Expression of metalloproteinases and interleukins on anastomoses in septic rats

ARTICLE *in* JOURNAL OF SURGICAL RESEARCH · MARCH 2013

Impact Factor: 1.94 · DOI: 10.1016/j.jss.2013.02.012 · Source: PubMed

5 AUTHORS, INCLUDING:

Márcio Sousa Jerônimo
University of Brasília
7 PUBLICATIONS **16** CITATIONS
SEE PROFILE

Ildinete Silva Pereira
University of Brasília
30 PUBLICATIONS **329** CITATIONS
SEE PROFILE

Anamelia L Bocca
University of Brasília
59 PUBLICATIONS **620** CITATIONS
SEE PROFILE

João Batista de Sousa
University of Brasília
68 PUBLICATIONS **197** CITATIONS
SEE PROFILE

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Expression of metalloproteinases and interleukins on anastomoses in septic rats

Silvana M. Silva, PhD,[a,*] *Márcio S. Jerônimo, PhD,*[b] *Ildinete Silva-Pereira, PhD,*[b] *Anamélia L. Bocca, PhD,*[b] *and João B. Sousa, PhD*[a]

[a] *Department of Surgical Clinics, School of Medicine, Universidade de Brasilia, Brasília, Brazil*
[b] *Department of Cellular Biology, Biology Institute, Universidade de Brasilia, Brasília, Brazil*

ARTICLE INFO

Article history:
Received 12 November 2012
Received in revised form
5 February 2013
Accepted 7 February 2013
Available online 4 March 2013

Keywords:
Surgical anastomosis
Colon
Sepsis
Metalloproteinase
Interleukin
Rats

ABSTRACT

Background: Anastomotic dehiscence is the most severe complication of colorectal surgery and its incidence increases in the presence of infection. It has been reported that immune factors or the activity of matrix metalloproteinases (MMP) may mediate the loss of anastomotic strength in the first postoperative days. In this study, we investigated the effects of abdominal sepsis on the *MMP* and interleukin *(IL)* gene expression in left colonic anastomoses in rats.

Materials and methods: Forty rats were divided into two groups of 20 animals according to the presence (group S) or absence (group N) of sepsis induction by cecal ligation and perforation during left colonic anastomosis. Each group was divided into subgroups for euthanasia on the third (N3 and S3) or seventh (N7 and S7) postoperative day (POD). A colonic segment containing anastomosis was removed for analysis of the expression of *MMP1a*, *MMP8*, *MMP13*, *IL1β*, *IL6*, *IL10*, *TNFα*, and *IFNγ* genes.

Results: The anastomoses with abdominal sepsis showed increased *MMP1a* gene expression and decreased *MMP8* gene expression both on the third and seventh POD. There was no change in the expression of *MMP13*. There was an increase in the expression of *IL10* only on the third POD and a negative modulation of *IL1β*, *IFNγ*, and *IL6* genes on both periods. The *TNFα* gene expression was negatively modulated on the third POD and became not modulated on the seventh POD.

Conclusion: Abdominal sepsis induced a specific inflammatory pattern with increased *MMP1a* and *IL10* gene expression and negative modulation of *MMP8*, *IL1β*, *IFNγ, and TNFα* gene expression in left colonic anastomoses in rats.

1. Introduction

Anastomotic dehiscence is the most severe complication of colorectal surgery and its incidence increases in the presence of infection [1].

Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent endopeptidases [2]. In terms of wound healing, MMPs play a role in angiogenesis, devitalized tissue removal, connective tissue remodeling, and epithelial cell and fibroblast migration [3]. It has been reported that the activity of MMPs is increased in the presence of sepsis [4] and it may mediate the loss of anastomotic strength in the first postoperative days (POD) by causing local degradation of collagen and other proteins of the extracellular matrix in the submucosal tissue near the suture [5,6].

* *Corresponding author*. School of Medicine, Universidade de Brasilia, SQS 405 Bloco B apt° 205, 70910-900 Brasília, Brazil. Tel.: +55 61 92961133; fax: +55 61 32444271.
E-mail address: silvismarques@yahoo.com.br (S.M. Silva).
0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.02.012

Healing impairment of intestinal anastomoses in the presence of sepsis may also be caused by immune factors. The antigens from the destroyed tissue activate the innate immune system by means of macrophages, neutrophils, natural killer cells, and endothelial cells. These activated cells produce cytokines and chemokines that interact in determining the intensity of the inflammatory response [7]. These interactions are complex, with the presence of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and interleukin 8 (IL-8); and anti-inflammatory cytokines, such as interleukin 4 (IL-4) and interleukin 10 (IL-10).

The objective of the present study was to evaluate the effects of abdominal sepsis on the metalloproteinase and interleukin gene expression in left colonic anastomoses in rats.

2. Materials and methods

Our study was approved by the Animal Ethics Committee of the Institute of Biological Sciences of Universidade de Brasília (UnBDOC protocol no. 67336/2009). All procedures are in accordance with Brazilian guidelines for the use of animals in research.

2.1. Animals

Forty healthy male Wistar rats (*Rattus norvergicus*) were included in the study. Their age ranged from 90–120 d of life and their baseline body weight was between 379 and 416 g.

Throughout the 2-wk preoperative period, the animals were housed in cages with five animals each and kept under a standard 12:12-h artificial light-dark cycle. Standard chow and water were provided. There were no preoperative fasting periods.

2.2. Experimental protocol

The rats were randomly assigned to two groups of 20 animals each for euthanasia on the third or seventh POD. Further randomization was performed, and the animals were allocated to subgroups of 10 rats, with (group S) or without sepsis (group N) (Fig.).

General anesthesia consisted of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (75 mg/kg) administered intramuscularly. All surgical procedures were performed by the same surgeon.

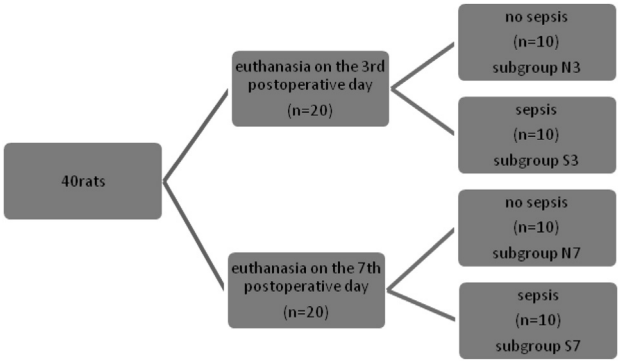

Fig. – Allocation of the animals to groups and subgroups.

The surgical site was shaved and disinfected with povidone-iodine. Nonsterile clean surgical instruments were used. A 4.0-cm-long midline laparotomy incision was made, starting 1.0 cm above the external genitalia. The distal colon was exposed, and a 0.5-cm-long segment of the left colon (2.5 and 3.0 cm above the peritoneal reflection) was resected, followed by end-to-end anastomosis of the resulting segment, in a single transmural plane, using continuous sutures of 6–0 polypropylene and a cylindrical needle.

After anastomosis, in the animals in the sepsis group abdominal sepsis was experimentally induced using the cecal ligation and perforation (CLP) method, as described by Rittirsch *et al.* [8]: One-half of the cecum was ligated using 3–0 silk thread (Shalon, Goiás, Brazil). The end of the cecum located distal to the ligation was punctured at 10 random sites using an 18G $\times$ 1.5 in (40 $\times$ 12 mm) venipuncture needle, and some fecal content was extruded. The abdominal wall was closed in two planes with simple running sutures (3–0 silk thread).

Those animals that died within the first 24 postoperative h were excluded from the study. Metamizole monohydrate (20 mg/kg) was added to the animals' water for analgesia until the day of euthanasia. No antibiotics were used.

Reoperation was performed on the scheduled day for each subgroup. After exposure, the abdominal cavity was assessed for peritonitis, abscesses, obstruction, or anastomotic dehiscence. A 4.0-cm-long colonic segment, with the anastomosis at the center, was removed *en bloc* with any adhering structures. Euthanasia was performed by means of an overdose of sodium thiopental (150 mg/kg), administered directly into the inferior vena cava.

2.3. Specimen preparation

After resection of the adherent structures, specimens were opened at the antimesenteric border and divided into longitudinal sections for later analysis of the expression of *MMP1a*, *MMP8*, *MMP13*, *IL1β*, *IL6*, *IL10*, *TNFα*, and *IFNγ*.

The anastomotic segment was macerated in TRI Reagent solution (Applied Biosystems ,Carlsbad, CA). RNA was isolated by phenol/chloroform extraction and treated with Promega DNAse in the presence of RNAse inhibitor (cat #M6101, lot #262753; Promega Inc, Madison, WI) in order to remove any traces of residual genomic DNA contamination, according to the protocol. Equal amounts of RNA (0.4 μg) were subjected to reverse transcription (High Capacity cDNA Reverse Transcription Kits; Applied Biosystems).

Real-time reverse transcription PCR (qRT-PCR) was performed using a 7500 Fast Real-Time PCR System (Applied Biosystems). Amplification of cDNA sequences was performed using the PCR Master Mix kit (Applied Biosystems) containing the intercalating agent SYBR Green, a fluorescent molecule that intercalates into double-stranded DNA after initial denaturation at 95°C for 15 s, followed by 60°C for 1 min.

In order to confirm the specificity of amplification, PCR products were analyzed by denaturation curve or melting curve. Comparison using the threshold cycle or crossing threshold [9] method with RPS9 to normalize values was used to assess the variation of fold-change expression of each gene.

The fold-change expression represents the septic animals compared with the nonseptic cohorts. Values above 2.0 were considered as positively modulated, whereas values below 0.5 were negatively modulated. We considered that there was no change in gene expressions between these values. Reactions were performed in triplicate for all genes analyzed. All oligonucleotide pairs were designed using Primer Express software (Applied Biosystems); they are listed in Table 1.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc, Chicago, IL). Fisher exact test was used for assessment of intraoperative findings. *P* value <0.05 was considered significant.

3. Results

There was no statistical difference between subgroups in terms of anastomotic dehiscence both on the third ($P > 0.99$) and seventh POD ($P < 0.01$). One animal in subgroup S3 had anastomotic dehiscence, with presence of feces in the abdominal cavity during reoperation. There was one case of anastomotic dehiscence blocked by the omentum in subgroup S7.

There was a predominance of peritonitis in animals subjected to CLP (subgroups S3 and S7), with significant differences both on the third ($P = 0.00$) and seventh POD ($P = 0.00$).

We found no difference between the groups in relation to the presence of paralytic ileus, intestinal obstruction, or intracavitary abscess in both periods investigated. One animal in subgroup S7 died from a cause not related to anastomotic complications.

Abdominal sepsis induction was associated with increased *MMP1a* gene expression and reduced *MMP8* gene expression both on the third and seventh POD. There was no change *MMP13* gene expression (Table 2).

The anastomoses of the animals subjected to CLP showed negative modulation of *IL6*, *IL1β*, and *IFNγ* both on the third and seventh POD. There was an increase in the expression of *TNFα* only on the seventh POD and of *IL10* gene expression on the third POD (Table 3).

Table 1 – Genes and oligonucleotides used in qRT-PCR.

Genes	Oligonucleotides
Cyclophilin	5′ - TAT CTG CAC TGC CAA GAC TGA GTG - 3′ F
	5′ - CTT CTT GCT GGT CTT GCC ATT CC - 3′ R
TNFα	5′ - AAA TGG GCT CCC TCT CAT CAG TCC - 3′ F
	5′ - TCT GCT TGG TGG TTT GCT ACG AC - 3′ R
IL6	5′ - TCC TAC CCC AAC TCC CAA TGC TC - 3′ F
	5′ - TTG GAT GGT CTT GGT CCY YAG CC - 3′ R
IL1β	5′ - CAC CTC TCA AGC AGA GCA CAG - 3′ F
	5′ - GGG TTG CAT GGT GAA GTC AAC - 3′ R
IL10	5′ - CAG ACC CAC ATG CTC CGA GA - 3′ F
	5′ - CAA GGC TTG GCA ACC CAA GTA - 3′ R
IFNγ	5′ - GAT CCA GCA CAA AGC TGT CA - 3′ F
	5′ - GAC TCC TTT TCC GCT TCC TT- 3′ R
MMP1a	5′ - AGG AGC ACT AAT GTT CCC CAG CT - 3′ F
	5′ - TGG GAT TTG GGG AAG GCC CAT A - 3′ R
MMP8	5′ - GGA GTG TGC CAT CAA CCC TGA CC - 3′ F
	5′ - TGT CAC CAT GGT CTC TTG AGA CGA - 3′ R
MMP13	5′ - ACC CAG CCC TAT CCC TTG ATG CC - 3′ F
	5′ - GGT GCA GAC GCC AGA AGA ATC TGT - 3′ R

4. Discussion

The CLP method is associated with polymicrobial sepsis and bacteremia. This procedure has become one of the most commonly used models of abdominal sepsis in pathophysiology studies, as well as in the treatment of abdominal sepsis and its systemic complications.

The incidence of anastomotic dehiscence increases in the presence of infection [1], but in this study there was no statistical difference between subgroups in terms of anastomotic dehiscence both on the third and seventh POD. This find might be attributed to the fact that in rodent and other animal models, the rate of spontaneous anastomotic failure in technically perfect anastomoses is very low and not comparable to colonic anastomoses under clinical conditions in humans. Therefore, in animal models, other criteria need to be utilized to determine the effectiveness of anastomotic colonic healing [10].

In a previous study [11], we evaluated the effects of CLP-induced abdominal sepsis on the left colonic anastomoses in rats. There was no difference between the groups in terms of variation of hydroxyproline concentration, collagen content in the line of the anastomosis, and histopathologic findings. On the third POD, the anastomoses of the animals with sepsis showed lower values of the strength required for anastomotic rupture than those of controls; however, on the seventh POD, we found higher rupture strength of the anastomoses performed in the animals with sepsis. The objective of the present study was to conduct a thorough investigation of the effects of sepsis on the healing of anastomoses.

On the third POD, the animals that underwent CLP showed increased expression of *MMP1a*. On the seventh POD, the expression of *MMP1a* remained increased. The expression of *MMP8* was negatively modulated in both periods. There was no change in the expression of *MMP13* in our sample. The authors believe that each *MMP* could have different importance depending on the phase of the healing process. In this way, the increased expression of *MMP1a* would be more important on the third POD, being responsible for decreasing the tensile strength of the anastomoses. On the seventh POD, *MMP8* would be the active *MMP* and because of this increased inhibition, the tensile strength was enhanced. This might explain the action mechanism of the sepsis that we found in our previous study [11]. So, the healing process could be increased by *MMP* gene modulation.

Under normal conditions, the expression of *MMP* is restricted to a few tissues showing intense remodeling, such as the endometrium during the menstrual cycle, healing wounds, and tissue undergoing inflammatory reaction [3,12]. However, *MMP* activity is intense in the first PODs. During the healing process, this action is progressively decreased, enabling the production of extracellular matrix and increased anastomotic resistance.

It has been reported that *MMP* activity is higher in the presence of bacterial peritonitis [4]. Under these conditions,

Table 2 – Fold-change expression of septic animals compared with the nonseptic cohorts assessed on the third and seventh postoperative days (metalloproteinases).

Subgroup	Gene		
	MMP1a	*MMP8*	*MMP13*
S3	21.2278 (± 12.0963)	0.0097 (± 0.0039)	0.9343 (± 0.2295)
S7	27.6051 (± 12.2790)	0.0059 (± 0.0025)	0.7665 (± 0.4835)

the degradation of the extracellular matrix by the *MMP* genes may increase even more, causing a more significant reduction of the anastomotic strength [6,13]. The cause of higher levels of the *MMP* gene in the anastomosis involving sepsis has not been clearly defined, but it could be explained by an increase in the number of inflammatory cells around the infected anastomosis, such as neutrophils, macrophages, and monocytes [4].

Patients with increased MMP1, MMP2, and MMP9 in perioperative colonic biopsies showed increased rates of anastomotic dehiscence [14,15]. Stumpf *et al.* [16] demonstrated increased MMP13 in intestinal samples of patients who had anastomotic dehiscence when compared with patients who did not have this complication, without any difference between the groups regarding MMP1. Pasternak *et al.* [15] measured the MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, and MMP13 genes in the intraperitoneal fluid of their patients after rectal anterior resection. There was an increase in MMP8 and MMP9 in patients who developed dehiscence. Evaluations based on immunohistochemistry and zymographic analysis demonstrated that MMP2, MMP3, MMP8, and MMP9 are increased in colonic anastomoses of rats [17,18], whereas MMP10 and MMP13 were not associated with the healing of intestinal anastomosis [19].

This study determined *IL* gene expression on perianastomotic tissue since after abdominal surgery, cytokines are primarily synthesized and released at the site of injury before they are released systemically [20]. The systemic response to surgery seems to be a secondary and minor reflection of local events [21]. Local wound levels of cytokines appeared to reflect the stage of wound healing, whereas systemic levels reflected clinical parameters, e.g., postoperative complications and tumor pathology [20].

The animals subjected to CLP showed negative modulation of *IL1β*, *IFNγ*, and *IL6* gene expression both on the third and seventh POD. There was an increased *IL10* gene expression and a negative modulation of *TNFα* gene expression only on the third POD. On the seventh POD, there was reduced expression of *IL10* and *TNFα* gene expression became not modulated.

IL1β is produced primarily by macrophages. It is the first warning sign of adjacent cells after tissue damage [22], and it also plays an important role in the attraction of neutrophils for removal of contaminating bacteria [23]. This cytokine induces secretion of *IFN-γ* by natural killer cells, activates macrophages, and induces *MMP* expression, leading to degradation of the extracellular matrix and migration of monocytes [24].

IL10 is one of the most widely known negative markers for inflammation. It has been reported that IL10 is the most important mediator responsible for inhibiting the inflammatory response [25]. This cytokine increases 24 h after surgical procedures, reducing proinflammatory cytokines, such as TNF-α and IL-6, as well as the activity of macrophages [26]. It may also participate in the suppression of the infiltration of inflammatory cells in the anastomotic segment [27].

IL6 is a fundamental mediator in several stages of inflammation. It plays an active role in the modulation of proliferation, differentiation, and maturation of several cell types [28]. Increased IL6 levels are a response to trauma, burns, and elective surgery and its elevations are proportional to the degree of tissue injury [28].

TNF-α is an inflammatory mediator that plays an active role in collagen synthesis and also provides metabolic substrate for wound healing in the inflammatory phase [29]. TNF-α is produced early at the anastomosis site; however, its levels are rapidly reduced, and it almost disappears 24 h after the surgery in a normal wound-healing process [26]. Increased TNF-α levels after this period are correlated with delayed healing [26]. Previous studies have demonstrated that TNF-α inhibits the gene expression of collagen, whereas it increases the collagenolytic activity, leading to impaired healing [30].

TNF-α and IL-6 may inhibit the healing process of intestinal anastomoses in animals with sepsis [26,31]. Ishimura *et al.* [27], in a study involving mice, used immunohistochemical staining to demonstrate that the expression of *IL6* in the anastomotic segment is suppressed after sepsis induction by means of intraperitoneal injection of lipopolysaccharide, suggesting that healing is impaired by the suppression of local reaction. Our date corroborated the suppression not only of

Table 3 – Fold-change expression of septic animals compared with the nonseptic cohorts assessed on the third and seventh postoperative day (interleukins).

Subgroup	Gene				
	TNFα	*IL6*	*IL1β*	*IFNγ*	*IL10*
S3	0.0008 (± 0.0005)	0.3431 (± 0.0332)	0.0240 (± 0.0193)	0.0198 (± 0.0167)	3.5582 (± 2.6583)
S7	0.6631 (± 0.2604)	0.4109 (± 0.0573)	0.0029 (± 0.0012)	0.0126 (± 0.0085)	0.0323 (± 0.0158)

IL6 gene expression but of all proinflammatory cytokines studied after CLP. In this model, decrease of inflammatory cytokine gene expression could be contributing to healing increase.

It is important to highlight, however, that the study of *MMP* activity using immunohistochemical staining or mRNA analysis does not necessarily mean that there will be degradation of the matrix, since the strict control of *MMP* activity is preserved when MMPs are released as inactive proenzymes, as well as by the presence of inhibitors [32]. In addition, the detection of cytokine gene expression or even cytokine levels in tissues or cytokine secretions does not always correlate with its biological activity. Cytokines may not have been produced, they may have been released as inactive proenzymes, or they may have already been degraded [33].

5. Conclusion

Abdominal sepsis induced a specific inflammatory pattern with increased *MMP1a* and *IL10* gene expression and negative modulation of *MMP8*, *IL1β*, *IFNγ*, and *TNFα* gene expression in left colonic anastomoses in rats.

Acknowledgment

The authors thank Marcos Vinícius Melo de Oliveira, Alexandre Malta Brandão, Pedro Henrique Alves de Morais, and Naiara Galvão da Silva for their contribution.

REFERENCES

[1] Thompson SK, Chang EY, Jobe BA. Clinical review: healing in gastrointestinal anastomoses, part I. Microsurgery 2006;26:131.
[2] Stamenkovis I. Extracellular matrix remodeling: the role of matrix metalloproteinases. J Pathol 2003;200:448.
[3] Parks WC. Matrix metalloproteinases in repair. Wound Repair Regen 1999;7:423.
[4] de Hingh IH, de Man BM, Lomme RM, van Goor H, Hendriks T. Colonic anastomotic strength and matrix metalloproteinase activity in an experimental model of bacterial peritonitis. Br J Surg 2003;90:981.
[5] Hendriks T, Mastboom WJB. Healing of experimental intestinal anastomosis. Parameters for repair. Dis Colon Rectum 1990;33:891.
[6] Thornton FJ, Barbul A. Healing in the gastrointestinal tract. Surg Clin North Am 1997;77:549.
[7] Len E, Calvano SE, Lowry SF. Inflammatory cytokines and cell response in surgery. Surgery 2000;127:117.
[8] Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009;4:316.
[9] Livrak KJ, Schmittger TD. Analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402.
[10] Hoeppner J, Wassmuth B, Marjanovic G, Timme S, Hopt UT, Keck T. Anastomotic sealing by extracellular matrices (ECM) improves healing of colonic anastomoses in the critical early phase. J Gastrointest Surg 2010;14:977.
[11] Silva SM, Oliveira MVM, Brandão AM, et al. Study on adhesion formation and the healing of colon anastomosis in rats with induced peritoneal sepsis. Acta Cir Bras 2011;26(Suppl 2):100.
[12] Borden P, Heller RA. Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit Rev Eukaryot Gene Expr 1997;7:159.
[13] Ahrendt GM, Gardner K, Barbul A. Loss of colonic structural collagen impairs healing during intra-abdominal sepsis. Arch Surg 1994;129:1179.
[14] Stumpf M, Klinge U, Wilms A, et al. Changes of the extracellular matrix as a risk factor for anastomotic leakage after large bowel surgery. Surgery 2005;137:229.
[15] Pasternak B, Matthiessen P, Jansson K, Andersson M, Aspenberg P. Elevated intraperitoneal matrix metalloproteinases-8 and -9 in patients who develop anastomotic leakage after rectal cancer surgery: a pilot study. Colorectal Dis 2010;12:e93.
[16] Stumpf M, Cao W, Klinge U, Klosterhalfen B, Kasperk R, Schumpelick V. Collagen distribution and expression of matrix metalloproteinases 1 and 13 in patients with anastomotic leakage after large-bowel surgery. Langenbecks Arch Surg 2002;386:502.
[17] Ågren MS, Andersen TL, Mirastschijski U, et al. Action of matrix metalloproteinases at restricted sites in colon anastomosis repair: an immunohistochemical and biochemical study. Surgery 2006;140:72.
[18] Shaper KR, Savage FJ, Hembry RM, Boulos PB. Regulation of matrix metalloproteinases in a model of colonic wound healing in a rabbit. Dis Colon Rectum 2001;44:1857.
[19] Vaalamo M, Karjalainen-Lindsberg ML, Puolakkainen P, Kere J, Saarialho-Kere U. Distinct expression profiles of stromelysin- 2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. Am J Pathol 1998;152:1005.
[20] Baker EA, El-Gaddal S, Williams L, Leaper DJ. Profiles of inflammatory cytokines following colorectal surgery: relationship with wound healing and outcome. Wound Repair Regen 2006;14:566.
[21] Wiik H, Karttunen R, Haukipuro K, Syrjala H. Maximal local and minimal systemic cytokine response to colorectal surgery: the influence of perioperative filgrastim. Cytokine 2001;14:188.
[22] Freedberg IM, Tomic-Canic M, Komine M, Blumenberg M. Keratins and the keratinocyte activation cycle. J Invest Dermatol 2001;116:633.
[23] Hantash BM, Zhao L, Knowles J, Lorenz HP. Adult and fetal wound healing. Front Biosci 2008;13:51.
[24] Ito A, Mukaiyama A, Itoh Y, et al. Degradation of interleukin 1 beta by matrix metalloproteinases. J Biol Chem 1996;271:14657.
[25] Rongione AJ, Kusske AM, Ashley SW, Reber HA, McFadden DW. Interleukin-10 prevents early cytokine release in severe intraabdominal infection and sepsis. J Surg Res 1997;70:107.
[26] Ishimura K, Moroguchi A, Okano K, Maeba T, Maeta H. Local expression of tumor necrosis factor and interleukin-10 on wound healing of intestinal anastomosis during endotoxemia in mice. J Surg Res 2002;108:91.
[27] Ishimura K, Tsubouchi T, Okano K, Maeba T, Maeta H. Wound healing of intestinal anastomosis after digestive surgery under septic conditions: participation of local interleukin-6 expression. World J Surg 1998;22:1069.
[28] Biffl WL, Moore EE, Moore FA, Peterson VM. Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? Ann Surg 1996;224:647.

[29] Lopes JV, Freitas LAM, Marques R, Bocca AL, Sousa JB, Gonçalves PG. Analysis of the tensile strength on the healing of the abdominal wall of rats treated with infliximab. Acta Cir Bras 2008;23:441.
[30] Buck M, Houglum K, Chojkier M. Tumor necrosis factor inhibits collagen 1 gene expression and wound healing in a murine model of cachexia. Am J Pathol 1996; 149:195.
[31] Martineau L, Shek P. Peritoneal cytokine concentrations and survival outcome in an experimental bacterial infusion model of peritonitis. Crit Care Med 2000;28:788.
[32] Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000;1477:267.
[33] Witte M, Barbul A. General principles of wound healing. Surg Clin North Am 1997;77:509.