



RESEARCH NOTE

Capsaicin from chili (*Capsicum* spp.) inhibits vascular smooth muscle cell proliferation [version 1; referees: 2 approved, 1 approved with reservations]

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Abstract

Accelerated vascular smooth muscle cell (VSMC) proliferation is implied in cardiovascular disease and significantly contributes to vessel lumen reduction following surgical interventions such as percutaneous transluminal coronary angioplasty or bypass surgery. Therefore, identification and characterization of compounds and mechanisms able to counteract VSMC proliferation is of potential therapeutic relevance. This work reveals the anti-proliferative effect of the natural product capsaicin from *Capsicum* spp. by quantification of metabolic activity and DNA synthesis in activated VSMC. The observed *in vitro* activity profile of capsaicin warrants further research on its mechanism of action and potential for therapeutic application.

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Referee Status:

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	1	2	3
version 1			
published	report	report	report
27 Jan 2015			

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

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Main text

Aberrant and accelerated VSMC proliferation is a main contributor to restenosis, the pathological re-narrowing of the vessel lumen after surgical interventions combating vascular stenosis. To overcome restenosis, drug-eluting stents have been developed, aiming at inhibiting VSMC growth by the release of anti-proliferative substances such as paclitaxel and rapamycin. However, these compounds display unresolved issues such as impaired re-endothelialization and subsequent thrombosis induction¹, which makes the characterization of other compounds able to suppress VSMC proliferation highly relevant. Plant-derived natural products are an excellent resource for identifying lead compounds². Here we examine the anti-proliferative potential of capsaicin, a bioactive component of chili peppers [*Capsicum* spp. (Solanaceae)], in VSMC.

To test whether capsaicin is able to inhibit proliferation of VSMC induced by PDGF, a major growth factor implied in the aberrant proliferative responses in restenosis³, the total amount of metabolically active cells was measured after 48 h of incubation by the resazurin conversion method⁴. Capsaicin indeed suppressed VSMC proliferation concentration-dependently with an IC_{50} of 5.36 μ M (Figure 1A). To confirm the anti-proliferative effect of capsaicin with a second experimental method, we measured DNA synthesis in VSMC by quantification of 5-bromo-2'-deoxyuridine (BrdU) incorporation into DNA. Capsaicin also inhibited PDGF-stimulated DNA synthesis in a concentration-dependent manner with an IC_{50} of 3.81 μ M (Figure 1B). To assure that the decreased number of VSMC upon treatment with capsaicin is not due to cytotoxicity, we quantified cell death by measuring cell membrane integrity estimated by lactate dehydrogenase (LDH) activity inside cells and in cell supernatants. No significant cytotoxicity was detected in the investigated concentration range (Figure 1C). In summary, capsaicin is identified as an inhibitor of VSMC proliferation. Further studies are prompted to elaborate the underlying mode of action of this natural product and to investigate its effect in advanced *in vivo* anti-restenotic models.

Rat aortic VSMC used in this study were purchased from Lonza (Braine-L'Alleud, Belgium) and cultivated in DMEM-F12 (1:1) medium supplemented with 20% fetal calf serum and gentamycin. Capsaicin and other chemicals were obtained from Sigma-Aldrich (Vienna, Austria).

For the resazurin conversion assay, VSMC were seeded in 96-well plates at 5×10^3 cells/well. 24 h later, cells were serum-starved for 24 h to render them quiescent. Quiescent cells were pretreated for 30 min with capsaicin or vehicle (0.1% DMSO) as indicated, and subsequently stimulated for 48 h with PDGF-BB (20 ng/mL). To measure the number of metabolically active VSMC by resazurin conversion⁴, cells were washed with PBS and incubated in serum-free medium containing 10 μ g/mL resazurin for 2 h. Total metabolic activity was measured by monitoring the increase in fluorescence at a wavelength of 590 nm using an excitation wavelength of 535 nm in a 96-well plate reader (Tecan GENios Pro).

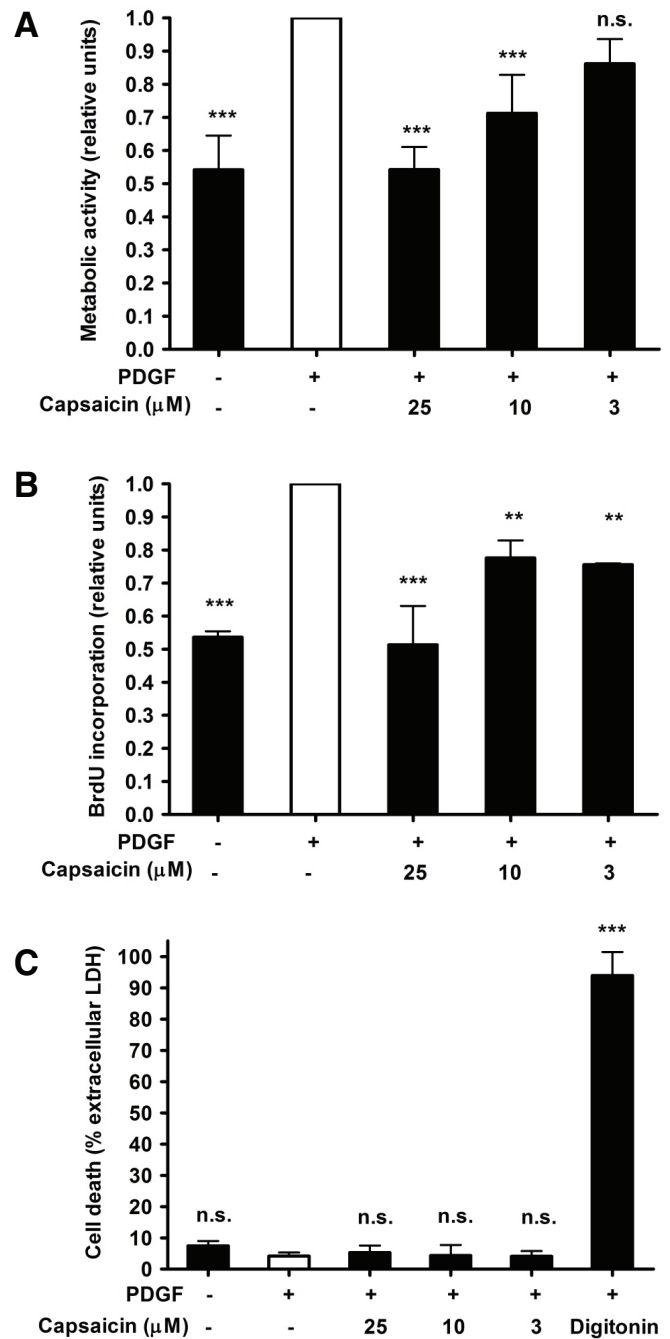


Figure 1. Effect of capsaicin on VSMC proliferation. Cell proliferation was estimated by quantification of metabolic activity (**A**) and DNA synthesis (**B**). Cell death was estimated by quantification of the percentage of extracellular LDH (**C**). Data represent mean $\pm$ SD from at least three independent experiments (n.s., not significant; *** $p < 0.001$; ** $p < 0.01$; ANOVA/Bonferroni).

For the BrdU incorporation assay, VSMC were seeded and starved as for the resazurin conversion assay. Quiescent cells were pretreated for 30 min with capsaicin, or vehicle as indicated and subsequently stimulated with PDGF-BB (20 ng/mL). To estimate *de novo* DNA synthesis in VSMC⁵, BrdU was added 2 h after PDGF stimulation, and the incorporated amount was determined 22 h afterwards with a BrdU ELISA kit according to the manufacturer's instructions (Roche Diagnostics).

For assessing cytotoxicity, VSMC were seeded and serum-starved as indicated above. The quiescent cells were pretreated for 30 min with capsaicin, or vehicle as indicated, and subsequently stimulated for 24 h with PDGF-BB (20 ng/mL). To quantify the loss of cell membrane integrity as a sign for cell death⁶, the supernatants of the treated cells were assessed for LDH activity. For estimation of the total LDH, identically treated samples were incubated for 45 min in the presence of 1% Triton X-100. The released and total LDH enzyme activity was quantified for 30 min in the dark in the presence of 4.5 mg/mL lactate, 0.56 mg/mL NAD⁺, 1.69 U/mL diaphorase, 0.004% (w/v) BSA, 0.15% (w/v) sucrose, and 0.5 mM 2-p-iodophenyl-3-nitrophenyl tetrazolium chloride (INT). The enzyme reaction was stopped with 1.78 mg/mL oxymate and the absorbance was measured at 490 nm in a 96-well plate reader (Tecan GENios Pro). Potential effects on cell viability were estimated as percentage of extracellular LDH activity. The cytotoxic natural product digitonin (100 µg/mL) was used as a positive control.

Statistical analysis was performed by ANOVA/Bonferroni test (GraphPad PRISM software, version 4).

Effect of capsaicin on vascular smooth muscle cell proliferation: raw data

3 Data Files

<http://dx.doi.org/10.6084/m9.figshare.1289721>

Data availability

Figshare: Effect of capsaicin on vascular smooth muscle cell proliferation: raw data. doi: [10.6084/m9.figshare.1289721](https://doi.org/10.6084/m9.figshare.1289721)⁷

Author contributions

RL, DG, VMD and AGA conceived the study. RL, EHH and AGA designed and analyzed the experiments. RL performed the experiments. RL and AGA prepared the first draft of the manuscript. All authors critically commented on and revised the draft manuscript and agreed to the final content.

Grant information

This work was supported by the EU-FP7 Marie Curie Fellowship 252881, and by the University of Vienna "Back-to-Research Grant" (both to R. Liu); as well as by the Austrian Science Fund (FWF): S10704, P25971-B23, and P23317-B11.

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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[Data Source](#)

Open Peer Review

Current Referee Status:



Version 1

Referee Report 02 February 2015

doi:10.5256/f1000research.6428.r7485



Karel Šmejkal

Department of Natural Drugs, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

The authors are describing the inhibitory activity of capsaicin on vascular smooth muscle cell proliferation. The article looks to be well written, with adequate data. I miss only a bit deeper discussion of reasons for capsaicin selection for testing.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Author Response 28 Apr 2015

Atanas Atanasov, Department of Pharmacognosy, University of Vienna, Austria

Thank you very much for taking the time to review our manuscript.

Aiming to identify new inhibitors of VSMC proliferation, we tested a range of natural compounds derived from medicinal plants traditionally used for the treatment of different inflammation-associated conditions (including cardiovascular disease). One of the tested compounds was capsaicin, which revealed activity and therefore was further characterized.

Competing Interests: No competing interests were disclosed.

Referee Report 02 February 2015

doi:10.5256/f1000research.6428.r7484



Goutam Brahmachari

Laboratory of Natural Products & Organic Synthesis, Department of Chemistry, Visva-Bharati University, Santiniketan, West Bengal, India

I have gone through the present manuscript entitled "Capsaicin from chili (*Capsicum* spp.) inhibits vascular smooth muscle cell proliferation" by Liu *et al.* with interest where the investigators demonstrated

the efficacy of natural capsaicin in overcoming restenosis by using a vascular muscle cell model (VSMC) *in vitro*.

Besides macrophages, vascular smooth muscle cells (VSMCs) are now also believed to play a significant role in generating foam cells that accumulate cytoplasmic droplets of cholesterol esters and triglycerides leading to atherosclerosis regarded as a prime cause of cardio- and cerebrovascular events. Proliferation of VSMCs thus is a common cause of restenosis among the patients who were previously undergone percutaneous transluminal coronary angioplasty or bypass surgery. Under this purview, the present work demonstrating promising *in vitro* anti-proliferative activity of natural capsaicin seems to be much interesting.

The experiments are straightforward and sufficient in arriving at the conclusions. Representation of facts and organization of the manuscript are praise-worthy. The manuscript may be accepted and indexed in its present form. At the same time, I have a suggestion for the investigators as mentioned below, which may be addressed to make the revised manuscript more demanding.

Suggestion

Rapamycin and paclitaxel are commonly used standard drugs to resist restenosis in patients although they suffer from certain serious health issues as mentioned by the authors in their present manuscript. Hence, search for better alternative(s) is warranted as a part of which the present work has been developed. Hence, I do suggest comparing the present results with those of at least any one of these two drugs (in addition to or in place of digitonin) to validate superiority of capsaicin.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Author Response 28 Apr 2015

Atanas Atanasov, Department of Pharmacognosy, University of Vienna, Austria

Thank you very much for taking the time to review our manuscript.

Based on the literature, as well as on our own data, the both suggested reference compounds, paclitaxel and rapamycin, are unfortunately more potent than capsaicin. Therefore with our short note we did not aim to claim superiority of action, but simply to report a new bioactivity of capsaicin, an interesting natural product with significant dietary and pharmacological relevance.

Competing Interests: No competing interests were disclosed.

Referee Report 30 January 2015

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Carsten Gründemann

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In the current manuscript, Rongxia Liu *et al* investigated the influence of the plant-derived natural product capsaicin to overcome restenosis by using a vascular muscle cell model (VSCM) *in vitro*.

The manuscript is well written and conclusion is precise. The experiments are straightforward and the presented cell-based model is adequate to investigate such question. Nevertheless, before indexation the comments below have to be improved in a revised version of the manuscript.

Major Comment:

Main text: The authors mentioned that paclitaxel and rapamycin are used as standard drugs to inhibit VSCM proliferation. For pharmacological testing, it is essential that the authors should compare the investigated capsaicin to a standard therapy (positive control) to get an idea about capsaicin's bio-activity.

Minor Comment:

Abstract: The authors have to depict more clearly why it is important to investigate new natural-derived compounds to overcome stenosis.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 28 Apr 2015

Atanas Atanasov, Department of Pharmacognosy, University of Vienna, Austria

Thank you very much for taking the time to review our manuscript.

While reporting a new bioactivity of the interesting natural product capsaicin, the purpose of our short note is not to claim superiority over existing VSMC inhibitors. Based on the literature, as well as on our own data, both suggested reference compounds, paclitaxel and rapamycin, are more potent than capsaicin. Nevertheless, the identification of new effective molecules (e.g., capsaicin) could be of potential interest, since it might serve as a starting point for the synthesis of more potent derivatives with superior bioactivity profiles (e.g., reduced adverse effects *in vivo*) in the future.

Competing Interests: No competing interests were disclosed.