

## Eicosanoid and Cytokine Levels in Acute Skin Irritation in Response to Tape Stripping and Capsaicin

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**We assessed the effects of physical and chemical irritants on a profile of acute inflammatory mediators in normal human skin. Skin damage in both cases is accompanied by a flux of inflammatory processes and repair mechanisms, which remain imprecisely understood. We used 10 sequential cellophane strips or topical application of 0.075% capsaicin as skin irritants and characterized the subsequent production and/or release of inflammatory mediators in suction blister fluids from human skin *in vivo*. In tape stripped skin, levels of prostaglandin E<sub>2</sub> and interleukin-1 $\alpha$  were increased 3.4-fold and 3.3-fold, respectively ( $p < 0.0001$ ;  $p < 0.02$ ), levels of tumour necrosis factor- $\alpha$  were decreased 3.0-fold ( $p < 0.01$ ), whereas levels of interleukin-6 and leukotriene B<sub>4</sub> in blister fluids remained relatively unchanged. For the capsaicin-treated skin, levels of mediators showed only minor differences when compared with matched controls. However, a correlation was observed between levels of prostaglandin E<sub>2</sub> and interleukin-1 $\alpha$  in capsaicin pre-treated blister fluids ( $r = 0.58$ ,  $p < 0.01$ ,  $n = 19$ ). These data are consistent with prostaglandin E<sub>2</sub> and interleukin-1 $\alpha$  playing key roles in acute skin responses to mild irritants. Key words: eicosanoids; cytokines; irritant stimuli; blister fluids.**

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Aberrant activity of eicosanoids and cytokines has been shown to be pivotal in the pathogenesis of a range of inflammatory dermatoses (1). However, the regulation of the acute inflammatory responses in normal human skin to mild stimuli remains poorly characterized. Superficial tape stripping of the skin (using 10 sequential cellophane strips) is a model of acute cutaneous barrier perturbation and inflammation, used widely on human and mouse skin (2–4). The tape strip procedure causes a small erythematous patch, which, during suction blister development, is subject to a mild stinging sensation. Topical application of the neuropharmacological agent capsaicin (an extract of chilli peppers) is widely used to treat pruritus, pain and some inflammatory dermatoses (5–7). Capsaicin gives rise to the classical cutaneous triple response reaction (itch/pain, flare and wheal) associated with C-fibre specific neurogenic inflammation (8). However, to date the non-neurogenic components that contribute to the capsaicin irritation have not been assessed.

In the present study we measured levels of 5 pro-inflammatory mediators, interleukins 1 $\alpha$  and 6 (Il-1 $\alpha$  and Il-6), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) in blister fluids following pre-treatment of forearm skin with capsaicin or by tape

stripping. This gives an indication of the factors that may be related to skin inflammatory responses *in vivo*, either by propagation or maintenance of responses to mild stimuli. Using these models of acute inflammation our observations suggest that PGE<sub>2</sub> and Il-1 $\alpha$  play key roles in skin responses to mild irritants.

### MATERIAL AND METHODS

#### *Skin perturbation*

For physical perturbation of the skin, the area of skin to be blistered was tape-stripped with cellophane sequentially 10 times. For chemical perturbation a working stock of 0.075% capsaicin (synthetic trans-8-methyl-N-vanillyl-6-nonenamide; 97% pure; Pflatz and Bauer, USA), solution was prepared in a vehicle of 80% ethanol/20% H<sub>2</sub>O. A 125  $\mu$ l aliquot was applied to and dried onto a 2.5 cm diameter Whatmann No. 1 filter disk (evaporation at room temperature). The dried filter disc was applied to the volar forearm skin with 125  $\mu$ l H<sub>2</sub>O, under occlusion, for 5 min prior to blistering of the skin at the concentration of capsaicin used in various medical preparations. However, penetration may be altered due to differences in vehicle and mode of application.

All subjects were pre-screened for their sensory and erythematous responses to topical capsaicin prior to blistering. Over a 15-min period, subjective sensory responses were recorded on a visual analogue scale and erythematous responses were measured using a Diastron Erythema Meter. Sensory data were expressed as the maximum intensity of the reaction as recorded on the Cutaneous Empirically Labelled Magnitude Scale (9) and as area under the curve for the time course of the response. Data from the Diastron Erythema Meter were recorded as the percentage increase in erythema due to capsaicin when corrected for the control site after 10 min and 15 min intervals.

#### *Skin sampling*

A total of 48 volunteers, who satisfied the inclusion and exclusion criteria and who gave witnessed, written informed consent for the procedures involved, were recruited into the study. All procedures were carried out in accordance with the Declaration of Helsinki (1964) and subsequent amendments. Only subjects with normal, healthy skin were blistered. Anyone indicating a medical history of skin disease (psoriasis, eczema, lupus, etc.) or skin disorders (keloid scar formation, chloasma, etc.) was excluded from the trial. Panellists recruited to the capsaicin trial were pre-screened for their response to topical capsaicin. The blister cup of the Dermovac Unit was placed on the volar forearm of the panellist and a negative pressure of approximately 250 mmHg applied for up to 2 h (10). Blistering was carried out in a temperature- and humidity-controlled environment (27°C and 40% humidity). In this manner blisters of 6 mm diameter were raised. Blister fluid was removed and assayed by immunoassay for eicosanoids and cytokines.

For the skin chamber technique, sequential tape-stripping, for a maximal 10 strips, was carried out on a site on the volar forearm adjacent to the blister site (11). Following the tape stripping a double adhesive (3 M Double-Stick Disc No. 2181) was placed over the lesion and this was used to hold a small (2.5 cm diameter) Perspex chamber in place. A volume of 500  $\mu$ l of sterile saline was placed into the chamber to collect the skin exudates. Both the blister and skin chamber techni-

ques are relatively non-invasive and innocuous. They can be carried out without local anaesthetic, cause little or no pain or discomfort, leave no scarring, and are relatively simple to perform.

#### Immunoassay

PGE<sub>2</sub> and LTB<sub>4</sub> were assayed by enzyme immunoassay (Amersham kits RPN 222 and RPN 223, respectively). Typically, aliquots of 10–30 µl blister fluid were used. In both cases it is an indirect assay based upon competition of antigen with a peroxidase-labelled antigen for a limited quantity of a specific antibody. Cytokine levels were measured by enzyme-linked immunosorbent assay of IL-1α, IL-6, TNF-α (British Biotechnology Quantikine Assay). Due to the low levels of cytokine in normal skin it is difficult to detect these cytokines using standard kits, thus high-sensitivity kits were used where possible. Protein concentration in the blister fluids was determined using the Bio-Rad microtitre plate assay. A dilution series of blister fluids was analysed to ensure linearity of response between protein and dye components of the assay. All mediators were expressed as pg analyte/mg protein. Variations in mediator levels due to perturbation of the skin were expressed relative to the level of mediator detected in matched untreated blister fluids. The mean and standard deviation are given for each subset of data, and the maximal and average effect on eicosanoid and cytokine levels observed for each type of acute response when compared with matched controls.

#### Statistics

Descriptive statistics (mean, standard deviation, median, etc.) were carried out using SigmaStat software package. The Mann-Whitney rank sum test was used to calculate significant difference between 2 groups. The Spearman rank order correlation was used to correlate levels of mediators in untreated, tape strip or capsaicin pre-treated blister fluids and to correlate mediator levels with sensory and erythematous responses to capsaicin.

## RESULTS

#### Effect of tape stripping

Tape stripping of the volar forearm prior to blistering was associated with a stinging response, which caused very little discomfort and usually diminished with time. An erythematous reaction was also observed. In the analysis of mediators in blister fluids from untreated and tape-stripped skin, PGE<sub>2</sub> was increased on average 3.4-fold ( $p < 0.0001$ ), from 193 to 655 pg PGE<sub>2</sub>/mg protein (Table I). The maximal increase observed in any donor fluid was a 25-fold increase above baseline. IL-1α also increased on average 3.3-fold ( $p < 0.02$ ), from 3.73 to 12.33 pg IL-1α/mg protein (Table I). Again the maximal increase was in the order of 20-fold, similar to the case for PGE<sub>2</sub>. Levels of TNF-α were decreased on average 3.0-fold ( $p < 0.01$ ), from 3.0 to

0.91 pg/mg protein. LTB<sub>4</sub> and IL-6 showed no significant change in levels detected in blister fluids (Table I).

#### Levels of mediators in skin chamber fluids

In skin chamber fluids the levels of PGE<sub>2</sub>, LTB<sub>4</sub>, IL-6 and TNF-α could not be measured by immunoassay due to dilution of the small amount of inflammatory exudate in a large volume (500 µl) of phosphate buffered saline. Taking 2 tape strips sequentially we observed a graded increase in IL-1α measured in skin chamber fluids, with a plateau effect observed at 10 tape strips (approximately 60 pg/ml saline). It also appears that the level of IL-1α is dependent on sampling time, as there was a gradual increase in levels of IL-1α when the saline was incubated in the skin chamber from 2 min to 15 min.

#### Effect of capsaicin

Capsaicin (0.075%) pre-treatment of skin caused a burn and erythema response in all panellists following application of the vacuum. This response occurs within 25 min of application of negative pressure. The burn persisted throughout the session, with minor variation in intensity of burn, possibly due to adaptation of sensory perception (12). The erythema also persists, apparently with little or no change during the session, although it was not possible to measure using an erythema meter due to the bulky presence of the blister cup. When the vacuum was removed the burning stopped immediately and the erythema began to fade over the following few minutes. The mechanisms of the "sensitization" of the burn receptors and the prolonged erythematous response under these conditions are unknown. A similar effect has been reported previously and is referred to as "shower shock", a condition in which the burn response is reactivated several hours after application of capsaicin in response to stimuli such as hot water or physical exercise (13).

Analysis of mediators in untreated and capsaicin pre-treated blister fluids showed on average only minor variations in levels (Table II). A significant correlation was observed between the levels of PGE<sub>2</sub> and IL-1α in capsaicin pre-treated blister fluids ( $r = 0.58$ ,  $p < 0.01$ ,  $n = 19$ , Fig. 1).

A correlation was observed between ΔPGE<sub>2</sub> levels (PGE<sub>2</sub> in capsaicin-treated blister fluids – levels in untreated site) and observed erythematous responses to capsaicin at the 10 min time interval ( $r = -0.548$ ,  $p = 0.018$ ,  $n = 18$ ). In the case of IL-1α a correlation was observed between levels in untreated blister fluids and sensory responses, expressed as area under the curve ( $r = 0.508$ ,  $p = 0.026$ ,  $n = 19$ ).

Table I. Effects of tape strip pre-treatment on levels of mediators in blister fluids. Data are expressed as mean values ± SD of mediator levels measured in blister fluids of 28 volunteers

Factor	Normal (pg/mg protein)	Tape strip (pg/mg protein)	Effects of tape strip pre-treatment	
			Maximum	Average
Interleukin-1α	3.73 ± 2.67	12.33 ± 21.6	18*	3.3*
Interleukin-6	0.56 ± 0.72	0.73 ± 0.97	8.2	ns
Tumour necrosis factor-α	3.0 ± 3.2	0.91 ± 0.78	(-5.5)*	(-3.0)**
Prostaglandin E <sub>2</sub>	193 ± 165	655 ± 454	25*	3.4**
Leukotriene B <sub>4</sub>	2.87 ± 1.42	1.86 ± 0.32	(-3.0)	ns

\*  $p < 0.05$ ; \*\*  $p < 0.01$ . ns, no significant change from matched control.

Table II. Effects of capsaicin pre-treatment on levels of mediators in blister fluids. Data are expressed as mean values  $\pm$  SD of mediator levels measured in blister fluids of 20 volunteers

Factor	Normal (pg/mg protein)	Capsaicin (pg/mg protein)	Effects of capsaicin pre-treatment	
			Maximum	Average
Interleukin-1 $\alpha$	2.35 $\pm$ 2.7	1.74 $\pm$ 1.71	4.5/(-3.5)	ns
Tumour necrosis factor- $\alpha$	0.83 $\pm$ 0.81	0.58 $\pm$ 0.45	(-4.5)	ns
Prostaglandin E <sub>2</sub>	207 $\pm$ 200	179 $\pm$ 161	6.0/(-6.0)	ns
Leukotriene B <sub>4</sub>	2.98 $\pm$ 1.58	2.18 $\pm$ 0.81	(-5.3)	ns

ns, no significant change from matched control.

## DISCUSSION

In some subjects high levels of PGE<sub>2</sub> were found in tape strip blisters and one might expect a strong inflammatory response to be associated with such levels. However, concentrations of up to 40-fold greater than this ( $\sim$ 1  $\mu$ g/ml) are required to elicit a response by intradermal injection of PGE<sub>2</sub>, so this level of activity clearly does not exceed that to which the skin can adapt and regulate (14). Levels of LTB<sub>4</sub> were slightly, but not significantly, decreased. This could be due to preferential utilization of arachidonic acid via the cyclo-oxygenase pathway, leading to decreased substrate availability for the lipoxygenase pathway, or a decrease in lipoxygenase activity.

A similar increase for Il-1 $\alpha$  in tape strip blister fluids may be the result of release of the Il-1 $\alpha$  stored in the epidermis or alternatively may represent a newly synthesized source of the mediator. However, it is difficult to state unambiguously which case applies as Il-1 $\alpha$  is sequestered in the epidermis at high levels (an estimated 300–900-fold higher than in other organs), thus one cannot exclude the possibility that leakage of Il-1 $\alpha$  contributes to levels measured in the suction blister fluids (15).

Levels of TNF- $\alpha$  were decreased in tape strip blister fluids. It has been reported previously that tape stripping of hairless mouse skin causes a time-dependent increase in TNF- $\alpha$  immunohistochemical detection and mRNA levels (4, 16). Although this is in contrast to our results, differences due to species, sampling method, immunoassay vs. immunostaining techniques,

and level of perturbation may account for this discrepancy. It is conceivable that the level of skin perturbation caused by the blister device may account for TNF- $\alpha$  being utilized continuously during our sampling thus yielding a decrease in observed levels. Levels of Il-6 in blister fluids remained relatively unchanged. In general, we observed only a slight increase in mediator levels of Il-6.

The inflammatory mediators in skin chamber fluids are present at very low concentrations due in part to the dilution of the sample with sterile saline. Thus without pooling and extraction it was not possible to detect Il-6, TNF- $\alpha$ , PGE<sub>2</sub> or LTB<sub>4</sub> by this method. Il-1 $\alpha$ , however, was readily detected in skin chamber fluids without pooling or extraction of the samples being required. Taking 2 tape strips sequentially we observed a graded increase in Il-1 $\alpha$  measured in skin chamber fluids, with a plateau effect observed at 10 tape strips (approximately 60 pg/ml saline). It also appears that the level of Il-1 $\alpha$  is dependent on sampling time, as there was a gradual increase in levels of Il-1 $\alpha$  when the saline was incubated in the skin chamber for 2–15 min. The very high concentrations of Il-1 $\alpha$  found in chamber fluids and the lack of significant sample-to-sample variation suggest that the release is non-specific and that bio-availability and/or bioactivity are not major contributors to the acute phase irritant response.

Capsaicin is a potent irritant, which has been observed to cause release of neuropeptides from sensory nerve fibres in skin, with eventual desensitization of the treated area (17). It is unlikely that the low level of capsaicin that we used caused a complete depletion of nerve fibres and their contents. However the negative pressure of the vacuum applied to the skin prolonged the hyperalgesia and burn sensation during the blister procedure, such that the nerve fibre network was mostly abolished, as determined by lack of confocal imaging using the pan-neuronal marker, PGP 9.5 (18). The hyperalgesia and prolonged erythematous response are likely to be due to the release of secondary mediators, as it apparently takes several hours to replace neuropeptide levels in skin following capsaicin depletion (19).

Only the levels of PGE<sub>2</sub>/Il-1 $\alpha$  were found to vary in capsaicin pre-treated fluids, ranging from a 6.0-fold increase to a 6.0-fold decrease for PGE<sub>2</sub>, and a 4.5-fold increase to a 3.5-fold decrease for Il-1 $\alpha$ . A significant correlation was observed between levels of PGE<sub>2</sub> and Il-1 $\alpha$  in capsaicin pre-treated blister fluids ( $r=0.58$ ,  $p<0.01$ ,  $n=19$ ; Fig. 1), an observation also made in the case of physical perturbation of the skin, in which case both were increased by similar levels (although this was not statistically significant). Subgroup analysis of levels of both mediators showed a varied response in inverse proportion to the concentration of mediator observed in the matched no treatment site (those with a high PGE<sub>2</sub>/Il-1 $\alpha$  in the matched

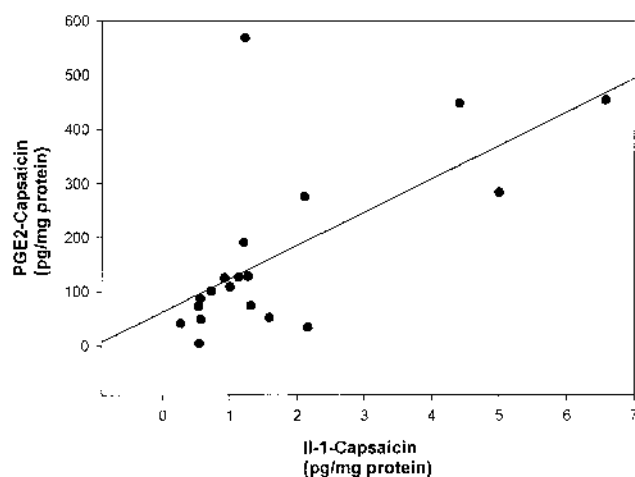


Fig. 1. The correlation between PGE<sub>2</sub> and Il-1 $\alpha$  in capsaicin-treated blister fluids. Using the Spearman rank order correlation fit, a positive correlation was observed between the levels of PGE<sub>2</sub> and Il-1 $\alpha$  measured in blister fluids pre-treated with capsaicin ( $r=0.58$ ,  $p<0.01$ ,  $n=19$ ).

no treatment site were found to decrease and vice versa, although there was no statistical significance following subgroup analysis) and thus a trend converging on a mean value was observed. These trends could not be explained in terms of age, gender or skin type. Thus, as in the case of tape strip perturbed skin, it is apparent that PGE<sub>2</sub> and IL-1 $\alpha$  are under similar regulatory control during both physically and chemically induced acute phase inflammatory responses.

Levels of TNF- $\alpha$  and LTB<sub>4</sub> both showed a decrease in capsaicin-treated blister fluids. Levels were decreased to a similar level for that observed in the case of tape stripping. Similar to the case of tape stripping, it is possible that skin perturbation caused by the continuous burn and erythematous response may account for the continual utilization of TNF- $\alpha$  and the concomitant decrease in blister fluids. As PGE<sub>2</sub> levels are not elevated to the extent observed following tape strip analysis, it is unlikely that the decreased LTB<sub>4</sub> is due to decreased substrate activity. Alternatively, it may reflect a decrease in lipoxygenase activity.

A correlation was observed between  $\Delta$ PGE<sub>2</sub> levels (PGE<sub>2</sub> in capsaicin-treated blister fluids – levels in untreated site) and observed erythematous responses to capsaicin at the 10 min time interval ( $r = -0.548$ ,  $p = 0.018$ ,  $n = 18$ ). This is not surprising as the role of PGE<sub>2</sub> in vasodilation and erythema has been well documented (20, 21). Intradermal injection of PGE<sub>2</sub> has been shown to cause local vasodilation and inhibition of PGE<sub>2</sub> synthesis has been shown to reduce vasodilation and UVB erythematous sensitivity in human skin (22, 23). In the case of IL-1 $\alpha$  a correlation was observed between levels in untreated blister fluids and sensory responses, expressed as area under the curve ( $r = 0.508$ ,  $p = 0.026$ ,  $n = 19$ ). The significance of this correlation is unknown, but it suggests that cytokines are important in the regulation of neural processing in skin.

The skin is equipped with a range of interactive and interdependent defence mechanisms for dealing with continuous exposure to irritant stimuli and associated acute inflammatory reactions. Although the response profile of inflammatory mediators in skin due to physical and chemical irritation is different, in these 2 specific cases, we have shown that differential regulation of the key mediators (PGE<sub>2</sub> and IL-1 $\alpha$ ) is associated with both types of acute inflammatory reaction. The mechanistic pathways giving rise to these different response profiles are not yet well understood, but it is possible that the tape strip-associated acute inflammatory response is regulated by release of keratinocyte cell-signalling molecules, whereas the capsaicin response is regulated by neuropeptide molecules, such as substance P and calcitonin gene-related peptide.

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