

Original Article

Dexmedetomidine alleviates the spinal cord ischemia-reperfusion injury through blocking mast cell degranulation

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Abstract: Objective: To investigate the neuro-protective effects of dexmedetomidine (dex) on I/R-induced spinal injury and potential mechanisms. Methods: sprague-Dawley rats in the treatment group received intraperitoneal injections of 25 mg/kg dexmedetomidine, MC stabilizer cromolyn (100 mg/kg), MCs stimuliser compound 48/80 (80 mg/kg), PBS at 24 h before IR. Underwent 5 minutes of aortic occlusion via median sternotomy, functional scores were recorded at 12, 24, 36 and 48 hours after reperfusion. Additionally, 3 mice underwent sham surgery with sternotomy and dissection of the aorta and subclavian artery with no occlusion. Spinal cords were examined for protein kinase B (AKT), CREB, and brain-derived neurotrophic factor (BDNF) following treatment alone or ischemia-reperfusion surgery. Collected the serum to observe the expression of pro-inflammation cytokines (TNF- α , INF- γ and IL-1 β) and anti-inflammation cytokines (TGF- β , IL-10 and IL-6). Then the MCs were harvested to test the expression surface molecular of Fc ϵ R and MCs' degranulation. Results: Pretreated the rats with dexmedetomidine has higher neurologic function at all time points after I/R injury. We collected the serum of rats then detected the pro-inflammation cytokines TNF- α , INF- γ and IL-1 β levels and anti-inflammation cytokines TGF- β , IL-10 and IL-6 levels, found that the pro-inflammation cytokines of dexmedetomidine group was decreased whereas the anti-inflammation cytokines was increased. At the same time the protect protein of AKT, CREB and mRNA BDNF were increased. They had the same results with cromolyn group, and opposite with the compound 48/80 group. We pretreated MCs with dexmedetomidine in vitro, and found that the activity surface molecular of MCs was down-regulation, and MCs degranulation was decreased. Conclusion: We thus demonstrate a possible mechanism by which dexmedetomidine alleviates spinal cord I/R injury through blocking the MCs degranulation.

Keyword: Dexmedetomidine, mast cell, degranulation, ischemia-reperfusion injury

Introduction

The ischemic insult to the spinal cord during thoracoabdominal aortic interventions continues to have devastating consequences in up to one fifth of high-risk patients [1]. Ischemia-reperfusion (IR) injury is complex and poorly understood. Neurons are the most vulnerable cells to ischemia and coupled with their inability to regenerate, spinal cord I/R is a significant threat to patients. The current management protocol for spinal ischemia is limited to supportive medical care. Recanalization following ischemia is the most effective method for treatment of acute spinal infarct and correction of

hypoxia, however, paradoxically could cause severe spinal ischemia-reperfusion (I/R) injury [2, 3]. Due to the lack of efficient neuroprotective therapies, the I/R injury is still a major medical problem which urgently needed to be further studied. It is therefore of major importance to prevent or alleviate I/R-induced injury in recanalizing patients, and perioperative treatment may be a potent protective strategy for the ischemic neuronal injury [4].

Dexmedetomidine (DEX) is an α -2 adrenoreceptor agonist and is used as a sedative and an analgesic especially in a neurointensive care unit [5, 6]. Dexmedetomidine has been found to

Dex alleviates the spinal cord IRI through blocking MC degranulation

be very effective as a premedication agent because of its sympatholytic, analgesic, anxiolytic, and sedative effects [7]. Previous studies have demonstrated that dexmedetomidine exhibited antiapoptotic and anti-inflammatory effects apart from its anesthetic features [8, 9]. In cerebral neurons, clonidine and other α -2 agonists have been shown to promote phosphorylation of neuroprotective pathways and up-regulation of the PI3K AKT pathway. Cyclic AMP response-element binding protein (CREB) is a transcription factor that is critical for neuron survival after ischemia supporting its role in the induction of ischemic tolerance. Among other functions, CREB induces expression of pro-survival proteins brain derived neurotrophic factor (BDNF) [10]. Moreover, studies in animals have reported organ protective effects of dexmedetomidine in ischemia-reperfusion injury [11, 12]. Dexmedetomidine promotes ischemic tolerance in a murine model of spinal cord ischemia-reperfusion [13]. But the mechanism is not understood.

Mast cells (MCs) participate in I/R injury in many organs, such as the heart, brain [14] and intestine. MCs take effect mainly through releasing chemicals stored within the MC granules, which include pro-inflammation cytokines and anti-inflammation cytokines, such as histamine [15], interleukin [16], and MC tryptase [17], and chymase [18]. Stabilizing the MCs membrane could alleviate the organs I/R injury. So we hypothesize that dexmedetomidine could stabilize the MCs' membrane and increase the protective signal protein to alleviate the spinal I/R injury.

Material and methods

Animals

All rats were purchased from Schleck Experimental Animals Co. (Shanghai, China). All rats were housed in a Specific pathogen free facility and used in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals of the Chinese Academy of Sciences.

Cell preparation

Rat bone marrow-derived mast cells were obtained as described previously [19]. The purity of MCs was assessed by measuring the expression of c-kit (CD117) and FC ϵ RI α using

flow cytometry. MCs were used at a purity of 95%.

Flow cytometry

MCs pretreated for 24 h with: PBS, 30 μ g/kg dexmedetomidine, MCs stabilizer cromolyn (100 μ g/kg), MCs stimulator compound 48/80 (80 μ g/kg), MCs were incubated with FC ϵ RI-PE antibodies for 30 min on ice. Flow cytometry analysis was performed using a FACS Calibur flow cytometer (BD BioSciences) equipped with Cell Quest software.

Mast cell degranulation assay

After treating MCs with PBS, dexmedetomidine, cromolyn, compound 48/80 for 24 h, MCs were preincubated with anti-DNP-IgE (100 ng/mL) for 24 h then challenged using DNP (200 ng/mL). After 1 h, conditional media from the IgE/Ag degranulated MCs was added to the culture system. After solubilization with 0.5% Triton X-100 in Tyrode's buffer, the enzymatic activity of β -hexosaminidase in both supernatants and cell pellets was measured using *p*-nitrophenyl-N-acetyl- β -D-glucosaminide in 0.1 M sodium citrate pH 4.5 at 37°C for 60 min. The reaction was stopped by addition of 0.2 M glycine pH 10.7 and the released *p*-nitrophenol was measured at 405 nm. The extent of degranulation was calculated as the *p*-nitrophenol absorbance of the supernatant divided by the total absorbance of the supernatant and detergent-solubilized cell pellet.

Aortic cross clamping: IR surgery

Rats were anesthetized using 2% isoflurane and placed in the supine position. Surgery was performed under normothermic conditions. The aortic arch was exposed using a cervico-thoracic approach as previously described [20]. Disruption of arterial flow to the spinal cord was achieved by placing vascular clamps on the aortic arch distal to the left common carotid artery and the subclavian artery for 5 minutes. To confirm at least 90% reduction in distal flow, a laser Doppler blood flow monitor (Moore Instruments, Devon, United Kingdom) was placed over the left femoral artery.

Medication administration

Rats in the treatment group received intraperitoneal injections of 25 mg/kg dexmedetomi-

Dex alleviates the spinal cord IRI through blocking MC degranulation

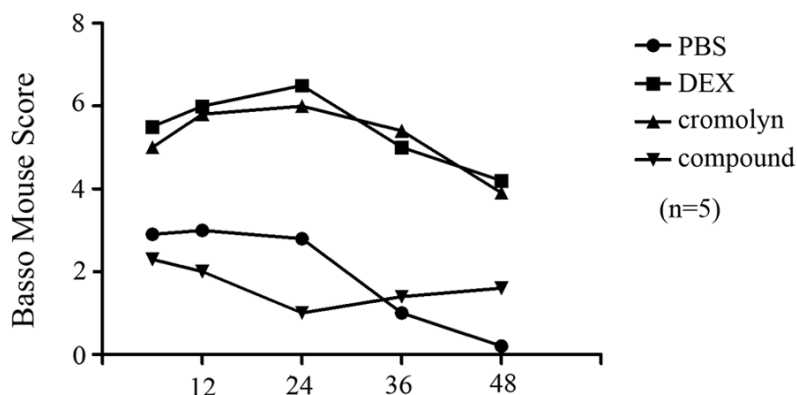


Figure 1. Time (h) following the ischemia. Following ischemia-reperfusion (IR) surgery with 5 minutes of aortic occlusion a significant functional deficit in hind-limb function was observed in all rats. 24 hours of pretreatment with dexmedetomidine (IR+ Dex) and cromolyn (IR+ cromolyn) resulted in significantly higher neurologic function at all time-point postoperatively. The rats pretreated with compound 48/80 had opposite result. Error bars represent mean \pm SEM. * $P < 0.05$.

dine, MC stabilizer cromolyn (100 mg/kg) MCs stimuliser compound 48/80 (80 mg/kg) or PBS ($n = 5$) at 24 h before IR. Additionally, 3 mice underwent sham surgery with sternotomy and dissection of the aorta and subclavian artery with no occlusion.

Functional scores

The Basso Mouse Scale for locomotion, which ranges from a score of 0 for complete paraplegia to a score of 9 for normal function, was used to quantify hind-limb function in mice after ischemia. Function was scored at 12, 24, 36, and 48 hours after reperfusion.

Quantitative polymerase chain reaction

Measurements BDNF (brain-derived neurotrophic factor) transcripts from treated and untreated spinal tissue without undergoing IR surgery were performed. Total RNA was isolated using the RNAqueous-4 PCR kit (Ambion, Austin, Tex) per the manufacturer's instructions. Real-time polymerase chain reactions using iQ mastermix (Bio-Rad, Hercules, Calif) on Bio-Rad CFX detection system (Hercules, Calif) were performed in duplicate using 50 ng complementary DNA. FAM-labeled probe/primer sets used to detect BDNF (Mm04230607_s1) were synthesized by Invitrogen (Grand Island, NY). The housekeeping gene β -actin (Mm00607939_s1) was also assayed for each sample using 5 ng complementary DNA. Cycle parameters used 95°C for 3 minutes followed

by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Expression levels were calculated using the delta-delta Ct method relative to β -actin.

Protein extraction and western blotting

Spinal cords were removed following pretreatment or IR surgery. Briefly, Protein extracts were obtained using lysis buffer (50 mM Tris HCl pH 8.0, 120 mM NaCl, 0.25% Nonidet P₄O, 0.1% SDS, protease inhibitors PMSF, aprotinin, leupeptin, and pepstatin (Roche) at a final concentration of 10

ng/mL, 1 mM DTT). A total of 60 mg of each protein sample was subjected to 15% SDS-PAGE and blotted onto a nitrocellulose membrane (Amersham Pharmacia Biotech, USA). Membranes were blocked with 5% non-fat dry milk in TBS-Tween (0.5%) and probed with either rabbit anti-Rat cyclic AMP response-element binding Protein (CREB) monoclonal antibody (2 mg/mL; Abcam) or rabbit anti-Rat β -actin monoclonal antibody (Bioworld, USA) followed by HRP-conjugated anti-rabbit IgG antibody (Amersham Pharmacia Biotech). Immunoreactive protein bands were detected with the ECL detection kit (Amersham Pharmacia Biotech).

Cytokines detection

Serum were harvested and bioactive TNF- α , INF- γ , IL-1 β , TGF- β , IL-10 and IL-6 were quantified by ELISA (eBiosciences) according to the manufacturer's instructions.

Statistical analysis

All statistical analysis was carried out using SPSS9.13 for Windows and expressed as the mean \pm SEM. One-way analysis of variance (ANOVA) was performed to determine the statistical significance of the data. Each sample was tested for homogeneity of variance with the Student-Newman-Keuls multiple comparison test for two-variable analysis. If the variance was not homogeneous, nonparametric statistical tests were performed. Differences were considered significant at the $P < 0.05$ level.

Dex alleviates the spinal cord IRI through blocking MC degranulation

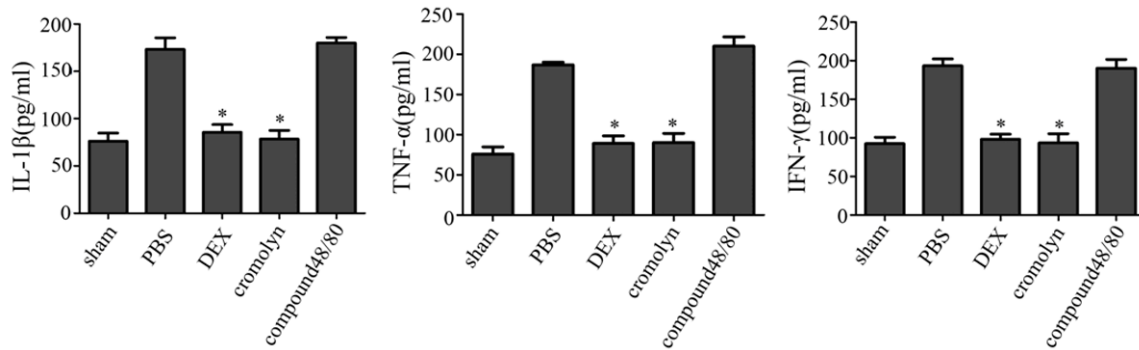


Figure 2. The expression of pro-inflammation cytokines. Dexmedetomidine suppressed I/R induced upregulation of TNF- α , INF- γ and IL-1 β levels expressions in spinal. ELISA was performed to detect the serum expression of TNF- α , INF- γ and IL-1 β levels 24 h following I/R insult ($n = 5$; * $P < 0.05$).

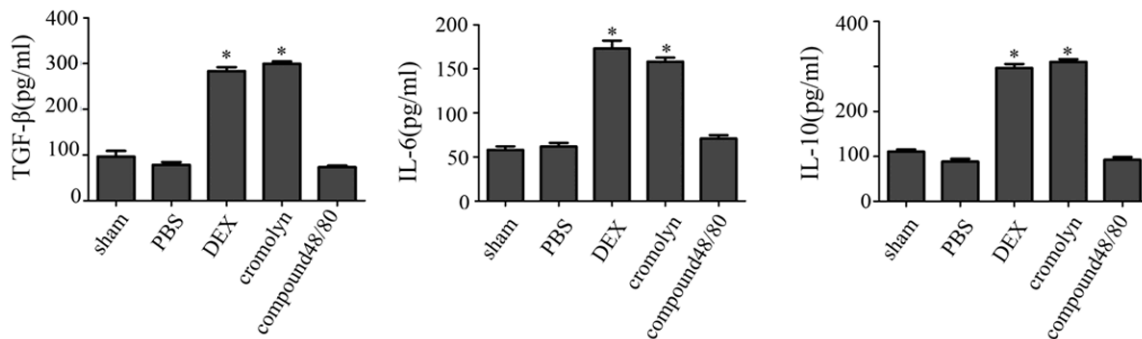


Figure 3. The expression of anti-inflammation cytokines. Dexmedetomidine induced upregulation of TGF- β , IL-10 and IL-6 levels expressions in spinal to alleviate the spinal I/R injury. ELISA was performed to detect the serum expression of TGF- β , IL-10 and IL-6 levels 24 h following I/R insult ($n = 5$; * $P < 0.05$).

Results

Functional outcomes

Mice in the sham group had no observable functional deficit at any time point in the post-operative period. Ischemia with 5 minutes of aortic occlusion produced a significant functional deficit in all mice. Twenty-four hours of pretreatment with dexmedetomidine, PBS, cromolyn, compound 48/80 resulted in a significant neurologic function ($P < 0.05$) at all time points postoperatively (**Figure 1**).

Effect of dexmedetomidine on serum of rats TNF- α , INF- γ and IL-1 β levels

To determine whether treatment with dexmedetomidine could reduce inflammation cytokines of MCs degranulation. ELISA assay was performed to evaluate the I/R induced changes in the protein levels of TNF- α , INF- γ and IL-1 β , which in part, responsible for the pathogenesis

of many inflammatory diseases. Results presented here showed that spinal I/R led to a significant increase in the TNF- α , INF- γ and IL-1 β levels at 24 h time point. Perioperative treatment with dexmedetomidine, MCs stabilizer cromolyn both significantly inhibited I/R injury induced up-regulation of TNF- α , INF- γ and IL-1 β production, there were no significant (**Figure 2**). The group of stimulate MCs degranulation compound 48/80 prompted the I/R injury. This suggested that dexmedetomidine might stabilize the MC membrane and displayed better anti-inflammatory effect in I/R model rats.

Effect of dexmedetomidine on serum of rats TGF- β , IL-10 and IL-6 levels

MCs degranulation could release pro-inflammation cytokines, whereas resting MCs could release anti-inflammation cytokines. To determine whether treatment with dexmedetomidine could stabilize MCs membrane, we detected the anti-inflammation cytokines TGF- β , IL-10

Dex alleviates the spinal cord IRI through blocking MC degranulation

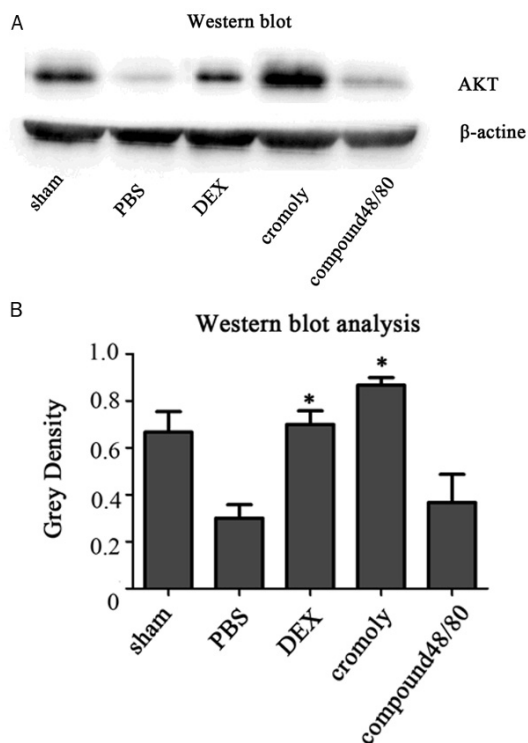


Figure 4. The protein of AKT expression. Dexmedetomidine induced enhancement in spinal AKT expression. A. Western blots assay was performed to evaluate AKT protein expression in the spinal 24 h following I/R insult. B. Bands corresponding to AKT and β-actin protein were quantified densitometrically. Results are expressed as the ratio of full-length AKT to β-actin. Data are means ± SD of three independent experiments performed in triplicate or a representative result. * $P < 0.05$.

and IL-6 which released by MCs (**Figure 3**). ELISA assay was performed to evaluate after I/R. The results showed the rats pretreated with dexmedetomidine and cromolyn that the TGF-β, IL-10 and IL-6 levels were high after I/R injury, the group of compound 48/80 had opposite result. So, this result suggested pretreated with dexmedetomidine made MCs release anti-inflammation cytokines to protect the spinal-cord from I/R injury.

Pretreatment and survival signaling

AKT and CREB phosphorylation are protect protein signaling after I/R injury of neural system. Spinal cord homogenates were analyzed for AKT and CREB phosphorylation. Following pretreatment with dexmedetomidine and cromolyn there were a robust and significant (* $P < 0.05$) increase in AKT and CREB phosphorylation (**Figures 4, 5**). To determine if CREB phosphory-

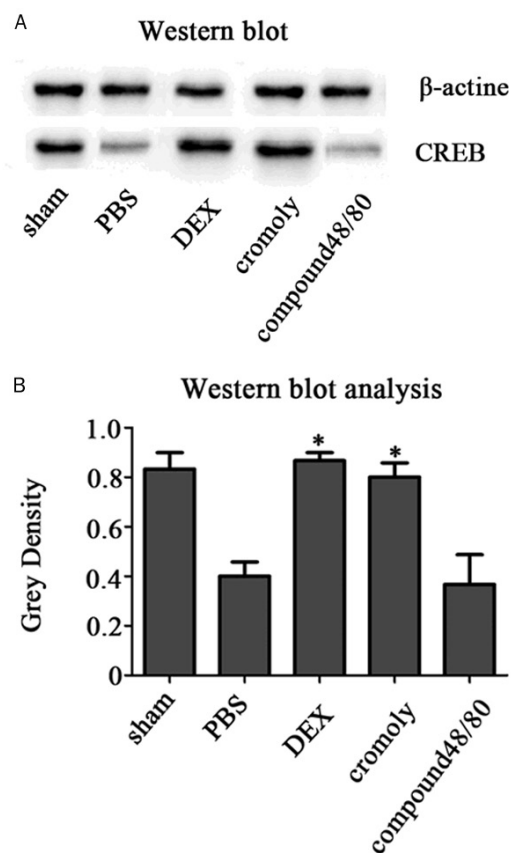


Figure 5. The protein of CREB expression. Dexmedetomidine induced enhancement in spinal CREB expression. A. Western blots was performed to evaluate CREB protein expression in the spinal 24 h following I/R insult. B. Bands corresponding to CREB and β-actin protein were quantified densitometrically. Results are expressed as the ratio of full-length CREB to β-actin. Data are means ± SD of three independent experiments performed in triplicate or a representative result. * $P < 0.05$.

lation resulted in a subsequent production of neuroprotective proteins with dexmedetomidine treatment, spinal cord tissue was tested by quantitative polymerase chain reaction for production of BDNF. Pretreatment with dexmedetomidine resulted in a significant BDNF when compared with the group of compound 48/80 and PBS (**Figure 6**). Dexmedetomidine treatment resulted in statistically significant elevations in BDNF expression following I/R surgery. There was no significant with the group of cromolyn.

Dexmedetomidine down regulation the FcεR of MCs

To examine the indirect effect of dexmedetomidine on MCs, we extracted the rat MCs from

Dex alleviates the spinal cord IRI through blocking MC degranulation

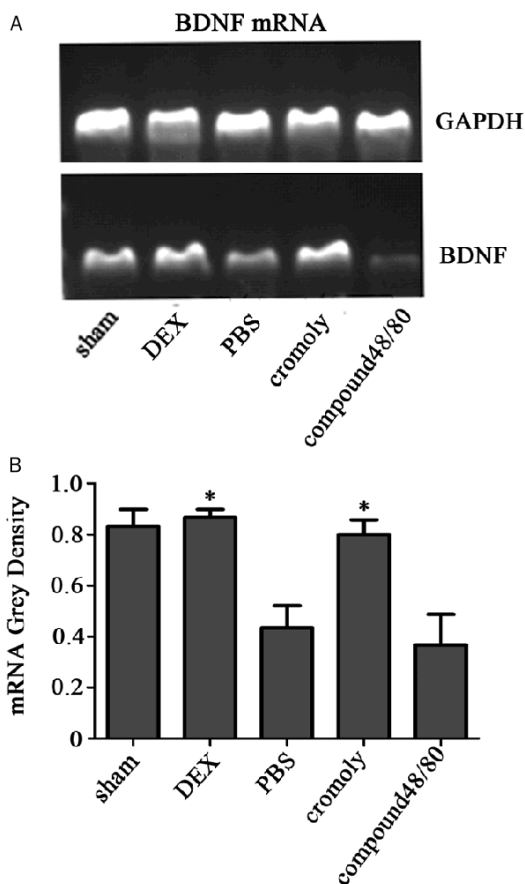


Figure 6. The mRNA of BDNF expression. A. Quantitative polymerase chain reaction for production of brain-derived neurotrophic factor (BDNF) transcripts was performed. Pretreatment with dexmedetomidine resulted in a significant up-regulation BDNF messenger RNA expression. B. Bands corresponding to BDNF and GAPDH were quantified densitometrically. Data are means \pm SD of three independent experiments performed in triplicate. * $P < 0.05$.

bone marrow (MCs in spinal and bone marrow are homologous), then, pretreated with PBS, dexmedetomidine, cromolyn, compound48/80 *in vitro*. After 24 h, expression of Fc ϵ R at the MCs surface was down-regulated (**Figure 7**), and there was no difference between the cromolyn group and DEX group. So, dexmedetomidine could alleviate the MCs activity to block them degranulation.

The degranulation of MCs pretreated with dexmedetomidine was decreased

MCs pretreated with PBS, DEX, cromolyn, compound 48/80 after 24 h. Using anti-DNP-IgE to stimulate MCs degranulation, we found the MCs pretreated with DEX, cromolyn they degranula-

tion were decreased, which compared with the group of PBS and compound 48/80 (* $P < 0.05$) (**Figure 8**). So, the dexmedetomidine could stabilize the MCs membrane.

Discussion

Ischemia-reperfusion (I/R) injury is inevitable in many surgery, vascular and musculoskeletal traumas, diseases, and free tissue transfers and during time-consuming reconstructive and transplantation surgeries [21]. The mechanism of organ damage after I/R injury has been studied extensively and consists of complex interactions of multiple pathways. The major contributors to I/R-induced injury included production of reactive oxygen species (ROS), release of proinflammatory cytokines that exacerbate inflammation and tissue damage and activation of immune cells to promote cell death by apoptosis or necrosis [22]. Some studies found that the dexmedetomidine could alleviate the I/R injury in rat skeletal muscle [23]. Experimental studies have shown that dexmedetomidine exerts an antioxidant effect in several organs, including brain, kidney, and heart [24]. In addition, dexmedetomidine has been shown to reduce the expression of I/R injury markers during upper extremity surgery using a tourniquet in human studies [25]. A previous study also showed that severe damage in the cerebral injury was accompanied by an increase in proinflammatory cytokines, which was restored after dexmedetomidine administration [26], and as α -2a adrenergic agonist could increase phosphorylation of protein kinase B and CREB and subsequent up-regulation of antiapoptotic factor brain-derived neurotrophic factor to protective mechanism in the spinal cord following ischemia [27, 28]. But the specific mechanism of the role of dexmedetomidine in I/R injury is not clear. MCs have long been thought to only possess effector cell functions, given that they store an armada of pro-inflammatory cytokines and chemokines in their granules. MCs have been shown to promote I/R injury in many organs, mainly through their degranulation [29, 30]. However, the role of MCs in spinal I/R injury is less clear. MCs have a bidirectional role, they can release much of proinflammatory cytokines to induce I/R injury through degranulation, and they also can release anti-inflammatory cytokine to alleviate the I/R injury in the resting state [31]. So, we pretreated the rats with dexmedetomidine, cromolyn (the stabilizer

Dex alleviates the spinal cord IRI through blocking MC degranulation

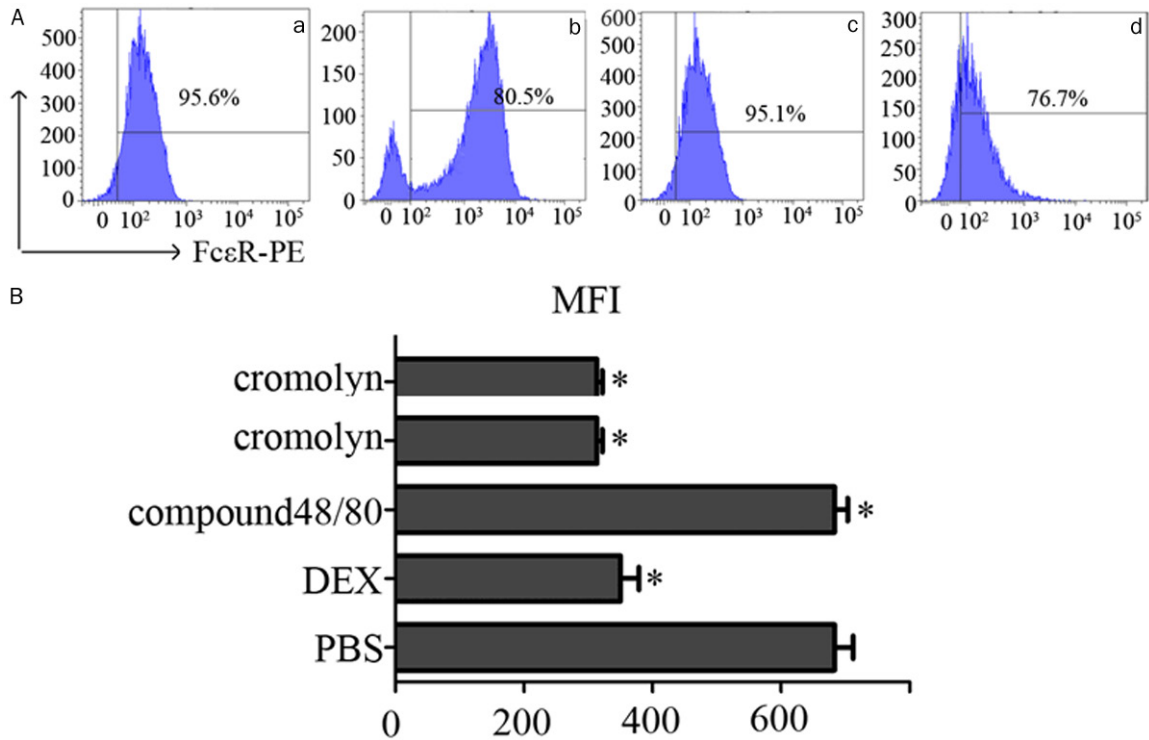


Figure 7. Expression of FcεR on the surface of MCs. MCs pretreated with PBS DEX cromolyn compound 48/80. A. FcεR expression on MCs decreased with DEX (b) and cromolyn (d). PBS (a) and compound 48/80 (c) had the opposite results. B. Expression of the FcεR on the MCs surface shown as mean fluorescence intensity (MFI) measured by flow cytometry after incubation for 24 h (with isotope control subtracted). Data are presented as the percentage MFI of MCs alone which was set as 100%. Data are means \pm SEM of three independent experiments. Statistical significance was calculated using the Student's t-test. * $P < 0.05$.

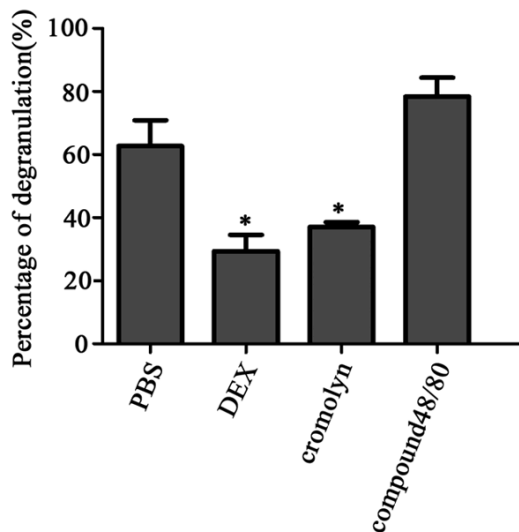


Figure 8. MCs degranulation induced by anti -DNP-IgE mAb and DNP-HAS was evaluated by 4-Nitrophenyl N-acetyl- β -D-glucosaminide after pretreatment. Compared with the control group, pretreatment with PBS, compound 48/80 obviously elicited MCs degranulation, whereas the DEX and cromolyn groups decreased the MCs degranulation, there were not

significantly different. Data are shown as the mean \pm SD of three independent experiments performed in triplicate. * $P < 0.05$.

of MCs membrane), compound 48/80 (inducer of MCs degranulation) to observe the mechanism of dexmedetomidine in spinal I/R injury. In our study, we found that pretreated the rats with dexmedetomidine has higher neurologic function at all time points after I/R injury. We collected the serum of rats then detected the pro-inflammation cytokines TNF- α , INF- γ and IL-1 β levels and anti-inflammation cytokines TGF- β , IL-10 and IL-6 levels, found that the pro-inflammation cytokines of dexmedetomidine group was decreased whereas the anti-inflammation cytokines was increased. At the same time the protect protein of AKT, CREB and mRNA BDNF were increased. They had the same results with cromolyn group, and opposite with the compound 48/80 group. So, we thought dexmedetomidine might stabilize the MCs membrane to alleviate the spinal I/R injury

Dex alleviates the spinal cord IRI through blocking MC degranulation

in vivo. To observe the indirect role of dexmedetomidine on MCs. We pretreated MCs with dexmedetomidine in vitro, and found that the activity surface molecular of MCs was down-regulation, and MCs degranulation was decreased. We thus demonstrate a possible mechanism by which dexmedetomidine alleviates spinal cord I/R injury through blocking the MCs degranulation.

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Disclosure of conflict of interest

None.

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References

- [1] Greenberg RK, Lu Q, Roselli EE, Svensson LG, Moon MC, Hernandez AV. Contemporary analysis of descending thoracic and thoracoabdominal aneurysm repair: a comparison of endovascular and open techniques. *Circulation* 2008; 118: 808-17.
- [2] Amaro S, Chamorro Á. Translational stroke research of the combination of thrombolysis and antioxidant therapy. *Stroke* 2011; 42: 1495-1499.
- [3] Chen YF, Tsai HY, Wu KJ, Siao LR, Wood WG. Pipoxolan ameliorates cerebral ischemia via inhibition of neuronal apoptosis and intimal hyperplasia through attenuation of VSMC migration and modulation of matrix metalloproteinase 2/9 and Ras/MEK/ERK signaling pathways. *PLoS One* 2013; 8: e75654.
- [4] Li J, Han B, Ma X, Qi S. The effects of propofol on hippocampal caspase-3 and Bcl-2 expression following forebrain ischemia-reperfusion in rats. *Brain Res* 2010; 1356: 11-23.
- [5] Demuro JP, Botros D, Nedea E. Use of dexmedetomidine for postoperative analgesia in spine patients. *J Neurosurg Sci* 2013; 57: 171-174.
- [6] Reardon DP, Anger KE, Adams CD. Role of dexmedetomidine in adults in the intensive care unit: an update. *Am J Health Syst Pharm* 2013; 70: 767-777.
- [7] Pandharipande PP, Pun BT, Herr DL, Maze M, Girard TD, Miller RR, Shintani AK, Thompson JL, Jackson JC, Deppen SA, Stiles RA, Dittus RS, Bernard GR, Ely EW. Effect of sedation with dexmedetomidine vs lorazepam on acute brain dysfunction in mechanically ventilated patients: the MENDS randomized controlled trial. *JAMA* 2007; 298: 2644-2653.
- [8] Sanders RD, Sun P, Patel S, Li M, Maze M and Ma D. Dexmedetomidine provides cortical neuroprotection: Impact on anaesthetic-induced neuroapoptosis in the rat developing brain. *Acta Anaesthesiologica Scandinavica* 2010; 54: 710-716.
- [9] Ayoglu H, Gul S, Hanci V. The effects of dexmedetomidine dosage on cerebral vasospasm in a rat subarachnoid haemorrhage model. *J Clin Neurosci* 2010; 17: 770-773.
- [10] Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA. cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *J Neurosci* 2002; 22: 3262-8.
- [11] Gu J, Chen J, Xia P, Tao G, Zhao H and Ma D. Dexmedetomidine attenuates remote lung injury induced by renal ischemia-reperfusion in mice. *Acta Anaesthesiologica Scandinavica* 2011; 55: 1272-1278.
- [12] Hanci V, Erol B, Bektas S. Effect of dexmedetomidine on testicular torsion/detorsion damage in rats. *Urologia Internationalis* 2010; 84: 105-111.
- [13] Okita Y. Fighting spinal cord complication during surgery for thoracoabdominal aortic disease. *Gen Thorac Cardiovasc Surg* 2011; 59: 79-90.
- [14] Jaggi AS, Singh M, Sharma A. Cardioprotective effects of mast cell modulators in ischemia-reperfusion-induced injury in rats. *Methods Find Exp Clin Pharmacol* 2007; 29: 593-600.
- [15] Biran V, Cochois V, Karroubi A. Stroke induces histamine accumulation and mast cell degranulation in the neonatal rat brain. *Brain Pathol* 2008; 18: 1-9.
- [16] Oldford SA, Haidl ID, Howatt MA. A critical role for mast cells and mast cell-derived IL-6 in TLR2-mediated inhibition of tumor growth. *J Immunol* 2010; 185: 7067-76.
- [17] Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* 2007; 217: 141-54.
- [18] Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005; 23: 749-86.
- [19] Kageyama Yahara N, Suehiro Y, Yamamoto T, adowaki KM. IgE-induced degranulation of mucosal mast cells is negatively regulated

Dex alleviates the spinal cord IRI through blocking MC degranulation

- via nicotinic acetylcholine receptors. *Biochem Biophys Res Commun* 2008; 377: 321-325.
- [20] Smith PD, Puskas F, Meng X, Cho D, Cleveland JC, Weyant MJ. Ischemic dose-response in the spinal cord: both immediate and delayed paraplegia. *J Surg Res* 2011; 7: 1-7.
- [21] Wang WZ, Baynosa RC, Zamboni WA. Therapeutic interventions against reperfusion injury in skeletal muscle. *J Surg Res* 2011; 17: 175-82.
- [22] Klune JR, Tsung A. Molecular biology of liver ischemia/reperfusion injury: Established mechanisms and recent advancements. *Surg Clin North Am* 2001; 90: 665-77.
- [23] Dong X, Xing Q, Li Y, Han X, Sun L. Dexmedetomidine protects against ischemia-reperfusion injury in rat skeletal muscle. *J Surg Res* 2014; 186: 240-245.
- [24] Gu J, Sun P, Zhao H. Dexmedetomidine provides renoprotection against ischemia-reperfusion injury in mice. *Crit Care* 2011; 15: R153.
- [25] Yagmurdur H, Ozcan N, Dokumaci F. Dexmedetomidine reduces the ischemia-reperfusion injury markers during upper extremity surgery with tourniquet. *J Hand Surg Am* 2008; 33: 941-7.
- [26] Grosu I, Lavand'homme P. Continuous regional anesthesia and inflammation, a new target. *Minerva Anesthesiol* 2015; 81: 1001-9.
- [27] Marshall T. Bell, Ferenc Puskas, Daine T. Bennett, Paco S. Herson, Nidia Quillinan, David A. Fullerton T. Brett Reece. Dexmedetomidine, an α -2a adrenergic agonist, promotes ischemic tolerance in a murine model of spinal cord ischemia-reperfusion. *J Thorac Cardiovasc Surg* 2014; 147: 500-6.
- [28] Kim KH. Safe Sedation and Hypnosis using Dexmedetomidine for Minimally Invasive Spine Surgery in a Prone Position. *Korean J Pain* 2014; 27: 313-320.
- [29] Abonia JP, Friend DS, Austen WG Jr. Mast cell protease 5 mediates ischemia-reperfusion injury of mouse skeletal muscle. *J Immunol* 2005; 174: 72-85.
- [30] Lin FS, Shen SQ, Chen ZB, Yan RC. Beta-estradiol attenuates reduced-size hepatic ischemia/reperfusion injury by inhibition apoptosis via mitochondrial pathway in rats. *Shock* 2012; 37: 183-90.
- [31] Schemann M, Kugler EM, Buhner S. The mast cell degranulator compound 48/80 directly activates neurons. *PLoS One* 2012; 7: e52104.