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RESEARCH LETTER

Glucocorticoids Do Not Affect the Vascular Component of TRP-Mediated Neurogenic Skin Inflammation

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Introduction

To support the clinical development of novel therapeutics in the context of inflammatory skin diseases, including atopic dermatitis or psoriasis, human in vivo skin inflammation models have been developed. For example, topical imiquimod application and intradermal lipopolysaccharide injection both induce local skin inflammation, manifesting as local skin heating, dermal vasodilation, and erythema. At a molecular level, this inflammatory reaction is characterized by a rapid cellular infiltration of, among others, neutrophils and monocytes, and the release of pro-inflammatory cytokines, including interleukin (IL)-6, IL-8, IL-10, and tumour necrosis factor α (TNFα). These local skin inflammation models have been validated by topical or systemic administration of glucocorticoids, which indeed attenuate the dermal vasodilation via inhibition of cytokine-induced nitric oxide synthase (iNOS) and a decreased monocyte infiltration.[1,2]

A very particular type of skin inflammation is neurogenic inflammation in which the inflammatory response is mediated by an interaction between sensory neurons, neuropeptides, cytokines, and immune cells. Key players in neurogenic inflammation are Transient Receptor Potential (TRP) channels, in particular TRP Vanilloid 1 (TRPV1) and TRP Ankyrin 1 (TRPA1). Both are Ca^{2+}-permeable ion channels, expressed on peripheral nerve endings, which detect harmful stimuli such as noxious heat (TRPV1) or chemical irritants, including capsaicin (TRPV1) and cinnamaldehyde (TRPA1). Upon activation of either TRP channel, neurogenic inflammation is initiated, resulting in vasodilation and erythema.[3] The current trial assessed whether the systemic administration of glucocorticoids also affects dermal vasodilation in the context of neurogenic skin inflammation as it does in the context of inflammatory skin diseases.

Materials and Methods

Approval from the Ethics Committee "Ethische Commissie Onderzoek UZ/KU Leuven" (S57829) and the Federal Agency for Medicines and Health Products (FAMHP [EudraCT 2014-004736-19]) was obtained. The study was conducted in accordance with the latest version (October 2013) of the Declaration of Helsinki and international guidelines on Good Clinical Practice (ICH/GCP, E6R2). Participants, all healthy male volunteers aged 18–45 years, gave written informed consent. During the screening visit, general eligibility was checked, and the pre-drug baseline dermal blood flow (DBF) response upon topical cinnamaldehyde and capsaicin application on the volar surface of the forearm was assessed. The main exclusion criteria were any skin abnormalities on the forearm, a history of gastrointestinal ulceration, smoking, daily intake of vasoactive drugs, or showing <100% increase from baseline in DBF to cinnamaldehyde or capsaicin application (ie, non-responders). The study period followed within 28 days after screening. Dexamethasone 10mg (2.5 tablets of Dexamethasone 4mg Jenapharm®, mibe GmbH Arzneimittel, Germany) was orally administered with 240 mL water. The DBF response was assessed at 18 ± 1 hours post-dose of dexamethasone intake. At least 14 days after the study period, a post-study visit to assess any adverse events concluded the trial. No vaccination was allowed during the entire trial.

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At least 24 hours prior to the vascular assessments, subjects refrained from any alcoholic or caffeinated beverages. In addition, subjects were in a fasting state for 3 hours prior to DBF measurements. The DBF was quantified using Laser Speckle Contrast Imaging (LSCI, PeriCam PSI, Perimed, Järfälla, Sweden) after an acclimatization period of at least 30 minutes in a temperature-controlled room (23 ± 1°C). After baseline DBF measurements, ie, pre-challenge baseline DBF, two 20 µL droplets of cinnamaldehyde 10% (v:v) and capsaicin 1000 µg were applied in two nitrile O-shaped quad rings (7.66 mm inner diameter, Polymax Ltd, Bordon, United Kingdom) proximally on the right and left forearms, respectively. In a distal ring, vehicle for cinnamaldehyde (96% ethanol, Tween-20, and purified water in a 3:3:1 ratio) and capsaicin (96% ethanol, Tween-20, and purified water in a 3:3:4 ratio) was applied. Every 10 minutes during 1 hour post-application, the DBF response was captured in an area of 3 × 3 cm² at the location of each of the rings. The DBF is expressed in arbitrary perfusion units (PUs), averaged within the area of challenge agent application within the ring (DBF_{ring}). In addition, also the flare area ($Flare_{area}$), ie, the total area in which the DBF exceeds the mean + 1 SD DBF at pre-challenge baseline, was calculated. Differences in DBF, at single points in time or using area under the curve ($AUC_{0\text{-}60min}$) calculations, were evaluated via linear mixed models in SAS® OnDemand for Academics (Fixed effect: dexamethasone treatment; random effect: subject; repeated statement [at single points in time]: time point). The significance level was set at 0.05.

Results

A total of 13 participants were recruited of which 12 were included in the trial. One subject was excluded due to a positive cotinine test. All included participants completed the trial as per protocol. Median ± IQR for age was 29 ± 8 years, and mean ± standard error of the mean (SEM) for the body mass index (BMI) was 23.2 ± 2.3 kg/m². Mean ± SEM for systolic blood pressure, diastolic blood pressure, and heart rate during the screening visit was 131 ± 8 mmHg, 76 ± 9 mmHg, and 63 ± 10 bpm, respectively. Vital signs 18 ± 1 hours after dexamethasone intake, prior to the vascular assessments, were not significantly different from measurements during the screening visit; 132 ± 6 mmHg ($p = 0.70$), 73 ± 6 mmHg ($p = 0.09$), and 75 ± 15 bpm ($p = 0.06$), respectively.

All DBF data are expressed as mean ± SEM. At screening, all participants displayed a robust DBF increase upon cinnamaldehyde as well as capsaicin application with a mean maximal change from pre-drug baseline (DBF_{ring}) of 95 ± 8 PUs (T_{20min}) and 83 ± 8 PUs (T_{40min}), respectively. At 18 ± 1 hours post-dose, dexamethasone significantly increased the pre-challenge baseline DBF on both forearms compared to the screening visit (averaged baseline DBF_{ring} 52 ± 3 versus 35 ± 1 PUs, respectively, $p < 0.001$). After correction for the difference in baseline, the maximal DBF_{ring} change from baseline to topical cinnamaldehyde application was unaffected by dexamethasone (95 ± 8 at screening versus 90 ± 7 PUs after dexamethasone, $p = 0.38$, Figure 1A). Similarly, also the maximal $Flare_{area}$ (T_{20min}) was comparable at pre-drug baseline versus after dexamethasone intake (483 ± 66 versus 474 ± 65 mm², respectively, $p = 0.84$, Figure 1A, B). The maximal DBF_{ring} increase upon capsaicin application was reduced, yet with no statistical significance (83 ± 8 PUs at screening versus 73 ± 8 PUs after dexamethasone, $p = 0.26$, Figure 1C). Similarly, the maximal $Flare_{area}$ (T_{30min}) was also slightly, yet not significantly lower after dexamethasone intake (532 ± 65 at screening versus 461 ± 64 mm² after dexamethasone, $p = 0.28$, Figure 1C, D).

Discussion

Neurogenic skin inflammation is a strictly regulated process that contributes to the protection of the body from noxious stimuli from the external environment. In particular, the vascular component is primarily the result of a close interaction between sensory neurons, neuropeptides and vascular smooth muscle cells, while the contribution of immune cells and cytokines is less obvious. Since glucocorticoids exert a strong anti-inflammatory effect by inhibiting cytokine release, proinflammatory prostaglandin formation, leukocyte infiltration, and mast cell degranulation, we assessed the effect of oral dexamethasone intake on capsaicin- and cinnamaldehyde-induced neurogenic inflammation.[4] Yet, dexamethasone did not affect the neurogenic-induced vasodilation upon TRPA1 or TRPV1 activation in the skin of healthy male volunteers.

Regarding the TRPV1-mediated vascular response to capsaicin, our results do not align with a previous report by Tafler R. et al (1993), who observed a small reduction in the capsaicin-induced vascular response after topical application of 3 g prednicarbate cream.[5] Yet, although statistically significant, the factual inhibition was low ($DBF_{ring} \approx 13\%$, $Flare_{area} \approx 18\%$). Thus, systemic treatment, even if highly dosed, might have failed to provide sufficient local levels of

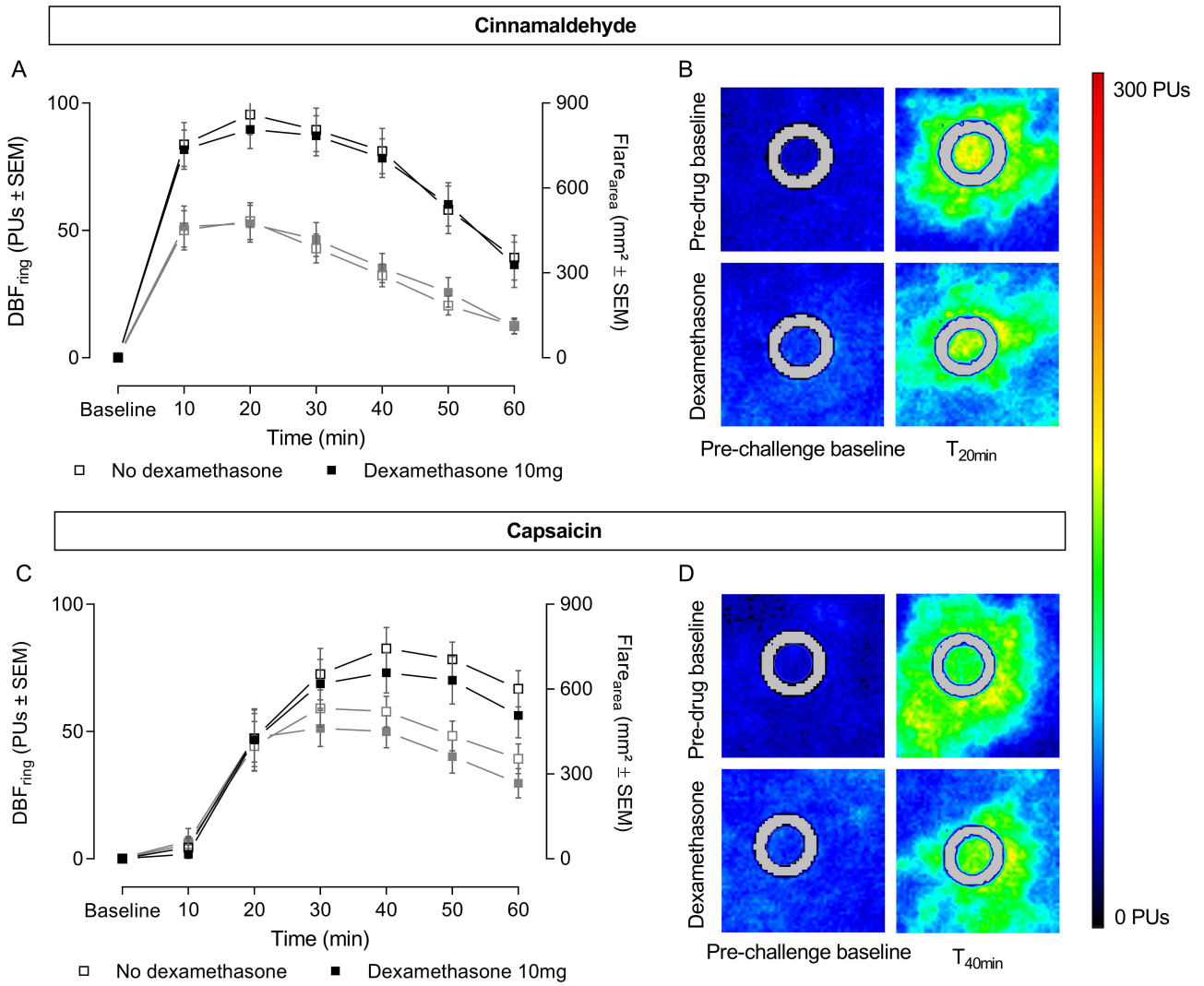

Figure 1 Vascular response upon topical cinnamaldehyde 10% (**A** and **B**) and capsaicin 1000µg/20µL (**C** and **D**) application on the forearm in 12 healthy male subjects. The vascular response is expressed over time (mean ± SEM) as dermal blood flow within the area of application (DBF_{ring}, perfusion units[PUs]) ((**A** and **C**) black) or $Flare_{area}$ (mm^2) ((**A** and **C**) grey) before, ie at pre-drug baseline, (blank) and 18 ± 1 hours after oral intake of dexamethasone 10mg (full). Statistical analysis using Linear mixed models (fixed effect: dexamethasone treatment; random effect: subject; repeated statement: time point), significance level set at 0.05. Representative images of the vascular response at pre-challenge baseline and respectively 20 or 40 minutes after cinnamaldehyde 10% (**B**) and capsaicin 1000µg/20µL (**D**) application.

dexamethasone to result in such subtle effects, especially since a small trend towards inhibition could be observed in this trial as well (DBF_{ring} ≈ 12%, $Flare_{area}$ ≈ 13%). Our results rather confirm that the capsaicin-induced vascular response is specifically mediated via neuropeptides including Calcitonin-Gene Related Peptide (CGRP) and substance P, with, if any, a minimal iNOS-mediated component.[3]

As with many aspects of TRPA1, however, the physiology upon its activation via topical cinnamaldehyde application and the resulting vasodilation is less straightforward. Cyclooxygenase-1 (COX-1) derived prostaglandins appeared crucial mediators, yet also CGRP and Substance P seemed to be involved, albeit to a lesser extent.[6] Since glucocorticoids inhibit cytokine-induced iNOS and reduce dermal mast cell degradation, our results exclude the involvement of either mechanism upon TRPA1 activation by cinnamaldehyde.[4,7] Whether endothelial (eNOS) or neuronal NOS (nNOS), both constitutively expressed and regulated via intracellular Ca^{2+} levels, are involved in the vasodilatory reaction downstream TRPA1 activation cannot be confirmed.[7] On the other hand, the fact that dexamethasone did not affect the cinnamaldehyde-induced vascular response adds to the evidence that COX-1 is not inhibited by glucocorticoids.[4,7]

Importantly, we assessed the effect of a single glucocorticoid administration, whereas prolonged treatment might affect the inflammatory response.[7] Besides, whether or not TRPV1- or TRPA1-mediated neurogenic skin inflammation also involves cytokine release and cellular infiltration in the first place, which might in turn be affected by glucocorticoids, cannot be confirmed as only the vascular response was evaluated.

Conclusion

In conclusion, oral dexamethasone intake did not affect the neurogenic vasodilation upon TRPA1 or TRPV1 activation in the skin. Although the resulting erythema is presumed to be part of a neurogenic inflammatory response, the vascular component is rather specific with limited involvement of inflammatory cytokines or cells.

Abbreviations

IL, Interleukin; TNF, tumour necrosis factor; NOS, nitric oxide synthase; TRP, Transient Receptor Potential; TRPV, Transient Receptor Potential Vanilloid; TRPA, Transient Receptor Potential Ankyrin; DBF, dermal blood flow; LSCI, laser speckle contrast imaging; PU, perfusion unit; AUC, area under the curve; SD, standard error; SEM, standard error of the mean; COX, cyclooxygenase.

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Disclosure

The authors report no conflicts of interest in this work.

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