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Development of a Model of Dermal Inflammation and Irritation (Urticaria) in Miniature Swine

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ABSTRACT

Histamine, the major mediator of mast cells, is known to induce a short-lived urticaria when applied intradermally in humans. The objective of this study is to develop a reproducible dermal urticaria model in miniature swine. Three female Hanford miniature swine, aged 3 to 18 months, were used in the study. The animals were pricked on their backs with a skin test device that was loaded with vehicle or histamine in vehicle. The irritation and wheal and flare responses of the dermis were evaluated with Draize scoring and with wheal-sized measurement. Histamine dose-dependently induced skin irritation at both 10 minutes and 20 minutes post-treatment. The most prominent erythema and edema (Draize score) responses were observed at 10 minutes post-treatment; the responses were slightly diminished at 20 minutes post-treatment. Histamine also caused skin wheals that ranged from 4 mm to 7 mm in diameter. Although wheal sizes increased over time following treatment, this change appeared unrelated to histamine effect. The optimal concentration of histamine to induce urticaria appeared to be 25 mg/mL. When urticaria was induced with 10 mg/mL histamine, topical antihistamine slightly inhibited both dermal irritation and wheal and flare, whereas topical hydrocortisone inhibited the wheal and flare only. When urticaria was induced with 25 mg/mL and 50 mg/mL histamine, topical antihistamine as well topical hydrocortisone inhibited both dermal irritation and wheal and flare. The inhibitory effects of antihistamine and hydrocortisone were observed at 25 minutes and 45 minutes post-dose. Histamine at 50 mg/mL was shown to induce urticaria with sustained dermal irritation and wheal and flare in Hanford miniature swine. In conclusion, a histamine-induced urticaria model has been established with the female Hanford miniature swine and can be used for the testing of topical treatments.

INTRODUCTION

Urticaria is dermal edema that results from vascular dilation and leakage of fluid into the skin in response to molecules released from mast cells. Histamine, the major mediator of mast cells, is known to induce a short-lived urticaria when applied intra-dermally in humans. Unlike other animals, the pig has a fixed skin tightly attached to the subcutaneous tissues similar to that in humans. In addition, pig and human skins share similar patterns of blood vessels. Biochemically, pig skin contains dermal collagen and has a dermal elastic content that is very similar to that of human skin. Pig and human skin have similar physical and molecular responses to various growth factors. These biological similarities between the skin of human and pig make miniature swine a preferable model for dermal studies. To date, it has not been known how histamine induces urticaria in swine.

EXPERIMENTAL PROCEDURES

Animals:

- Three female Hanford miniature swine
- Ages: 3, 6, and 18 months

Procedures:

- Histamine (Sigma, St Louis, MO) solutions were freshly prepared in 50% glycerin saline.
- Hair was clipped and skin was cleaned.
- Animals were temporarily anesthetized with isoflurane/O_2 for skin prick.
- Backs were pricked with a skin test device (Lincoln Diagnostics Inc, Decatur, IN) that was loaded with histamine solutions.
- Treatment sites were placed 2 cm away from dorsal midline and spaced 4 cm between sites.
- Sites were treated topically with placebo, hydrocortisone (Cortaid®), or antihistamine (Allegra®) creams at 15 minutes following histamine challenge.
- Draize scoring and wheal sizes were measured at different time points.

RESULTS

Table 1a. Effects of Different Concentrations of Histamine on Dermal Irritation (Draize) in a Hanford Miniature Swine (Swine 1)

Treatments	Control (50% Glycerin)		Histamine (3mg/mL)		Histamine (10 mg/mL)		Histamine (25 mg/mL)	
Time Points (Minutes)	10	20	10	20	10	20	10	20
Mean (n=16)	1	1	3	2	5	3	5	4
SD (n=16)	0	1	0	1	1	1	0	0

Table 1b. Effects of Different Concentrations of Histamine on Wheal Diameter (mm) in a Hanford Miniature Swine (Swine 1)

Treatments	Control (50% Glycerin)		Histamine (3mg/mL)		Histamine (10 mg/mL)		Histamine (25 mg/mL)	
Time Points (Minutes)	10	20	10	20	10	20	10	20
Mean (n=16)	2	3	4	5	5	6	6	7
SD (n=16)	1	1	0	0	1	1	1	1

Table 2a. Effects of Different Test Formulations on Histamine-Induced Dermal Irritation (Draize) in a Hanford Miniature Swine (Swine 2)

Histamine	Time Points	10 Min (n=3)		25 Min (n=3)		40 Min (n=3)	
	TEST ARTICLE	MEAN	SD	MEAN	SD	MEAN	SD
10 mg/mL	Placebo	2.0	0.0	2.0	0.0	2.0	0.0
	Cortaid®	2.0	0.0	2.0	0.0	2.0	0.0
	Allegra®	2.0	0.0	1.3	1.2	1.0	1.0
25 mg/mL	Placebo	3.0	0.0	3.3	0.6	2.3	0.6
	Cortaid®	3.0	0.0	2.7	0.6	1.7	0.6
	Allegra®	3.0	0.0	2.3	0.6	1.7	0.6

Table 2b. Effects of Different Test Formulations on Histamine-Induced Wheal Diameter (mm) in a Hanford Miniature Swine (Swine 2)

Histamine	Time Points	10 Min (n=3)		25 Min (n=3)		40 Min (n=3)	
	TEST ARTICLE	MEAN	SD	MEAN	SD	MEAN	SD
10 mg/mL	Placebo	4.7	0.6	5.7	1.2	4.7	0.6
	Cortaid®	2.0	0.0	4.7	0.6	3.7	0.6
	Allegra®	2.7	1.2	2.0	2.0	1.3	1.2
25 mg/mL	Placebo	2.7	0.6	5.0	0.0	4.0	0.0
	Cortaid®	6.0	0.0	4.7	0.6	2.0	1.7
	Allegra®	6.3	0.6	5.0	0.0	1.7	1.5

Figure 1. Urticaria of pig skin challenged with different concentrations of histamine for 10 minutes and 20 minutes (Swine 1)

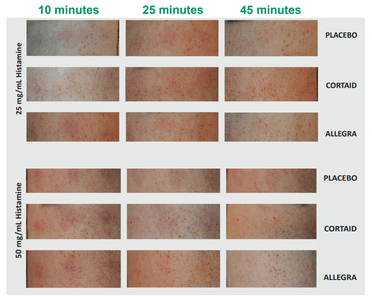

Figure 2. Effects of different test formulations on skin urticaria induced by 25 mg/mL and 50 mg/mL histamine (Swine 3)

Table 3a. Effects of Different Test Formulations on Histamine-Induced Dermal Irritation (Draize) in a Hanford Miniature Swine (Swine 3)

Histamine	Time Points	10 Min (n=3)		25 Min (n=3)		40 Min (n=3)	
	TEST ARTICLE	MEAN	SD	MEAN	SD	MEAN	SD
25 mg/mL	Placebo	3.0	0.0	3.7	0.6	2.0	0.0
	Cortaid®	4.0	0.0	2.7	0.6	1.0	0.0
	Allegra®	3.3	0.6	2.0	0.0	0.3	0.6
50 mg/mL	Placebo	4.3	0.6	5.3	0.6	4.0	0.0
	Cortaid®	4.0	0.0	3.7	0.6	1.0	0.0
	Allegra®	4.0	0.0	2.7	1.2	1.0	0.0

Table 3b. Effects of Different Test Formulations on Histamine-Induced Wheal Diameter (mm) in a Hanford Miniature Swine (Swine 3)

Histamine	Time Points	10 Min (n=3)		25 Min (n=3)		40 Min (n=3)	
	TEST ARTICLE	MEAN	SD	MEAN	SD	MEAN	SD
25 mg/mL	Placebo	7.0	0.0	7.0	0.0	6.3	1.2
	Cortaid®	7.0	0.0	6.3	1.2	4.7	0.6
	Allegra®	6.3	1.2	5.7	1.2	2.7	2.3
50 mg/mL	Placebo	10.3	1.2	11.0	0.0	10.3	1.2
	Cortaid®	10.3	1.2	10.3	1.2	7.0	0.0
	Allegra®	8.3	1.2	7.7	1.2	7.0	0.

DRAIZE SCORING SYSTEM

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-drained erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet red) to slight eschar formation (injuries in depth)	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined)	2
Moderate edema (raised approximately 1mm)	3
Severe edema (raised more than 1mm and extracting beyond the area of exposure)	4
Total highest possible score for irritation	8

SUMMARY

- Histamine dose-dependently induced skin urticaria at both 10 minutes and 20 minutes after treatment.
- Both Allegra® and Cortaid® inhibited urticaria that was induced by 25 mg/mL histamine. The inhibitory effects of Allegra® and Cortaid® were reproducible in two pigs.
- Histamine at 50 mg/mL induced a much more intensive urticaria response than did 20 mg/mL histamine. At the same time, Allegra® and Cortaid® were observed to have more robust effects on reducing urticaria on skin treated with 50 mg/mL histamine.

CONCLUSION

A histamine-induced urticaria model has been successfully established with female Hanford miniature swine.

CONFLICT OF INTEREST

All authors declare that they are employees (or were at the time of the conducted study) of either Altasciences or Sinclair Bio Resources, LLC as denoted on the poster.