To the Editor,

Ultraviolet radiation (UVR) induces acute skin inflammation, characterized clinically by erythema, histologically by dermal leukocyte infiltration and biochemically by upregulation of pro-inflammatory eicosanoids (1). Metabolism of arachidonic acid (AA) released from membrane lipids enhances eicosanoid production, with notable elevation of prostaglandin (PG)E₂ and 12-hydroxyeicosatetraenoic acid (12-HETE), major metabolites of cyclooxygenase (COX) and 12-lipoxygenase (12-LOX) pathways, respectively. At low concentrations, PGE₂ has roles in skin homeostasis, while UVR-upregulated PGE₂ has a significant vasodilatory effect in the erythemal response (2). A 12-fold increase in 12-HETE occurs in UVR-inflamed skin (1), and this monohydroxy fatty acid has potent leucocyte chemoattractant properties ex vivo (3,4) and in animal models (5). Its role as a chemoattractant for neutrophils and lymphocytes in human skin is supported by their pronounced infiltration following intradermal injection of 12-HETE (6); 12-HETE may also increase leukocytic infiltration in lesional psoriatic skin (3,4).

Cyclooxygenase inhibitors are widely employed to treat inflammatory conditions including sunburn. Their therapeutic properties are principally attributed to reduction of PGE₂ synthesis from AA (2). However, while COX inhibition reduces PGE₂ concentration and erythema in UVR-inflamed skin (2), reduced metabolism of AA via COX may potentially result in an enhanced availability of AA for LOX metabolism (7), paradoxically increasing aspects of UVR inflammation through augmentation of chemoattractant 12-HETE (Fig. 1a). Accordingly, we have examined the impact of a COX inhibitor on PGE₂ and 12-HETE levels, and clinical and histological outcomes, in UVR-induced inflammation in human skin.

Ethical approval was granted (Greater Manchester North NREC; reference 11/NW/0567) and the study was performed in accordance with Declaration of Helsinki principles (Seoul 2008); all volunteers gave written informed consent. Overall, the study involved 13 healthy individuals (phototype II/III; 20-39 years; 8 female, 5 male). Initially, in n = 10, one-cm-diameter sites of previously photoprotected buttock skin were exposed to 3× each individual’s minimal erythema dose (MED) of UVR (TL 20W/12 lamp, Philips GmbH, Hamburg, Germany; 270–400 nm) and indomethacin gel (1%; Indomet-ratiopharm® Gel, Ratiopharm GmbH, Germany) was applied to UVR-exposed and unexposed skin for 3 h under Tegaderm™ dressing on three occasions, immediately post-UVR, after 24 and 48 h. Changes in haemoglobin (Hb) index were measured via reflectance spectrophotometry (CM-600d, Konica Minolta Sensing Europe B.V.), at indomethacin-treated and control untreated sites, in both UVR-exposed and unexposed skin at 24, 48 and 72 h post-UVR, prior to suction blister fluid sampling. Eicosanoid levels were analysed by LC-MS/MS (described in Ref. (8,9)).

Topical indomethacin significantly reduced Hb index post-UVR compared to untreated skin at 24, 48 and 72 h (all p < 0.001; Fig. 1b). Skin PGE₂ level (n = 5 for technical reasons) was almost completely abolished after indomethacin treatment in unexposed skin (mean 97% reduction; p < 0.05) and at 24 h (96% reduction, p < 0.01) and 72 h post-UVR (94% reduction, p < 0.01) (Fig. 1c). In contrast, levels of 12-HETE (n = 10) increased substantially with indomethacin application, at both 24 h (92% increase) and 72 h (74% increase) post-UVR compared to untreated skin (both p < 0.05); no significant increase was seen in unexposed skin (Fig. 1d). While an increase in 12-HETE production has been noted following COX inhibitor application in vitro (s1) including in human psoriatic epidermal extracts (s2), to our knowledge we present the first report of elevated 12-HETE levels in skin in vivo resulting from COX inhibition.

In the light of the significantly increased 12-HETE, we assessed the impact of topical indomethacin on the UVR-induced inflammatory cell infiltrate. Thus, n = 4 volunteers (including one who participated in the blister study) underwent the same protocol as above, but provided skin punch biopsies (5 mm) from UVR-exposed and unexposed skin at 24 and 72 h post-UVR, at both indomethacin-treated and untreated sites. Immunohistochemistry was performed on frozen skin sections (5 μm) using mouse primary antibodies for neutrophil elastase (Dako, Cambridgeshire,
their time-point of maximum infiltration, the difference in CD8+ T-cell infiltration between indomethacin-treated and untreated skin at 72 h was statistically significant, mean 46.2 (SEM 10.32) versus 15.04 (3.9) in indomethacin-treated vs untreated skin, respectively.

COX-2, in contrast to COX-1 (s3), is strongly upregulated in the UVR-induced inflammatory cell infiltrate. As skin COX-2, in contrast to COX-1 (s3), is strongly upregulated by UVR, these effects are anticipated to be largely due to COX-2 inhibition. Increased synthesis of LOX-produced mediators, such as 12-HETE, could potentially contribute to adverse effects of COX inhibitors, as proposed for NSAID exacerbation of psoriasis (s4) and of asthma by aspirin (s5). Studies in mice also implicate the 12-LOX pathway in pathogenesis of atherosclerosis and vascular inflammatory reactions in diabetes (s6, s7). There are potential further complexities surrounding the impact of NSAIDs, which in the sunburn response may include effect of the PGE2 reduction on immunosuppressive cytokine IL-10 levels (s8) and on reparative activities of the response. Future research could explore these, as well as examine impact on histological findings in a larger group and potentially also in polymorphic light eruption patients, where dysregulated leukocytic infiltration is reported (s9).

Our study provides human in vivo data showing significant rise in skin chemoattractant 12-HETE following application of a COX inhibitor, supporting a speculative role of the 12-LOX pathway in adverse reactions associated with therapeutic COX inhibition. We highlight that indomethacin treatment of sunburn may be limited in its scope and that changes in UVR-erythema do not, as commonly assumed, necessarily reflect histological leukocytic infiltration in this acute inflammatory response.

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**Conflict of interests**

The authors have declared no conflicting interests.

**Supporting Information**

Additional supporting data may be found in the supplementary information of this article.

**Appendix S1 References.**

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