

Hyperbaric oxygen on the healing of ischemic colonic anastomosis - an experimental study in rats

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ABSTRACT

The aim of the present study was to evaluate the effect of hyperbaric oxygen therapy (HBO₂) on the healing process of ischemic colonic anastomoses in rats. Forty Wistar rats were divided into four groups: control (Group I), control and HBO₂ (Group II), ischemia (Group III), ischemia and HBO₂ (Group IV). Ischemia was achieved by clamping four centimeters of the colonic arcade. On the eighth therapy day, the anastomotic region was removed for quantification of hydroxyproline and immunohistochemical determination of metalloproteinases 1 and 9 (MMP1, MMP9). The immunohistochemical studies showed significantly larger metalloproteinase-labeled areas in Group IV compared with Group III for both MMP1 and MMP9 ($p < 0.01$). This finding points to a higher remodeling activity of the anastomoses in this experimental group. Additionally, animals subjected to hyperbaric oxygen therapy showed both a reduction in interstitial edema and an increase in hydroxyproline concentrations [at the anastomotic site]. Therefore, we conclude that HBO₂ is indeed beneficial in anastomotic ischemia.



INTRODUCTION

The incidence of colonic anastomotic dehiscence is influenced by various factors, including the mechanical preparation of the colon, the surgical technique applied [1-4], the use of certain pharmacological agents, the patient's general condition [5], as well as the vascularization and oxygen tension in the tissue around the anastomosis [6,7].

The synthesis and development of collagen fibers and, consequently, the appropriate tension of the intestinal wall are directly associated with the partial oxygen pressure [8]. Thus, administration of oxygen is crucial under conditions that potentially impair healing processes. In addition to the concomitant loss of tensile force, ischemic anastomoses are characterized by diminished levels of hydroxyproline and rise in the amount of free radicals [9]. Clinical studies have demonstrated that hyperbaric oxygen therapy (HBO₂) increases proliferation of fibroblasts

[10] and enhances the angiogenetic potential of ischemic tissues. Hence, HBO₂ promotes healing processes by optimizing the mechanism through which polymorphonuclear leukocytes combat infections [11,12]. Therefore, HBO₂ can aid healing processes after an anastomosis has suffered ischemia, as in the case of patients with post-operative hemodynamic instability due to technical difficulties during the surgery, which in turn culminated in anastomotic ischemia. Using rats, Hamzagoul *et al.* [13] have demonstrated that application of HBO₂ augmented the partial oxygen pressure (ppO₂) as well as the oxygen tension in the tissues around normal and ischemic colonic anastomoses, which promoted cicatrization under ischemic conditions.

The aim of the present study was to evaluate the effect of HBO₂ on healing processes of ischemic colonic anastomoses.

TABLE 1

	GROUPS		$p^{(*)}$
	I	II	
ADHESIONS			
<i>missing</i>	9 (81.82)	2 (18.18)	
<i>slight</i>	1 (16.67)	5 (83.33)	< 0.01
<i>moderate</i>	0 (0.00)	2 (100.00)	
COLONIC DISTENSION			
<i>missing</i>	10 (100.00)	0 (0.00)	
<i>slight</i>	0 (0.00)	5 (100.00)	< 0.01
<i>moderate</i>	0 (0.00)	2 (100.00)	
<i>intense</i>	0 (0.00)	2 (100.00)	
TOTAL	10	9	

(*) p -value of Fisher's Exact test

TABLE 1 – Frequency distribution of adhesions and colonic distensions in experimental Groups I (control) and II (control + HBO₂) observed in the macroscopic examination of the anastomoses.

MATERIALS AND METHODS

Forty healthy Wistar rats from the bioterium of the School of Medicine of Ribeirão Preto, University of São Paulo, Brazil, were employed in the experiments. All animals were subjected to colonic anastomosis with continuous suture with monofilamentar thread 6.0 at 2 centimeters above the peritoneal reflection. Subsequently, the rats were divided into four groups of 10 animals each, which were then submitted to different experimental treatments:

- Group I - control group (anastomosis);
- Group II - control + HBO₂ (anastomosis and HBO₂);
- Group III - ischemia (ischemic anastomosis);
- Group IV - ischemia + HBO₂ (ischemic anastomosis and HBO₂).

In Groups III and IV, ischemia was achieved by ligating 4 centimeters of the colonic arcade. HBO₂ was initiated in animals belonging to Groups II and IV immediately after the surgery and consisted of one daily therapy session during the first seven post-operative days. HBO₂ sessions lasted two hours: 15 minutes were employed for chamber pressurization, followed by 90 minutes at maximum pressure (2 atm absolute pressure), and 15 minutes of depressurization.

During the entire post-operative period, the animals were kept in cages (maximum of five animals per cage) at the Laboratory of Technical and Experimental Surgery of the Department of Surgery and Anatomy of the Medical School of Ribeirão Preto, University of São Paulo, Brazil, with laboratory standard food and water *ad libitum*. All the animals were sacrificed on the eighth post-operative day. Thereafter, the occurrence of colonic adhesions and dehiscences, as well as the distension of the colon were evaluated macroscopically. Subsequently, the anastomotic region was removed for quantification of hydroxyproline and immunohistochemical determination of metalloproteinases 1 and 9 (MMP1, MMP9).

The results were analyzed using one-way ANOVA and Fisher's Exact test. The level of significance was set at $p \leq 0.05$.

RESULTS

The animals belonging to the ischemia groups (Groups III and IV) featured abdominal distensions and displayed apathic behavior. During the post-operative period, one rat in each ischemia group died prior to the end of the experiment. The necropsy of these animals revealed general peritonitis and necrosis of the anastomoses.

TABLE 2

	GROUPS		
	III	IV	$p^{(*)}$
ADHESIONS			
<i>missing</i>	1 (50.00)	1 (50.00)	0.811
<i>slight</i>	6 (60.00)	4 (40.00)	
<i>moderate</i>	3 (42.86)	4 (57.14)	
COLONIC DISTENSION			
<i>missing</i>	1 (100.00)	0 (0.00)	0.99
<i>slight</i>	4 (57.14)	3 (42.86)	
<i>moderate</i>	2 (40)	3 (60.00)	
<i>intense</i>	3 (50.00)	3 (50.00)	
TOTAL	9	9	

(*) p -value of Fisher's Exact test

TABLE 2 – Frequency distribution of adhesions and colonic distensions in experimental Groups III (ischemia) and IV (ischemia + HBO₂) observed in the macroscopic examination of the anastomoses.

After sacrifice on the eighth post-operative day, macroscopic examination showed a significantly higher number of colonic adhesions in Group II rats (HBO₂-treated) compared with Group I (control) ($p < 0.01$, *Table 1, facing page*). In the ischemia groups (Groups III and IV), adhesions also tended to occur more frequently in the HBO₂-treated animals (Group IV) compared with the untreated rats (Group III). In this case, however, the difference between the two groups was not statistically significant (*Table 2, above*). Concerning the occurrence of colonic dehiscence, there were no significant differences among the experimental groups. A total of three dehiscences were detected, one in Group III and two in Group IV. Details of the macroscopic evaluation are listed in Tables 1 and 2.

Analysis of hydroxyproline concentration in the colonic tissues evidenced significantly increased levels of this amino acid in Group II compared with Group I ($p < 0.01$). Similarly, hydroxyproline concentrations were more elevated in Group IV compared with Group III; nevertheless, the difference between the two ischemia groups was not statistically significant ($p = 0.49$).

The immunohistochemical analysis of the anastomoses, accomplished for evaluation of MMP1 and MMP9 indicated significantly augmented expression of both

these metalloproteinases in Group IV compared with Group III ($p < 0.01$ for MMP1 and MMP9) (*Figures 1-2*). The results concerning analyses of hydroxyproline concentrations and metalloproteinase expressions can be visualized in Figures 3-5 (*Pages 408-409*).

DISCUSSION

The healing of anastomoses involves a variety of biochemical and cellular processes, among which the conformation of collagen plays a crucial role. In cicatrization processes, the concentration of collagen in the extracellular matrix increases gradually, yet most intensely between the fourth and twelfth day, whereas metalloproteinases degrade this protein. The balance between collagen synthesis and degradation plays a crucial role in healing processes. The concentration of collagen in tissues can be quantified by measuring hydroxyproline levels, once this amino acid is one of the major components of collagen. In the present study, a higher number of colonic adhesions was detected in animals belonging to the HBO₂-treated groups (Groups II and IV), compared with the groups that did not receive such treatment (Groups I and III). This increase in adhesions was probably due to the formation of new blood vessels (neovascularization), stimulated by HBO₂.

FIGURE 1

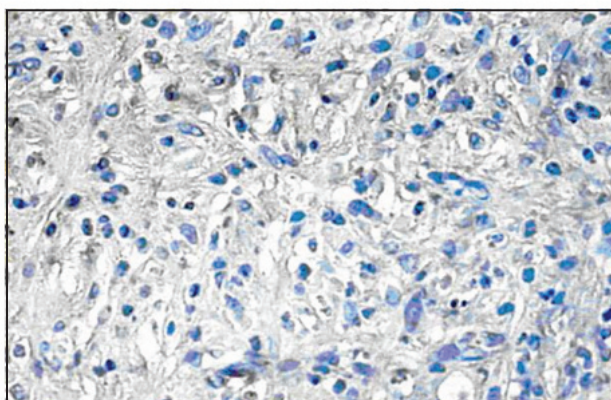


FIGURE 1 – Expression of metalloproteinase 1 in Group IV (ischemia + HBO₂)

One beneficial effect of HBO₂ is indicated by our findings concerning the hydroxyproline levels in the anastomotic region. Accordingly, the tissues of the animals that had been subjected to HBO₂ treatment (Groups II and IV) contained elevated levels of this amino acid compared with the untreated groups (Groups I and III).

In the present study, the biochemical analysis of collagen concentration in the cicatricial tissue was performed indirectly by measuring the hydroxyproline concentration. In general, low concentrations of this amino acid are associated with slow healing processes. Hamazaoglu *et al.* (1998) [13] found higher concentrations of hydroxyproline in the cicatricial tissues of ischemic colonic anastomoses in rats subjected to HBO₂ compared with untreated animals. In a recent study, Guzel and co-authors (2006) [14] obtained similar results. In addition to the elevated hydroxyproline levels, these authors reported significantly increased concentrations of immunomodulators in the anastomotic region of HBO₂-treated rats compared with untreated animals, after both groups had been subjected to ischemic colonic anastomoses. Yagci *et al.* (2006) [15] compared the influence of HBO₂ on healing processes of both normal and ischemic colonic anastomoses. These authors demonstrated that the concentration of hydroxyproline increased in all HBO₂-treated groups, especially in those that had undergone therapy in both pre- and post-operative periods. Furthermore, Ereoglu *et al.* (2003) [16] described a rise in hydroxyproline concentrations in colonic anastomoses of rats after HBO₂, even when the animals had been additionally submitted to chemotherapy with 5-fluoracil.

FIGURE 2

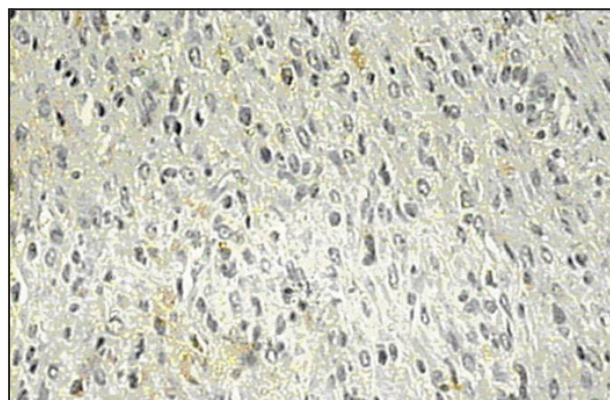


FIGURE 2 – Expression of metalloproteinase 9 in Group IV (ischemia + HBO₂)

Rocha *et al.* (2007) [17] reported that HBO₂ has no beneficial effects on ischemic anastomoses. Nevertheless, these authors analyzed the effect of HBO₂ without the use of sectioning or anastomosis and accomplished only four HBO₂ sessions and assessed the results by the “Pressure of rupture by liquid distension resistance test,” which is a method of little accuracy when compared with the biochemical approach.

In this study, the anastomoses were examined on the eighth post-operative day. Our results, which demonstrate a beneficial effect of HBO₂ on the healing of ischemic colonic anastomoses by inverting the deleterious effects of ischemia, are in accordance with previous investigations (13-16).

In addition to the macroscopic and biochemical evidences mentioned above, a beneficial effect of HBO₂ was observed by the immunohistochemical analysis of the anastomotic region. Higher concentrations of both MMP1 and MMP9 were measured in Group IV animals (ischemia and HBO₂) compared with Group III (ischemia). Since the presence of these enzymes indicates collagen degradation, it can be concluded that, despite the ischemia, higher quantities of collagen were present in the anastomotic tissues of Group IV rats compared with Group III.

Burrow *et al.* (2007) [18] reported that non-healing diabetic foot wounds displayed a pathologically elevated activity of metalloproteinases. Furthermore, Chen *et al.* (2007) [19] found an increased activity of matrix metalloproteinases and metalloproteinase inhibitors in the skin of both embryos in advanced gestational stage and adults

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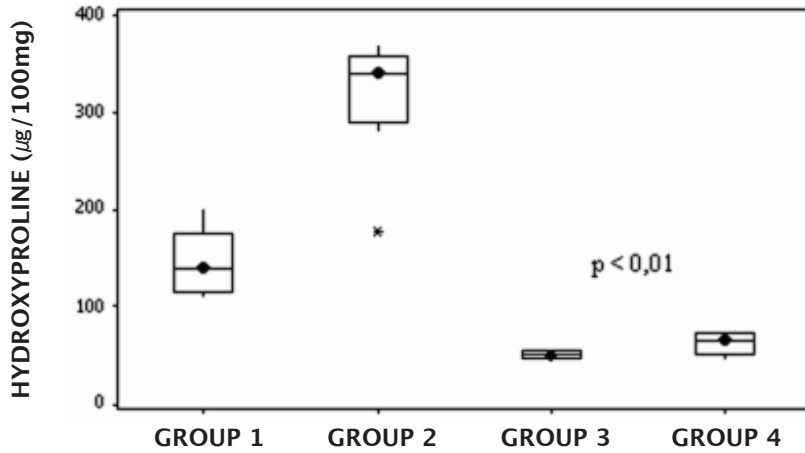


FIGURE 3 – Boxplot of the hydroxyproline concentrations measured in the different experimental groups:
 Group I: control;
 Group II: control + HBO₂;
 Group III: ischemia;
 Group IV: ischemia + HBO₂.
 Asterisk indicates significant difference (One-way ANOVA; $p \leq 0.05$) between the indicated groups.

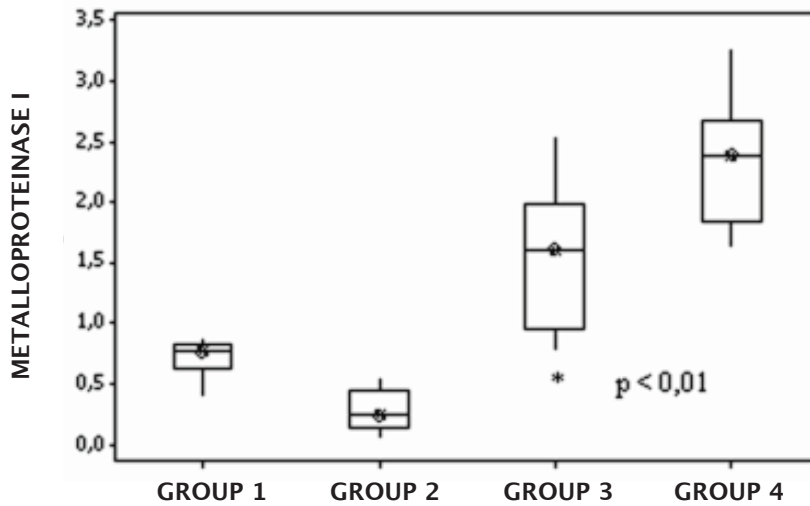


FIGURE 4 – Boxplot of the concentrations of metalloproteinase 1 (MMP1) measured in the different experimental groups
 Group I: control;
 Group II: control + HBO₂;
 Group III: ischemia;
 Group IV: ischemia + HBO₂.
 Asterisk indicates significant difference (One-way ANOVA; $p \leq 0.05$).

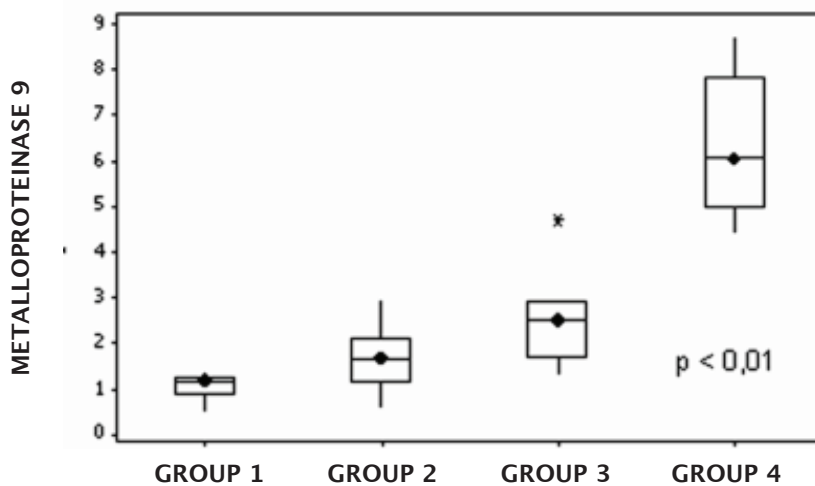


FIGURE 5 – Boxplot of the concentrations of metalloproteinase 9 (MMP9) in the different experimental groups:
 Group I: control;
 Group II: control + HBO₂;
 Group III: ischemia;
 Group IV: ischemia + HBO₂.
 Asterisk indicates significant difference (One-way ANOVA; $p \leq 0.05$).

compared with the skin of embryos at the beginning of gestation. These findings clearly demonstrate the importance of the metalloproteinases for the maintenance of the extracellular matrix. In addition, Takeyama *et al.* (2007) [20] studied whether and how the administration of hyperbaric oxygen affects gene expression of metalloproteinases in lacerated knees of rats. These authors demonstrated diminished lytic action of the metalloproteinases after treatment with oxygen, which indicates that HBO₂ can indeed be beneficial for the surgical treatment of ligament injuries.

The impact of HBO₂ on healing processes of ischemic tissues is an ample field of research of great importance and relevance for clinical practice. Consequently, it sparks interest in new findings and information concerning this topic. Immunohistochemistry represents a new tool for evaluation of anastomoses, since it allows for a meticulous study of cicatrization and enables detailed analysis of the expression and distribution of substances that are important for the complex chain of events in the course of a healing process. New antibodies are currently being evaluated for their potential application in more complex biochemical analyses of cicatrization processes.

HBO₂ has been demonstrated to be beneficial for healing processes under ischemic conditions, as it leads to enhanced oxygenation of tissues, thereby improving collagen synthesis as well as promoting balance between collagen synthesis and degeneration – as demonstrated by the elevated concentrations of the metalloproteins 1 and 9 in HBO₂-treated animals.

CONCLUSION

From the results of our study, it can be concluded that application of HBO₂ in ischemic anastomoses leads, under the chosen experimental conditions, to an increase in the concentration of hydroxyproline and to a significant elevation in the concentration of the metalloproteinases 1 and 9. These findings strongly point to the beneficial effect of HBO₂ on healing processes. ■

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