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Romana Mammerler

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Abstract

Transdermal drug delivery (TDD) has become an alternative to conventional routes, like oral or nasal administration, because of its avoidance of their side effects. The principal barrier to TDD, the *stratum corneum* (SC) in human skin, makes penetration enhancers (PEs) like essential oils (EOs) necessary for drugs to cross the outermost layer. EOs can penetrate through the skin and thus play a major role in enhancing drug permeability through human skin because of their interaction with the lipid bilayers. This review deals with different essential oils, their constituents - especially monoterpenes and sesquiterpenes with long chain alkyl functionality – how and why they enhance the penetration of special drugs, their mechanism of action and their advantages in contrast to oral administration.

Zusammenfassung

Die transdermale Arzneimittelgabe wurde zur Alternative zu herkömmlichen Applikationsarten, wie oraler oder nasaler Verabreichung, aufgrund der Umgehung von deren Nebenwirkungen. Die Hauptbarriere in der transdermalen Applikation stellt das Stratum Corneum in der menschlichen Haut dar, es macht Penetrationsverstärker wie ätherische Öle erforderlich, um Drogen das Passieren der äußersten Hautschicht zu ermöglichen. Ätherische Öle können durch die Haut dringen und spielen somit wegen ihrer Interaktion mit der Lipiddoppelschicht beim Erleichtern der Arzneimittelpermeabilität durch die Haut eine große Rolle. Diese Literaturübersicht beschäftigt sich mit verschiedenen ätherischen Ölen, deren Bestandteilen – insbesondere Monoterpene und Sesquiterpene mit langer Alkylketten-Funktionalität – wie und warum sie die Penetration bestimmter Arzneimittel unterstützen, deren Wirkmechanismen und deren Vorteile im Gegensatz zu oraler Applikation.

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Introduction

Essential oils (EOs) as carrier oils are receiving more and more attention in the last years. As it is already known EOs can be obtained from aromatic plants and consist of terpenes, phenylpropanoids and some other components. In comparison to many other drugs the mono- and sesquiterpenes, the main components of the complex aromatic volatile mixtures are rather small molecules with low molecular weights and thus they are able to overcome the skin barrier and penetrate through human skin. As the terpenes reversibly reduce the barrier function of the *stratum corneum* (SC), they enable drugs with higher molar mass to diffuse into lower skin layers. There are different pathways for percutaneous penetration, caused by penetration enhancers, recognized that the intercellular lipid domain of the SC is the main pathway, which will be discussed properly afterwards. This paper also focuses on the chemical structures and the lipophilicity and hydrophilicity of both, penetration enhancers and drugs and how these influence their interaction. Many medical treatments can be improved with the transdermal drug delivery (TDD), because of the positive effects TDD provides, like avoiding the first pass effect, reducing the gastrointestinal side effects or decreasing the frequency of administration. For that reason transdermal drug delivery gains in importance concerning different diseases and therapies. (Jiang et al., 2017; Chen et al., 2016; Herman et al., 2014; Fung Chye Lim et al., 2009)

Main part

1. Essential oils in general

Essential oils are volatile mixtures of many different components with diverse structures and low molecular weights from aromatic plants. They are complex, multi-component systems in which terpenes play the main part besides some other non-terpene components like phenylpropanoids and fatty acids. The natural agents can be used in many different domains like anti-inflammatory therapy, wound healing, inhalation, aromatherapy, anticancer therapy, cosmetic usage and some others because of their numerous biological activities (antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, antidiabetic and so on). Thus they are applied oral, nasal or topical as pure essential oils or formulations like emulsions, gels or solutions to treat but also prevent many human diseases. To extract the essential oils from aromatic plants following techniques are possible: steam or water distillation as well as expression under pressure of the peels of citrus fruits. (Herman et al., 2014; Cal et al., 2005; Edris et al., 2007; Pharm. Eu., 2008)

2. Essential oils as penetration enhancers

Essential oils (EOs) and their active constituents can be used as natural, safe and clinically acceptable penetration enhancers for hydrophilic and lipophilic drugs because of their physico-chemical properties, their structure, their activity on the skin layers and the advantages they cause. They show a better safety profile in comparison to some other chemical penetration enhancers like azone, sulphoxides, pyrrolidones, alcohols or surfactants, that often cause skin erythema and edema. Terpenes are one of the main constituents but remaining in EOs they penetrate slower through the skin than as pure substances. EOs facilitate drug delivery through the skin and induce at best a reversible, temporary reduction in the *stratum corneum* (SC) barrier function without damaging viable cells. Characteristics that absolutely influence the penetration are concentration of active compounds, polarity, molecular weight (<500Da), solubility of molecule in water and oil and composition of formulation. What is also crucial to obtain the optimal penetration enhancement effectiveness is the volatility (0,759 ~ 1,67 mg/h/cm²), which can be determined by weight loss. If the volatility is too high, it is not possible to

achieve good interaction with SC lipids because of the rapid evaporation. It can be assumed that enhancers to be classified as promising penetration enhancer with good activity should be easy removable from the skin, not lead to loss of body fluids or electrolytes and must not cause irritation, allergy or toxicity, besides they should possess good solvent properties, be combinable with other drugs and should not bring color, taste or odor. (Jiang et al., 2017; Herman et al., 2014; Fung Chye Lim et al., 2009; Cal et al., 2005; Feng et al., 2015)

3. Terpenes

3.1. Classes & complexes

Mono- and sesquiterpenes consisting of only carbon, hydrogen and oxygen atoms are mostly volatile and extracted from medicinal plants. Those terpenes are formed by a number of isoprene units (C_5H_8), which are repeated in an appropriate number. Monoterpenes (C_{10}) are built from 2 isoprene units, sesquiterpenes (C_{15}) from 3 isoprene units, those are the two most important penetration enhancer classes. Monoterpenes seem to be a little more effective as they do not possess the bulky structure like sesquiterpenes do. So they are able to overcome the biological barriers easily as they constitute small, lipophilic and unionized molecules. It should be noted that many of the terpenes used as penetration enhancer are oxygen-containing terpenes thus they can form hydrogen bonds while interacting with the drugs enhanced and so they possess higher ability to support drug permeation. The drug-terpene complex formed by hydrocarbon terpenes needs either donor/acceptor interactions, van der Waals forces or hydrogen-bond-donor- π interactions. These formed complexes increase the SC partition of the drug. Furthermore terpenes can loosen the hydrogen bonding network of the SC simply by offering a functional group that can donate or accept a hydrogen bond, hence increase the fluidity of SC lipids. (Jiang et al., 2017; Chen et al., 2016; Fung Chye Lim et al., 2009; Cal et al., 2005)

3.2. Hydrophilic, lipophilic, amphiphilic terpenes

Natural terpenes in comparison to conventional synthetic penetration enhancers (PEs) possess higher enhancement activity of hydrophilic and lipophilic compounds and show lower skin irritation potential. As the natural terpenes are nonirritating and non-toxic they can be regarded as promising PEs with a 'Generally Recognized As Safe' (GRAS)

status. Highly lipophilic drugs require lipophilic terpenes, so called hydrocarbon terpenes to increase their penetration through the skin while terpenes with polar groups show a better enhancement effect for hydrophilic drugs. However, amphiphilic terpenes show the best penetration enhancement effect for most of the drugs, because they are able to disrupt the highly organized lipid packing in the *stratum corneum* due to their amphiphilic structure. (Jiang et al., 2017; Chen et al., 2016; Herman et al., 2014)

3.3. Structure of terpenes

Another factor that influences the penetration enhancement activity is the structure of the terpenes. A long chain alkyl structure increases the enhancement effect better than a ring structure. Furthermore, farnesol, which represents a chain molecule, in comparison to cyclic terpenes possess better enhancement effects for hydrophilic drugs because of its lower vaporization energy as it can be supposed. In contrast ring structure containing terpenes like menthol and camphor show less of an effect. The size and degree of long chain alkyl functionality correlates to the degree of SC lipid disorder. (Jiang et al., 2017; Chen et al., 2016)

3.4. Boiling point of terpenes

What acts a part as well is the boiling point. It relates to the penetration enhancement effect of the terpene. The lower the boiling point is the more effective is the terpene. With a low boiling point come relatively weaker cohesive forces. Thus it is easier for the functional groups of terpenes to react with the skin ceramides through competitive hydrogen bonding, as the oxygen of the functional groups is mostly free. Therefore, by weak cohesiveness or self-association of the molecules of the terpenes, they are able to interact with the SC more easily and modify the barrier function. (Jiang et al., 2017; Chen et al., 2016)

3.5. Concentration of terpenes

Besides it is important to choose the right terpene-concentration, which is arranged between 1% - 5% whereas 3% is considered to be the most effective concentration for many drugs. If the concentration is chosen too low the optimal effect is not going to be achieved, on the other hand if the concentration is too high side effects like skin irritation have to be expected, but this theme will be dealt with in detail in 6.1.

Therefore, it is necessary to find the optimal balance between potency and safety and to keep it constant. (Chen et al., 2016; Herman et al., 2014)

3.6. Increase of terpenes

The combination of terpenes can lead to an increase of the permeation enhancement effect just as physical enhancers do in combination with terpenes. Ultrasound as well as iontophoresis show synergistic effects when combined or rather pretreated the skin with (mono-) terpenes. (Jiang et al., 2017; Fung Chye Lim et al., 2009)

3.7. Vehicles of terpenes

The vehicle system in which the EO respectively the terpene will be carried also affects the penetration enhancement effect. Based on the physico-chemical properties of the vehicles and their interaction with the SC they cause a difference concerning the penetration. Some organic solvents like ethanol or propylene glycol even show penetration enhancing function hence induce synergistic effects, but work as the formulation of the vehicle to dissolve the drug and the EO. Invasomes, which describe novel vesicles for transdermal drug delivery, consist of phospholipids, little amounts of ethanol and terpene mixtures as enhancers. In comparison to liposomes or ethosomes they cause a higher penetration rate. These vesicles are proven to possess the positive effects of both, liposomes as potential carriers and terpenes as they are able to modify the skin barrier. Vehicles are needed because of the difficulties that occur while dissolving lipophilic EOs in water and the possible skin irritation when directly applying the EOs on the skin. Namely the accumulation of the terpenes, when applied as pure EOs is considerably higher than in vehicles, although proportionality to the penetrant concentration is not discovered. When not applied as pure EOs, there are a few possibilities of dermatological formulations like o/w emulsions, suspensions, oily solutions, hydrogels, ointments or multi-layer transdermal patch. If grape seed oil is used as vehicle for EOs like linalool, linalyl acetate, terpinen-4-ol, citronellol or α -pinene the EOs are present completely dissolved compared to the o/w emulsion, where they are either dissolved in the oily internal phase or in micelles of surfactants or emulsified with the surfactants and forming an internal oily phase. (Jiang et al., 2017; Chen et al., 2016; Herman et al., 2014; Fung Chye Lim et al. 2009; Cal et al., 2005)

Furthermore, in formulations terpenes can be combined with other active and synergistic compounds and do not only appear as drugs themselves. But not only the formulation ingredients affect the activity of the penetration enhancers, also pH values and skin type are co-dominant. (Cal et al., 2005; Chen et al., 2016)

4. Skin

The skin represents the most accessible and biggest organ of the body with a surface area of about 1.72m². There are many functions it has to fulfill like protect the organism from the outer environment, microorganisms and damage, regulate the body temperature, avoid transepidermal water loss and act as organ of perception. However, the main task of the skin is protecting the inner organism, it is also able to absorb agents that are applied on the skin through hair follicles or reversibly disrupted skin barrier. This causes increasing interest in transdermal drug delivery. (Herman et al., 2014; Fung Chye Lim et al. 2009)

4.1. Layers

There are 3 main layers in which the skin can be divided namely the epidermis, dermis and subcutis. The epidermis itself consists of 5 other layers which are identified as *stratum corneum* (SC), the outermost skin barrier, *stratum lucidum*, *stratum granulosum*, *stratum spinosum* and *stratum basale*. For the topical application of drugs the SC is the most interesting layer which has to be bridged. (Fung Chye Lim et al., 2009)

4.2. Stratum corneum (SC)

The *stratum corneum* that belongs to the epidermis and builds the outermost layer of the skin can be considered as significant transport barrier and rate-limiting layer which hinders the diffusion of compounds into and out of the host. It consists of 15-20 layers of non-viable, flattened cells embedded in a lipid domain and its arrangement can be described as “brick-and-mortar”, where the keratin-filled corneocytes represent the bricks and the intercellular lipid bilayers, which are composed of 50% ceramides, 25% cholesterol, 15% free fatty acids and some phospholipids, can be seen as mortar that causes the permeability properties of SC. Due to this structure the SC is presumed to be a protective and impermeable barrier to drug diffusion which justifies the usage of

penetration enhancers to overcome the barrier. (Chen et al., 2016; Fung Chye Lim et al., 2009; Chen et al., 2015)

4.3. Skin models

To test the penetration of active compounds through the skin some *in-vitro* and *in-vivo* models are necessary. Although human skin is considered as the most reliable model, animal models are needed, because of the low availability, the limited access and the high costs of human skin. Instead of human skin, animal skin of rat, mouse, guinea pig, snake and some others can be used, considering the differences in skin structure. (Herman et al., 2014)

4.4. Franz cell

For the *in vitro* permeation experiments Franz-type diffusion cells can be used. Therefore the prepared skin surface of about 1.7 cm² is mounted between the donor and receptor chambers with the SC-side facing the donor compartment. The donor compartment is filled with vehicle containing drugs and penetration enhancers while the receptor phase consists of buffer pH 7.4, maintained at 37°C and stirred at about 500-600rpm. To find out whether the penetration enhancer is helpful for the drug to overcome the SC barrier the experiment is carried out with and without it and the drug concentration of the collected samples from receptor compartment are compared. (Jiang et al., 2017; Feng et al., 2015; Brito et al., 2009; Fang et al., 2004)

5. Advantages

5.1. Advantages of natural penetration enhancers

Natural penetration enhancers (PEs) such as EOs, which come into operation in transdermal drug delivery are an alternative to synthetic PEs like azone, alcohols, pyrrolidones, sulphoxides, fatty acids, solvents and surfactants. Natural PEs are more and more preferred on the one hand because of their relatively low price and on the other hand because of their promising enhancement activities and their hardly existent adverse effects when administered at low concentrations (1-5%). They facilitate the penetration of both hydrophilic and lipophilic drugs and besides they can be considered as safe PEs due to their metabolism, which is quite rapid. So the EOs are fast eliminated

and excreted and thus do not accumulate in the organism. Besides they cause less skin irritation and higher permeation flux determined by the enhancement ratios. (Chen et al., 2016; Edris et al., 2007; Feng et al., 2015; Herman et al., 2014; Jiang et al., 2017)

5.2. Advantages of transdermal drug delivery

Transdermal drug delivery in general represents a positive option to the conventional drug administration routes, such as oral and nasal routes when not administrated invasive. It causes avoidance of the hepatic first pass effect and differences in gastrointestinal absorption and metabolism what normally leads to low bioavailability when administrated orally. Thus an improved bioavailability as well as a steady-state plasma level with minimal fluctuations can be attained with transdermal drug application. Beside the reduction of the gastrointestinal side effects, the administration frequency can be decreased hence the patients' compliance is improved. (Chen et al., 2016; Fung Chye Lim et al., 2009; Chen et al., 2015)

6. Side effects of natural penetration enhancers

6.1. Skin irritancy & toxicity

EOs and their components do not only show the advantages of an increased skin permeability and a higher drug absorption by disturbing the skin barrier, the disruption of the SC can also lead to cytotoxicity, skin irritation and allergic reaction when applied at too high concentration although natural PEs are less toxic than synthetic PEs. Furthermore, sesquiterpenes seem more potent compared to monoterpenes because of their chemical structure and their potential in interrupting the SC barrier. To reduce or avoid the adverse reactions it is important to apply the lowest possible, still efficacious concentration of diluted EO. (Chen et al., 2016; Herman et al., 2014)

6.2. Trans epidermal water loss (TEWL)

To check and demonstrate the health of the skin barrier function TEWL provides an effective index, where the skin irritation can be determined with as well. Skin irritancy is a reaction to substances, that cannot be tolerated by the skin and provoke inflammation of skin and itchiness. Non-oxidized terpenes and terpenes at an appropriate concentration do not cause any irritation. (Chen et al., 2016)

7. Mechanism of action

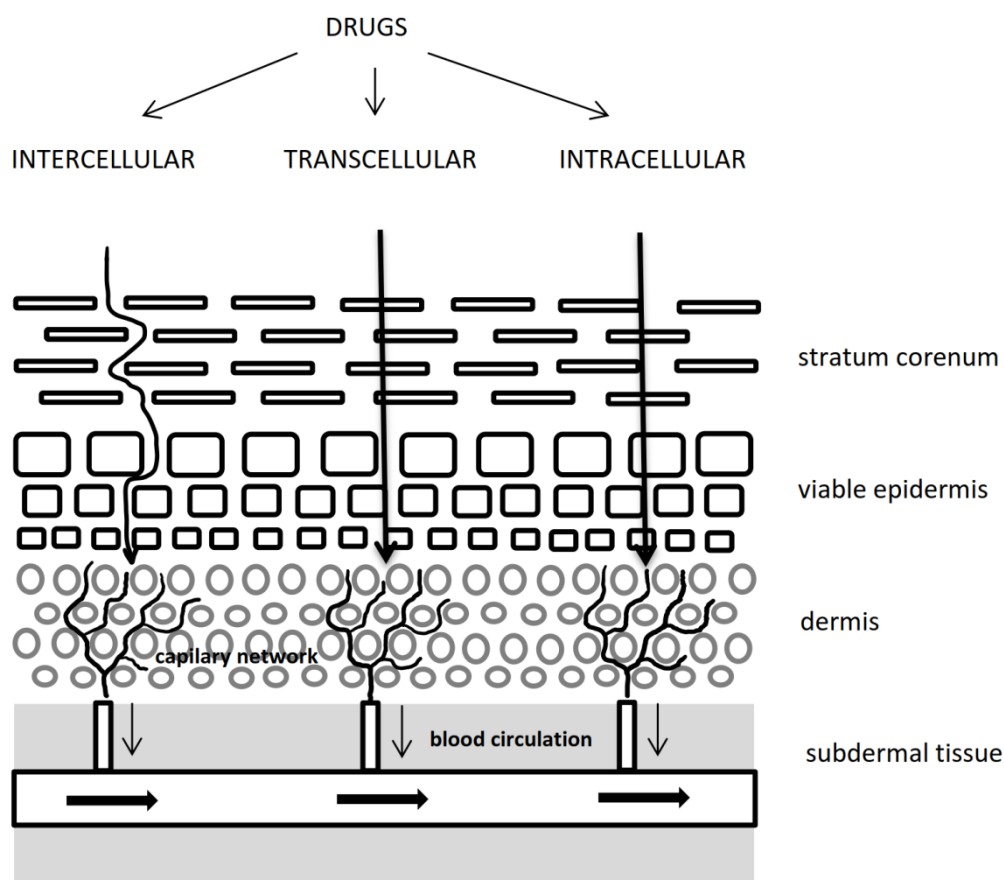
As the natural agents enhance the penetration of drugs through the skin, their main task is changing the structure of the SC and interacting with its lipids to reduce the barrier resistance and increase the drug diffusivity. To improve the permeation of drugs, that are normally poorly absorbed, incorporation of PEs into drug formulations is conducive. Drug permeation enhancement can be induced either by increasing the permeability of the drug into the SC or by reducing the tortuous pathway in the SC as well as using both options. (Brito et al., 2009)

PEs use 4 different mechanisms of action namely (1) disrupting the highly ordered intercellular lipid structure between corneocytes in SC via extraction, fluidization, polarity alteration and phase separation what leads to higher permeability, (2) interacting with intercellular domain of keratinized protein to induce their conformational modification, (3) increasing the partitioning – several solvents alter the SC properties and thus force up the partitioning of a drug and (4) enhancers acting on desmosomal connections between corneocytes or altering metabolic activity within the skin. There are also 3 possible pathways that can be used which include intracellular diffusion across the corneocytes of SC, penetration through the SC intercellular lipid spaces and appendage penetration across hair follicle, sebaceous and sweat glands. The intracellular pathway is normally chosen by hydrophilic compounds while lipophilic permeants prefer the intercellular route. Although most molecules cross the SC via both routes, the intercellular lipid domain of the SC describes the main pathway thus the extraction of SC lipids can be seen as one of the key mechanisms. (Jiang et al. 2017; Chen et al., 2016; Herman et al., 2014; Fung Chye Lim et al., 2009)

To elucidate which mechanisms take place different analytical techniques like DSC (differential scanning calorimetry) and FTIR (fourier transform infra-red) spectroscopy can be used. These techniques will be examined in detail in 7.5.

Beside the 4 main mechanisms respective the enhancement of permeability mentioned above, there are some other possible modes of action. Among those ranks the inhibition of detoxifying enzyme CYP 450 what leads to a delayed metabolism and excretion so the drug remains in the organism and shows its effect longer. (Tak et al., 2017)

Skin layers & pathway



Adapted from Herman et al. (2014) and newly drawn by Romana Mammerler

7.1. Effect on stratum corneum lipids

As it is known lipids are composed of a polar head and lipophilic tails. Therefore, interactions between terpenes and SC lipids are possible at two sites, as there are the polar head groups in the polar transcellular pathways and the lipoidal intracellular pathways as well as the lipophilic tails in the intercellular lipid pathways. Depending on the biophysical alterations of the skin barrier it is about extraction or fluidization. To elucidate which of the two options occurs attenuated total reflection-fourier transform infrared spectroscopy (ATR-FTIR) – explained in detail in 7.5. – can be applied. (Chen et al., 2016)

7.2. Effect on hydrogen bond networks

Hydrogen bonding describes the manner how ceramides are held together tightly in the SC. The hydrogen bond networks are built at the head of ceramides and are the reason for the stability and strength of the lipid bilayer and thus cause the barrier function.

Terpenes with functional groups have the ability to loosen the ceramide network as they are able to accept or donate a hydrogen bond. As terpenes build new hydrogen bonds with the ceramides, the existing hydrogen bond network between ceramide head groups will be disintegrated and thus the permeation of the drug will be facilitated. Beside the increase in diffusivity of the SC caused by the hydrogen-bonding potential of terpenes with functional groups, they are also able to affect the conductivity as they build new polar channels near the head groups of SC lipids that can be passed by ions and polar molecules. (Chen et al., 2016; Fung Chye Lim et al., 2009)

7.3. Effect on SC partition of drugs

With transdermal drug delivery comes first the partition of the drug in the SC and this process is improved when terpenes are existent in the intercellular lipid domain in dissolved form. The terpene uptake correlates with an increased drug partition coefficient and the enhanced partition coefficient is induced by the interaction between terpenes and drugs via hydrogen bonding – mentioned in 7.2.. This procedure builds the basis for penetration enhancement. (Chen et al., 2016)

7.4. Affecting factors

There are a lot of factors that affect the penetration enhancement effect of the terpenes namely the skin type and origin to start with the treated organ. Besides the pretreatment, the vehicle system and the ingredients, their structure, concentration and polarity, as well as pH values are crucial. Terpenes themselves can also influence their activity as some of them – for example menthol – are able to induce physiological reactions in the living skin like increase of skin temperature and vasodilatation. They also have to be suitable for the drug they enhance as polar terpenes are beneficial for hydrophilic drugs and hydrocarbon terpenes go with lipophilic drugs. Not only the terpenes influence their effects, the drugs are co-decisive too. Drug lipophilicity is presumed to be a predominant factor affecting the skin permeability of drugs, not to forget the molecular weight, where the upper limit seems to be about 500 and the melting point. To get information whether a molecule is able to penetrate through the lipophilic SC barrier or not and how it can partition between the SC and hydrophilic or lipophilic vehicle it is important to know the octanol-water partition coefficient, normally presented as logP (logarithm). The optimal logP for percutaneous penetration is in the range of 1-3 obtained by less lipophilic compounds that penetrate into the skin easily, while a logP >

4 caused by highly lipophilic compounds that permeate less through the skin is not desired. (Chen et al., 2016; Herman et al., 2014; Cal et al., 2005)

Physical methods can affect the skin morphology and thus make it easier for the drugs to permeate. There are a few methods such as low-frequency ultrasound, also known as sonophoresis and electrical current, namely iontophoresis and electroporation. By increasing the free volume space in between the lipid lamellae cavitation is induced by ultrasound, while iontophoresis works with current defects through which ionized drug molecules can get and electroporation causes aqueous pore formation for enhancing drug permeation. (Fung Chye Lim et al., 2009)

7.5. Screening-techniques

To investigate the mechanism of action and the biophysical alterations of the SC barrier different analytical techniques come into operation.

One of the most frequently used techniques is the ATR-FTIR (attenuated total reflection-fourier transform infrared) spectroscopy also known as FT-IR spectrometry. It is often used due to its ability of obtaining the information how SC lipids and keratins are arranged and how their conformation will be changed after application of penetration enhancers. All the alterations can be seen on the infra-red spectra bands of the SC, which are referred to the lipid or protein molecular vibrations. After application of terpenes stretching peaks near 2850cm^{-1} (C-H symmetric stretching absorbance frequency peak) and 2920cm^{-1} (C-H asymmetric stretching absorbance frequency peak) can be noticed because of hydrocarbon chains of SC lipids that give rise and near 1540cm^{-1} (amide 2) and 1640cm^{-1} (amide 1) because of SC proteins that give rise to CN stretching and NH bending vibrations. The shift to a higher frequency of C-H stretching peaks, that signifies perturbation of SC lipids and further lipid fluidization can be noticed in case methylene groups of the SC lipid alkyl chains change from a trans to a gauche conformation, that is more energetic. Shifts to a high frequency mean strong perturbation. Also the two C-H peak areas and heights act proportional to the SC lipid amount and thus the lipid extraction caused by a penetration enhancer results in a decrease of peak area and height. In contrast to the C-H peaks and shifts that stand for the alteration of SC lipids, the amide peaks represent the change in protein conformation. With FTIR findings it is proven that partial extraction is a major mechanism for hydrocarbon, alcoholic and oxide terpenes. Further it can be seen that

increased drug permeation correlates with delipidization. (Chen et al., 2016; Fung Chye Lim et al., 2009)

DSC (differential scanning calorimetry) is the other technique that is often applied due to its sensitivity to the thermal effects that come with phase changes like melting of lipids at 65°C, melting of lipid-protein complexes at 75°C and protein denaturation at 95°C or transitions of the components of the SC layer. Phase separation denotes a weakened SC resistance caused by the formation of interfacial defects in the lamellae. With the phase separation a decrease in lipid melting transition can be detected. (Fung Chye Lim et al., 2009)

8. Combination of penetration enhancers and drugs

8.1. Essential oils and anti-inflammatory drugs

Anti-inflammatory drugs, more precisely non-steroidal anti-inflammatory drugs (NSAID) like ibuprofen, diclofenac, indomethacin, salicylic acid and so on come into operation when suffering from inflammation and pain based on dysmenorrhea and rheumatic disease for example due to their analgesic, antipyretic and anti-inflammatory activity. Unfortunately, the oral application of NSAIDs can provoke a lot of gastrointestinal adverse effects such as ulceration, perforation of the stomach and intestines as well as bleeding. Therefore, the topical application of NSAIDs presents a safer potential option to oral therapy with fewer side effects. Though the poor skin permeability of the NSAIDs and the associated low therapeutic blood concentrations build an obstacle to transdermal drug delivery that needs to be conquered with EOs as penetration enhancers. Hydrophilic terpenes are supposed to be the best penetration enhancers for NSAIDs under which alcohol terpenes are the most effective followed by ketone and oxide terpenes. (Chen et al., 2015; Akbari et al., 2015)

Chuanxiong oil and ibuprofen

Chuanxiong oil is obtained from Rhizoma Chuanxiong, exactly *Ligusticum chuanxiong* a member from the Umbelliferae family, which is often used in traditional Chinese medicine to cure gynecological and cardiovascular diseases. The EO is extracted by steam distillation and consists of many phthalide components under which ligustilide with 41.28% forms the major component that is able to attenuate pain. Thus the EO shows analgesic activity itself and causes synergistic effects beside its penetration

enhancement effect. Chuanxiong oil with its phthalides is suggested to be the best appropriate penetration enhancer for the lipophilic ibuprofen – which possesses the poorest bioavailability among NSAIDs – due to the increased steady state flux and the highest obtained permeation rate of $52.05 \pm 7.83 \mu\text{g}/\text{cm}^2/\text{h}$ compared with the control rate of $14.57 \pm 3.47 \mu\text{g}/\text{cm}^2/\text{h}$. To facilitate the penetration of ibuprofen in order to obtain an effective blood level Chuanxiong oil disrupts and extracts the SC lipid structure and makes it possible for ibuprofen to overcome the barrier through intercellular spaces. So, the EO and the drug make a good combination to treat dysmenorrhea as they reduce cramping pain and writhing times – attributed to decreased Ca^{2+} levels, increased NO levels and the reduction of the pro-inflammatory prostaglandins – when applied at the abdominal region. (Chen et al., 2015)

Rosemary essential oil and Na-diclofenac

Rosemary, also known as *Rosmarinus officinalis* belongs to the Lamiaceae family and is mostly located in Mediterranean and Iran regions. From the evergreen plant which shows anti-inflammatory, antiseptic, antioxidant, anti-aging, healing, anti-rheumatic as well as antispasmodic activity Rosemary EO can be obtained. The EO that is rich in monoterpenoids – identified as 1,8-cineol (16.0%), α -pinene (13.4%), camphor (7.9%), verbenone (5.8%), borneol (5.2%) and camphene (5.0%) beside some other co-major constituents like bornyl acetate (6.5%) and (*E*)-caryophyllene (3,8%) – represents a good candidate in enhancing the penetration of Na-diclofenac that counts among NSAIDs. With this formulation inflammation of skin tissues as well as supporting structures of the body-bones, joints, ligaments, tendons and muscles can be treated. The best analgesic effect was noticed with the combination of 1% rosemary essential oil and 1% Na-diclofenac, however the most enhancing effect could be seen at the concentration of 0.5 and 1% of the EO. Rosemary EO helps Na-diclofenac permeate through the skin by vasodilatation, increased disorder of the SC lipids and complex formation between the enhancer and the drug or structures of the SC. (Akbari et al., 2015)

Alpinia oxyphylla essential oil and indomethacin

Alpinia oxyphylla, a plant that belongs to the Zingiberaceae family was often used in treatment of diarrhea and gastralgia and also in neuroprotection because of its anti-angiogenetic and anti-oxidative effects. With hydrodistillation from fruits and leaves

the EO can be isolated. With this method both EOs show a better enhancement effect than an extract obtained with organic solvents like acetone, because of the synergistic effects between the constituents. Both the fruit oil and the leaf oil can act as penetration enhancer, however the fruit oil supports the more lipophilic drugs because of its constituents. The fruit oil consists of 14 hydrocarbon terpenes that make 40.4% of the total content and 15 oxygenated terpenes that make 35.6%. Besides nootkatone was found with 3.9%, a constituent with beneficial properties. In comparison the leaf oil contains 27.7% hydrocarbon terpenes, 50.8% oxygenated terpenes and 1.4% nootkatone. Thus for indomethacin as lipophilic drug beneath NSAIDs *A. oxyphylla* fruit oil is the better candidate to enhance the diffusion through the skin because of the higher content of hydrocarbon terpenes. Furthermore, it is proven that with pretreatment with the EO better results like increase in skin flux values of drug and cumulative amount can be achieved due to the directly acting on the skin and avoiding co-solvent effects on the thermodynamic activities of the drug. The concentration of the EO is also crucial, as the enhancement effect and the reduction of lag time depend on it. So 3% fruit oil with an enhancement ratio (E_r) of 10.16 exhibits the best effect followed by 5% fruit oil with E_r of 5.25 and 3% leaf oil with E_r of 4.61, mentioned that the differences in E_r can be explained by the different boiling points of the terpenes as well as their molecular weights.. However, 5% level shows an increase in lag time while 3% forms the optimum concentration for both EOs to decrease the lag time to reach steady state flux. The enhancement effect of the *A. oxyphylla* EO may be attributed to disruption of the SC barrier. (Feng et al., 2015)

***Lippia sidoides* essential oil and salicylic acid**

Lippia sidoides Cham belongs to the Verbenaceae family and is known for its antimicrobial and larvicidal activity as well as for gastrointestinal treatment. The essential oil basically consists of thymol (59.7%), (*E*)-caryophyllene (10.6%) and p-cymene (9.1%). The *L. sidoides* EO (LSEO 1%) as enhancer makes a good formulation in combination with propylenglycol (PG:phosphat buffer 1:1) as co-solvent to facilitate the penetration of salicylic acid. Due to propylenglycol enhanced solubility of LSEO and its diffusion into SC is possible, as well as LSEO causes enhanced PG penetration by its incorporation between the intercellular lipids. The enhancer provokes the disruption of the lipid bilayer and the extraction of SC lipids, as it can be demonstrated with FTIR spectroscopy. Thus the flux and permeability coefficients of salicylic acid

are increased when applied in combination with the penetration enhancer. (Brito et al., 2009)

Sweet basil oil and indomethacin

Sweet basil oil comes from *Ocimum basilicum* that belongs to the Lamiaceae family and is not only a culinary herb but also a medicinal plant that is the host of many EOs. There are two fractions of sweet basil oil, namely the lower-polarity fraction OB-1 and the higher-polarity fraction OB-2. OB-1 consists predominantly of hydrocarbon components, only estragol the major constituent with 76.7% belongs to the oxygenated components. Because of the lower polarity OB-1 at the concentration of 1%, 3% or 5% is an adequate penetration enhancer for the lipophilic indomethacin – that prefers a non-polar pathway like intercellular lipids of the SC – and further enables the transfer to the circulation. In comparison to that OB-2 possesses higher polarity due to oxygenated terpenes that outbalance in this fraction, under which phytol presents the major component with 52.3%. OB-2 shows a relative low permeation enhancement, but it enables the drug to retain within the skin reservoir. What influences the penetration and the skin reservoir of indomethacin is the pretreatment with 3% of OB-1 or OB-2 in 25% EtOH for 1 hour that results in avoidance of co-solvent effects on the thermodynamic activities of the drug while directly acting on the skin structure. The presence of EtOH beside the enhancer leads to accumulation in the tissue and a higher partitioning of the drug because of the affinity the drug shows to the solvent. The partitioning of the EOs to the SC can lead to decreased polarity of the SC what further causes an enhanced penetration of indomethacin, a lipophilic drug into the skin. Thus the partitioning is considered to be the main mechanism beside the disruption of the SC lipids. (Fang et al., 2004)

Zanthoxylum bungeanum essential oil and indomethacin / 5-fluorouracil

see in 8.4.

8.2. Essential oils and antiseptic drugs

1,8-Cineole and chlorhexidine

1,8-Cineole, a monoterpene cyclic ether is the main constituent of the eucalyptus oil that is known for its penetration enhancement of lipophilic drugs. Because of the ability of

the EO to enhance the absorption of chlorhexidine and its antimicrobial activity, but due to the variability of its constitution a purified solution of 1,8-cineole should represent a potent alternative with synergistic effect. Chlorhexidine comes to usage with skin antiseptics however the SC barrier and the poor diffusion into the skin, caused by the large molecular size and its binding to intercellular lipids in the SC hinder the treatment of endogenous microorganisms in deeper skin layers. Therefore 1,8-cineole should facilitate the penetration of chlorhexidine to eradicate the pathogens and lower the risk of infection as it interacts with and disorders the lipids of the SC. In comparison to the application of the combination of 2% (w/v) chlorhexidine with 70% (v/v) isopropyl alcohol but without the penetration enhancer 2% (v/v) 1,8-cineole it can be seen that with 1,8-cineole the concentration of chlorhexidine in the skin is on average 33.3% higher although the size of the effect does not show significant differences demonstrated by the depth of penetration. Thus according to the higher concentration it is proven that 1,8-cineole promotes the permeation of chlorhexidine. (Casey et al., 2017)

8.3. Essential oils and vesicular carriers

Vesicular carriers are promising candidates for transdermal drug delivery as they encapsulate drugs that are not able to achieve the desired effects or cause side effects like gastrointestinal irritation when administered orally. There are different types of vesicular formulations namely transferosomes, liposomes, ethosomes and glycerosomes. Liposomes often come with the disadvantage of low encapsulation efficiency, instability and limited drug delivery efficiency although they increase the drug accumulation within the tissue very well. Ethosomes correlate to liposomes with addition of high concentration of short-chain alcohols like ethanol. The alcohols lead to improved deformability of vesicles however skin irritation is possible. Glycerosomes that are named after the key-component glycerol, a harmless short-chain alcohol also improve the deformability and enhance the penetration. Combined with EOs it is easier for the encapsulated drugs to permeate through the skin and release from the vesicles, mentioned that all vesicular formulations are supposed to increase transdermal flux more than tinctures. (Zhang et al., 2017; Rajan et al., 2012)

Terpenes and ultradeformable liposomes of sodium fluorescein

Ultradeformable liposomes in combination with terpenes attract attention as they are able to encapsulate and deliver drugs – here sodium fluorescein to detect the effect –

through the skin by using follicular pathways to bypass the outermost layer of the skin that forms a barrier to transdermal drug delivery. The fluidity of these flexible vesicles that consist of phospholipids and surfactant is increased as small, lipophilic monoterpenes like 1,8-cineole or limonene are added. The terpenes alter the fluidity at the C16 atom, the lipophilic region of the acyl chain of the phospholipid bilayer. With the increased fluidity and the reduced liposomal size due to the higher amount of terpenes localized in the outer layer the flux and thus the penetration of drugs is improved as the permeation through hair follicles becomes the preferred pathway of the ultradeformable liposomes with terpenes. (Thirapit et al., 2015)

Terpenes and liposomes of antisense oligonucleotide

As liposomes represent common carriers for transdermal drug delivery they gain interest respective a promising method of cancer therapy namely gene therapy as they can deliver antisense oligonucleotide (AsODN) for treating lung cancer. The vesicle enables the uptake via endocytosis and thus avoids the difficult penetration through the SC. The addition of terpenes – in this case 1,8-cineole – promotes the liposomal gene delivery by fluidizing the bilayer and thus improves the specific activity of AsODN. 1,8-Cineole is able to increase the enhancement effect which is dependent on concentration and chemical structure of the enhancer up to 40 times, although the storage of the lipophilic terpene in the lipid bilayers causes reduction in the encapsulation efficiency. (Saffari et al., 2016)

Eucalyptus oil and transfersomes of ketoconazole

Transfersomes represent synthetic vesicles that surround drugs and imitate cell vesicles so they enable drug delivery by simply crossing barriers that cannot be passed by drugs themselves. To define it, they improve defective transdermal permeation. Transfersomes form a vesicle with an aqueous core covered with complex lipid bilayers, so they are very flexible, ultra-deformable and stress responsive. If the transfersome incorporates ketoconazole, a broad-spectrum antifungal agent life-threatening fungal infections can be treated, what turned out to be difficult when applied orally because of its incomplete absorbance. The inclusion of a penetration enhancer into the transfersomal gel formulation, in this case eucalyptus oil, is advising because it facilitates the release and permeation of ketoconazole as it increases the diffusion rate. (Rajan et al., 2012)

***Speranskia tuberculata* essential oil and glycerosomes of paeoniflorin**

Glycerosomes are carriers with vesicle structure for transdermal drug delivery that possess the ability to encapsulate drugs with poor diffusion rate and poor bioavailability and transfer them through the skin barrier. They are formed by 5% (w/v) phospholipids – with their concentration increases the encapsulation efficiency – 0.6% (w/v) cholesterol – causing membrane rigidification – and 10% (v/v) glycerol – which influences the glycerosome particle size as it becomes bigger with the amount of glycerol in the water phase. If this vesicular formulation encapsulates paeoniflorin a potent alternative to treat rheumatoid arthritis – a long-lasting autoimmune disease that leads to synovium inflammation and lesions in joints – is built. Paeoniflorin is known for its anti-inflammatory and immune-regulatory effects and derives from *Paeonia lactiflora* Pall (Paeoniaceae). As monoterpene glucoside it is a hydrophilic drug that shows poor bioavailability with oral application and a low encapsulation rate. To facilitate the transdermal delivery of paeoniflorin in the glycerosomes it is recommendable to add 2% (v/v) *Speranskia tuberculata* EO (STEO). This EO consists of 14 compounds, predominantly sesquiterpenes that are able to disturb the lipid bilayers. Thus glycerosomes packed with paeoniflorin and STEO – characterized by their spherical shape and uniform size – produce the best results in transdermal performance as they enhance the transdermal flux and increase the accumulation of paeoniflorin in the inflamed synovium and even remain the drug concentration at high level after a long time. (Zhang et al., 2017)

8.4. Essential oils and cytostatic drugs

With minimizing the serious adverse effects like gastrointestinal problems, hair loss, leukopenia, cardiotoxicity, nephrotoxicity, loss of body mass, immunosuppression as well as drug resistance and many others that come with anti-cancer therapy the transdermal application of cytostatic drugs with EOs gains attention. There are some advantages to commend the transdermal usage of cytostatic drugs, namely improved efficacy with decreased dose, upkeep of the same effect with less toxicity, reduced drug resistance development as well as potential synergistic effects. Thus EOs promote the effectiveness of chemotherapeutic drugs. (Amaral et al., 2016)

***Myrica rubra* essential oil and doxorubicin**

Myrica rubra from the Myricaceae family delivers the EO that comes to operation in treating cancer cell lines with the cytostatic doxorubicin. It consists mostly of sesquiterpenes which are known to promote skin permeability, namely β -caryophyllene oxide, α -humulene, trans-nerolidol and valencene. The constituents do not only enhance the penetration of doxorubicin, they possess anti-cancer and anti-proliferative activity themselves. So they can act synergistically and improve the effect of doxorubicin as they increase its efficacy and accumulation beside the increase of reactive oxygen species formation. For doxorubicin it is important to be enhanced by the EO and its components, because many cancer cell lines react with resistance by increasing drug efflux, more precisely by increasing the expression of ATP-binding cassette transporters that can be inhibited by the sesquiterpenes. However, the effect of the sesquiterpenes from *M. rubra* EO and doxorubicin depends on the cancer cell lines. Sesquiterpenes can only influence doxorubicin efficacy in the sensitive and partly resistant cancer cells, but not in completely resistant cells. (Ambrož et al., 2017)

***Mentha x villosa* essential oil and 5-fluorouracil**

The essential oil from *Mentha x villosa* Hudson which belongs to the Lamiaceae family possesses cytotoxic activity beside antimicrobial, antinociceptive, cardiovascular, spasmolytic and some other effects. Together with 5-fluorouracil they make a promising combination for treating tumors. In comparison to 5-fluorouracil alone at highest dose (25 mg/kg/d) the combination causes similar effects with less side effects. As it can be seen with the tumor growth inhibition rate the combination of the EO (50 or 100 mg/kg/d) and 5-FU (10 mg/kg/d) leads to a higher inhibition rate as 5-FU (10 mg/kg/d) alone while the combination shows the same effect with higher dosed 5-FU (25 mg/kg/d) and less severe leukopenia, what represents a great benefit. Furthermore, the large doses of the chemotherapeutic drug that are required due to the limited availability in cancer tissues and the repeated treatment that leads to resistance can be reduced with the enhancer *M. x villosa* EO. Also the severe side effects like the loss of body mass and the alterations of spleen weight, liver aspartate transaminase (AST) and renal function are decreased, despite the higher antitumor activity when treated with the association. (Amaral et al., 2016)

***Zanthoxylum bungeanum* essential oil and 5-fluorouracil (5-FU) / indomethacin**

The EO from *Zanthoxylum bungeanum* Maxim., that belongs to the Rutaceae family, consists of oxygenated monoterpenes and monoterpene hydrocarbons, exactly of 48 compounds among which the following 3 are the major constituents: terpinen-4-ol, 1,8-cineole and limonene. These compounds are able to enhance both, polar (5-FU) and non-polar drugs (indomethacin) as they work with different mechanisms. For the hydrophilic 5-FU lower concentrations (1 or 3%) of the EO come to operation and alter the thermodynamic activity of the drug what leads to higher saturated solubility. In contrast the lipophilic indomethacin requires a higher EO concentration (10%) to increase the SC/vehicle partition coefficient and the saturated solubility. Mentioned that the alterations of the drug properties described above are relatively weak the main mechanism of improving the drug permeation is suggested to be changing the SC skin barrier by disturbing and extracting SC lipids. Thus the drug delivery is promoted in concentration-dependent manner and causes higher flux, shorter T_{lag} which decreased with increased EO concentration and higher cumulative amount. Summarized *Z. bungeanum* EO exhibits enhancement activity for hydrophilic drugs with a long T_{lag} and lipophilic drugs with a short T_{lag} , however efficiency for hydrophilic drugs is higher. (Lan et al.; 2014)

8.5. Essential oils and cardiovascular drugs

***Zanthoxylum bungeanum* essential oil and osthole / tetramthylpyrazine / ferulic acid / puerarin / geniposide**

As noted above the essential oil from *Z. bungeanum* Maxim. enhances polar and non-polar drugs and to face the enhancement activities from the EO to the major constituents terpinen-4-ol, 1,8-cineole and limonene they are tested in combination with drugs for cardiovascular treatment which possess different polarity namely osthole, tetramethylpyrazine, ferulic acid, puerarin and geniposide. The physicochemical properties like molecular size, solubility and lipophilicity specify whether a drug permeates the skin well or not whereat the lipophilicity is the most crucial factor. To facilitate the permeation of the different polar drugs adequate penetration enhancers have to be chosen as the lipophilicity of the enhancer and the drug should match. *Z. bungeanum* EO – followed by limonene – possesses the best enhancement permeation capacity as it causes the greatest steady state fluxes and cumulative amounts for all five

model drugs, although the EO preferably promotes the absorption of hydrophilic drugs and limonene primarily enhances the moderate lipophilic drugs. In contrast terpinen-4-ol and 1,8-cineole show enhancement activity for more lipophilic drugs like osthole and tetramethylpyrazine, however their enhancement activities are relatively low. The drug absorption is mainly facilitated by changing the skin barrier structure as the enhancers disorder and extract the SC lipids, not to forget that with lipophilic drugs the enhancers also cause alteration of their thermodynamic activities what leads to increased saturation solubilities. All together the EO in total is the best choice for promoting the skin diffusion of the five model drugs. (Lan et al., 2014)

Eucalyptus oil and tetramethylpyrazine

Eucalyptus oil, isolated from *Eucalyptus globulus*, a member of the Myrtaceae family, consists of more than 80% cineole and represents a promising penetration enhancer that acts on the skin barrier as it disrupts the SC bilayers thus improving the partitioning and permeation of small polar molecules. 2,3,5,6-Tetramethylpyrazine (TMP), a lipophilic calcium channel antagonist isolated from *Ligusticum wallichii* - a member from the Apiaceae family - and used for treating cardiovascular disorders exhibits some problems when administered orally. The first-pass-metabolism causes low bioavailability besides a short half-life that leads to a high frequent dosing. To avoid the side effects that come with oral administration, a reservoir-type transdermal delivery system (TDS) that incorporates the drug and the penetration enhancer is produced namely a transdermal delivery system patch. For creating such a reservoir-type TDS for TMP the following compounds are required: Carbopol gel for gelling, an EVA membrane for rate-control and silicone adhesive for pressure-sensitivity. With these components an effective TDS can be formed to reach an appropriate clinical concentration. Further, the TMP patch with a clinical surface area of 20cm² consists of 5% eucalyptus oil, what turned out to be the best concentration for promoting the drug permeation. The penetration enhancer causes a 17-fold higher permeation rate compared to an application without enhancer as well as a lower C_{max} is needed for a prolonged steady-state concentration, T_{max} and mean residence time. The reservoir-type transdermal delivery system presents a promising alternative route to oral administration as it improves the permeation and thus the compliance. (Shen et al., 2013)

Basil oil and labetalol hydrochloride

Basil oil, extracted from *Ocimum basilicum*, a plant that belongs to the Lamiaceae family, contains alcoholic terpenes and exhibits a low boiling point what is good for interacting with the SC lipids and leads to a higher enhancement rate. Thus it is an adequate penetration enhancer for a hydrophilic drug like labetalol hydrochloride (LHCl) which is a combined alpha- and beta-blocker that comes to operation while treating hypertension. LHCl presents a good candidate for transdermal application due to the high first-pass metabolism and the poor bioavailability which comes with oral administration. Only few patients fully benefit from the oral medication because of the fluctuated plasma concentration and the uncontrolled drug release and for that reason the transdermal route attracts attention for antihypertensive agents. For overcoming the skin barrier basil oil and 5% (w/v) terpenes respectively are crucial and in combination with the vehicle for LHCl synergistic effects are provided. Greater steady-state flux and a decreased lag time are achieved due to the disruption of the SC barrier and the increase in partitioning and diffusion coefficient. Thus lower activation energy for LHCl is needed for maximum permeation. (Jain et al., 2008)

Anethol, menthone, eugenol and valsartan

Anethol, menthone and eugenol are oxygen containing terpenes that act as penetration enhancers for valsartan, a lipophilic specific angiotensin 2 receptor blocker used for treating hypertension. Valsartan should be delivered through human skin due to its low oral bioavailability, its low melting point and molecular weight. For the terpenes 1% (w/v) concentration is the optimum to increase the flux by interacting with the SC lipids whereas anethol, the most lipophilic enhancer causes the maximum lipid extraction and thereby improves the drug enhancement rate 4.4 times, followed by menthone. It is always important that penetration enhancers and drug conform with each other in polarity, thus eugenol, the least lipophilic enhancer leads to the lowest permeation rate of the highly lipophilic valsartan. Anethol and eugenol promote the drug penetration by keratin denaturation and lipid extraction while menthone only works with lipid extraction. Overall it is supposed that the following terpenes are preferred over the other ones, because of the better enhancement effects they provoke, namely: liquid terpenes and terpenes with higher Log P values due to the better mixture properties from lipophilic terpenes with SC intercellular lipids. (Ahad et al., 2016)

Basil oil, Petit grain oil, Thyme oil and nitrendipine

The EOs like basil oil, petit grain oil and thyme oil are able to enhance the permeation of nitrendipine 10 to 12 times by altering the solubility properties and improving the partitioning of the drug within the SC. Nitrendipine, also known as lipophilic 1,4-dihydropyridine derivative calcium channel blocker represents a potent vasodilator able to decrease blood pressure. Because of its high first-pass effect and the following low bioavailability when administered orally a transdermal patch is a good alternative for nitrendipine medication, mentioned that transdermal drug delivery is always a good choice to treat chronic disorders that require long-term dosing. Due to its poor skin permeation activity a penetration enhancer is needed to overcome the SC barrier. Basil oil, followed by petit grain oil and thyme oil turned out to be the best penetration enhancer among the other essential oils as it exhibits the highest increase in relative activity value due to increased thermodynamic activity and solubility of nitrendipine in the SC. The only enhancer that shows better results in facilitating the permeation of nitrendipine is oleic acid, an unsaturated fatty acid that is more effective than other saturated fatty acids. The similar structure of the oleic acid to the SC enables the fast penetration through the barrier. (Mittal et al., 2008)

9. Essential oils and the influence of temperature

9.1. Borneol, osthole and increasing temperature

Borneol is a cyclic terpene alcohol that is isolated from *Cinnamomum camphora* - a member of the Lauraceae family - and represents a penetration enhancer for osthole, a relative lipophilic drug extracted from the fruit of *Cnidium monnieri* which belongs to the Apiaceae family. Disturbance of the ordered SC lipids is the main mechanism caused by borneol. This permeation mechanism can be influenced by temperature, an external factor. Increase in temperature leads to changes in the lipid bilayers of SC, lipids become shorter and frizzier and therefore they are more flexible and molecular movement is improved. The area per lipid increases while the thickness and order of lipids decrease what results in synergism affecting the permeation of osthole. Thus the penetration enhancement of borneol is promoted with increased temperature as the diffusion rate and the speed of permeation raised. However, the temperature has to be increased carefully, because with too high temperature in combination with borneol the

SC structure gets ruined as a water pore is built and the micelle reversed, caused by 5% borneol at 323K or 10% borneol at 310K. (Yin et al., 2017)

10. Essential oils and the effect on Cytochrome P450

10.1. *Zataria multiflora* essential oils with cancer chemopreventive effect

Zataria multiflora Boiss. belongs to the Lamiaceae family and possesses cytotoxicity, antioxidant and chemopreventive effects beside some others. 1,2-Dimethylhydrazine is suggested to be a potent colon specific carcinogen as it is metabolized by cytochrome P450 to active intermediates that cause colon cancer in the broadest sense. *Z. multiflora* EO is able to inhibit the tumor formation as it interacts with and decreases the activity of CYP450 and thus hinders the metabolism induced by the cytochrome. Proven that EOs can suppress the activity of CYP450, an enzyme that is of importance related to drug metabolism it can be suggested that with this inhibition effect EOs can slow down the drug metabolism and increase and prolong the drug plasma level. Thus EOs not only improve the medication effect by interacting with the SC barrier and enhancing the drug permeation but also by limiting the degradation of drugs by inhibiting CYP450 activity. (Dadkhah et al., 2014)

11. Essential oils and their synergistic effects

11.1. 1,8-Cineole and camphor

Rosemary oil, isolated from *Rosmarinus officinalis* – a member of the Lamiaceae family – possesses 1,8-cineole and camphor as major constituents. The two compounds do not only exhibit insecticidal activity but also penetration enhancing effects. In comparison to the application of 1,8-cineole or camphor alone that may cause partial or incomplete activity, the two major compounds administered as binary mixture show same or better activity with lesser amounts, as synergy takes place. The synergy mechanism can be declared as a multi-target effect where the two compounds attack different sites, ameliorate solubility or bioavailability, that take place caused by pharmacokinetic or physicochemical effects or interactions with resistance mechanisms. Thus it comes to synergy as 1,8-cineole enables camphor a better permeation by interacting with the lipid layer. The interaction with each other leads to lowered surface tension, increased solubility of camphor and higher mobility what finally causes enhanced penetration. As

the two compounds possess insecticidal activity, it can be seen that with the described synergy effect their toxicity over a larvae of the moth *Trichoplusia ni*, the cabbage looper, is improved in contrast to individual application of higher terpene amounts. (Tak et al., 2015)

Conclusion

EOs and their terpenes cause many effects among which the function as carrier oils is the most interesting discussed in this overview. Due to their properties EOs show the ability to cross the SC barrier of the epidermis by disordering and loosening the lipid packing thus facilitating the delivery of drugs through human skin that cannot sufficiently overcome the barrier themselves to achieve therapeutic plasma levels. The conformance of polarity between penetration enhancer and drug as well as the adequate concentration of the enhancer and the right vehicle are important factors that influence the enhancement effect. Mixing terpenes can lead to synergism and increased temperature can improve the permeation activity of carriers and drugs. All those involved factors make transdermal drug delivery and the required therapy whether according inflammation or cancer treatment possible without inducing adverse effects in contrast to drug administration via conventional routes. Mentioned that the main mechanism of EOs and their constituents is affecting the SC properties, a further possibility to improve and prolong the drug remaining in the organism is acting on the metabolism via cytochrome P450 inhibition. So whatever mechanism is induced with the enhancement of EOs less drug concentration as well as less frequent dosing is required to achieve the same therapeutic effect with reduced side effects. All together the transdermal drug delivery makes medication more acceptable for the patients and therefore improves their compliance.

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