Body Temperature Drops as a Humane Endopoint in Sanke Venom - Lethality Neutralization Tests

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INTRODUCTION

Snakebite envenoming is a neglected and life-threatening disease that constitutes a public health problem worldwide. Evaluation of the antivenom potency is performed by analyzing the neutralization of venom-induced lethality in mice. Since venom-neutralizing potency tests are performed on animals, it is essential to follow ethical conducts and guidelines that guarantee minimum animal suffering. Much concern has been expressed about the suffering of animals during these procedures, and several actions have been proposed to mitigate it, including: (i) reducing the number of animals used in the assays; (ii) reducing the time of the lethality assessment; (iii) the use of analgesia; and (iv) the application of a humane endpoint [1,2]. Variation in body temperature in rodents has been suggested as a criterion for establishing a humane endpoint in a variety of models [3,4]. A drop in body temperature is an indicator of disease or toxemia in animals that has been correlated with death in infectious diseases models. We assessed the body temperature of animals during venom-neutralizing potency tests to determine if this parameter could be used as a predictor of mortality. BALB/c mice were exposed to venom from ***Bothrops asper*** or ***Lachesis stenophrys*** mixed with polyvalent antivenom. We observed that, based on the temperature change from **baseli**ne, it is possible to predict which animals will survive during the first 3 h after inoculation.

MATERIALS and METHODS

Animals -
Female BALB/c mice, weighing 20–22 g, were provided by the INDICASAT Animal Facility. Animals were maintained with a 12 h light/dark cycle, at a constant temperature of 24°C with free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of INDICASAT (IACUC-22-005).

Mixtures of venom and anti-venom
were prepared by adding a constant amount of venom (corresponding to 4xLD50 for each venom per animal) with variable concentrations of antivenom. The proportions were 2, 3, 4.5, and 6.75 mg of venom/mL of antivenom. Mixtures were incubated in a bath at 37°C for 30 min. A total of five animals per group (four groups) were inoculated intraperitoneally (ip) with 0.5 mL of the mixtures. An additional control group inoculated only with venom resuspended in PBS was included. Death was recorded for a period of 48 h.

Temperature Measurement
A non-contact infrared thermometer was used to measure the surface temperature of animals in the perianal region. The temperature was measured just before inoculation (baseline), 1, 2, and 3 h post-inoculation and additionally, for the surviving animals, at 24 and 48 h.

RESULTADOS

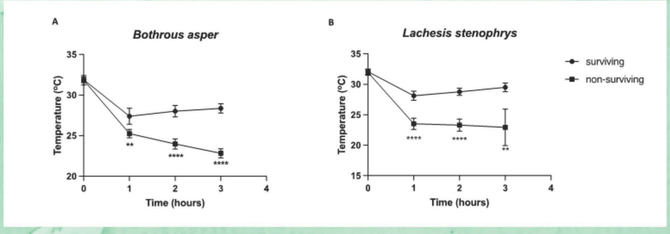

Figure shows the temperature differences between surviving and non-surviving mice. Graphs represent mean ± 95% CI of temperature from surviving (n = 40 for *B. asper* (A); n = 39 for *L. stenophrys* (B)) and non-surviving (n = 35 for *B. asper* (A); n = 35 for *L. stenophrys* (B)) mice. Animals that did not survive had significantly lower temperatures at all time points (except at 0 h) relative to animals that survived, (** $p < 0.01$; **** $p < 0.0001$). Results show that the pattern of temperature change across time points in surviving and non-surviving animals was similar for both venom types. Significant differences between surviving and non-surviving animals were evident at each time point.

	Time of temperature measurement	Mean temperature change (SD)	95% CI
Outcome			
Survived	1 h	3.87 (0.38)	3.11, 4.63
	2 h	3.26 (0.32)	2.62, 3.90
	3 h	2.95 (0.30)	2.35, 3.54
Did not survive			
	1 h	6.88 (0.47)	5.95, 7.80
	2 h	7.91 (0.39)	7.13, 8.69
	3 h	8.88 (0.36)	8.15, 9.60

Table shows for animals that survived, at 1 h, the average temperature drop was 3.9°C (95% CI: 3.1–4.6). At this time point, the animals that would not survive had a temperature variation relative to a baseline of 6.9°C (95% CI: 6.0–7.8). These results suggest that, for this experimental set up of using the venoms of *B. asper* and *L. stenophrys*, animals that reach a drop in temperature equal to or greater than 6°C will not survive.

CONCLUSIONS

Our results show that drop in temperature equal or greater than 6°C within the first 3 h of inoculation with a combination of *B. asper* or *L. stenophrys* venoms and antivenom is a predictor of mortality in BALB/c mice.

REFERENCES

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