Experimental Hypothermia Murine Model for Spinal Cord Injury Studies

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ABSTRACT

Given the complexity of hypothermal trial systems in murines, they are expensive. Our objective was to evaluate if the exogenous hypothermal model used in our laboratory for ocular hypothermia was useful for a significant reduction in medullar spine temperature in adult murines. **Materials and Methods:** 36 60-day-old adult male Sprague-Dawley rats were used. They were separated into two groups: a normal temperature group at 24 °C (n=18) and a hypothermia group in a cold chamber at 8 °C for 180 minutes (n=18). **Results:** The mean rectal temperature was 37.71 ºC ± 0.572 in the normothermia group and 34.03ºC ± 0.250 in the hypothermia group (p <0.0001). The mean medullar temperature was 38.8 ± 0.468 ºC in the normothermia group and 36.4 ± 0.290 ºC in the hypothermia group (p <0.0001). **Conclusion:** Using systematic hypothermia in lab rats seems to be promising to evaluate physiologic and pathological mechanisms triggered in the medullar spine. Exposure to cold in the external chamber produces significant medullar hypothermia in adult rats. Results suggest this might be an adequate and inexpensive medullar hypothermal model.

Key words: Hypothermia; murines; spinal cord.

Level of Evidence: III

Modelo de hipotermia experimental en murinos para estudios de lesión medular

RESUMEN

Introducción: Los ensayos de hipotermia sistémica en murinos son costosos, debido a la complejidad de los sistemas. El objetivo de este estudio fue evaluar si el modelo de hipotermia sistémica exógena utilizado en nuestro laboratorio para la hipotermia ocular es útil para reducir significativamente la temperatura de la médula espinal en ratas adultas. **Materiales y Métodos:** Se utilizaron 36 ratas Sprague-Dawley albinas macho de 60 días, distribuidas en dos grupos: grupo normotermia a 24 ºC (n = 18) y grupo hipotermia (n = 18) en cámara fría a 8 ºC durante 180 minutos. **Resultados:** La temperatura rectal promedio fue de 37,71 ± 0,572 °C en el grupo normotermia y 34,03 ± 0,250 °C en el grupo hipotermia (p <0,0001). La temperatura medular promedio fue de 38,8 ± 0,468 ºC en el grupo normotermia y de 36,4 ± 0,290 ºC en el grupo hipotermia (p <0,0001). **Conclusiones:** El uso de hipotermia sistémica en ratas de laboratorio parece ser un método prometedor para evaluar los mecanismos fisiológicos y patológicos que se desencadenan en la médula espinal. La exposición al frío en cámara genera hipotermia medular significativa en ratas adultas. Los resultados sugieren que podría ser un modelo adecuado de hipotermia medular de bajo costo.

Palabras clave: Hipotermia; murinos; médula espinal.

Nivel de Evidencia: III

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INTRODUCTION

Several studies conducted since the 1990s have shown that hypothermia reduces central nervous system damage in an experimental model of severe perinatal asphyxia in rats, with encouraging results in medullary, ocular, and brain pathology.[1-4] These studies were conducted primarily in models with newborn rats. To evaluate adults, it was necessary to develop new models of systemic hypothermia that were easy to apply and low cost, since the usual models have some technical difficulty and a high economic cost for our field.

In our laboratory, we used a model of exogenous systemic hypothermia in adult rats used for eye injury (8 ºC of ambient temperature), which triggers a sufficient stimulus for the increase in the expression of a particular type of cold-inducible proteins with neuroprotective effect.[5] It is proposed that this model developed for eye injuries could be suitable as a model of hypothermia for spinal cord injuries.

The objective of this study was to evaluate whether the exogenous systemic hypothermia model used in our laboratory for ocular hypothermia is useful for significantly reducing spinal cord temperature in adult rats.

MATERIALS AND METHODS

The procedures for handling and treating animals were carried out in accordance with the care guidelines for laboratory animals[6] and CICUAL UBA standards.[7]

60-day-old adult male albino Sprague-Dawley rats were used. The animals were kept under standard laboratory conditions. In total, 36 rats were used, which were distributed in two groups: the normothermia group at 24 ºC (n = 18) and the hypothermia group (n = 18). For hypothermia, the animals were placed in a cold chamber at 8 ºC for 180 min, with hydration *ad libitum*.[5] Temperature was measured with a digital thermometer (TES-1300, TES Electrical Electronic Corp., Taipei, Taiwan) connected to a K-type thermocouple (TPK-01).

The animals of the hypothermia group were euthanized immediately after being removed from the refrigerator. All animals were euthanized by decapitation with a guillotine. Immediately afterward, intramedullary and intrarectal temperatures were measured.

The results are presented as mean ± standard deviation. Comparisons were made using Student's t-test for independent samples. A p-value <0.05 was considered significant.

RESULTS

The average rectal temperature was 37.71 ± 0.572 °C in the normothermia group and 34.03 ± 0.250 °C in the hypothermia group. The difference was statistically significant (p <0.0001). The average medullary temperature was 38.8 ±0.468 ºC in the normothermia group and 36.4 ±0.290 ºC in the hypothermia group (p <0.0001). The average temperature difference between the groups was 3.68 ºC in the rectum and 2.4 ºC in the marrow (Table and Figure).

A greater gradient between rectal and intramedullary temperatures was observed in the hypothermia group (+1.1 ºC in the normothermia group and +2.4 ºC in the hypothermia group).

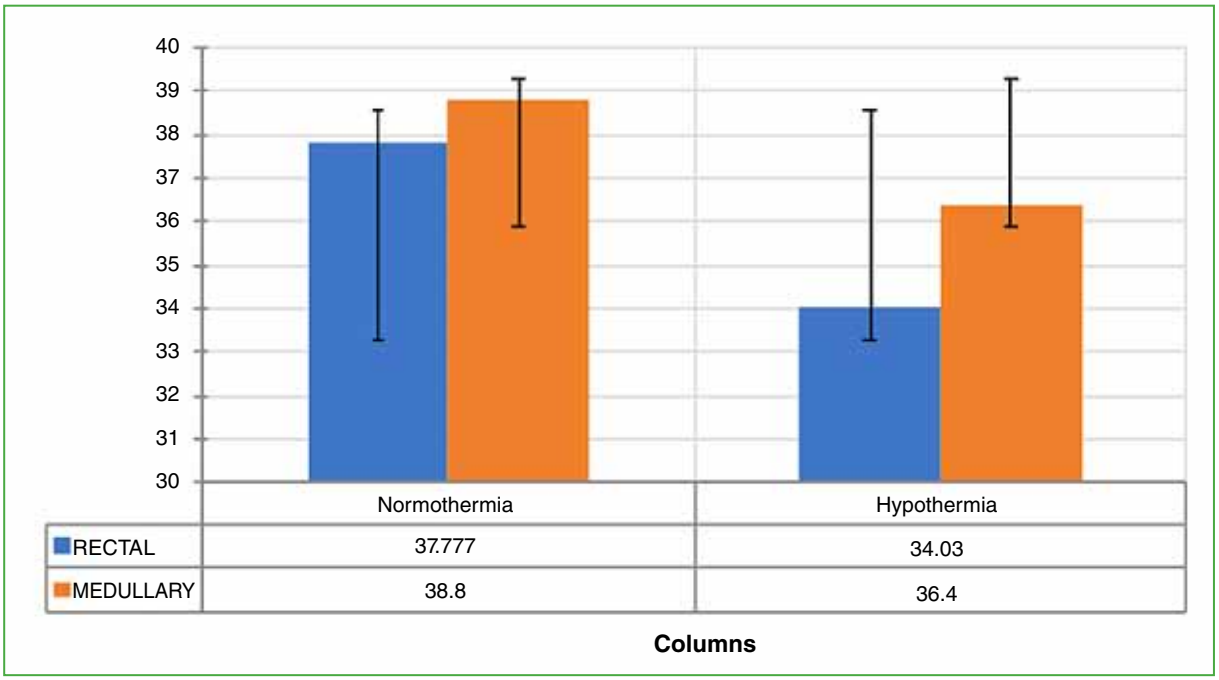

Figure. Contingency table with standard deviations of rectal and medullary temperature in normothermia and hypothermia.

Table. Rectal and medullary temperature values in normothermia and hypothermia.

Normothermia		Hypothermia	
Rectal	Medullary	Rectal	Medullary
38	38.8	34.4	36.8
36.3	37.8	34.5	36.9
38.3	39.3	33.8	36.6
37.2	38.9	34.2	36.3
37.6	39	34	36.2
38	39.1	34	36.6
38	39.2	33.8	36.4
38.2	38.5	34.1	35.9
38.1	38.6	33.9	36.5
38.3	38.8	33.7	36
36.7	37.9	34.2	36.1
36.9	38	33.5	36.4
37.8	39	33.9	36.6
37.7	39.1	34.2	36.1
38.1	39.3	34.3	36.2
38	39.2	34	36.8
37.7	38.9	34.1	36.6
37.9	39	34	36.3
37.71111111	**38.8**	**34.03333333**	**36.40555556**
38.3	**39.3**	**34.5**	**36.9**
36.3	**37.8**	**33.5**	**35.9**

DISCUSSION

From the experimental point of view, induced systemic hypothermia has neuroprotective properties in cerebral[8] and medullary[9] ischemia. In asphyxia, at the medullary level, the overproduction of nitric oxide manifests itself as a structural and functional injury. This situation can be prevented by inhibiting the enzyme nitric oxide synthase by using hypothermia in neonates.[1,10]

Systemic hypothermia assays in experimental murine models pose great difficulties due to acquisition costs, the complexity of the systems and the coolers by the circulation of ice water,[11] or the cold chamber-induced coma,[12]or even transrectal cooling systems that require several hours.[13]

Based on the published experience,[5] we decided to evaluate the possibility of applying a simple and low-cost method to obtain a medullary cooling model that was useful for laboratory tests. The results of this research show data in favor of the usefulness of a model of exogenous systemic hypothermia that is usually used in our laboratory for ocular hypothermia in adult rats. We observed that the simple application of cold in the chamber at 8 ºC for 3 hours managed to significantly reduce the temperature of the spinal cord.

The use of hypothermia has been proposed as a neuroprotective treatment in humans. In term infants with hypoxic-ischemic encephalopathy, benefits were observed from selective cooling of the head (10 °C), mild systemic hypothermia (34.5-35 °C), and also from the combination of both methods.[14,15]

Scientific evidence also suggests using therapeutic hypothermia in patients with acute spinal cord injury.[9] Due to the medical costs associated with spinal cord injuries and the lack of effective treatments, there is an ongoing need to evaluate new therapies that can be initiated in the acute period of the injury to limit secondary injury mechanisms and improve functional outcomes in this patient population.[16-18]

Cold therapies can be local or systemic. Regional medullary hypothermia through epidural cooling provided cytoprotection in the prophylaxis of medullary ischemia during aortic surgery,[19] but due to the complexity of the procedure, systemic hypothermia was chosen.[20] In cases of spinal cord injury, relatively mild levels of systemic hypothermia after the injury improve function and reduce histopathological damage.[21,22] Moderate systemically induced hypothermia is protective in a variety of spinal cord injury models and therefore deserves consideration for clinical application.

Despite its potential applications, hypothermia poses a problem: the difficulty of applying cold to some specific organs or regions of the organism due to the ability of homeothermic organisms to regulate temperature.[23] To apply hypothermia to internal organs, such as the brain or marrow, it is necessary to cool the blood with an external circulation system,[24] while, in newborns, a fairly elaborate device is necessary and that is not easily affordable for hospitals or dispensaries in developing countries.[25]

CONCLUSION

Exposure to cold in a chamber at 8 °C for 3 hours generates significant medullary hypothermia in 60-day-old male albino Sprague-Dawley rats. The results suggest that it could be a suitable model of low-cost medullary hypothermia.

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