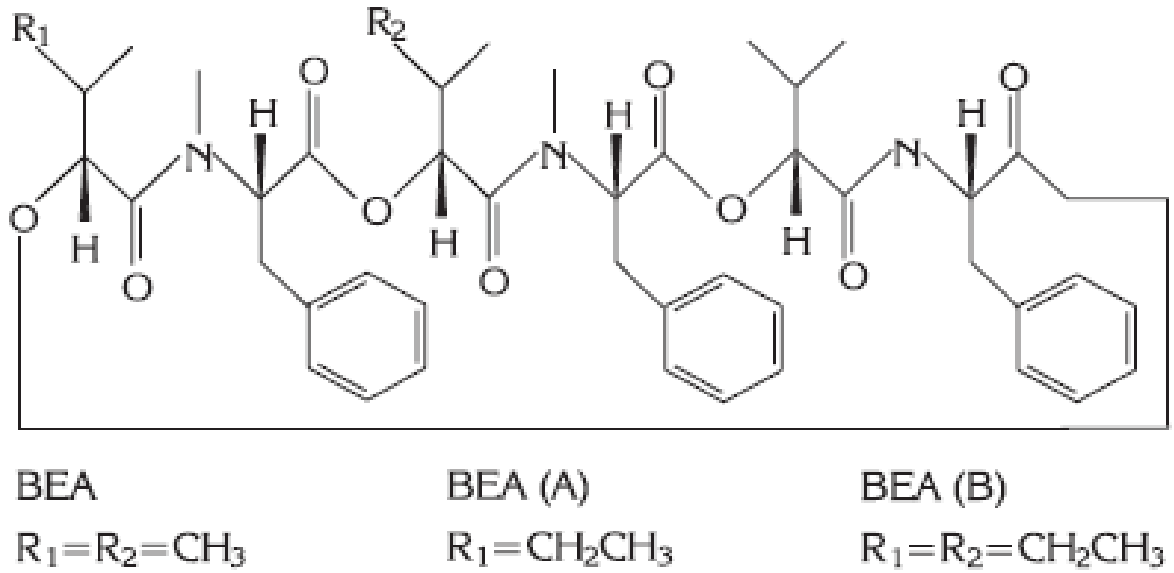
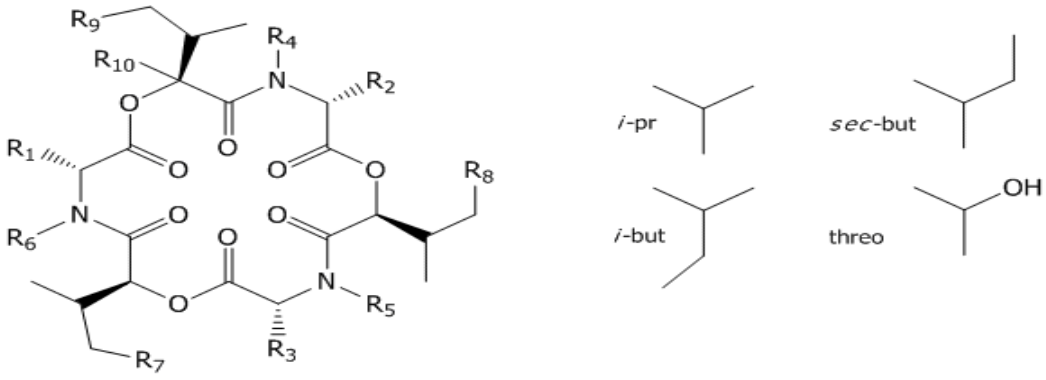


Figure 16: Chemical structure of BEA, and its analogues BEA (A) and BEA (B) (63).

Different biological properties have been described for BEA, including antimicrobial, insecticide, cytotoxic, and proapoptotic activity (63). Further, BEA was shown to be a highly effective synergizer of antifungal activity when applied in combination with e.g. ketoconazole, which itself has only limited fungicidal effect (64). BEA revealed antiangiogenic activity, for which sublethal concentrations are required. Moreover, the cyclic peptide inhibits the migration of cancer cells, including metastatic prostate and breast cancer cells (65). The proapoptotic properties of BEA in non-small lung carcinoma cell lines were shown to be based on mitochondria-mediated effects, since mitochondrial modifications like down-regulation of Bcl-2, changes in the membrane potential, release of cytochrome c from mitochondria to cytosol, and increased caspase-3 activity was detected (66). Similarly, BEA-associated apoptosis based on comparable mitochondrial modifications was also found in human acute lymphoblastic leukemia cells and was related to changes in calcium concentrations (67). Moreover, this mycotoxin is a specific blocker of the enzyme cholesterol-

acyltransferase. Its biological activity is principally due to its ionophoric properties. BEA has a high affinity to form complexes with monovalent and bivalent ions in cell membranes, such as sodium, calcium, and potassium cations, which results in altered membrane permeability for these cations and consequently affects the homeostasis at cellular level (63). BEA has been identified in corn in many countries, where it commonly co-occurs with other mycotoxins, such as ochratoxin A and fumonisins. However, despite its widespread occurrence and well-established cellular effects, exact implications of exposure to BEA related to human health remain widely unknown and require further elucidation (63).

Enniatins are a relatively large group of mycotoxins, comprising 28 different derivatives identified to date (Figure 17). The main producing species of this substance class originate from the fungal genuses *Fusarium, Halosarpheia* and *Verticillum.* Structurally, compounds of this group consist of six acid residues that are connected into an 18-membered cyclic structure by alternating ester and amide groups. Out of the six residues, three are D-hydroxy acids, and the other three are L-amino acids (most commonly N-methylated branched amino acids valine, leucin and isoleucin). Specifically, in enniatins formed in *Fusarium* species, other N-methylated amino acids have been identified, including alanine, threonine and aminobutyric acid (68).



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	Molecular weight
enniatin A	*sec*-but	*sec*-but	*sec*-but	Me	Me	Me	H	H	H	H	681
enniatin A1	*i*-pr	*sec*-but	*sec*-but	Me	Me	Me	H	H	H	H	667
enniatin A2	*sec*-but	*sec*-but	*i*-but	Me	Me	Me	H	H	H	H	681
enniatin B	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	H	H	H	H	639
enniatin B1	*i*-pr	*i*-pr	*sec*-but	Me	Me	Me	H	H	H	H	653
enniatin B2	*i*-pr	*i*-pr	*i*-pr	H	Me	Me	H	H	H	H	625
enniatin B3	*i*-pr	*i*-pr	*i*-pr	H	H	Me	H	H	H	H	611
enniatin B4/D	*i*-but	*i*-pr	*i*-pr	Me	Me	Me	H	H	H	H	653
enniatin C	*i*-but	*i*-but	*i*-but	Me	Me	Me	H	H	H	H	681
enniatin E	*i*-pr	*i*-but	*sec*-but	Me	Me	Me	H	H	H	H	667
enniatin F	*i*-but	*sec*-but	*sec*-but	Me	Me	Me	H	H	H	H	681
enniatin G	*i*-pr	*i*-but	*i*-but	Me	Me	Me	H	H	H	H	667
enniatin H	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	H	H	Me	H	653
enniatin I	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	H	Me	Me	H	667
MK1688	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	Me	Me	Me	H	681
enniatin J1	*i*-pr	*i*-pr	Me	Me	Me	Me	H	H	H	H	611
enniatin J2	*i*-pr	*sec*-but	Me	Me	Me	Me	H	H	H	H	625
enniatin J3	*i*-pr	*Me*	*sec*-but	Me	Me	Me	H	H	H	H	625
enniatin M1	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	Me	H	Me	OH	683
enniatin M2	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	H	Me	Me	OH	683
enniatin N	*i*-pr	*i*-pr	*i*-pr	Me	Me	Me	Me	Me	Me	OH	697
enniatin O1	*i*-but	*i*-pr	*i*-pr	Me	Me	Me	Me	H	H	H	669
enniatin O2	*i*-but	*i*-pr	*i*-pr	Me	Me	Me	H	Me	H	H	669
enniatin O3	*i*-but	*i*-pr	*i*-pr	Me	Me	Me	H	H	Me	H	669
enniatin P1	*i*-pr	*i*-pr	threo	Me	Me	Me	H	H	H	H	664
enniatin P2	*i*-pr	*i*-but	threo	Me	Me	Me	H	H	H	H	664

Figure 17: Possible substitutes of enniatins (68).

Analogous to BEA, enniatin's biologic activity is proposed to be based on its ability to integrate in cell membranes, resulting in formation of membrane pores and subsequent changes in cellular ion levels. However, the nature of this process was shown to be different between sub-types of enniatins (A_1, B, and B_1), suggesting chemical structure to play an important determining role (69). Ambiguous effects of exposure to enniatin have been found -

low doses were shown to have growth promoting effects that might relate to concomitant tumor promotion, whereas high doses were found to be suppressive against human cancer cells with underlying cytotoxic and cytostatic properties (70). Anti-helmintic activity was demonstrated for semi-synthetic enniatin derivates targeted against *Haemonchus contortus*, a nematode commonly occurring in parasitic form (71). Furthermore, both enniatin and BEA were shown to have antiresorptive and antitumor properties in bones (72). BEA and enniatin effects on ion homeostasis, along with oxidative phosphorylation, and mitochondrial swelling were shown to be the underlying causes of impaired mitochondrial functionality in rat liver cells (73).

III Interactions between ABC-transporters and secondary fungal metabolites

3.1 Interactions between lovastatin and ABC-transporters

3.1.1 Lovastatin and P-gp

Lovastatin interacts with P-gp *in vitro* in an inhibitory manner (74, 75, 76). The statin drug decreases the transport of well-established P-gp substrates, such as daunorubicin (74), rhodamine 123 (74, 76), and calcein-acetoxymethylester (75). Its inhibitory potential is more evidently targeted towards P-gp coded by the human MDR-1 gene as compared to the rodent gene, apparent by the IC_{50} value of 26 µM versus 102 µM, respectively (74). Moreover, the extent of inhibition also depends on the chemical form of the drug – lovastatin lactone exhibits a clearly stronger inhibition of the ABC transporter (IC_{50} set at approximately 10 µM) than the respective acid form (IC_{50} more than 100 µM) (Figure 18). Similar discrepancies in

P-gp inhibition in dependence from the underlying chemical structure were observed for other statins, including simvastatin and atorvastatin. This might be explained by different physicochemical features of the two chemical forms, e.g. lipophilicity and basicity (75). Along with P-gp, chemical structure of statins also plays a role in determining the extent of CYP-enzymes inhibition. As for the ABC-transporter, these enzymes are generally inhibited more strongly by the lactone than the acid form (77).

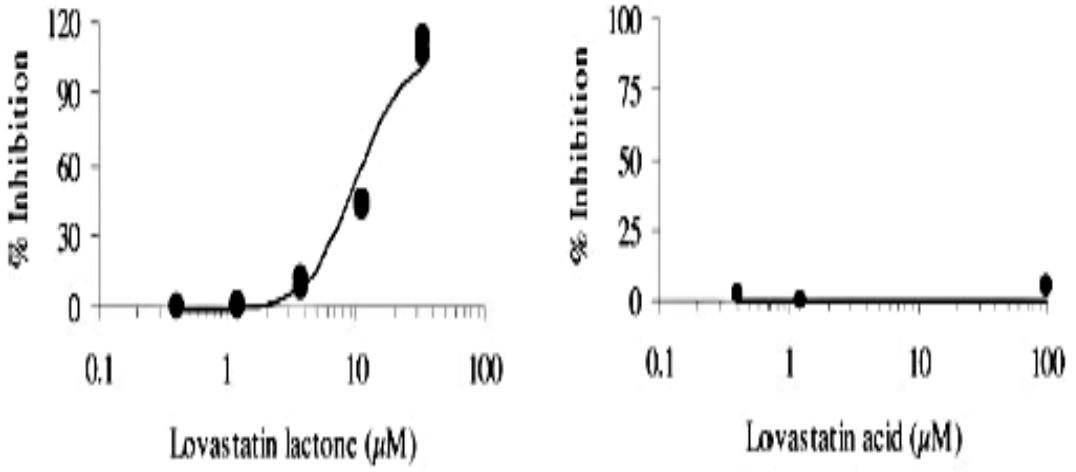


Figure 18: Inhibitory effect of lovastatin in lactone and acid form on the P-gp mediated efflux of calcein-AM in MDCK-MDR1 cells (75).

Furthermore, lovastatin is a substrate of P-gp (74, 75). The extent of this interaction is weak to moderate as compared to quinidine and prazosine, which are good and moderate P-gp substrates, respectively. Like the inhibitory potential, the substrate nature of lovastatin is dependent from its chemical form – the lactone form stimulates the hydrolytic activity of P-gp already at low concentrations, while the acid form has no effect (75). Lovastatin containing red yeast rice products are also potent inhibitors of P-gp, and their inhibitory potential is even stronger than the one of pure lovastatin. Moreover, lovastatin is more effectively absorbed from such products in comparison to the pure drug with same underlying dose, as evident in Caco-2 cells as intestinal model (78).

Lovastatin also interacts with P-gp *in vivo*. In healthy men, lovastatin has major impact on the pharmacokinetics of verapamil, a drug that is a substrate of P-gp and CYP3A4. A combined oral intake of verapamil and lovastatin leads to a significant increase in bioavailability of verapamil as compared to the calcium channel blocker alone. Precisely, the area under the plasma-time curve (AUC) and maximum plasma concentration (C_{max}) of verapamil increase by roughly two and one third in the presence of lovastatin, respectively. However, half-life of verapamil is not significantly influenced by lovastatin. Besides stimulating its absorption, lovastatin also reduces the first-pass effect of verapamil, as apparent by its metabolite norverapamil. Unlike verapamil, the AUC of its metabolite was not significantly affected by lovastatin (79). As in humans, lovastatin significantly alters the pharmacokinetics of verapamil and norverapamil in rats. An increase in AUC and C_{max} of both the parental compound and its metabolite was noticed, but the ratio of two compounds was not significantly affected by lovastatin (80). Similarly, lovastatin leads to significant increases in AUC, C_{max} and bioavailability of diltiazem in rats, and decreases the ratio of the metabolite desacetyldiltiazem to the parent compound. This way, lovastatin has a potential to increase absorption and decrease the first-pass effect of a second calcium channel blocker, which might be explained by inhibitory interactions between the statin and P-gp in intestinal, and with CYP3A4 in intestinal and/or hepatic cells (76). Interactions between lovastatin and P-gp are dependent on the chemical form of the drug also *in vivo*. In rats, P-gp has an evident impact on the brain penetration of the lactone form, but not of the active acid form. Moreover, P-gp has no major influence on the liver disposition of lovastatin (81). Despite evident inhibition of P-glycoprotein and CYP450 enzymes *in vitro*, a red yeast rice product had no major impact on the pharmacokinetics of nifedipine *in vivo* in human individuals (78). Also, no significant influence of two ABCB1 gene haplotypes (c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T) on pharmacokinetic features of lovastatin was found, and the same was observed for fluvastatin, pravastatin, and rosuvastatin (82).

A range of isoprenoid compounds are generated in the mevalonate pathway that not only serve to produce cholesterol, but are also key points in regulating cell growth. Manipulating this process might therefore be a treatment strategy in some malignant conditions (83). Lovastatin leads to an arrest of cells in the G_1 phase of cell cycle of both normal and tumor cells. This is probably due to loss of a compound normally generated in the mevalonate pathway that is vital for cell progression in the G_1 phase (84). In one case of a patient suffering from acute myeloblastic leukemia, lovastatin was able to control the growth of leukemic blast cells when applied at a double dose as compared to the one normally used in treating blood lipid disorders (85). However, in a phase I clinical trial, the absolute majority of individuals diagnosed with solid tumors did not respond to a cyclic treatment with lovastatin on monthly basis during two and a half years regime, although a clear set back in cholesterol and ubiquinone levels as products of the mevalonate pathway was observed (86). However, *in vitro* experiments have confirmed lovastatin to be effective in treating different forms of cancer cells, such as acute myeloid leukemia (87), pediatric solid tumors, cervical cancer, juvenile myelomonocytic leukemia, and head and neck squamous cell carcinoma (88). Lovastatin's cytotoxicity against human neuroblastoma cell lines is especially targeted against P-gp overexpressing cells. This is particularly relevant in patients suffering from metastatic neuroblastoma at advanced stage, as they often show resistance towards chemotherapeutic treatment, to which P-gp could contribute. Consequently, lovastatin could represent a better alternative to standard treatment of neuroblastoma. Lovastatin's cytotoxicity can further be potentiated by dibutyryl cyclic AMP, an effect that probably involves an increase in cellular AMP concentrations (89). A correlation between HMG-CoA reductase expression and chemoresistance was found also in leukemic cells. Precisely, lovastatin treatment induces the expression of the HMG-CoA reductase gene in cells that do not contain P-gp. To the contrary, most cell lines characterized by chemoresistance do not react to lovastatin exposure in the

same way. These findings advocate a potential use of lovastatin in treating leukemic malignancies (90). Similarly, lovastatin induces apoptosis of ovarian cancer cells through a p53-independent mechanism. The statin drug also potentiates the cytotoxic effects of the chemotherapeutic drug doxorubicin through a mechanism not involving HMG-CoA reductase inhibition, but probably by inhibiting P-gp mediated drug elimination from malignant cells (91). P-gp expression is associated with increased susceptibility to lovastatin's cytostatic effect to arrest myeloid leukemia cells in the G_0/G_1 cycle. In such cells, lovastatin induces apoptotic and necrotic changes. To the contrary, cells that acquire chemoresistance through MRP expression are insensitive to lovastatin exposure and do not undergo apoptosis or necrosis (92).

3.1.2 Lovastatin and other ABC-transporters

Lovastatin interacts with the human MRP-2 transporter *in vitro*, and similar interactions are seen with the rodent transporter. As in the case of P-gp, lovastatin inhibits the MRP-2 mediated transport of a substrate compound. The extent of such effect is dependent from the chemical form – lovastatin lactone has a more pronounced effect as compared to the active acid form (Figure 19) (75).

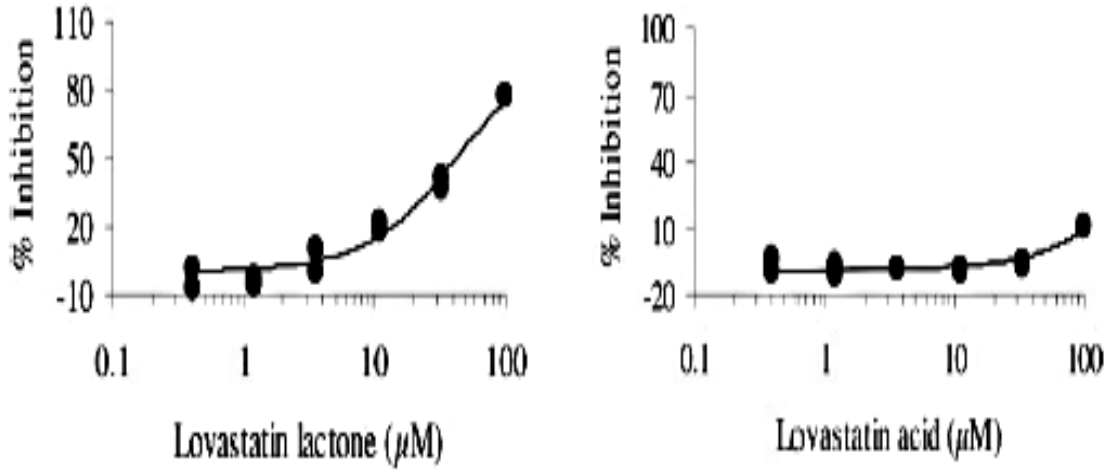


Figure 19: Inhibitory effect of lovastatin in lactone and acid form on the MRP-2 mediated efflux of calcein-AM in MDCK-MRP-2 cells (75).

To the contrary, the transport of lovastatin was not shown to involve MRP-2, BCRP or P-gp in Caco-2 cells regardless of the underlying chemical form. The same was observed for simvastatin, while other statins, including atorvastatin, fluvastatin, and rosuvastatin were transported by the three ABC-transporters (93).

However, in chronic myeloid leukemia cells, lovastatin's ability to potentiate the cytotoxic effects of chemotherapeutic drugs also involves BCRP, along with P-gp. Lovastatin reduces the eliminatory potential of the ABC-transporters, but the effect can be entirely reversed when cholesterol is added. This indicates an important role of cholesterol in determining the activity of P-gp and BCRP as to eliminating compounds from cells. The application of lovastatin leads to increased cellular concentrations of the drug imatinib in murine and human cell lines, but also in primary cells originating from individuals suffering from the malignant condition at different stages (94).

Lovastatin is associated with overcoming resistance to treatment not only in cancer cells, but also in infectious diseases. In animals infected with antimony resistant strains of the parasite *Leishmania donovani,* the outcome is mostly fatal despite treatment with sodium antimony gluconate. However, co-administration of the drug with P-gp and MRP-1 blocking agents, such as a combination of verapamil and probenecid or lovastatin, leads to elimination of drug resistant parasites and survival. This way, inhibition of P-gp and MRP-1 becomes an important mechanism in overcoming antimony resistance in treating parasitic leishmania infections (95).

Lovastatin has a treatment potential in cases of X-ALD. The statin drug, along with the mevalonate pyrophosphate decarboxylase blocker sodium phenylacetate, can reduce pathologically elevated levels of VLCFA in skin fibroblast cells of X-ALD (96). Lovastatin's ability to reduce elevated plasma concentration of fatty acids in X-ALD affected individuals

was also demonstrated *in vivo* (97, 98). The mechanism for this effect in *in vitro* cells involves the activation of sterol regulatory element-binding proteins (SREBPs) upon reduction of cellular cholesterol content. SREBP can subsequently bind to a sterol regulatory element located in the promoter region of the ABCD2 gene, which results in increased transporter expression. ABCD2 can then compensate the deficient ABCD1 transporter, and therefore normalize the metabolism of VLCFA (99).

3.2 Interactions between ergot alkaloids and ABC-transporters

Based on their pharmacological use, ergot alkaloids have to penetrate into the CNS to exert a great part of their effects. Ergotamine was detected in the cerebrospinal fluid (CSF) at a concentration of 0.40 ± 0.03 ng/ml 1-2 hours following oral administration of 2mg of ergotamine tartrate (100). However, no ergotamine was detected in the CSF following intramuscular or rectal application in a small number of hospitalized patients (101). Yet, ergot alkaloids can efficiently be transported across the blood-brain barrier *in vitro.* Ergometrine, ergocristine, and ergotamine are transported across the blood-brain barrier in a porcine cell model, implicating their ability to penetrate into the CNS. However, only 8-(R) isomers seem to be able to cross the endothelial barrier, while the 8-(S) isomer can successfully penetrate into the cells, but stays captured inside. ABC-transporters are involved in the transport of ergometrine across the cells, but do not mediate the transport of ergocristine or ergotamine. Although P-gp also seems to be involved, BCRP is the predominant transport mediating the transport (102).

Similar was observed for ergotamine in a cancer cell line. Ergotamine and the reduced derivate dihydroergotamine have no influence on expression levels of BCRP in a cancer cell line. Moreover, these ergot alkaloids cannot reverse cellular chemoresistance by inhibiting BCRP (103).

A range of ergot alkaloids were characterized for their ability to inhibit P-gp. Ergometrine is a weak inhibitor of P-gp coded by the human MDR1 gene with a inhibition constant (K_i) value of 115 μM. A slightly stronger inhibitory effect is attributable to ergocornine (K_i = 105 μM). However, this effect can be potentiated by inserting a brome atom into the structure, as the semisynthetic derivate of ergocornine, bromocriptine exhibits a clearly stringer inhibition of P-gp with a K_i value of 2.81 μM. The length of the C_5-positioned rest has a substantial role in determining the inhibitory potential of the compound towards human P-gp, as the strongest inhibition is seen with α-ergocryptine (K_i = 12.2 μM), as compared to ergocristine (42.8 μM) and ergocornine (105.2 μM), which have shorter residues at the given position. The length of the C_3-rest is also important, as reducing the chain length of ergocristine to ergotamine from methyl-ethyl to methyl group results in roughly half of inhibitory potential (42.8 and 98.9 μM, respectively). Furthermore, the inhibitory potential of ergot alkaloids can be effectively changed by reducing the double bond between C_9 and C_{10} of lysergic acid. Moreover, dihydroergocryptine is a substrate of human P-gp, and the same is observed for the mouse mdr1a product (104).

A clavine alkaloid, lysergol can potentiate the antibacterial activity of tetracycline against *E. coli* strains that acquired multidrug resistance. Lysergol, along with two of its derivates, inhibits bacterial ABC-transporters (105).

3.3 Interactions between cyclosporine A and ABC-transporters

3.3.1 Cyclosporine A and P-gp

Nephrotoxicity is one of the main side effects of the immunosuppressive therapy with cyclosporine A and has been reported *in vivo* (106, 107, 108). Several mechanisms are involved in this pathological process, including increased concentrations of procollagen alpha

I mRNA in the renal cortex (109), that precedes typical morphological changes of kidneys, such as tubular atrophy, interstitial fibrosis and arteriolar hyalinosis (110).

Cyclosporine A can modulate renal P-gp expression, which probably serves as a protective detoxifying tool. Renal biopsies of transplant recipients who received immunosuppressive cyclosporine A treatment revealed a clear correlation between the presence of cyclosporine A and P-gp expression in the kidneys. Moreover, cyclosporine A increased the number of P-gp expressing cells *in vitro* following 7 and 60 days of exposure (111). Cyclosporine A was shown to stimulate the expression of P-gp *in vitro* in proximal tubule cells obtained from rats in a concentration-dependent manner following one week of treatment (112).

The influence of a low-salt diet regime was examined in mice treated with cyclosporine A in relation to renal P-gp expression patterns. Low-salt diet is associated with stronger manifestation of lesions typical for cyclosporine A-induced chronic nephrotoxicity. In parallel, exposure to cyclosporine A induces P-gp overexpression across the renal tubular system in a time-dependent fashion. Specifically, P-gp expression levels are slightly higher in mice maintained on the low salt dietary regime. Kidneys of mice exposed to cyclosporine A are also more susceptive to deposition of angiotensin II, with the effect being time-dependent. A negative correlation exists between hyaline arteriopathy and periglomerular and peritubular fibrosis and overexpression of P-gp. In mice with evident nephrotoxicity of severe degree, the same correlation is found between the overexpression of P-gp and deposition of angiotensin II in the kidneys. These findings again suggest that P-gp is an important protective mechanism in kidneys, and its role might be connected to pathways through which toxic products derived from cyclosporine A metabolism are eliminated from cells (113).

However, P-gp was also reported not to be associated with cyclosporine A-induced nephrotoxicity. Contrary to other reports where induction of renal P-gp expression in kidneys has protective implications in the context of cyclosporine A-related toxicity, this mechanism

can also be associated with negative outcomes, such as acute tubular necrosis or rejection of the transplant organ. Moreover, increased P-gp levels in immune cells that infiltrate kidneys are associated with organ rejection (114).

Cyclosporine A induces stronger nephrotoxic effects than its analogues and metabolites. Compared to these compounds, cyclosporine A exhibits the strongest affinity towards human renal P-gp, as evident by competitive inhibition of incorporation of labeled azidopine molecules into the transporter. Precisely, cyclosporine A has a 2-fold stronger inhibitory potential in comparison to cyclosporine G, and a 25-fold than cyclosporine C. The extent of inhibitory potential of its metabolites 1, 17, and 21 is 10-fold lower, whereas the metabolites 8 and H exhibit no competitive inhibition of azidopine incorporation. Cyclosporine A along with C and G has the same effect in inhibiting P-gp labeling in multidrug resistant cells. Also, these three compounds exert a stronger inhibition of P-gp mediated transport of a range of compounds in multidrug resistant cells, which are known to be substrates of the ABC-transporter (115). The ability of cyclosporine A to block the photolabeling of renal P-gp is also higher than the one of chemotherapeutics vinblastine, doxorubicin and daunorubicin, as well as of verapamil or quinidine (116). However, the nature of this interaction in multidrug resistant cell seems to be non-competitive, i.e. cyclosporine A binds on another site in P-gp than azidopine (117).

Genetic features of the underlying ABCB1 gene can contribute to variability in pharmacokinetics of cyclosporine A. The single nucleotide polymorphism (SNP) 3435T in the ABCB1 gene, along with the T-G-C haplotype originating from three investigated SNPs (C1236T, G2677T/A, and C3435T) in individuals who carry CYP3A5*3 SNP, and the CYP3A5*3 SNP are factors contributing to pharmacokinetic variations during oral immunosuppressive therapy with cyclosporine A in the first 30 days in individuals who received bone marrow or hematopoietic stem cells transplants. Analysis of these genes prior

to such therapy can thus support efforts in developing individual dosage patterns for immunosuppressive cyclosporine A therapy, particularly in the context of required initial dose (118). Similar observations were also found in patients who received kidney transplantation (119, 120) and also received the calcium blocker diltiazem besides cyclosporine A (121). However, no influence of ABCB1 was found in stable patients who received kidney transplants (122).

Furthermore, cyclosporine and its non-nephrotoxic and non-immunosuppressive analogue PSC-833 are modulators of P-gp in drug-selected cell lines. Modulation of P-gp activity in eliminating chemotherapeutic drugs from cells reveals a promising potential of cyclosporine A in combating drug resistance in malignancies, e.g. as leukemia, to which P-gp contributes together with other ABC-transporters (123). Apart from cyclosporine A, two other immunosuppressive drugs (sirolimus and tacrolimus) have a similar effect (124). However, modulation of P-gp with cyclosporine A was not shown to have a positive outcome in patients suffering from chronic myeloid leukemia currently in the blast phase (125).

Cyclosporine A can also interact with the brain P-gp. The compound leads to increased brain uptake of a radioligand that binds on serotonin 5-HT1A receptors in rodents through a mechanism that involves P-gp inhibition (126). Cyclosporine A can also increase the brain uptake of labeled verapamil, a P-gp substrate, by inhibiting its activity in the blood-brain barrier in humans (127), and rats (128).

Cyclosporine A can effectively be transported by P-gp coded both by the human MDR1 and rodent mdr1a gene. Moreover, P-gp has a crucial role in determining the distribution of cyclosporine A (Table 1). Precisely, P-gp deficient mice have significantly higher levels of cyclosporine A in the brain (17-fold), testis (2.6-fold), small intestine (1.9-fold), heart and colon (1.6-fold), and spleen (1.5-fold) as compared to the non-deficient counterparts when measured 4 hours after intravenous application. Discrepancies in brain concentration of cyclosporine A grow significantly over the time course (26.3 and 55.2 after 8 and 24 hours for

P-gp deficient and non-deficient mice, respectively). Significant increases in cyclosporine A organ concentration after 24 hours are found also in other organs, such as muscles, heart, and kidneys. Plasma concentration of cyclosporine A is also significantly higher (1.9-fold) in P-gp lacking mice when measured after 24 hours (129).

Tissue	*mdrla* (+/+)	*mdrla* (−/−)	Ratio (−/−):(+/+)
Brain	2.5±0.9	138±6	55.2§
Muscle	5.8±2.0	23±6	4.0‡
Heart	21±6	43±3	2.1‡
Kidney	88±17	135±14	1.5*
Liver	857±280	1164±73	1.4
Gall bladder	753±134	3491±402	4.6‡
Lung	33±12	70±8	2.1*
Stomach	401±174	461±284	1.1
Small intestine	161±46	1334±204	8.3§
Colon	153±36	252±32	1.7*
Testis	63±6	116±35	1.8
Spleen	66±3	140±46	2.1*
Thymus	163±43	200±58	1.2
Lymph nodes	108±24	196±10	1.8‡
Plasma	3.4±0.7	6.4±0.7	1.9‡

Table 1: "Tissue Levels of Radioactivity in mdr1a (+/+) and (-/-) Mice 24 h after Intravenous Injection of [^{3}H]Cyclosporin A (1mg/kg)"."Results are expressed as means ± SD (n-1) in ng/g tissue ([^{3}H]Cyclosporin A equivalent). Three mice were analyzed in each group. * $P < 0.05$; ‡ $P < 0.01$; § $P < 0.001$." (both quoted from ref. 129)

However, cyclosporine A not only modulates P-gp expressed in the CNS, but also in peripheral nerves. This drug induces significant increases in peripheral nerve concentrations of the cytotoxic compounds doxorubicin and vinblastine in P-gp lacking mice. Concomitant

immunosuppressive therapy with cyclosporine A and chemotherapy with these drugs might therefore be dangerous and lead to increased neurotoxicity (130).

Similarly, a concomitant administration of doxorubicin with cyclosporine A in mice leads to increased concentrations of the chemotherapeutic drug in the inner ear and consequent hearing problems. This suggests that cyclosporine A inhibits P-gp expressed in the blood-inner ear barrier (131), similar to mice deficient in P-gp (132). Apart from doxorubicin, cyclosporine A exposure can also lead to increased levels of vinblastine in the inner ear and other organs, including brain (133).

In addition, cyclosporine A can increase intracellular concentrations of other chemotherapeutic compounds in chemoresistant cells, such as of daunorubicin (134), and increase the sensitivity of such cells to other anthracyclines, particularly mitoxantrone and aclacinomycin (135).

Cyclosporine A was proven to be a transport substrate for the human P-gp, the nature of the transport process being saturable (136). However, transport of the immunosuppressive drug across intestinal cells by mammalian P-gp is not a determinant of its bioavailibity when administered by oral route, as demonstrated *in vivo* in dogs (137).

The *in vitro* activity against *Plasmodium vivax* has already been reported for cyclosporine A and its non-immunosuppressive derivate (138). There is a possible contribution of interactions between P-gp and similar cyclosporine A derivates that show no or only weak affinity towards cyclophillin in determining their activity targeted against the parasites *Cryptosporidium parvum* (139) and *Toxoplasma* (140).

The use of chemotherapeutics in treating intraocular retinoblastoma malignancies is often not effective, which might be associated with P-gp overexpression. Cyclosporine A can be effectively used in treating patients suffering with intraocular retinoblastomas. The drug can complement the use of chemotherapeutics, e.g. vincristine, teniposide, and carboplatin, as evident by positive outcomes of the treatment, e.g. high relapse-free rates both in pre-treated

patients, as well as those who had previously received no treatment (141). However, the inhibition of P-gp by cyclosporine is not of major clinical relevance in MRP co-expressing retinoblastoma (142).

3.3.2 Interactions between cyclosporine A and other ABC-transporters

Contradictory reports exist on the potential of cyclosporine A to modulate BCRP activity. Cyclosporine A exhibits only weak modulation of BCRP as compared to the vesicular monoamine transporter blocker reserpine, which can effectively increase the susceptibility of BCRP-expressing cells towards the cytotoxic agent CPT-11 (143). Unlike P-gp, BCRP-mediated transport of compounds is unaffected by cyclosporine A. Analogously, the drug does not interfere with the photolabelling of the transporter, nor does it affect its basal or substrate-related hydrolytic activity. Additionally, human P-gp, but not BCRP is involved in the transport of cyclosporine A (144).

However, contrary studies also exist. Along with P-gp, cyclosporine A was also reported to modulate the activity of MRP-1 and BCRP in drug selected cell lines, which frequently co-determine chemoresistance in malignant cells. Its modulatory effect on BCRP is not only directed towards the wild-type form, but also towards mutated transporter forms, in which arginine in position 482 is exchanged by glycine and threonine. However, its analogue PSC-833, that is not associated with immunosuppression or the typical cyclosporine A-induced nephrotoxicity does not exhibit a modulatory effect towards MRP1 nor the wild-type and mutated forms of BCRP (123). The ability of cyclosporine A to block wild-type BCRP and the threonine-containing mutant is generally lower than the one observed for P-gp (145).

As already described for P-gp, cyclosporine A, together with tacrolimus and sirolimus can also increase intracellular concentrations of the cytotoxic drug mitoxantrone by modulating

MRP1 and BCRP activity. These compounds modulate BCRP activity by competitively binding on the drug docking site of the transporter (124). However, these three drugs are not substrates of BCRP, but can block BCRP-mediated transport of compounds. Furthermore, such a modulation can restore cell sensitivity to chemotherapeutics mitoxantrone and topotecan, which had been previously lost due to BCRP activity (146).

In humans, cyclosporine A-associated modulation of brain ABC-transporters does not affect the uptake of carbon labeled morphine into the brain (147).

Immunosuppressive treatment with cyclosporine A is associated with adverse changes in lipid metabolism. In renal transplant patients, blood levels of cyclosporine A show significant correlation with cholesterol levels, including HDL and HDL3 cholesterol, apolipoproteins AI and B, LDL cholesterol, as well as the cholesterol/HDL cholesterol ratio (148), and similar results were discovered in bone marrow transplant recipients (149). These effects might be related to the inhibitory potency of cyclosporine A towards the ABCA1 transporter, which is involved in transporting cholesterol and phospholipids on the surface of apolipoprotein AI, amongst other apolipoproteins. The immunosuppressive agent does not reduce the expression of the transporter, but rather increases its levels, as demonstrated in mouse macrophage cells. However, cyclosporine A inhibits cell-apolipoprotein AI interactions, e.g. its uptake. Furthermore, the drug reduces plasma concentrations of HDL in mice (150).

However, the underlying mechanism for the cyclosporine A inhibition of ABCA1-mediated transport of lipids on apolipoprotein AI is controversial as to whether it involves a direct inhibition of the transporter or an indirect inhibition through a secondary cellular pathway.

On one side, this inhibitory process does not seem to involve a direct interaction with the transporter protein itself. Cyclosporine A inhibits this process rather by affecting Ca^{2+}-dependent cellular pathways, i.e. blocking of calcineurin prevents the binding of apolipoprotein AI. In addition, inhibition of calcineurin reduces the phosphorylation of JAK2,

a recognized signaling mechanism in transport of cholesterol on the acceptor lipoprotein (151). To the contrary, cyclosporine A and PSC-833 were similarly effective in reducing the elimination of cholesterol from cells in a process involving direct inhibition of ABCA1 transporter (152).

The association of cyclosporine A with cholestasis has already been reported (153, 154), and interactions with ABC-transporters are suggested to contribute to this pathological process. Localized along the bile canaliculus in the liver, the bile salt export pump (BSEP), also known as sister of P-gp, plays a substantial role in exporting bile salts in mammals, such as taurochenodeoxycholate, tauroursodeoxycholate, glycocholate and others (155). Human BSEP has strong similarities with the respective mouse and rat transporter (156).

Cyclosporine A, but not the immunosuppressive compound tacrolimus (FK506), induces a change in localization patterns of the rat Bsep in the liver canaliculi (Figure 20). Precisely, there is an evident shift of the export pump into the cell following exposure to cyclosporine A. Furthermore, such treatment disrupts the pericanalicular F-actin cytoskeleton (157).

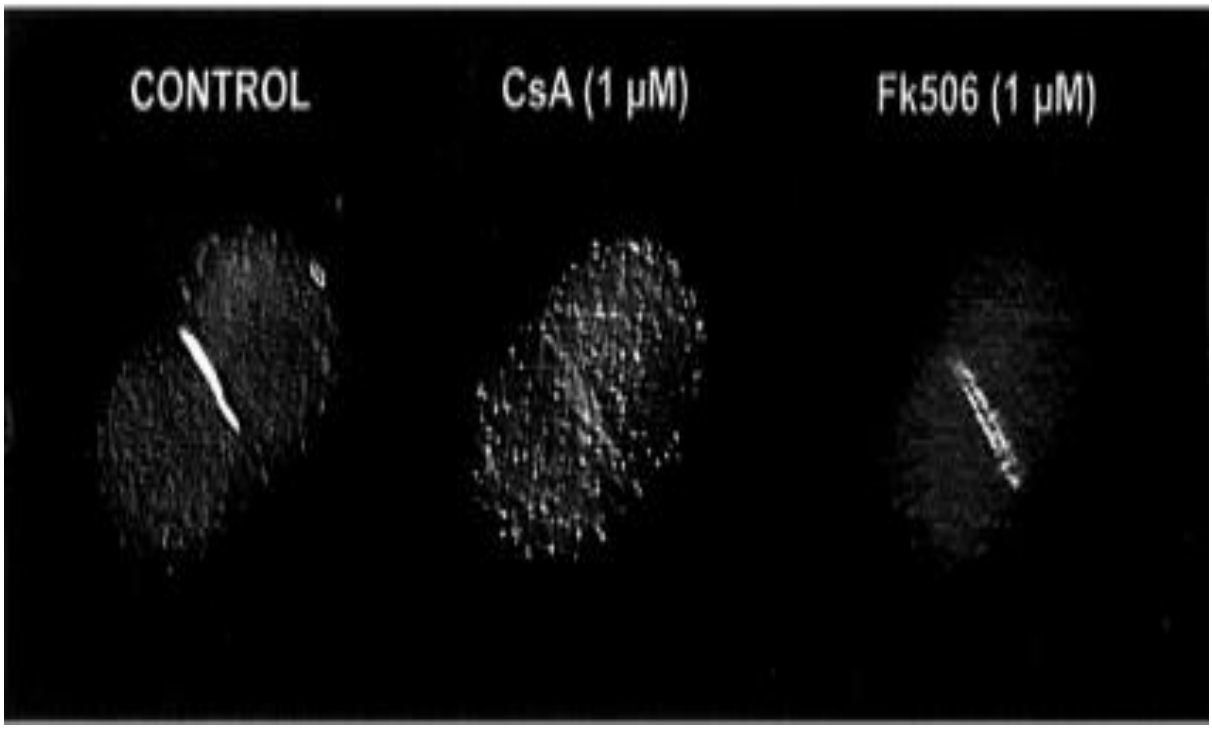


Figure 20: "FITC-labeled Bsep localization in rat hepatocyte couplets treated with DMSO (control), CsA (1 μM), or FK506 (1 μM) for 15 min. White indicates the highest intensity of fluorescein measured." (quoted from ref. 157)

Cyclosporine A and its derivate PSC-833 can both reduce the transport of compounds across the liver canaliculi membranes, which is believed to be caused by their inhibition of ABC-transporters. In rat membrane vesicles, cyclosporine A is more effective in interrupting the transport of the bile salt taurochalate, and LTC4; while the transport of the antracycline chemotherapeutic drug daunorubicin is more effectively inhibited by PSC-833 (158). As demonstrated in rats, cyclosporine A together with other tested drugs has a cis-inhibitory potential towards Bsep, unlike 17β-glucuronide-conjugated estradiol, which can only inhibit this transporter after its excretion into the bile canaliculi performed by the Mrp2 transporter, i.e. it exhibits a trans-inhibition of Bsep (159). Cyclosporine A inhibits the ATP-requiring transport of the bile salt taurochalate also in the human liver, and is slightly more effective than tacrolimus in doing so. It is suggested that this could be performed in a competitive manner, as evident in rat liver (160).

However, pre-administration of hydrophilic bile salts has a protective potential against cyclosporine A-associated cholestasis. Namely, infusion of these salts prior to cyclosporine A treatment can diminish adverse drug-induced effects, such as decreased fluidity of the canalicular hepatocellular membrane, and leads to subsequent overexpression of some ABC-transporters, e.g. Bsep and Mrp2. However, the treatment does not enhance P-gp or Mdr2 levels (161).

Apart from bile salts, treatment with cyclosporine A is also associated with changes in bilirubin metabolism. These changes encompass slightly increased levels of serum bilirubin along with other liver function parameters, i.e. alkaline phosphatase and aminotransferase (162). Moreover, two cyclosporine A metabolites of second generation are eliminated through the same transport mechanism as bilirubin into the bile. Consequently, elevated total serum

levels of bilirubin can be indicative of defect elimination of the immunosuppressive drug (163). On the other side, bilirubin is associated with a nephroprotective effect, and can lessen cyclosporine A-related renal pathological changes, such as tubular damage, by decreasing oxidative stress and cell apoptosis (164).

As for the bile salts, ABC-transporters play a recognized role in biliary secretion of bilirubin. However, they are also potential cross points of cyclosporine A-associated bilirubin changes. Conjugated bilirubin (mono- and bisglucuronosyl) is transported by the human and rat MRP2 transporter. The transport of monoglucuronosyl bilirubin by this carrier can be inhibited by other substrates, i.e. LTC4 and 17β-glucuronosyl estradiol, but also by cyclosporine A, which inhibits the transporter in a competitive manner. However, the inhibitory potential of the drug towards the human transporter is only half of the one towards the rat orthologue (respective K_i values of 21 and 10 μmol/L). Moreover, cyclosporine A is more effective in inhibiting the human MRP2 transporter in comparison to the substrate hormone conjugate (K_i = 30 μmol/L), but still roughly 10-fold less effective than LTC4 (K_i = 2.3 μmol/L) (165). However, cyclosporine A was also reported to have stronger apparent inhibitory potency of bilirubin uptake in human hepatocytes as compared to rat cells (166).

3.3.3 ABC-transporters mediated interactions between cyclosporine A and mycophenolic acid

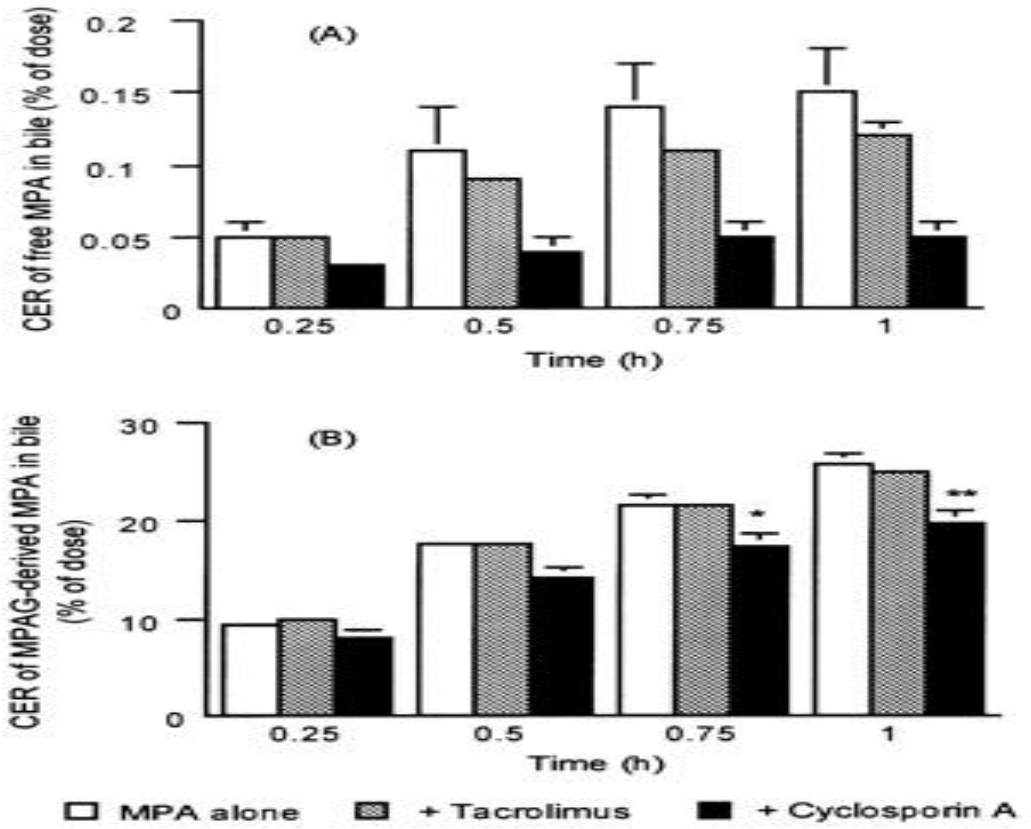
During post-transplant immunosuppressive therapy, blood levels of mycophenolic acid were reported to be approximately two times lower in renal transplant patients who received only the corticosteroid prednisone in addition to mycophenolat mofetil as compared to those who were also treated with cyclosporine A as third immunosuppressive drug (167). Similar effects were reported also in comparison to other immunosuppressive drugs, including tacrolimus

(168). Subsequent withdrawal of cyclosporine A from the treatment results in increasing levels of mycophenolic acid (169).

Mycophenolic acid is converted into glucuronide metabolites in the body, including the inactive phenolic 7-O-conjugate (170), which is excreted into urine with a high rate of 70% within the first 48 hours (171). Cyclosporine A reduces the enterohepatic circulation of mycophenolic acid as well as of the inactive metabolite in a dose-dependent manner (172). ABC-transporters are suggested to contribute to such interactions between cyclosporine A and mycophenolic acid as well as its conjugates.

Human MRP2 can effectively replace the rat transporter (Mrp2) in the liver, as demonstrated in genetically deficient mice, e.g. in terms of expression patterns. Also, it can substitute Mrp2 clearance function in transporting compounds from blood into the bile (173). Human and rat MRP2 display similar specificity to substrates (174), suggesting that general similarities exist between these two transporters.

Biliary elimination of mycophenolic acid as glucuronide conjugate is evidently impaired in Mrp-2 deficient mice. It reaches only 0.5 % of the administered dose within the first hour,



compared to 21-26% in non-deficient mice, indicating a clear involvement of Mrp-2 in this process. Cyclosporine A induced a reduction in the biliary excretion of the conjugate in non-deficient mice (Figure 21). The reduction was constant in time over 1 hour upon administration, and was significant when measured at time points 45 min and 60 min. Cumulative biliary excretion (60 min) of the glucuronide conjugate reached 26% in non-treated mice, and roughly 20% in cyclosporine A treated animals. Tacrolimus did not achieve the same effects, i.e. it did not reduce the biliary elimination of mycophenolic acid glucuronide. Accordingly, co-exposure to cyclosporine A, but not to tacrolimus, increases significantly the plasma concentration of glucuronide-derived mycophenolic acid (175).

Figure 21: "Cumulative biliary excretion of free (A) and MPAG-derived MPA (B) after a bolus intravenous administration together with CsA or tacrolimus to Wistar rats. MPA (5mg/ml), dissolved in polyethylene glycol 400, was administered into the jugular vein at a dose of 5 mg/kg. CsA or tacrolimus was administered at 5 min

before MPA administration via the jungular vein. The dose of CsA and tacrolimus was 5 and 0.1 mg/kg, respectively. In the control (MPA alone), saline was administerd instead of CsA and tacrolimus. Each column represents the mean ± S.E. of three to four experiments, although some standard error bars are hidden by columns. *, $p < 0.05$; **, $p < 0.02$, significantly different from the control." (quoted from ref. 175), MPA = mycophenolic acid, MPAG = mycophenolic acid glucuronide, CsA = cyclosporine A

Mrp2 transporter is suggested to be the only carrier involved in excretion of the ether conjugate into the bile, and contributes partly to the transport of the acyl conjugate. Precisely, Mrp2 deficiency in mice is associated with reduced biliary elimination of the acyl conjugate (by 71.8%), but more strongly with that of the ether glucuronide (by 99%). In Mrp2 expressing mice, cyclosporine A decreases the excretion of both conjugated forms (176).

Apart from Mrp2, P-gp could also contribute to the metabolism of mycophenolic acid, e.g. plasma concentrations after oral intake, and its interactions with cyclosporine A.

Mdr1a-coded mouse P-gp is suggested to be involved in the transport of mycophenolic acid in this species. Drug plasma concentrations show a significant 4-5 fold increase in Mrp-2 (1.94 μg/ml) and P-gp (2.64 μg/ml) deficient mice in comparison to non-deficient animals (0.52 μg/ml) (Figure 22). However, these discrepancies are only present within the first 30 min upon administration of the prodrug mycophenolat mofetil, and disappear in two subsequent measurements (1 and 2 hours). This indicates that more than one transporter determines plasma concentrations of the immunosuppressive drug. In non-deficient mice, co-treatment with cyclosporine A leads to a significant 2-fold increase in plasma concentration of mycophenolic acid (1.07 vs 0.52 μg/ml). However, this disproportion diminishes with time, so that drug levels are higher in non-cyclosporine A co-treated animals after 1 and 2 hours after administration. To the contrary, plasma concentration of the conjugated metabolite is consistently at higher levels in cyclosporine A exposed deficient and non-deficient mice, albeit these differences again decrease with time. Moreover, mice genetically deficient in P-gp have significantly greater cerebral levels of mycophenolic acid compared to Mrp2

deficient or wild-type mice when measured after 30 min. This is in accordance with plasma concentrations of mycophenolic acid (177).

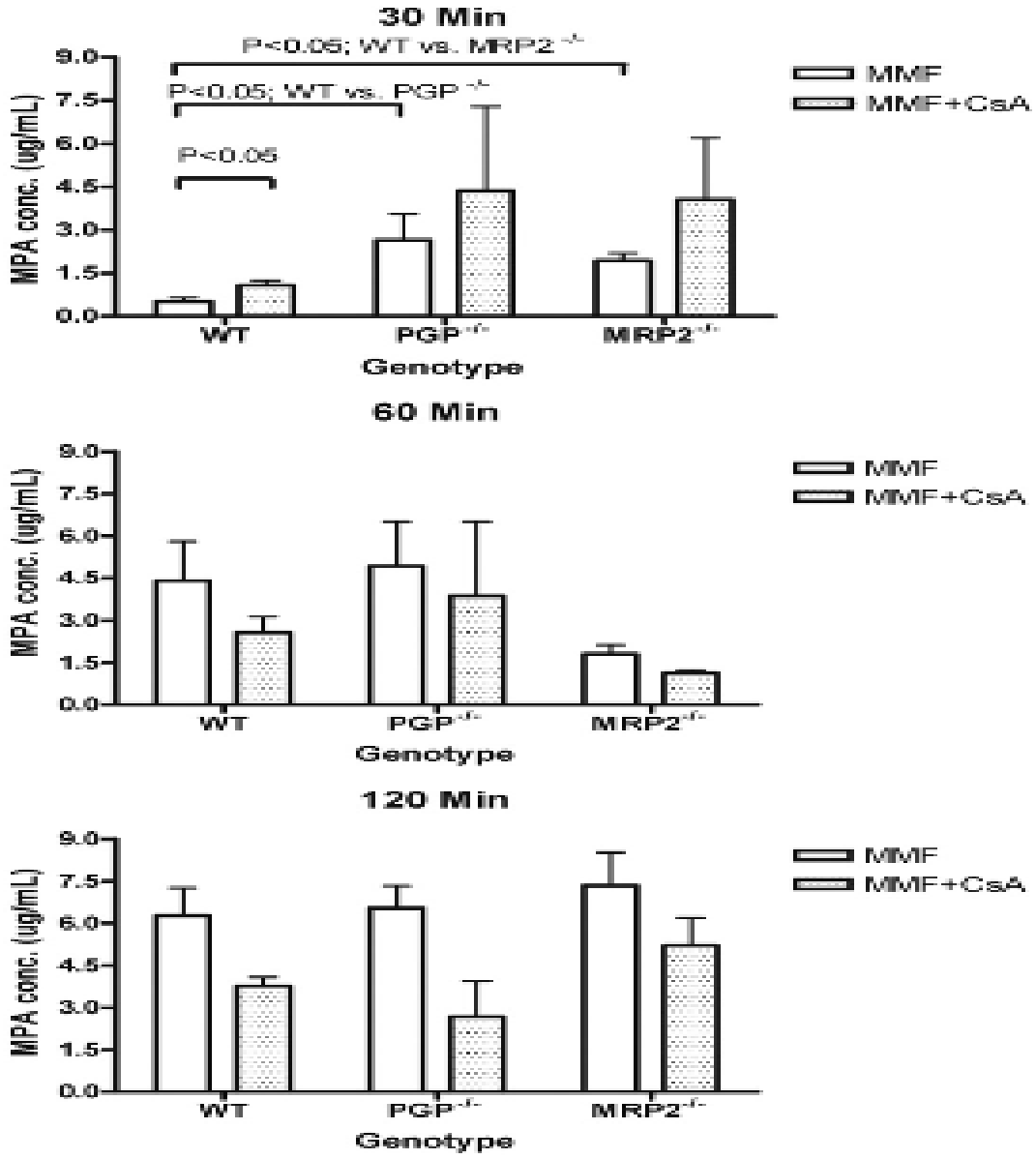


Figure 22: "Plasma concentrations of MPA at 30, 60, 120 min after oral administration of the MMF in wild-type, Mdr1a$^{-/-}$ and Mrp-2$^{-/-}$ mice. MMF was given to animal with a final single dose of 60 mg/kg by oral gavage. CsA (10 mh/kg) was administered 0.5 prior to MMF treatment. 5% dextrose was administered as placebo in control group. Each column represents the mean ± S.D. of 4-6 animals each group. Differences within a time period are based on two-way ANOVA." (quoted from ref. 179); MMF =mycophenolat mofetil

However, experimental studies using the human transporter could not confirm results obtained in mice. That is, cyclosporine A does not block MRP-2 mediated transport of the mycophenolic acid phenol glucuronide, although the conjugate is clearly a substrate for the transporter (178).

3.4 MRP2 gene as determinant of mycophenolic acid pharmakokinetics

As in the case of cyclosporine A, genetic features of proteins involved in metabolism of mycophenolic acid play a role in contributing to pharmacokinetics.

A SNP in the human MRP2 gene, 24T>C, co-determines the pharmacokinetics of the immunosuppressive drug. In detail, heterozygous expression of this SNP in combination with an additional SNP in the conjugating enzyme glucuronosyltransferase (UGT) increases AUC of mycophenolic acid in pediatric renal transplant cases (178). Similarly, polymorphisms in the respective genes are associated also with pharmacokinetic variability of the conjugate metabolite. In healthy individuals, a genetic polymorphism in MRP2 promoter induces elevated levels of the acyl glucuronide by one fourth, and combined with a polymorphism in the UGT gene by 169% (180), but the MRP2 promoter polymorphism has no significant effect on pharmacokinetics of the 7-O-glucuronide metabolite or mycophenolic acid itself in renal transplant recipients (181). However, heterozygous mutant alleles in the MRP2 polymorphism G1249A predispose patients to higher AUC values of the acyl glucuronide (182). Concomitant treatment with tacrolimus and sirolimus can decrease the AUC of mycophenolic acid in patients with patients carrying a specific SNP (183). Furthermore, genetic polymorphisms in the MRP2 gene are associated with increased enterohepatic circulation of mycophenolic acid (184).

3.5 Interactions between aflatoxins and ABC-transporters

3.5.1 AFB_1 and P-gp

Treatment of rats with AFB_1 induced an increased expression of P-gp in the liver, as evident by mRNA levels. Similar effects were seen with other compounds, including synthetic drugs such as phenothiazine (185). AFB_1 clearly induces the expression of one of P-gp coding rat genes (mdr1b) both in normal and cancer liver cells. However, this process does not involve a regulatory element on the promoter that is specific for the mycotoxin. The carcinogenic compound rather acts through the basal promoter element of the gene, or very similar sequences (186). Besides enhancing mRNA levels of the coding gene, AFB_1 leads to accordingly higher levels of membrane-embedded P-gp protein by 3 to 4 times (187).

However, the induction of the P-gp coding gene seems to have different implications than protecting the cell from very potent AFB_1 toxicity. Precisely, mouse fibroblast cells transfected with the mdr1b gene are not any less sensitive to cytotoxic effects of AFB_1 than control cells, suggesting that the encoded P-gp does not contribute to a substantial extent in protecting these cells from AFB_1-induced damage (Figure 23). Moreover, inhibition of P-gp by verapamil does not further enhance cell sensitivity to the mycotoxin. As oppose to AFB_1, the P-gp blocker increases cell sensitivity to chemotherapeutic drugs, e.g. colchicine, taxol, and vinblastine. The calcium channel blocker verapamil, as well as the alkaloid chemotherapeutic colchicine, decreased P-gp mediated elimination of its fluorescent substrate, but AFB_1 had no effect. Taken overall, AFB_1 is not a substrate of P-gp. Consequently, this ABC-transporter does not display cytoprotective effects against the mycocarcinogen (187). Similarly, apart from AFB_1, P-gp is not the primary transport mechanism when the mycotoxin is conjugated with a GSH moiety (188).

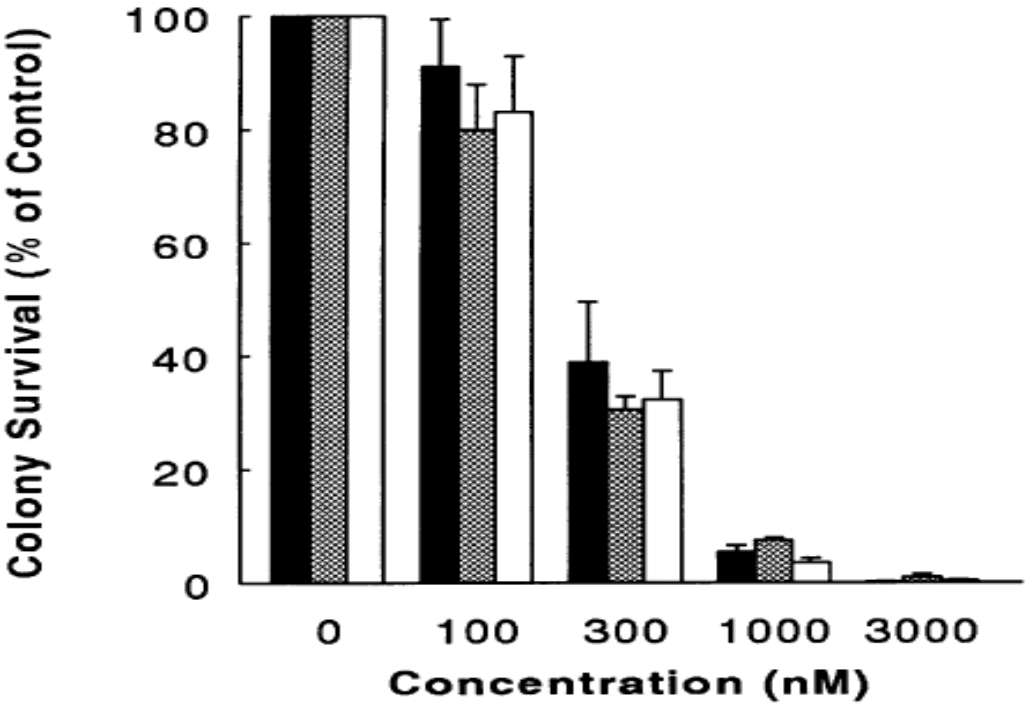


Figure 23: Colony survival of cells after exposure to different concentrations of $AFB_{1,}$ expressed as % of survival in cells that were not treated. Solid bars represent parental cells, hatched bars empty vector-transfected cells, and open bars mdr1b transfected mouse fibroblast cells. (187)

3.5.2 AFB_1 and other ABC-transporters

3.5.2.1 Interactions in the placenta

Human placenta disposes over a variety of transporters, out of which P-gp and BCRP have the most recognized function in drug transport (189). The transport of AFB_1 in human placental samples has been confirmed. Placental tissue is also able to convert AFB_1 into aflatoxicol, which is the only metabolite found (190). Aflatoxicol is a very important metabolite – it is also associated with carcinogenicity (191), can be converted back into AFB_1 by the human liver through dehydrogenation (192), and could be indicative of how susceptible a species is to AFB_1-related carcinogenicity (193). The influence of AFB_1 on expression levels of several ABC-transporters has been assessed in human choriocarcinoma cell (JEG-3) as placental

model. However, P-gp expression in this cell line was very low, with mRNA levels being even non-detectable (194). This restricts further analysis of P-gp expression levels in response to AFB_1 exposure.

Evident changes were observed in expression levels of ABCC2 (MRP2) as consequence of AFB_1 exposure. However, these changes were only noted after 96 hours of exposure to AFB_1. Precisely, a significant increase in levels of ABCC2 mRNA expression was seen, in cases when 2 μM and 6 μM of AFB_1 were used, with an enhancement of 4 and 14 times with respect to control, respectively. Interestingly, when the same concentrations of AFB_1 were applied together with 4 μM finrozole (CYP19A1), the mRNA expression levels remained completely unchanged. A subsequent Western blot revealed that ABCC2 protein expression increased dose-dependently, but the induction was not as great as the one observed in mRNA. Furthermore, ABCC2 protein expression was enhanced with finrozole, and the same effect was observed when low concentrations of AFB_1 were added together with the CYP19A1 inhibitor. To the contrary, the amount of ABCC2 protein was reduced in cells which were exposed to finrozole in combination with high concentrations of AFB_1, whilst the expression of mRNA was completely blocked. The inhibitory potency of finrozole on the expression of ABCC2 mRNA implies that CYP19A1-generated aflatoxicol could stimulate this expression (194). Finrozole was already reported to have a cytoprotective effect in JEG-3 cells against AFB_1-induced toxicity, which was evident at mycotoxin concentrations exceeding 1 μM (195). Combining the results of both studies, it can be concluded on a basis of the time consumed (96 hours) that a secondary pathway or a metabolite of AFB_1 can induce changes in expression levels of ABCC2, which can be interpreted as a cytoprotective mechanism. Aflatoxicol is most likely one of the responsible metabolites in this process, as CYP19A1 blockade by finrozole abolished the mRNA inducing effects. That is, aflatoxicol is cytotoxic

to placental cells, but the cell responds to its cytotoxicity by enhancing primarily mRNA levels of ABCC2.

Changes in mRNA expression levels of ABCG2 (BCRP) in JEG-3 cells are not in concordance with protein levels. Precisely, levels of expression of mRNA rose in cells which were treated with AFB_1 higher concentrations (2 and 6 μM). The same was observed when AFB_1 was applied in low concentration (0.6 μM) combined with finrozole. However, subsequent protein levels analysis revealed a decreased expression of ABCG2 when 0.6, 2 and 6 μM of AFB_1 alone were used, in a dose-dependent manner; and the same was noted for the combination with the CYP19A1 inhibitor (194). Such a discrepancy in expression levels of mRNA versus protein cannot be clearly interpreted and requires further elucidation.

Less apparent changes were observed as to shifts in expression of ABCB4 and ABCC1. Precisely, AFB1 (in 0.6 μM and 6 μM concentrations) enhanced levels of mRNA expression of ABCB4 when applied together with 0.4 μM finrozole. However, no changes in protein levels were observed. Levels of mRNA expression of ABCC1 did not change to a significant extent, with only a small increase noted when 6 μM of AFB_1 was used, while no alterations in protein levels were found (194). These results are not indicative of AFB_1 influence and require further investigation.

3.5.2.2 Other interactions between aflatoxins and ABC-transporters

BCRP is highly expressed in the mammary gland of lactating females, with similar existing patterns in humans, cows and mice. Here, it mediates transport of compounds into breast milk, including both pharmacologically used substances, such as the chemotherapeutic topotecan, as well as carcinogenic compounds occurring in the diet (196). Aflatoxins have already been detected in human breast milk of lactating mothers, including AFM1 (197, 198), AFM2 (198), and AFG1 (199). In addition, the occurrence of aflatoxins has also been

demonstrated in cow milk (200, 201) as well as cow feed samples (201). In this relation, it is highly relevant to inspect the influence of interactions between BCRP and aflatoxins as to their potential implications for human health.

A canine cell line (MDCKII) was used in order to investigate the involvement of Bcrp1 (normally expressed in mice) and the human BCRP transporter in AFB_1 transport *in vitro*, using a concentration of 2 μM (Figure 24). Apical and basolateral transport of labeled AFB_1 in parental cells did not differ substantially. MRP-2 introduction into cells did not change the pattern of transport in comparison to parental cells. However, in Bcrp1 transfected subclones, a great increase was observed in apical transport, along with a strong reduction in rates of basolateral transport. The same pattern was observed in cells transfected with human BCRP, despite the relatively low rates of its cellular expression. The observed transfer pattern was entirely abolished following the application of Ko143, a selective blocker of Bcrp1, and GF120918, which additionally can block P-glycoprotein. This resulted in very similar transport to the one observed in non-transfected cells (202).

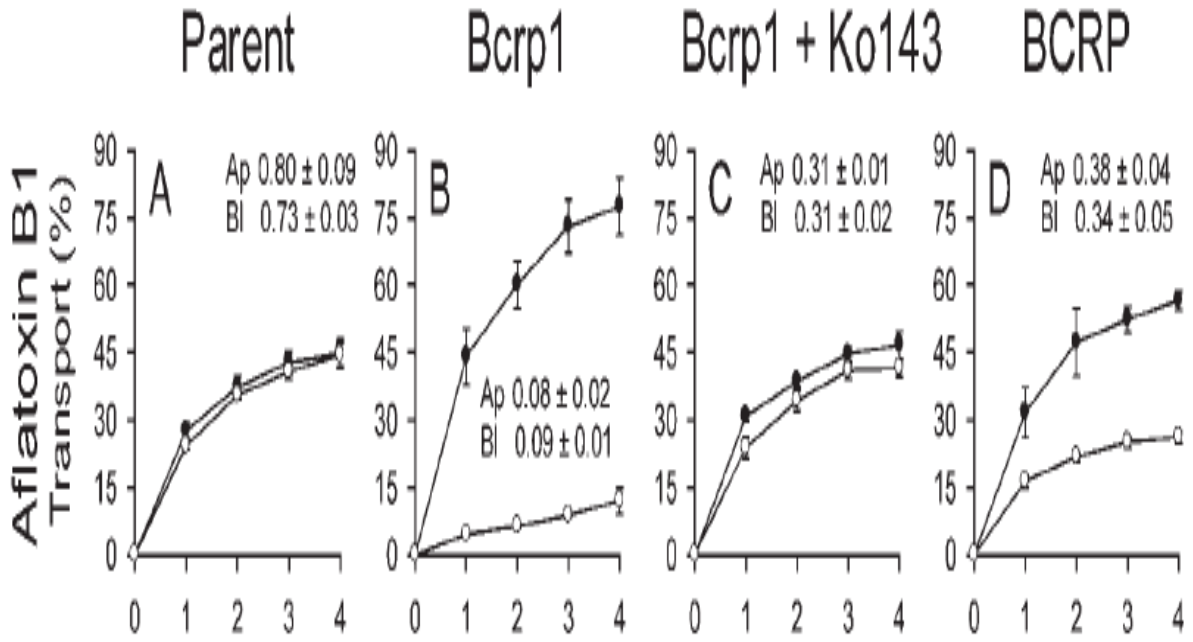


Figure 24: Transport of (^{3}H)-AFB$_1$ in epithelial cells; in parent MDCK-II cells (A), Bcrp1-transfected subclone cells alone (B) or with Bcrp-1 blocker (C), and BCRP-transfected MDCK-II cells (D). (^{3}H)-AFB$_1$ was added to the apical or basolateral side, and levels of radioactivity in percentage were measured on the respectively opposite side at t=1, 2, 3 and 4 hours. Percentage of radioactivity on the opposite side measured after 4 h is shown in numbers within each graph. Closed circles mark apically directed transport; open circles mark basolaterally directed transport (202).

Levels of accumulation of the radioactively labeled AFB$_1$ in the cell were evaluated after 4 hours of transport. (^{3}H)-AFB$_1$ concentrations in Bcrp-1 transfected cells were reduced up to 10 times in comparison to non-transfected cells. In addition, P-gp was examined for its ability to transport (^{3}H)-AFB$_1$ in another cell line – LLC-PK1 (parental, transfected with mice or human P-gp gene). Rates of apical and basolateral transport were not found to be different. However, rates of apical transport of radioactive aflatoxin were significantly higher, and rates of basolateral transport significantly lower in LLC-PK1 cells transfected with murine Bcrp-1 transporter when compared to non-transfected cells. Conclusively, both murine Bcrp-1 and human BCRP transporter can effectively mediate the transport of AFB$_1$ *in vitro (202)*.

In order to evaluate the relevance of the priory conducted *in vitro* measurements for conditions *in vivo*, radioactively labelled AFB$_1$ was given to male mice that were either

deficient in Bcrp-1 transporter (Bcrp-1 $^{-/-}$), or had wild-type genetic features. AFB_1 was applied either orally or intravenously, and levels of radioactivity in plasma were determined 30 min thereafter. Following oral administration, plasma levels of (^{3}H)-AFB_1 were 1.7 times higher in Bcrp-1 deficient mice than in the wild-type mice. For intravenous administration, deficient mice had 1.4 times higher plasma levels (Figure 25). According to these measurements, Bcrp-1 can effectively reduce levels of systematic exposure of AFB_1 (202).

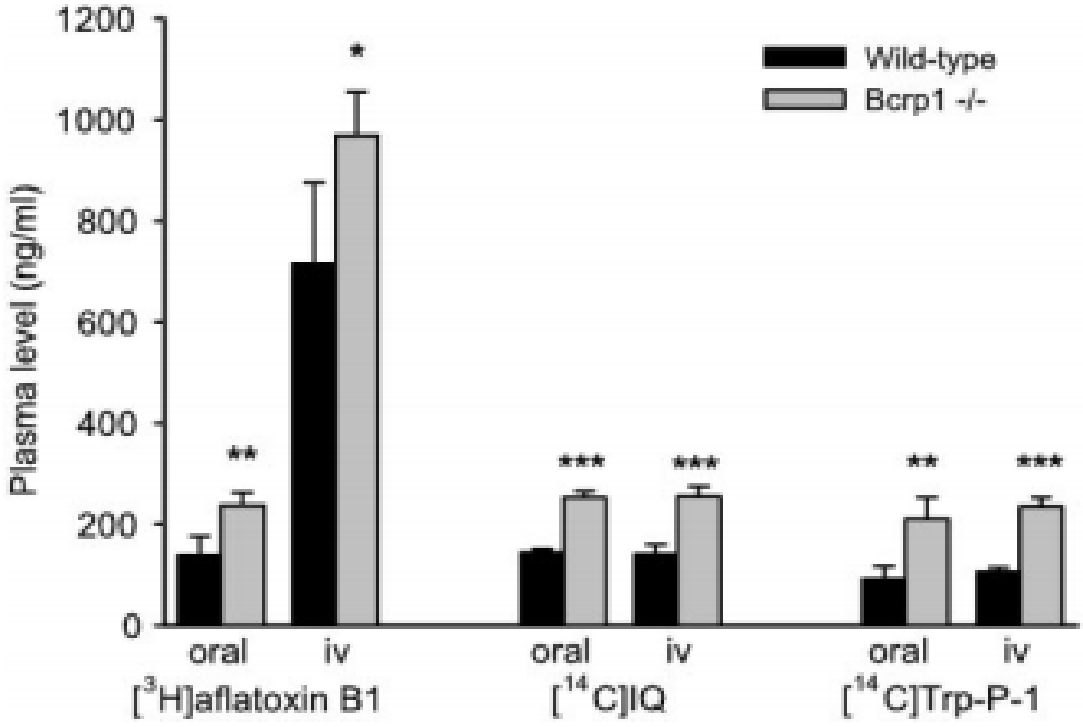


Figure 25: Measured plasma concentrations expressed in ng-equivalent/ml of (^{3}H)-AFB_1 (in addition to other examined compounds). The administered dose both orally and i.v. was 1 mg/kg. Higher concentrations are found in Bcrp-1 deficient, compared to wild-type mice. * P <0.05, ** P <0.01 (202).

Additionally, levels of AFB_1 in different tissues (liver and kidneys) were determined 60 min after administration in order to characterize its distribution pattern. Very similar levels of radioactivity were found in the liver of Bcrp-1 deficient and wild-type mice (concentrations 0.75 ± 0.06 μg/g for wild type and 0.85 ±0.25 μg/g for Bcrp-1 $^{-/-}$, P=0.44). However, renal levels of radioactivity were different, and the tissue concentration of (^{3}H)-AFB_1 in Bcrp-1 $^{-/-}$

(0.61± 0.20 μg/g) was 2 times higher than the one in wild-type mice, which had 0.31 ± 0.03 μg/g (202).

In order to examine how the expression of Bcrp-1 in mammary gland cells of lactating female mice (BCRP is equally expressed in human females during lactation) affects the excretion of AFB_1 into breast milk, radioactively labeled AFB_1 was applied intravenously (1 mg/kg dose) to female Bcrp-1 $^{-/-}$ and wild-type mice during lactation period (Figure 26). Murine milk and plasma were sampled 30 min thereafter. The milk/plasma concentration ratio was 3.8 ± 0.5 times lower in the Bcrp-1deficient than in the wild-type mice. These measurements suggest that Bcrp-1 is clearly involved in the transport of AFB_1 into breast milk (202).

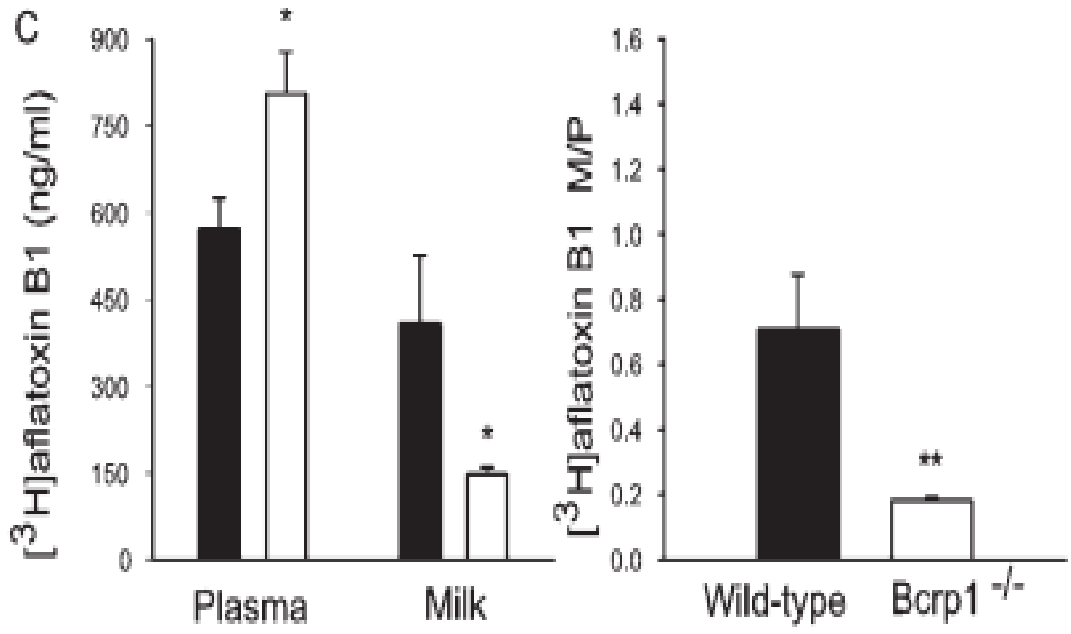


Figure 26: Concentrations (ng/ml) measured in plasma and milk, and milk/plasma concentration ratio of (^{3}H) AFB_1 in wild-type (black bars) and Bcrp-1 deficient (white bars) mice. * $P < 0.05$, ** $P < 0.01$ (202).

Results of this study are important. AFB_1 is clearly a substrate compound for Bcrp-1. It was shown that Bcrp-1 can have a protective role, by efficiently reducing levels of systematic exposure to this carcinogenic mycotoxin. It was also demonstrated that Bcrp-1 is involved in secretion of AFB_1 into breast milk of lactating mice. As similar expression pattern is seen in

cows and humans, this could have adverse health-related implications for milk and dairy product consumers, and also for breastfed infants, by indirectly exposing them to AFB_1. Thus, BCRP transporter could have ambiguous implications related to human health - it protects humans from the mutagenic and carcinogenic AFB_1, but on the other hand, its involvement in the transport of this natural toxin into breast milk is potentially associated with adverse health consequences (202).

3.5.2.3 Transport of AFB_1 by MRP and involvement of GSH

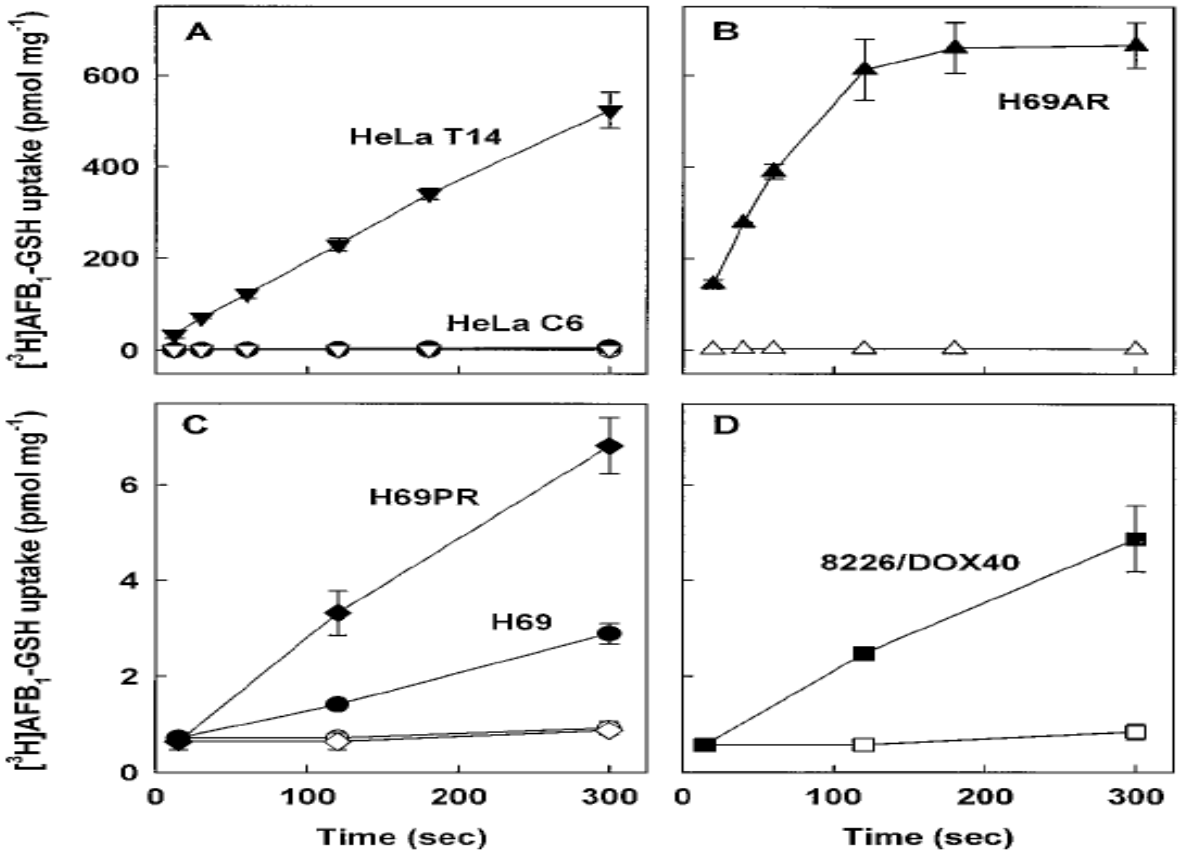
GST plays an important protective role against AFB_1 associated toxicity (203). Mice have 52-fold higher activity of this cytoprotective enzyme in their liver as compared to rats, and are not susceptible to carcinogenicity of AFB_1. Depletion of GSH from the liver results in increased formation of DNA-AFB_1 adducts (204). However, emerging evidence indicate that GSH-conjugates can also be cytotoxic, mutagenic and genotoxic (205). GSH itself is not transported in ATP-requiring process involving MRP (206). In view of these findings, it is relevant to investigate how MRP interacts with AFB_1 and what role GSH plays in this process.

In MRP-containing membrane vesicles, ATP-dependent transport of ^{3}H-AFB_1-GSH is constantly greater than the one noted in MRP-free vesicles, and follows a linear increase pattern until reaching steady state (Figure 27). ATP plays crucial importance in the transport, as addition of the non-hydrolysable nucleotide AMP markedly decreases the transport rate. With a relatively low K_m value of 189 nM, ^{3}H-AFB_1-GSH substrate affinity towards MRP exceeds the one observed with other conjugates, such as 17β-estradiol-17-(β-D-glucuronide). Furthermore, rates of ^{3}H-AFB1-GSH evidently correlate with expression levels of MRP, i.e. in vesicles prepared from MRP-overexpressing cells, the transport rate was roughly 3-fold higher during the initial linear phase as compared to non-overexpressing vesicles. As shown

also for other conjugates, ^{3}H-AFB$_1$-GSH transport was markedly inhibited by a specific antibody that recognizes a conformation-dependent epitope on MRP, but only minimally by another antibody directed against a linear MRP epitope (188).

Figure 27: Time dependent uptake of ^{3}H-AFB$_1$-GSH transport by membrane vesicles prepared from 6 different cell lines, in the absence of ATP (closed symbols) or AMP (open symbols). T14 vesicles contain MRP, C6 do not. H69AR cells overexpress MRP, as oppose to H69PR and H69; DOX40 cells overexpress P-gp, but also express MRP (188).

Further on, a range of GSH derived and steroid-based glucuronide compounds were tested for



their inhibitory potential directed at MRP-mediated transport of ^{3}H-AFB$_1$-GSH. GSSG (a GSH-like compound that is a substrate of MRP) inhibited also the transport of ^{3}H-AFB$_1$-GSH (at approximately 100 µM concentration). LTC4 also had a potent inhibitory effect (more than 85% inhibition) at a concentration of 10 µM, as well as 17β-estradiol 17-(β-D-glucuronide) at the applied concentration of 100 µM. The inhibitory effect of alkyl GSH-derivates was demonstrated to correlate with the chain length of the alkyl rest – 10 µM of S-methyl-GSH

had no inhibitory effect, whilst a 96% inhibition was attributable to S-decyl-GSH applied in aquimolar concentration. As for steroid-based glucuronides, their inhibitory potential was associated with the extent of their cholestatic effect – 17 or 16β-D-glucuronides (conjugated on D-ring) have a stronger transport-inhibitory and cholestatic potential than 3-β-D-glucuronides, conjugated on A-ring (188).

As evident in Figure 28, stereochemistry plays a minimum role in determining the rate of transport of GSH-conjugated AFB_1 epoxides by MRP, as the exo and endo-epoxide were transported almost with the same efficiency. Similar to AFB_1-GSH, the transport of AFB_1-GSH epoxides was dependent from ATP (188).

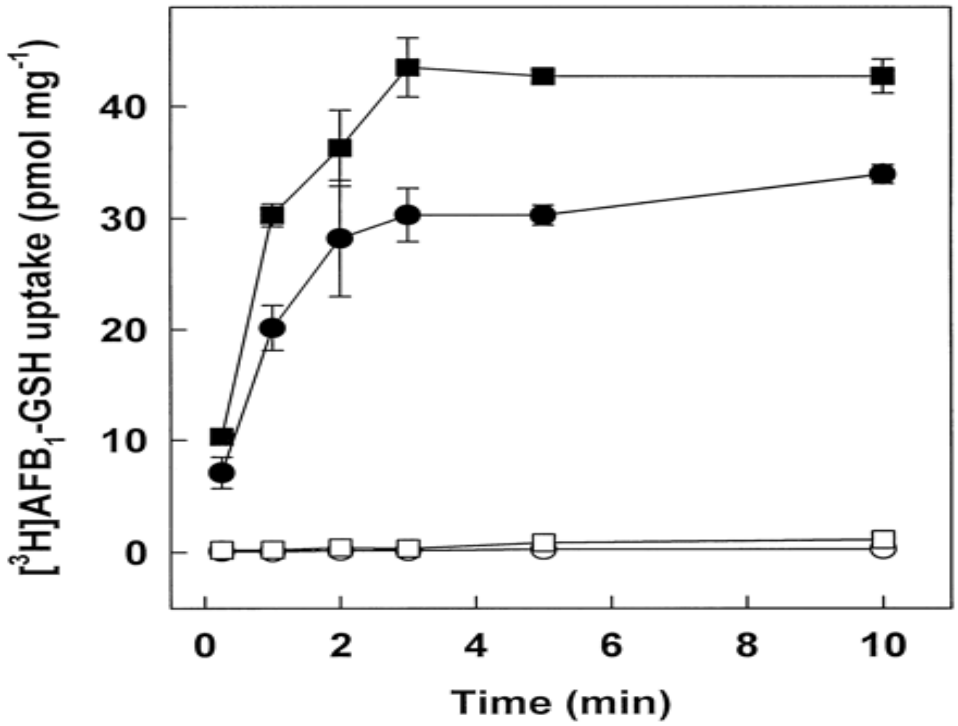


Figure 28: Effect of stereochemistry on the transport rate of ^{3}H-AFB1-GSH-exo-8,9-epoxide (circles) and ^{3}H-AFB1-GSH-endo-8,9-epoxide (squares) in MRP-containing vesicles (T14), in the presence of AMP (open symbols) or ATP (closed symbols) (188).

Non-conjugated ^{3}H-AFB1 was also shown to be transported in MRP-containing vesicles (Figure 29). Moreover, it was demonstrated that GSH is not crucial in the transport of AFB_1

by MRP. However, when GSH was applied at physiologically found concentrations, transport rate of ^{3}H-AFB_1 increased roughly two-fold at steady state. Such an increase was not seen in MRP-lacking vesicles. The antibody which recognizes conformation-dependent epitope had an inhibitory effect also on the transport of not-conjugated AFB_1. The addition of radioactively unlabeled AFB_1 at significantly excess concentrations resulted in a total inhibition of GSH-associated ^{3}H-AFB_1, but the effect was observed only in MRP containing vesicles in conjugation with ATP. On the other hand, GSH conjugate was also shown to reduce the transport of unconjugated AFB_1 when applied at 16x higher concentration (inhibitory effect 59 ± 7%). A mutual inhibition in the MRP-mediated transport of unconjugated and GSH conjugated AFB_1 indicates a potential interaction with similar or identical ligand pockets. Moreover, when unconjugated AFB_1 was applied in combination with GSH in respective concentrations of 100 μM and 5 mM, this resulted in reduction of ^{3}H-AFB_1-GSH initial transport in the presence of ATP (inhibition 76 ± 1%). Only a combination of these two compounds seems to be relevant with respect to inhibition of GSH-conjugated

AFB_1 transport, as AFB_1 alone led only to a 20 ± 4%, and GSH to a 27 ± 3% reduction in transport rates (188).

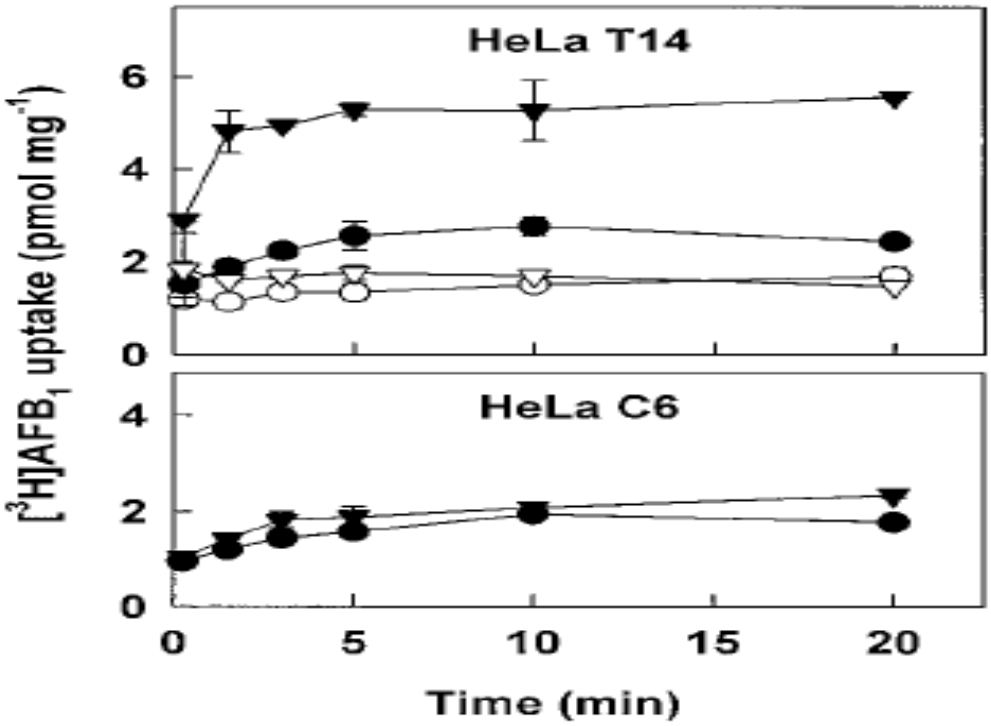


Figure 29:

Transport of non-conjugated ^{3}H-AFB1 in T14 and C6 vesicles. *Top*: T14 vesicles in the presence of 250 nM ^{3}H-AFB_1 and AMP or ATP, with or without GSH (open arrow: AMP + GSH, filled arrow: ATP + GSH, filled circle: only ATP, open circle: only AMP). *Bottom*: C6 vesicles in the presence of ^{3}H-AFB_1 and ATP (filled circles), or ATP and GSH (filled arrow). In both experiments, GSH was applied at concentration of 5mM, AMP or ATP at 4 mM (188).

To clarify the role of glutathione in the MRP-mediated transport of AFB_1, three reducing compounds were applied (2-mercaptoethanol, dithiotreitol, and L-cysteine) in order to

evaluate the contribution of the reducing nature of glutathione in this process. None of the three compounds affected the transport as GSH, emphasizing the specificity of GSH in the transport process. Furthermore, the GSH conjugate of AFB_1 was most probably formed by enzymatic reactions, as conditions applied in this study are normally not associated with non-enzymatic formation of the conjugate. No metabolites of AFB_1 were formed, i.e. all transport was attributable to AFB_1 (188).

Taken together, results of this important study suggest that MRP is an effective transporter of GSH-conjugated and unconjugated AFB_1, together with its toxic epoxide metabolites; and underlined the contribution of GSH in this process with no clear explanation of its exact involvement. Therefore, MRP may play an important role in protecting tissues in which it is expressed from the highly toxic and cancerogenic potential of this mycotoxin (188).

3.6 Interactions between ochratoxin A and ABC-transporters

Absorption of ochratoxin A at intestinal levels was studied using Caco-2 cell line, in which important members of the ABC-transporters family are expressed, including P-gp and MRP (207). The influence of non-physiological versus physiological conditions, e.g. in pH value and presence of the biggest plasma protein fraction albumin was investigated. In addition, impact of ABC-transporters in determining the nature of transepithelial transport of this important mycotoxin was evaluated.

The transport of ochratoxin A over the apical membrane exceeds the basolatelly directed transport at pH=7.4, translating into an excretion of the mycotoxin from epithelial cells into the intestinal lumen. However, when Caco-2 cells are exposed to physiological pH conditions, i.e. when pH value of 6.0 is applied to the apical side and 7.4 to the basolateral compartment with reference to intestinal lumen and basolaterally facing tissue compartments, respectively,

there is an appreciable change in levels of both accumulation in the cell and transport across cell membrane. In detail, these parameters increase, and ochratoxin A is predominantly transported to the basolateral compartment, i.e. it is absorbed from the intestinal lumen. These results are probably based on the chemical features of ochratoxin A – the decrease in apical pH value (from 7.4 to 6.0) prevents ochratoxin A to dissociate, and the mycotoxin can be more effectively absorbed by diffusion processes in non-dissociated form (208).

Moreover, absorption rates can be increased by adding albumin to the basolateral side – free mycotoxin concentration is reduced, and the albumin-ochratoxin A complex cannot be transported across cell membranes causing it to be trapped into the bloodstream. H^+-driven peptide transporter and organic anion transporter are not suggested to be a part of the carrier system responsible for the transepithelial transport of ochratoxin A at intestinal level (208).
However, ABC-transporters are suggested to play a valuable contribution in intestinal transport of ochratoxin A. Firstly, metabolic blockers markedly alter the pattern of its transport in Caco-2 cells. The application of these compounds resulted in increased transport over the basolaterally directed membrane, and decreased transport over the apical membrane (208), implying the importance of ATP generated in cell metabolism in secretion of the mycotoxin into the intestinal lumen.

Secondly, contrary to secretion, the permeability observed in absorption transport of ochratoxin A is not a saturable process, and does not decrease with increasing concentrations of the mycotoxin. These findings clearly suggest an involvement of a carrier system in the intestinal secretion of ochratoxin A. In opposite, the absorption of ochratoxin A at intestinal level is not mediated by a saturable carrier system, such as the ABC-transporters (209).
MRP transport is suggested to be an important tool in decreasing rates of intestinal absorption of this mycotoxin, as demonstrated in Caco-2 cells *in vitro*. Precisely, compounds with

inhibiting effect that is not specifically targeted against MRP transporters have an increasing effect on rates of ochratoxin A transport into the basolaterally located compartment while decreasing rates of apical transport (Figure 30). They also induce intracellular accumulation of the mycotoxin. Similar effects on the transmembranous transport are achieved through specific inhibition of transporters of the MRP-family. However, inhibition of the enzyme γ-glutamylcysteine synthetase has no evident effect on transport of ochratoxin A in Caco-2 cells (208). As inhibition of this enzyme does not influence ochratoxin A transport, and as previously demonstrated that AFB_1-GSH conjugates are transported by MRP (188), this suggests that ochratoxin A is not transported in conjuction with GSH moiety across membranes of intestinal cells.

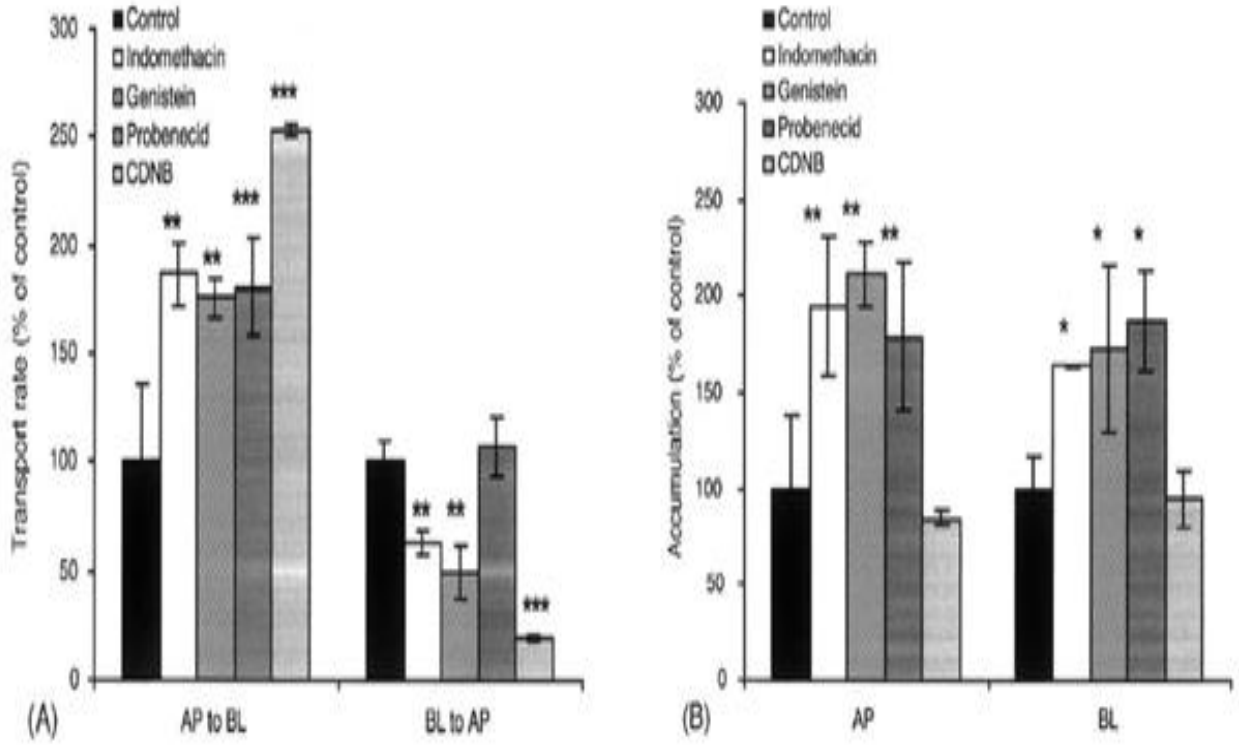


Figure 30: Transepithelial transport (A) and intracellular accumulation (B) of ochratoxin A in the presence of selected ABC-transporter inhibitors, and under physiological pH conditions in Caco-2 cells. A: Inhibitors were either added to both compartments, or not added at all (control values). Transport rates shown (%) are normalized against control measurements. ** P <0.01, *** P <0.001, with respect to control measurements. B: Accumulation of ochratoxin A in Caco-2 cells. Accumulation rates (%) shown are normalized against control measurements. * P < 0.05, ** P <0.01, with respect to control measurements (208).

Furthermore, the rate of ochratoxin A transport by transporter of the MRP-family in both directions changes accordingly to the concentration of the applied specific blocker. That is, the decreasing tendency of the secretory transport becomes more pronounced with greater concentrations of the inhibitor (209).

However, these findings cannot be easily interpreted, as a concomitant inhibition of P-gp and MRP2 by cyclosporine A decreases the secretory transport only at high concentrations, while not affecting at all the absorptive transfer of ochratoxin A (209).

Yet, the role of intestinal P-gp towards ochratoxin A is tentative. Inhibition of this ABC-transporter does not affect transport of the mycotoxin across apical or the basolateral

membrane (208, 209). However, it results in slightly enhanced intracellular concentrations of ochratoxin A (208).

BCRP is also suggested to be involved in intestinal transport of the mycotoxin. As shown in Figure 31, selective inhibition of this transporter by the blocker Ko143 decreases significantly the secretion of ochratoxin A, although the effect was seen only at higher concentrations. Concomitantly, functional inhibition of BCRP with higher concentrations of the blocker resulted in slightly, but significantly increased absorptive transport of this fungal metabolite (209).

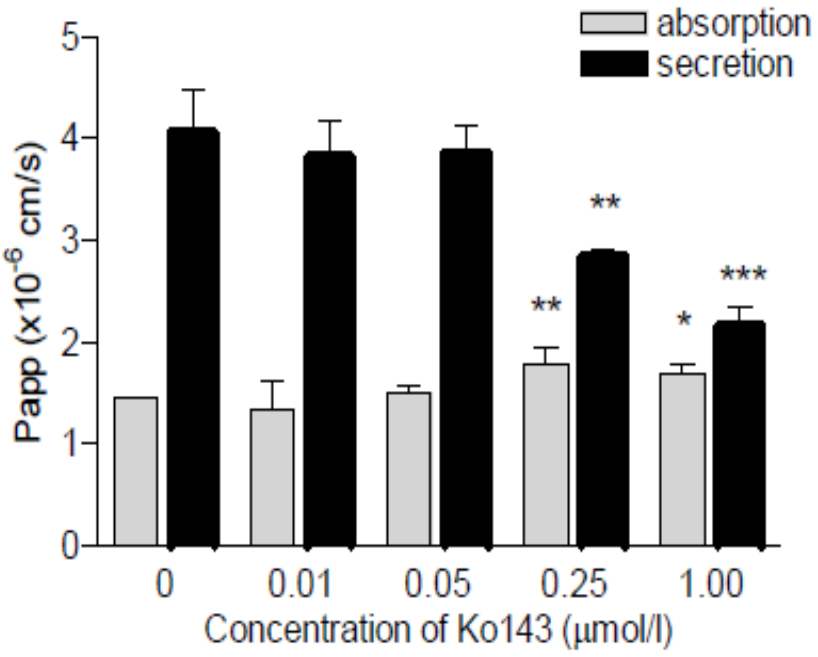


Figure 31: Changes in secretory and absorptive permeability of ochratoxin A at increasing concentrations of Ko143 inhibitor. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (209).

The MRP2 transporter is expressed in the apical membrane of proximal tubular cells in rats (210), and in humans (211). Ochratoxin A disrupts the renal transport of para-amino-hippurate (PAH) across the basolateral, and more evidently the apical membrane (212).

In vitro evidence suggests that MRP transporters could contribute to these interactions. As shown in Figure 32, ochratoxin A interferes with the transport of PAH by MRP1 and MRP2

with similar potency, as the IC_{50} values for these two ATP-ases are set to 53 and 58 µmol/l, respectively (213).

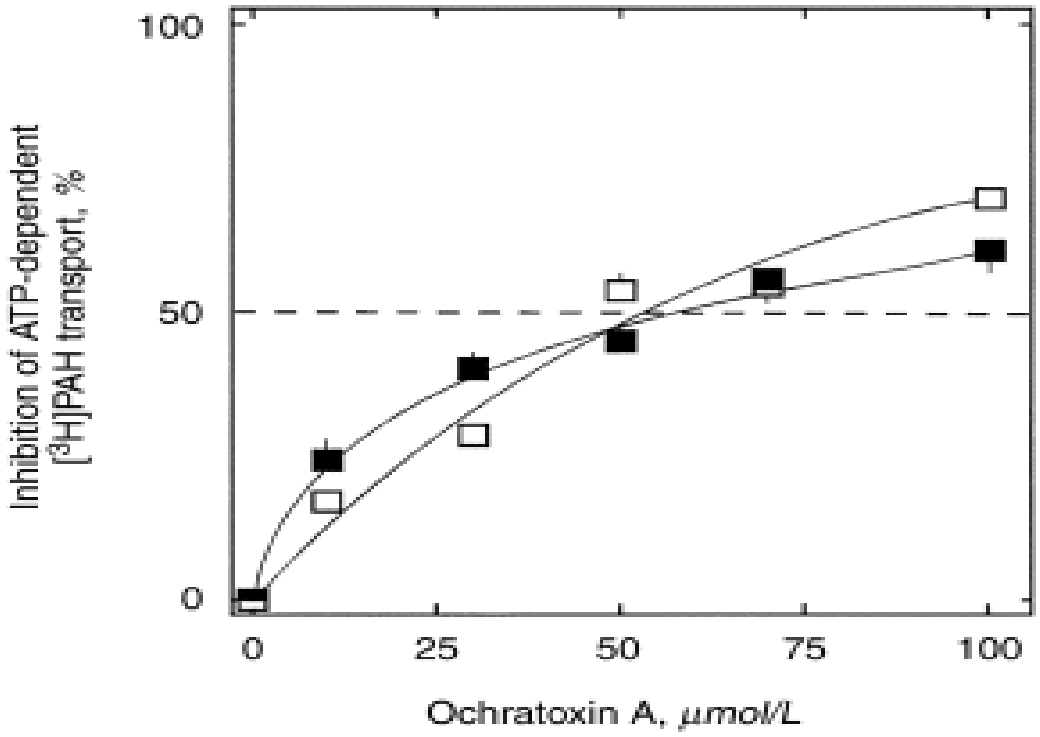


Figure 32: Inhibitory effect of ochratoxin A on MRP-1 (white points) and MRP-2 (black points) mediated transport of PAH. Inhibitory effect was determined for all indicated concentrations of ochratoxin A (213).

The inhibitory potency of ochratoxin A towards MRP-mediated transport of PAH could be explained by the fact that this mycotoxin is effectively transported by MRP-2 (213), and the same was already reported in Caco-2 cells (208, 209).

3.7 Interactions between trichothecenes and ABC-transporters

Nivalenol can be effectively absorbed across intestinal epithelial cells, with passive transport being the most likely mechanism. Its absorption is a slow process, and these rates do not reach the ones attributable to secretory transport. However, this slowness is not accounted for by retention of nivalenol in cells, as only minimal levels of intracellular accumulation are seen.

The slowness of absorption and a minimal tendency towards accumulation in the cell is more probably due to an effective elimination pathway. Apparent activation energy values for secretory transport indicate it to be an active process and this is confirmed by decreasing rates of secretion in the presence of metabolic blockers, which led to depletion of cellular ATP. Indeed, the contribution of ABC-transporters in mediating the efflux of nivalenol from cells was confirmed *in vitro*. P-gp and MRP2 are both suggested to be involved in the transport, but not BCRP (214).

As shown in Figure 33, valspodar (P-gp inhibitor) reduced the rate of secretory nivalenol transport by 51% in Caco-2 cells. At the same time, inhibition of the MRP-family by MK571 displayed a reducing effect by 24%. Interestingly, combined inhibition of these transporters by cyclosporine A inhibited the transport to a smaller extent than of P-gp alone, producing a result of 46% reduction. However, synchronized inhibition of these transporters by their individual inhibitors has an additive effect, exhibiting the greatest inhibitory value of 55%. As opposed to these findings, inhibition of BCRP had no effect at all on the transepithelial transport rates of nivalenol, implying that BCRP is not involved in its transport across intestinal epithelium (214).

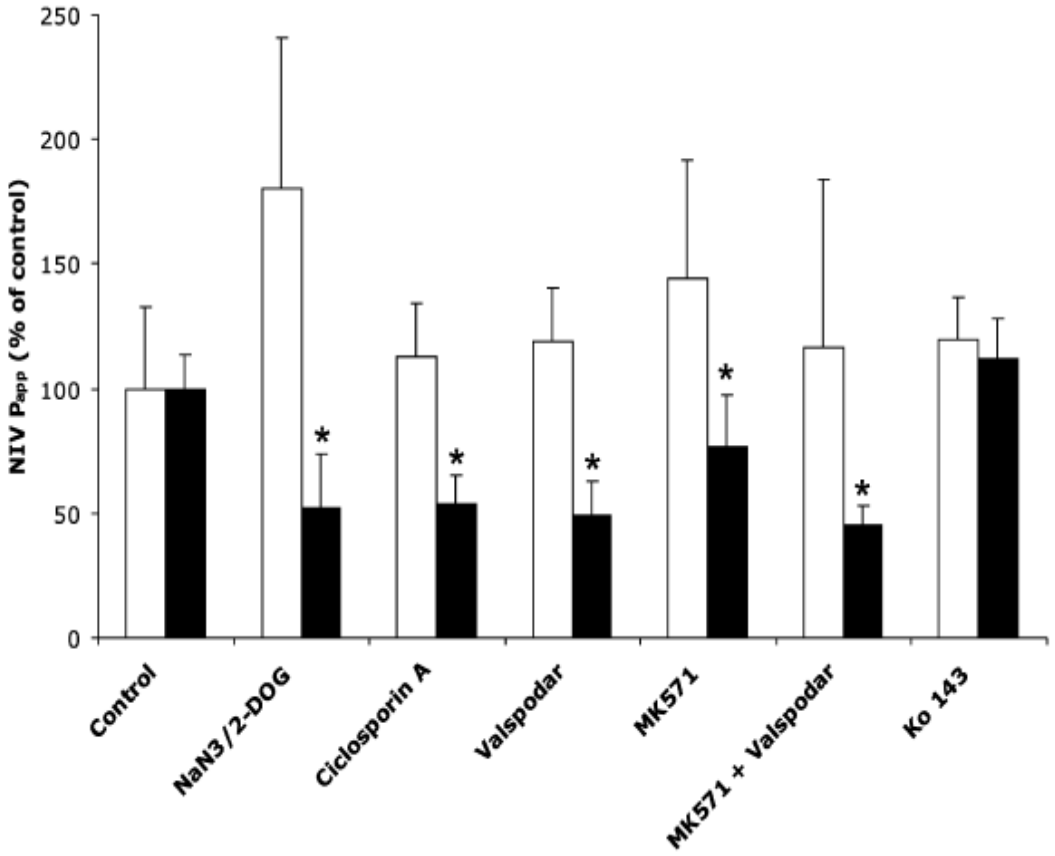


Figure 33: Effect of inhibitors of ATP production and ABC- transporters on transepithelial transport of nivalenol in Caco-2 cells, expressed as apparent permeability (P_{app}). Open bars represent absorptive transport, filled bars secretory transport. * $P < 0.05$, compared to control cells (214).

ABC-transporters exert highly similar effects in relation to secretion of another trichotecene, **deoxynivalenol** (Figure 34). Moreover, secretion of this mycotoxin from Caco-2 involves the very same transporters – P-gp and MRP2. Again, BCRP did not contribute to secretion, as its inhibition does not alter deoxynivalenol transport patterns at all (215).

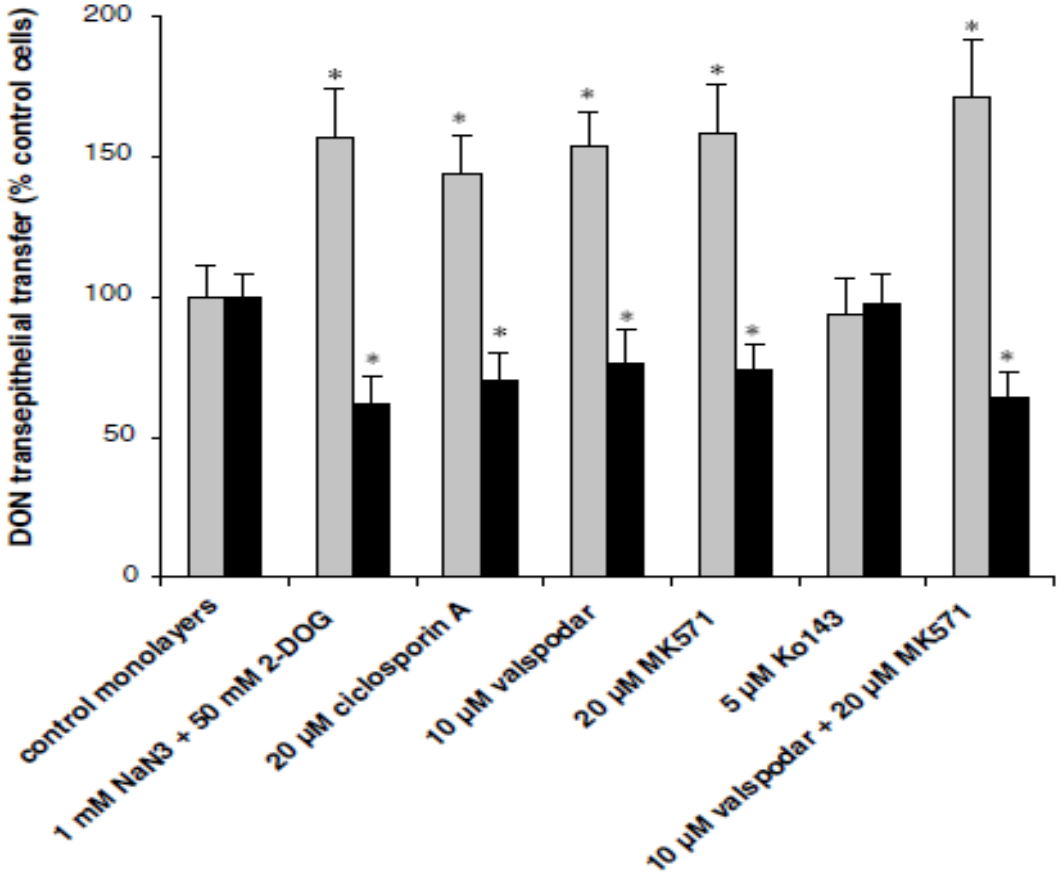


Figure 34: Effect of different metabolic and ABC-transporter inhibitors on absorptive (gray bars) and secretory transport (black bars) of deoxynivalenol in Caco-2 cells, expressed as % control value. * $P < 0.05$ (215).

Further, inhibition of these two ABC-transporters markedly increased the intracellular accumulation of the mycotoxin to similar extent (3.4 and 3.5-fold for P-gp and MRP2, respectively). Combined inhibition accordingly results in even higher rates of intracellular accumulation (4.6-fold). Concordantly, the importance of these two carriers in maintaining low intracellular levels of this mycotoxin became apparent when these levels were compared in cells transfected with the encoding genes. Namely, these cells contained 35-45% lower levels of deoxynivalenol compared to non-transfected cells (215).

Translating the results of these studies (214, 215) into *in vivo* conditions, they clearly suggest a protective role of P-gp and MRP2 against potential toxicity associated with exposure to these two mycotoxins. By effectively mediating secretion of these compounds from intestinal

cells, they can decrease rates of absorption after oral consumption of e.g. deoxynivalenol and nivalenol-contaminated foodstuffs.

However, *in vitro* results suggested that ABC-transporters expressed in placental tissue do not play a protective role against the T-2 toxin, which exhibits a strong toxic potential, and its metabolite HT-2 toxin. These toxins are readily absorbed across the placental barrier through active transport (T-2 toxin) or passive diffusion (HT-2 toxin) (216).

3.8 Interactions between fumonisin B_1 and its metabolites with ABC-transporters

The importance of P-gp in protecting the organism from fumonisin B_1-associated toxicity was studied *in vivo* in mice. Parameters associated with toxicity of this secondary fungal metabolite were investigated on molecular, cellular and organ levels. These were compared in mice deficient in both genes coding for P-gp (mdr1a and mdr1b) and wild-type mice (217).

In detail, exposure to fumonisin B_1 led to an increase in liver size of both mice strains. To the contrary, no changes were observed in hematological parameters, i.e. in red and white blood cell counts. Levels of plasma circulating liver enzymes – alanine transaminase (ALT) and aspartate transaminase (AST), which are frequently used indicators of liver injury, were affected similarly in both mice groups. In further relation, they are coherent with the histopathological image. Nephropathy as a result of exposure to fumonisin B_1 is apparent in both strains. Hepatic and renal levels of free sphingosine and sphinganine as indicators of fumonisin B_1 inhibition of the target enzyme ceramide synthase were greater in both strains treated with the mycotoxin than with control. Specifically, hepatic increase was somewhat greater in mice lacking P-gp coding genes. As for sphingosine and sphinganine concentrations originating from complex sphingolipids, which normally deplete from cells in fumonisin B_1-related cytotoxicity, a decrease in hepatic levels for the prior and increase in nephrotic levels

for the later was noted in both strains. Hepatic expression levels of TNFα-coding mRNA, which is thought to be one of the mediators of fumonisin's toxicity, were increased in both strains treated with the mycotoxin. CNS neurotransmitters and their metabolites did not undergo any apparent concentration changes, except for a single dopamine metabolite, for which a similar increase in both mice strains was observed. Taken together, double-knockout mdr1a/mdr1b mice that consequently do not express P-gp were not any more susceptive to toxic-related effects of fumonisin B_1. Therefore, the absence of P-gp does not seem to have a crucial impact on the transport of this *Fusarium* mycotoxin across cell membranes and brain barriers (217).

Although it is not directly protective against fumonisin B_1-related toxicity, P-gp could have an indirect protective effect by decreasing rates of absorption of one of its important and often occurring metabolites formed by total hydrolization, aminopentol. Namely, fumonisin B_1 is incapable of passing across the intestinal epithelium, as demonstrated in Caco-2 cells. However, aminopentol can be transported both apically and basolaterally, although with strong discrepancies in rates of transport to favor of apical transport. Overall, its intestinal absorption seems to be dependent on the dose present. Substantial quantities of aminopentol were not found in the cell, most likely due to activity of P-gp in eliminating the toxin from the cell. That is, inhibition of P-gp abolishes the transport of aminopentol from the basolateral compartment into the apical, i.e. into intestinal lumen. Unlike, P-gp, MRPs do not seem to play such a role, since their inhibition was not associated with changes in transport pattern of this metabolite (218).

3.9 Interactions between zearalenone and ABC-transporters

The primary concern related to zearalenone-associated toxicity is based on its ability to bind to estrogen receptors with strong similarities to physiologically occuring estradiol (219). This mycoestrogen was reported to have detrimental effects on the reproductive system in male mice, resulting in significant and dose-dependent reduction in numbers of sperm cell, impaired sperm production and decreased testosterone levels (220). Moreover, exposure to this toxin not only predisposed males towards infertility, but also females, including their offspring. Importantly, similar effects in offspring were provoked by mothers' exposure to the fungal toxin during pregnancy and exposure at early life stage (221). The importance of ABC-transporters in the highly protective blood-testis barrier has already been recognized (222), as well as the potential of endocrine disruptors to interfere with their activity (223). In view of these findings, it becomes highly necessary to investigate how zearalenone interacts with ABC-transporters of the reproductive system, and what are the implications of these interactions for the reproductive health.

Long-term modulations of ABC-transporters by zearalenone were explored *in vivo* in male rats, which were treated in the first 5 days after birth. In addition, *in vitro* experiment was conducted using a cell model of Sertoli cells, which are an important integral part of the blood-testis barrier (224).

Firstly, it was investigated how zearalenone (at a high dose of 100 µg/kg/day) impacts the distribution of several ABC-transporters, in particular that of Abcc4 and Abcc5, two members whose localization and cellular distribution in testis still remains uncertain. Abcc5 localization was found across testicular samples, and specifically in Sertoli and Leydig cells, as well as in peritubular myoid and endothelial cells (Figure 35A). Abcc4 was concentrated in Leydig cells, peritubular myoid cells, as well as around spermatocytes and spermatids (Figure 35B). For the greatest part of inspected ABC-transporters, no changes in cellular distribution patterns were seen (Abcb1, Abcc1, Abcc5, and Abcg2). However, zearalenone caused major

shifts in cellular distribution of Abcc4, as the transporter was detected to its greatest proportion in spermatocytes and round spermatids, i.e. in cells that already underwent meiosis (Figure 35C) (224).

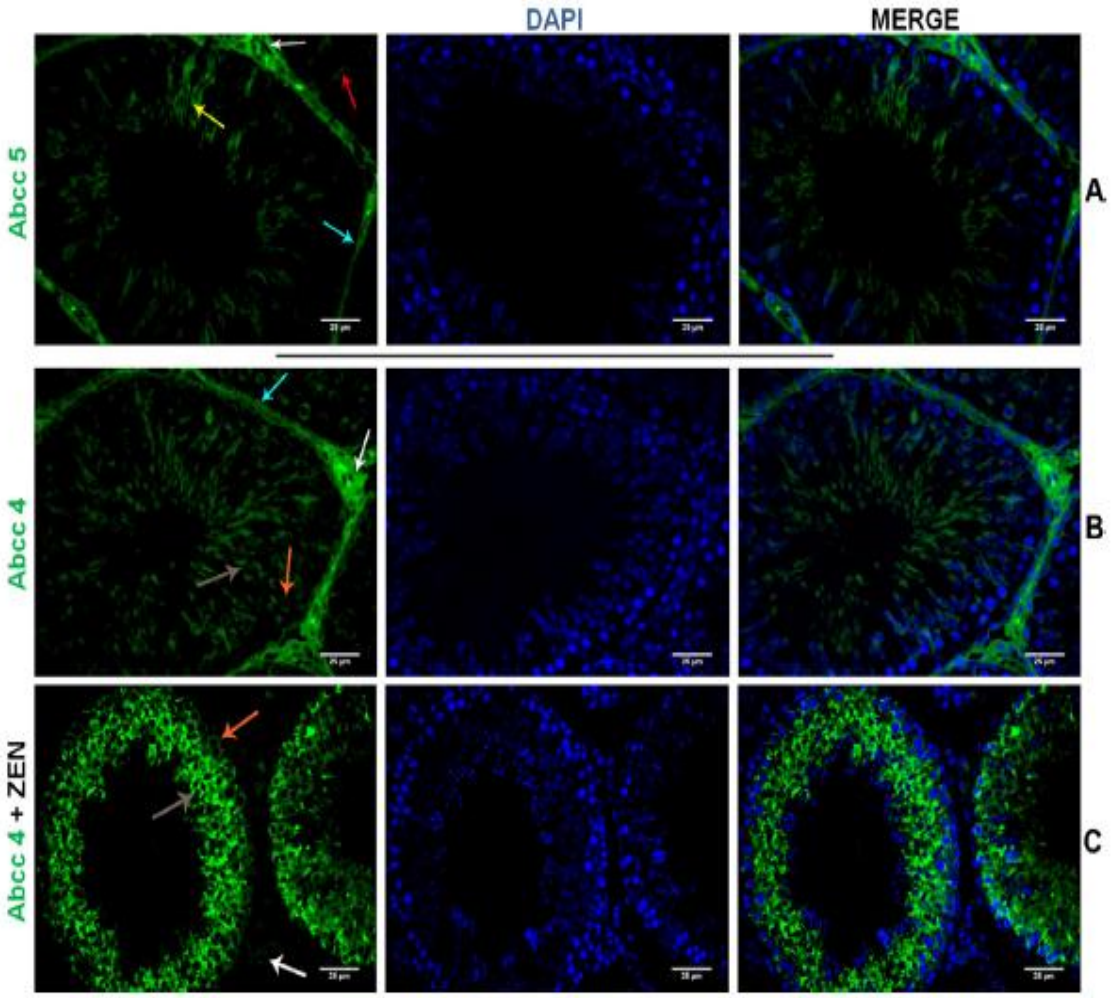


Figure 35: Testicular distribution of Abcc4 and Abcc5 in adult rats and effects of high dose zearalenone treatment on Abcc4. Immunohistochemical analysis was used – FITC antibodies were stained with nuclei-showing DAPI to resemble the localization of Abcc5 (A), Abcc4 (B), and Abcc4 following zearalanone treatment (C). Arrows show ABC-transporters in interstitium: white – Leydig cells, blue – peritubular myoid cells, red – endothelial cells; or seminiferous tubules: yellow – Sertoli cells, orange – spermatocytes, gray – round spermatids (224).

This finding is important as it implicates potentially altered gametogenesis as consequence due to the importance of this ABC-transporter in delivering the necessary nucleotides. Moreover, as the expression of Abcc4 was only sparsely found in Leydig cells, which are

essential for normal male fertility, this might additionally contribute to pathologically altered spermatogenesis, and lead to decreased production of testosterone in the testis (224).

The effects of zearalenone and estradiol benzoate as comparison on Abcg2 expression *in vivo* were of high similarity, while no comparison was possible *in vitro*, since this ABC-transporter is not expressed in Sertoli (SerW3) cell line, which was used. However, the two compounds differently affected all other studied ABC-transporters *in vivo*, as well as *in vitro*. In the case of Abcg2, similarities were observed in terms of effects on mRNA and protein levels both for fusariotoxin and the estrogenic drug, implying the involvement of transcription effects, as already demonstrated in several studies that compounds with estrogenic activity have regulatory effects on transcription of the Abcg2 gene. Discrepancies exist in effects of zearalanone and estradiol benzoate on mRNA and protein levels of Abcb1, Abcc4 and Abcc5. These could be based on microRNAs – small RNA molecules which don't carry a code, but are involved in regulation of post-transciptional gene expression. Also, different doses that were used put a constraint on directly comparing these compounds. Namely, estrogenic substances rarely show linear dose-response curves, but rather U- or inverted U-shaped curves, which complicate the comparison of effects of different doses. Overall, zearalenone and estradiol benzoate caused similar effects, indicating the involvement of zearalenone's estrogenic properties in modulating the expression of these ABC-transporters. However, expression levels of Abcc1 parameters were not changed *in vitro* by any of the two compounds, implying that the estrogenic activity of zearalenone is not the underlying mechanism of modulation of this ABC-transporter (224).

Results obtained *in vitro* using an anti-estrogenic compound, which clearly reversed the effects of zearalenone, imply that its modulation of Abcb1, Abcc4 and Abcc5 expression is to a certain part surely associated with its estrogenic properties. In general, the potential of

zearalanone to modulate the expression rate of ABC-transporters was demonstrated (224). An up-regulation of ABC-transporters, especially in Sertoli cells, is potentially beneficial, as it might result in enhanced protective function of the testis-blood barrier against harmful toxic agents. On the other hand, a down-regulation could make sensitive cells more vulnerable, as it would result in their higher exposure to the influence of toxicants (224).

In relation to zearalenone's effects on fertility of females and their offspring, it is also relevant to investigate how this mycotoxin affects placental ABC-transporters.
A study was conducted in a choriocarcinoma cell line (BeWO), which is a validated model due to its morphological and biochemical similarities with placental trophoblast cells, and the fact that these cells can differentiate spontaneously into scyncytiotrophoblasts. Scyncytiotrophoblasts play a central role in differentiation of trophoblast cells (225).

It was shown that the effects of zearalenone on differentiation of BeWO cells go hand in hand with changes in expression levels of some ABC-transporters. Firstly, zearalenone was shown to have a profound impact on the differentiation of BeWo cells both in terms of morphology and biochemical features, as it induced the formation of syncytium and modulated the secretion of hCG, respectively. The expression of the MRP2 gene was only mildly up-regulated, as detected via protein levels, implying that this ABC-transporter is not significantly affected by zearalenone-induced differentiation. As for BCRP and MRP1, their expression was increased in the presence of zearalenone, which might be attributable to its role on cell differentiation directly or its estrogenic activity. However, forskolin, a physiological inductor of the syncytilisation process also generated a comparable effect. Therefore, it is likely that cell differentiation is an important determinant in expression of these two ABC-transporters. The increase in levels of BCRP might be explained both by the affinity of zearalenone to bind to estrogen receptors, which occur in BeWo cells and are

clearly involved in regulation of BCRP expression; as well as by the differentiation process associated with zearalenone, since comparable effects were obtained with the differentiation inducing forskolin. In general, zearalenone was found to have a modulating effect on the tested ABC-transports, and although the observed increases were not in large-scale proportions (maximum of double increase), they could have serious implications in determining fetal or infant exposure particularly to harmful toxic compounds (225). However, zearalanone seems to have different effects on human trophoblast cells as compared to the reduced metabolites (α and β zearalenol). Zearalenone, but not its metabolites, can induce differentiation process in these cells, although not by interacting with the PPARγ receptor. In addition, the involvement of several nuclear receptors in the modulation of trophoblast ABC-transporters by these compounds has been suggested (226).

It is suggested that the importance of the extent to which zearalenone and its metabolites impregnates exposed tissues is small. The time factor seems to be more important, i.e. short-term and long-term modulation exist. However, this factor is not unique, as the origin of tissue, i.e. from mother or fetus, and type of tissue (mothers' uterus reacts with the greatest sensitivity), also seems to be important. As to the short-term modulation (4 hours upon exposure) of mothers, there was a decrease in expression of Mrp-1 and Abcc3 in uterus, but an up-regulation in levels of these transporters was seen when significantly more time passed (24 hours). To the contrary, the time factor was not determinant of how zearalenone exposure affected uterus levels of P-gp encoded by both rat genes (Abcb1a and Abcb1b), as well as of Bcrp, as their expression was always stimulated. Results in fetuses are not that clear-cut. Hepatic levels of P-gp, product of the Mdr1a gene, were always decreased, although there was an increase in mRNA levels of this gene at long-term exposure (227).

3.10 Interactions between cyclohexadepsipeptides and ABC-transporters

Enniatins and beauvericin can affect intracellular concentrations of Ca^{2+}, and the effect is sensitive to alterations in cellular ATP levels, as an exogenous ATP source was able to reverse the effect. These cyclohexadepsipeptides have also the ability to modulate the activity of ABC-transporters. Firstly, ABCG2 overexpression was associated with a mild, but significant protective effect against beauvericin-induced short-term cytotoxicity, but no effect was seen with regards to enniatins. To the contrary, cells characterized by an overexpression of MRP1 and P-gp were not protected against cytotoxicity. Inhibitors of ABCG2 were able to reverse cell resistance to beauvericin. ABCG2, together with P-gp was effective in protecting cells from long-term cytotoxicity of both fusariotoxins, particularly enniatins. The interaction between the two mycotoxins and ABC-transporters (ABCG2, ABCB1) was also confirmed by their inhibitory effect on the transport of well-established substrates of these two ABC-transporters, along with evident effects on the ATP-ase activity and photoaffinity labelling. Cells selected with the fusariotoxins developed resistance to enniatins at double rates compared to the parental cell line, whereas only minimal changes were reported for beauvericin. Concomitantly, an increase in expression levels of all studied ABC-transporters, except for ABCB1 was noted in selected cells, and they also developed resistance to two other cytotoxic agents – cisplatin and beauvericin. In general, a mild, but significant protective effect against cytotoxicity induced by enniatins and beauvericin was found for ABCG2 and ABCB1. In parallel, the ability of these fusariotoxins to interfere with ABCB1 and ABCG2-mediated transport was also proven, which might be of importance in the context of bioavailibity of substances, including drugs (228).

Additional *in vitro* evidence suggests that intestinal brush border ABC-transporters are effective means in mediating the transport of enniatin B_1 from enterocytes back into the intestinal lumen. Namely, this transport exceeds many-fold the one across the basolaterally

facing membrane. However, inhibition of P-gp and MRP2, but not of BCRP, induced a shift in basolateral transport rates, resulting in a significant increase (229). With some difference to what was shown in the previous study (228), overexpression of these three ABC-transporters confered resistance to cells against enniatin B_1 (229).

3.11 Interactions between austocystin D and P-gp

Austocystins were isolated in 1974 from *Aspergillus ustus* (230). Austocystin D was reported to have a cytotoxic potential directed against P-gp overexpressing cells (231), suggesting it to be a promising compound in overcoming the often occurring overexpression of this transporter in tumor cells.

However, this could not be confirmed later on. Austocystin D is a substrate of P-gp. Like other compounds (verapamil and vinblastine), which are well recognized substrates of P-gp, austocystin D was shown to have a stimulating effect on the ATPase activity of P-gp, indicating that it is also a substrate of the transporter. Moreover, this stimulating effect is completely suppressed when cyclosporine A, a P-gp inhibitor, is applied (232).
However, the cytotoxic potential of austocystin D is not attributable to its interaction with P-gp (Figure 36) – the profile of this interaction is very similar to the one seen in verapamil, which has a significantly lower cytotoxic activity (232).

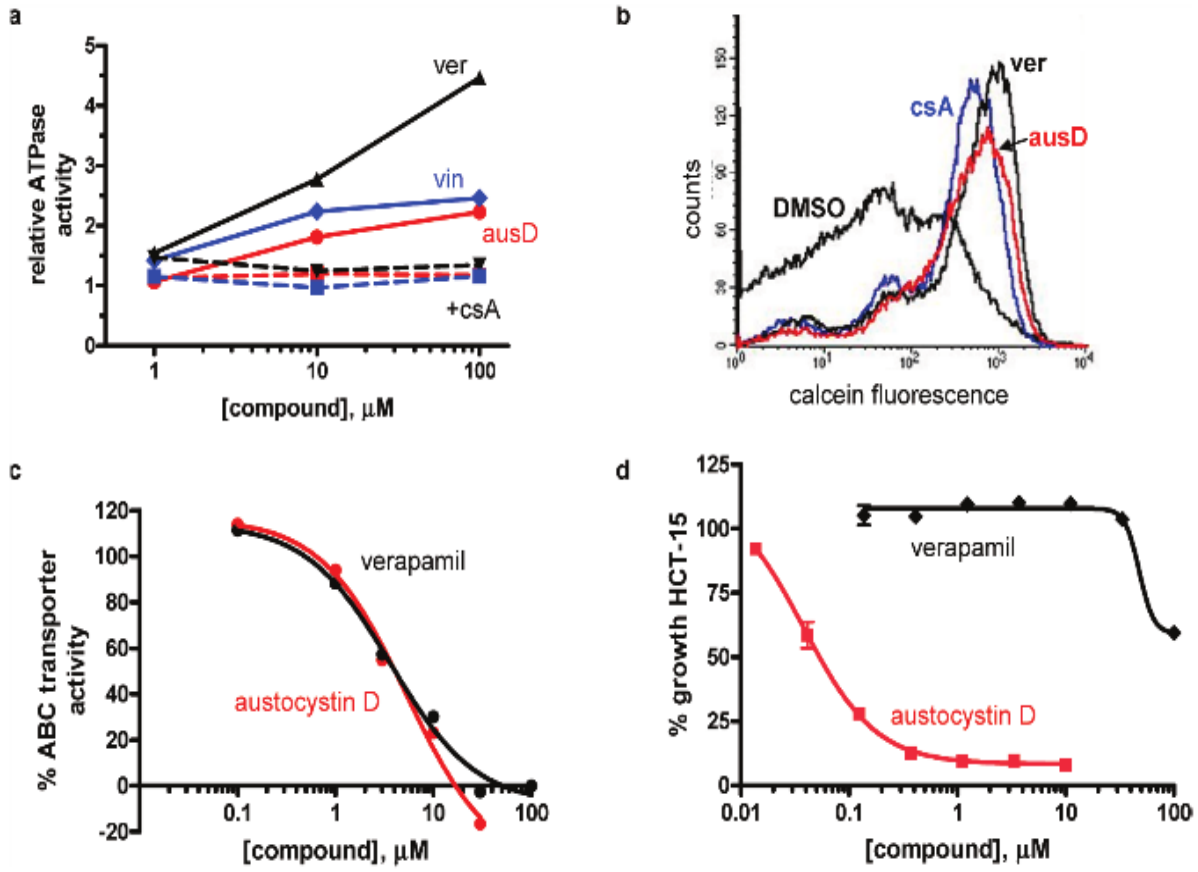


Figure 36: Effect of austocystin D on the activity of P-gp. (a) Austocystin D (ausD), verapamil (ver) and vinblastine (vin) stimulation of ATPase activity of P-glycoprotein (solid lines). Cyclosporine diminishes the stimulatory effect of all three compounds (dashed lines). (b) Calcein-acetoxymethylester assay: Austocystin D shows a similar effect as cyclosporine and verapamil, leading to measurable fluorescence (relative to vehicle). (c) Verapamil and austocystin D lead to intracellular accumulation of calcein-AM by inhibiting its P-glycoprotein mediated efflux. (d) Austocystin D has a much higher growth inhibiting effect on the tested HCT-15 cells than verapamil (232).

The cytotoxicity of austocystin D was also not proven to correlate with the expression of other ABC-transporters, including MRP1 and BCRP (232).

Conclusion

The importance of ABC-transporters is being increasingly recognized. Apart from their important physiological roles and pathological importance, the relevance of this widely

distributed transporter class in pharmacotherapy is emerging. Along with other protein classes, ABC-transporters are determinant of the pharmacokinetic profile of numeuros drugs. Their effects on the absorption, distribution, and elimination of drugs are mostly due to their expression pattern. Namely, they are abundantly found in tissues and organs that are major determinants of pharmacokinetics, i.e. in barrier tissues (intestinal epithelium, blood-brain barrier), the liver, and kidneys. Drugs derived from the secondary metabolism of fungi can interact with ABC-transporters in different ways, e.g. as inhibitors (cyclosporine A, ergot alkaloids). However, these interactions are often not clear-cut in that a secondary fungal metabolite can act both as a substrate and inhibitor (e.g. lovastatin). Consequently, such interactions reflect in plasma concentration and tissue distribution pattern of drugs, as in the case of cyclosporine A. Moreover, drug-drug interactions are often based on ABC-transporters (e.g. lovastatin-verapamil, cyclosporine A-chemotherapeutic drugs). Genetic variability in ABC-transporters is associated with variations in pharmacokinetic parameters of drugs, for instance of cyclosporine A and mycophenolic acid. Apart from the pharmakokinetics, interactions between ABC-transporters and secondary fungal metabolites also have a pharmacodynamical perspective. They can be directly responsible for the therapeutic potential of the drug (e.g. lovastatin in malignant conditions, cyclosporine A in intraocular retinoblastoma). In addition, they can indirectly contribute to the therapy when combined with the acting drug, as in the case of lovastatin that helps to overcome antimony resistance in parasitic leishmania infections. However, interactions with ABC-transporters can sometimes also be the cause of side effects of a drug. For example, cholestasis and negative changes in blood lipids associated with cyclosporine A immunosuppressive therapy are most likely mediated by its interactions with ABC-transporters. These transporters can be overexpressed in response to drug’s toxicity, but this effect can also have negative implications for therapy (e.g. cyclosporine A and renal expression of P-glycoprotein).

ABC-transporters also interact with mycotoxins. However, these interactions are not as well-established and are often difficult to interprete, particularly in terms of potential implications for human health. For example, aflatoxin B_1, the most prominent mycotoxin due to its well recognized toxic, mutagenic and hepatocarcinogenic effects, interacts with ABC-transporters at several levels (mRNA, protein), and comparable effects are also observed with the mycoestrogen zearalenone. Although a protective effect seems to be mediated by such interactions (e.g. aflatoxin B1, cyclohexadepsipeptides), they are also associated with negative health implications. For instance, BCRP mediates the excretion of aflatoxin B_1 to breast milk, leading to higher exposure to the mycocarcinogen. ABC-transporters' interactions with mycotoxins which interfere with the endocrine system (e.g. zearalenone) potentially carry a strong implication for the reproductive health, both male and female. Furthermore, ABC-transporters could be determinant of the intestinal absorption rate for mycotoxins with diverse structural properties (e.g. ochratoxin A, trichothecenes) and this way contribute to protecting the organism from their possible toxic potential. Additionally, these interactions might also display in elimination pathways (e.g. ochratoxin A in proximal tubulus). Such interactions in absorptive and eliminatory organs could have a profound effect on the pharmacokinetics of a large number of drugs which also interact with ABC-transporters. This implies that food commodities contamined with mycotoxins could not only affect human health, but could also lead to unpredictable pharmacokinetic profile of drugs during therapy. Generally, ABC-transporters interact strongly with many secondary fungal metabolites, but the exact effects and implications of these interactions still remain tentative.

Abbreviations

ABC-transporters - ATP-binding cassette transporters

ACV - δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine

ADP - adenosine diphosphate

AFB_1 - aflatoxin B_1 (analogues: AFB2, AFM1, AFM_2, AFG_1, AFG_2, $AFGM_1$, $AFGM_2$, AFQ_1, AFP_1)

ALA - alimentary toxic aleukia

ALD - adrenoleukodystrophy

ALT - alanine transaminase

AST - aspartate transaminase

ATP - adenosine triphosphate

AUC - area under the plasma-time curve

BCRP - breast cancer resistance protein

BEA - beauvericin

BSEP - bile salt export pump

cAMP - cyclic adenosine monophosphate

cDNA - complementary DNA

CHD - coronary heart disease

C_{max} - maximum plasma concentration

CNS - central nervous system

CSF - cerebrospinal fluid

CTFR - cystic fibrosis transmembrane conductance regulator

DAC - desacetylcephalosporin C

DAOC - desacetoxycephalosporin C

DNA - desoxyribonucleic acid

E. coli - *Escherichia coli*

GSH - glutathione

GST - glutathione-S-transferase

H. influanzae - Haemophilus influanzae

HDL - high-density lipoprotein

HIV - human immunodeficiency virus

HLA I - human leukocyte antigen I

HMG-CoA - 3-hydroxy-3-methyl-glutaryl-coenzyme A

IC_{50} - half maximal inhibitory concentration

IMPDH - inosine-5'-monophosphat-dehydrogenase

K_i - inhibition constant

JNK – c-JUN N-terminal kinase

L-α-AAA - L-α-aminoadipinic acid

LDL - low-density lipoprotein

LSD - lysergic acid diethylamide

LTC4 - leukotriene C4

MDR1 - multidrug resistance protein 1

MHC I - major histocompability complex I

MRP - multidrug resistance-associated protein

NBD - nucleotide-binding domain

PAH - para-amino-hippurate

PBP - periplasmic binding protein

PFIC - progressive familial intrahepatic cholestasis

P-gp - P-glycoprotein

PPAR - peroxisome proliferator activated receptor

S. aureus - *Staphylococcus aureus*

S. typhimurium - *Salmonella typhimurium*

SNP - single nucleotide polymorphism

SREBP - sterol regulatory element-binding protein

SUR - sulfonylurea receptor

T. inflatum - *Tolypocladium inflatum*

TAP - transporters associated with antigen presentation

TMD - transmembrane domain

TNF α - tumor necrosis factor α

UGT - uridine 5'-diphospho-glucuronosyltransferase

VLCFA - very long chain fatty acids

X-ALD - X-linked adrenoleukodystrophy

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Publikationen

1. 'Nutrition and NCDs in North Africa', N. Mokhtar, T. Becic; presentiert in '20th International Congress of Nutrition', 15-20 Sept 2013, Granada, Spanien; Abstrakt publiziert in: Annals of Nutrition and Metabolism, An Official Journal of International Union of Nutritional Sciences (IUNS) and Federation of European Nutrition Societies (FENS), 2013, 63 (suppl 1), Seite 52

2. 'International Atomic Energy Agency activities in infant and child nutrition', N. Mokhtar, T. Becic; presentiert in '13th scientific meeting of the Commonwealth Association of Paediatric Gastroenterology and Nutrition (CAPGAN 2013)', 6-7 Dez 2013, Colombo, Sri Lanka; Abstrakt publiziert in 'Proceedings of CAPGAN 2013'

Technische Kompetenzen

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