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Cytokine expression correlates with differential sensory perception between lye and no-lye relaxers

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Synopsis

Differences in perceived sensory scalp discomfort between guanidine carbonate/calcium hydroxide (no-lye) and sodium hydroxide (lye) relaxer technologies have been reported by users for decades. However, the biochemical processes responsible for the perceived differences have not been fully studied. We have used an *in vitro* three-dimensional skin model with well-developed epidermis to explore the expression of cytokines that may partially explain the biological response resulting in differences in sensory perception. The cytokines selected were prostaglandin E_2 (PGE_2), interleukin-1α (IL-1α), and IL-1 receptor antagonist (IL-1ra) because they have been shown to participate in irritant-induced discomfort.

We show that lye relaxer induced over 350% increase in PGE_2 expression over untreated control compared to 200% by no-lye in the early phase (4 h) postexposure epidermal response. Expression of IL-1α in the early phase showed no significant difference between lye and no-lye; however, no-lye induced higher levels ($p < 0.0001$) in 24 and 48 h. Concomitantly, no-lye induced increased expression of IL-1ra compared to lye at all time points.

Given the association of PGE_2 with nociceptive activation, these findings suggest that the perceived variation in sensory discomfort reported by consumers between lye and no-lye relaxers may be associated with differences in induced PGE_2 expression.

INTRODUCTION

Hair relaxers are complex cosmetic formulations consisting of many ingredients and are designed to permanently straighten curly hair. The active ingredient in these emulsions is the hydroxide ion, which can be quantified by measuring the pH of the formulation. Lye and no-lye are the two main types of relaxers used in the United States to straighten curly hair. Lye relaxers contain sodium hydroxide as the source of hydroxide ion. No-lye relaxers contain calcium hydroxide and guanidine carbonate, which when combined provides the source of hydroxide ions. Hair relaxers are not designed to be in direct contact with the scalp, but through the process of use, contact may occur. Although measures including, but not limited to, the application of petrolatum (basing) to the scalp are

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practiced to limit contact with the scalp, complaints of discomfort still arise (1,2). It is common for cosmetic products to induce discomfort including itching, stinging, and burning sensations if used incorrectly (1). Although some of these complaints are associated with overuse or misuse, there remains a difference in consumer-perceived discomfort potential between the two most widely used relaxers in the United States.

A population-based *in vivo* study showed that majority of relaxer users perceive no-lye relaxer as less irritating than lye relaxer regardless of whether the discomfort is rated as severe, moderate, or mild (2). Results reported were based on comfort/discomfort evaluations of over a thousand salon patrons as an indication of irritation potentials of the two types of relaxers. While reported, these differences are not linked to formulations or biochemical processes through scientific experimentation. However, there are good reasons to believe that cytokines may be involved as several studies provide evidence of the role of cytokines in inducing sensory discomfort and inflammation (3–5). Acute inflammatory pain is characterized by hypernociception due to sensitization of primary sensory neurons. Furthermore, it has been shown that specific primary cytokines are released after tissue injury to act on membrane sensory receptors to trigger sensory activation. The nature and type of mediators released depend on the nature of tissue injury. Chen *et al.* (6) have demonstrated the mechanical sensitization of cutaneous C-fiber nociceptors by prostaglandin E_2 (PGE_2) in the rat, which provides the basis for studying its possible role in relaxer-induced discomfort. However, no comparative profiling of cytokines responsible for these perceived differences between lye and no-lye relaxers has been done to date.

In this investigation, we used a well-developed three-dimensional reconstructed human epidermis (EpiSkin™; Figure 1) to examine the types and quantity of cytokines associated with sensory irritation that may provide a partial explanation for the perceived differences in discomfort between lye and no-lye relaxers. The cytokines selected in this study are those suggested and confirmed by many authors (7–9) as being good predictive indicators in both short- and long-term exposure response by the epidermis.

MATERIALS AND METHODS

MATERIALS

Two separate lots of EpiSkin™ model (1.07 cm^2) supplied as a 12-well kit and all necessary accessories were purchased from SkinEthic Laboratories (Lyon, France). Upon arrival, tissue inserts were removed from agar and placed in 12-well culture plate containing 2 ml of maintenance medium. Transferred tissues were incubated at 37°C, 5% CO_2, and 95% relative humidity for a period of 24 h before using for all experiments. Consumer lye (Mizani, Chicago, IL) and no-lye (Optimum Care, New York, NY) relaxers were purchased and used according to instructions in the product insert.

PRODUCT APPLICATION

Separate EpiSkin™ tissues were topically exposed to75 µl each of medium strength lye relaxer or no-lye relaxer for exactly 15 min. Treated tissues were washed three times, each

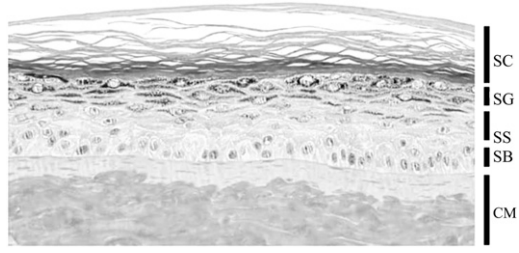

Figure 1. Histology of EpiSkin™ showing epidermal structures. A cross section of reconstructed human epidermis stained with hematoxylin and eosin. The model consists of human-derived epidermal keratinocytes that have been grown on bovine collagen coated with collagens 1 and 5. Cultures are raised in air interface to form a multilayered structure of stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and basal layer (SB).

with 25 ml phosphate-buffered saline (PBS) containing Ca^{++} and Mg^{++} and incubated at 37°C, 5% CO_2, and 95% relative humidity for a period of 4, 24, or 48 h. Cytokines were extracted from the tissues into the media by shaking at 300 rpm for 15 min, and collected aliquots were kept frozen at −20°C until assayed.

CYTOKINE ASSAY

A panel of proinflammatory cytokines {PGE_2, interleukin-1α (IL-1α)} and antiinflammatory cytokine {IL-1 receptor antagonist (IL-1ra)} in a 2-plex kit was purchased from Millipore Corporation (Billerica, MA). The ELISA kit for PGE_2 was obtained from the R&D Systems (Minneapolis, MN). The concentration of mediators was determined by using a Luminex-based multiplex assay system (Luminex Corp, Austin, TX). All assays were performed by following manufacturer's instructions.

STATISTICS

Each of two separate experiments using a skin model with different lot number was performed in triplicate, and all measurements were in duplicate. The student's *t*-test was used to determine significant difference where $p \leq 0.05$ was considered significant. Results were calculated as a difference between the normalized control (100%) and observed value at each time period and are given as mean ± SE.

RESULTS

LYE AND NO-LYE RELAXERS DIFFERENTIALLY EXPRESS PGE_2

Prostaglandins are produced as by-products of arachidonic acid metabolism and are known to induce sensory discomfort in humans and animals (4,6). To explore the possible involvement of the cytokine in the differential sensory perception of discomfort between lye and no-lye relaxers, its expression was examined. As shown in Figure 2, both relaxers induce a statistically significant higher level of PGE_2 compared to the control at each

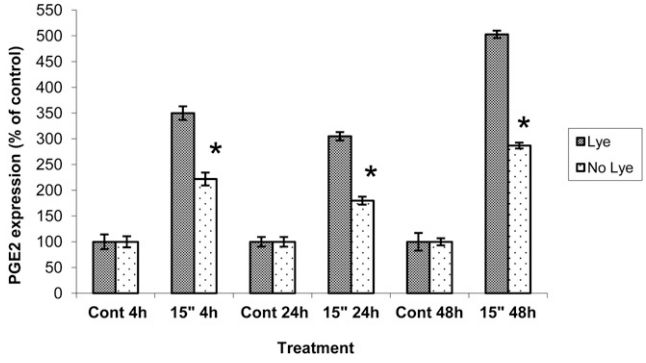

Figure 2. Hair relaxers differentially induce expression of PGE_2. Lye or no-lye relaxer was topically applied on EpiSkin™ model for exactly 15 min and washed thoroughly with PBS and incubated in fresh media as described in the section Materials and Methods. PGE_2 was extracted, and concentration was determined by ELISA. Each data point represents the mean of six tissues run in duplicate ± SE showing significant differential expression of the cytokine (*$p < 0.05$).

time point. However, the increase is statistically higher for the lye relaxer at each time period after exposure when compared with no-lye ($p < 0.001$).

IL-1α INDUCTION LEVELS ARE INFLUENCED BY RELAXER TYPE

As the main initiator of epidermal response to injury, IL-1α induction was determined for up to 48 h following application of lye and or no-lye relaxer on EpiSkin™. As with PGE_2, the expression of the cytokine was increased versus control with both relaxer types at each time interval. However, in this case, it is the no-lye relaxer that elicits a higher response that is statistically different at the latter two time points ($p < 0.01$) (Figure 3).

Expression of the cytokine after exposure to lye relaxer was 368%, 256%, and 201% over 4, 24, and 48 h, respectively, over untreated control. Comparatively, no-lye induced expression levels of 436%, 468%, and 338% under the same conditions and over the same period, respectively (Figure 3).

EXPRESSION OF IL-1ra BY RELAXERS

Excessive production of IL-1α due to injury causes significant side effects (10–13); therefore, the epidermis has developed an exquisite mechanism to counteract activities of the cytokine to maintain homeostasis and protect itself by the expression of IL-1ra (8). Figure 4A shows how the two types of relaxers affect induction of IL-1ra. No-lye relaxer was better able to cause the induction of IL-1ra than lye by over 140% in the early phase of EpiSkin response, and at 24 h postapplication, this difference has significantly increased to over fourfold. The ratio of IL-1ra to IL-1α expression has been used in other reported studies to determine the control of an inflammatory response (8). We therefore examined if the ratio of IL-1ra to IL-1α, as a result of exposure to the two relaxers correlated with differences in perceived level of discomfort. As Figure 4B shows, both relaxers have similar

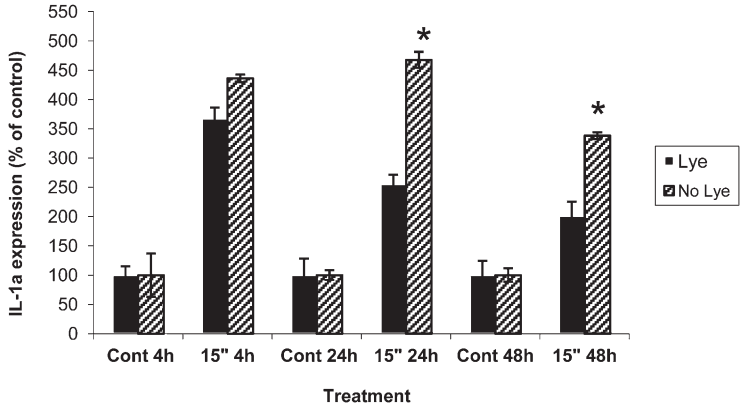

Figure 3. Post-relaxer induced expression of IL-1α by EpiSkin. IL-1α was extracted from tissue into the medium and determined by ELISA after treatment with relaxer as described. Each data point represents the mean of six tissues run in duplicate ± SE showing increased IL-1α expression by no-lye at 24 and 48 h (*$p < 0.05$).

ratios in the early and late phases of epidermal response. However, no-lye relaxer treatment results in higher ratio compared to lye at 24 h suggesting better control of the inflammatory response.

DISCUSSION AND CONCLUSION

Given the well-known biological activity of PGE_2 and other prostaglandins, we hypothesize that the increased expression resulting from exposure to lye relaxer may in part explain the perceived difference in the level of discomfort associated with the use of the relaxer. In this work, we show that in the early phase (4 h) of epidermal response, lye relaxer induced over 1.5-fold higher level in PGE_2 than no-lye relaxer (Figure 2). Our hypothesis as stated above is in agreement with other authors that showed dose-dependent induction and intensity of sensory discomfort by PGE_2, which is a by-product of arachidonic acid metabolism (14–17). These authors suggested that the level of discomfort and the time it takes to feel the sensation are time and dose dependent. It is therefore tempting to speculate that perceived differences in discomfort levels between the two relaxers may be due in part to higher level of PGE_2 induced by lye relaxer. This level probably exceeded the threshold needed to saturate and activate nociceptive receptors on peripheral nerves compared to no-lye relaxer induced level. To our knowledge, this is the first time an expression of a specific mediator has been associated with discomfort induced by hair relaxers. Our data also showed that although both types of relaxers induce expression of selected cytokines associated with irritation, kinetics and the levels of released cytokines are different, which may influence the intensity of discomfort.

IL-1α has consistently been shown by *in vitro* and *in vivo* studies to be the main cytokine produced by keratinocytes that initiates and propagates epidermal inflammatory response to irritants (5–8,18). It is able to act in an autocrine and paracrine fashion to influence production of other inflammatory cytokines from keratinocytes and other immune cells. We show in our study that both lye and no-lye relaxers induce expression of IL-1α; however, no-lye induces slightly higher level in the early phase of keratinocyte response along

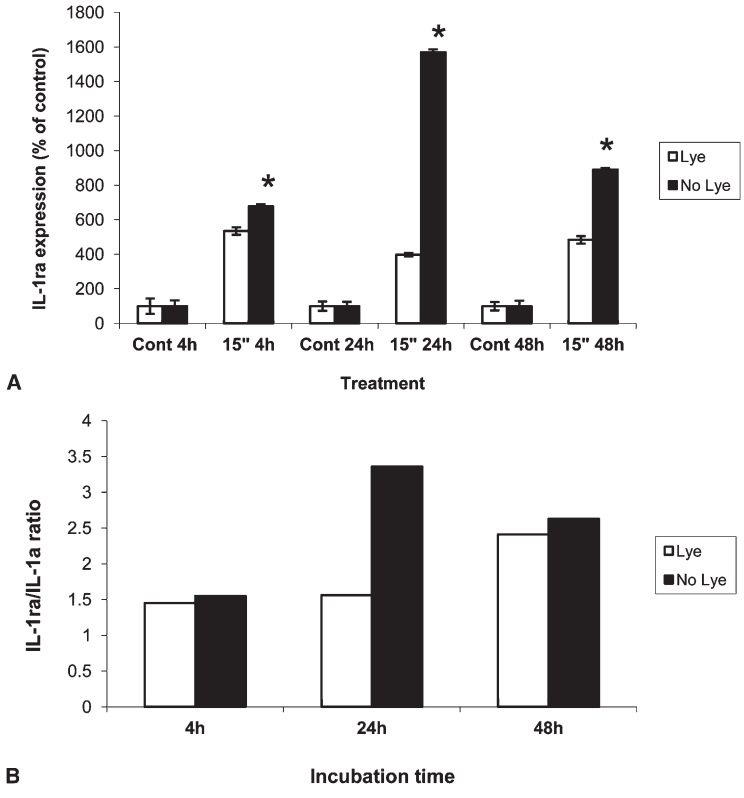

Figure 4. Hair relaxers induce expression of IL-1ra. Exposure of EpiSkin™ to no-lye relaxer induced significantly higher level of IL-1ra compared to lye (A) at all time points. Each data point represents the mean of six tissues run in duplicate ± SE (*$p < 0.05$). (B) IL-ra/IL-1 ratio was determined, which shows that no-lye is better able to control IL-1α–induced acute epidermal response.

with a significant increase of the cytokine at 24 h (Figure 3). As may be expected, no-lye relaxer with a greater ability to induce the expression of IL-1α results in higher production of IL-1ra. Both relaxer types in fact show increased levels of IL-1ra; however, no-lye relaxer elicits a statistically significant higher level at all time intervals. With this result, we show IL-1ra/IL-1α ratio is higher with no-lye than lye relaxer at 24 h (Figure 4B), suggesting it is better able to control the inflammatory process compared to the lye. However, this difference is absent at 4 and 48 h indicating that both relaxers elicit similar control at those time points. This seems contrary to the fact that the no-lye is reported to be less irritating to the scalp in the early phase. Therefore, one may conclude that the irritation experienced by the users of lye relaxer, which occurs within 20 min of contact is not directly related to the level of IL-1α induced.

Both lye and no-lye relaxers are high pH complex formulations so it is not known at this time if a specific ingredient or pH is responsible for the perceived phenomenon. A difference in pH may be suspected to influence the level of sensory response as it is known that alkaline reagents at pH 12 or higher are corrosive to mammalian tissues. Regular strength lye relaxers have an alkalinity of 0.51 meq/g compared with 0.65 meq/g for regular strength no-lye relaxers. Since no-lye relaxers have higher alkalinity but are perceived to be less irritating, the differences in sensory perception are not likely related to differences

in alkalinity. This observation suggests that other mechanisms may be involved in differences in sensory perception between the two relaxers as reported by consumers. It is likely that further studies will be required to explore the role of individual ingredients to answer the question.

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