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Dexmedetomidine Ameliorates Histological and Neurological Outcomes after Transient Spinal Ischemia in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author TG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author YT involved in the design of the manuscript and data collection. All authors read and approved the final manuscript.

Short Research Article

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ABSTRACT

Aims: Dexmedetomidine, an$\alpha_2$adrenergicagonist, provides neuroprotection in various cerebral ischemia models and against anesthesia-related neurotoxicity. Dexmedetomidine also improves paraplegia induced by intrathecal morphine after short-term spinal ischemia. In this preliminary study, we investigated whether dexmedetomidine provides spinal protection against transient spinal ischemia in rats.
Methodology: Adult male Sprague-Dawley rats were randomly divided into the following 3 groups: 1) intravenous infusion of 0.9% NaCl at a rate of 0.5 mL/h (control), 2) dexmedetomidine 1 μg/kg/h, and 3) intravenous infusion of 0.9% NaCl without spinal ischemia (sham). The rats received saline solution or dexmedetomidine 30 min before spinal cord ischemia and for 24 h. Spinal cord ischemia was induced by intra-aortic balloon occlusion combined with proximal arterial hypotension for 10 min. Ischemic injury was assessed by the neurological deficit score and by the number of viable motor nerve cells in the anterior spinal cord at 24 h of reperfusion.
Results: The neurological deficit score was significantly lower in the dexmedetomidine group compared to the control group ($p< 0.05$). The number of viable motor nerve cells in the dexmedetomidine group was significantly greater than was that in the control group ($p< 0.05$), but was lower than was that in the sham group.

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Conclusion: Our findings suggest that continuous administration of dexmedetomidine ameliorates short-term neurological and histological outcomes induced by transient spinal cord ischemia and reperfusion in rats; thus, dexmedetomidine appears to protect the spinal as well as the brain.

Keywords: Spinal protection; spinal ischemia; dexmedetomidine; α_2 adrenergic agonist.

1. INTRODUCTION

Dexmedetomidine, an α_2 adrenergicagonist, has been widely used clinically as an adjuvant of anesthesia and in intensive care because it has sedative and analgesic effects [1,2]. Dexmedetomidine has also been shown to have a protective effect on the brain in various ischemia models, such as focal [3-5], global [6-8], and incomplete cerebral ischemia [9-11]. Previous studies suggested the following mechanisms for these neuroprotective effects: decreased activation of focal adhesion [12], imidazoline 1 receptor-extracellular-regulated kinase pathways [12,13], activation of protein kinase C [14], anti-oxidant effect [7,15], excitatory neurotransmitter suppression [10,16], anti-inflammatory effect [7], and anti-apoptotic effect [7,11,17].

Transient spinal ischemia can occur under various conditions during surgery, especially thoracic aortic surgery, and spinal ischemia can induce detrimental paraplegia in some circumstances. Pharmacological adjuncts need to reduce and prevent the incidence of paraplegia following spinal cord ischemia.Based on the previous reports that dexmedetomidine prevented morphine-induced paraplegia following short-term transient spinal ischemia [18] and reduced spinal cord ischemia-reperfusion injury in mice [19], administration of dexmedetomidine would affect spinal cord injury caused ischemic insult as well as brain protective effects. Thus, the aim of this preliminary study was to evaluate the neuroprotective effect of dexmedetomidine on spinal cord ischemia and reperfusion injuryin rats.

2. MATERIALS AND METHODS

2.1 Preparation of Rats

Male Sprague–Dawley rats weighing 350 to 450 g were used in this study. The rats had free access to food and tap water before the experiment. None of the animals had any neurological abnormality before anesthesia and surgery. On the day of surgical preparation, the rats were weighed and were administered a continuous flow of 4% halothane and 60% nitrous oxide in oxygen in an acrylic plastic box. Anesthesia was maintained with 0.75% to 1.5% halothane with 30% oxygen with a non-sealing facemask device. The rectal and paravertebral muscle temperatures were maintained at approximately 37.0ºC with a heating lamp and an underbody heating pad. Surgical preparation was conducted as previously described [20]. The femoral vein was cannulated with a PE-50 catheter to infuse dexmedetomidine or saline. The catheter was tunneled subcutaneously and was exteriorized through a swivel sutured over the dorsal mid-thorax, which allowed the rat to move freely in the cage after emergence from anesthesia. The tail artery was cannulated with a PE-50 catheter for monitoring the distal arterial pressure (DAP). A PE-60 catheter was inserted into the right carotid artery for monitoring the proximal arterial pressure (PAP), and the catheter was connected to an external blood

reservoir to reduce the mean PAP during aortic occlusion to 40 mm Hg. Spinal ischemia was induced by a balloon catheter via the femoral artery, as previously described [20,21]. Briefly, the right femoral artery was exposed and a Fogarty 2F balloon-tipped catheter (Edwards Life sciences, Irvine, CA) was advanced into the thoracic descending aorta (11 cm from the site of insertion). Immediately after performing arterial cannulation, 200 U of heparin (0.2 mL) was injected into the tail artery. The catheter balloon was inflated with 0.05 mL saline and was maintained for 10 minutes. The efficiency of the occlusion was confirmed by a decrease in the DAP measured at the tail artery. The PAP was decreased to 40 mm Hg during occlusion by drawing blood from the carotid artery containing 1 mL of 7% sodium bicarbonate solution. The balloon was deflated after ischemia, and the collected blood was administered to the animals through the carotid artery catheter within 2 min. The rats were brought out of the anesthesia 30 min after reperfusion. The animals were returned to the cage if their hemodynamic variables were stable after all catheters, except for the femoral vein catheter, were removed. The incisions were subsequently closed.

2.2 Groups

The rats were randomly divided into 3 groups as follows: 0.9% NaCl solution (control group; n = 8), dexmedetomidine at a rate of 1 μg/kg/h (dex group; n = 8), and the sham surgical group (n = 6). The drug-infusion or saline-infusion volume was adjusted to a rate of 0.5 mL/h and was administered from 30 min before aortic occlusion until the end of the subsequent 24-h reperfusion. The rats in the sham surgical group received saline through catheters that were inserted in the same manner, without the induction of spinal cord ischemia.

2.3 Measurements

2.3.1 Hemodynamics

Hemodynamic variables (PAP, DAP, and heart rate) were continuously monitored and were recorded after surgical preparation for up to 5 minutes after reperfusion. Arterial blood gas and blood glucose levels were determined immediately before aortic occlusion and 5 min after reperfusion.

2.3.2 Neurological evaluation

At 24 h after reperfusion, the neurological deficit score (NDS) of the animals was assessed according to previously described grading systems [20]. The NDS was quantified as shown in Table 1. The NDS was calculated for each rat as the sum of the ambulation, placing/stepping reflex scoresand sensory score; the maximal score was 8. The assessments were made by a blinded observer (YT).

2.3.3 Histological evaluation

After scoring neurological function at 24 h, the animals were anesthetized with 4% halothane in an acrylic plastic box. A high dose of pentobarbital (80 mg/kg) was administered by intraperitoneal injection. After the administration of a direct left ventricular bolus of 0.2 mL heparin, each rat was transcardially perfused with 100 mL heparinized saline followed by 150 mL of 4% paraformaldehyde in phosphate buffer (pH 7.4). The

lumbar spinal cord was removed and was post-fixed in the same fixative for another 48 h. Post-fixation, the L4 spinal segment was dissected and was embedded in paraffin; subsequently, serial transverse sections of 3-μm thickness were prepared. The slides were stained with hematoxylin and eosin for quantitative evaluation. Analysis of the degree of ischemic cell injury was based on the number of viable neurons in the ventral area of the gray matter (anterior to a transverse line drawn through the central canal) with × 100 magnification in each group. Intact cells that contained Nissl substance in the cytoplasm, loose chromatin, and prominent nucleoli were considered viable neurons. The assessments were made by a blinded observer (YT).

Table 1. Neurological deficit score

Motor and sensory function		Score
Ambulation	**Normal**	**0**
	toes flat beneath the body when walking but presence of ataxia toes	1
	knuckle walking	2
	unable to knuckle walk but some movement of the lower extremities	3
	no movement of the lower extremities	4
Placing/stepping reflex	**Normal**	**0**
	weak	1
	no stepping	2
Sensory	**Normal**	**0**
	weak	1
	none	2

2.4 Statistical Analyses

The physiological variables and the number of normal ventral cells are expressed as the mean ± SD. Comparisons among groups were made with 1-way analysis of variance for multiple comparisons followed by a Bonferroni post-hoc test. The NDS data for the animals are expressed as the median with the range in parentheses. The differences were determined by nonparametric analysis using the Kruskal-Wallis test. P values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

The physiological variables and arterial blood gas data are presented in Table 2. No differences were found between the experimental groups with respect to the proximal MBP, Distal MBP, heart rate, and paravertebral muscle temperaturebefore and after ischemia.Proximal and distal MBP in the saline group after ischemia were higher than that before ischemia. The blood chemistry values before aortic occlusion were within the normal range, with no significant differences between the groups. The blood glucose and pH levels after reperfusion were significantly higher and lower, respectively, compared to those before ischemia and sham group($p< 0.05$).

As shown in Fig. 1, the medians (range) of the NDS were significantly lower in the DEX groups compared to the control group ($p< 0.05$).

Table 2. Physiological variables 10 min before, during and after ischemia

	Before ischemia			During ischemia			After ischemia		
	Sham (n=6)	**Control (n=8)**	**Dex (n=8)**	**Sham (n=6)**	**Control (n=8)**	**Dex (n=8)**	**Sham (n=6)**	**Control (n=8)**	**Dex (n=8)**
Proximal MBP (mm Hg)	89±11	77±9	81±11	99±9	42±4*#	41±2*#	97±9	111±17*	91±12
Distal MBP (mmHg)	84±11	72±10	79±10	97±10	7±3*#	6±1*#	99±10	109±20*	89±14
Heart Rate (bpm)	367±27	335±28	339±18	363±19	256±94#	310±21#	371±18	320±32	347±12
Paravertebral temperature (℃)	37.9±0.3	37.9±0.2	37.7±0.2	38.0±0.3	38.0±0.3	37.7±0.1	37.9±0.2	37.8±0.2	37.9±0.1
pH	7.43±0.02	7.412±0.05	7.339±0.06				7.433±0.01	7.314±0.04*	7.282±0.02*#
PaO_2 (mmHg)	141±19	123±23	107±23				136±11	135±19	114±14
$PaCO_2$ (mmHg)	43±2	42±3	54±9				43±2	49±4	57±5#
Blood glucose (mg/dL)	110±13	110±20	121±26				108±15	222±18*#	197±56*#
Hb (g/dL)	14.7±0.8	14.6±1.0	14.5±0.7				14.6±0.3	14.5±0.8	13.9±0.7

Data are expressed as mean ± SD. MBP = mean arterial blood pressure. DEX = dexmedetomidine. p<0.05 vs before ischemia in the group. # p<0.05 vssham group*

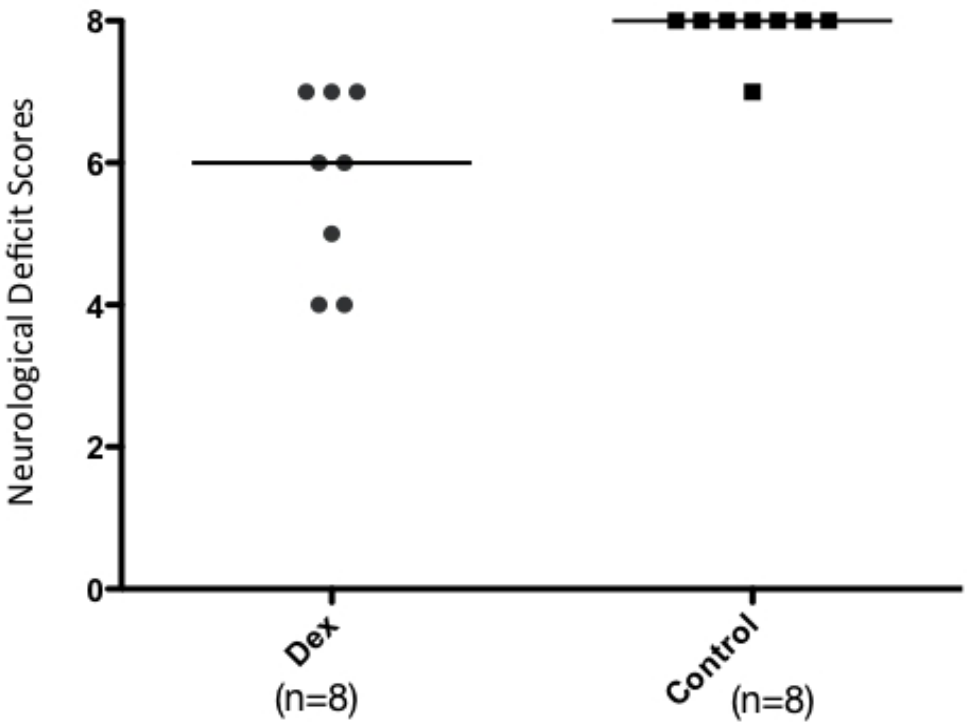

Fig. 1. Neurological deficit score (NDS) 24 hours after transient spinal ischemia in rats

The median value was significantly lower in the dex group compared to that in the control group at 24 hours after ischemia. The score in the sham group was zero.dex = dexmedetomidine

Representative photomicrographs of the ventral area of hematoxyline and eosin-stained transverse sections taken from the L4 spinal segment are shown in Fig. 2. The number of viable motor nerve cells in the ventral area of the gray matter in the dexmedetomidine group (39.2 ± 6.5) was significantly greater than was that in the control group (20.2±12, $p< 0.05$), but was lower than was that in the sham group (58 ± 6, $p< 0.05$).

This preliminary study showed that dexmedetomidine improved the neurological and histological outcomes after 24 h of transient spinal ischemia and reperfusion. These findings are consistent with a previous report demonstrating that dexmedetomidine provided neuroprotection against brain ischemia [3-11].

Many previous reports regarding the neuroprotective effects of dexmedetomidine focused on its ability to protect the brain against various insults such as transient focal [3-5], forebrain [6-8], and incompleteischemia [9-11], anesthesia-related neurotoxicity [22,23], and various other conditions [15,24,25]. Although there were many studies which referred the drug-induced spinal protection against spinal cord ischemia for example beta-blocker [20], statin [26], and rolipram [27], few studies that dexmedetomidine prevented morphine-induced paraplegia following short-term transient spinal ischemia [18] and reduced spinal cord ischemia-reperfusion injury in mice [19], existed.

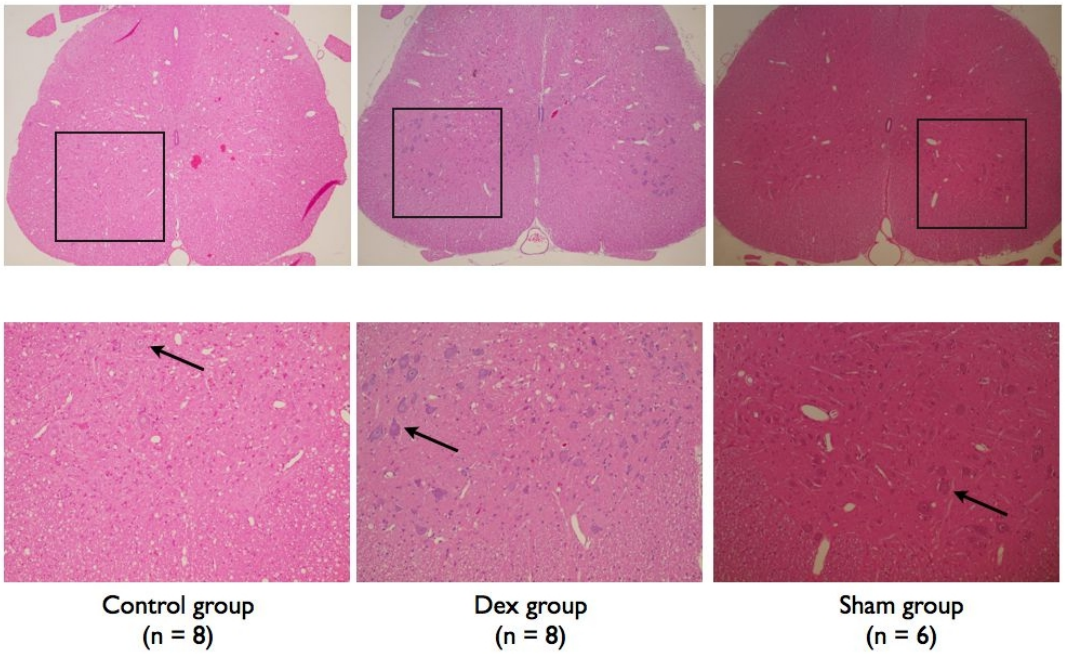

Fig. 2. Representative photomicrographs (×40; upper figures, ×100; lower figures) of the ventral area of hematoxyline and eosin-stained transverse section 24 hours after transient spinal ischemia

Arrows indicated viable motor nerve cells

In this study, only short-term results (i.e., 24 hours) were assessed. A previous study showed that transient spinal cord ischemia-induced motor dysfunction deteriorated into paraplegia for several days [28]. Neural degeneration was shown to progress gradually after an ischemic insult, with the number of necrotic neurons peaking at 2 days after the insult [29]. Moreover, hind-limb motor function might gradually recover during the 2 weeks following spinal cord ischemia [29]. Therefore, a further study that includes long-term observation is needed.

We did not examine the mechanisms of dexmedetomidine-induced spinal protection in this study. Many previous studies demonstrated the protective effects of dexmedetomidine on the brain against transient focal ischemia [3-5], transient forebrain ischemia [6-8], and incomplete brain ischemia [9-11]. Further, numerous reports have proposed mechanisms for the neuroprotective effects of dexmedetomidine, including decreased activation of focal adhesion [12], imidazoline 1 receptor-extracellular-regulated kinase pathways [12,13], activation of protein kinase C [14], anti-oxidant effect [7,15], excitatory neurotransmitter suppression [10,16], anti-inflammatory effect [7], and anti-apoptotic effect [7,11,17]. Therefore, we can only speculate on the mechanisms responsible for the protective effects of dexmedetomidine against transient spinal ischemia.

Although we did not evaluate the mechanisms or the long-term effects of dexmedetomidine against transient spinal cord ischemia, the results from this preliminary study suggest that dexmedetomidine exerts short-term protective effects against spinal cord ischemia-reperfusion injury. Further studies are warranted to investigate the detailed

action, mechanisms, and therapeutic time window of dexmedetomidine-induced spinal protection.

4. CONCLUSION

In summary, we investigated the effect of pre-administered dexmedetomidine after transient spinal cord ischemia in rats by performing neurological and histological evaluations. Dexmedetomidine improved the short-term neurological and histological outcomes, suggesting that dexmedetomidine exerts protective effects on the spinalcord as well as on the brain.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Animal Subjects Committee of Akita University Graduate School of Medicine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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