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The Blend of Taurine and Aloe Vera Extract Boosts Action Against Skin Irritation: *In Vitro* and Clinical Evaluations

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Synopsis

Regular usage of cosmetic products and drugs in dermatological vehicles may cause irritant contact dermatitis. For example, aluminum chloride ($AlCl_3$), the most efficacious antiperspirant salt to treat hyperhidrosis, shows high irritancy potential. To mitigate the irritant contact dermatitis caused by topical application of products containing $AlCl_3$, we investigated the anti-irritating effects of aloe extract and taurine *in vitro* and *in vivo*. In an *in vitro* experiment, reconstructed human epidermis model, EpiDerm, was tested with $AlCl_3$ in the presence or absence of taurine and aloe extract. In a human clinical study, 12 adult subjects were tested with two products, a commercial $AlCl_3$ antiperspirant product and a prototype 12% $AlCl_3$ formulation containing 0.1% taurine and 0.1% aloe extract. Skin irritation potential *in vitro* and *in vivo* was measured by the release of pro-inflammatory cytokine, IL-1α, and chemokine, IL-8. Taurine and aloe extract significantly ($p < 0.05$) reduced IL-lα and IL-8 production *in vitro* and *in vivo* after topical application of formulations containing $AlCl_3$. The blend of taurine and aloe extract demonstrated boosted anti-irritation benefits on $AlCl_3$ irritated skin both *in vitro* and *in vivo*. These results suggest that the combination of these anti-irritating actives may possibly be effective in mitigating irritant contact dermatitis caused by other dermatological vehicles containing irritating agents, but further research is warranted to assess their effects.

INTRODUCTION

In the United States alone, approximately 15.2 million people are affected by dermatitis (1) and 50% of the population has sensitive skin with reduced irritation threshold (2). Application of cosmetics and drugs in dermatological vehicles can induce skin dryness and irritation of the uppermost layer of the epidermis (3). Irritation and sensitization of the stratum corneum can lead to the development of irritant contact dermatitis, which limits the usage of products containing such compounds. Irritant contact dermatitis is the inflammation of the skin which leads to erythema, dryness, itching, burning, and stinging and is caused by a physical or chemical agent that damages the stratum corneum. It is, therefore, important to identify compounds that can reduce skin irritation to develop efficacious formulations that mitigate irritant contact dermatitis in consumers. However, the biochemical mechanisms and triggers of skin irritation are complex and not fully

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understood. Current data and models have shown that irritants trigger inflammatory processes by inducing the release of pro-inflammatory cytokines and chemokines, such as IL-1α, PGE_2, IL-6, and IL-8 (4). Commonly used cosmetic products or topical prescription drugs can upregulate the expression of these pro-inflammatory cytokines. Thus, identification of compounds that inhibit the production of these cytokines is crucial in mitigating irritant contact dermatitis.

Aluminum chloride is a highly effective active ingredient used to treat hyperhidrosis, a medical condition in which a person sweats excessively and uncontrollably, but regular usage results in irritant contact dermatitis (5). Aluminum chloride is well-known to cause skin irritation because of its low pH range of 2–3 and topical application leads to the release of cytokines. Aluminum (Al^{3+}) ion also generates reactive oxygen species (ROS) and induces cytotoxic lipid peroxidation in human skin fibroblasts, which leads to cell damage (6,7). Thus, the addition of anti-irritating compounds, such as taurine and aloe extract, into formulations containing aluminum chloride has become necessary to protect against irritation and maintain skin homeostasis.

Taurine, 2-aminoethanesulfonic acid, is an amino sulfonic acid that is sometimes considered as amino acid that is synthesized in the liver from cysteine and methionine. Taurine is a β-amino acid and exists mainly as the zwitterion taurine species at biological pH values. Because of the predominance of the zwitterion species, it contributes immensely to the overall lipophilicity of taurine and allows for it to be absorbed through biological membranes (8). It serves important physiological roles, such as membrane stabilization, regulation of calcium levels, and acts as an osmoregulator and a neuromodulator in the brain (9). In the skin, taurine has been previously found to inhibit inflammation and irritation responses triggered by irritating compounds, such as sodium lauryl sulfate, and it has been added to many topical formulations in recent years (10). It is known that keratinocytes in the outermost granular layer of the stratum spinosum accumulate taurine through the taurine transporter to exert its antioxidant, anti-inflammatory, and cell-proliferating effects in the skin (11–13). Although taurine alone has been previously found to exhibit anti-irritating effects, supplementation of additional anti-irritating agents into formulations with high irritation potential will improve taurine's efficacy in mitigating irritant contact dermatitis.

Aloe extract is already widely used in many cosmetic and personal care products because of its ability to reduce irritation and promote skin repair. Aloe extract contains various natural ingredients, essential amino acids, minerals, and vitamins that are known to inhibit COX-1 activity, a major enzyme that plays an important role in the production of prostaglandins (14,15). Aloe is also known for its skin-penetrating enhancement effects and has been found to increase the skin permeation of transdermal drugs in formulations (16,17).

Our study aims to investigate the boost in anti-irritating effects that the blend of taurine and aloe extract exhibit with regard to aluminum chloride–induced skin irritation while maintaining the acidic pH necessary for high efficacy. Although both of these compounds are known to exhibit anti-irritating effects and beneficial effects on skin health, investigation into their combined effects has been limited. We hypothesize that aloe extract increases the permeation and penetration of taurine into the skin, which may explain the significant boost in anti-irritating effects observed *in vitro*. A multilayered human keratinocyte skin model, EpiDerm (MatTek Corporation, Ashland, MA), was used to assess

percutaneous absorption of taurine with or without the addition of aloe extract *in vitro*. EpiDerm human skin model was also used to measure the release of inflammatory cytokines and chemokines, such as IL-1α and IL-8, to assess for skin irritation *in vitro*. To confirm *in vitro* results, a human *in vivo* study was conducted to assess skin irritation after the topical application of prototype $AlCl_3$ products containing taurine and aloe extract.

MATERIALS AND METHODS

MATERIALS AND FORMULATION

The compounds tested in these experiments were acquired from a variety of vendors that are listed as follows: Hexaaqua 99.5% Aluminum Chloride Hexahydrate (Alfa Aesar, Ward Hill, MA), Aloe Vera Freeze Dried Powder 200× (Mexi Aloe, Campeche, Mexico), and Taurine 99% (Sigma-Aldrich, St. Louis, MO). $AlCl_3$, aloe vera extract, and taurine (w/w) solutions were prepared by dissolving compounds into phosphate-buffered saline (PBS) solution pH 7.4 (Life Technologies, Merelbeke, Belgium). Final solutions were prepared by combining 0.5 ml of $AlCl_3$ solution and 0.5 ml aloe extract and/or taurine solutions. Aqueous solutions of $AlCl_3$ for initial irritation testing had 14% (w/w) $AlCl_3$ concentration. The initial concentration of 14% $AlCl_3$ was selected to induce high levels of irritation to observe significant differences after the addition of anti-irritating actives, taurine and aloe extract. Subsequent sets of experiments had 12% $AlCl_3$ concentration because the commercial product selected for irritation testing contains 12% $AlCl_3$ as active concentration. Prototype products containing 12% $AlCl_3$ (w/w) were oil-in-water emulsion formulations.

EPIDERMIS MODEL

The EpiDerm skin model (SIT-200 skin irritation model, MatTek Corporation, Ashland, MA) is a multilayered, highly differentiated model of the human epidermis composed of normal, human epidermal keratinocytes. EpiDerm consists of organized layers analogous to *in vivo* epidermis. It contains the basal, spinous, granular, and cornified layers that are found *in vivo*. The EpiDerm tissue are cultured on cell culture inserts and shipped in a 24-well plate on agarose. The manufacturer ships PBS solution and culture media made specifically for the EpiDerm tissue. On arrival, tissue inserts were removed and placed onto 1.0 ml of culture medium for 24 h before testing.

MTT CELL CYTOTOXICITY ASSAY

Cytotoxicity was evaluated by mitochondrial metabolic activity using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) MTT-reduction assay. In this study, a modified method of MTT assay was used. MTT solution was prepared as 1.0 mg/ml in PBS prior to usage. After treating tissues with test products by following the protocol described previously, MatTek EpiDerm tissue were placed in a 24-well tissue culture plate 24 h after treatment and 500 µl of the MTT solutions were added to each well and incubated at 37°C for 2 h. The cell survivability was analyzed by measuring

the ability of the cell's mitochondria to reduce the yellow MTT to a purple formazan (crystals) end product. The reaction was terminated by the removal of the MTT solution. Addition of 1.0 ml of 0.04 M HCl in isopropanol to each well was used to dissolve the intracellular MTT formazan crystals. The contents of each well were mixed gently in an orbital shaker at room temperature for 1 h and the absorbency at 570 nm was measured by an enzyme-linked immunosorbent assay (ELISA) microplate reader (SpectraMax M5 Multi-Mode Microplate Readers; Molecular Devices, San Jose, CA). Cell viability data obtained from the MTT assay were used to normalize IL-1α and IL-8 levels.

CYTOKINE RELEASE MEASUREMENTS

To assess the irritancy potential of aluminum-containing solutions and formulations, *in vitro* experiments were conducted using MatTek EpiDerm tissue model to measure the release of inflammatory cytokines. *In vitro* skin samples were treated topically with 30 µl of solutions or products for 1 h in an incubator at 37°C and 5% CO_2. Following the incubation, skin samples were washed with PBS and placed in culture medium, provided by MatTek Corporation, and continued to incubate for 24 h. After 24 h, cell culture media were collected for IL-1α release assay. IL-1α levels were analyzed by the ELISA assay kit (R&D Systems, Minneapolis, MN). IL-8 levels were analyzed using immunoassay multiplex kit (Millipore, Billerica, MA) on a Luminex X200 (Luminex Corporation, Austin, TX).

PERCUTANEOUS ABSORPTION OF TAURINE

Percutaneous permeation of taurine was evaluated *in vitro* by using human keratinocyte skin model, EpiDerm 200-X. A standard permeation device, MatTek Permeation Device was used to approximate the permeability of taurine through the skin. 5% taurine solution with or without 5% aloe extract (w/w) was applied topically to EpiDerm tissue. After set time points the EpiDerm tissue, donor and receiver solutions were collected and analyzed for taurine concentration. Receiver solution was 5.0 ml PBS solution and stored at −20°C.

DETERMINATION OF CELLULAR TAURINE CONCENTRATION

High-pressure liquid chromatography (HPLC) was used to determine cellular taurine concentration after treating MatTek EpiDerm tissue with a 5% (w/w) taurine solution or 5% (w/w) taurine and aloe extract solution. Before analysis, tissue samples were lysed using an ultrasonic tissue lyser, Qiagen Retsch MM300 (Retsch Inc, Newtown, PA) in 500 µl of 5× Extraction Buffer (Enzo Life Sciences Inc., Farmingdale, NY). Tissue lysates were pooled together from the same treatment groups ($n = 2$) to ensure the adequate volume needed to perform the taurine analysis. Taurine does not absorb UV/Vis radiation adequately; thus, a pre-column derivatization reaction is necessary to allow for detection by HPLC. 4-fluoro-7-nitrobenzofurazan (NBD-F) was used as a fluorescent reagent to produce derivatives of primary and secondary amines. NBD-taurine derivative has a

maximum UV/Vis intensity at 470 nm and has high specificity. Precolumn derivatization was performed as described in Pencheva et al. (18). Separation was performed using an isocratic elution with TBAB/phosphate (0.01 M/0.08 M) buffer: acetonitrile (70:30, v/v) mobile phase with a flow rate of 1 ml/m at a 7 m runtime. Chromatographic separations were performed on a Zorbax C_{18} (4.6 × 150 mm, 5 μ) column. Data were collected with an Agilent 1200 G1310A Isocratic pump, G1329A Autosampler, G1314B Variable Wavelength Detector. Analysis was performed with Agilent Chemstation software (Agilent Technologies, Inc., Wilmington, DE). Concentration of taurine was calculated based on peak area by using a regression curve constructed from a six-point (10.43–208.8 ppm) taurine calibration curve. Standard solutions were prepared from a stock taurine solution and regression curve had a correlation coefficient of 0.991.

SIZE-EXCLUSION CHROMATOGRAPHY (SEC–HPLC)

To ensure taurine and aloe extract did not affect the high efficacy of $AlCl_3$ antiperspirant salt, the size distribution of aluminum salt in aqueous solutions was monitored by SEC or SEC–HPLC. Retention times for each of the peaks may vary depending on experimental conditions but they remain relative to each other. Water®600 analytical pump and controller, Rheodyne®7725I injector using a Protein-Pak® 125 (Waters) column, and Waters 2414 Refractive Index Detector were used to collect SEC data. The mobile phase was a 5.56 mM nitric acid solution, pH of 2.3 (with KNO_3), with a flow rate of 1.0 ml/min. Analysis was conducted using Waters® Empower software (Waters Corporation, Milford, MA). Peak distribution for aluminum salt in our prototype $AlCl_3$ formulation was observed to be similar to a 1.2% $AlCl_3$ in DI water solution, which suggests that taurine and aloe extract would not affect the high efficacy of $AlCl_3$ salt. When the extraction of $AlCl_3$ from our prototype product for SEC was conducted, the concentration of $AlCl_3$ is reduced. However, the elution time for the aluminum peaks are the same, which indicates that the aluminum chloride is intact. If taurine and aloe extract hydrolyze aluminum chloride to large, insoluble aluminum hydroxide species, we would expect multiple peaks to elute before the aluminum chloride peak but we only observe one aluminum peak, peak 5, at a retention time of *ca.* 9.0 min (Figure 1).

CLINICAL STUDY DESIGN

A human clinical study was conducted to compare the anti-irritating properties of taurine and aloe extract in a 12% $AlCl_3$ antiperspirant prototype product to a 12% commercial $AlCl_3$ antiperspirant product. The study enrolled six male and six female subjects ($n = 12$) and lasted 4 days. Each subject was treated with both products (one on each designated forearm area) in a randomized design. A 5 cm by 5 cm area was marked on both forearms where the prototype product and benchmark product were applied. Dosage for both products was 3 mg/cm^2. Products were applied once a day, for four consecutive days in the mornings and were left on the skin overnight. Skin surface samples were collected at least 24 h after the fourth application to analyze for IL-1α levels. Skin surface samples were collected by using the cup-scrubbing method. A hollow glass cylinder (8.5 cm^2) was

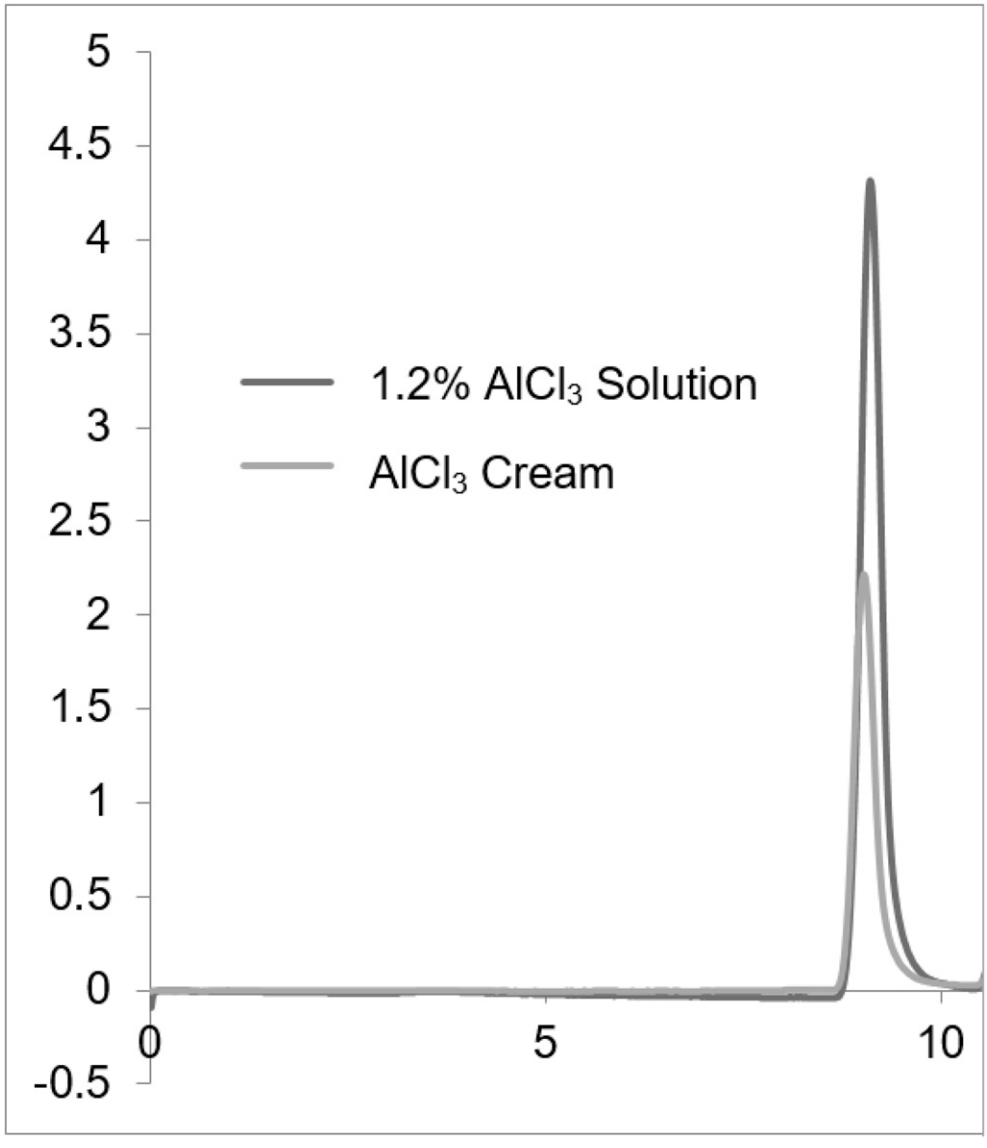

Figure 1. Size-exclusion chromatography aluminum salt size distribution profile of 1.2% $AlCl_3$ solution and prototype 12% $AlCl_3$ formulation with 0.1% taurine and 0.1% aloe extract (w/w).

placed on the skin surface of the forearm and 1 ml of PBS was pipetted onto the skin. The PBS was stirred with a glass rod for 60 s. Afterwards the extract was collected in an Eppendorf tube.

STATISTICAL ANALYSIS

Statistical analysis was performed using the two-tailed Student's T-test. *p*-values below 0.05 were considered as significant.

RESULTS AND DISCUSSIONS

DETERMINING THE OPTIMAL CONCENTRATION OF TAURINE AND ALOE EXTRACT

Per cent $AlCl_3$ (w/w) is the highest concentration allowed by the Food and Drug Administration in an over-the-counter product (19). Fourteen percent $AlCl_3$ concentration was selected to test for irritation because it is the highest concentration of $AlCl_3$ we found in over-the-counter products. $AlCl_3$ treatment alone resulted in a 5.0-fold increase in IL-1α levels compared with the untreated control. MTT assay to measure cell viability after treatment was used to normalize IL-1α data. In tissue that are co-treated with taurine or aloe extract at either 0.1% or 0.5% concentrations we observed drastic reductions in IL-1α levels, up to a 40% reduction (Figure 2). However,

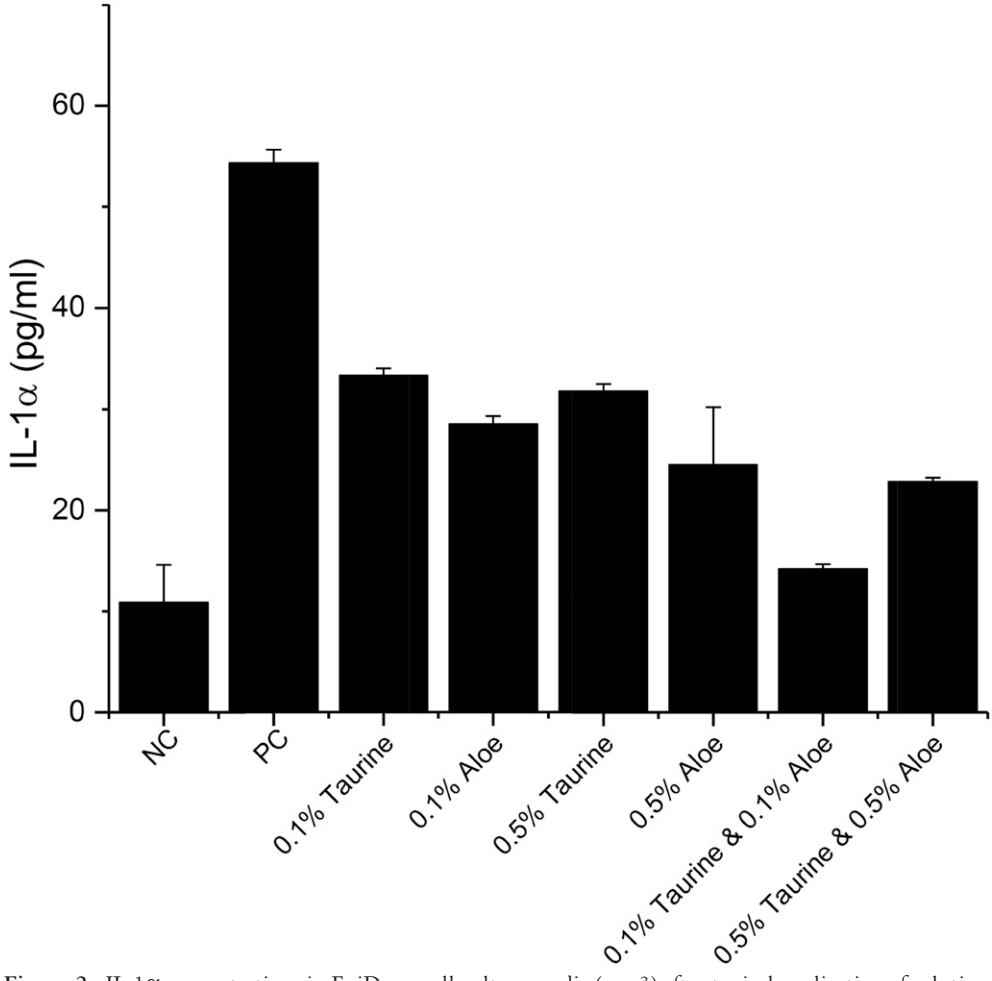

Figure 2. IL-1α concentrations in EpiDerm cell culture media ($n = 3$) after topical application of solutions containing $AlCl_3$ and actives for 1 h. Negative control (NC) was untreated tissue and positive control (PC) was tissue treated with 14% $AlCl_3$ solution without taurine or aloe extract. EpiDerm tissues were treated with 14% $AlCl_3$ solution containing either taurine or aloe extract or both at varying concentrations to determine the optimal ratio and concentration of actives.

when tissue cultures were co-treated with both taurine and aloe extract at 0.1%, there is an even further reduction in IL-1α levels compared with the control and tissue treated with only taurine or aloe extract. Treatment with taurine and aloe extract at 0.1% concentrations resulted in a statistically significant ($p < 0.05$) reduction in IL-1α levels compared with treatment with just taurine or aloe extract at either 0.1% or 0.5% concentrations. Although EpiDerm tissue treated with 0.1% taurine and 0.1% aloe extract released lower levels of IL-1α compared with tissue treated with only taurine or aloe extract at 0.1% or 0.5% concentrations, the combined, boosted effects were not observed in EpiDerm tissue co-treated with 0.5% taurine and 0.5% aloe extract. We found that at higher concentrations of aloe extract, the pH of the aqueous solution and formulations decreased significantly because of the high percentage of malic and citric acid (>18%). Thus, the concentration of aloe extract must be careful selected with regards to the pH. From this study, we were able to conclude that the 0.1% taurine and 0.1% aloe extract concentration would be the ideal concentration for our test products.

BOOSTED ANTI-IRRITATING EFFECTS OF TAURINE AND ALOE EXTRACT BLEND

After experiments were conducted to determine the optimal ratio and concentration of taurine and aloe extract, which was found to be a 1:1 ratio at 0.1% (Figure 2), we proceeded to assess the irritancy potential of $AlCl_3$ in a simple aqueous solution by measuring the levels of pro-inflammatory cytokine, IL-1α, after treatment with or without the addition of the anti-irritating actives. $AlCl_3$ treatment alone resulted in a 3.4-fold increase in IL-1α levels compared with the untreated control. MTT assay to measure cell viability after treatment was used to normalize IL-1α data (Figure 3). In tissue that are treated with 0.2% taurine or 0.2% aloe extract, we observed drastic reductions in IL-1α levels, up to a 50% reduction (Figure 4). However, when tissue cultures were co-treated with 0.1% taurine and 0.1% aloe extract, there is an even further reduction in IL-1α levels compared with the tissue treated with only taurine or aloe extract. Co-treatment with 0.1% taurine and 0.1% aloe extract resulted in a statistically significant ($p < 0.05$) reduction in IL-1α levels compared with treatment with 0.2% taurine or 0.2% aloe extract. These results suggest that the blend of taurine and aloe extract leads to a significant boost in their anti-irritating effects.

TAURINE AND ALOE EXTRACT REDUCE IRRITATION POTENTIAL OF PROTOTYPE $ALCL_3$ PRODUCT

To further test the boosted anti-irritating effect of the taurine and aloe extract blend, prototype antiperspirant formulations containing $AlCl_3$ with or without taurine and aloe extract was assessed for irritation potential. A commonly used 12% $AlCl_3$ over-the-counter product was selected as a benchmark for irritation testing. Figures 5 and 6 illustrate the results of IL-1α and IL-8 release assays after treatment of EpiDerm tissue with our prototype product and controls. Topical application of the benchmark product, 12% $AlCl_3$ solution and prototype product results in significantly increased levels of IL-1α and IL-8. However, when tissues are treated with prototype product containing 0.1% taurine and 0.1% aloe extract, we observed statistically significant reductions in IL-1α

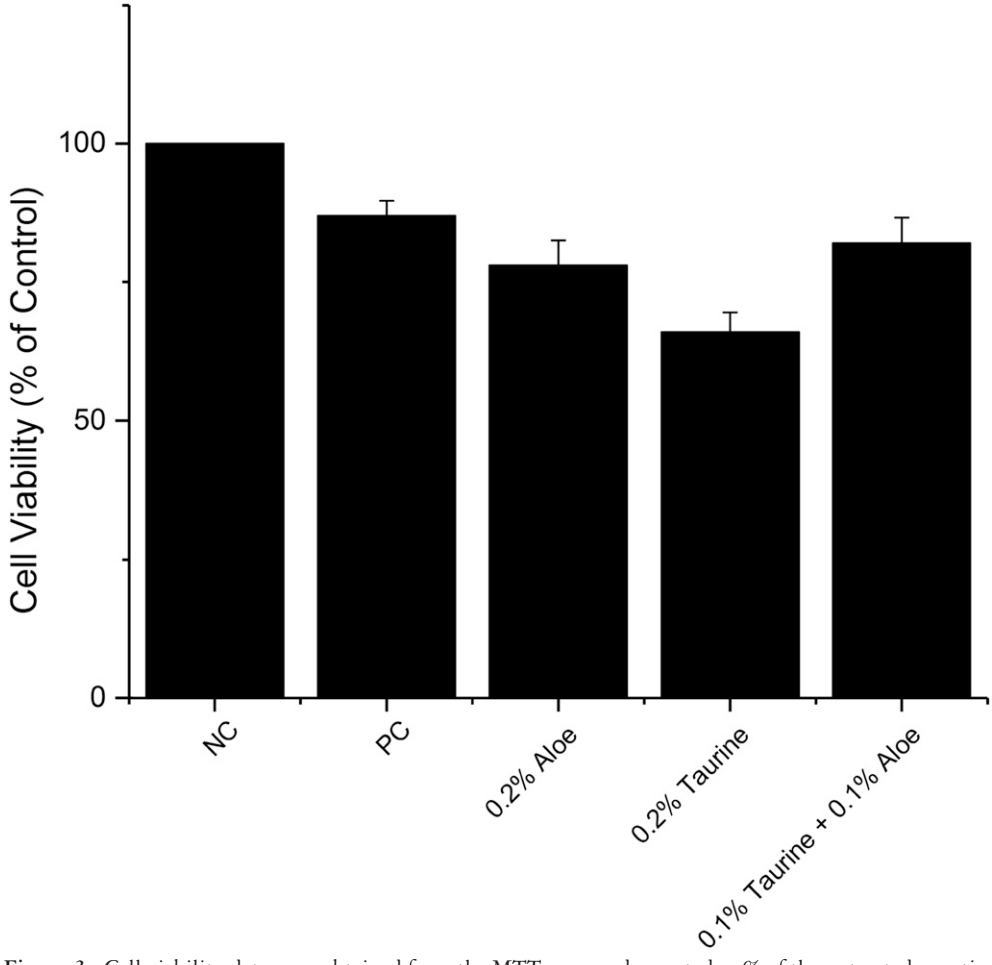

Figure 3. Cell viability data were obtained from the MTT assay and reported as % of the untreated negative control (NC).

and IL-8 levels compared with formulations without taurine and aloe ($p < 0.05$). These results suggest that the boosted anti-irritating effect of the taurine and aloe extract blend are kept intact even in dermatological vehicle.

CELLULAR TAURINE ACCUMULATION

To investigate the mechanism of the interaction between taurine and aloe extract, accumulation of taurine in EpiDerm keratinocytes was assessed. EpiDerm tissue was lysed after set time points and taurine concentration was measured by HPLC. After 2 h of treatment with solutions containing only taurine or both taurine and aloe extract, similar intracellular taurine levels were observed (Figure 7). However, after 6 h of treatment, differential intracellular taurine levels were observed and after 24 h there is a twofold, statistically significant difference in intracellular taurine levels ($p < 0.05$). Our results reveal increased taurine accumulation in keratinocytes co-treated with aloe extract. Therefore, it

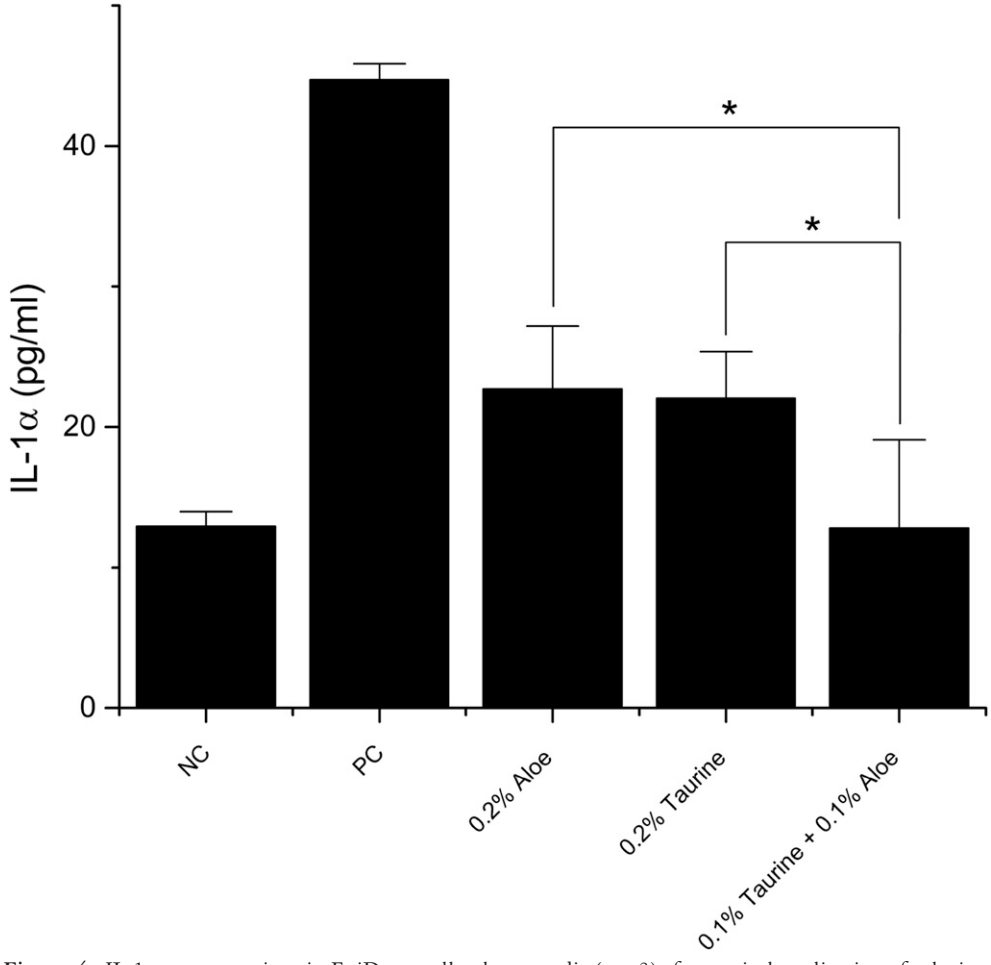

Figure 4. IL-1α concentrations in EpiDerm cell culture media ($n = 3$) after topical application of solutions containing $AlCl_3$ and actives for 1 h. Negative control (NC) was untreated tissue and positive control (PC) was tissue treated with 14% $AlCl_3$ solution without taurine or aloe extract. EpiDerm tissue was treated with 14% $AlCl_3$ solution containing either taurine or aloe extract or both. $*p < 0.05$.

suggests that aloe extract increases the absorption of taurine by keratinocytes, which offers an explanation for the observed, significant increase in anti-irritating effects.

HUMAN CLINICAL STUDY DATA

A clinical study involving 12 subjects was conducted with a prototype $AlCl_3$ antiperspirant formulation containing 0.1% taurine and 0.1% aloe extract to assess the predictability of our *in vitro* results *in vivo*. Subjects were treated with two products, a commercial 12% $AlCl_3$ antiperspirant product and a 12% $AlCl_3$ test product containing taurine and aloe extract. These two products were the same products that were assessed *in vitro* using the EpiDerm model. After repeated application on the same area for a period of 4 d, we observed a statistically significant difference in IL-1α levels between the two products

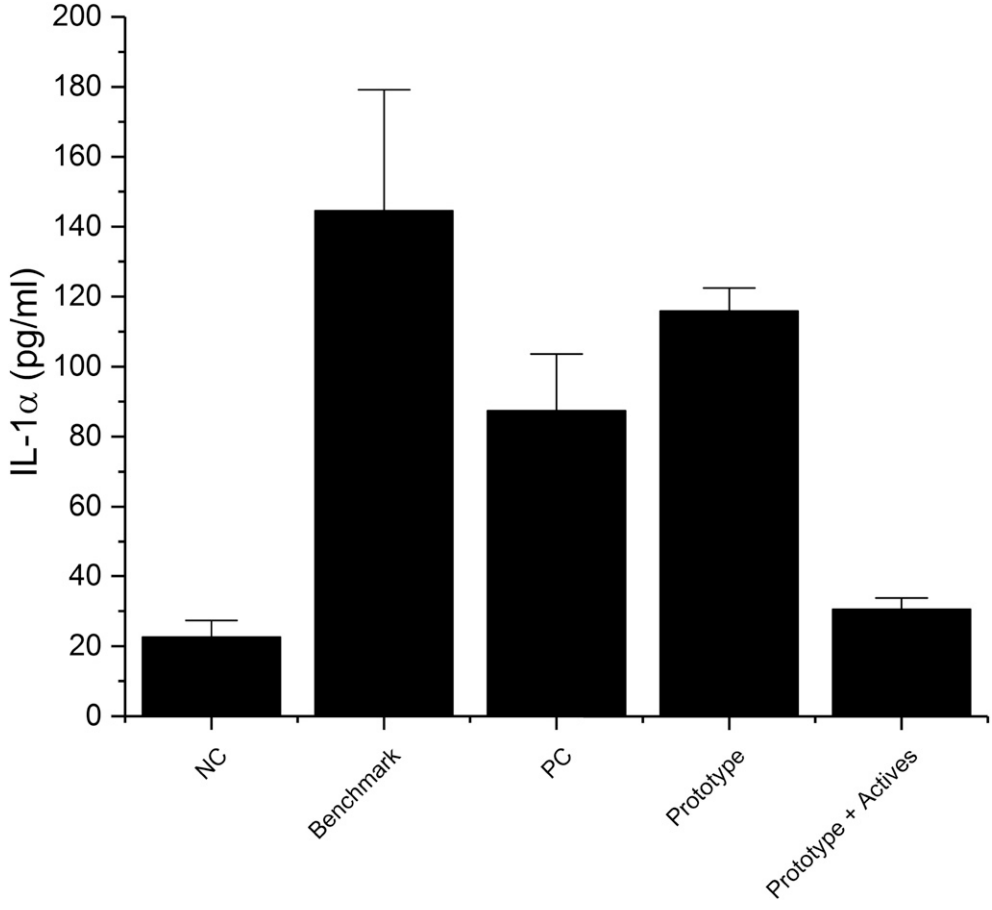

Figure 5. IL-1α concentrations in EpiDerm cell culture media ($n = 3$) after topical application of $AlCl_3$ products for 1 h. Negative control (NC) was untreated tissue and positive control (PC) was tissue treated with 12% $AlCl_3$ solution without taurine or aloe extract. A commercial 12% $AlCl_3$ antiperspirant product was used as a benchmark product to assess irritancy potential. Prototype 12% $AlCl_3$ product with and without 0.1% taurine and 0.1% aloe extract actives were tested.

($p < 0.05$) (Figure 8). Topical application of the benchmark product resulted in higher IL-1α levels compared with our prototype formulation containing taurine and aloe extract. Our results are analogous to those demonstrated in the *in vitro* EpiDerm model (Figure 5) and reveal the applicability of the anti-irritating effects of taurine and aloe extract *in vivo*.

CONCLUSIONS

$AlCl_3$ is a strong skin irritant and is known to cause the formation of ROS and induce lipid peroxidation (6,7). The low pH of $AlCl_3$ containing formulations leads to the release of pro-inflammatory cytokines, chemokines and causes tissue damage. However, the low pH is necessary to maintain the efficacy of $AlCl_3$. Higher pH environments result in the formation of larger, ineffective aluminum species (19). Therefore, the addition of

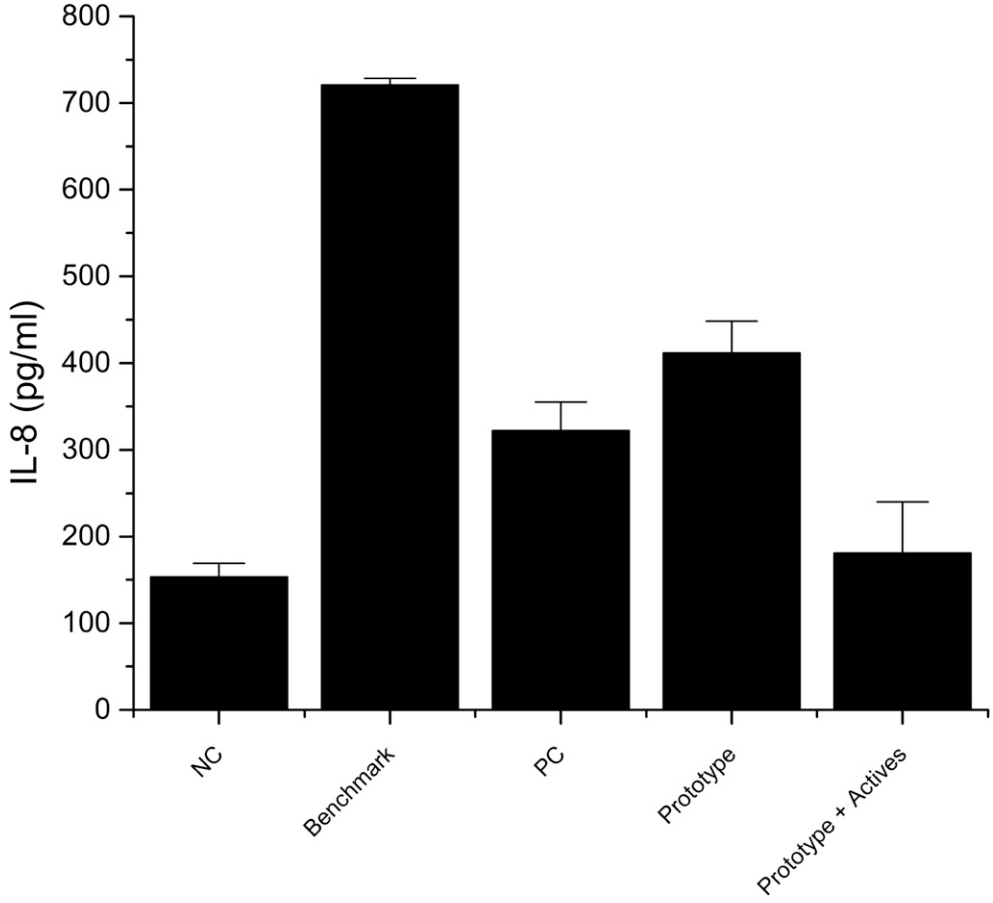

Figure 6. IL-8 concentrations in EpiDerm cell culture media ($n = 3$) after topical application of $AlCl_3$ products for 1 h. Negative control (NC) was untreated tissue and positive control (PC) was tissue treated with 12% $AlCl_3$ solution without taurine or aloe extract. A commercial 12% $AlCl_3$ antiperspirant product was used as a benchmark product to assess irritancy potential. Prototype 12% $AlCl_3$ product with and without 0.1% taurine and 0.1% aloe extract actives were tested.

anti-irritating agents into dermatological vehicles has become necessary to mitigate the contact irritant dermatitis that arises from the usage of antiperspirants containing $AlCl_3$.

Our results have revealed the existence of a boost in anti-irritating effects that the taurine and aloe extract blend demonstrate on topical application. We observed that co-treatment with taurine and aloe extract in a PBS solution results in further inhibition of IL-1α production compared with treatments with only taurine or aloe extract in human epidermis model. EpiDerm tissue treated with 0.1% taurine and 0.1% aloe extract released lower levels of IL-1α compared with tissue treated with only 0.2% taurine or 0.2% aloe extract (Figure 4). IL-1α is a primary cytokine that is constitutively expressed by keratinocytes and plays a major role in the onset of inflammatory responses in the skin (20). IL-1α release from human epidermal equivalents is commonly measured to predict the irritation potential of consumer products and provides reliable *in vivo* correlation (21).

We next addressed whether the anti-irritating effects of taurine and aloe extract would still be observable in oil in water emulsion formulation containing 12% $AlCl_3$. Our

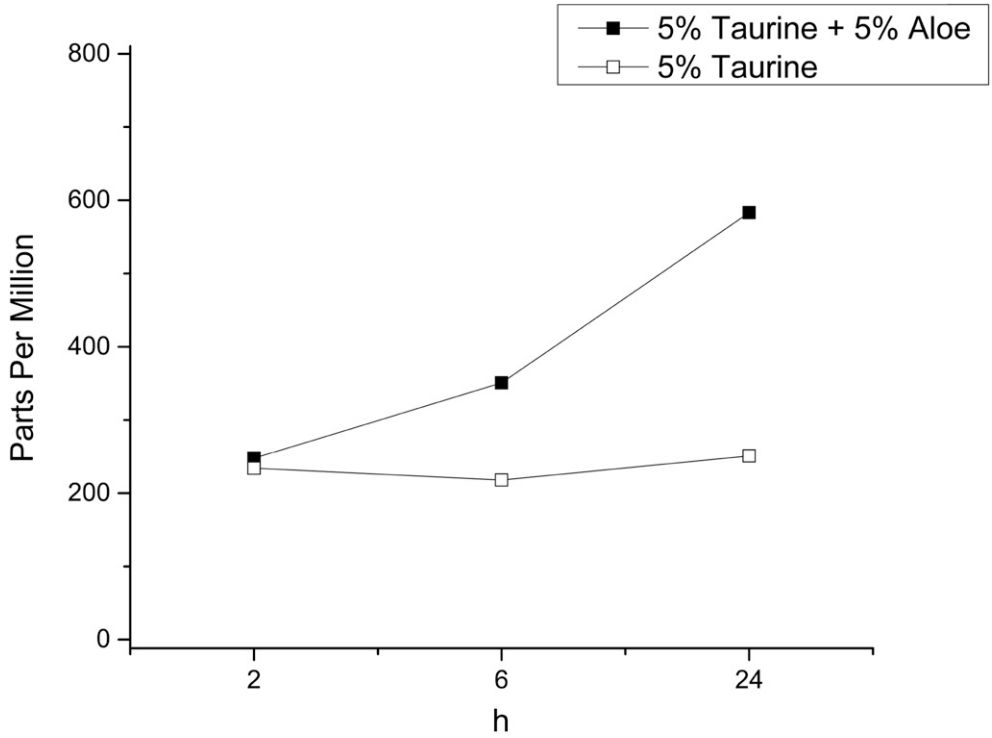

Figure 7. EpiDerm tissue was lysed at set time points and intracellular taurine concentration was measured using HPLC. Tissues were treated with either PBS solution containing only taurine or PBS solution with both taurine and aloe extract to assess the permeation enhancing effects of aloe extract on keratinocytes.

prototype antiperspirant product containing 0.1% taurine and 0.1% aloe extract was found to significantly reduce the production of IL-1α and IL-8 compared with controls in EpiDerm tissue (Figures 5 and 6). IL-8 is a chemokine that exerts chemotactic effects on neutrophils and lymphocytes. Increased expression of IL-8 by keratinocytes is a non-specific response to tissue damage due to chemical or physical agents (22). A human clinical study was conducted to assess the irritancy potential of our prototype product, and the results demonstrated that our prototype product significantly reduced the production of IL-1α compared with a benchmark product (Figure 8). A commercial 12% $AlCl_3$ antiperspirant product was used as a control and benchmark for our irritation experiments. These results demonstrated the applicability of taurine and aloe extract in reducing skin irritation to dermatological vehicles. Data obtained from SEC revealed that the additional of taurine and aloe extract into formulation did not alter the size distribution of Al^{3+}. This suggests these anti-irritating agents do not affect the polymerization of $AlCl_3$, which suggests the sweat reduction efficacy would remain intact (Figure 1). From our studies, we were able to develop an efficacious, antiperspirant formulation with reduced irritation potential.

Finally, we sought to understand the mechanism of the boosted anti-irritating effect of the taurine and aloe extract blend. Taurine has two largely separated pKa, a very low pK_1 (1.5) from the sulfonic acid group and a high pK_2 (9.08) from the amino group which give rise to a zwitterion over a wide pH range. Under our experimental pH range

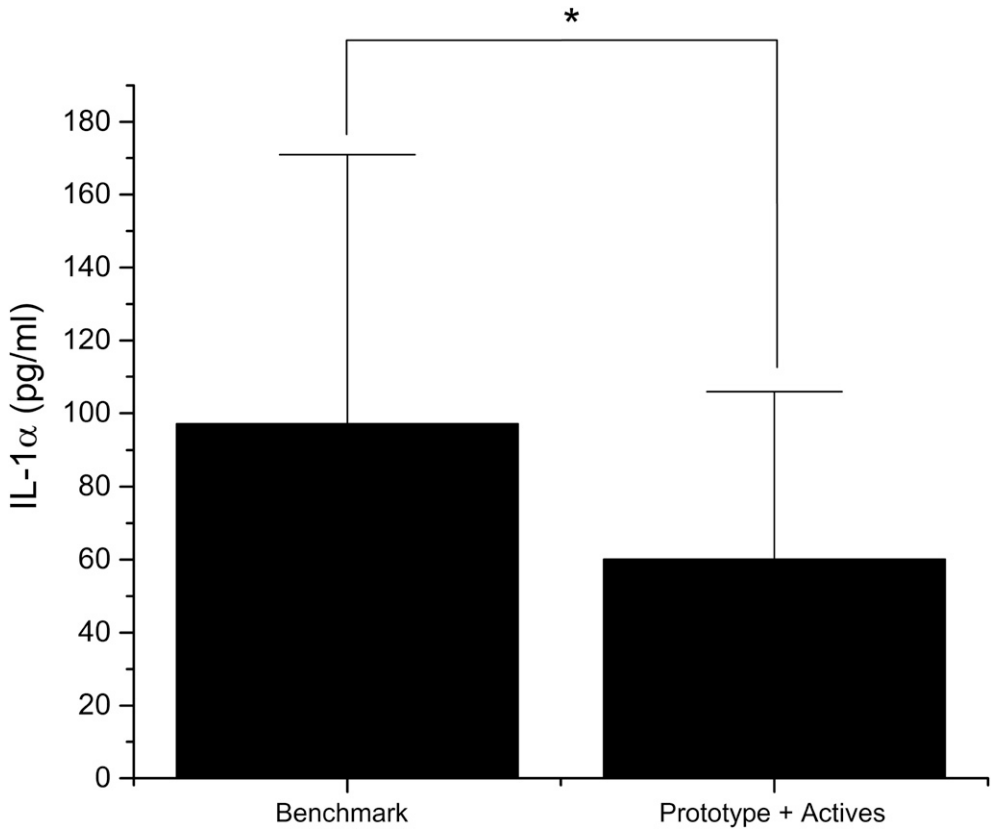

Figure 8. Subjects ($n = 12$) were treated with a commercial 12% $AlCl_3$ antiperspirant benchmark product and a prototype 12% $AlCl_3$ product containing 0.1% taurine and 0.1% aloe extract (one on each designated forearm area). IL-1α concentration was measured after four consecutive days of dermal exposure from obtained skin samples. $*p < 0.05$.

(2.5 < pH < 4), taurine exists as the zwitterionic form with high water solubility (104.8 g/l) (Figure 9) (12).

Compared with the cationic and anionic forms, the zwitterionic form readily penetrates the skin barrier into keratinocytes (8). Previous research has disclosed that taurine is predominantly localized to the stratum granulosum and stratum spinosum in the epidermis (12). Aloe vera extract has been previously found to enhance the transport of various drugs and compounds across skin and intestinal epithelial cells (23). There are several hypotheses as to how aloe vera enhances the permeation of certain compounds.

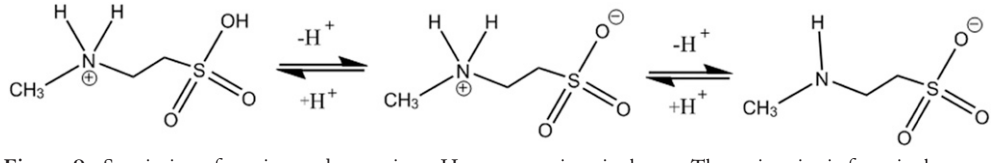

Figure 9. Speciation of taurine under varying pH concentrations is shown. The zwitterionic form is the most predominant form at biological pH values.

We hypothesized that aloe extract enhanced the permeation of taurine in the stratum corneum and increased cellular uptake. To confirm this assumption, a designed study demonstrated that over time keratinocytes in the EpiDerm tissue had increasing intracellular taurine concentration when co-treated with aloe extract (Figure 7). One proposed mechanism is that components of aloe vera form complexes with the drug or compound to increase the permeation by size exclusion and pull effect (24). Lower molecular weight drugs are able to form complexes with aloe vera components and are pulled down into the deeper layers of the epidermis. We hypothesized that the permeation enhancement effects were due to the presence of alpha-hydroxyacids in the aloe vera extract. Alpha-hydroxyacids, such as lactic acid, have been found to significantly increase the penetration and intracellular accumulation of hydrophilic molecules in the epidermis (25). Malic acid is an alpha-hydroxyacid that is found in the aloe vera extract used in our studies. Further studies would need to be conducted to assess the penetration enhancement effects of major components of aloe extract alone.

In conclusion, aloe extract and taurine blend demonstrates a boosted effect in reducing the skin irritation potential of irritating dermatological actives, such as $AlCl_3$. The production of pro-inflammatory cytokines and chemokines, such as IL-lα and IL-8, was statistically significantly decreased by the treatment of aloe extract and taurine in both *in vitro* and *in vivo* studies. Our data demonstrate the boosted, anti-irritating capabilities of aloe extract and taurine blend. This discovery does not only lead to new antiperspirant formulations with reduced irritation potential, but possibly other dermatological vehicles and cosmetic products with reduced tendency in causing irritant contact dermatitis after usage.

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