Injury, Int. J. Care Injured 47 (2016) 605–608

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

The effect of induced hypertension on neurological outcome in forebrain ischaemia model in rats

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ARTICLE INFO

Article history:
Accepted 2 November 2015

Keywords:
Apoptosis
Brain ischaemia
Hypertension
Necrosis
Phenylephrine

ABSTRACT

Introduction: The present study investigated the effects of induced hypertension on hippocampal cell death after forebrain ischaemia in rats.
Materials and methods: In this study, forebrain ischaemia was induced in 20 Sprague-Dawley rats by clamping the bilateral common carotid arteries to induce systemic hypotension for 8 min. All rats then underwent reperfusion during which the induced hypertension group ($n = 10$) received intermittent intravenous injections of phenylephrine (5 μg) to maintain their mean arterial blood pressure at 20 mmHg above baseline for 10 min and the control group ($n = 10$) did not receive any treatment. In both groups, the numbers of viable and apoptotic neuronal cells in the cornu ammonis 1 (CA1) area of the hippocampus were evaluated 7 days after the induction of ischaemia.
Results: The mean percentage of viable neuronal cells was higher in the induced hypertension group than in the control group (35% vs. 26%, respectively; $p = 0.004$), but there was no significant difference in the proportion of apoptotic neuronal cells between the groups (57% vs. 43%, respectively; $p = 0.165$).
Conclusions: Induced hypertension significantly attenuated necrotic cell death in the hippocampal CA1 area, but apoptotic cell death was not affected.

Introduction

Accurate prediction of cerebral ischaemia is nearly impossible. Thus, many studies have focused on post-ischaemia treatment options. A number of studies have demonstrated the neuroprotective effects of ischemic post-conditioning, remote ischemic post-conditioning, and the administration of volatile anaesthetics following ischemic insult [1–5]. Additionally, several experimental studies have shown that induced hypertension also exerts neuroprotective effects [6–8]. In a model of focal cerebral ischaemia, induced hypertension increased blood flow to the ischemic penumbra, improved cerebral oxygen metabolism, reduced cerebral oedema, and decreased mortality [7]. Similarly, following temporary occlusion of the unilateral carotid artery in rats in another study, induced hypertension increased cerebral blood flow, ameliorated cytotoxic cerebral oedema, and decreased total infarct volume [8]. However, whether induced hypertension influences cell death after ischaemia remains unclear. Thus, the present study investigated the effects of induced hypertension on cell death within the cornu ammonis 1 (CA1) area of the hippocampus after forebrain ischaemia in rats. The present study was approved by the Institutional Animal Care and Use Committee.

Materials and methods

Rats were housed under a 12-h light/dark schedule and fasted for 12 to 16 h prior to the procedure with free access to water. For establishment of transient ischaemia, anaesthesia was induced by oxygen and Zoletil, and a needle thermistor was subcutaneously inserted under the temporalis muscle to monitor the maintenance of the pericranial temperature at 37.0 °C by heating or cooling (model TCAT-2 Temperature Controller; Harvard Apparatus, Holliston, MA, USA). The tail artery was cannulated to monitor

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http://dx.doi.org/10.1016/j.injury.2015.11.003
0020–1383/

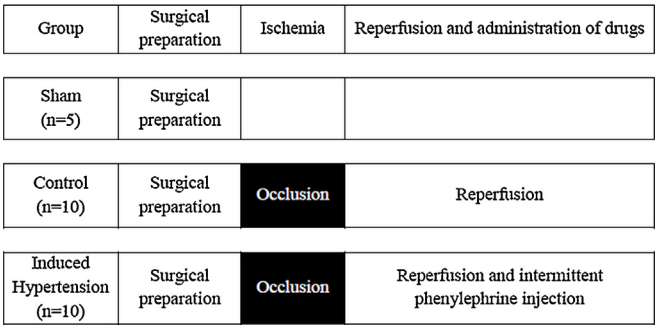

Fig. 1. Overview of the experimental procedure.

Table 1
Physiological variables 10 min before and after ischaemia/reperfusion.

		pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Haemoglobin (g/dl)	Blood glucose (mg/dl)
Sham	Before	7.35 ± .02	43 ± 2	303 ± 21	13.6 ± 0.5	120 ± 9
	After	7.36 ± .01	42 ± 2	335 ± 17	12.9 ± 0.2	
Control	Before	7.32 ± .01	48 ± 2	320 ± 13	13.9 ± 0.4	124 ± 7
	After	7.34 ± .02	47 ± 2	336 ± 14	13.4 ± 0.2	
IHT	Before	7.34 ± .01	46 ± 2	326 ± 9	13.6 ± 0.3	132 ± 7
	After	7.33 ± .03	42 ± 3	305 ± 15	12.1 ± 0.7	

Data are expressed as mean ± SEM.

blood pressure and obtain serial blood samples. Next, skin antisepsis was performed with a 10% povidone–iodine solution, and the right internal jugular vein and bilateral common carotid arteries were exposed. The right internal jugular vein was catheterised with a silicone catheter for drug infusion and blood withdrawal, and strings were applied to the bilateral common carotid arteries to induce ischaemia. After stabilizing for 30 min, the rats were heparinised (50 U), and forebrain ischaemia was induced by clamping both carotid arteries and drawing blood to decrease the mean arterial blood pressure (MAP) to 30 mmHg for 8 min. A reperfusion procedure was subsequently performed in which the arteries were declamped and autologous blood was transfused.

The rats were randomly allocated into a control group (n = 10) and an induced hypertension group (n = 10). A third sham group (n = 5) underwent anaesthesia and surgical preparation procedures identical to those in the induced hypertension group, but ischaemia was not induced. During the reperfusion period, the induced hypertension group received intermittent intravenous administrations of phenylephrine (5 μg) to maintain the MAP at 20 mmHg above baseline for 10 min; the control group did not receive any treatment (Fig. 1). Arterial blood gas and haemoglobin levels were measured 10 min prior to and after the ischaemia procedure. Following the completion of the experimental procedures, the incision was sutured and anaesthesia was maintained for another hour. After a 7-day post-ischaemia recovery period, the rats were humanely killed via perfusion of normal saline (100 ml) and 10% formalin (200 ml) and then kept refrigerated overnight. The next day, the brain of each rat was separated from the skull and fixed with formalin. The bilateral hippocampus was sectioned along the coronal planes and stained with haematoxylin and eosin (H&E) and terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick-end labelling (TUNEL). TUNEL staining was performed using an ApopTag Peroxidase In Situ Apoptosis Detection Kit (S7100; Millipore Corp., Billerica, MA, USA) according to the manufacturer's instructions. The H&E-stained sections of the hippocampal CA1 were examined under a microscope (400×) to evaluate the presence of necrotic and viable neuronal cells. Cells with a round nucleus and a prominent nucleosome were regarded as viable cells, whereas cells showing nuclear swelling, chromatin flocculation, and/or the loss of nuclear basophilia were identified as necrotic cells. Cell apoptosis was also microscopically assessed (400×), and apoptotic cells were identified as TUNEL-positive neurons with brown-stained nuclei. The percentages of necrotic and apoptotic cells to the total number of neuronal cells were calculated, respectively.

All data are expressed as mean ± standard error of the mean. All physiological variables were analysed with repeated-measures analysis of variance, and statistically significant differences among the groups were analysed with the Kruskal–Wallis test when comparing three groups and the Mann–Whitney U test when comparing two groups. A p value of <0.05 was considered to indicate statistical significance.

Results

The blood glucose level, haemoglobin level, pH, and partial pressures of carbon dioxide and oxygen were measured before and after the forebrain ischaemia/reperfusion procedure. Prior to ischaemia, there were no significant differences between the induced hypertension and control groups in these variables (Table 1) or the average MAP (81 ± 9 and 83 ± 9 mmHg, respectively). However, 2 min after reperfusion, the MAP of the control group was similar to the pre-ischaemia value, whereas the MAP of the induced hypertension group remained at 20 mmHg above the baseline value (Table 2).

All rats survived until 7 days after the transient forebrain ischaemia procedure, at which time a histological evaluation was performed. The mean percentage of viable cells was higher in the induced hypertension group than in the control group (35% vs. 26%, respectively; p = 0.004) (Fig. 2) and in the sham group versus the control group ($p < 0.01$) (Fig. 2). The proportion of apoptotic cells was significantly higher in the control group than in the sham group ($p < 0.01$) (Fig. 3), but there was no significant difference in the ratio of apoptotic cells between the induced hypertension group and control group (57% vs. 43%, respectively; p = 0.165) (Fig. 3).

Discussion

The present study demonstrated that the application of induced hypertension resulted in a decreased number of necrotic cells in the hippocampal CA1 area 7 days after an ischaemia procedure. In contrast, apoptotic cell death was not affected by induced hypertension at this time. The neuroprotective effects exerted

Table 2
Mean arterial blood pressure before and after ischaemia/reperfusion.

	Before	After ischaemia/reperfusion									
		1 min	2 min	3 min	4 min	5 min	6 min	7 min	8 min	9 min	10 min
Control	83 ± 9	67 ± 14	78 ± 16	83 ± 19	84 ± 20	85 ± 19	85 ± 17	85 ± 16	86 ± 17	86 ± 19	84 ± 16
Induced hypertension	81 ± 9	91 ± 15	102 ± 15	115 ± 22	111 ± 17	129 ± 17	126 ± 19	127 ± 14	120 ± 14	121 ± 16	108 ± 12

Data are expressed as mean ± SD (mmHg).