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EXPERIMENTAL THIRD DEGREE FROSTBITE IN HANFORD MINIATURE SWINE

JULY 1987

US Army Research Institute of Environmental Medicine
Natick, Massachusetts 01760

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Epithelial necrosis and/or regeneration were seen consistently, but not until 1-2 weeks after injury. Vascular injury was absent for the 1 minute freeze, but was seen by 48 hours for the 5, 10, and 20 minute freezes. Sweat gland injury was seen by 12 hours for all freezes except the 1 minute freeze. Subcutis changes were seen 1-2 weeks after injury.

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Technical Report

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by

Polly Schoning, DVM, Ph.D., and Murray P. Hamlet, DVM

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FORWARD

This experiment was done in 1986 as part of the U. S. Army Summer Faculty Research and Engineering Program administered by Battelle. In addition, Kansas State University has played a major role in providing support for continuation of the project. Kansas State has provided salary to the principal investigator, laboratory space, and clerical and photographic support.

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ABSTRACT:

Third degree frostbite was produced in five Hanford Miniature Swine exposed to chilled air($-75^{o}C$) for 1, 3, 5, 10, or 20 minutes. Biopsies were taken at 0, 3, 6, 12, 24, and 48 hours and 1 and 2 weeks. The 200 slides were evaluated microscopically: epithelial necrosis and regeneration were recorded as absent or present. Vascular degeneration, thrombosis, sweat gland injury, and subcutis injury were ranked from 0-5; 0 = no change and 5 = severe change.

Epithelial necrosis and/or regeneration were seen consistently, but not until 1-2 weeks after injury. Vascular injury was absent for the 1 minute freeze,but was seen by 48 hours for the 5, 10, and 20 minute freezes. Sweat gland injury was seen by 12 hours for all freezes except the 1 minute freeze. Subcutis changes were seen 1-2 weeks after injury.

INTRODUCTION

Frostbite injury is divided into four categories: first degree is characterized by erythema and edema after thawing; vesicles do not occur. Second degree frostbite is characterized by erythema and edema after rewarming and with vesicle formation within 24-48 hours. Third degree frostbite is characterized by necrosis involving all layers of the skin and extending into the subcutis. Fourth degree frostbite involves the entire thickness of an extremity and eventual loss.[1]

In order to test topical ointments that could be used for treatment or prevention of frostbite, it is necessary to be able to repetitively produce frostbite with similar degree of injury. In this experiment, frostbite was produced with chilled air (-75°C), because it most closely duplicates spontaneous frostbite of exposed body areas.

This report documents the development of third degree frostbite from 0 hours to 2 weeks for freezes of 1, 3, 5, 10, and 20 minutes. Only the salient features necessary to access third degree injury are discussed: epithelial necrosis and regeneration, vascular degeneration and thrombosis, sweat gland injury, and subcutaneous injury.

MATERIALS AND METHODS

Five, male, 4-6 month old Hanford Miniature Swine (Charles River Laboratories, Wilmington, MA) weighing 50-65 lbs were used. Prior to experiments, each pig was given an initial dose of .025 mg/lb of atropine and 4 mg/lb of xylazine intramuscularly, followed in 30 minutes by 6 mg/lb of ketamine HCl. Xylazine and ketamine were given alternately as needed for the

duration of the experiment. Each pig was placed in lateral recumbency, shaved, and scrubbed with surgical soap; then a thin coat of iodine solution was applied.

Lesions were produced by cold air ($-75^{\circ}C$) delivered to the skin of the cranial halves of both sides of pigs.[2] The caudal halves were used to test ointments and results are not included in this report. Once the skin thermocouple reached $0^{\circ}C$ or lower, as recorded by the computer, the cold air was continued for 1, 3, 5, 10, or 20 minutes. At the end of that time, the skin was rewarmed with warm air ($25^{\circ}C$) until the computer recorded a skin temperature of $25^{\circ}C$. The freezing cup was then transferred to another skin site and the procedure repeated. One freezing run would produce a column of five one-inch lesions with the 1 minute freeze dorsal and the 20 minute freeze ventral.

The 40 frostbite lesions per pig were scheduled over a three week period so that biopsies could be taken at 0, 3, 6, 12, 24, and 48 hours and 1 and 2 week intervals. One elliptical biopsy was taken from the center of each lesion and fixed in 10% buffered neutral formalin. It was trimmed, embedded, sectioned, and stained with hematoxylin and eosin (HE).

Two hundred slides were evaluated. The following parameters were used:

Epithelial necrosis and regeneration were recorded simply as present (+) or absent (-). Vascular degeneration, thrombosis, sweat gland injury, and subcutaneous injury were evaluated using the following scale:

0 = no change

1 = mild change

2 = mild to moderate change

3 = moderate change

4 = moderate to severe change

5 = severe change

RESULTS

Forty slides were examined for each freeze of 1, 3, 5, 10, and or 20 minute duration. Results are summarized in Tables 1-5.

1 Minute Freeze (Table 1)

Epithelial necrosis was present in 4 skin sections. Epithelial regeneration was present in 9 sections. Vascular regeneration and thrombosis were absent. Severity of moderate to severe was seen 6 times for sweat gland injury and 4 times for subcutaneous injury.

3 Minute Freeze (Table 2)

Epithelial necrosis was present in 7 skin sections. Epithelial regeneration was present in 9 sections. Severity of moderate to severe was seen 6 times for vascular degeneration, 4 times for thrombosis, 21 times for sweat gland injury, and 7 times for subcutaneous injury.

5 Minute Freeze (Table 3)

Epithelial necrosis was present in 12 skin sections. Epithelial regeneration was present in 8 sections. Severity of moderate to severe was seen 11 times for vascular degeneration, 9 times for thrombosis, 25 times for sweat gland injury, and 15 times for subcutaneous injury.

10 Minute Freeze (Table 4)

Epithelial necrosis was present in 6 skin sections. Epithelial regeneration was present in 5 sections. Severity of moderate to severe was seen 14 times for vascular degeneration, 14 times for thrombosis, 27 times for sweat gland injury, and 10 times for subcutaneous injury.

20 Minute Freeze (Table 5)

Epithelial necrosis was present in 11 skin sections. Epithelial regeneration was present in 5 sections. Severity of moderate to severe was seen 16 times for vascular degeneration, 19 times for thrombosis, 20 times for sweat gland injury, and 7 times for subcutaneous injury.

DISCUSSION

In this experiment, frostbite was consistently duplicated by exposing the skin of Hanford Miniature Swine to cold air ($-75^{\circ}C$) for 1, 3, 5, 10, or 20 minutes. However, the changes seen with third degree frostbite - necrosis of all skin layers - were not recognized until 1 - 2 weeks following insult. Tissues examined during the early hours following injury had few changes. It is well documented that clinical assessment of early frostbite is also difficult. Treatment, other than initial thawing, is usually conservative during the early stages.[3-7]

Epithelial necrosis and/or regeneration were seen in all of the 1 and 2 week biopsies with 6 exceptions: 2 had no epithelium to evaluate, 2 had severe epithelial degeneration, and 2 had only mild changes. Fewer necroses were seen in the 1 minute freeze, but more regeneration was present. This finding suggests that milder injury stimulates regeneration, possiblv because the basal layer is intact.

Vascular degeneration and thrombosis were absent from the 1 minute freezes. Injury that was moderate to severe was seen by 48 hours for the 5, 10, and 20 minute freezes.

Altered sweat glands were seen earlier than epithelial necrosis and regeneration and vascular degeneration and thrombosis. For the 3, 5, 10, and 20 minute freezes, injury of moderate to severe severity was seen by 12 hours. Sweat gland injury of moderate severity was not seen before 1 week for the 1 minute freeze.

Alterations of the subcutis were seldom seen before 1 week following injury and were more common in the 5, 10, and 20 minute freezes. Subcutaneous changes were inflammatory and assumed to be in response to the injured skin.

CONCLUSIONS

1) Third degree frostbite was produced repetively using cold air ($-75^{o}C$).

2) However, third degree frostbite can not be determined, microscopically, until 1-2 weeks after injury. Biopsies taken before that time are not severely changed.

3) There is still a need for an animal model for second degree frostbite.

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Table 1 - Degree of injury in pig skin frozen with cold air (-75 C) for 1 minute.

	Biopsy Time																					
	hours																		weeks			
	0					3					6					12					24					48					1					2				
Epithelial necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	+	0	0	0	0	0	-
Epithelial regeneration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	0	+	+	+	+	+
Vascular degeneration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Thrombosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	0	0
Sweat gland injury	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	2	0	0	1	0	2	4	4	3	2	3	0	0	3	0	3	1
Subcutis injury	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	3	4	-	3	0	0	1	-	-	3

(+) = present, (-) = absent; 0 = no change, 1 = mild change, 2 = mild to moderate, 3 = moderate, 4 = moderate to severe, 5 = severe change.

Table 2 - Degree of injury in pig skin frozen with cold air (-75°C) for 3 minutes.

	Biopsy Time																					
	hours																		weeks			
	0					3					6					12					24					48					1					2				
Epithelial necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	+	+	+	0	+	0	-
Epithelial regeneration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	+	+	0	+	+	+	+	+	+	+
Vascular degeneration	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	3	4	0	0	0	0	3	4	4	3	0	0	0	0	0	0	0	0
Thrombosis	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	4	0	0	0	0	3	4	0	3	0	0	0	0	0	0	0	0
Sweat gland injury	0	0	0	0	0	0	1	2	0	3	0	0	1	0	0	3	3	3	2	0	3	3	2	3	3	3	4	4	4	4	4	5	3	3	2	0	3	3	4	3
Subcutis injury	-	-	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	2	2	2	0	0	3	3	2	0	4	4	0	3	3	2	0	0	0	3

(+) = present, (-) = absent; 0 = no change, 1 = mild change, 2 = mild to moderate, 3 = moderate, 4 = moderate to severe, 5 = severe change.

Table 3 - Degree of injury in pig skin frozen with cold air (-75°C) for 5 minutes.

	Biopsy Time							
	hours						weeks	
	0	3	6	12	24	48	1	2
Epithelial necrosis	0 0 0 0 0	0 0 0 0 -	0 0 0 0 0	0 0 0 0 +	0 0 0 0 +	0 0 0 0 +	+ + + + +	+ + 0 + +
Epithelial regeneration	0 0 0 0 0	0 0 0 0 -	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 +	+ + 0 + +	+ + + 0 0
Vascular degeneration	0 0 0 0 0	0 0 0 2 0	0 0 0 2 2	1 0 0 2 0	2 0 0 3 0	0 0 3 3 4	4 3 5 4 4	4 - 0 0 5
Thrombosis	0 0 0 0 0	0 0 0 2 2	0 0 0 2 2	2 0 0 2 2	2 0 0 3 0	0 0 3 4 4	4 3 2 4 4	4 - 0 0 0
Sweat gland injury	1 0 0 2 0	2 2 1 3 3	0 2 1 3 3	3 2 3 3 3	3 2 3 4 3	3 3 4 5 4	4 5 5 5 4	- - 3 5 5
Subcutis injury	0 0 0 0 0	0 0 0 0 0	2 0 0 3 0	0 0 3 3 0	0 0 3 3 2	2 3 0 2 1	3 3 4 3 3	4 5 2 3 3

(+) = present, (-) = absent; 0 = no change, 1 = mild change, 2 = mild to moderate, 3 = moderate, 4 = moderate to severe, 5 = severe change

Table 4 - Degree of injury in pig skin frozen with cold air (-75°C) for 10 minutes.

	Biopsy Time							
	hours						weeks	
	0	3	6	12	24	48	1	2
Epithelial necrosis	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 + + +	+ + 0 - +
Epithelial regeneration	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 + +	- 0 + + +
Vascular degeneration	0 0 0 0 0	0 0 0 0 3	0 0 0 2 3	0 2 2 2 3	2 0 2 2 4	4 4 4 3 4	4 4 4 3 5	- - 0 - 0
Thrombosis	0 0 0 0 0	0 0 0 2 3	0 0 0 2 3	2 2 2 2 3	2 0 0 3 4	4 4 3 3 4	4 3 3 2 5	- - 0 - 0
Sweat gland injury	0 0 0 0 0	2 3 1 2 3	3 3 - 2 3	3 3 3 4 4	3 3 2 4 4	4 4 4 - 4	4 5 5 5 5	5 5 4 5 -
Subcutis injury	0 0 0 0 0	0 0 0 0 0	1 0 0 0 0	0 0 0 2 0	0 0 - 2 0	0 0 0 3 0	4 4 3 0 3	4 5 3 4 4

(+) = present, (-) = absent; 0 = no change, 1 = mild change, 2 = mild to moderate, 3 = moderate, 4 = moderate to severe, 5 = severe change

Table 5 = Degree of injury in pig skin frozen with cold air (-75°C) for 20 minutes.

	Biopsy Time							
	hours						weeks	
	0	3	6	12	24	48	1	2
Epithelial necrosis	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	- 0 0 0 0	0 0 0 0 0	+ 0 + 0 +	+ + + + +	- + - + +
Epithelial regeneration	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	- 0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 + +	- + - + +
Vascular degeneration	0 0 0 0 0	0 0 0 0 2	0 1 0 2 3	0 3 3 3 3	2 2 - 2 2	4 2 4 3 4	5 5 - 4 4	5 3 5 - 0
Thrombosis	0 0 0 0 2	0 0 0 2 3	0 1 0 2 3	3 3 3 3 3	2 2 - 3 3	4 2 4 3 4	5 5 - 4 4	0 3 5 - 0
Sweat gland injury	0 0 1 0 -	0 1 2 2 -	3 3 2 2 -	3 3 - 3 4	3 3 - 3 -	4 3 4 - -	5 5 5 5 -	- 5 5 4 5
Subcutis injury	0 0 0 0 0	0 0 0 0 0	1 0 0 0 0	0 0 0 0 0	0 0 - 0 0	0 0 0 0 0	4 0 0 0 0	5 5 5 4 4

(+) = present, (-) = absent; 0 = no change, 1 = mild change, 2 = mild to moderate, 3 = moderate, 4 = moderate to severe, 5 = severe change

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