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Dihydrocapsaicin-induced Hypothermia after Asphyxial Cardiac Arrest in Rats

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Abstract

Cardiac arrest (CA) is one of the leading causes of mortality and morbidity in the world. Fast, reversible and controllable pharmaceutical-induced hypothermia (PIH) is strongly desired to treat ischemia-reperfusion brain injury. Dihydrocapsaicin (DHC), an agonist of transient receptor potential vanilloid type 1 cation channel (TRPV1), is an emerging candidate for PIH. Its capability to lower body temperature has been validated in both healthy and stroke animal models. However, DHC has shown cardiovascular effects and its safety and feasibility in a CA model has not been tested. Additionally, activated TRPV1 has multiple functions in addition to regulating body temperature and its effect on neurological recovery needs to be evaluated. In this study, we compared two methods of DHC administration, bolus injection and infusion via the femoral vein. We found that cardiovascular effects were only seen with a large dose DHC bolus injection. Then, we applied DHC-induced hypothermia in an asphyxial-CA rat model. We showed that DHC-treated rats were viable. Four-hour infusion of DHC at a rate of 0.75 mg/kg/h after CA maintained a body temperature of about 34 °C for at least 8 hours. DHC-treated rats had higher electrical activity during the first 4 hours after CA and had better neurological recovery during the 3 days after CA compared with normothermia rats. Additional pathway investigation of DHC administration following CA will further uncover the benefits of DHC-induced hypothermia.

I. Introduction

Cardiac arrest (CA) is one of the leading causes of mortality and morbidity in the world. Therapeutic hyperthermia (TH) is the most effective treatment for CA patients [1]. At present, clinical TH protocols involve methods of physical cooling, of which one of the shortcomings is the shivering response that must be countered by sedatives and mechanical ventilation, which complicate the prognosis and recovery [2]. Therefore, fast, reversible and

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controllable pharmaceutical-induced hypothermia (PIH) would provide a good alternative methodology.

Several candidates targeting distinct signaling pathways have been tested for PIH including a neurotensin receptor agonist HPI201/ABS201 in stroke and TBI models [3, 4], a cannabinoid receptor agonist WIN55, 212-2 in a ventricular defibrillation CA model [5], the A1 adenosine receptor agonist 8-SPT in an asphyxial-CA model [6], the muscarinic cholinergic partial agonist U-80816E in the gerbil brief bilateral carotid occlusion ischemia model [7], a dopaminergic agonist Talipexole in stroke model [8], a serotonergic agonist S14671 in a stroke model [9] and the transient receptor potential cation channel vanilloid type 1 (TRPV1) agonist dihydrocapsaicin (DHC) in a stroke model [10].

Body temperature is often dysregulated after CA, embodied by either fever or spontaneous hypothermia. TRPV1 has a role in body temperature sensation and regulation [11]. TRPV1 is widely expressed in all tissues and activated by heat (> 43 °C), low pH and some chemicals such as capsaicin and ethanol. DHC, a capsaicin analog, is roughly 65% more effective than capsaicin in producing hypothermia [12], and is consequently a promising candidate for PIH. The capability of DHC to lower body temperature has been validated in healthy mice, rats, cows and monkeys [13, 14] and in stroke mice [10]. However, DHC has displayed cardiovascular effects [15], and whether CA victims could tolerate DHC has not been determined. Since activating TRPV1 may be protective or detrimental in neuronal injury [10, 16, 17], it is unclear whether or not CA victims could benefit from DHC administration.

In this study, we tested the applicability of DHC-induced hypothermia in an asphyxial-CA rat model by comparing the electrical activity during the first 4 hours after CA and the neurological deficit score (NDS) during the 3 days after CA, between DHC-treated rats and the vehicle-treated rats.

II. Methods

The experimental protocols were approved by the University of Maryland Animal Care and Use Committee. A total of nine adult Wistar rats (300–350 g) were used. Three rats were used to test the DHC administration methods. Six rats were randomly allocated into two groups (DHC-treated CA vs normothermia CA vehicle control, n=3 per group) for the asphyxial-CA model.

A. Asphyxial-CA model

The experimental procedure to induce CA and resuscitation was modified from previously published papers [18–20]. Briefly, rats were implanted with cortical electrodes 3 days before CA. On the day of CA, rats were anesthetized by 1.5% isoflurane and intubated. The femoral artery and vein were cannulated to administer drugs and monitor mean arterial blood pressure (MAP). Before CA, there was a 2-min anesthesia washout period with 100% oxygen, then the rats were paralyzed via an i.v. bolus injection of vecuronium (2 mg/kg) followed by 3 min of room air ventilation. CA was induced by clamping the breathing circuit and disconnecting the ventilator for 9 min. The time of CA was recorded when pulse

pressure <10 mmHg. Cardiopulmonary resuscitation (CPR) was initiated immediately after 9 min of asphyxia with oxygenation, sternal chest compressions, epinephrine and $NaHCO_3$ until MAP > 60 mmHg, which we defined as return of spontaneous circulation (ROSC). The femoral arterial blood gases (ABG) were measured before CA and 20 min after ROSC. Electroencephalogram (EEG) and electrocardiogram (ECG) were monitored by electrodes connected to a TDT System3 data acquisition system (Tucker-Davis Technologies, Alachua, FL). Core temperature was measured with a rectal probe and recorded periodically.

B. DHC-induced hypothermia

DHC (Cayman, Ann Arbor, MI) was dissolved in dimethylsulfoxide (DMSO) to 33 mg/ml as stock solution and stored at −20 °C. On the day of CA, the DHC solution was diluted in 2% Tween-20 saline to a final concentration of 0.75 mg/ml [14]. First, two methods were employed to deliver DHC via the femoral vein. The bolus injection method was only applied to healthy isoflurane-anaesthetized rats. Either 750 μg/kg or 100 μg/kg was administered per injection for a maximum dose of 3 mg/kg to test the cardiovascular effect of DHC. In the syringe pump method, the DHC solution was infused from 10 min to 4 h after ROSC at a rate of 0.75 mg/kg/h. Normothermia CA control rats were infused the same way with the vehicle solution.

Before CA, the rectal temperature of rats was maintained at 37 ± 0.5 °C by a heating lamp. After CA, for the normothermia CA control rats, the environmental temperature was controlled at 28 ± 0.5 °C with a heating lamp. For DHC rats, the environmental temperature was maintained at 28 ± 0.5 °C with the heating lamp after the rectal temperature of rats reached 34 °C. Eight hours after ROSC, rats were put in a neonatal incubator at 28 °C to prevent spontaneous hypothermia overnight.

C. EEG quantification and NDS evaluation

The EEG signals were examined for noise, both in the time domain using TDT software and in the frequency domain using MATLAB (Mathworks, Natick, MA). Artifacts were excluded in the final analysis. Entropy-based quantitative EEG informational quantity (qEEG-IQ) was used to objectively track the cerebral electrical activity recovery after CA [21]. The qEEG-IQ was computed every 30 min after ROSC and normalized to baseline. An IQ of 0 was assigned to dead animals and a higher IQ indicated better recovery.

The NDS of each animal ranged from 0–80 based on a series of behavioral tests [18] at 6 h, 24 h, 48 h and 72 h after ROSC. The NDS examination was performed by a trained examiner blinded to groups and the functional outcome was defined by the 72-hr NDS score.

D. Statistical analysis

The data was analyzed by SPSS (version 22, Armonk, NY). Group values that are parametric (i.e. body temperature and qEEG-IQ) are reported as mean ± SEM. Nonparametric variables (i.e., NDS) are reported as median (25^{th}–75^{th}, percentile). Univariate analysis was used to compare aggregated qEEG-IQ during the 4 hours after ROSC and the averaged qEEG-IQ at the indicated time intervals.

III. Results

A. Comparison of bolus injection and infusion: the two methods of DHC administration

Bolus administration of the drug was preferred for convenience. We first tested whether bolus injection of DHC was feasible in two healthy anesthetized rats. A 750 μg/kg/bolus i.v. injection of DHC caused breathing and the heart to stop, which was reversed by CPR in a timely manner. Rats responded to 100 μg/kg/bolus injections of DHC with relatively mild cardiovascular and blood pressure effects. Specifically, there was a transient increase of MAP for about 1 min, and an episode of cardiac arrhythmia lasting tens of seconds (Fig 1). Infusion of DHC in a healthy anesthetized rat at a rate of 750 μg/kg/h had no noticeable cardiovascular effect. Thus, we decided to administer DHC via infusion in the subsequent CA rats.

B. Body temperature management with DHC

Next, we tested the hypothermia effect of DHC on a rat CA model. DHC was infused from 10 min to 4 h after ROSC. The target temperature of 32–34 °C was reached in 80 ± 15 min. After the infusion was completed, hypothermia was continuously maintained for at least 4 more hours. The normothermia CA vehicle-control rats had a normal body temperature throughout the experiment (37 ± 0.5 °C) (Fig. 2).

C. Effect of DHC-induced hypothermia on qEEG-IQ after CA

The aggregated qEEG-IQ of DHC-treated rats were higher than that of normothermia CA control rats (0.73 ± 0.04 vs 0.60 ± 0.04, $p = 0.04$) during the first 4 hours after ROSC. The averaged qEEG-IQ of DHC-treated rats were significantly higher than that of normothermia CA control rats (0.64 ± 0.05 vs 0.43 ± 0.05, $p = 0.033$) at the early time interval of 30–60 min after ROSC. The qEEG-IQ was similarly higher in DHC-treated rats than control rats at 60–90 min ($p = 0.052$) and 90–120 min ($p = 0.065$) after ROSC, but the differences were not significant with the current cohort (Fig. 3).

D. Effect of DHC-induced hypothermia on the neurological recovery after CA

At 72 h, the mortality rate of DHC-treated rats was the same as the normothermia CA control rats (1 out of 3 in each group). NDS was compared between normothemia CA control and DHC-treated CA rats at 6 h, 24 h, 48 h and 72 h after ROSC (Fig. 4). Due to the small number of experimental animals in this pilot study, statistical analysis of NDS between groups was not the current focus. Overall, the median NDS was better in DHC-treated CA rats (67) than normothermia CA control rats (47).

IV. Discussion

This is the first study to test the feasibility of DHC-induced hypothermia in treating post-resuscitation rats in an asphyxial-CA model. We showed that DHC-treated rats were viable. DHC steadily decreased the body temperature below 34 °C within 80 ± 15 min, and after DHC infusion, the body temperature was maintained around 34 °C for at least 4 more hours (Fig 2). There were no notable complications with DHC administration in post-resuscitation animals. The NDS of DHC-treated rats tended to be better, with a higher median NDS than

that of normothermia rats (Fig 4) and comparable with surface cooling controlled hypothermia (data not shown). Therefore, DHC is a promising PIH candidate to treat CA patients.

It was a concern that DHC might induce the Bezold-Jarisch reflex and airway reflex that cause tachycardia, hypotension, cough and bronchoconstriction [15]. However, we showed that the negative effect of DHC was related to the administration method. The large bolus injection (0.75 mg/kg) induced respiratory and heart stoppage, and the small bolus injection (100 μg/kg) caused mild tachycardia and hypotension, however, infusion at a rate of 0.75 mg/kg/h had no cardiovascular effects. Fosgerau et al reported that DHC infusion at the rate of 0.65 mg/kg/h induced several episodes of transient tachycardia and hypotension, which is contradictory to our findings. This may be related to the composition of infusion solution, strain of rats or the recovery status of CA rats. In our case, 0.75 mg/kg/h infusion rate was safe for Wistar rats.

In addition to thermal sensation and regulation [22], TRPV1 is also involved in sensation of pain, susceptibility to infection [23] and vascular-related pathologies including hypertension and ischemia-reperfusion injury [24]. These many functions must be considered to effectively evaluate the overall effect of DHC on TRPV1. In stroke mice, DHC-activated TRPV1 decreased infarct volume and improved neuro-function [10]. In our pilot study, DHC-treated CA rats showed an early increase in cerebral electrical activity in the early recovery period and improved median NDS at 72 h after CA. However, it is reported that capsaicin activated TRPV1 caused a blood-brain barrier disruption in the rat [25]. Stroke activated TRPV1 exacerbates neurological and motor deficits and infarction [16]. These conflicting effects of TRPV1 on neuronal function may depend on the method of activation, degree of activation and severity of brain injury.

The mechanisms of TRPV1 regulation after CA and TH are unknown. Impaired thermoregulation (mainly spontaneous hypothermia) is common after CA [26], and high incidence of fever (41–89%) was reported after TH in post-CA patients [27]. We have observed occasional fever from 24 h to 72 h in post-CA rats under normothermia, surface cooling hypothermia, and DHC-induced hypothermia (data not shown). In future work, DHC involved pathway, which contributes to the post-resuscitation recovery, will be of our primary interest.

V. Conclusion

In this pilot study, we showed that DHC-induced hypothermia was applicable to CA and showed its benefits to neurological recovery. Further studies on the alteration of TRPV1 after CA, at both the expression and activity levels, are needed to elucidate the mechanism of DHC’s protective role in brain ischemia-reperfusion injury.

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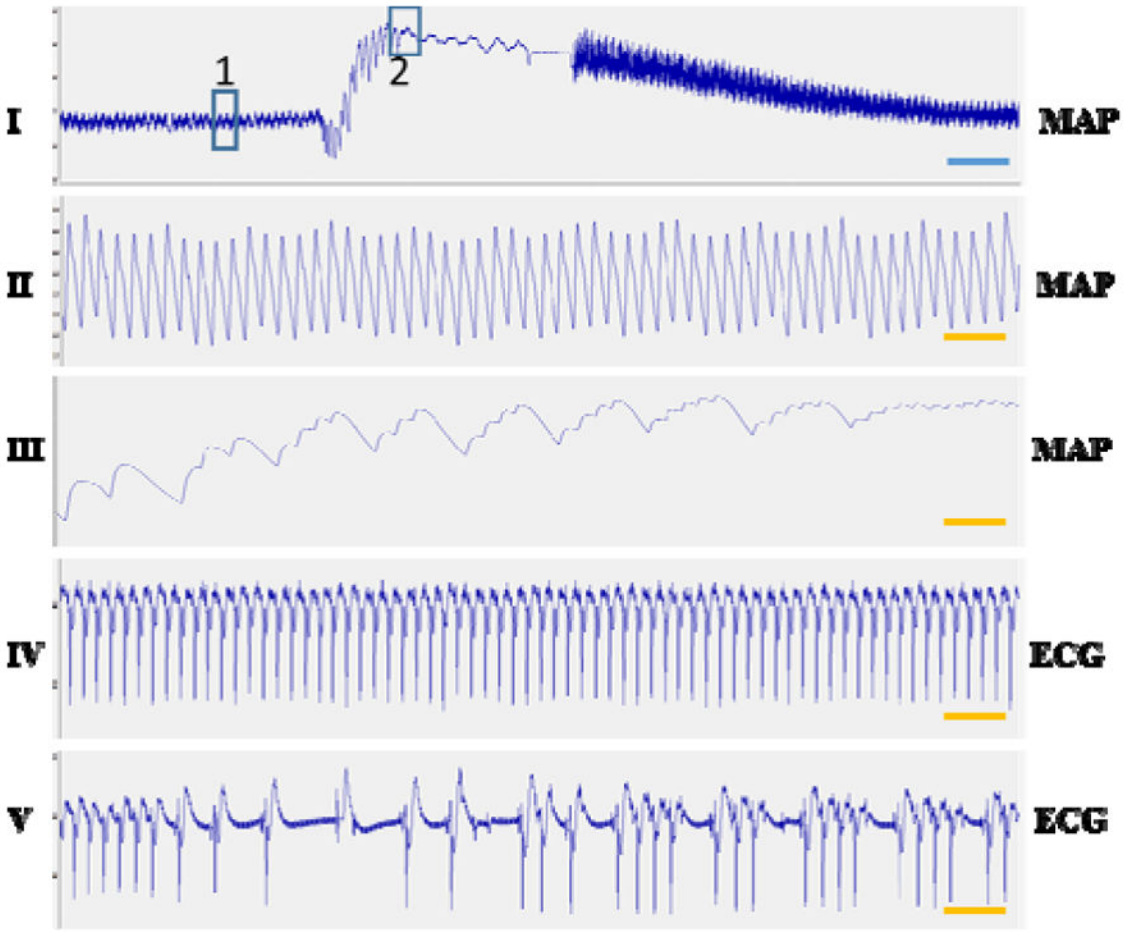

Fig 1.

Cardiovascular effect of DHC by bolus intravenous injection at the dose of 100 μg/kg in healthy rat. I: a 3 min recording of MAP. II: Enlarged MAP of box 1 in I. III: Enlarged MAP of box 2 in I. IV: ECG at the same time interval of box 1. V: ECG at the same time of box 2. Box1 is before and box 2 is after the injection of DHC. Blue bar indicates 20 sec, orange bar indicates 2 sec. There is a transient increase of MAP (III compare with II) and arrhythmia (V compare with IV)

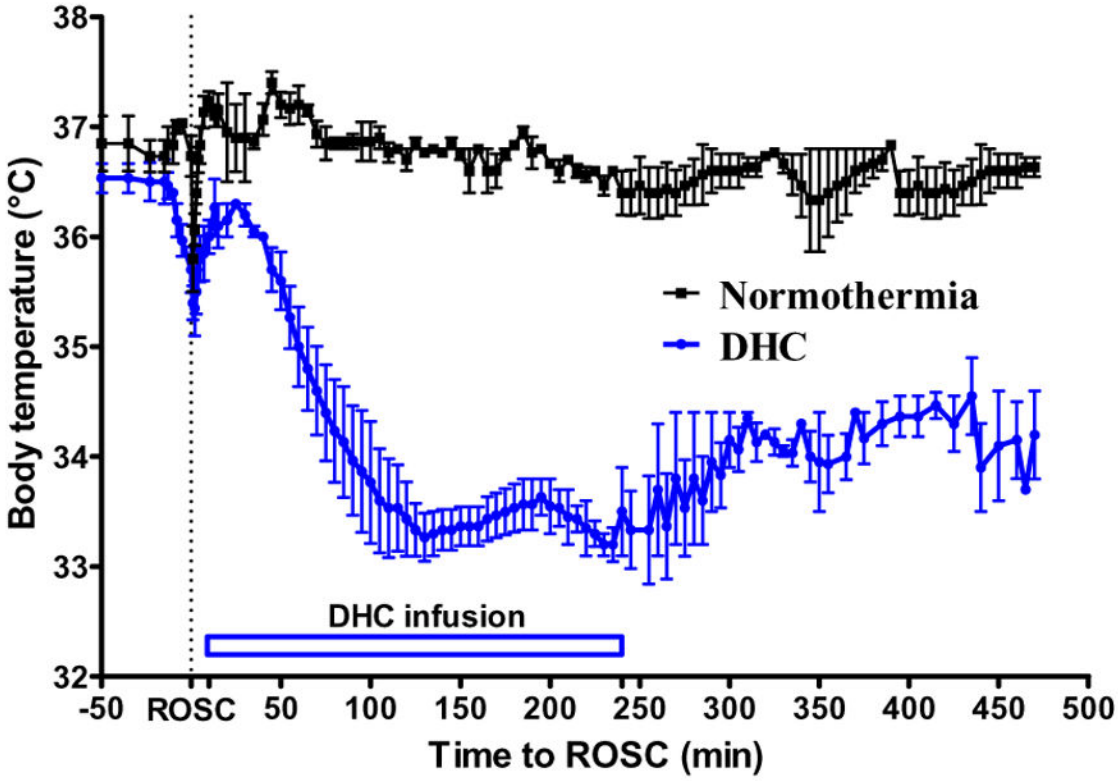

Fig 2.

DHC-induced hypothermia in CA model. DHC was infused from 10 min until 4 h after ROSC.

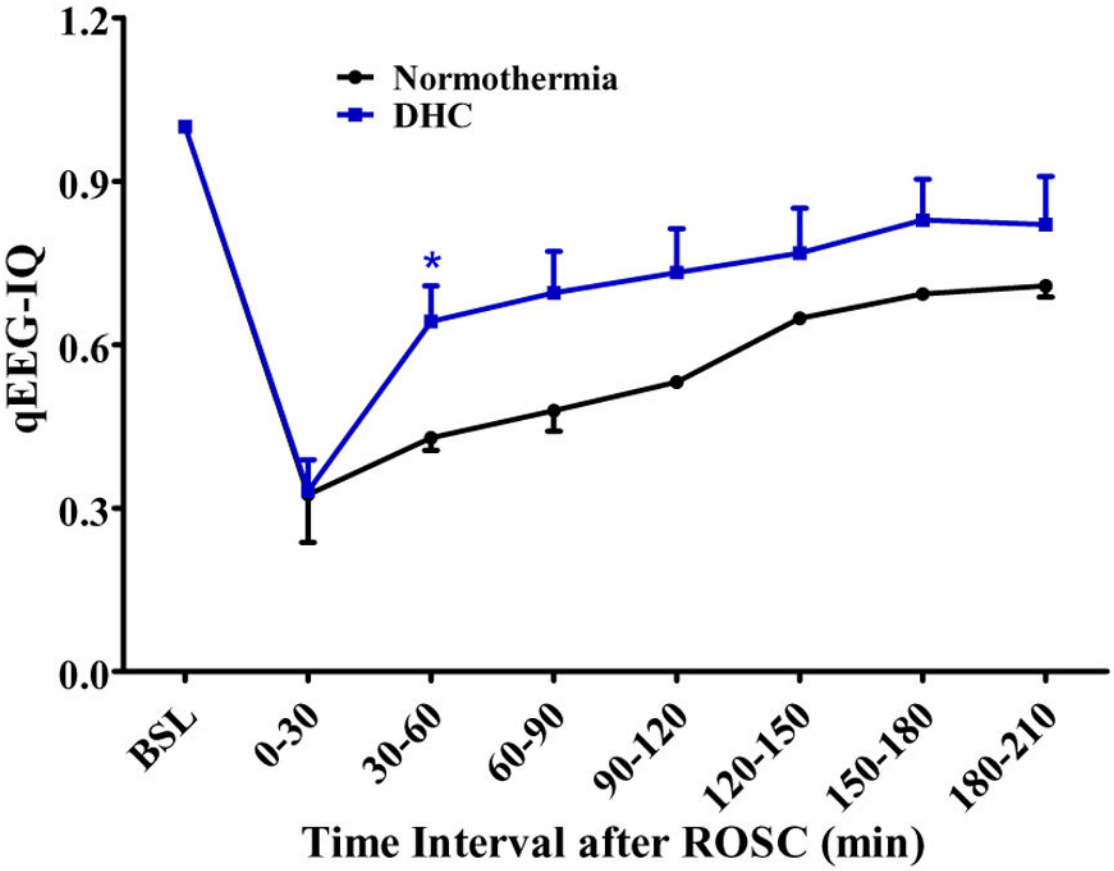

Fig. 3.
Comparison of qEEG-IQ between normothermia CA control rats and DHC-treated CA rats.

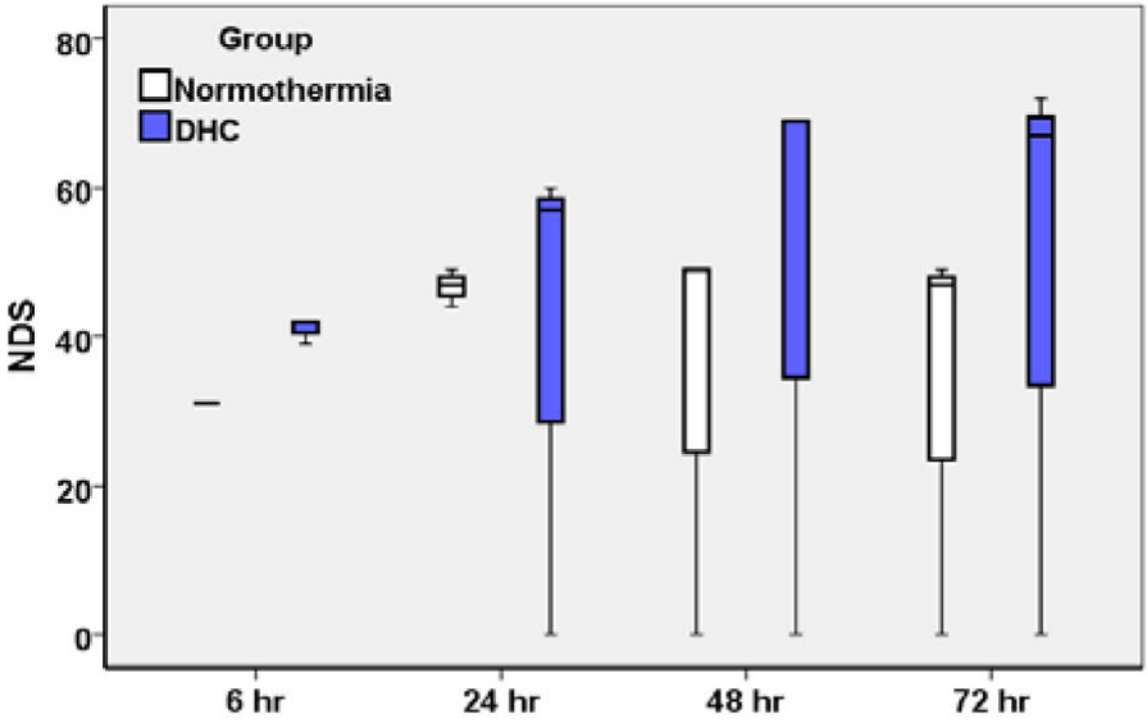

Fig 4.

Comparison of NDS between normothermia CA control rats and DHC-treated CA rats. Box-plot of median and interquartile range (25^{th} and 75^{th} percentile,).