Chinese Journal of Traumatology 2011; 14(2):92-95

Protective effects of ischemic postconditioning on intestinal mucosa barrier function in rabbits with crush injury of hind limb: an experimental study

*DING Jun-tao and ZHANG Lian-yang**

【**Abstract**】**Objective:** To explore the protective effects of two types of ischemic postconditioning (IP) on intestinal mucosa barrier in rabbits with crush injury of the hind limb.

Methods: This study was conducted between August and December 2008 in the Department of Trauma Surgery, Daping Hospital, Third Military Medical University, Chongqing, China. The model of crush injury to the hind limb of rabbits was firstly developed by a 25 kg object with the right hind limbs fixed by wooden splints, and then two types of IP were established, including occluding/opening the common iliac artery and vein alternatively (traditional IP, IP A) and binding/loosening the proximum of the injured hind limb alternatively (modified IP, IP B). Thirty-six male New Zealand white rabbits were randomly divided into three groups: IP A group, IP B group and control group, with 12 rabbits in each group. The serum levels of diamine oxidase (DAO) and intestinal fatty acid-binding protein (I-FABP) were detected at 2, 6, 12 and 24 hours after injury. Pathological changes of ileum were examined at 24 hours after injury.

Results: The serum levels of I-FABP at 2, 6, 12 and 24 hours after injury in both IP A and IP B groups had a significant decrease, compared with control group. DAO levels also showed the same change trend at 2 and 6 hours after injury, but showed no significant difference between two IP groups. No difference in pathological changes of ileum was found among the three groups.

Conclusions: IP can protect intestinal mucosa barrier function on the model of hind limb crush injury in rabbits. Meanwhile the modified IP B shows the same protection as the traditional IP A, and is worth applying in clinic.

Key words: ***Ischemic postconditioning; Crush syndrome; Intestinal mucosa***

Chin J Traumatol 2011; 14(2):92-95

Traumatic rhabdomyolysis, which is common in natural disasters such as earthquake and hurricane, refers to rhabdomyolysis caused by crush injuries, especially those in the limbs. Its clinical manifestations are muscular fatigue, pain and weakness, dark urine and other typical symptoms of muscular injury. However, due to the non-specific or mild symptoms, clinical and laboratory findings are easily neglected at the early phase of the illness. And the complications of rhabdomyolysis are life threatening, thus it is vital to make an early diagnosis and interventions.

Ischemic postconditioning (IP) is a new measure raised recently to decrease the damage of ischemia-reperfusion. Some reports have proved that IP can prevent acute ischemic renal failure in rats.[1] But there are few reports on the first aid of crush injury. After limb crush injury, body fluids flow to the third interstice, which leads to blood volume depletion and metabolic disorder. Intestinal mucosa is very sensitive to ischemia. It is proved that intestinal chorioepithelium will disappear after ischemia for 2 hours (by occlusion of mesenteric arteries).[2] However, because the traditional measure requires dissection of the main artery that supplies the organ, and is not feasible in the field first aid of trauma. This study aimed to explore the protective effects of two types of IP (the traditional IP and a modified IP, which is similar to the remote IP mode raised by Yang et al[3]) on intestinal mucosa barrier in a rabbit model of hind limb crush injury.

DOI: 10.3760/cma.j.issn.1008-1275.2011.02.005
Trauma Center of PLA, State Key Laboratory of Trauma, Burns and Combined Injury, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing 400042, China (Ding JT and Zhang LY)

*Corresponding author: Tel: 86-23-68757991, Email: dpzhangly@163.com

METHODS

Animal model

This study was conducted between August and December 2008 in the Department of Trauma Surgery, Daping Hospital, Third Military Medical University, Chongqing, China after being approved by the Third Military Medical University Council on Animal Care in accordance with the Guidance of Experimental Animal Welfare. A total of 16 male New Zealand white rabbits weighing 2.03 to 2.55 kg from the Experimental Animal Center of Daping Hospital were randomized into two groups with 8 rabbits in each group. They were fed routinely. All the rabbits were anesthetized with 30 mg/kg pentobarbital sodium injected into the marginal ear vein. Then, their right hind limbs were fixed by wooden splints and crushed with a 25 kg object. The rabbits were divided as 4-h group and 10-h group according to the duration of crush. Before and 12 hours after crush, 3 ml blood was obtained from the central ear artery, and centrifuged at 3 200 r/min for 5 minutes with equal volume of heparin. Then the supernatant was utilized for the detection of creatinine kinase MB isoenzyme (CK-MB, goat anti-rabbit CK-MB ELISA kit; ADL Inc. USA). Similarly, 5 ml sterile urine was taken and centrifuged at 3 200 r/min for 5 minutes. The obtained supernatant was utilized for the detection of myoglobin (Myo, goat anti-rabbit Myo ELISA kit, ADL Inc. USA). The results are shown in Table 1. In the 4-h group, the serum CK-MB and urine Myo levels were not significantly elevated after crush (P>0.05), while in the 10-h group, the increase was significant (P<0.05). The 10-h group was thus inferred to have a serious crush injury and the experimental condition was adopted for the subsequent experiment in the study.

IP models

IP A: occlude/open the common iliac artery and vein alternately Dissect the right common iliac artery and vein before crush. Then alternately occlude and open the common iliac artery and vein at 60 s/time for 3 times.

IP B: bind/ loosen alternately the proximum of the injured hind limb Bind the proximum of right limb by tourniquet (till the pulse of dorsal artery of right foot cannot be touched) before crush. Then bind/ loosen alternately the proximum of the injured hind limb at 60 s/time for 3 times.

Grouping

In the subsequent experiment, a total of 36 male New Zealand white rabbits weighing 2.05-2.67 kg, averaged 2.33 kg±0.18 kg, were included after fed conventionally for one week. The diet and rearing environment were concordant with the national standard (GB 14924.4-2001; GB 14925-2001, China). The rabbits were randomly divided into three groups: IP A group, IP B group and control group (12 rabbits in each group).

Pathological and laboratory findings

Before and 2, 6, 12 and 24 hours after crush, blood samples were collected and sent for laboratory examination. The samples (3 ml from each rabbit) were centrifuged at 3 200 r/min for 20 minutes with equivalent volume of heparin. The supernatant was then taken and preserved at -20 °C. The serum diamine oxidase (DAO) and intestinal fatty acid-binding protein (I-FABP) concentrations were detected at the end of the experiment with goat anti-rabbit DAO ELISA kit and goat anti-rabbit I-FABP ELISA kit respectively (ADL Inc. USA). Twenty-four hours after crush, the rabbits were executed and ileum specimens (size of 1 cm×1 cm and 5 cm from the ileocecal junction) were sampled. The samples were soaked in 4% formalin for one week and stained with HE for pathological examination by a microscope (x100, Leica, DM2500, Germany). The 12 rabbits in control group received the same pathological and laboratory examinations simultaneously.

Statistical analysis

The data were expressed as $\bar{x}\pm s$. Student's t test was performed to analyze serum DAO level and I-FABP level, and Mann-Whitney U test to process the enumeration data by SPSS 16.0. Data were recognized as significant difference if the P value was less than 0.05.

RESULTS

The serum DAO level began to increase at 2 hours and peaked at 6 hours after crush, and it was lower in groups IP A and IP B, compared with controls (P<0.05). At 12 and 24 hours after crush, the differences in DAO levels showed no significance among the three groups (P>0.05, Table 2).

The serum I-FABP level began to rise at 2 hours and peaked at 12 hours after crush, and the level was much lower in groups IP A and IP B, compared with

controls in the whole laboratory process (P<0.05). There was no significant difference for I-FABP levels between groups IP A and IP B (P>0.05, Table 3).

There was no significant difference in the microvilli of ileum specimens under a microscope in all groups (x100, Leica, DM2500, Germany). The microvilli injury was regarded as grades 1 to 3 based on the standard of Chiu et al's[4], and it revealed no significant difference in all groups by Mann-Whitney U test (Figure 1).

Table 1. CK-MB and Myo concentrations before and after crush ($\bar{x}$±s, ng/ml)

Groups	Serum CK-MB		Urine Myo	
	Before crush	After crush	Before crush	After crush
4-h group (n=8)	37.73±21.71	40.31±13.54#	273.39±171.98	390.19±169.36#
10-h group (n=8)	34.15±20.38	61.64±12.97*	279.63±163.00	995.19±296.19*

#P>0.05 and *P<0.05, compared with the values before crush.

Table 2. Serum DAO concentrations at various time points (n=12 for each group, $\bar{x}$±s, U/ml)

Groups	Time after crash injury				
	0 h	2 h	6 h	12 h	24 h
Control	7.42±0.50	9.81±0.75	13.44±1.11	7.99±0.44	7.30±0.99
IP A	7.43±0.58●	7.48±0.46▲	8.69±0.69▲	7.67±0.44●	7.35±0.60●
IP B	7.52±0.60●	7.43±0.46▲*	8.70±0.70▲*	7.67±0.40●*	7.23±0.57●*

●P>0.05 and ▲P<0.05, compared with controls; *P>0.05, compared with IP A group.

Table 3. Serum I-FABP concentrations at various time points (n=12 for each group, $\bar{x}$±s, ng/ml)

Groups	Time after crash injury				
	0 h	2 h	6 h	12 h	24 h
Control	100.76±3.19	120.70±4.96	133.78±3.45	161.24±5.69	120.97±4.68
IP A	99.96±3.09●	108.75±4.81▲	117.04±3.70▲	125.52±4.22▲	109.12±6.95▲
IP B	99.42±3.25●	108.62±3.05▲*	117.83±3.45▲*	127.93±3.86▲*	106.59±3.92▲*

●P>0.05 and ▲P<0.05, compared with controls; *P>0.05, compared with IP A group.

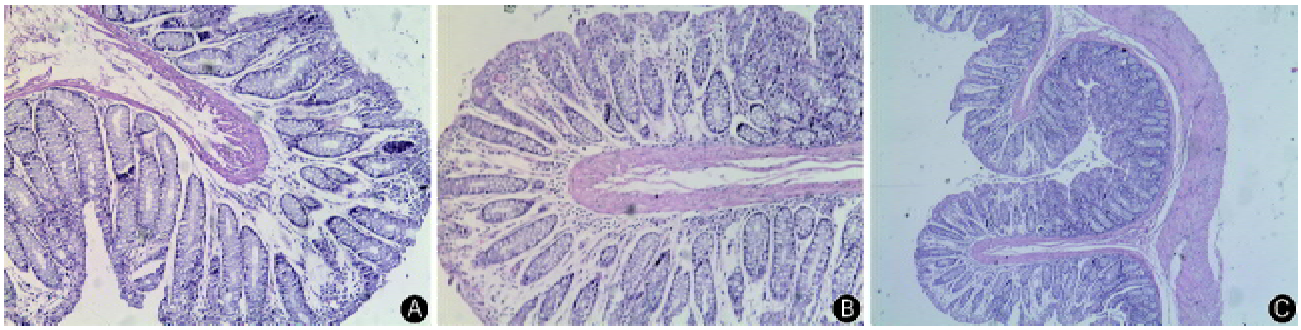

Figure 1. HE stain of the ileum microvilli specimens from IP A (**A**), IP B (**B**) and control groups (**C**) under a microscope (×100, Leica, DM2500, Germany), which shows no significant difference among three groups according to Mann-Whitney U test.

DISCUSSION

It has been proved that trauma can lead to atrophy of the intestinal mucosa in rats. Endotoxemia (lipopolysaccharide) damages the intestinal barrier function,[5] so how to relieve this damage in trauma is very worth exploring. In this study, we choose DAO and I-FABP as the indexes of intestinal function. DAO, an enzyme which deaminates histamine and polyamines, is very active in the intestinal mucosa in most mammalian species, including humans.[6] It is found almost exclusive in the small intestine. The activity of DAO is closely correlated with intestine nucleic acid and protein synthesis, and its plasma level reveals mucosal DAO content and structural change in the intestinal mucosa. Plasma DAO activity can reflect the development of mucosal injury in severe trauma. I-FABP is specific for intestinal mucosa and sensitive to intestinal ischemia. The serum I-FABP combined with DAO is an early, sensitive and specific biochemical marker in the diagnosis of intestinal mucosal barrier injury after ischemia-reperfusion.

In this study, we found that the activities of DAO and I-FABP in the IP groups significantly increased, suggesting that both types of IP have protective effects on intestinal barrier function after crush injury. And the level of DAO returned to normal earlier than that of I-FABP, which was still higher than that of the control. The microvilli of ileum specimens were not significantly different under a microscope in all groups, however it needs further studies to determine whether the intestinal mucosa changed morphologically or had already repaired.

There are many factors that may lead to intestinal mucosa damage in trauma. IP is a new measure raised recently to decrease the damage. It was firstly raised in the studies on ischemia-reperfusion injury in heart, and was proved effective in protecting the functions of heart and kidney on some animal models.[7-10] But the traditional measure requires dissection of the main artery that supplies the organ, thus it is not feasible in the first aid of locus in quo remedy in trauma. To solve this problem, we explored a new method of IP: to bind/loosen alternately (60 s/time, 3 times) the proximum of the injured hind limb (IP B), which showed the same protection for intestinal barrier function as the traditional one (IP A). The new measure has notable protective effects and is feasible; therefore it is worth applying in clinic. But the best combination of time for perfusion-reperfusion and number of repetition need to be studied in-depth.

REFERENCES

1. Wang W, Tang T, Zhang P, et al. Postconditioning attenuates renal ischemia-reperfusion injury by preventing DAF down-regulation. J Urolo 2010;183(6):2424-2431.

2. Blikslager AT, Roberts MC, Rhoads JM, et al. Is reperfusion injury an important cause of mucosal damage after porcine intestinal ischemia? Surgery 1997;121(5): 526-534.

3. Yang XM, Proctor JB, Cui L, et al. Multiple brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. J Am Coll Cardiol 2004;44(5):1103-1110.

4. Chiu CJ, Mcardle AH, Brown R, et al. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970;101(4): 478-483.

5. Ding LA, Li JS, Li YS, et al. The intestinal barrier damage caused by trauma and lipopolysaccharide. Chin J Exp Surg 2004; 21(6):697-699.

6. Peng X, Yan H, You ZY, et al. Effects of enteral supplementation with glutamine granules on intestinal mucosal barrier function in severe burned patients. Burns 2004;30(2): 135-139.

7. Szwarc I, Soullier S, Gayrard N, et al. Ischemic postconditioning prevents ischemic acute renal failure. Transplant Proc 2007;39(8):2554-2556.

8. Shi FW, Cai WQ, Chen ZY, et al. Effect of ischemic postconditioning on apoptosis and fas expression, Caspase-3 activity in pig myocardial cells during ischemic reperfusion. Chin J Exp Surg 2006;23(6):709-712.

9. Przyklenk K, Maynard M, Darling CE, et al. Aging mouse hearts are refractory to infarct size reduction with post-conditioning. J Am Coll Cardiol 2008;51(14):1393-1398.

10. Taskapilioglu MO, Alkan T, Goren B, et al. Neuronal protective effects of focal ischemic pre- and/or postconditioning on the model of transient focal cerebral ischemia in rats. J Clin Neurosci 2009;16(5):693-697.

(Received September 25, 2010)
Edited by LIU Gui-e