Simulation of Limb Frostbites in Air Medium

B. A. Paramonov, I. I. Turkovskii, V. N. Taranova, K. P. Pomorski, and V. Yu. Chebotaryov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 3, pp. 358-360, March, 2009
Original article submitted February 7, 2008

The clinical course of frostbites of the limbs was studied in 3 animal species (swine, dog, rabbit) with frostbites of different intensity and duration. Animals of different species exhibited different sensitivity of their limb tissues to cold. The rabbit proved to be the most adequate animal for frostbite simulation. Frostbites of similar area and depth of lesions can be induced by cooling according to different protocols.

Key Words: *cooling; frostbites*

Frostbites develop only after exposure to cold within a certain period of time; the length of this period, in turn, depends on the cooling characteristics of the environment: the more intense is the cooling - the shorter is the exposure inducing tissue damage [1]. The severity of lesions in humans depends to a certain measure on individual sensitivity to cold [3]. Animals of different species are characterized by different cold sensitivity. Experimental studies are needed for the development and optimization of methods for the treatment of frostbites. High degree of reversion of the pathological process in pathogenetically justified therapy is characteristic of frostbites. It is therefore essential to develop protocols for inducing frostbites of the needed depth and size [2]. Another important problem is to select the model animal fit for studies of the pathogenesis of a local cold injury.

We studied the course of a local cold injury in swine, dogs, and rabbits with frostbites induced by different cooling protocols.

MATERIALS AND METHODS

The study was carried out at Experimental Animal Clinic No. 2, Military Medical Academy, and at vivarium of St. Petersburg Medical Academy for Continuous Education. Experiments were carried out on animals of 3 species: mini sibs swine (2 groups, 3 animals per group), mongrel dogs (5 groups of 5-7 animals, a total of 26 dogs), and random-bred rabbits (9 groups of 6-8 animals, a total of 56 rabbits).

In order to induce frostbites of the limbs in laboratory animals, similar to those under which frostbites of the hands emerge in humans, a special device was designed for simulation of cooling according to different protocols (Table 1) and wind of different velocity. Frostbites in this device are caused without contact by cold air flow, aimed at the animal limb. The device consists of a freeze box with holes in its upper cover, through which the limbs of experimental animal are put inside, and a cooling block. The cooling block consisted of a compressor, receiver, filter, cooling battery, generator regulator, and a temperature control tap. There is a ventilator with controlled rotation velocity in the freeze chamber and a control panel outside. The temperature in the freezer is monitored with an electric thermometer. After the device is switched on, the temperature in the freeze box reduces due to the cooling block's work. After attaining the desired temperature (-10°C, -20°C) the animal is fixed to the box surface with its limb in the box. The temperature is maintained in an automated mode. The limbs are placed at a distance of 50 cm from the box walls. Airflow velocity inside the box was 0.4 m/sec.

High Technologies Firm, St. Petersburg, Russia. ***Address for correspondence:*** gloria@home.ru. B. A. Paramonov

0007-4888/09/1473-0381

One day before the experiment the fur on the limbs was shaven. The animals were fixed to the device, the limb was put (through the hole in the cover) into the freezer, in which the temperature needed for experiment was created using the temperature controller. The limb was exposed in the cold during the preset time. Rectal temperature was measured with TPEM-1 electric thermometer throughout the entire exposure, the general status and behavior were recorded. After exposure of needed duration the animal was set free and clinical symptoms were evaluated: skin color of the limb exposed to cold, soft tissue consistency, mobility of the joints, status of subcutaneous veins, presence of visible borderline of injury, skin temperature in standard sites (on the sole, shin, crural joint, knee joint, and hip). Skin temperature was measured by applying a mercuric low-temperature thermometer to the skin integument.

The following parameters were evaluated during the reactive period of the injury (24 h after experiment): skin color, soft tissue consistency, mobility of joints, presence and dissemination of edema, formation of wounds, necrotic areas, pre-

TABLE 1. Conditions of Cooling in Experimental Groups

Animals, *n*		Cooling conditions	
		temperature, °C	exposure, h
Swine	3	-20	3
	3	-20	4
Dogs	5	-20	0.5
	5	-20	1
	5	-20	2
	7	-20	3
	5	-20	4
Rabbits	6	-20	1
	6	-20	2
	6	-20	3
	6	-20	4
	8	-20	5
	6	-10	2
	6	-10	4
	6	-10	6
	6	-10	8

TABLE 2. Differences in Clinical Picture of Frostbites in Rabbits and Dogs after Cooling of the Limb at -20°C

Duration of cooling	Signs by which differences were detected	Animals, *n*		*p*
		dogs	rabbits	
2 h	Edema during reactive period	0/5	6/6	0.025
3 h	Changed consistency of soft tissues	0/7	8/8	0.025
	Immobility of fingers	0/7	8/8	0.025
	Difficult passive movements of the talocrural joint	0/7	8/8	0.025
	Edema during reactive period	0/7	8/8	0.025
	Soft tissue necrosis during reactive period	0/7	8/8	0.025

Note. Numerator: presence of the sign; denominator: number of animals.

TABLE 3. Time Course of Rabbit Limb Skin Temperature after Exposure to Cold ($M \pm m$, °C)

Cooling conditions		After cold exposure		On day 2		
temperature, °C	duration, h	shin	hip	sole	shin	hip
-20	1	27.2±2.3	31.3±1.6	35.0±1.4	32.7±1.4	No data
-20	2	20.5±1.9	26.8±1.7	30.3±1.3	32.9±1.4	34.3±1.5
-20	3	11.0±2.0	16.0±3.0	20.0±2.0	32.8±1.2	33.0±3.0
-20	4	10.1±3.3	15.6±2.1	17.5±1.5	23.0±3.0	32.5±2.5
-20	5	7.0±2.0	15.0±2.5	18.0±1.8	20.0±2.1	30.5±1.4
-10	2	26.5±1.6	29.4±1.2	30.4±1.3	32.4±1.6	35.2±1.4
-10	4	17.3±1.6	24.3±2.2	30.6±1.8	31.8±1.5	35.2±1.4
-10	6	10.8±2.5	15.7±2.4	20.5±1.5	30.0±1.4	34.0±2.0
-10	8	6.2±1.5	13.5±1.6	20.5±1.0	25.1±2.0	31.3±1.3

sence and level of visible borderline of injury, skin temperature, and the way the animal used the limb during walking. These data were recorded in the file (standard case history). The observation was carried out during 20 days.

The significance of differences was evaluated using Student's t test ($p<0.05$). The differences were identified by methods of nonparametrical statistical analysis: analysis of dispersions (Fisher's test), Mann—Whitney's test, and χ^2 test (Pirson's test).

RESULTS

Simulation of frostbites in the air showed differences in the sensitivity of animals of different species to cooling. Swine proved to be unfit for studies of frostbites in humans, though their skin structure is similar to human. Dog limb tissues were more sensitive to local cold exposure in comparison with swine, but were less sensitive than the rabbit limb soft tissues (Table 2).

The clinical picture of frostbite in rabbits (Table 3) was the most close to that in humans. Measurement of skin temperature directly after cold exposure showed freezing of the soles in all groups. Based on the results, we developed protocols for inducing frostbites of the needed size and depth.

Clinical manifestations of cold injury increased with prolongation and intensification of cold exposure; in other words, frostbites with tissue injuries of similar size and depth can be obtained by different cooling protocols.

Hence, protocols of the rabbit limb cooling were developed, using which it is possible to induce frostbites of different depth and size.

REFERENCES

1. A. Barton and O. Edholm, *Human under Conditions of Cold* [in Russian], Moscow (1957).
2. V. P. Kotel'nikov, *Frostbites* [in Russian], Moscow (1988).
3. E. V. Maistrakh, *Thermoregulation Physiology, Manual of Physiology* [in Russian], Leningrad (1984), pp. 181-218.