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Evaluation of liver regeneration diet supplemented with omega-3 fatty acids: experimental study in rats

Avaliação da regeneração hepática com dieta suplementada com ácidos graxos ômega-3: estudo experimental em ratos

Rosilda Mendes da Silva[1,2]; Osvaldo Malafaia, ECBC-PR[1]; Orlando Jorge Martins Torres, TCBC-MA[2,3]; Nicolau Gregori Czeczko, TCBC-PR[1]; Carlos Hespanha Marinho Junior[1]; Ronaldo Kiviatcoski Kozlowski[1]

A B S T R A C T

Objective: to evaluate liver regeneration in rats after partial hepatectomy of 60% with and without action diet supplemented with fatty acids through the study of the regenerated liver weight, laboratory parameters of liver function and histological study. **Methods**: thirty-six Wistar rats, males, adults were used, weighing between 195 and 330 g assigned to control and groups. The supplementation group received the diet by gavage and were killed after 24h, 72h and seven days. Evaluation of regeneration occurred through analysis of weight gain liver, serum aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltranspeptidase, and mitosis of the liver stained with H&E. **Results**: the diet supplemented group showed no statistical difference (p>0.05) on the evolution of weights. Administration of fatty acids post-hepatectomy had significant reduction in gamma glutamyltransferase levels and may reflect liver regeneration. Referring to mitotic index, it did not differ between period of times among the groups. **Conclusion**: supplementation with fatty acids in rats undergoing 60% hepatic resection showed no significant interference related to liver regeneration.

Key words: Hepatectomy. Liver Regeneration. Fatty acids. Fatty acids, Omega-3.

INTRODUCTION

The early evolution of liver surgery was in 1716, with the partial liver resection in a trauma patient. However, the first successful liver resection was performed in 1888 by Langenbuch and the technique of vascular control, with great improvement on the procedure, was introduced by Pringle in 1908[1].

In the last decades, the surgical safety increased considerably because of new techniques, new equipment and materials, reducing the morbidity and mortality of patients submitted to hepatectomy[2].

In recent years elements involvement on liver regeneration process took place on the scenario, such as the hepatocyte growth, the alfa growth transformer and the epidermal growth of fibroblasts, which determine mitogen stimulus affecting other liver cells[3].

The regeneration of the liver is a cellular phenomenon that confers special responsiveness to injurious stimuli. It differs from other types of regeneration. Under normal circumstances, the liver remains with a very low basal cell renewal, where the average lifetime of an adult hepatocyte ranges from 200 to 300 days. After hepatectomy happens quick increase levels of interleukin 6 (IL–6) and tumor necrosis factor (TNF- α)[4].

It is known that the nutritional status of the patient influences of the regenerative capacity of the liver, as well as, it has an important role in the nutritional regulation, metabolization, distribution and use the nutrients[5]. The changes in the nutritional status of patients with cirrhosis, especially the malnutrition, may contribute to the low resistance to infections, fluid retention and delayed healing, increasing morbidity and mortality after surgical procedures[6].

Some specific nutrients, denominated pharmaconutrients, showed in clinical and laboratory studies to have ability to modulate the immune and inflammatory responses in animals and humans. Among them can be cited: arginine, glutamine, fatty acids and nucleotides[7].

The use of diets enriched with fatty acids showed benefits on liver regeneration in rats that were submitted at partial hepatectomy of 90%[8]. Thus, it is interesting to study the relationship between supplemented immunonutrition diet with fatty acids and liver regeneration. Reduction of pro-inflammatory cytokines and increase expression of cytokine, as anti-inflammatory agent, can

1. Programa de Pós-graduação em Princípios da Cirurgia da Faculdade Evangélica do Paraná/Hospital Universitário Evangélico de Curitiba, Curitiba, PR, Brasil; 2. Hospital São Domingos, São Luis, MA, Brasil; 3. Universidade Federal do Maranhão, São Luis, MA, Brasil.

retard the process of acute liver failure and can promote the liver regeneration process[8].

The objective of this study was to evaluate liver regeneration after 60% partial hepatectomy in rats with and without action of diet supplemented with fatty acids evaluating regenerated liver weight, laboratory parameters of liver function and histological findings.

METHODS

This study was approved by the Ethics Commitee and Animal Experimentation, Universidade Estadual do Maranhão, São Luis, MA, Brazil under protocol 036/12. It were used 36 Wistar rats (*Rattus norvegicus albinus*, Rodentia mammalia), males, adults, weighing between 195 and 330 g. The survey was conducted in the laboratory of experimental surgery of the Federal University of Maranhão. The animals were accommodated three for cage receiving water and standard ration for species (Purina®Labina), at temperatures of 23±2°C, in a cool environment, without noise and dark/light cycle at 12/12 hours. It were randomly divided in two groups of 18, and all of them were submitted the same hepatectomy.

The first was the control group; the animals received oral nutrition in adequate quantities for their species, age and weight. To determine this amount, an experiment was done for 24 hours where the rat was fed freely, weighting the feed before administration and also at the end. It is noteworthy that at the end of 24 hours the ration was weighed again including the rest that was deposited at the bottom of the cage. Thus, it was obtained the exact amount that the animal would consume in a day. After knowing this value, the control group was fed daily with this amount of ration, and water added with saline solution (1ml/100g) freely.

In the second group, besides being fed with ration, was administered 15 minutes before the surgical procedure 1ml/100g of lipid emulsion 10%, with the formulation: (10%), carboxymethyl cellulose(0,2%), Tween (50%), distilled water q.s.p (100%). The equal dose was taken every 24h until the date of death, administered by gavage to ensure full administration of the nutrient; what characterized each subgroup was the time of preoperative supplementation on 24h, 72h and seven days.

The weight control was performed in the beginning of the experiment after acclimatization and daily at pre- and postoperative phases. The measurements were used in order to perform the calculation of diet daily doses to be supplemented.

The liver resection was standardized according to the animal weight and liver weight. This was established after test in four rats out of study. They were weighed and defined the average weight. After it, they were anesthetized, sacrificed and their livers removed for weighing and set the ratio rat/liver. After the completion of the cavity, they liver was located and then was made the hepatic resection ligament. The resected segment was weighed on a precision balance. The calculation of the weight-based regeneration was done by Kwon[9]: % of regeneration = D/E x 100 (D=liver weight per 100g of animal weight on the day of death; E = R/0,7; E = the estimated resected liver per 100g before hepatectomy, which is calculated by the weight of the resected liver (R).

The resected liver lobes were preserved in 10% formalin and later sent for histopathologic study, staining the histological sections by H&E. The analysis was performed by a single pathologist blinded to the study groups. After the surgical procedure, each rat was placed alone in a cage for recovery from anesthesia and kept in ambient air until full recovery. Postoperative analgesia was made with application of 0.1 ml dipyrone 500 mg/ml, intramuscularly in the left hind limb.

Six hours after surgery, they had free access to water, food; after 12 hours, they were put at the same conditions of temperature and light as in preoperative phase. Weight, behavioral conditions and appearance of the surgical wound were checked-out daily.

In each subgroup was collected 4ml of blood from caudal vena cava, placed in a test tube for analysis of: albumin, total protein, globulin, total direct and indirect bilirubin, urea, creatinine, AST, ALT, gama-GT, glucose and alkaline phosphatase.

For statistical analysis, the data were analyzed using SPSS for Windows 20 (2011). Initially it was done the Shapiro Wilk normality test, and only the initial weight of rat variables, percentage of late liver regeneration, glucose, creatinine and alkaline phosphatase showed normal distribution ($p<0,05$). The remaining distributions were asymmetric ($p>0,05$). In the variables with normal distribution was applied multivariate analysis of variance (MANOVA) with two factors (group and time) and, after, the Tukey test to post-hoc comparison over time. In the variables that did not showed normal distribution, the nonparametric Mann-Whitney test to evaluate the effect of time was applied. The significance level for rejecting the null hypothesis was 5%, it was considered as significant value of<0, 05.

RESULTS

In the omega-3 group, one animal died within the first 24 hours after operation. When the initial weights of the liver and the liver resected fragment were analyzed, they were comparable ($p<0, 05$) (Table 1).

Evaluating the results of liver weight gain in relation to time of death, using the formula of Kwon[9], it was observed that there was no difference between the group and the control (pÃ0,05) (Table 2).

It was made the evaluation of liver function in relation to groups by laboratory parameter settings. It was observed alterations of gama-GT in both groups (Table 3).

However, the omega-3 group showed lower levels compared with the control (p<0,05).

When performed the control group and comparison on mitotic number (Table 4), the results showed no differences between the two groups (p=0,215). On the days of death, both control and omega-3 group, showed no significant differences (p>0,05) (Figures 1 e 2).

DISCUSSION

The use of pre- and postoperative supplementation in rats, in this study, was based on clinical and experimental evidence that it can interfere beneficially in liver regeneration but not clearly understood[7,10]. The daily supplementation in this experiment was performed by gavage to ensure that the correct administration of the dietary doses, calculated by the weight of the animal, was done. Problems with this process in animal models, such stress by the procedure in itself or injury to the mouth, esophagus and stomach are described. Through previous training, use of sedation and standardization of the procedure, the gavage could be performed safely without noticeable complications. To reduce the risk of regurgitation, the rat was maintained with cephalic portion in higher position after gavage.

Several studies have shown benefit from the use of supplementation with arginine, fatty acids and nucleotides in the reduction of infectious complications and the time of hospital stay in critical and surgical patients[11-13].

The process of liver regeneration after being triggered can be evaluated by several methods: liver weight, number of mitosis, components of deoxyribonucleic acid, synthesis rate of nuclear antigens, immunohistochemistry, gene expression, changes in serum protein levels, serological tests, enzymatic determination of proliferation markers and flow cytometry[14].

It was observed using the formula of Kwon for the weight gain evaluation, there was no difference between and control group (p>0,05), as it can be observed in table 2. The variable weight was not different in either group (both the control group and the group supplemented with fatty acids) for 24h, 72h and seven days. This fact is consistent with the literature showing that the weight in perioperative nutrition is not superior to the one in preoperative period, considering group without nutritional deficits[15].

In the evaluation of liver regeneration by laboratory tests between groups (table 3), only gama-GT showed significant differences (p>0,05) and was observed that in the group it was even in a lower level.

Statistical analysis of the values obtained for total protein, albumin and globulin between the groups showed no significant difference. Serological reference values considered in this study were similar to those obtained in the control group.

Table 1 - Evaluation of the initial liver weight and percentage of hepatectomy between the control and omega-3 group.

Group	Control		Omega 3		p
	N	Median	N	Median	
Initial liver weight	18	4,8	18	5,4	0,141
% of hepatectomy	18	51,5	18	56,6	0,181

Teste of Mann-Whitney in relation to group.

Table 2 - Analysis of the percentage of liver regeneration using the Kwon[9] formula.

Group	Time	N	Median	Mean rank	p
Control		18	101.5	19.1	0.715
	24 h	6	1.1	5.3	0.013
	72 h	6	80.0	8.8	
	7 dias	6	144.1	14.3	
Omega-3		18	78.9	17.9	
	24 h	6	0.2	5.7	0.086
	72 h	6	115.8	12.2	
	7 dias	5	81.0	10.7	

Test of Kruskal-Wallis in relation to group

Table 3 - Evaluation of liver regeneration by laboratory tests between control groups and omega-3 group.

Group	Control		Omega 3		p
	N	Median	N	Median	
Urea (mg/dl)	18	54.0	17	53.0	0.684
AST (U/l)	18	243.5	17	314.0	0.118
ALT (U/l)	18	164.5	17	155.0	0.443
Gama-GT (U/l)	18	5.0	17	3.0	0.025
Total protein (g/dl)	18	1.3	17	1.3	0.684
Albumin (g/dl)	18	0.9	17	0.9	0.525
Globulin (g/dl)	18	0.4	17	0.4	0.546
Total bilirubin (mg/dl)	18	0.9	17	0.9	0.546
Indirect bilirubin (mg/dl)	18	0.3	17	0.3	0.083
Direct bilirubin (mg/dl)	17	0.6	17	0.6	0.339

Test of Mann-Whitney

Table 4 - Analysis of mitosis.

Group	Time	N	Median	Mean rank	p
Control		18	4.0	20.1	0.215
	24 h	6	7.5	10.3	0.088
	72 h	6	10.5	12.4	
	7 days	6	2.0	5.8	
Omega-3		17	3.0	15.8	
	24 h	6	2.5	9.1	0.105
	72 h	6	4.5	11.8	
	7 days	5	1.0	5.5	

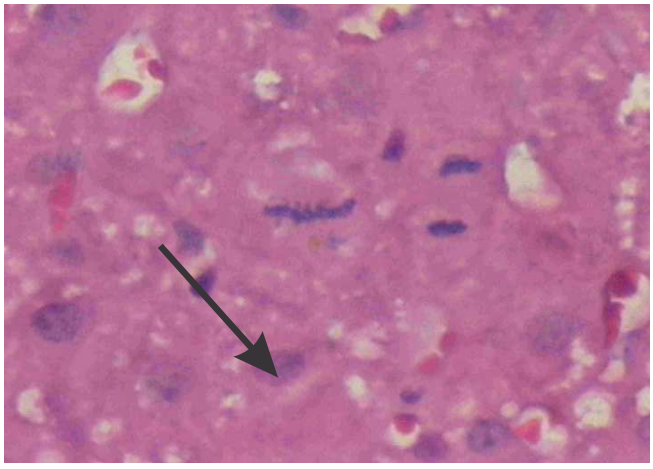

Figure 1 - *Photomicrograph of liver section from group stained with H&E: presence of mitosis (arrow).*

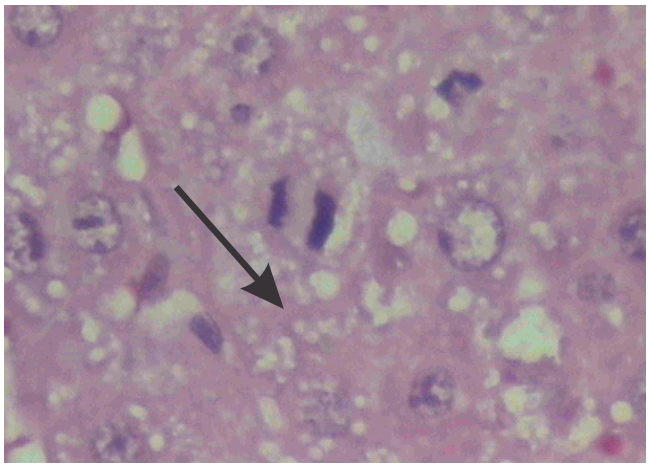

Figure 2 - *Photomicrograph of liver section from control group stained by H&E: presence of mitosis (arrow).*

Some biochemical tests can also be used to observe the hepatic profile as dosage ALT, AST, AST/ALT, gama-GT, alkaline phosphatase,prothrombin time, bilirubin and albumin. However severe liver disease can still have normal liver enzyme levels, or cause changes of 1.5 to three times above the reference levels[16,17].

The counting of mitosis process stained by H&E is one of the most commonly used parameters for evaluation of liver regeneration by being easy to replicate at low cost and thus is considered the reference method for experimental studies[14]. For evaluation of liver regeneration, assessed by mitotic index in this study, it was found no alterations among 24h, 72h and seven days groups.

The administration of fatty acids in rats after partial hepatectomy of 60% in 24h, 72h and seven days periods of observation did not play a significant role in liver regeneration.

R E S U M O

Objetivo: *avaliar a regeneração hepática em ratos submetidos à hepatectomia parcial de 60% com e sem ação de dieta suplementada com ácidos graxos ômega-3 através do estudo ponderal do fígado regenerado, parâmetros laboratoriais da função hepática e estudo histológico.* ***Métodos:*** *foram usados 36 ratos machos, distribuídos em dois grupos: grupo controle e grupo ômega-3. Cada um foi subdividido em mais três subgrupos com óbito em 24h, 72h e sete dias. O grupo ômega-3 recebeu água e dieta padrão suplementada com emulsão lipídica de ácidos graxos ômega-3 a 10% e o controle solução fisiológica a 0,9%. Em todos os subgrupos foi feita análise da regeneração hepática através da fórmula de Kwon, estudo da função hepática: dosagem de AST, ALT, gama-GT, bilirrubina total, bilirrubina indireta e indireta e albumina, e análise de mitose celular pela coloração de Hematoxilina-Eosina.* ***Resultados:*** *o grupo com dieta suplementada não apresentou diferença estatística (p>0,05) quanto à evolução dos pesos. Administração de ácidos graxos ômega-3 pós-hepatectomia mostrou que os níveis de gama-GT tiveram redução significante, podendo refletir na regeneração hepática. Na avaliação do índice mitótico não houve diferença entre os momentos estudados.* ***Conclusão:*** *a suplementação com ácidos graxos ômega-3 em ratos submetidos à ressecção hepática a 60% não apresentou papel expressivo relacionados à regeneração do fígado.*

Descritores: *Hepatectomia. Regeneração Hepática. Ácidos graxos. Ácidos Graxos Ômega-3.*

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Mailing address:
Rosilda Mendes da Silva
E-mail: rosildamendes@terra.com.br