Infant skin barrier damage inflicted by fecal enzymes and ways to mitigate: Why water is insufficient

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Introduction

Maintaining healthy skin in the diapered region is critical to the overall well-being of an infant. Digestive enzymes found in feces, particularly trypsin and chymotrypsin, are potent skin irritants with the ability to break down the skin barrier and are a primary cause of diaper rash. Trypsin and chymotrypsin function at optimal pH ranges of 7-9[1] and 7.8-8.0[2], respectively. Gentle and effective removal of these enzymes from skin is critical in keeping the skin barrier intact.

While water alone has long been used to clean infant diapered skin, water generally has a neutral pH (pH 7), thus providing an optimal environment for the activity of fecal enzymes that are irritating to skin. On the other hand, properly formulated disposable baby wipes have a slightly acidic pH that complements infant skin pH (pH 5.5[3]), which helps reduce the activity of irritating enzymes present in feces.

In this work, we aimed to evaluate the gentleness and effectiveness of a properly formulated disposable wipe to reduce the activity of enzymes present in feces. Results were compared to water alone or to a water-like wipe.

Methods

A) Impact of fecal enzymes on skin:

In this *in vitro* study, 3D skin equivalents developed with cells derived from neonatal foreskin (MatTek Corp., MA) were exposed to trypsin and chymotrypsin (fecal enzymes), or phosphate buffered saline, for 4 hours. Skin barrier damage was determined via transepithelial electrical resistance using an epithelial voltohmmeter (World Precision Instruments, Cat #EVOM2). Inflammatory response was evaluated by measuring the pro-inflammatory cytokines IL-1α and GM-CSF using a magnetic bead-based enzyme linked immunosorbent assay. Cytokine levels were measured in culture medium at 24 and 48 hour post-treatment collection times.

B) Impact of cleansing products on fecal enzyme activity:

Wipes and water at different pH values, were tested for impact on trypsin/chymotrypsin activity. Four layers of a wipe, cut into 8 mm rounds, or 100µL of water, were added to a well containing fecal enzymes. An enzyme indicator, Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA) was added to each well. Contents were mixed for 10 minutes. After this, enzymatic activity was monitored by measuring absorbance at 405nm with a spectrophotometer.

C) *In vivo* evaluation of wipes gentleness:

30 female adult subjects were tape-stripped on the volar forearm until they reached a TEWL level representative of compromised skin[4] on day 1. On days 1 (after tape-stripping), 2, 3, and 4, test sites were wiped over four sessions with wipes or cloth and water, for a total of 240 wipes/day. Prior to wiping each day, TEWL and colorimetry were collected. A final assessment was taken on day 5; no wiping was conducted on the final visit.

Results

Fecal enzymes induced skin barrier damage in a dose-dependent manner

A dose-dependent increase in barrier damage was observed when skin samples were treated with increasing concentrations of a trypsin and chymotrypsin mixture (corresponds to increased activity of enzymes per cm² of skin) (**Figure 1**).

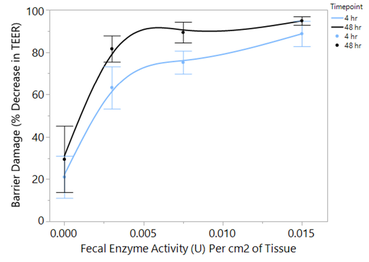

Figure 1: Standard curve of damage to barrier of a full thickness skin model (measured by percent decrease in TEER) with respect to fecal enzyme activity per square centimeter of skin. Barrier damage was assessed immediately after treatment (4 hr) and after a recovery period (48 hr). Data shown as mean ± SD.

Results

Fecal enzymes induced the production of key inflammatory cytokines in a dose-dependent manner

A dose-dependent increase in GM-CSF and IL-1α cytokine production was observed when skin samples were treated with increasing concentrations of a trypsin and chymotrypsin mixture (**Figure 2**).

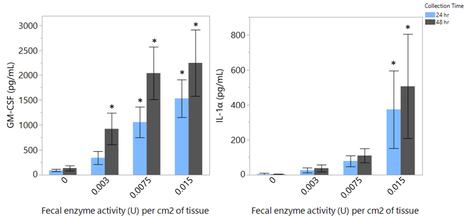

Figure 2: Expression of inflammatory cytokines, GM-CSF and IL-1α, into culture media collected at 24 and 48 hours, by skin equivalents treated with varying concentrations of fecal enzyme mixtures for 4 hours. Error bars shown as mean ± SD. Data shown is representative of three independent experiments. *p<0.05 treatment compared to respective PBS controls (0 U/cm²) at 24 hr or 48 hr, One-Way ANOVA, post-hoc: Dunnett's multiple comparison.

Water alone was not able to significantly inhibit fecal enzyme activity

Water alone did not significantly inhibit fecal enzyme activity (**Figure 3**). At a pH of 7, fecal enzyme activity was high. When the pH of water was reduced to 4.5, a reduction in fecal enzyme activity was observed. However, this pH reduction still resulted in significantly higher fecal enzyme activity compared to formulated wipes at pH 4.5.

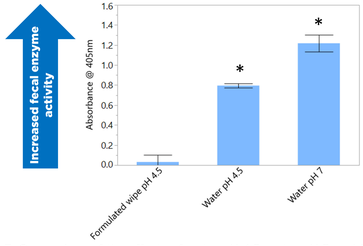

Figure 3: Comparison of the effects of water pH 4.5, water pH 7, and formulated wipe at pH 4.5 on fecal enzyme activity. Increased absorbance at 405nm was proportional to increasing amounts of active enzyme present. Data shown as mean ± SD. *p<0.0001 compared to formulated wipe at pH 4.5, One-Way ANOVA, Tukey's HSD.

Formulated wipes at pH 4.5 significantly inhibited fecal enzyme activity compared to formulated water wipe at pH 7

Formulated wipes at pH 4.5 significantly reduced fecal enzyme activity compared to formulated wipes at a pH 7 (**Figure 4**).

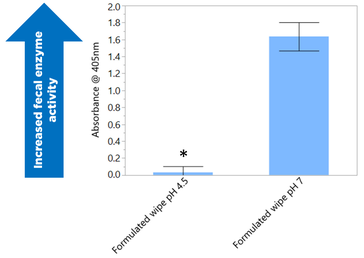

Figure 4: Comparison of the effects of formulated wipes at pH 4.5 and formulated wipes at pH 7 on fecal enzyme activity. Increased absorbance at 405nm was proportional to increasing amounts of active enzyme present. Data shown as mean ± SD. *p<0.0001 compared to formulated wipe at pH 7, Student's t-test.

Wipes properly formulated at pH 4.5 were more gentle on compromised skin than cloth and water or formulated water wipes at pH 7

On days 2, 3, 4, and 5, forearm sites that were repetitively wiped with formulated wipes at pH 4.5 had statistically lower TEWL values compared to forearm sites wiped with cloth and water or formulated water-like wipes at pH 7 (**Figure 5**).

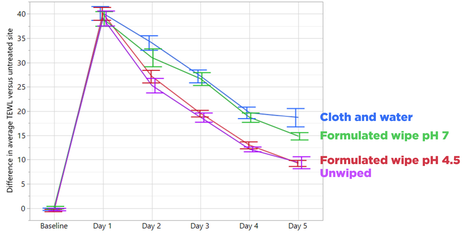

Figure 5: Difference in average TEWL versus untreated site over 4 days of repetitive wiping with different products. No wiping was conducted on day 5. Data shown as mean ± SE. Results were analyzed using an ANOVA model fitted to subject, location, and code effects. Pairwise differences between codes were declared statistically significant at the 95% confidence level.

Compromised skin wiped with formulated wipes at pH 4.5 had lower erythema scores than cloth and water and formulated wipes at pH 7

On day 5, forearm sites that were repetitively wiped with formulated wipes at pH 4.5 had lower erythema values compared to forearm sites wiped with cloth and water or formulated wipes at pH 7 (**Figure 6**).

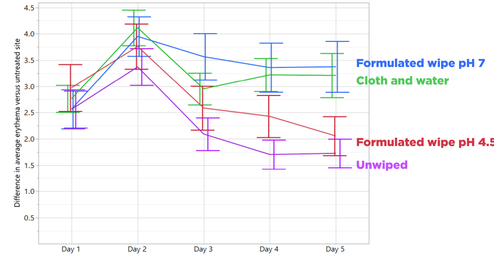

Figure 6: Difference in average erythema versus untreated site over 4 days of repetitive wiping with different products. No wiping was conducted on day 5. Data shown as mean ± SE. Results were analyzed using an ANOVA model fitted to subject, location, and code effects. Pairwise differences between codes were declared statistically significant at the 95% confidence level.

Conclusions

- **Water alone and formulated water-like wipes were not effective** at reducing activity of fecal enzymes and were less gentle on compromised skin, resulting in significantly increased TEWL and erythema from baseline.
- **Wipes formulated at pH 4.5 were found to be more gentle than water and cloth on compromised skin** and provided superior ability to inhibit fecal enzyme activity.
- **Wipes formulated at pH 4.5 did not impact compromised skin barrier** after exaggerated wiping for 4 days, as evidenced by an unchanged TEWL or erythema, compared to baseline.

Acknowledgments

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References

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