Healing of the abdominal wall after parcial hepatectomy

Cicatrização da parede abdominal após hepatectomia parcial

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ABSTRACT

Objective: To evaluate the wound healing of the abdominal wall incision in hepatectomized rats as for the concentration of collagen, inflammatory reaction and angiogenesis. **Methods**: We used 48 rats randomly assigned to laparotomy with or without hepatectomy. The scars were studied in the 3rd, 7th and 14th postoperative days. We analyzed the density of collagen by the histochemical method and angiogenesis, by immunohistochemistry. **Results**: The analysis showed a lower total collagen concentration in skin and subcutaneous tissue in the abdominal scars of the Experiment group (p_3 = 0.011, p_7 = 0.004 and p_{14} = 0.008). The density of collagen I was lower in the hepatectomy group, especially in the third day, in the skin, subcutaneous tissue (p = 0.038) and in the aponeurotic plane (p = 0.026). There was a lower concentration of collagen III in the two abdominal wall layers studied, although not statistically significant. The inflammatory response was similar at all times in both groups. It was found that angiogenesis was developed earlier in the Control group (p_3 = 0.005 and p_7 = 0.012) and later in the Experimental group (p_{14} = 0.048). **Conclusion**: Hepatectomy leads to a delay in the healing process, interfering with collagen synthesis and angiogenesis.

Key words: Liver. Regeneration. Hepatectomy. Wound healing.

INTRODUCTION

Failure in healing of the abdominal wall remains a problem for surgeons. Despite technological advances, problems such as dehiscence and incisional hernias continue highly incident. In the United States 200,000 surgical repairs of incisional hernias are held each year[1]. The type and magnitude of the operative act are among the known risk factors for suture dehiscence of abdominal wall. Emergency operations, perioperative periods of hemodynamic instability, procedures involving the biliary tree, liver disease and surgical treatment of aneurysm of the aorta are associated with increased incidence of failed of acute wound healing[2].

Gómez *et al.*[3] reported incisional hernias in 11.6% of patients undergoing liver transplantation, Müller *et al.*[4] in 12%, Piazzese *et al.*[5] in 4.9% and Vardanian *et al.*[6] in 4.6%. Similar situation is described for patients undergoing partial hepatectomies. Rudow *et al.*[7] reported an incidence of 20%, while D'Angelica *et al.*[8], 9.8%. According to Van't *et al.*[9], most severe situations with evisceration led 25% of patients to death within 60 days. Among those who survived, 69% of them have developed incisional hernias.

Living donor hepatic transplant, when the donor cedes up to 60% of his/her liver, has been increasingly stimulated. This is possible because the remaining liver can regenerate itself, although the term "hepatic regeneration" is not biologically appropriate, since there is no regeneration of resected lobes, but hyperplasia and hypertrophy of the remaining ones (compensatory growth). This term has been consecrated by the literature[10,11].

All liver cells, hepatocytes, endothelial cells, Küpffer cells, Ito cells, and ductal cells, proliferate. However, as hepatocytes constitute 90% of the parenchyma and 60% of the total number of cells, most studies of regeneration monitor these cells[10,11].

During the proliferation of hepatocytes there is release of growth factors, such as: hepatocyte growth factor (HGF), transformative growth factor alpha (TGF-á), epidermal growth factor (EGF) and fibroblast growth factor (FGF)[11].

The EGF stimulates the synthesis of DNA in the majority of epithelial cells and in hepatocytes[10,11]. The levels of messenger RNA for the synthesis of EGF are elevated at the beginning of liver regeneration, indicating that this growth factor promotes gene expression and hepatic growth[11].

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TGF-á is able to stimulate mitoses by autocrine and paracrine signalling mechanisms. Its potential effect on hepatocytes can be part of a mitogenic signal that directs the stroma of adjacent cells towards proliferation[10]. The HGF was the first mitogenic factor identified in blood in high concentrations during regenerative process, being considered the most potent stimulator of liver proliferation[12,13].

Transforming growth factor beta (TGF-â) is able to reversibly stimulate the growth of fibroblasts[11]. TGF $â_1$ and TGF $â_2$ are important mediators of acute phase tissue repair, increasing wound resistance[14,15]. The presence of TGF-â is important to start and sustain tissue healing[16]. HGF has its effect fully inhibited by TGF-â[10]. This factor proved a potent inhibitor of hepatocyte proliferation *in vitro*[10]. It was demonstrated *in vivo* that there is increased expression of TGF-â after toxic injury of non-parenchymatous liver cells, i.e. Küpffer cells, stellate cells and endothelial cells, but there is no increase in hepatocytes. During liver regeneration, elevation of TGF-â levels does not occur until most part of the hepatocytes proliferation ends.

It was shown that, during liver regeneration, levels of TGF-$â_1$ decrease and the expression of HGF increases, stimulating the proliferation of hepatocytes[17]. It is interesting to note that the levels of TGF-â rise during the normal process of tissue healing.

Kuhn *et al*. verified, in a study done on rats, that there was a deficiency of abdominal scars resistance during liver regeneration. They found high levels of HGF and low TGF-$â_2$. This finding led them to suggest that there may be prioritization of liver regeneration over abdominal wall scar fibroplasia[18].

Considering the high incidence of complications of abdominal wound healing and changes of concentrations of growth factors, the study of the healing of abdominal wall after hepatectomies becomes important. The understanding of the causes that lead to suture dehiscence and failures in healing is necessary to conceive methods of prevention and correction of these complications.

This study aimed at examining the wound healing process of the abdominal wall of hepatectomized rats and comparing it with the wound of non-hepatectomized rats.

METHODS

The project that gave rise to this study was evaluated by the Committee of Ethics in Research with Animals of the Health Sciences Sector of the Federal University of Paraná and approved with protocol number A.N. 009.005.07.09.

We used 48 male rats (*Rattus norvegicus albinus, Rodentia mammalia*) aged between 100 and 120 days and weight of 250 ± 50 grams, from the Central Animal Facility of UFPR. We kept them in quarantine for a week before starting the study, and throughout the research in the laboratory of Experimental Surgery and surgical technique of UFPR they were housed in groups of three to five animals per box suitable for the species. The temperature was 20 ± 2°C, light/dark cycle of 12 hours and relative humidity of the environment itself. They received *ad libitum* water and proper chow.

The rats were randomly assigned, 21 of them composing the Control Group (C) and 27 the Hepatectomized (or Experiment) Group (H). These groups were again randomly divided in the subgroups C3, C7 and C14 and H3, H7 and H14, according to the dates set for the evaluation of the experiment, three, seven and 14 days. The subgroups of the Control Group had seven rats and the ones of the Hepatectomized one, nine each.

After being weighed and marked, they were subjected to anesthesia by intramuscular injection of 0.2 ml/100 g body weight of a mixture of one milliliter of ketamine (50 mg) with a milliliter of xylazine (20 mg).

After depilation of the ventral abdominal wall a median laparotomy was performed with 4 cm in length, starting immediately below the xiphoid process. In the Group H we performed a partial hepatectomy by resecting the median lobe with its central portions, along with the left lateral lobe. This resection represents 67 to 70% of the hepatic mass[19].

Once revised the hemostasis, we proceeded to closure with two plans of continuous-type suture with 5.0 monofilament nylon. The first plan encompassed the peritoneum, the muscle and aponeurosis, and the second, the skin.

After recovery from anesthesia, the mice were returned to their cages with free access to water and food. They received sodium diclofenac 10 mg/kg intramuscularly immediately after the operative act, with anti-inflammatory and analgesic purposes[19]. For euthanasia we made a lethal dose of intraperitoneal sodium thiopental (120 mg/kg).

After the animals' death, the ventral portion of the abdominal wall, containing the scar in its central region, was withdrawn, leaving 2 cm laterally and 1 cm above and below it. We separated the skin of the peritoneum-musculoaponeurosis, extended both on filter paper and discarded half centimeter both at the top and bottom of the two flaps. The remainder was fractionated into three portions with 1 x 4 cm, constituting the fragments A, B and C (Figure 1).

Fractions A and C were fixed in 10% formalin and forwarded to histopathology. They were sliced in 4-ìm-thick cuts, which were mounted on slides and stained with hematoxylin-eosin (HE) and Picrosirius, as well as by the method of Immunohistochemistry with anti-CD 34 antibody.

General morphological evaluation of scar was carried out with the HE slides. The inflammatory process was evaluated by reading three fields with magnification of 400 times, according to a standardization described by Vizzotto *et al*.[20] (Table 1). For counting of cells we adopted

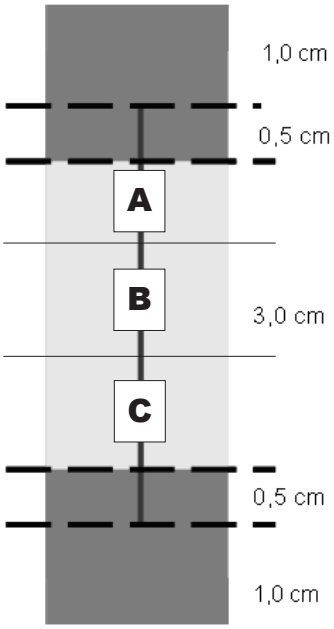

Figure 1 – *Scheme of the scar fragments used for the study.*

the following scale: no cell = 0; up to 50 cells = 1; 50 to 100 cells = 2; and more than 100 cells = 3, positive for mononuclear and negative for polymorphonuclear cells[20].

After attribution of the values, they were summed, so that each group of animals had a final score, thus classifying groups in three phases of inflammatory process (Table 2)[20].

Coloring by Picrosirius was used to recognize the density of collagen in the SCAR and the fractions of collagen I and III under the microscope with polarized light. The thicker and strongly birefringent fibers are colored in shades of orange to red (collagen I) and the smallest, dispersed and weakly birefringent fibers are stained in green (collagen III)[21].

Angiogenesis was evaluated by immunohistochemical method; the number of vessels was counted in three large fields. CD 34 is a glycosylated protein unique to the membrane, which is expressed by immature blood cells and endothelial cells. The anti-CD 34 recognizes the molecule CD 34 and thus enables the identification of cells that possess them.

For a comparison of Experiment and Control groups on each day of assessment, we used the non-parametric Mann-Whitney test. Comparison of the moments of evaluation within groups was made with the non-parametric test of Kruskal-Wallis. Statistical significance was indicated by $p < 0.05$.

RESULTS

Total collagen was present in less concentration in the abdominal scars of the Experiment group at all times in the skin and subcutaneous tissue ($p_3 = 0.011$; $p_7 = 0.004$ and $p_{14} = 0.008$). There was lower density of total collagen in the aponeurosis only on the 7th and 14th days ($p_7 = 0,017$ and $p_{14} = 0.022$) (Figures 2 and 3).

The fraction of collagen I displayed lower concentration in the Experiment group in the skin and subcutaneous tissue in the 3rd and 14th days ($p_3 = 0.038$ and $p_{14} = 0.002$), and in the aponeurosis in the 3rd and 7th days ($p_3 = 0.026$ and $p_7 = 0.017$).

The fraction of collagen III was lower in the Experiment group three times, having been significant in 7th day in skin and subcutaneous tissue ($p = 0.026$). There was no significant difference for the aponeurosis.

The inflammatory reaction on the 3rd day was predominantly of acute type ($p = 0.461$) and on the 7th day of chronic type in the Control group, while in the Experiment Group there were subacute and chronic-type reactions ($p = 0.192$). The same situation was observed on the 14th day ($p = 0.103$).

Tabela 2 - Characterization of the phase of the inflammatory process according to the final score.

Final score	Phase of inflammatory process
- 9 a - 3	Acute
- 2,9 a +3	Subacute
+ 3,1 a +9	Chronic

Table 1 – Methodology of quantification of histological findings in cuts colored by hematoxylin-eosin.

Inflammatory Parameters	Intensity			
	High	Moderate	Discreet	Absent
Polymorphonuclear	-3	-2	-1	0
Mononuclear	3	2	1	0
Edema	-3	-2	-1	0
Congestion	-3	-2	-1	0
Granulation tissue	3	2	1	0
Fibrosis	3	2	1	0

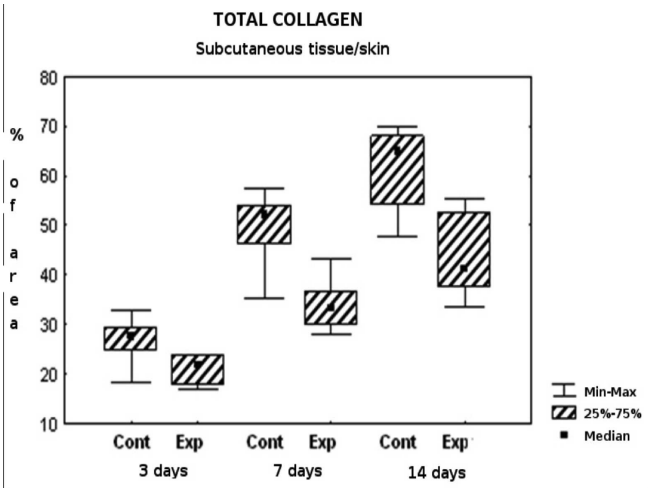

Figure 2 – *Percentage average of examined areas corresponding to total collagen in three, seven and 14 days in the Control and Experiment groups on skin/subcutaneous tissue.*

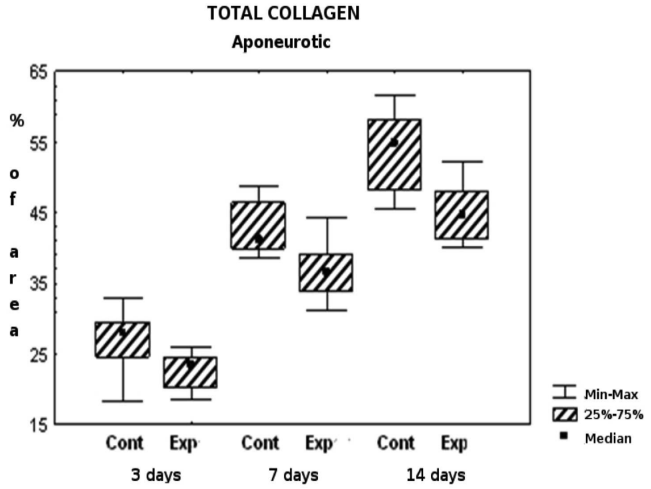

Figure 3 – *Percentage average of the areas examined represented by total collagen at three, seven and 14 days in the Control and Experiment groups in the aponeurosis.*

Analysis of angiogenesis demonstrated greater number of vessels in the scars of the Control group at days 3 ($p = 0.011$) and 7 ($p = 0.038$). On the 14th day we observed a tendency to larger number of vessels in the Experiment group ($p = 0.181$) (Figure 4).

DISCUSSION

In spite of the progress of surgical techniques and better postoperative follow-up, literature information shows that the incidence of healing failures has not changed in recent years[3,4,9]. Works suggest that post-hepatectomy scars are less resistant than for other surgical procedures[4,7,18].

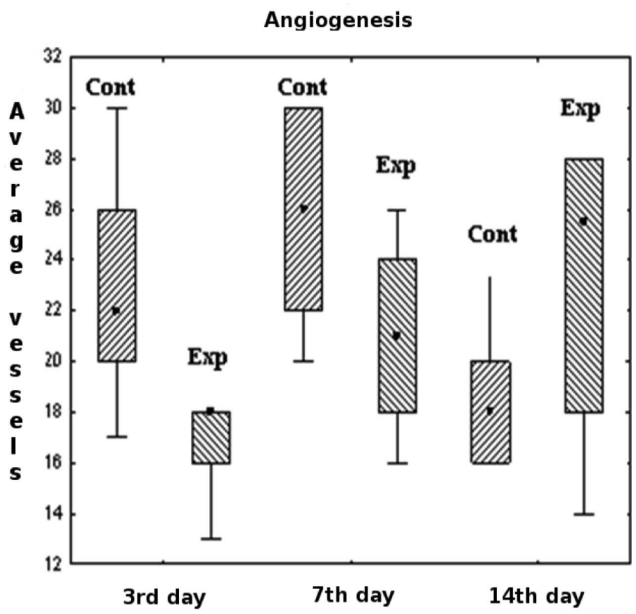

Figure 4 – *Average number of vessels by field.*

It was demonstrated that there is an increase in collagen synthesis in the remaining liver after partial hepatectomy until the 7th day, this difference no more existing from the 14th day on. Furthermore, there is decrease in collagen degradation, suggesting that this process favors the accumulation of this protein during early liver regeneration[22].

The analysis of collagen made in the present study showed lower density of collagen in the skin scars of skin/subcutaneous tissue and Aponeurosis. One can also verify that this difference was due to lower density of collagen I, especially in the initial period, which leads to assume that there is delay in this protein synthesis by fibroblasts. One can argue that this lower density can lead to reduced scars' resistance and be responsible for the larger number of dehiscences and incisional hernias observed in hepatectomized individuals[3-9].

The inflammatory response of the Hepatectomized Group has proved to be prolonged, but not so significant. Prolonged inflammatory reaction could be a good rationale to explain the lower density of collagen. However, it was not possible to confirm this information in this study. Perhaps a larger sample can clarify this doubt.

The inflammatory process slows the progression of healing stages, possibly by changes in signaling held by growth factors. Literature data show that the level of TGF-β_2 responsible for the proliferation of fibroblasts and initiation and maintenance of scar response only increases after the proliferation of hepatocytes. Hyperplasia and hepatic mass recovery are prioritized over to healing[18].

The hepatectomy and liver regeneration lead to delayed recovery of the abdominal wall incision, possibly by inhibiting the secretion of some cytokines (TGF-β_2) over others (HGF), as well as different cell mobilization, demonstrated by increased inflammation[12,18].

It is not known if a process of healing a tissue interferes with other in the same body, but we know of the importance of cytokines in healing or repair process and that they are expressed in quantities and at different times in each tissue. Some authors suggest that simultaneous procedures lead to increased risk of failure and may hinder the recovery of the abdominal wall[1,3,5].

Considering the high incidence of complications of abdominal wound healing and changes of concentrations of growth factors, we note the importance of studying the healing of abdominal wall after hepatectomies. Further studies with dosages of the main factors involved are necessary to better understanding and possibly reversing the healing delay caused by liver regeneration.

It was observed on the 3rd and 7th days that both type I collagen and angiogenesis were increased in the Control group. This fact may be explained by the increased concentration of growth factors and oxygen in the site, stimulating fibroblasts.

It would be interesting to test the strength of scars, but for technical reasons this was not held at this time, being the object of subsequent studies.

The data analyzed in this study suggest that hepatectomy leads to delayed healing process, interfering with the synthesis of collagen and angiogenesis.

R E S U M O

Objetivo: *Avaliar a cicatrização da ferida incisional da parede abdominal de ratos hepatectomizados quanto à concentração de colágeno, reação inflamatória e angiogênese.* ***Métodos:*** *Utilizaram-se 48 ratos distribuídos aleatoriamente para laparotomia com e sem hepatectomia. As cicatrizes foram estudadas no 3°, 7° e 14° dia de pós-operatório. Analisou-se a densidade do colágeno por método histoquímico e a angiogênese por método imunohistoquímico.* ***Resultados:*** *A análise do colágeno total mostrou menor concentração no plano da pele e da tela subcutânea, nas cicatrizes abdominais do grupo experimento (p_3=0,011; p_7=0,004 e p_{14}=0,008). A densidade de colágeno I foi inferior no grupo hepatectomizado, principalmente no 3° dia, tanto na pele e tela subcutânea (p=0,038) quanto no plano aponeurótico (p=0,026). Houve menor concentração de colágeno III nos dois planos estudados, embora não significante. A resposta inflamatória foi semelhante em todos os tempos, nos dois grupos. Verificou-se que a angiogênese desenvolveu-se mais precocemente no grupo controle (p_3=0,005 e p_7=0,012) e mais tardiamente no grupo experimento (p_{14}=0,048).* ***Conclusão:*** *A hepatectomia leva ao atraso do processo cicatricial, interferindo na síntese do colágeno e na angiogênese.*

Descritores: *Fígado. Regeneração. Hepatectomia. Cicatrização de feridas.*

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