

Use of 2-octyl cyanoacrylate adhesive in rat liver induced lesion¹

Uso adesivo de 2-octil cianoacrilato em lesão induzida de fígado de ratos

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ABSTRACT

PURPOSE: To evaluate the healing process of rat traumatic liver lesion corrected with the use of 2-octyl cyanoacrylate adhesive, compared to the use of biologically absorbable chromed catgut thread suture.

METHODS: Thirty male adult rats were divided into two groups (15 per group) according to the used method for liver lesion correction as follows: adhesive group (AG), and catgut group (CG); each group being divided into three subsets of five animals (7th, 14th, and 21st day), respectively, according to post-surgery evaluation. All animals were submitted to homogeneous lesion applying synthetic bonding to AG and using chromed catgut suture to CG for lesion correction. Macroscopic and microscopic parameters of healing processes were evaluated.

RESULTS: Both groups of animals showed excellent abdominal wall healing, with no evidence of infection, and no abdominal cavity peritonitis or abscess. The presence of adherence was observed in both groups with no statistically significant difference. As to macroscopic evaluation, there was statistically significant difference with respect to specific factors of clinical inflammation (ischemic inflammation and giant cellular inflammatory reaction) between animals evaluated on the 10th day (ischemic necrosis and giant cellular inflammatory reaction) among animals evaluated on the 14th day (A14 versus C14).

CONCLUSION: Applying 2-octyl-cyanoacrylate adhesive for correcting rat liver lesion does not change healing process when compared to the use of chromed catgut stitch.

Key words: Wound Healing. Liver. Adhesives. Rats.

RESUMO

OBJETIVO: Avaliar o processo de cicatrização de lesão induzida em fígado de rato utilizando adesivo de 2-octil cianoacrilato em comparação com a sutura com catgut cromado.

MÉTODOS: Utilizou-se 30 ratos adultos, machos, distribuídos em dois grupos de 15 animais denominados de grupo adesivo (GA) e grupo catgut (GC); cada grupo foi dividido em três subgrupos, cada um com cinco animais, de acordo com a data marcada para avaliação pós-operatória (sétimo, décimo quarto e vigésimo primeiro dia). Todos os animais foram submetidos à lesão homogênea, aplicando o adesivo sintético no GA e utilizando sutura com catgut cromado no GC para correção das lesões. Foram avaliados parâmetros de macroscópicos e microscópicos do processo de cicatrização.

RESULTADOS: Em ambos os grupos os animais evoluíram com boa cicatrização da parede abdominal, sem evidência clínica de infecção, sem abscessos ou peritonite na cavidade abdominal; a presença de aderências foi observada em ambos os grupos sem diferenças estatisticamente significantes; quanto à avaliação microscópica só houve diferença estatisticamente significativa quanto aos parâmetros específicos de inflamação crônica (necrose isquêmica e reação gigantocelular) entre os animais avaliados no décimo quarto dia (A14 vs. C14).

CONCLUSÃO: O emprego de adesivo de 2-octil cianoacrilato na correção de lesão em fígado de rato não altera o processo de cicatrização quando comparado ao uso de sutura com fio de catgut cromado.

Descritores: Cicatrização. Fígado. Adesivos. Ratos.

Introduction

The abdominal cavity holds most of the digesting organ system as well as the adjunct glandules, being part of the urogenital system and autonomous plexus¹. The increasing urban violence coupled with driving accidents has increased abdominal trauma incidence. Being the major abdominal human cavity glandule, the liver is a main lesion target. Therefore, liver traumatism is quite frequent, accounting to a high mortality rate².

Liver trauma merits special attention as to its diagnosis and management. It is assumed that around 45% of closed abdominal trauma and 40% of open abdominal traumas involving the liver. Regarding closed abdominal trauma 84% due traffic accidents followed by 8% trauma by falling, and 2% by physical attack³.

Though presenting under several aspects, most liver traumas provoke small liver lesions that at times do not need specific management and when they do, they are solved with minor procedures. Liver lesions can be treated in several manners depending on their severity varying from simple stitching, drainage, regradas and widening resections, or even transplant. In spite of hospital improved infrastructure, aseptic means, material, technical and anatomical know-how, mortality rate is still elevated for subjects with lever lesions, though such organ is protected by the costal gradil^{4,5}.

Lately, several treatment alternatives have been tried for liver lesion such as collagen haemostatic agent application, cellulose gelatin, as well as the use of base synthetic bondings diffused in spleen and digestive system, notably in experimental studies⁶.

There is scant information in the literature referring to this bonding use in parenchimatous bleeding. Aiming at collecting more information, we set out to study the use of 2-octyl cyanoacrylate in rat liver provoked lesions to evaluate the healing process of the target organ lesions, comparing the use of the bonding agent with the long traditional chromed catgut suture, to evaluate both microscopic and macroscopic aspects.

Methods

The project was evaluated and approved by the by the Ethics in Research Committee at the Federal University of Maranhao (UFMA), protocol n° 00386/2004. The study followed the guidelines for animal research as set out by the Brazilian College of Animal Experimentation (COBEA).

The sample was made up of 30 male Wistar rats (*Rattus*

norvegicus albinus, Rodentia mammalia), supplied by the UFMA vivarium. It was between 40 and 60 days old (average age 46 days). The weight was between 119g and 286g (average weight 219.1g). They were kept in groups of five per standard cage for the species at the Research Laboratory of the Physiology Department where the light-dark cycle was 12 hours and humidity was the same as a normal environment without any artificial regulation. The temperature was a constant $20 \pm 2^\circ\text{C}$. The animals were fed standard commercial food and had free access to water throughout the experiment.

An anesthetic by an intramuscular injection of 0.2ml/100g of weight from a mixture of 1ml of ketamine (50mg) and 1ml of xylazine (20mg). The trychotomy of the ventral abdominal wall was done and anti-sepsis with polyvinylpyrrolidone-iodine.

The surgical procedure consisted of a median laparotomy with four centimeters. The injury in the liver was done at 2.5cm from the peritoneal reflection, followed by simple synthesis with catgut cromate (two stitches) on the catgut groups and synthesis with cianocrilate adhesive and adhesive groups. We then moved on to the laparorrhaphy at two levels: the first, peritoneal-muscularaponeurotic; the second, of the skin, with continuous synthesis, utilizing 5.0 monofilament nylon (Figures 1 and 2).



FIGURE 1 - Median laparotomy and injury in the liver.

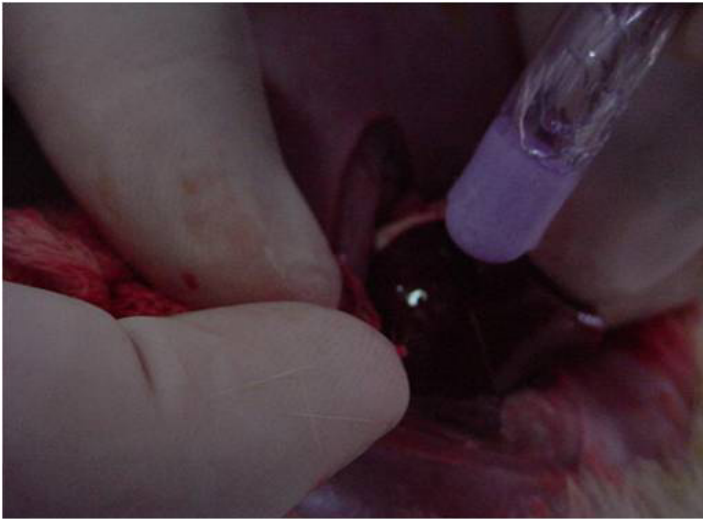


FIGURE 2 - Synthesis with cianocrilate adhesive.

After recuperating from the anesthetic, the rats were returned to their cages, where they had free access to standard commercial food and water. Immediately after surgery they were given an intramuscular dose of sodium diclofenac at a dosage of 10 mg/kg, as an analgesic and anti-inflammatory drug.

The animals were divided in two groups: adhesive group (AG) and catgut group (CG). Each group subdivided at random into three subgroups according to the date of euthanasia: catgut 7 (C7), catgut 14 (C14), catgut 21 (C21), adhesive 5 (A7), adhesive 5 (A14) and adhesive 21 (A21). The numbers correspond on the seventh, fourteenth and twenty one days after surgery. For euthanasia, a lethal dose of intraperitoneal sodium thiopental was used at 120mg/kg.

After euthanasia, another laparotomy was performed, enabling us to observe the abdominal cavity, the existence of peritonitis or abscess, adhesences and the integrity of the synthesis (Figure 3).

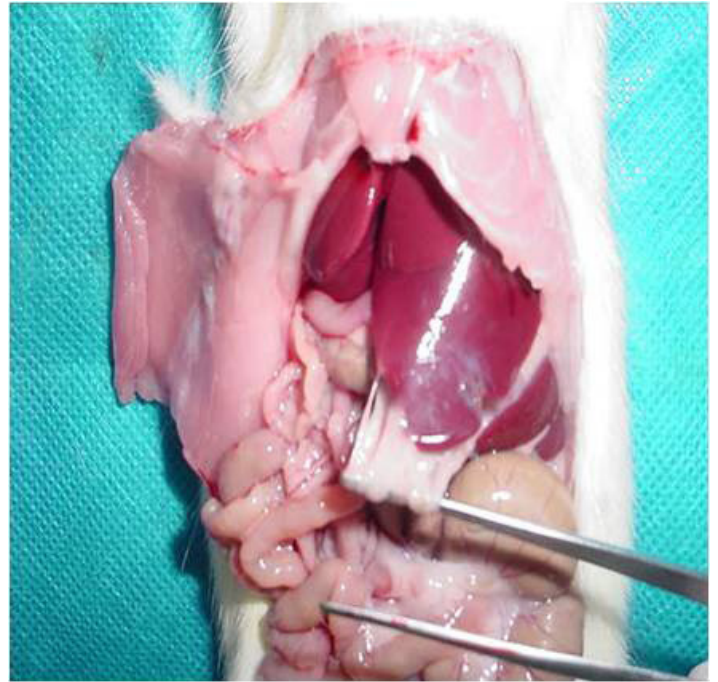


FIGURE 3 – Macroscopic observation of the existence of adhesences and the integrity of the synthesis in the abdominal cavity.

After this evaluation, the surgical parts were opened lengthwise and extended on filter paper and set in 10% formalin and forwarded for histopathological study.

Results

Macroscopic evaluation

All animals from the experiment had their clinical course classified as good during the post-surgery period, up to the programmed of death day.

The animals presented satisfactory healing of surgical wound, with no evidence of infection or dehiscence. No animals from either studied groups presented abscess or swelling upon abdominal cavity evaluation. The presence of bonding with adjoining structures evaluated according to the Nair score varied in both groups, showing a greater frequency on the abdominal wall and lower small intestine. On the other hand, there was statistically significant difference on day 7th only among animals from the adhesive and catgut group, with predominating grade 1 for the C7, and grade 0 for the A7, $p=0.036$; there was no statistically significant difference between C14 and A14, and between C21 and A21 (Table 1).

TABLE 1 - Impact of inter-group in adhesions seven, 14 and 21 days of PO by score nair.

Adhesions	Catgut			Adhesive			p		
	C7	C14	C21	A7	A14	A21	C7 x A7	C14 x A14	C21 x A21
Score							0.036	0.530	0.601
0	1	2	4	5	2	5			
I	3	1	1	0	3	0			
II	1	2	0	0	0	0			
III	0	0	0	0	0	0			
IV	0	0	0	0	0	0			

Mann-Whitney Test

p - value with statistical significance

Microscopic evaluation

There was no statistically significant difference on the temporal (intergroup) evaluation on the incidence of adhesive on the catgut group (C7 versus C14 versus C21), $p=0.177$, however, upon evaluation of adhesive on the catgut group, statistically significant difference was observed (A7 versus A14 versus A21), $p=0.030$.

Acute inflammation intensity was markedly accentuated for the C7 and discrete on the A7 with no statistically significant difference, $p=0.250$. It showed to be moderate on the 14th day for the CG and largely discrete for the AG; on day 21st it showed predominantly moderated on both evaluated groups. There was no statistically significant difference between the two groups neither on the 14th or 21st day with $p=0.117$ and $p=0.676$, respectively (Table 2).

TABLE 2 - Evaluation of the intensity of acute inflammatory reaction in inter-group seven, 14 and 21 days of PO.

Histological Parameters	Catgut			Adhesive			p		
	C7	C14	C21	A7	A14	A21	C7 x A7	C14 x A14	C21 x A21
Acute Inflammation							0.250	0.117	0.676
Absent	0	0	0	0	0	0			
Discrete	2	0	1	3	3	1			
Moderate	0	5	4	2	2	3			
Intense	3	0	0	0	0	1			

Mann-Whitney Test

p - value with statistical significance

As to the intergroup evaluation there was no statistically significant difference for both chromed group with $p=0.627$, and bonding group with $p=0.259$.

Chronic inflammation intensity was largely absent on the C7, discrete and moderate on the A7 with no statistically

significant difference, $p=0.296$. It was absent in most animals from the C14, whereas there was a discrete intensity on the A14 with no statistically significant difference, $p=0.117$. On day 21st it presented predominantly discrete for both CG and AG with no statistically significant difference, $p=0.403$ (Table 3).

TABLE 3 – Evaluation of the intensity of chronic inflammatory reaction in inter-group seven, 14 and 21 days of PO.

Histological Parameters	Catgut			Adhesive			p		
	C7	C14	C21	A7	A14	A21	C7 x A7	C14 x A14	C21 x A21
Chronic Inflammation							0.296	0.117	0.403
Absent	3	3	1	1	0	2			
Discrete	1	2	3	2	5	3			
Moderate	1	0	1	2	0	0			
Intense	0	0	0	0	0	0			

Mann-Whitney Test

p - value with statistical significance

There was no statistically significant difference for the catgut group ($p=0.438$), nor for the adhesive group ($p=0.274$) on the intergroup assessment.

Discussion

Due to its easy availability, handling and accommodation, resistance to manipulation, surgical aggression, and to infectious processes, the rat was used for evaluation of liver healing course.

In the present study the animals were divided into two groups denominated catgut group (CG) and adhesive group (AG), each group was further subdivided into three subgroups, according to the stipulated date for the proposed parameters evaluation (C7, C14 and C21; and A7, A14, and A21), with observations taking place on the 7th, 14th, and 21st days. The 7th day was chosen for the first observation for being a still acute period for the inflammatory response^{7,8}. The 2nd observation took place on the 14th day to evaluate inflammatory response and the intermediary stage of the healing parameters⁹. The third observation was done on the 21st day aiming at evaluating the inflammatory standard and late healing¹⁰.

Because of their sealing properties, cyanoacrylate adhesive and their derivatives, and fibrin glue, came to be used in animals and in almost all organic tissues in healing studies¹¹.

Among the cyanoacrylate derivatives, we chose to use the 2-octyl cyanoacrylate adhesive due to its having lower exothermal reaction compared to others, seeing that as the number of radical carbon increases, tissue reaction diminishes. It also has sealing and bactericide properties¹². Its bactericide properties are

of major importance, since the second main cause of death by liver traumatism is represented by intra or extra-abdominal sepsis, seconded only by hemorrhage⁵

Cyanoacrylate adhesive was applied to the liver diaphragmatic surface of the adhesive group (AG) according to the manufacturer (Dermabond®, Ethicon) recommendations, obtaining satisfactory haemostatic activity. The need for compression and site cleansing for adequate bonding polymerization must be highlighted¹³.

Simple suture with chromed catgut 4-0(Ethicon®) thread was used on the catgut group (CG) lesion, the choice being made because of its widespread use for correcting liver trauma lesions, thus observing adequate lesion haemostasia with no perceptible differences with respect to the adhesive group⁹.

As to macroscopic evaluation the presence of abdominal cavity bondings was not seen in the greater majority of animals where the bonding was used, contrasting with studies, where increased intra-cavity bonding was found¹¹. We observed, however, statistically significant difference between the subgroups A7 and C7, with a lower bonding incidence for the A7 group.

Inflammation is divided into chronic and acute standards. Acute inflammation has a relatively short duration, varying from hours to a few days, and its main aspects are fluid and plasmatic protein exudation and the emigration of leukocytes, neutrophils predominately. On the other hand, chronic inflammation has longer duration and it is histologically associated to the presence of lymphocytes and macrophages, blood vessel proliferation, tissue fibrosis and necrosis. Therefore, we opted to use the very same protocol used for the histological evaluation of the study pieces,

excluding the reepitelization, coaptation of suture edges, and wall infiltrate extension items, present in the original protocol¹⁴.

It is assumed that acute inflammation characterizes the stage 1 or inflammatory phase of the healing process upon healing evaluation, whereas indicators for chronic inflammation correspond to stage 2, or proliferative phase of healing process¹⁵. Hematoxylin-eosin dyeing was used in the present study to evaluate the proposed parameters⁹. However, the degree of fibrosis (collagen) was evaluated by Trichrome of Massom dyeing, not performing, nonetheless, the identification and quantification of collagen type.

The inflammatory reaction intensity degree could be of uttermost importance for the healing process, since the quantity of polymorphonuclear (PMN) in the wound has a close relationship with proliferation, activation and cellular movement, and the production of inflammatory process mediating cytokines¹⁶.

When evaluating histological changes in liver fragments on the respective groups, and comparing the groups with their respective dates of observation, one can notice statistically significant difference only on evaluating specific aspects of chronic inflammation (ischemic necrosis and alien body type giant cellular inflammatory reaction between the A14 and C14 groups, where there was an increase of those parameters in the C14 group with respect to the A14 group. Both with respect to acute inflammation and chronic inflammation, we did not observe statistically significant difference between the two groups as to the other inter-group parameter evaluation.

Both for acute inflammation assessment and chronic inflammation parameters an average variance between mild and moderate within the described evaluation periods was observed, thus favoring the adequate healing process since the absence or excessive inflammatory response markedly compromises tissue healing.

Upon intergroup (A7 versus A14 versus A21 and C7 versus C14 versus C21) assessment, statistically significant difference was observed only for the catgut group when fibroblastic proliferation and capillary neo-formation were studied, contrasting with what was expected for both groups, which would be a decreased acute inflammatory intensity, and temporal progression of the chronic one.

Conclusion

Applying 2-octyl-cyanoacrylate adhesive for correcting rat liver lesion does not change healing process when compared to the use of chromed catgut stitch.

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