The PAR2 Antagonist Larazotide Can Mitigate Acute Histamine-Stimulated Epithelial Barrier Disruption in Keratinocytes: A Potential Adjunct Treatment for Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin condition with evidence of defects in the barrier properties of the epidermis. Changes in the permeability properties of the tight junction have been reported in AD, and reversing this leaky tight junction may be a potential treatment for AD. This study aimed to determine the effect of larazotide, an antagonist of the protease-activated receptor 2, on the permeability and barrier properties of the tight junctions in keratinocyte monolayers. Normal human epithelial keratinocytes were grown in culture on permeable supports. The effects of larazotide on transepithelial resistance and permeability properties of keratinocyte monolayers were studied before and after histamine challenge. Larazotide mitigated the disruptive effect of histamine on epithelial permeability by increasing the electrical resistance and decreasing epithelial permeability. Larazotide may be beneficial as a topical therapeutic for AD; however, the permeability properties of the short-peptide larazotide through the uppers layers of the epidermis is currently unknown. In conclusion, the protease-activated receptor 2 antagonist larazotide has a protective effect on keratinocyte monolayers and may be useful as an adjunct therapeutic agent to enhance barrier function and promote epidermal healing in AD.

Keywords: Histamine, PAR2, Paracellular, Permeability, Tight junction

INTRODUCTION

In the skin, both the terminally differentiated keratinocytes in the stratum corneum and keratinocytes in the stratum granulosum provide a protective barrier to water loss, microbe entry, and allergen permeability from the environment (Proksch et al, 2008). In the stratum granulosum, tight junctions between the keratinocytes form the permeability barrier and provide a size-selective filter for the regulation of molecular permeability (Furuse et al, 2002). Changes in the integrity of the epithelial barrier function are thought to have a key role in the etiology of many inflammatory diseases, including inflammatory bowel disease, celiac disease, and atopic dermatitis (AD) (Akdis, 2021).

Histamine has previously been shown to increase the permeability of keratinocyte monolayers in culture (Gschwandtner et al, 2013). It has also been reported that tissue from individuals with AD has a defect in the permeability properties of the tight junction (De Benedetto et al, 2011). These observations support the hypothesis that an impaired skin barrier could be a critical feature in the pathogenesis of AD.

The protease-activated receptor 2 (PAR2) is highly expressed in epidermal keratinocytes of the stratum granulosum layer in normal and AD skin, where it is thought to contribute to the pathophysiology of AD (Steinhoff et al, 1999). PAR2 is activated by its tethered aminoterminal sequence, upon cleavage by proteases, which are released together with histamine upon mast cell degranulation (Henehan and De Benedetto, 2019). PAR2 is an inflammatory mediator by nature and has been shown to decrease epidermal barrier function and increase permeability (Nadeau et al, 2018). Antagonism of PAR2 may be a useful therapeutic target to resolve the symptoms of AD such as inflammation and pruritus (Bohannon et al, 2020).

In this study, we investigate the effect of the PAR2 antagonist larazotide on keratinocytes in a histamine-stimulated model of keratinocyte epithelial barrier disruption. Larazotide is a synthetic 8 amino-acid peptide (sequence GGVLVQPG) that stabilizes and decreases the permeability of the tight junction through mechanisms not yet fully understood. Larazotide has been shown to increase the

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Abbreviations: AD, atopic dermatitis; ERK, extracellular signal–regulated kinase; HBSS, Hanks' Balanced Salt Solution; NHEK, normal human epidermal keratinocyte; PAR2, protease-activated receptor 2; TEER, transepithelial electrical resistance

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electrical resistance of gastrointestinal epithelia and decrease the permeability of larger molecules across the cultured gastrointestinal cell lines (Gopalakrishnan et al, 2012; Slifer et al, 2021), although there are no data on its effect on keratinocyte epithelial cells.

Our hypothesis (H_a) is that the PAR2 antagonist larazotide will restore the epithelial permeability barrier in a histamine-stimulated model of keratinocyte barrier disruption. The null hypothesis (H_0) is that larazotide will have no effect on the barrier properties of keratinocytes.

RESULTS

Histamine has a powerful effect on the disruption of the barrier properties of human epidermal keratinocytes. Application of 100 μM histamine to the solution bathing the keratinocyte monolayers decreased transepithelial electrical resistance (TEER) from 352.7 ± 72.1 Ω.cm^2 to 258.6 ± 19.8 Ω.cm^2, a decrease of 25.3 ± 9.4% ($P < .05$, n = 4) of the control value in 120 minutes (Figure 1a). Conversely, addition of 10 μM larazotide stabilized resting normal human epidermal keratinocyte (NHEK) monolayers and increased TEER from 192.4 ± 23.5 Ω.cm^2 to 294.7 ± 40.8 Ω.cm^2, an increase of 53.9 ± 19.3% ($P < .05$, n = 3) of control value in 120 minutes (Figure 1a).

In proof-of-concept experiments, larazotide increased keratinocyte transepithelial resistance and protected against the histamine-induced increase in epithelial permeability. Initially, we showed that larazotide (10 μM) increased the TEER of a resting keratinocyte monolayer from 205.8 ± 28.7 Ω.cm^2 to 286.6 ± 15.7 Ω.cm^2, an increase of 40.4 ± 12.1% ($P < .01$, n = 3) over control level after 90 minutes. Subsequently, even after 60 minutes of histamine (100 μM) exposure, the TEER only decreased to 233.7 ± 31.6 Ω.cm^2, still 13.7 ± 4.9% ($P = .05$, n = 3) higher than the control value (Figure 1b). In a second set of experiments with a reversal of the order of drug addition, 100 μM of histamine decreased the monolayer TEER from 302.4 ± 31.1 Ω.cm^2 to 222.0 ± 6.5 Ω.cm^2, a decrease of 26.1 ± 7.7% after 60 minutes ($P < .05$, n = 3). Addition of 10 μM larazotide restored the TEER back to the control value of 300.4 ± 13.4 Ω.cm^2 ($P = .9163$, n = 3) after an additional 90 minutes (Figure 1b).

The effect of sequential addition of histamine and larazotide was tested on multiple replicates of human keratinocyte monolayers (Figure 2). The keratinocyte monolayers were grouped into high-resistance or low-resistance cohorts and analyzed independently. Addition of 100 μM histamine to the high-resistance NHEK monolayers (solid squares) decreased TEER by 10.1 ± 2.9%, from 1458 ± 107 Ω.cm^2 to 1311 ± 57 Ω.cm^2 ($P < .05$, n = 4). After 90 minutes of larazotide (10 μM) exposure, the resistance of the monolayer recovered to 1441 ± 107 Ω.cm^2 ($P = .605$, n = 4), similar to that of the control TEER. Addition of 100 μM histamine to the low-resistance NHEK monolayers (solid circles) decreased TEER by 15.8 ± 8.7%, from 282 ± 65 Ω.cm^2 to 234 ± 46 Ω.cm^2 ($P < .05$, n = 4). After 90 minutes of larazotide (10 μM) exposure, the resistance of the monolayer increased to 362 ± 95 Ω.cm^2 ($P = .091$, n = 4) similar to the control TEER (Figure 2a). The data were normalized to the control TEER (time = 0) measured in Hanks' Balanced Salt Solution (HBSS) and plotted on the same graph (Figure 2b). Addition of 100 μM histamine to the NHEK monolayers disrupted the barrier properties and decreased TEER to 87.1 ± 6.9% ($P < .01$, n = 8) of control after 60 minutes (Figure 2b). Transepithelial resistance was restored and increased to 114.5 ± 24.7% (nonsignificant, $P = .1403$, n = 8) of control, 90 minutes after larazotide addition (Figure 2b).

In addition to a decrease in electrical resistance, histamine exposure also increased paracellular permeability to larger molecules such as fluorescein (0.33 kDa). After a 60-minute exposure to 100 μM histamine, basolateral fluorescein fluorescence increased to 151.6 ± 21.9% ($P < .01$, n = 5) of control (Figure 2c). Incubation for a further 90 minutes after the addition of 10 μM of larazotide decreased the basolateral compartment fluorescein fluorescence to 119.9 ± 23.2% (nonsignificant, $P = .1269$, n = 5), which was similar to control (Figure 2c).

Taken together, we have shown that the PAR2 antagonist larazotide had an acute and robust effect on increasing keratinocyte transepithelial resistance and can protect against the detrimental effect of histamine on increased paracellular permeability.

DISCUSSION

In this study, we show that acute exposure of keratinocytes to the tight junction–modifying peptide larazotide increased the transepithelial resistance and decreased the epithelial permeability to larger molecules. We also provide evidence that larazotide can mitigate the detrimental effect of histamine on keratinocyte barrier function and reverse the increase in epithelial permeability triggered by histamine.

Histamine-releasing mast cells have been shown to be abundant in the skin of individuals with AD, and this correlates with increased histamine concentration measured in the tissue (Damsgaard et al, 1997). Histamine disrupts the barrier function of keratinocytes, and excess histamine release from mast cells likely contributes to the pathophysiology of diseases of the epidermis such as AD (Gschwandtner et al, 2013). Chronic increased histamine levels deteriorate the skin barrier by decreasing the expression of cutaneous structural proteins and disrupting tight junctions (Beck et al, 2022; De Benedetto et al, 2015; Gschwandtner et al, 2013).

Our findings from this study confirm the observations of others (Bergmann et al., 2020; Fujikawa et al, 2022) and additionally show that acute increased histamine levels could contribute to skin barrier defects by altering the properties of the tight junction, after only a few minutes. Hence, attempts to repair the leakiness of the keratinocyte layer by modulating the properties of the tight junction might be an attractive strategy to treat inflammatory disorders of the epidermis, such as AD. Our data showed that the PAR2 antagonist larazotide stabilized and strengthened the epithelial barrier in the resting state and also mitigated the detrimental effect of histamine on epithelial permeability properties. Thus, antagonism of PAR2 appears to be a dominant regulator of keratinocyte permeability, which may possibly overpower the effects of mast cell–released histamine on epithelial permeability properties.

Endogenous protease-dependent activation of PAR2 could exert its effect on the tight junction through PAR2 itself or

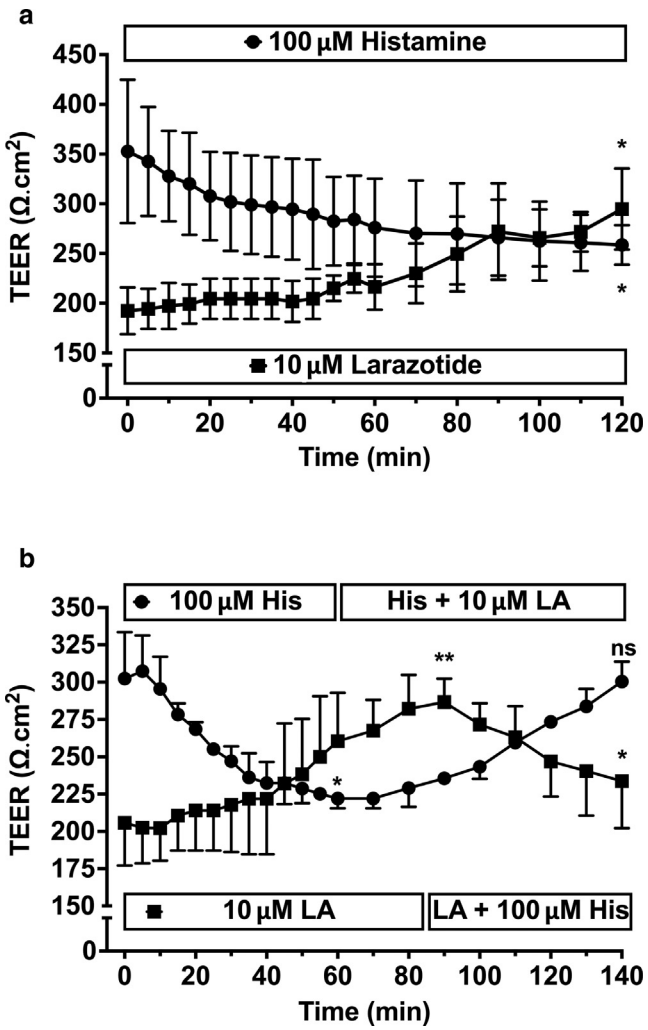

Figure 1. Histamine disrupts and larazotide strengthens the barrier properties of keratinocyte epithelial monolayers. (**a**) Histamine (100 μM) decreased the TEER from 352.7 ± 72.1 Ω.cm^2 to 258.6.7 ± 19.8 Ω.cm^2 ($P <$ .05, n = 4) of the control (time = 0) in 120 minutes (●, upper trace). Larazotide (10 μM) increased the TEER from 192.4 ± 23.5 Ω.cm^2 to 294.7 ± 40.8 Ω.cm^2 ($P <$ .05, n = 3) of the control (time = 0) over 120 minutes (■, lower trace). The epithelial monolayer resistance was measured every 5 minutes for the first 60 minutes and then every 10 minutes for the next 60 minutes. Data are presented as mean ± SD, and data at 120 minutes were compared with the sample mean at time = 0, using a paired 2-tailed *t*-test. These experiments were performed on different keratinocyte monolayers from 3 different biological replicates. The asterisks (*) at time = 120 minutes on both datasets signify a difference from time = 0 with significance of *$P <$ .05. (**b**) In proof-of-concept experiments, larazotide reversed and prevented the histamine-induced barrier disruption in a keratinocyte monolayer. In the upper trace, histamine (100 μM) was added before larazotide (10 μM). At time = 0, the TEER was 302.4 ± 31.1 Ω.cm^2 and decreased to 222.0 ± 6.5 Ω.cm^2 (*$P <$ .05, n = 3) after 60 minutes in the presence of histamine (100 μM), a decrease of 26.1 ± 7.7%. After addition of larazotide (10 μM), the TEER recovered to 300.4 ± 13.4 Ω.cm^2 (ns, P = .9163, n = 3), similar to control value (●). These experiments were performed on 3 different keratinocyte monolayers from 2 different biological replicates. In the lower trace, larazotide (10 μM) was added to the monolayers before histamine (100 μM). Larazotide increased the TEER from 205.8 ± 28.7 Ω.cm^2 to 286.6 ± 15.7 Ω.cm^2 (**$P <$ .01, n = 3) after 90 minutes, an increase of 40.4 ± 12.1%. Addition of histamine decreased TEER to 233.7 ± 31.6 Ω.cm^2 (*$P <$ .05) after 60 minutes (■). These experiments were performed on 3 different keratinocyte monolayers from 3 different biological replicates. The frequency of TEER measurements was similar to that in the panel above. Data are presented as mean, with either upper or lower SD for clarity, and data were compared with the sample mean at time = 0, using a paired 2-tailed *t*-test. The asterisk (*) at time = 60 minutes on the Histamine first dataset (●) signifies a difference from time = 0 with a significance of $P <$ .05, whereas ns at 140 minutes indicates not significantly different from time = 0. The double asterisk (**) at time = 90 minutes on the Larazotide first dataset (■) signifies a difference from time = 0 with a significance of $P <$ .01, whereas the single asterisk (*) at time = 140 minutes indicates a difference from time = 0 with a significance of $P <$ .05. min, minute; ns, not significant; TEER, transepithelial electrical resistance.

through PAR2-induced transactivation of the EGFR (Figure 3) (Tripathi et al, 2009). The intracellular effects of PAR2 activation occur through multiple kinases, including the ROCK and MAPK pathway, including the extracellular signal–regulated kinases (ERKs) (Ossovskaya and Bunnett, 2004). A second regulatory pathway occurs by PAR2-mediated transactivation of EGFR, also triggering the ERK kinases in keratinocytes (Jacob et al, 2005; Rattenholl et al, 2007). Hence, stimulation of PAR2/EGFR results in depolymerization of cytoskeletal fibers and a disruption of the tight junction through the ROCK/ERK pathways (Figure 3) (Enjoji et al, 2014; Sturgeon and Fasano, 2016; Tripathi et al, 2009; Veres-Székely et al, 2023).

Histamine is also a very powerful disrupter of tight junctions, and in keratinocytes, it likely has its effect through the histamine receptor 1 (Ashida et al, 2001; Gschwandtner et al, 2013). It is thought that histamine also impairs tight junctions through the activation of the ERK or ROCK pathways (Figure 3) (Jain et al, 2016; Kugelmann et al, 2018). Hence, the second messenger intracellular kinase pathways of all 3 cell-surface receptors (PAR2/EGFR and histamine receptor 1) overlap to some extent and have a common pathway that disables the tight junction, increasing the paracellular permeability (Figure 3).

Even though the exact mechanism of the action of larazotide is not fully understood, it has been proposed to function as a PAR2 antagonist (Fasano, 2011). Under this scenario, larazotide will prevent activation of the PAR2 and also prevent transactivation of EGFR (Tripathi et al, 2009). Larazotide will function by inhibiting the PAR2 ligand activation of the ROCK/ERK pathways and restore tight junction integrity (Figure 3b) (Enjoji et al, 2014; Wang et al, 2023; Zeze et al, 2022). If PAR2 is spontaneously active, then larazotide will increase epithelial resistance and repair barrier properties. Alternatively, in the presence of histamine and histamine-induced tight junction disruption, then inactivating the ROCK/ERK kinase cascades with the PAR2 antagonist larazotide may somehow interfere with the histamine receptor 1 kinase pathway because they are in common (Figure 3b).

In summary, our data show that larazotide has a rapid and powerful effect on stabilizing and repairing the tight junction in keratinocytes to maintain epithelial integrity and mitigate histamine-induced epithelial barrier disruption.

In conclusion, we propose that topically applied larazotide should be investigated in human studies because it may be useful as an adjunct in the treatment of AD in a

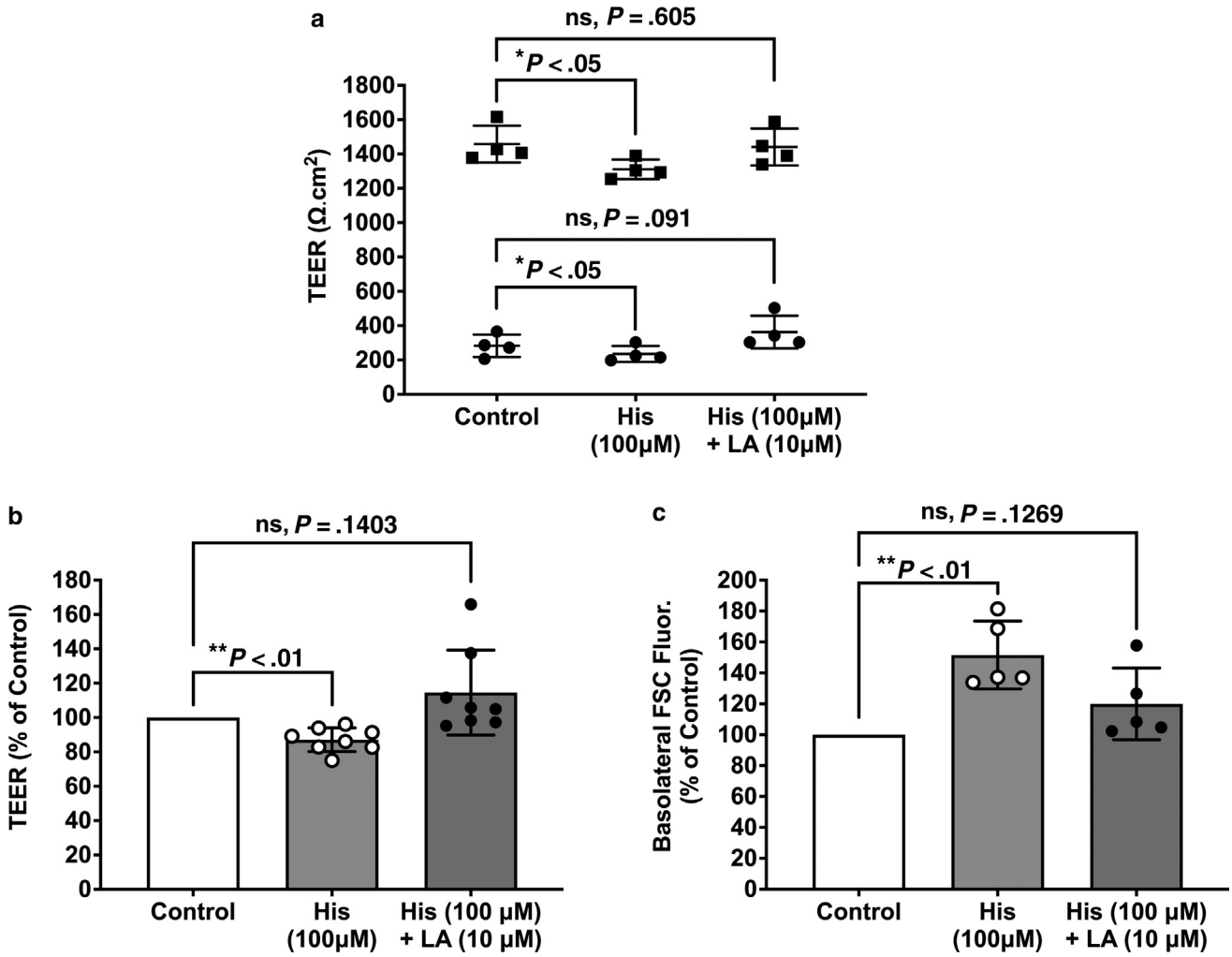

Figure 2. Larazotide mitigates the histamine-induced increase in epithelial permeability. (**a**) The keratinocyte monolayers have been grouped into high-resistance or low-resistance cohorts and analyzed independently. Addition of 100 μM histamine to the high-resistance NHEK monolayers (■) decreased TEER by 10.1 ± 2.9%, from 1458 ± 107 Ω.cm^2 to 1311 ± 57 Ω.cm^2 (*P < .05, n = 4). After 90 minutes of larazotide (10 μM) exposure, the resistance of the monolayer recovered to 1441 ± 107 Ω.cm^2 (ns, P = .605, n = 4), similar to the control TEER. Addition of 100 μM histamine to the low-resistance NHEK monolayers (●) decreased TEER by 15.8 ± 8.7%, from 282 ± 65 Ω.cm^2 to 234 ± 46 Ω.cm^2 (*P < .05, n = 4). After 90 minutes of larazotide (10 μM) exposure, the resistance of the monolayer increased to 362 ± 95 Ω.cm^2 (ns, P = .091, n = 4) similar to the control TEER. Data are presented as mean ± SD, and all experimental data were compared with the control mean (monolayer bathed in HBSS) using a paired 2-tailed *t*-test. The experiments were performed on 8 separate keratinocyte monolayers from 4 different biological replicates. (**b**) The high-resistance and low-resistance cohorts were normalized to the control mean (monolayer bathed in HBSS) and plotted together. Addition of 100 μM histamine to the NHEK monolayers decreased TEER to 87.1 ± 6.9% (**P < .01, n = 8) of control after 60 minutes. The further addition of 10 μM larazotide increased the transepithelial resistance to 114.5 ± 24.7% (ns, P = .1403, n = 8) of control after 90 minutes. These experiments were performed on 8 separate monolayers from 4 biological replicates, and all experimental data were compared with the sample mean normalized to 100% using a 1-sample *t*-test. (**c**) Fluorescein transport was also measured in the same keratinocyte monolayers. After a 60-minute exposure to 100 μM histamine, basolateral fluorescein fluorescence increased to 151.6 ± 21.9% of control (**P < .01, n = 5), incubated for a further 90 minutes in 10 μM larazotide, and reduced the basolateral compartment fluorescein fluorescence to 119.9 ± 23.2% of control (ns, P = .1269, n = 5). These experiments were performed on 5 separate keratinocyte monolayers from 2 different biological replicates. Data are presented as mean ± SD, and all experimental data were compared with the sample mean normalized to 100% using a 1-sample *t*-test. NHEK, normal human epidermal keratinocyte; ns, not significant; TEER, transepithelial electrical resistance.

complementary approach with immunosuppressive and anti-inflammatory therapies.

MATERIALS AND METHODS

Keratinocyte cell culture

Pooled NHEKs isolated from the foreskin (PromoCell, Heidelberg, Germany) were grown in Keratinocyte Basal Media 2 (phenol red free) supplemented with the Keratinocyte Supplement Pack that included bovine pituitary extract, epidermal GF, insulin, hydrocortisone, epinephrine, transferrin, and a low calcium concentration of 0.06 mmol/l of calcium chloride (PromoCell). The NHEKs were used between subcultures 2 and 4. Cells were plated at 125,000 cells/ml on Snapwell (1.12 cm^2) permeable supports (Corning Life Sciences, Corning, NY) and grown for 6 days. Monolayers were then switched to a high-calcium differentiation medium CnT-PR-3D (ZenBio, Durham, NC), and experiments were performed on days 18–24 of culture.

Histamine phosphate and larazotide were purchased from MedChemExpress (Monmouth Junction, NJ) and were dissolved as 1000-fold stock solutions of 100 mmol/l and 10 mmol/l, respectively, and

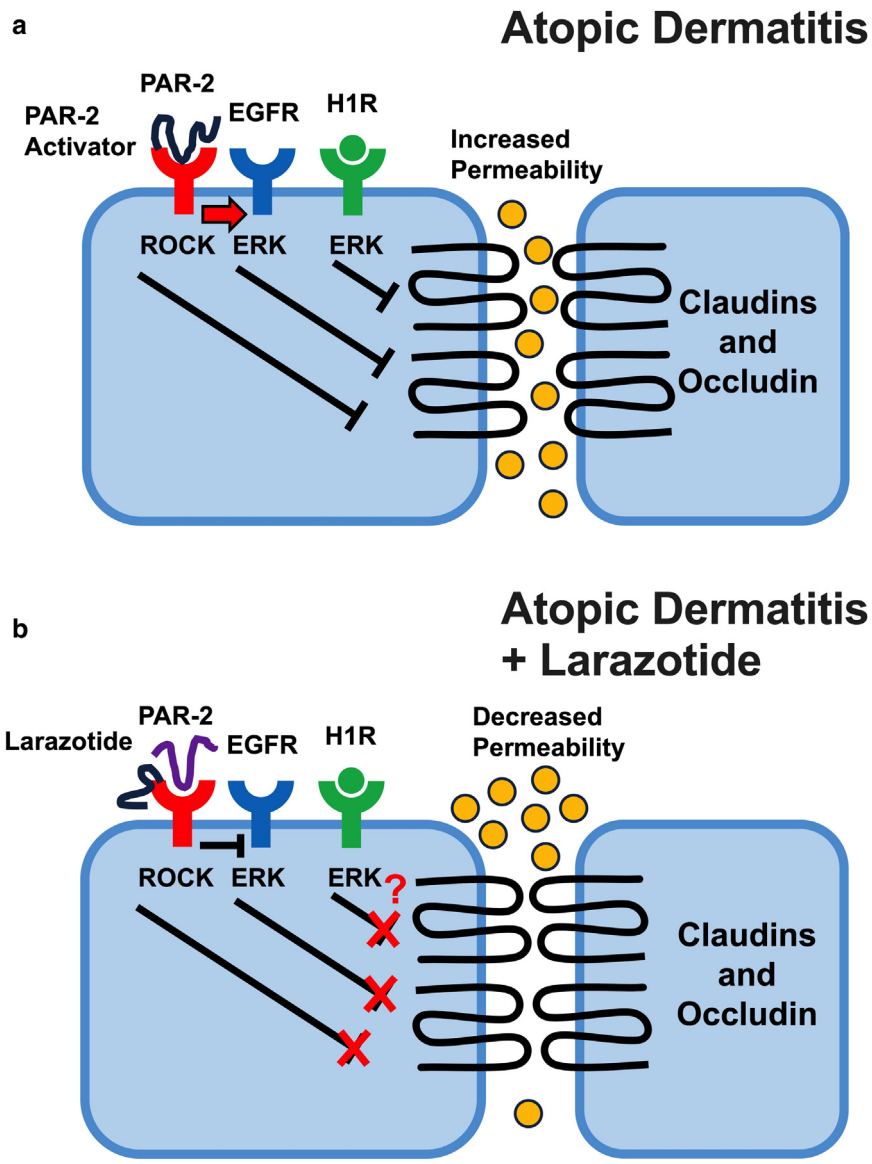

Figure 3. A highly schematic cartoon depicting the proposed pharmacological action of larazotide in atopic dermatitis. (**a**) In atopic dermatitis, a PAR2-activating protease or ligand will stimulate the PAR2 and transactivate (red arrow) the EGFR. This stimulation will have a negative and disruptive effect on the tight junction through multiple kinase pathways, including ROCK and ERKs and likely others not shown here. Histamine (green circle) binding to the histamine receptor (histamine receptor 1) will also disrupt the tight junction through similar ERK kinase pathways. These kinase pathways lead to a disassembly of the tight junction and increase the epithelial permeability, allowing increased penetration of larger molecules (orange circles) that can contribute to inflammation. (**b**) In the presence of the PAR2 antagonist larazotide (purple peptide), the kinase pathways (ROCK and ERK) linked to the PAR2 and EGFR will be repressed. If the PAR2 inhibitory pathway has a dominant effect compared with the histamine pathway, then this on its own may be sufficient to reverse the disruptive effect of histamine on the tight junction. An additional hypothesis is that the PAR2 antagonist larazotide can inhibit the H1R kinase pathways because it has a common link with the PAR2/EGFR kinase cascades. ERK, extracellular signal–regulated kinase; HBSS, Hanks' Balanced Salt Solution; HBBS, H1R, histamine receptor 1; PAR2, protease-activated receptor 2.

stored frozen at −20 °C until use. We did not perform a dose–response of the effect of histamine and larazotide on NHEK monolayer integrity and electrical resistance. However, previous studies in human epidermal keratinocytes have shown a dose–dependent decrease in electrical resistance in the range of 1–1000 μM histamine (Fujikawa et al, 2022). Hence, we chose 100 μM histamine to have a near-maximal effect on keratinocyte monolayer electrical resistance. Larazotide has been shown to have a barrier restorative effect on ischemic intestine at concentrations of 1 μM (Slifer et al, 2021). Again, we chose to use 10 μM larazotide to show a maximal effect in this initial proof-of-concept study. All transepithelial resistance and fluorescence permeability experiments were performed at room temperature in HBSS (Gibco, Grand Island, NY), and all drugs were applied to both the apical and basolateral membrane compartments of the culture inserts.

TEER measurements

The TEER of NHEK cell monolayers grown on Snapwell permeable supports was measured using a DVC-1000 epithelial voltage clamp (WPI, New Haven, CT) and an EndOhm concentric Ag/AgCl electrode (WPI, Sarasota, FL). TEER was measured at room temperature in HBSS with a ±5 mV transepithelial membrane clamp and calculated using Ohm's Law. All transepithelial resistances were adjusted for empty filter resistance and the filter support area and

recorded as $\Omega.cm^2$. The resistance of biological replicate batches of NHEK monolayers varied from about 200 to 1600 $\Omega.cm^2$. This variability may be related to original seeding density, confluence density upon switch to high-calcium solution, batch of switch media, or an unknown variable. For this reason, we report our data as low-resistance and high-resistance cohorts separately and also as percentage change over control monolayer. Nonetheless, histamine and larazotide always showed the same physiological effects whether tested on a low- or high-resistance monolayer.

Epithelial permeability measurements

Epithelial permeability was measured over 30 minutes by apical to basolateral transport of 0.5 mg/ml fluorescein disodium salt (Thermo Fisher Scientific, Waltham, MA). Fluorescein has been shown to be a valid marker of paracellular transport in several epithelia (Krug et al, 2009). The apical fluid compartment on the filter was replaced with 0.5 mg/ml fluorescein dissolved in a total volume of 0.3 ml HBSS experimental solution. The basolateral compartment contained 2 ml of HBSS experimental buffer. After 30 minutes, the basolateral fluid compartment was removed, and fluorescence measurements were taken in triplicate and averaged for a single data point, from a single NHEK monolayer. Fluorescein fluorescence was measured using a Synergy HTX 96-well fluorescent plate reader (BioTek, Winooski, VT) with an excitation of 485/20 nm and emission of 528/20 nm.

Experimental procedure

For the initial proof-of-concept experiments, cell culture media was replaced with HBSS containing either 100 μM histamine or 10 μM larazotide, and TEER was measured every 5 minutes for the first 60 minutes and then every 10 minutes for the next 60–90 minutes (Figure 1a and b). Sequential additions of both 10 μM larazotide and 100 μM histamine combined with 10 μM larazotide were tested on the same monolayer (Figure 1b, lower trace). The reverse protocol where 100 μM histamine was added before the combination of both 100 μM histamine and 10 μM larazotide can be seen in Figure 1b (upper trace).

In the sequential drug-addition experiments (Figure 2a–c), each monolayer served as its own control for the 3 experimental conditions. Experiments were performed sequentially on the same epithelial monolayer in the order of control (HBSS), 100 μM histamine, and 100 μM histamine plus 10 μM larazotide. Briefly, the culture media was replaced with HBSS, and a control TEER and apical-to-basolateral 0.5 mg/ml fluorescein transport was measured. The transepithelial resistance measurement and a 30-minute fluorescein flux assay were performed after each experimental condition. Transepithelial resistance was measured in $\Omega.cm^2$ and normalized to the control monolayer resistance. Basolateral compartment fluorescein fluorescence was measured in arbitrary fluorescence units and normalized to the control monolayer fluorescence level. The number of experimental replicates and biological replicates are shown in the figure legends. We define a biological replicate as a unique vial of cells cultured from the stocks stored in liquid nitrogen.

Statistical analysis

Our primary hypothesis was that histamine will disrupt the barrier properties of NHEK monolayers and that larazotide will stabilize the transepithelial barrier, potentially reversing the effects of histamine. Concurrent permeability changes were a secondary associated finding to support our transepithelial resistance results.

In our a priori analysis to estimate the experimental sample size required to achieve a power of 80% with an alpha = 0.05, the SD and effect size were based on our preliminary data (Figure 1). After 45 minutes, histamine decreased resistance to about 80% of the control with an SD of 10%, and after 90 minutes, larazotide increased TEER to about 140% of control with an SD of 12%. On the basis of this, a sample size of 6 monolayers would be required to meet our statistical power using a 1-sample *t*-test compared with a mean of 100%. The sample size was calculated using software G*Power, version 3.1.9.6 (Faul et al, 2007).

Data are presented as multiple technical replicates from different keratinocyte monolayers, which are taken from 2 to 4 different biological replicates (keratinocytes between subculture 2 and 4). The raw data measured in $\Omega.cm^2$ were analyzed using a paired 2-tailed *t*-test, with an $\alpha = 0.05$. The normalized data were analyzed using a 1-sample *t*-test compared with a constant sample mean of 100%, with an $\alpha = 0.05$. Data were plotted using Prism 10.2.0 (GraphPad Software, San Diego, CA).

DATA AVAILABILITY STATEMENT

No large datasets were generated or analyzed during this study. Minimal datasets necessary to interpret and/or replicate data in this paper are available upon request to the corresponding author.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: DMG, GGM; Data Curation: DMG, GGM; Formal Analysis: DMG, GGM; Investigation: DMG, GGM; Methodology: DMG, GGM; Project Administration: GGM; Supervision: GGM; Visualization: DMG, GGM; Writing - Original Draft Preparation: DMG, GGM; Writing - Review and Editing: DMG, GGM

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) OR LARGE LANGUAGE MODELS (LLMs)

The author(s) did not use AI/LLM in any part of the research process and/or manuscript preparation.

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