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Effects of intraperitoneal nitroglycerin on the strength and healing attitude of anastomosis of rat intestines with ischemia–reperfusion injury

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ABSTRACT

Background: Ischemic conditions in the intestine result in deterioration of anastomosis healing process. In this study, our aim was to evaluate the possible effects of intraperitoneal nitroglycerin on the intestinal anastomosis healing and anastomosis burst pressures in rats with ischemia and reperfusion injury (I/R). **Materials and Methods:** Fifty four Wistar albino rats were divided into six groups. In the first two groups, the rats underwent I/R. In the Group 1, the rats had normal saline (S) and in Group 2, the rats had nitroglycerin (N) injection. In the 3rd and 4th groups, an intestinal anastomosis was made at the 10 cm proximally to the ileocecal valve. In Group 3, S and in Group 4, N were injected. In Group 5, the rats received I/R, intestinal anastomosis and intraperitoneal S injection. I/R, intestinal anastomosis and intraperitoneal N injection were made in Group 6 rats. All nitroglycerin (50 μg/kg) injections were made at postoperative days of 0, 1, 2, 3, 4, 5 consecutively. On the sixth day, all rats were killed. In all rats with anastomosis, anastomotic burst pressure (ABP) was measured. Histopathological specimens were collected from all rats and evaluated under light microscopy. **Results:** Serious tissue damage was only detected in the Group 1 histopathologically (8 rats had grade 4 damage). In Group 2, there was a decrease in tissue damage according to histopathologic examination (5 rats had grade 1 damage). The effect onto the healing was similar in S and N groups. Nitroglycerin was noted to have a positive effect on collagen production. Nitroglycerin increased the ABP levels in rats both with and without I/R (the means are 17.93, 21.10, 14.67, and 17.63 in Groups 3, 4, 5, and 6, respectively). **Conclusion:** I/R may weaken the strength of intestinal anastomosis. Intraperitoneal application of nitroglycerin may prevent the histopathologic changes within a limited degree. Intraperitoneal nitroglycerin has also positive effects on the healing of intestinal anastomosis of rats with and without I/R. It may increase the fibroblast proliferation and the strength of the anastomosis.

Key words: Anastomotic burst pressure, intraperitoneal nitroglycerin, ischemia–reperfusion

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INTRODUCTION

Ischemia/reperfusion (I/R) injury is an important phenomenon in clinical practice. It is also the main problem of intestine in disorders such as necrotizing enterocolitis, midgut volvulus, and intussusception.[1,2] Intestinal ischemia causes depletion of cellular energy stores. Afterwards, accumulation of toxic metabolites results in cell damage. Reperfusion exacerbates mucosal injury via the reactive oxygen species such as superoxide and initiates the activation of neutrophils and other chemotactic agents. These cells penetrate into intestinal epithelium and increase the bowel permeability. Free oxygen radicals (FOR) are responsible for the reperfusion injury. The main source of the oxygen radicals is the post-ischemic endothelium.[3-5] Preoperative or intraoperative I/R may also affect the wound healing and decrease the anastomotic strength.[6,7]

Many materials and drugs have been used to establish a secure anastomosis in I/R injury. Nitroglycerin (N) is a potent vasodilator to all vessels and available as an exogenous nitric oxide donor. It has been used in the treatment of angina pectoris since many decades.[8,9] The hypothesis of this study is that intraperitoneal use of nitroglycerin at anastomosis site might provide an increased blood flow on the anastomosis resulting in increased healing process and increased strength of the anastomosis in conditions with I/R.

MATERIALS AND METHODS

This study was performed with the approval of animal research committee. Fifty four Wistar-Albino rats were divided into six groups [Table 1]. In Table 1, a summary of study groups were shown. Rats were 10-weeks old with the mean weight of 220 g (200–250 g). They were kept under normal laboratory conditions in 22°C. All rats underwent laparotomy via midline incision. In I/R groups, arterial clips were used to obstruct the mesenteric vessels for 45 min to produce an ischemia and then the clips were relaxed for reperfusion.[10]

After I/R, normal saline or 50 μg/kg nitroglycerin was injected intraperitoneally on the postoperative days of 1, 2, 3, 4 and 5 in the first two groups. In Groups 3, 4, 5, and 6, an intestinal anastomosis was performed. In Groups 5 and 6, the anastomosis had been performed after I/R. A transection of ileum was performed at 10 cm proximally to ileocecal valve, and an end to end anastomosis was made by using 6/0 polyglactin sutures. In Groups 5 and 6, normal saline (S) or nitroglycerin (N) were applied. As in a previous study of Ugurlu and Turan, the rats were killed at the 6th day of operation.[11]

Histopathological study

Histopathological specimens were collected from all rats and fixed with 10% formaldehyde.

All slides were stained with Haematoxylin–Eosin (HE) and trichome and examined under light microscope. Intestinal wall impairment grades after I/R were investigated according to Chiu scoring[12] [Table 2]. Anastomosis healing was evaluated through collagen deposition parameters according to Phillips *et al.*[13] [Table 3].

Table 1: Experimental groups

Group 1	I/R + S
Group 2	I/R + N
Group 3	A + S
Group 4	A + N
Group 5	I/R + A + S
Group 6	I/R + A+ N

I/R = Ischemia + reperfusion, S = Normal Saline, N = Nitroglycerin, A = Anastomosis

Table 2: Evaluation of histopathological damage in the intestinal wall

Grade 1	Dilatation of subepithelium with capillary congestion at the villus tip
Grade 2	Dilatation of subepithelium with the moderate separation of epithelial space
Grade 3	Complete separation of epithelium
Grade 4	Damaged villus with dilatated capillary
Grade 5	Broken in lamina propria and hemorrage and ulceration

Anastomotic burst pressure measurement

Anastomotic strength was evaluated by determining the burst pressure. In all groups with anastomosis, we measured anastomotic burst pressure (ABP) via a pressure transducer and monitor after five days of operation.

For this purpose, we resected the intestinal segment of approximately 5 cm in length containing the anastomosis. The segmental blood flow was protected. The lumen of the intestine was cleaned by gentle washout with saline. Eight Fr catheters were fixed at both ends of intestine. The distal catheter was connected to a pressure transducer (Omega, Pressure Transducer DP-41, USA). While we insuffulated nitrogen gas through the proximal catheter, the pressure was recorded at the distal catheter in kilopascal (kPa). The pressure at the time of rupture was recorded as ABP.

Statistical analysis

After normality analysis, one way ANOVA, Kruskal Wallis analysis of variance, t-test; Chi-square test, Mann Whitney U test and Fisher's exact test were used for the statistical evaluation of the groups.

RESULTS

Histopathological results

No rat had macroscopically detected anastomosis leak. Tissue damage was more significant in Group 2 than in Group 1 ($P<0.05$) (Chi-square test and Fisher's exact test). In Groups 1 and 2, the number of rats with serious tissue damage is significantly more in groups with saline than in groups with nitroglycerin [Figure 1] (Chi-square test and Fisher's exact test). The collagen accumulation has apparently greater amount in Group 4 than Group 3, and in Group 6 than Group 5. However, the difference was not significant [Figures 2 and 3] (Chi-square test and Fisher's exact test).

Anastomotic burst pressure

In rats with nitroglycerin administration, the mean

Table 3: Grading of collagen deposition at the anastomosis

Grade 0	No
Grade 1	Rare
Grade 2	Mild
Grade 3	Moderate
Grade 4	Severe

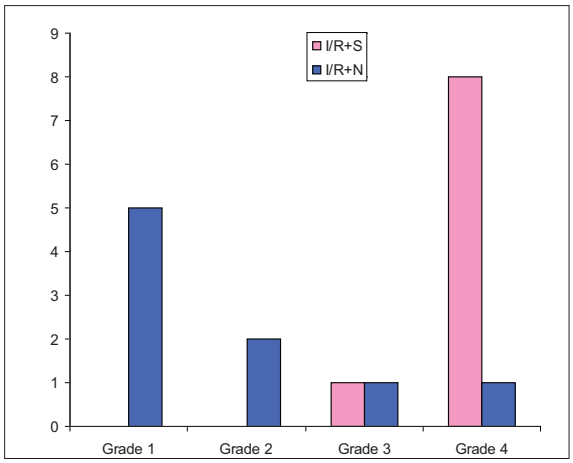


Figure 1: Number of rats according to the grade of damage in Groups 1 and 2. (I/R+S: Ischemia + reperfusion + normal saline, I/R+N: Ischemia + reperfusion + nitroglycerin)

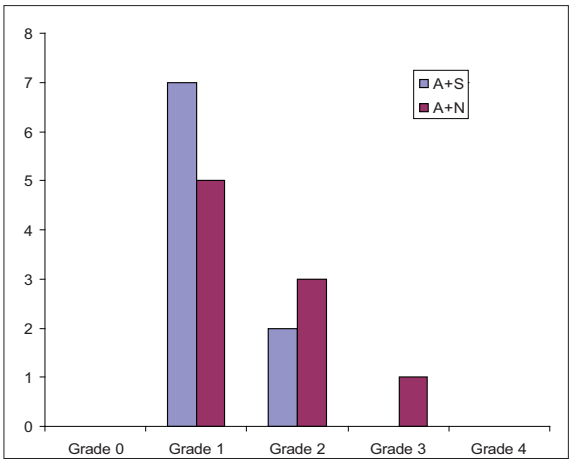


Figure 2: Number of the rats with collagen accumulation in the group 3, 4. (A+S: anastomosis + normal saline, A+N: anastomsis + nitroglycerin)

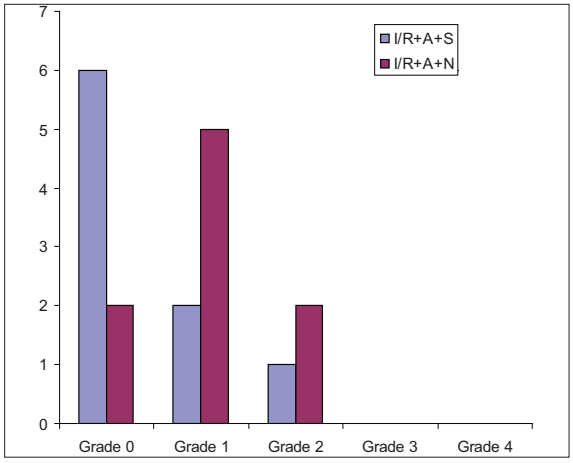


Figure 3: Number of the rats with collagen accumulation in the group 5, 6. (I/R+A+S: ischemia/reperfusion + anastomosis + normal saline, I/R+A+N: ischemia/reperfusion + anastomosis + nitroglycerin)

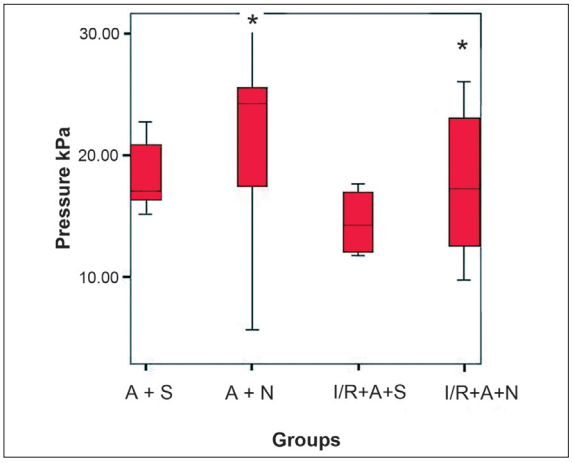


Figure 4: Mean ABS in groups with anastomosis in kPa. (A+S: anastomosis + normal saline, A+N: anastomosis + nitroglycerin, I/R+A+S: ischemia/reperfusion + anastomosis + normal saline, I/R+A+N: ischemia/reperfusion + anastomosis + nitroglycerin). (*) sign indicates statistical significance.

ABP was significantly higher than the normal saline groups both in rats with and without I/R ($P<0.05$) (ANOVA, Kruskal Wallis analysis of variance, *t*-test, Mann Whitney U test). This finding indicates nitroglycerin may strengthen the power of anastomosis [Table 4 and Figure 4]. Nitroglycerin leads to increase in ABP when compared with normal saline with or without I/R.

Table 4: The mean and standard deviation of the anastomotic burst pressure of the groups 3, 4, 5, and 6

ABP (kPa)	Mean	SD
A + S	17.926	6.531
A + N	21.101	8.170
I/R + A + S	14.670	5.867
I/R + A+ N	17.627	6.163

(A+S = Anastomosiz + normal saline, A+N = Anastomosiz+nitroglycerin, I/R+A+S = Ischemia/reperfusion + anastomosiz+ normal saline, I/R+A+N = Ischemia/reperfusion + anastomosiz + nitroglycerin), ABP = Anastomotic burst pressure

DISCUSSION

Intestinal ischemia/reperfusion (I/R) may occur in a number of gastrointestinal surgical disorders, and anastomosis of the resected necrotic bowel becomes risky in ischemic conditions.[8,14-17]

Many methods to reduce the I/R-induced intestinal injury were investigated so far.[13,18-20] However demand on an effective method to prevent I/R injury continues.

Nitric oxide (NO) is a reactive compound produced endogenously by nitric oxide synthase. It is synthesized from the guanidino group of L-arginine.[21,22] NO has been shown that it is a protector of I/R-related tissue damage.[23] NO shows its protective effects against I/R by means of the scavenge of free-oxygen radicals and it inhibits neutrophil activation, stimulates the cell

regeneration, and inhibits the platelet aggregation.[24-28] NO also stimulates the collagen synthesis, probably leading to earlier healing of the wound.[29]

There are some exogenous donors of NO such as nitroglycerin and nitroprusside. Nitroglycerin has been used in many disorders such as angina pectoris, congestive heart failure, and acute myocardial infarction.[30,31] It has also local effects leading to increased blood flow and in some instances it enhances wound healing.[32]

In this study, we showed that nitroglycerin has protective effect in conditions of I/R on the bowel. It decreases the grade of intestinal damage as well. Administration of nitroglycerin had significantly enhanced the strength of the anastomosis when compared to the groups of those without nitroglycerin. However the expected maximum level of normal pressures in normal bowel in rats is not clear. Therefore it is rather difficult to suggest that nitroglycerin may prevent the burst of anastomosis. We were only able to suggest that nitroglycerin may strengthen the anastomosis when compared those without it. We also showed that the grade of collagen accumulation increased in groups with anastomosis and nitroglycerin.

The mechanism of alleviating property of nitroglycerin is complex in I/R-related intestinal damage. This phenomenon may be related to the anti-oxidant and anti-inflammatory effects of nitric oxide. As in our results, some other studies suggested that nitric oxide has therapeutic potential following the I/R.[33-35]

As conclusion, I/R injury had histopathologic alterations on the rat intestine as well as it weakens the strength of the intestinal anastomosis. Intraperitoneal use of nitroglycerin may prevent the histopathologic changes with a limited degree, but the nitroglycerin enhances the strength of the intestinal anastomosis. Especially in risky circumstances such as extensive bowel necrosis, the intraperitoneal application of nitroglycerin may be beneficial for the security of the intestinal anastomosis.

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