Original article

Role of preoperative retrograde enema on inflammatory and healing parameters in colonic anastomosis: experimental study in dogs☆

Carlos Renato Sales Bezerra a,*, Francisco Sérgio Pinheiro Regadas b, Wellington Ribeiro Figueiredo b, Miguel Augusto Arcorverde Nogueira b, Péricles Cerqueira de Sousa a, Claudio Manuel Gonçalves da Silva Leite c, Kaline Fortes e Silva d, Erbert Portela Martins Filho e, José Alberto Dias Leite b

a Universidade Federal do Ceará (UFC), Fortaleza, CE, Brazil
b School of Medicine, Universidade Federal do Ceará (UFC), Fortaleza, CE, Brazil
c School of Medicine, Centro Universitário Christus (UNICHRISTUS), Fortaleza, CE, Brazil
d School of Medicine, DeVry, FACID, Teresina, PI, Brazil
e Universidade Federal do Piauí (UFPJ), Teresina, PI, Brazil

ARTICLE INFO

Article history:
Received 10 June 2014
Accepted 10 July 2014
Available online 10 September 2014

Keywords:
Enema
Wound healing
Inflammation
Collagen

ABSTRACT

Objective: The purpose of this experimental study was to compare the inflammatory and wound healing response of dogs submitted to colonic anastomosis with and without preoperative retrograde enema.

Methods: The study included two groups of 31 female dogs (Canis familiaris). G-I (control): no preoperative bowel preparation; G-II (study): preoperative retrograde enema using a 10% glycerin solution. All the animals were submitted to laparotomy and colotomy at 20 cm from the anal verge, followed by closure with a running extramucosal single-layer suture (Prolene® 000). The animals were then anesthetized and euthanized on the 7th (n = 10) or 21st (n = 20) postoperative day (POD) to remove the anastomosed colon segment for histological and immunohistochemical analysis evaluating the parameters: anastomatic edema, vasoproliferation, abdominal adhesions, type I and III collagen, nitric oxide and myeloperoxidase. The observed differences were analyzed with the Mann–Whitney test for nonparametric data and Student’s t test for unpaired samples and parametric data.

Results: One animal from G-I and one from G-II died on POD 7 and POD 10 due to anastomotic complications and sepsis, respectively. The groups did not differ significantly with regard to inflammatory and healing parameters, although the levels of mature collagen were significantly lower in the animals submitted to preoperative bowel preparation.

☆ Study carried out at Post-graduation program stricto sensu in Surgery, Surgery Department, Faculty of Medicine, Universidade Federal do Ceará (UFC), Fortaleza, CE, Brazil.
* Corresponding author.
E-mail: piripiri@uol.com.br (C.R.S. Bezerra).
http://dx.doi.org/10.1016/j.jcol.2014.08.007
2237-9363/© 2014 Sociedade Brasileira de Coloproctologia. Published by Elsevier Editora Ltda.
Este é um artigo Open Access sob a licença de CC BY-NC-ND
Conclusion: It has been shown that both procedures are safe to be used, however, the group with bowel preparation showed a lower amount of mature collagen in the immediate postoperative period and may be constituted a preventive factor for surgical complications for this type of surgical procedure, although no evidence in this study could be determined.

© 2014 Sociedade Brasileira de Coloproctologia. Published by Elsevier Editora Ltda.
Este é um artigo Open Access sob a licença de CC BY-NC-ND

Introduction

Historically, colorectal surgical procedures have been associated with postoperative complications, especially prior to the advent of antibiotics in the 20th century.1,2 The practice of bowel preparation was first introduced in 1950 and was further popularized in the 1970s when cathartic substances were combined with oral antibiotics (macrolids) and metronidazole. Nevertheless, bowel preparation is also associated with complications, including dehydration and excessive flatulence.3 In fact, the use of bowel preparation (primarily enema) to prepare patients for colorectal procedures has been the object of much discussion in the literature over the past few years.3

The purpose of this experimental study was to compare the inflammatory and wound healing response of dogs submitted to colonic anastomosis with and without preoperative retrograde enema using a 10% glycerin solution.

Methods

The number of the protocol approved by the Research Ethics Committee was 65/2010 and the research was conducted in accordance with the ethical standards required.

The study included two groups of 31 female dogs (Canis familiaris) with an average weight of 12.01 kg (range: 5.75–25.0).

Group I (control): no preoperative bowel preparation administered.

Group II (study): preoperative bowel preparation (retrograde enema) using a 10% glycerin solution on the day before the procedure and on the day of the procedure.

Following anesthesia with ketamine hydrochloride, all the animals were submitted to individual digital rectal examination to determine bowel status according to the classification

The first study was conducted in 1966 and showed an increase in the number of complications with the use of bowel preparation.

The results were consistent with previous studies, showing a decrease in the number of complications with the use of bowel preparation.

© 2014 Sociedade Brasileira de Coloproctologia. Published by Elsevier Editora Ltda.
Este é um artigo Open Access sob a licença de CC BY-NC-ND

Papel do enema pré-operatório em parâmetro de inflamação e cicatrização em anastomose cólica: estudo experimental em cães

RESUMO

Objetivo: O objetivo deste estudo experimental foi comparar a resposta inflamatória e cicatrização de feridas em cães submetidos a anastomose cólica com e sem enema retrógrado pré-operatório.

Métodos: O estudo incluiu dois grupos de 31 cães fêmeas (Canis familiaris). G-I (controle): sem preparo intestinal pré-operatório; G-II (estudo): Enema retrógrado pré-operatório com uma solução de glicerina a 10%. Todos os animais foram submetidos à laparotomia e colotomia a 20 cm da borda anal, seguido de fechamento em sutura extramucosa contínua (Prolene ®000). Os animais foram anestesiados e, em seguida, submetidos à eutanásia no 7º (n = 10) ou 21 (n = 20) pós-operatório (DPO) para remover o segmento de cólon anastomosado para análise histológica e imunohistoquímica avaliando os parâmetros: edema da anastomose, vasoproliferação, aderências abdominais, colagénio tipo I e III, o óxido nítrico e a mieloperoxidase. As diferenças observadas foram analisadas com o teste de Mann–Whitney para os dados não paramétricos e teste t de Student para amostras não pareadas e dados paramétricos.

Resultados: Um animal do G-I e um do G-II morreu no dia 7 e 10º DPO devido a complicações de anastomose e sepse, respectivamente. Os grupos não diferiram significativamente em relação aos parâmetros inflamatórios e de cura, embora os níveis de colágeno maduro foram significativamente menores nos animais submetidos ao preparo intestinal pré-operatório.

Conclusão: Demonstrou-se que ambos os procedimentos são seguros para serem usados, no entanto, o grupo com a preparação do intestino mostrou uma menor quantidade de colágeno maduro no período pós-operatório imediato, podendo ser constituído um fator preventivo para complicações cirúrgicas para este tipo de procedimento cirúrgico, embora nenhuma evidência neste estudo.
proposed by O’Dwyer (1989) (excellent = absence of feces; good = presence of minimal fecal residue; acceptable = presence of liquid feces; soiled = presence of solid feces). The surgical procedure consisted of a laparotomy using a midline transumbilical incision, identification of the descending colon at 20 cm from the anal verge and a full thickness circumferential colotomy, followed by an end-to-end manual colonic anastomosis using a single-layer extramucosal running suture with polypropylene 3-0. On the 7th (n = 10) and the 21st (n = 20) postoperative day (POD), the animals were euthanized, a second laparotomy was performed through the previous abdominal incision and the anastomosis was evaluated for anastomotic complications (fistula and dehiscence). Adhesions were measured according to Knightly’s classification: 0 = no adhesions, 1 = single thin and easily separable adhesion, 2 = less extensive but weak adhesions which withstand traction poorly, 3 = extensive visceral adhesions extending to abdominal wall, 4 = numerous extensive and visceral adhesions involving the mesentery, bowel, omentum and abdominal wall. Then a 6-cm colon segment including the anastomotic site was removed for analysis (Fig. 1).

The resected segment of colon including the anastomotic area was prepared for histological analysis and stained with hematoxylin-eosin. Edema and vasoproliferation were evaluated according to Sousa’s classification. Collagen was evaluated with Picro-Sirius Red under a polarized light microscope (Type I collagen stains red, yellow and orange; type III collagen stains green) (Fig. 2). The histological sections were submitted to immunohistochemistry to evaluate the direct and indirect inflammatory markers (myeloperoxidase and induced nitric oxide synthase, respectively).

All animals were evaluated with regard to the parameters weight, postoperative clinical recovery, intra-abdominal adhesion grade, anastomotic edema, vasoproliferation, type I and III collagen, myeloperoxidase (MPO) and induced nitric oxide synthase (iNOS). The results of the two groups were compared on POD 7 and POD 21 (Fig. 3).

Statistical analysis

Differences between the groups were analyzed with Student’s t test (unpaired samples) and parametric data or with the Mann–Whitney test (nonparametric data). The level of statistical significance was set at 5% (p < 0.05).

Results

In G-II, bowel preparation was considered good (n = 18) or excellent (n = 12), according to O’Dwyers classification. In G-I, 23 animals had solid stools and 7 had liquid stools identified by rectal digital exam.

The groups did not differ significantly with regard to average weight on POD 7 (G-I = 11.45 kg; G-II = 10.86 kg; p = 0.493) or POD 21 (G-I = 13.04 kg; G-II = 11.86 kg; p = 0.306).

One animal from G-I died on 7th POD due to anastomotic dehiscence, and one animal from G-II died on 10th POD due to infection of the abdominal incision, necrotizing fasciitis and sepsis, despite an intact anastomosis.

The groups did not differ significantly with regard to abdominal adhesions, neither on POD 7 (p = 0.734) nor on POD 21 (p = 0.568). However, a significant difference was observed between POD 7 and POD 21 in both G-I (p < 0.0001) and G-II (p = 0.039).
On POD 7, 3 animals in G-I were classified as Knightly grade IV, 6 grade III, and one grade II. On POD 21, 7 were grade II, 5 grade III, 4 grade I, 3 grade IV and 1 grade 0 (no adhesions). In G-II, 5 animals were classified as Knightly grade III, 4 animals grade IV and 1 animal grade II on POD 7. In contrast, on POD 21, 5 animals were grade I, II and III, 4 grade IV, and 1 grade 0 (no adhesions).

The groups did not differ significantly regarding to edema at the anastomosis site, but within each group a significant difference was observed between POD 7 and POD 21 (G-I: \( p = 0.046 \); G-II: \( p = 0.010 \)).

Regarding to vasoproliferation, G-I did not differ significantly from G-II on POD 7 (\( p = 0.196 \)) and POD 21 (\( p = 0.075 \)). However, on POD 7, vasoproliferation was moderate (\( n = 7 \)) and severe (\( n = 3 \)) in G-I, compared to mild (\( n = 3 \)), moderate (\( n = 5 \)) and severe (\( n = 2 \)) in G-II. On POD 21, vasoproliferation was mild (\( n = 17 \)), moderate (\( n = 3 \)) in G-I, compared to mild (\( n = 12 \)), moderate (\( n = 7 \)) and severe (\( n = 1 \)) in G-II. However, within G-I, POD 7 differed significantly from POD 21 (\( p = 0.0001 \)).

G-I and G-II did not differ significantly with regard to type I and III collagen levels on POD 7 (\( p = 0.891 \); \( p = 0.915 \)) or POD 21 (\( p = 0.271 \); \( p = 0.008 \)). When POD 7 and POD 21 were compared, a significant difference in type I collagen concentrations was observed in both G-I (\( p = 0.009 \)) and G-II (\( p = 0.002 \)). Finally, when comparing type I to type III collagen, a significant difference was found in G-I on both POD 7 (\( p = 0.0077 \)) and POD 21 (\( p = 0.0003 \)). In G-II, the two types of collagen only differed on POD 7 (\( p = 0.002 \)).

The groups did not differ with regard to MPO or iNOS on POD 7 (\( p = 0.580 \) and \( p = 0.089 \)) and on POD 21 (\( p = 0.775 \) and \( p = 0.394 \)) respectively. When comparing POD 7 to POD 21, iNOS levels differed significantly in G-II (\( p = 0.003 \)), whereas MPO levels differed in both G-I (\( p = 0.0004 \)) and G-II (\( p < 0.0001 \)).

Discussion

The use of preoperative retrograde enema is still a matter of controversy. However, many surgeons perform the procedure routinely, mainly in order to reduce exposure of the surgical site (anastomosis) to fecal matter and thereby minimize local inflammatory reactions.

Female mongrel dogs (Canis familiaris) were chosen for this study because of the convenient characteristics of the pelvic cavity. The suture was made with monofilament polypropylene 000 thread because of the low indices of inflammatory reactions with which it is associated. The type of suture used (running single-layer extramucosal suture) is considered better than continuous suture with interruptions with regard to vascularization and healing at the anastomotic site. Preoperative retrograde enema was performed using 10% glycerin (rather than magnesium hydroxide, mannitol or polyethylene glycol/sodium phosphate) due to its low cost, ease of acquisition and low indices of morbidity.

In the study group, bowel preparation was rated very good or excellent in all cases according to O’Dwyer’s classification: on digital examination, no feces were observed which might compromise the experiment. Likewise, during colotomy with colorectal anastomosis, no feces were detected in the lumen. In the control group, despite the absence of bowel preparation, no spillage, abdominal contamination or technical difficulties occurred, as previously suggested by Feres et al.

Despite changes in weight between POD 7 and POD 21, the groups did not differ when compared on the same day. The results of the present study are therefore not likely to have been influenced by differences in weight, as shown elsewhere.

The anastomotic development observed in this study match descriptions in the literature, with strong inflammatory response early in the healing process which subside toward the end of the study period (POD 21). Inflammation promotes the migration of the epiloon and surrounding structures to the site of inflammation, leading to the formation of abdominal adhesions. As the grade of adhesions is proportional to the intensity of inflammation in the serous membrane at the anastomotic site, adhesion grade is used as a parameter to quantify the inflammatory response in the anastomosis site.

The absence of a significant difference between the groups with regard to edema on both POD 7 and POD 21 suggests bowel preparation had no influence on the intensity of inflammation. Studies based on other animal models have yielded similar results.

On POD 7, the two groups did not differ with regard to vasoproliferation, which was moderate to severe in G-I. On POD 21, vasoproliferation was predominantly mild in both groups. However, when comparing POD 21 to POD 7 within G-I, vasoproliferation was significantly higher on POD 7 than on POD 21, indicating that the presence of feces in the lumen may be a determining factor for vasoproliferation due to greater bacterial contamination in the early postoperative stage. As shown in another experimental study, the greater the vascularization, more viable will be the anastomosis.

Types I and III collagen levels were similar in the two groups on both POD 7 and POD 21, despite exposure to feces and bacteria in G-I, as also suggested in a previous study.

Within each group, the type I collagen levels were significantly lower on POD 21 than on POD 7. Other researchers reported increased levels of type III collagen in the first four days after surgery, followed by a decrease, while type I collagen levels remained high until the 14th postoperative day.

The difference between type I and type III collagen in G-I on POD 21 may be explained by the exacerbation of inflammatory response due to the presence of higher amount of fecal matter and bacteria at the anastomotic site, which, however, did not interfere with the healing process. In another study, the proliferation of type I and type III collagen was more evident in animals submitted to bowel preparation due to lower inflammatory response and better interweaving of fibers of both types.

In G-II, intense staining for iNOS was observed significantly more often (70% of the animals) on POD 7 than on POD 21. No such difference was observed in G-I, indicating that the absence of bowel preparation was associated with high postoperative nitric oxide levels until the 21st postoperative day. iNOS levels (as evaluated by immunohistochemical analysis) may be high inside macrophages for up to one month after the initial surgery. The same may be observed for endothelial cells in anastomoses, even in the absence of macrophages.
The groups did not differ with regard to myeloperoxidase on POD 7 or POD 21, but when comparing POD 7 to POD 21, myeloperoxidase levels differed in both groups due to higher inflammatory activity in the early postoperative stage.

The incidence of postoperative complications was similar in the two groups, suggesting that the use of preoperative retrograde enema had no influence on this parameter.

**Conclusion**

It has been shown that both procedures are safe to be used on the pre-operative colorectal surgical procedures, however, the group with bowel preparation showed a lower amount of mature collagen in the immediate postoperative period and may be constituted a preventive factor for surgical complications for this type of surgical procedure, although no evidence in this study could be determined.

**Conflicts of interest**

The authors declare no conflicts of interest.

**REFERENCES**