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• *BRIEF REPORTS* •

Effects of L-arginine on serum nitric oxide, nitric oxide synthase and mucosal *Na⁺-K⁺-ATPase* and nitric oxide synthase activity in segmental small-bowel autotransplantation model

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Abstract

AIM: To explore a simple method to create intestinal autotransplantation in rats and growing pigs and to investigate the effect of L-arginine supplementation on serum nitric oxide (NO), nitric oxide synthase (NOS) and intestinal mucosal NOS and Na^+-K^+-ATPase activity during cold ischemia-reperfusion (IR) in growing pigs.

METHODS: In adult Wistar rat models of small bowel autotransplantation, a fine tube was inserted into mesenteric artery via the abdominal aorta. The superior mesenteric artery and vein were occluded. Isolated terminal ileum segment was irrigated with Ringer's solution at 4 ℃ and preserved in the same solution at 0-4 ℃ for 60 min. Then, the tube was removed and reperfusion was established. In growing pig models, a terminal ileum segment, 50 cm in length, was isolated and its mesenteric artery was irrigated via a needle with lactated Ringer's solution at 4 ℃. The method and period of cold preservation and reperfusion were described above. Ten white outbred pigs were randomly divided into control group and experimental group. L-arginine (150 mg/kg) was continuously infused for 15 min before reperfusion and for 30 min after reperfusion in the experimental group. One, 24, 48, and 72 h after reperfusion, peripheral vein blood was respectively collected for NO and NOS determination. At the same time point, intestinal mucosae were also obtained for NOS and Na+-K+-ATPase activity measurement.

RESULTS: In adult rat models, 16 of 20 rats sustained the procedure, three died of hemorrhage shock and one of deep anesthesia. In growing pig models, the viability of small bowel graft remained for 72 h after cold IR in eight of 10 pigs. In experimental group, serum NO level at 1 and 24 h after reperfusion increased significantly when compared with control group at the same time point (152.2±61.4 μmol/L *vs* 60.8±31.6 μmol/L, $t = 2.802$, $P = 0.02<0.05$; 82.2±24.0 μmol/L *vs* 54.0±24.3 μmol/L, $t = 2.490$, $P = 0.04<0.05$). Serum NO level increased significantly at 1 h post-reperfusion when compared with the same group before cold IR, 24 and 48 h post-reperfusion (152.2±61.4 μmol/L *vs* 75.6±16.2 μmol/L, $t = 2.820$, $P = 0.02<0.05$, 82.2 ±24.0 μmol/L, $t = 2.760$, $P = 0.03<0.05$, 74.2±21.9 μmol/L, $t = 2.822$, $P = 0.02<0.05$). Serum NOS activity at each time point had no significant difference between two groups. In experimental group, intestinal mucosal NOS activity at 1 h post-reperfusion reduced significantly when compared with pre-cold IR (0.79±0.04 U/mg *vs* 0.46±0.12 U/mg, $t = 3.460$, $P = 0.009<0.01$). Mucosal NOS activity at 24, 48, and 72 h post-reperfusion also reduced significantly when compared with pre-cold IR (0.79±0.04 U/mg *vs* 0.57±0.14 U/mg, $t = 2.380$, $P = 0.04<0.05$, 0.61±0.11 U/mg, $t = 2.309$, $P = 0.04<0.05$, 0.63±0.12U/mg, $t = 2.307$, $P = 0.04<0.05$). In control group, mucosal NOS activity at 1 and 24 h post-reperfusion was significantly lower than that in pre-cold IR (0.72±0.12 U/mg *vs* 0.60±0.07 U/mg, $t = 2.320$, $P = 0.04<0.05$, 0.58±0.18 U/mg, $t = 2.310$, $P = 0.04<0.05$). When compared to the normal value, Na^+-K^+-ATPase activity increased significantly at 48 and 72 h post-reperfusion in experimental group (2.48±0.59 μmol/mg *vs* 3.89±1.43 μmol/mg, $t = 3.202$, $P = 0.04<0.05$, 3.96±0.86 μmol/mg, $t = 3.401$, $P = 0.009<0.01$) and control group (2.48±0.59 μmol/mg *vs* 3.58±0.76 μmol/mg, $t = 2.489$, $P = 0.04<0.05$, 3.67±0.81 μmol/mg, $t = 2.542$, $P = 0.03<0.05$).

CONCLUSION: This novel technique for intestinal autotransplantation provides a potentially consistent and practical model for experimental studies of graft cold preservation. L-arginine supplementation during cold IR may act as a useful adjunct to preserve the grafted intestine.

Key words: Intestine transplantation; Nitric oxide; L-arginine; Animal model

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INTRODUCTION

Small intestinal transplantation may eventually become the most logical definitive treatment for short bowel syndrome. One of the determining factors in intestinal transplantation is the extreme sensitivity of bowel to cold IR injury[1,2]. Apart from rejection-related events, the manifestation of IR injury remains a major problem, hampering success in human small bowel transplantation (SBT)[3]. Moderate hypothermia throughout intestinal IR injury reduces multiple organ dysfunction[4]. Ischemic preconditioning provides a way of protecting organs from damage inflicted with prolonged IR[5,6]. It has been shown that L-arginine and molsidomine application prior to reperfusion significantly reduced the preservation and reperfusion-related injury after small bowel transplantation. The architecture of intestinal mucosae was best preserved after treatment with L-arginine and methylprednisolone[7]. NO is an important mediator of both physiological and pathological responses. Its dual role in ischemia-reperfusion syndrome is still controversial[8].

At present, small intestinal transplantation models in rats and pigs has frequently been used in experimental investigations. However, the surgical technique involved in intestinal transplantation in rats and pigs is rather difficult with a lower successful rate[9,10]. Cold preservation and vascular anastomosis of graft are very important for successful transplantation[11]. In the current study, the aim was to explore a simple method to create intestinal autotransplantation in rats and pigs and to investigate the effects of L-arginine on serum NO, NOS and intestinal mucosal NOS and Na^+-K^+-ATPase activity after cold IR in growing pig models.

MATERIALS AND METHODS

Creation of intestinal autotransplantation model in rats

Healthy inbred Wistar rats of both sex, weighing 250-320 g, from Experimental Animal Center of Shandong University were used in the present study. The rats were provided with standard laboratory chow and water and housed in accordance with institutional animal care policies. Twelve hours prior to experimentation, animals were fasted with free access to water. All animals were anesthetized with intraperitoneal ketamine (15 mg/kg) and maintained with intermittent boluses of intraperitoneal ketamine (10 mg/kg). Animals were placed on a heating pad set at 37 ℃. Through a mid-abdominal incision, the aorta below the renal artery level was carefully dissected with microsurgical technique. A fine tube was inserted into mesenteric artery via the dissected aorta. The superior mesenteric artery and vein were occluded with non-traumatic vascular clamps. The mesenteric artery of isolated terminal ileum segment was irrigated with Ringer's solution at 4 ℃, and the fluid outflowed through a leak of mesenteric vein. Then the irrigated bowel segment *in vivo* was stored for 60 min in a plastic bag filled with Ringer's solution at 4 ℃. Hypothermia was maintained at 0-4 ℃ with ice below the water bag. The tube and clamps were removed, the leaks of aorta and vein were repaired with 9-0 non-traumatic suture. Reperfusion of the intestine (graft) was confirmed by the return of pulsatile mesenteric blood flow.

Creation of intestinal autotransplantation model in growing pigs

Ten white outbred pigs of both sex, from Animal Center of Nanjing General Hospital of Nanjing Military Command, four weeks of age, weighing 4.5-7.0 kg, were used to create intestinal autotransplantation model. Prior to the experiments, pigs were fasted for 24 h with free access to water. Atropine (0.02 mg/kg) and ketamine (20 mg/kg) were given intramuscularly 30 min before anesthesia. Sodium thiopental (20%) was given intravenously to maintain proper anesthesia. After laparotomy, 50-cm distal ileum was isolated and its mesenteric artery was irrigated with lactated Ringer's solution at 4 ℃. The mesenteric artery and vein were blocked with non-traumatic vascular clamps. At the same time, a 3-mm incision of the mesenteric vein was performed to drain the irrigation fluid. Cold preservation method and period were described above, and graft lumen was perfused with metronidazole solution at 4 ℃. During cold ischemia, the leaks of mesenteric vein and artery were repaired using microsurgical technique. When the cold ischemia ended (60 min), the vascular clamps were removed and the graft was re-vascularized. Both proximal and distal ends were exteriorized as stomas (Thiry-Vella loop), and the remaining bowel anastomosis was performed to restore its continuity.

Experimental protocol

Ten growing white outbred pigs, from Animal Center of Nanjing General Hospital of Nanjing Military Command, four weeks of age, weighing 5.0-7.5 kg, were randomized into two groups with five animals in each. Intestinal cold IR and autotransplantation model was established as described above. In experimental group (EG), intravenous L-arginine (150 mg/kg) was given for 15 min before reperfusion and for 30 min after reperfusion. In control group (CG), intravenous normal saline of the same volume was given.

Sampling procedure

One, 24, 48 and 72 h after reperfusion of the graft, each pig was anesthetized with ketamine intramuscularly, and 2 mL of peripheral blood sample was collected. The serum was separated and immediately stored at -20 ℃ for NO and NOS activity analysis. At the same time points, relaparotomy was performed and experimental bowel segment was sampled. The mucosae (about 1 g each time) were obtained, washed with cold normal saline, and stored immediately at -20 ℃ for NOS and Na^+-K^+-ATPase activity determination.

Assessment of serum NO and NOS, mucosal NOS and Na^+-K^+-ATPase activity

The assay kits of NO, NOS, and Na^+-K^+-ATPase activity were supplied by Nanjing Jiancheng Bioengineering

Table 1 Serum NO contents (μmol/L) at different time points of IR (mean±SE)

	n	Pre-IR	1h post-IR	24 h post-IR	48 h post-IR	72 h post-IR
EG	5	75.6±16.2[a]	152.2±61.4	82.2±24.0[a]	74.2±21.9[a]	95.6±25.7
CG	5	72.5±12.4	60.8±31.6[c]	54.0±24.3[c]	58.6±24.1	74.8±35.0

[a]$P<0.05$ *vs* 1 h post-IR in the experimental group (EG); [c]$P<0.05$ *vs* the value at the same time point in EG.

Table 2 Serum NOS activity (U/mL) at different time points of IR (mean±SE)

	n	Pre-IR	1h post-IR	24 h post-IR	48 h post-IR	72 h post-IR
EG	5	1.93±0.91	2.53±1.43	1.39±0. 42	1.49±0.40	1.31±1.01
CG	5	1.86±0.93	2.62±1.07	2.37±1.37	1.59±0.62	1.63±0.57

Table 3 Mucosal NOS activity (U/mg) at different time points of IR (mean±SE)

	n	Pre-IR	1h post-IR	24 h post-IR	48 h post-IR	72 h post-IR
EG	5	0.79±0.04	0.46±0.12[b]	0.57±0.14[a]	0.61±0.11[a]	0.63±0.12[a]
CG	5	0.72±0.12	0.60±0.07[c]	0.58±0.18[c]	0.64±0.17	0.62±0.17

[a]$P<0.05$ *vs* pre-IR in EG; [b]$P<0.01$ *vs* pre-IR in the experimental group (EG); [c]$P<0.05$ *vs* pre-IR in control group (CG), respectively.

Institute (Nanjing, China). The serum NO content and NOS activity were monitored according to the manufacturer's instructions. The stored mucosae were homogenized and centrifuged at 4 000 r/min for 20 min at 4 ℃. The mucosal NOS and Na+-K+-ATPase activity were detected according to the provider's instructions.

Statistical analysis

All data were presented as mean±SE. Significance was determined using *t* test. *P* less than 0.05 was considered statistically significant.

RESULTS

Successful creation of new bowel autotransplantation model in rats and pigs

In adult rat models, 16 of 20 rats sustained the procedure, three died of hemorrhage shock and one of deep anesthesia. In growing pig model, the viability of the small bowel graft was remained for 72 h after cold IR in 8 of 10 pigs, 2 died of either deep anesthesia or cold IR.

Serum NO contents and NOS activity

In experimental group, serum NO level at 1 and 24 h post-reperfusion increased significantly when compared with control group (152.2±61.4 μmol/L *vs* 60.8±31.6 μmol/L, $t = 2.802$, $P = 0.02<0.05$; 82.2±24.0 μmol/L *vs* 54.0±24.3 μmol/L, $t = 2.490$, $P = 0.04<0.05$). Serum NO level increased significantly at 1 h post-reperfusion when compared with the same group before cold IR, 24 and 48 h post-reperfusion (152.2±61.4 μmol/L *vs* 75.6±16.2 μmol/L, $t = 2.820$, $P = 0.02<0.05$, 82.2±24.0 μmol/L, $t = 2.760$, $P = 0.03<0.05$, 74.2±21.9 μmol/L, $t = 2.822$, $P = 0.02<0.05$) as showed in Table 1. Serum NOS activity at each time point had no significant difference between two groups as shown in Table 2.

Intestinal mucosal NOS and Na^+-K^+-ATPase activity

In experimental group, intestinal mucosal NOS activity at 1, 24, 48, and 72 h reduced significantly when compared with pre-cold IR (0.79±0.04 U/mg *vs* 0.46±0.12 U/mg, $t = 3.460$, $P = 0.009<0.01$, 0.57±0.14 U/mg, $t = 2.380$, $P = 0.04<0.05$, 0.61±0.11 U/mg, $t = 2.309$, $P = 0.04<0.05$, 0.63±0.12U/mg, $t = 2.307$, $P = 0.04<0.05$). In control group, NOS activity at 1 and 24 h post-reperfusion was significantly lower than that in pre-cold IR (0.72±0.12 U/mg *vs* 0.60±0.07 U/mg, $t = 2.320$, $P = 0.04<0.05$, 0.58±0.18 U/mg, $t = 2.310$, $P = 0.04<0.05$). But the difference of mucosal NOS activity at the same time point between two groups did not reach significance (Table 3). When compared to the normal value, Na^+-K^+-ATPase activity increased significantly at 48 and 72 h post-reperfusion in experimental group (2.48±0.59 μmol/mg *vs* 3.89±1.43 μmol/mg, $t = 3.202$, $P = 0.04<0.05$, 3.96±0.86 μmol/mg, $t = 3.401$, $P = 0.009<0.01$) and in control group (2.48±0.59 μmol/mg *vs* 3.58±0.76 μmol/mg, $t = 2.489$, $P = 0.04<0.05$, 3.67±0.81 μmol/mg, $t = 2.542$, $P = 0.03<0.05$). There was no significant difference in Na^+-K^+-ATPase activity between the two groups at the same time point (Table 4).

Table 4 Mucosal Na^+-K^+-ATPase activity (μmol /mg) at different time points of IR (mean±SE)

	n	24 h post-IR	48 h post-IR	72 h post-IR
EG	5	3.29±0.79	3.89±1.43[a]	3.96±0.86[b]
CG	5	3.17±0.64	3.58±0.76[a]	3.67±0.81[a]

Note: Normal value of ileum at the same site sampled before IR (2.48±0.59 μmol /mg, $n = 5$). [a] $P<0.05$ *vs* normal value; [b]$P<0.01$ *vs* normal value.

DISCUSSION

Intestinal transplantation, either alone or in combination

with liver transplantation, is a preferred therapy for patients with irreversible failure of the intestine. However, in comparison with solid organ transplantations, such as kidney and liver transplantation, there has been slower progression from experimentation towards routine clinical practice. Improved results are only expected with newer immunosuppressive agents, better antiviral prophylaxis and treatment, and improved preservation and surgical techniques[9,12]. Patients with short-bowel syndrome and secondary TPN-related hepatic dysfunction need small-bowel transplantation combined with a liver graft. Only a few patients with short bowel syndrome are candidates for non-transplant procedures, such as autologous gastrointestinal reconstruction[13], bowel rehabilitation and combined trophic therapy for intestinal adaptation using growth hormones[9,14]. Glycyl-glutamine-enriched long-term total parenteral nutrition attenuates bacterial translocation and selectively improves graft mucosal structure and function following small bowel transplantation in pigs[9].

In recent years, several technical modifications in experimental and clinical gut or liver transplantation have been reported. Wu *et al*, adopted arterial anastomosis by microvascular end-to-side anastomosis between the donor aortic segment with superior mesenteric artery and the recipient abdominal aorta, and performed a "cuff" vein anastomosis between the donor portal vein and the recipient left renal vein. This improved vascular anastomosis technique simplifies the surgical procedure, reduces the operation time, and increases the survival rate of small intestinal transplantation in rats. Li *et al*[10], used endoscopic monitoring instead of re-laparotomy to detect gut mucosal changes after intestinal transplantation.

The main advantage of our model is that the operation is simplified by inserting a fine catheter into the mesenteric artery to accomplish cold irrigation and preservation. This new technique may lead to a higher successful rate of autotransplantation. However, a limitation of this model is that only segmental jejunum or ileum rather than total small bowel can be used for autotransplantation. In small animal models, the incidence of massive hemorrhage during tube inserting is relatively high (3/20, 15%). But in pig models, no massive hemorrhage has occurred. It is suggested that these surgical modifications are feasible and reliable.

Many factors, such as moderate hypothermia[15], matrine[16], leflunomide[17], carbon monoxide at a low concentration[18], adenosine[19], and exotic melatonin[20] have been investigated to prevent intestinal or hepatic and cold IR. To elucidate the effect of L-arginine on cold IR and preservation of the small bowel graft in pigs, serum NO contents, NOS activity and graft mucosal NOS and Na^+-K^+-ATPase activity were studied. When compared to the normal value, mucosal Na^+-K^+-ATPase activity increased significantly at 48 and 72 h post-reperfusion both in experimental group and in control group. Intestinal mucosal NOS activity at 1, 24, 48, and 72 h in experimental group reduced significantly when compared with pre-cold IR. Meanwhile, the graft mucosal NOS activity in control group at 1 and 24 h post-reperfusion was significantly lower than that of pre-cold IR.

L-arginine in a storage solution to supplement the nitric oxide synthesizing pathway (NOSP) protects the grafted liver during ischemia-reperfusion in orthotopic liver transplantation. In liver transplantation, the NOSP may be a critical determinant of successful organ transplantation, thus providing a useful pharmacological approach to enhancing liver preservation[8]. Our study showed that in experimental group, serum NO level at 1 and 24 h after reperfusion increased significantly when compared with the control group at the same time point. Serum NO level increased significantly at 1 h post-reperfusion when compared with the same group before cold IR, 24 and 48 h post-reperfusion.

In conclusion, the new intestinal autotransplantation technique provides a potentially consistent and practical model for experimental studies about intestinal graft cold preservation. L-arginine administration during intestinal cold IR immediately enhances serum NO production and mucosal Na^+-K^+-ATPase activity, reduces mucosal NOS activity. L-arginine supplementation may act as a useful adjunct to preserve intestinal grafts.

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REFERENCES

1 **Li Y,** Li J, Jiang J, Li N, Wang X, Wang Z, Wu B, Liu F. Glycyl-glutamine-supplemented long-term total parenteral nutrition selectively improves structure and function in heterotopic small-bowel autotransplantation in the pig. *Transpl Int* 2003; **16**: 866-871

2 **Urata K,** Nguyen B, Brault A, Lavoie J, Rocheleau B, Huet PM. Decreased survival in rat liver transplantation with extended cold preservation: role of portal vein clamping time. *Hepatology* 1998; **28**: 366-373

3 **Massberg S,** Leiderer R, Gonzalez AP, Menger MD, Messmer K. Carolina rinse attenuates postischemic microvascular injury in rat small bowel isografts. *Surgery* 1998; **123**: 181-190

4 **Parkinson EJ,** Townsend PA, Stephanou A, Latchman DS, Eaton S, Pierro A. The protective effect of moderate hypothermia during intestinal ischemia-reperfusion is associated with modification of hepatic transcription factor activation. *J Pediatr Surg* 2004; **39**: 696-701

5 **Ferencz A,** Szanto Z, Borsiczky B, Kiss K, Kalmar-Nagy K, Szeberenyi J, Horvath PO, Roth E. The effects of preconditioning on the oxidative stress in small-bowel autotransplantation. *Surgery* 2002; **132**: 877-884

6 **Montero EF,** Abrahao MS, Koike MK, Manna MC, Ramalho CE. Intestinal ischemia and reperfusion injury in growing rats: hypothermia and N-acetylcysteine modulation. *Microsurgery* 2003; **23**: 517-521

7 **Muller AR,** Platz KP, Hausler M, Heckert C, Lobeck H, Neuhaus P. L-arginine administration improves mucosal structure in the early phase of reperfusion of small intestine transplants. *Langenbecks Arch Chir Suppl Kongressbd* 1998; **115**: 601-605

8 **Diao TJ,** Yao XP, Ji B, Yang JM, Wu MC, Zhang SG. Effects of L-arginine during ischemia reperfusion injury in rat orthotopic liver transplantation. *Huaren Xiaohua Zazhi* 1998; **6**: 291-295

9 **Tzakis AG,** Tryphonopoulos P, Kato T, Nishida S, Levi DM, Nery JR, Madariaga J, De Faria W, Mittal N, Thompson JF, Ruiz P. Intestinal transplantation: advances in immunosuppression and surgical techniques. *Transplant Proc* 2003; **35**:

1925-1926
10 **Li YS,** Li JS, Li N, Jiang ZW, Li YX, Li XH. Endoscopic monitoring in small bowel transplantation. *China Nati J New Gastroenterol* 1997; **3**: 137-138
11 **Sola A,** De Oca J, Gonzalez R, Prats N, Rosello-Catafau J, Gelpi E, Jaurrieta E, Hotter G. Protective effect of ischemic preconditioning on cold preservation and reperfusion injury associated with rat intestinal transplantation. *Ann Surg* 2001; **234**: 98-106
12 **Song WL,** Wang WZ, Wu GS, Dong GL, Ling R, Ji G, Zhao JX. Evaluation of perioperative serum cytokine level in acute rejection in human living-related small bowel transplantation. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 401-404
13 **Fu T,** Wang X, Xu M. Bowel elongation technique by using omentum as vascular pedicle in beagle dogs. *Zhongguo Xiufu Chongjian Waike Zazhi* 1997; **11**: 106-108
14 **Su ZD,** Qin HL. Effects of growth hormone on intestinal adaptation of rat with short bowel syndrome. *Shijie Huaren Xiaohua Zazhi* 2004; **12**: 646-649
15 **Vinardi S,** Pierro A, Parkinson EJ, Vejchapipat P, Stefanutti G, Spitz L, Eaton S. Hypothermia throughout intestinal ischaemia-reperfusion injury attenuates lung neutrophil infiltration. *J Pediatr Surg* 2003; **38**: 88-91; discussion 88-91
16 **Qiu YD,** Zhu XH, Shi MK, Ding YT. Protective effect of matrine on sinusoidal endothelial cells of rat liver isograft. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 1156-1159
17 **Wang X,** Yang JM, Yin GF, Xu ZC, Yan YQ, Wu MC. Effect of leflunomide pretreatment on Kupffer cells in transplanted rat liver. *Xinxiaohua Bingxue Zazhi* 1997; **5**: 487-488
18 **Nakao A,** Kimizuka K, Stolz DB, Seda Neto J, Kaizu T, Choi AM, Uchiyama T, Zuckerbraun BS, Bauer AJ, Nalesnik MA, Otterbein LE, Geller DA, Murase N. Protective effect of carbon monoxide inhalation for cold-preserved small intestinal grafts. *Surgery* 2003; **134**: 285-292
19 **Chen XH,** Bao MS, Li ZZ. Effects of adenosine and its mechanism in ischemic preconditioning in rat liver *in vivo*. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 298-299
20 **Li JY,** Zhang WH, Zhou Y, Yang J, Qin YM. Protective effect of melatonin on liver ischemia reperfusion injury in rats. *Shijie Huaren Xiaohua Zazhi* 2004; **12**: 880-885

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