The relationship between vasopressor dose and anastomotic leak in colon surgery: An experimental trial[☆]

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ABSTRACT

Background: The effect of vasopressors on the healing of gastrointestinal anastomoses is still controversial. The purpose of our study was to research the relationship between dose of dopamine, which is used generally as a vasopressor in shock status, and anastomotic leak in colonic surgery.
Methods: Forty-two male New Zealand rabbits were included in the study. Under general anesthesia, the right colon was identified, incised, and divided 5 cm distal to the ileocecal valve. Colonic integrity was then established with end-to-end anastomosis in all animals. The animals were randomized into 6 groups. While group 1 was not given any vasopressors, groups 2, 3, 4, 5, and 6 were administered 5, 10, 15, 20, and 25 $\mu g\,kg^{-1}\,h^{-1}$ dopamine infusions, respectively, for 2 h. On the 4th postoperative day, relaparotomy was performed under general anesthesia. The bursting pressures of anastomoses (BPA) were measured in situ, and then the lines of anastomoses were excised. The levels of hydroxyproline and collagen were measured in this tissue.
Results: When compared with the control group (140 ± 39 mmHg), BPA were found to be statistically increased only in group 5 (238 ± 91 mmHg) ($p = 0.03$) and group 6 (277 ± 64 mmHg) ($p = 0.002$). There were no differences between groups in terms of the hydroxyproline and collagen levels in the tissue ($p > 0.05$).
Conclusions: Although vasopressors appeared to increase the risk of anastomotic leakage as a result of splanchnic vasoconstriction, deterioration of microcirculation, and local hypoxia, we found that BPA were increased with high doses of vasopressor. We speculated that the use of vasopressors without shock might increase blood supply to the anastomotic line by increasing cardiac output.

1. Introduction

Despite extensive experiences in surgical and postoperative care, even in the hands of experienced colonic surgeons, anastomotic leaks are dreaded complications of gastrointestinal (GI) surgery because of their high rate of morbidity and mortality. Anastomotic leakage after colorectal surgery is observed in 5–15% of cases, and causes a marked increase in mortality, morbidity, and frequency of local recurrence, and a marked decrease in 5-year survival rate and quality of life.[1] Both local and systemic factors play a role in the anastomotic healing of patients who undergo colorectal surgery. Increased catecholamine and dopamine release, and decreased motiline levels are observed during and after laparotomy.[2] As a result, the increased sympathetic activity leads to decreased GI tract motility after surgery with delayed gastric emptying and increased distention. These are some of the main risk factors for anastomotic leakage following surgical procedures involving the GI tract.[2–4]

Nowadays, the patients submitted to surgery are older, have increased comorbidities, and have also undergone more extensive operations compared to earlier cohorts.[5] In current patients, vasopressors and vasoactive agents are used more frequently in the preoperative, intraoperative and intensive care periods. It is

proposed that the use of these agents may have adverse effects on the recovery of GI anastomoses. To date, there is only anecdotal evidence of the risk of administering vasoconstrictive drugs that might cause ischemia in recently created GI anastomosis. In a recent retrospective clinical study, Zakrison et al. noted that the use of vasopressor agents in the preoperative period increases the rate of anastomotic leakage three times and causes a change in all clinical results.[6]

One of the most basic and widely accepted principles in surgery is the need to maintain adequate blood supply to a recently created GI anastomoses to prevent leaks or dehiscence.[7] Animal studies demonstrate that vasopressor-induced constriction of the splanchnic vessels leads to shunting of the microcirculation and local hypoxia.[8,9] The effects of vasopressor agents used to support the circulation and to correct hypotension, on recovery of GI anastomoses needs further investigation.

Dopamine is one of the agents widely used for treatment of patients with shock. Dopamine affects different types of receptors depending on its dosage. In this study, we aimed to investigate the dose-dependent effect of dopamine on the bursting pressure of colonic anastomoses and the levels of hydroxyproline and collagen content of anastomoses which considered as indicators of potential anastomotic leakage in a rabbit model.

2. Material and methods

2.1. Animal preparation

This study design was approved by the Institutional Local Ethics Committee. Forty-two male New Zealand white rabbits weighing 1854–2750 g were used in this study. The New Zealand Rabbits were chosen for the study because hemodynamic monitorization is easy in those animals. Moreover it enables researchers to take multiple samples of blood for arterial gas analysis which cannot be readily managed using other small animals. Before the experiment, the rabbits were acclimatized for a minimum of 72 h and carefully checked for pre-existing diseases. The diseased animals were excluded. The daily food ration was not withdrawn until the procedure. All the procedures were performed between 16:00 and 20:00 h. On the day of the experiment, anesthesia was induced with 30 mg kg^{-1} i.m. ketamine (Ketasol 10%, Richter Pharma AG, Wels, Austria) and 10 mg kg^{-1} i.m. xylazine (Alfazyne 2%, Alfasan International BV, Woerden, Netherlands). After cannulation into the right-ear marginal veins of the animals, anesthesia was maintained intravenously with 10 mg kg^{-1} h^{-1} ketamine. The left-ear marginal artery was cannulated for mean arterial pressure (MAP) and heart rate (HR) measurements (Petas KMA 800, Professional Electronic Industry and Tic. AS, Turkey) and samples were taken for serial blood gas analysis. The rabbits were placed in the supine position and were warmed to maintain a constant body temperature. Their tracheas were not intubated, and the animals breathed room air spontaneously. All animals received prophylactic antibiotic (cephamezine 20 mg kg^{-1}, IV).

2.2. Surgery

All animals were placed in a supine position. They were then prepared and draped, and peritoneal access was gained using midline laparotomy. Equal length of incision was used in all animals. The right colon was identified, incised, and divided 5 cm distal to the ileocecal valve. Colonic integrity was then established with end-to-end anastomoses in all animals. Atraumatic 4/0 Vicryl Rapid® interrupted stitch was used for colon anastomoses. The procedures were performed by one surgeon with a standardized technique, blinded to the study groups. The right colon was selected

for the healing anastomoses model because we wanted to be sure the distance of the anastomoses is standard for all animals.

Animals were monitored with an intra-arterial catheter during the procedure. They were randomly assigned into six groups with seven animals in each group chosen by computer-generated random numbers. Animals in group 1 were not given any vasopressor; those in groups 2, 3, 4, 5, and 6 were administered 5, 10, 15, 20, and 25 µg kg^{-1} h^{-1} dopamine infusions (for 2 h, starting with the abdominal incision and continued during the early postoperative period), respectively.

On the fourth postoperative day, relaparotomy was performed under general anesthesia by another surgeon who was unaware of the groups. Gross observation of the circumferential healing of anastomotic lines was documented. The bursting pressures of anastomoses (BPA) were measured in situ by another anesthetist blinded to the study groups. The anastomotic segment was dissected from the adhering tissue, opened at the mesenteric side, and a 1-cm long segment containing the complete suture line was excised and washed gently with a saline solution. The levels of hydroxyproline and collagen were measured in this sample tissue.

2.3. Bursting pressure measurement

Measurements were done in vivo; whilst the intestinal flow intact. The colon was ligated 3 cm distal to the anastomotic line, which was kept open. A 14 G silicon double-lumen catheter was inserted from the proximal end of the colon, and this end was ligated 3 cm above the anastomoses over the catheter with a silk stitch. Normal saline solution was infused via one lumen of this catheter at a rate of 10 ml min^{-1}. The hub of the second lumen of the catheter was attached to the transducer (Sasan pressure set, Sasan, Ankara, Turkey) for BPA measurement (the maximum pressure recorded just before sudden loss of pressure).

After the procedures, the animals were euthanized using thiopental (120 mg kg^{-1}, i.v.).

2.4. Biochemical analyses

Tissue samples (10 mg) were homogenized and stored at −40 °C. Autoclave was preferred for the hydrolysis of the specimens. Chloramine-T was added to provide oxidation at room temperature. Finally, Erlich reactive was also used to stain samples with a 550-nm spectrophotometer.[10] Hydroxyproline levels were measured by using standard graphics (0.05–1.5 mmol L^{-1}). Collagen concentrations were also measured (µg mg^{-1}). These assessments were provided by biochemists blinded to the study.

2.5. Statistical analysis

Statistical analyses were carried out using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Because this trial is pilot study, we estimated that seven animals were adequate. Seven animals are adequate sample size for the non-parametric ANOVA tests (Mann–Whitney U-tests and Kruskal–Wallis tests). In addition, the previously published another pilot study data[11] revealed that seven animals were required in each group.

Results were expressed as mean ± SD. Because each group had $n < 30$ and was independent of each other, and variables had been measured at equal intervals, statistical analyses were performed with non-parametric tests. In all the groups, the mean values of BPA, and the levels of hydroxyproline and collagen were analyzed with Mann–Whitney U-tests and Kruskal–Wallis tests. Mann–Whitney U-test with Bonferroni's correction and Wilcoxon signed rank sum test were used to assess the differences found among the six groups. A p-value <0.05 was set as the significance level.
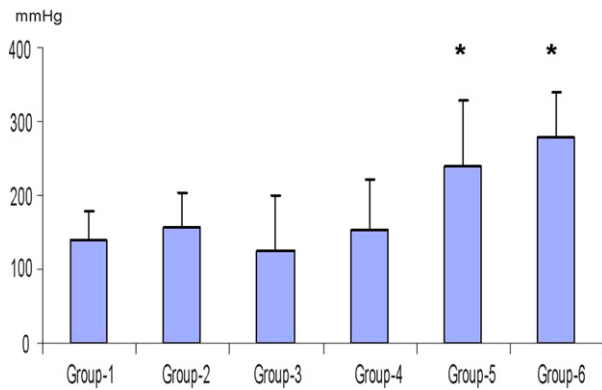
Fig. 1. When compared with the control group, BPA were found to be statistically increased only in group 5 and group 6. *$p < 0.05$.

3. Results

The mean BPA were measured at 140 ± 39 mmHg (93–195) for group 1 animals, 158 ± 46 mmHg (77–207) for group 2, 125 ± 76 mmHg (0–220) for group 3, and 152 ± 71 mmHg (63–230) for group 4. The differences among these groups were not statistically significant ($p > 0.05$). However, the mean BPA were measured at 238 ± 91 mmHg (100–362) and 277 ± 64 mmHg (197–343) for group 5 and group 6 animals, respectively. The dosages of dopamine in these two groups have more apparent vasoconstrictive effects. In both groups, the BPA were statistically higher than in the control groups ($p = 0.004$) ($p = 0.03$ in group 5 vs. control; $p = 0.002$ in group 6 vs. control) (Fig. 1).

No statistically significant difference was detected among all groups when the tissue hydroxyproline and collagen levels were compared ($p > 0.05$) (Table 1).

4. Discussion

In the present study, although there were no differences seen among the groups in terms of hydroxyproline and collagen levels, higher anastomotic bursting pressures were observed in the high dopamine doses.

Anastomotic leakage is of the most devastating complication in GI surgery. It can be defined as partial or total detachment of the anastomoses due to different causes, which can be determined in the postoperative period by means of clinical and/or radiological methods. Several local and systemic factors affect anastomotic leakage after colorectal surgery. In a patient undergoing laparotomy, the motility of the colon is decreased by the effects of increasing catecholamine and dopamine; subsequently it returns to normal after 48 h or more. Such an increase in vasopressors has been suggested to cause a wide spectrum of effects ranging from abdominal distention to anastomotic leakage.[2,4] Moreover external vasopressor exposure of the patient for blood pressure maintenance may have adverse effects on anastomotic healing. In a retrospective study, Zakrison et al. have suggested that the vasopressors used during the preoperative and postoperative intensive care periods may be a risk factor for anastomotic leakage

of GI surgery. The authors speculated that the vasopressor dose used and the exposure time to the vasopressor may be correlated with the rate of anastomotic leakage.[6]

Dopamine was used as vasopressor agent in the study because it is widely used. The clinical effects of dopamine, a nonselective direct and indirect adrenergic agonist, vary markedly with the dose. Small doses ($\leq 2\,\mu g\,kg^{-1}\,min^{-1}$) have minimal adrenergic effects but activate dopaminergic receptors. Stimulation of these non-adrenergic receptors (specifically, dopamine-1 receptors) vasodilates the renal vasculature and promotes diuresis. At moderate doses (≤ 2–$10\,\mu g\,kg^{-1}\,min^{-1}$), β_2-stimulation increases myocardial contractility, heart rate, and cardiac output. Myocardial oxygen demand typically increases more than the supply. Alpha-1 effects become prominent at higher doses (≥ 10–$20\,\mu g\,kg^{-1}\,min^{-1}$), causing an increase in peripheral vascular resistance and a decrease in renal blood flow. The indirect effects of dopamine, which manifest at doses above $20\,\mu g\,kg^{-1}\,min^{-1}$, are due to the release of norepinephrine.[12] Dopamine is generally given at doses ranging from 2 to $20\,\mu g\,kg^{-1}\,min^{-1}$. Because we wanted to produce a greater vasopressor effect and study the dose-dependent effect on colonic anastomotic healing, a supra-therapeutic dose of dopamine ($25\,\mu g\,kg^{-1}\,min^{-1}$), rarely given in a clinical setting,[13] was used in the trial.

In the experimental study which we performed to study the effects of dopamine at various doses on the recovery of anastomoses in colon surgery, the anastomotic bursting pressures, and the tissue hydroxyproline and collagen levels in the anastomotic line were measured. Reddy et al.[10] have determined the hydroxyproline level in biological tissues by means of alkaline hydrolysis. The presence of the hydroxyproline amino acid inside the collagen (12.5%) is a distinctive characteristic because hydroxyproline is an amino acid that is only present in collagen and elastin (1%). Due to this peculiarity, hydroxyproline can be used to determine the amount of collagen in tissue. For this reason, the recovery of the anastomotic line was evaluated through the hydroxyproline level.

However, we could not find any difference in terms of tissue collagen and hydroxyproline levels which are indicators of anastomotic healing. Anastomotic leakage is generally determined in 5th–7th postoperative days.[14] Whereas the strength of anastomoses decreases markedly during the first 3–4 days due to changes in the enzymatic structure of collagen bundles. After the 4th day the strength of anastomoses increases with the prominence of collagen production and accumulation.[15,16] As anastomotic leakage is clinically determined in the 5th–7th postoperative days, the re-laparotomies of the experimental animals were performed on the 4th postoperative day, during which collagen production begins to increase while the strength of anastomoses is still poor. For this reason, the time needed for collagen development might be not enough. In the previous experimental studies, anastomotic bursting pressures and tissue collagen levels were generally evaluated between 7th and 14th postoperative days. But, during this time either anastomotic leakage is already formed or healing is completed. On this occasion, we performed re-laparotomies and measured BPA at 4th postoperative day in which anastomotic leakage had not developed yet.

Table 1
The levels of hydroxyproline and collagen were measured in the anastomotic tissues.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Hydroxyproline levels (mmol L^{-1})	1.16 ± 0.8	0.35 ± 0.2	0.26 ± 0.1	0.32 ± 0.09	0.69 ± 0.6	0.46 ± 0.26
Collagen levels (μg mg^{-1})	12.2 ± 8.9	3.7 ± 2.2	2.8 ± 1.1	3.5 ± 0.9	7.3 ± 6.9	4.8 ± 2.7

5. Conclusion

According to our data, although there was no difference seen among the groups in terms of hydroxyproline and collagen levels, higher anastomotic bursting pressures were observed with the increase in dopamine dose. In addition, while dopamine did not show any effect on the healing process of anastomoses, it increased the strength of the anastomotic line. This is in contrast to our expected finding that the use of vasopressors would scale down the strength and recovery of the anastomotic line. This situation may be explained by the fact that the vasopressor was administered to animals without hemodynamic collapse and shock. Consequently, we speculated that not only vasopressor use but shock itself may be responsible for anastomotic leakage. Although vasopressors are likely to increase the risk of anastomotic leakage as a result of splanchnic vasoconstriction, microcirculatory disturbances, and local hypoxia, we considered the possibility that their use prior to fully developed shock increases perfusion in the anastomotic line by increasing the cardiac flow rate. Further studies are needed to evaluate the strength of the anastomotic line after the use of vasopressors by establishing shock intraoperatively and post-operatively in an animal model.

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