Effect of oral suplementation of vitamin C on intestinal anastomotic resistance

Efeito da suplementação oral de vitamina C na resistência anastomótica intestinal

Andy Petroianu,TCBC-MG[1]; Luiz Ronaldo Alberti[2]

A B S T R A C T

Objective: To compare the resistance of anastomosed and intact jejunal segments of rats submitted to administration of vitamin C in different postoperative periods. **Methods:** Fifty Wistar rats underwent enterotomy followed by end-to-end anastomosis of the jejunal segment, 10 cm from the duodenojejunal flexure. The animals were divided into two groups (n = 25): Group I – control; Group II – administration of oral vitamin C 100 mg/kg. We evaluated the bursting pressures of the anastomotic and the intact jejunal segments in the third, fifth, seventh, 21st and 28th postoperative days. **Results:** The rats that received vitamin C had higher anastomotic bursting pressure in the fifth, seventh and 28th postoperative days. The same happened with the bursting pressures of intact jejunal segments. **Conclusion:** Vitamin C increased the resistance of jejunal anastomoses in rats, both in the immediate and in late postoperative periods. In addition, the final resistance of intact jejunal segments of rats under administration of vitamin C was significantly higher than in the control group.

Key words: Wound healing. Ascorbic acid. Anastomosis, surgical. Rats, wistar.

INTRODUCTION

In the healing process, under the influence of hypoxia and tissue macrophages, fibroblasts synthesize collagen, which polymerize in its passage into the extracellular space. During collagen synthesis, proline is incorporated into the polypeptide chain and undergoes initial action from peptidyl proline-hydroxylase and molecular oxygen to become hydroxyproline. The same occurs with lysine. The polymerization of collagen peptides requires removal of the terminal peptides and lysine condensation[1-4].

According to the literature, ascorbic acid acts as electron donor for the process of hydroxylation of proline during collagen synthesis, a fact that leads to the suspicion of an increased demand for tissue repair[5-8].

Recent studies have revealed various functions of ascorbic acid other than those already described in wound healing. Acting as an antioxidant, the acid is able to capture the free oxygen resulting from cellular metabolism, preventing it from binding with free radicals, which causes cell damage [9-12]. It is likely that ascorbic acid is also involved in maintaining intracellular integrity, with participation in capillary integrity, immune responses, allergic reactions and increased iron absorption[13-17].

According to recent studies, the surrounding intestine participating in the reaction to injury of jejunal anastomosis loses a large part of its collagen by lysis, thus having its resistance reduced[11]. These data suggest an important role of vitamin C on the healing process. However, its administration to further improve the healing process is still debated.

Within a research line[3,4,12,18], the objective of this study was to evaluate the influence of oral supplementation of ascorbic acid in the tension of anastomotic and intact jejunal segments of rats at different postoperative periods.

METHODS

We studied 50 male albino Wistar rats, weighing between 250 and 320 grams. Animals from the Central Animal Veterinary School, UFMG, were kept in cages in number of five, where they received specific chow and water *ad libitum*. They remained in observation and adaptation of health status for 20 days. We conducted thorough examination of each animal to be sure of the absence of signs of illness.

The rats were anesthetized with an intramuscular injection in the buttock of 5% ketamine at a dose of 35 mg/kg (0.7 ml/kg) combined with 2% xylazine at a dose of 6 mg/kg (0.3 ml/kg) and 1% acepromazine maleate at a

Work done at Santa Casa de Belo Horizonte. Department of Surgery, Faculty of Medicine. Belo Horizonte, Minas Gerais, Brazil.
1. Professor, Department of Surgery, Faculty of Medicine - UFMG - Belo Horizonte, BR; 2. Associate Professor, Department of Surgery, UFMG - Belo Horizonte, BR.

dose of 2 mg/kg (0.2 ml/kg). When necessary, half of the initial dose was readministered. Then the animals underwent laparotomy (3 cm in length). Once the jejunal segment was identified at 10 cm from the duodenojejunal flexure, its section was performed. Jejunal stumps were termino-terminally anastomosed with separated 4-0 Vicryl®. The abdominal wall was closed in two layers with 3-0 polypropylene suture. The rats recovered spontaneously from the anesthesia.

The animals were randomly divided into two groups (n=25): Group I, control, animals only subjected to the operation; Group II, study, operation with oral administration of vitamin C in doses of 100mg/kg/d throughout the Postoperative follow-up period.

Vitamin C was added to water given to the animals according to results of a pilot project that determined the average amount of water that the rats ingested per day (80 ml). The animals received pure water (Group I) or water plus vitamin C (Group II) and were fed at will. They were monitored daily, received the same type and amount of nutrition and remained divided into cages with the same number of animals (n = 5).

Each group of animals was divided into five subgroups (n=5), to be studied in the 3rd, 5th, 7th, 21st and 28th postoperative days. After the time of monitoring, the animals' death was induced with an inhaled lethal ether dose after general anesthesia with ketamine and xylazine at the same doses previously used.

After carefully dividing the adhesions, we removed two jejunal segments, measuring 10 cm each. The first contained the anastomosis in its central part. The second consisted of the jejunum immediately distal to the first segment. The distal portions of these segments were tied with 2-0 silk and the proximal part connected to an electronic tensiometer that measured the rupture tension of the intestinal segment.

In addition, the following parameters were evaluated: presence of general and anastomotic postoperative complications and histological assessment of scar tissue.

The macroscopic abdominal evaluation verified the presence of the following: dehiscence, adhesions, infection (characterized by the presence of pus). Adhesions were classified as few (less than three), moderate (between three and five) and many (more than five).

Histological studies were conducted on preparations stained with hematoxylin-eosin and Gomori trichrome. We measured the thickness of the fibrous scar tissue neoformation in three different regions (near the cranial end, in the middle and near the caudal border). Histopathology and histomorphometry was performed using conventional optical microscopy and polarized light. Analysis of inflammatory cells with differential quantification and analysis of fibrosis, with quantification and observation of the degree of intermingling of collagen fibers, was performed by medium-magnification fields (100 X) and of collagen by high-magnification fields (400 X).

As for quantity of collagen, we assigned a score 0 to anastomoses in which collagen was negligible; 1 for the presence of collagen in small quantities; 2, in moderate quantity; 3, when large amounts of collagen were found. Regarding the distribution of collagen fibers, we observed two distinct structural patterns: fibers in parallel or intertwined. It is considered that intermingling gives greater tensile strength (resistance) in the segment in question. The inflammatory infiltrate was semi-quantitatively evaluated according to the following parameters: grade 0, absence of inflammatory infiltrate, 1 for mild infiltrate, 2 for moderate infiltrate and 3, intense.

Descriptive analysis (mean and standard deviation of the mean) and the Student t test were applied to compare the rupture tension between the groups. The presence of adhesions was analyzed by Fisher exact test. Differences were considered significant for values corresponding to $p<0.05$.

RESULTS

The animals recovered satisfactorily. The return of the digestive transit was confirmed since the first postoperative day by the presence of feces in the cages. No anastomotic leak was observed.

Table 1 shows the mean pressure values and standard deviation of anastomotic disruption in different postoperative periods. Group II showed higher tensile strength values in the fifth, seventh and 28th postoperative days.

The analysis of resistance in the third day showed tension equal to 0 for both groups. The most significant difference was for the 28th postoperative day, when the rats submitted to administration of vitamin C had significantly higher tension than the control group ($p = 0.037$). The resistance after 28 days was higher than that found in earlier periods for both groups.

Table 1 also shows the median rupture tension values of the intact jejunal segment. It is observed that the tensions of Group II were higher than those in Group I, although the difference was significant only in the 28th postoperative day ($p = 0.032$). After seven days there were no differences between the resistances of the anastomoses and the intact segments ($p>0.05$).

At necropsy, there were no evident signs of dehiscence, intra-abdominal and surgical site infection in any animal. Adhesions were observed in both groups from the seventh postoperative day, but with no differences between groups ($p = 0.41$). (Table 2)

As for histological evaluation, a heterogeneous morphology was noted in the third postoperative day, with inflammatory reaction and lower number of

fibroblasts and collagen bundles. On the fifth day, there was increased amount of collagen fibers, inflammatory infiltrate composed of neutrophils, plasma cells, lymphocytes and macrophages, and vascular congestion. On the 21st day there was more homogeneous morphology in relation to the first week in all groups, with a better arrangement of fibroblasts interspersed with firmer and orderly collagen fibers. By the 28th day, the collagen bundles were thicker compared to the initial two weeks, with more orderly distribution and parallel to each other.

The presence of acute inflammatory infiltration had a mean of 1.45 ± 0.45 for Group 1 and 1.33 ± 0.38 for Group 2 (p = 0.41). In relation to the concentration of collagen, it was observed, in the seventh day, 1.83 ± 0.14 and 2.46 ± 0.23 in Group 2 (p = 0.021). Group 2 had 87% of interwoven collagen fibers, while Group 1 had 62% (p = 0.016).

DISCUSSION

Factors affecting anastomotic healing have been continuously researched[1-4,12,18]. However, results of experimental studies investigating the healing of intestinal anastomoses are conflicting[5,9]. Although there are studies assessing the influence of steroids and other substances on healing phenomena[3,9], little research has been done on ascorbic acid.

In this study, the dose of ascorbic acid was chosen based on previous studies that have found that these are the minimum concentrations that could affect wound healing and that could be used in humans without leading to toxic and harmful effects[18].

The use of this substance since three days before surgery and continued until the day the animals were killed followed the literature and guidelines aimed at ensuring its pharmacological effect at the beginning of tissue reactions to trauma[18].

Table 1 - Mean and standard deviation of the rupture tensions (cmHg) of the anastomoses and intact jejunal segments.

Postoperatives Days	Groups			
	I		II	
	Intact Segment	Anastomosis	Intact Segment	Anastomosis
3º	22,7 ± 3,4	0	24,3 ± 2,1	0
5º	24,6 ± 2,8	13,7 ± 0,9*	26,5 ± 2,4	17,9 ± 1,4*
7º	23,1 ± 1,4	11,5 + 1,3	28,8 ± 3,0	23,4 ± 2,5+
21º	22,5 ± 2,6	24,5 ± 3,2	20,8 ± 2,4	18,7 ± 1,3
28º	24,7 ± 2,1**	32,8 ± 4,1++	43,1 ± 3,7**	47,3 ± 4,2 ++

Group I: control
Group II: Oral supplementation of vitamin C
** Different from the other group on the same postoperative day (p = 0,021)*
*** Different from the other group on the same postoperative day (p = 0,032)*
+ Different from the other group on the same postoperative day (p = 0,006)
++ Different from the other group on the same postoperative day (p = 0,037)

Table 2 - Gradation of the presence of intra-abdominal adhesions in the groups studied.

Postoperatives Days	Groups					
	I			II		
	Presence of adhesions (number of animals)					
	Few	Moderate	Many	Few	Moderate	Many
3º	2	1	-		1	-
5º	2	2	-	1	2	-
7º	-	2	1	1	-	1
21º	2		1	3		1
28º	1	1			1	1

Few: up to 3 adhesions
Moderate: from 3 to 5 adhesions
Many: more than 5 adhesions
Group I: control
Group II: Oral supplementation of vitamin C

Dehiscence of intestinal anastomosis is a common complication, the collagen being of fundamental importance in maintaining tension on the intestinal wall[10]. Investigations with enteric anastomoses in various animals showed marked changes in the concentrations of collagen, occurring mainly in the first postoperative week. The results of this study show a significant increase of the scar tension, both immediate and late postoperative, after ingestion of vitamin C.

Another major factor is the negative influence of microorganisms able to produce collagenolytic enzymes that would lead to an imbalance between collagen synthesis and degradation[6,7,11]. This suggests that, in addition to surgical technique and perioperative care, administration of supplemental vitamin C might be beneficial in order to reestablish metabolic balance. This hypothesis could help explain in part the higher tensions found in the groups that were given vitamin C.

By seven days follow-up, all groups showed scar tensions below the ones found at day 21. At first, the scar tension is still tenuous, which complicates the analysis of the effect of ascorbic acid. Still, it was observed that in the groups treated with vitamin C the tension was higher when compared to the control group. However, our data do not explain the pathophysiology of these findings.

According to Chowcat *et al.*[5], collagenase is synthesized on demand and is controlled by a metalloproteinase tissue inhibitor. The supplementation of vitamin C, even though exerting no influence on these enzymes, provides greater substrate flow, which could be responsible for chemical reactions inducing collagen synthesis[10].

The translation of experimental findings into medical reality should be cautious. Although some studies showed a beneficial effect of vitamin C, others reported that inflammatory mechanisms (cells and chemical mediators) are the responsible for collagen synthesis[6,16,17].

The results of this study suggest a positive effect of vitamin C, both in early and late phases of the healing process. Taking into account that most complications (dehiscence, fistula) occur in the immediate postoperative period, we deem appropriate to assume that taking vitamin C may be beneficial, even when it comes to chronic or degenerative diseases.

Another aspect that deserves consideration is the administration of vitamin C leading to a significantly higher rupture tension of the late intact jejunal segment when compared to the control group. In this study, we found no histological explanation for this result. Therefore, this resistance also requires more study to be understood.

Oral supplementation of vitamin C increased the resistance of jejunal anastomotic and intact segments in rats, both in the immediate and late postoperative periods.

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R E S U M O

Objetivo: *Comparar a resistência cicatricial de anastomoses e de segmentos íntegros jejunais de ratos, submetidos à administração de vitamina C, em distintos períodos pós-operatórios.* ***Métodos:*** *Foram estudados 50 ratos Wistar, submetidos a enterotomia seguida de anastomose término-terminal de segmento jejunal, a 10 cm da flexura duodenojejunal. Os animais foram distribuídos em dois grupos (n=25): Grupo I - controle; Grupo II - administração de vitamina C oral 100 mg/kg. Avaliaram-se as pressões de ruptura anastomótica e do segmento íntegro jejunal nos 3º, 5º, 7º, 21º E 28º dias do pós-peratório.* ***Resultados:*** *Os ratos que receberam vitamina C apresentaram pressão de ruptura anastomótica maior nos 5º, 7º, e 28º dias pós-operatórios. O mesmo ocorreu com as pressões de ruptura do segmento íntegro jejunal dos ratos.* ***Conclusão:*** *A vitamina C aumentou a resistência das anastomoses jejunais dos ratos, tanto no pós-operatório imediato quanto no tardio. Além disso, a resistência final dos segmentos jejunais íntegros dos ratos submetidos à administração de vitamina C foi significativamente maior do que no Grupo Controle.*

Descritores: *Cicatrização. Ácido ascórbico. Anastomose cirúrgica. Ratos wistar.*

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Correspondence to:
Prof. Andy Petroianu
E-mail: petroian@medicina.ufmg.br