

# HEPATOTROPHIC FACTORS REDUCE HEPATIC FIBROSIS IN RATS

Bruno **COGLIATI**<sup>1</sup>, Hélder de Moraes **PEREIRA**<sup>2</sup>, Maria Lúcia Zaidan **DAGLI**<sup>1</sup>, Osório Miguel **PARRA**<sup>3</sup>, José Roberto Machado Cunha da **SILVA**<sup>4</sup> and Francisco Javier **HERNANDEZ-BLAZQUEZ**<sup>2</sup>

**ABSTRACT** – *Context* - Hepatic fibrosis occurs in response to several aggressive agents and is a predisposing factor in cirrhosis. Hepatotrophic factors were shown to stimulate liver growth and to restore the histological architecture of the liver. They also cause an improvement in liver function and accelerate the reversion of fibrosis before it progresses to cirrhosis. *Objective* - To test the effects of hepatic fibrosis solution composed by amino acids, vitamins, glucose, insulin, glucagon and triiodothyronine on hepatic fibrosis in rats. *Methods* - Fibrosis was induced in rats by gastric administration of dimethylnitrosamine (10 mg/kg) for 5 weeks. After liver biopsy, the rats received either hepatotrophic factors solution (40 mg/kg/day) or saline solution for 10 days by intraperitoneal injection. Blood samples and liver fragments were collected for hepatic function analysis, standard histopathology evaluation, and morphometric collagen quantification. *Results* - Rats in the hepatotrophic factors group showed a decrease of the histopathological components of fibrosis and an increase of their hepatic mass (12.2%). There was no development of neoplastic lesions in both groups. Compared with the saline group, the hepatotrophic factors group also had a decrease of blood levels of hepatic-lesion markers (AST, ALT) and a decrease of collagen content in the portal spaces (31.6%) and perisinusoidal spaces (42.3%), as well as around the hepatic terminal vein (57.7%). Thus, hepatotrophic factors administration in the portal blood promoted a regenerative hepatic response, with an overall reduction of the volumetric density of collagen, improved hepatic function, and a general improvement in the histopathological aspects of fibrosis. *Conclusion* - Taken together, these results suggest the potential therapeutic use of this hepatotrophic factors solution to treat chronic liver diseases.

**HEADINGS** – Liver cirrhosis, experimental, drug therapy. Rats.

## INTRODUCTION

Hepatic fibrosis results from a shift in the constant balance between aggressive and regenerative processes, in which the primary characteristic is morphological alteration of the hepatic parenchyma<sup>(14, 25)</sup>. Fibrosis is an essential component in the development of cirrhosis, characterized by the presence of regeneration nodules that form irregular islets of hepatocytes surrounded by scarred connective tissue<sup>(27, 39, 43)</sup>. The final consequence of cirrhosis is the impairment of liver function<sup>(18, 28, 34, 38)</sup>.

Several clinical and surgical treatments have been proposed to reduce fibrosis and cirrhosis<sup>(13, 19, 34, 36, 37)</sup>. Modification of the disturbed histology in fibrotic livers by stimulation of regenerative processes, such as partial hepatectomy, has been proposed<sup>(17)</sup>. However, this can increase the functional deficit of an already failing organ<sup>(3, 4, 42)</sup>. Currently, the only definitive treatment when fibrosis progresses to advanced cirrhosis is liver transplant; but this procedure has several technical and physiological limitations<sup>(5, 8, 9, 11, 26)</sup>.

A different approach to stimulate hepatic tissue and improve the quality of liver recovery is the use of exogenous hepatotrophic factors<sup>(10, 19, 35, 44)</sup>. Parra et al.<sup>(31, 32, 33)</sup> developed an experimental model in rats based on administration of hepatotrophic factors (HF) solution. They can influence the growth, trophism, and histological architecture of the liver, and this solution consist of several amino acids, vitamins, glucose, insulin, glucagon, and triiodothyronine (T<sub>3</sub>). Administration of HF into the portal circulation of healthy rats promoted growth of additional liver mass, with an increase from 34.5 to 149%<sup>(31, 32)</sup> and a decrease of 37.5% in the volumetric density of collagen fibers<sup>(33)</sup>. These results raised the possibility of using hepatotrophic substances to directly stimulate liver regeneration in patients with fibrosis, thus improving liver function and reducing or reversing fibrosis.

The aims of this study were to test the effects of HF solution developed by Parra et al.<sup>(31, 32, 33)</sup> on liver fibrosis in rats. This therapy promotes a decrease of collagen

<sup>1</sup>Departments of <sup>1</sup>Pathology and <sup>2</sup>Surgery, School of Veterinary Medicine and Animal Science, University of Sao Paulo; <sup>3</sup>Department of Clinical Surgery, School of Medicine, University of Sao Paulo Medical School; <sup>4</sup>Department of Cellular and Developmental Biology, Biomedical Sciences Institute, University of Sao Paulo, São Paulo, SP, Brazil. Correspondence: Dr. Bruno Cogliati - Department of Pathology, School of Veterinary Medicine and Animal Science, University of Sao Paulo - Av. Prof. Dr. Orlando Marques de Paiva, 87 - Cidade Universitária - 05508-900. São Paulo, SP, Brazil. E-mail: bcogliati@usp.br

content in liver parenchyma, as well as improvements in liver histopathology and the blood hepatic-lesion markers.

## METHODS

### Animal protocols and induction of fibrosis

Seventy 2-month-old female Wistar rats weighing  $220 \pm 10$  g were used in this study. They were fed with a standard rat diet and water ad libitum and housed in cages with controlled temperature and humidity. The rats were provided by the animal facility of the Department of Pathology, School of Veterinary Medicine, University of São Paulo, SP, Brazil. All rats were cared for according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals<sup>(21)</sup>; the experimental procedure was approved by Bioethics Committee of our institution (protocol n°. 63/02). Fibrosis was induced by gastric gavage with an aqueous solution (10 mg/kg) of dimethylnitrosamine (DMN, Sigma-Aldrich, St Louis, Missouri, USA) twice a week for 5 weeks, followed by a resting period of 4 weeks. The mortality rate during the 9 weeks of treatment was 10%. After, the rats were anesthetized with isoflurane and a biopsy sample was surgically collected for histopathological analysis before treatment. All rats survived biopsy and subsequent treatments until the end of the experiment.

### Treatment with hepatotrophic factors and saline solution

Fifteen days after the biopsies, rats ( $n = 30$ ) in the HF group received intraperitoneal injections of HF solution (40 mg/kg/day), as described by Parra et al.<sup>(31, 32, 33)</sup>, divided into two daily doses for 10 days. The HF solution is composed by: glucose (104 g), amino acids solution (200 mL), pyridoxine (2 mg), calcium pantothenate (2 mg), thiamine (30 mg), riboflavin phosphate (4 mg), potassium chloride (1.43 g), sodium bicarbonate (1.5 g), nicotinamide (50 mg), monopotassium phosphate (750 mg), magnesium sulfate (500 mg), vitamin C (500 mg), insulin (62.5 IU), glucagon (0.625 mg), folic acid (2.5 mg), vitamin B12 (31.25 mg), zinc sulfate (3.125 mg) and distilled water (500 mL). The aminoacid solution contains: L-phenylalanine (1.080 mg), L-isoleucine (740 mg), L-leucine (1.960 mg), L-lysine acetate (1.180 mg), L-methionine (1.060 mg), L-threonine (980 mg), L-tryptophan (360 mg), L-valine (1.060 mg), L-arginine base (1.060 mg), L-histidine base (920 mg), L-alanine (2.060 mg), L-asparagine (790 mg), L-aspartic acid (540 mg), L-glutamic acid (500 mg), L-cystine (30 mg), L-ornithine (520 mg), L-proline (1.680 mg), L-serina (500 mg), L-tyrosine (320 mg), glycine (1.600 mg) in 200 mL of water. Triiodothyronine (2.26 µg/200 g rat weight) in ethyl alcohol was injected separately. Rats ( $n = 15$ ) in the saline (S) group were treated with saline solution (0.85 NaCl in distilled water) under the same conditions as the HF group.

### Biometrics, necropsy, and histopathology

The animals were weighed and euthanized in a CO<sub>2</sub> chamber. Live body weight (LBW), carcass weight (CW, body weight of the animal eviscerated), and liver weight (LW) were

measured. The hepatosomatic index [ $HSI = (\frac{LW}{LBW} * 100)$ ] and hepatocarcass index [ $HCI = (\frac{LW}{CW} * 100)$ ] were calculated. Fragments from each liver were fixed in bouin fixative for 12 h, embedded in paraffin wax, and 5-µm tissue sections were stained with hematoxylin-eosin, picosirius-hematoxylin, and picosirius alone<sup>(22)</sup>. Histological sections obtained from biopsies of each rat were compared with histological sections from necropsy samples from the same rat after treatment. Ten animals from each group were analyzed for the following lesions: regenerative nodules, fibrosis and fibrotic bridges, inflammatory infiltrate with oval cells, proliferation of bile ducts, and megalocytosis. The intensity of each lesion was classified as light (1 point), moderate (2 points), or severe (3 points).

### Serum biochemistry analysis

Blood samples from all animals were obtained by cardiac puncture just before euthanasia. Serum albumin (bromocresol green method), total protein (biuret method), AST and ALT enzyme activity (Biosystems® kits, BioSystems S.A, Barcelona, Spain) were analyzed on a BST-379 plus Automatic Analyzer. A group of 30 healthy reference rats was also studied (R group) in order to obtain reference values for the experiment.

### Collagen morphometry

Morphometric analysis of collagen was carried out on samples obtained from 10 rats from each group. The volumetric density of perisinusoidal collagen fibers in liver parenchyma was calculated using Kontron Zeiss morphometry software KS 400.3 (Carl Zeiss Vision GmbH, Halldergmoos, Germany). Briefly, the total microscopic-field area of a picosirius-stained section was measured with a 100× objective. The images were captured in black and white with a green filter in order to increase the contrast of the red collagen fibers. In the same field, the fibers were separated from the background by a threshold macro and the area of the collagen fibers was determined. This procedure was repeated 10 times on different areas of the same histological section. The areas of all 10 fields from each section were summed, as were the collagen areas from each field. The proportion of collagen relative to the field area in one section was calculated. The procedure was repeated on 10 histological non-serial slides for each rat, and the mean and standard deviation were determined. The resulting average proportion of area occupied by collagen in the liver parenchyma of each rat is considered an accurate estimate of the collagen volumetric density in the parenchyma<sup>(1, 41)</sup>.

In the next step, the collagen around the perivascular spaces (portal space and central veins) was quantified. However, the size of the target vessels was variable and the quantity of surrounding collagen may have reflected this variability. Thus, in order to normalize the amount of collagen in perivascular spaces, the collagen area measured around each blood vessel was related to the blood vessel area (portal vein in the portal space or the central vein). Pearson's correlation coefficient was used to measure the linearity of the relationship between the variables collagen area and vessel lumen area. Forty vessels

of each type were measured in histological sections from each rat, both in biopsy and in treated samples of liver. The images were obtained with a 20× objective. Results were expressed as the index of perivascular collagen [ $IPC = (\frac{PCA}{VLA})$ ], which indicates units of perivascular collagen (PCA) area relative to the units of the vessel luminal area (VLA).

**Statistical analysis**

Different comparison tests were performed between experimental groups. Analysis of variance (Levene test) and Student’s *t* test was used to test differences between means. The Wilcoxon matched-pairs signed-ranks test was used to intragroup (biopsy and treatment) comparisons. A 1-way analysis of variance (ANOVA) test with Tukey post-test was used to analyze differences among the means. Pearson’s coefficient of correlation was carried out between collagen area and area of the blood vessel lumen means. The results were expressed as mean ± SD. The Minitab 15 statistical software was used, and a *P* value less than .05 was considered significant.

**RESULTS**

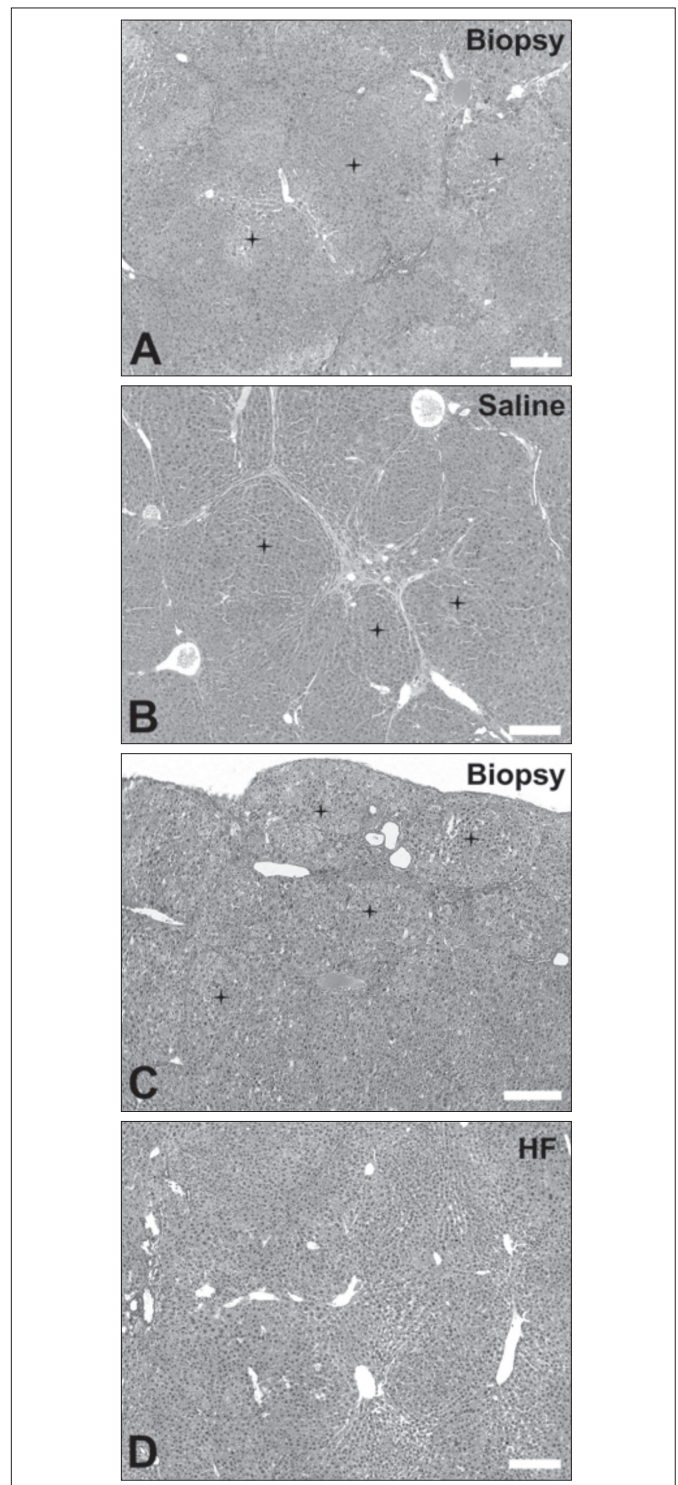
**Biometry and histopathology**

The LW, HSI, and HCI increased by 15.5%, 14.2%, and 21.6%, respectively, in the HF group compared to the S group (Table 1). Histopathology examination of biopsies obtained before treatment showed the presence of hepatic fibrosis with diffuse regenerative nodules, irregularly disseminated, bridges of fibrous connective tissue surrounding the nodules, and accentuated periportal fibrosis (Figure 1A–C). A multifocal inflammatory infiltrate was also observed, especially in the portal space, with oval cells, proliferation of bile ducts, and megalocytosis of hepatocytes. There were no neoplastic lesions in animals of both groups. Comparison of the paired biopsy and S liver samples showed a 27% reduction of periportal fibrosis, a 20% reduction of inflammatory infiltrate with oval cells, and a 15% reduction of bile-duct proliferation 5 weeks after interruption of DMN treatment. In the livers of rats treated with HF, the reduction of periportal fibrosis and fibrotic bridges (60%), inflammatory infiltrate with oval cells (36%), and bile-duct proliferation (36%) was much greater than in the biopsies previously obtained from each animal (Figure 1D, Table 2). Regenerative nodules were observed in biopsy samples, but disappeared in the HF group after treatment (Figures 1D, 2C).

**TABLE 1.** Comparison between means of anatomical parameters of rats after 10 days of treatment with saline (S) or hepatotrophic factors (HF) solution

	LBW (g)	LW (g)	HSI (%)	HCI (%)
S group	259.9 ± 15.8	7.1 ± 0.6	2.8 ± 0.3	3.7 ± 0.3
HF group	254.5 ± 21.6	8.2 ± 1	3.2 ± 0.4	4.5 ± 0.6
<i>P</i>	0.348	0.002	0.002	<0.001
Increase in HF group (%)	-	+ 15.5	+ 14.2	+ 21.6

Data are mean ± SD (S group, n = 15; HF group, n = 30). Student’s *t*-test was used to test for differences between means. LBW = live body weight at euthanasia; LW = liver weight; HSI = hepatosomatic index; HCI = hepatocarcass index



**FIGURE 1.** Histology of fibrotic rat livers stained with hematoxylin-eosin. A, B. Sections from the same rat before and after treatment with saline solution, showing regenerative nodules (star), thickened fibrotic bridges and disorganization of the lobular architecture. C, D. Sections from the same rat before (C) and after (D) treatment with hepatotrophic factors. Note that the fibrotic bridges and regenerative nodules (star) observed in A–C have disappeared in D. Scale bar = 200 μm

**TABLE 2.** Histopathological classification of liver lesions in the saline (S) and hepatotropic factors (HF) groups

	Periportal fibrosis and fibrotic bridges		P	Inflammatory infiltrate with oval cells		P	Bile-duct proliferation		P
	Biopsy	Treatment		Biopsy	Treatment		Biopsy	Treatment	
S group	2.9 ± 0.3	2.1 ± 0.3	<0.001	2.5 ± 0.5	2 ± 0.6	0.07	2 ± 0	1.7 ± 0.4	0.1
HF group	2.9 ± 0.1	1.2 ± 0.4	<0.001	2.2 ± 0.4	1.4 ± 0.5	0.031	2.2 ± 0.4	1.4 ± 0.5	0.03
Decrease in HF group (%)	- 42.8			- 30			- 17.6		

The intensity of each lesion was ranked as light =1, moderate =2, or severe = 3. Data are mean ± SD (n = 10). The Wilcoxon matched-pairs signed-ranks test was used to for intragroup (biopsy and treatment) comparisons

**Serum biochemistry analysis and collagen morphometry**

Serum albumin and total protein concentrations increased 23.6% and 14.7%, respectively, in HF-treated animals. Serum AST and ALT activities were reduced 36 and 49.2% in rats from the HF group (Table 3). All comparisons of collagen morphometry were done in paired

samples of liver biopsy and necropsy of the same rat after treatment with either saline or HF solution. The volumetric density of perisinusoidal collagen fibers decreased 43% in rat liver parenchyma after HF treatment (Figure 2). The correlation coefficients between the perivascular collagen area and the portal-vein and central-vein areas were high (Table 4). The IPCs measured around the central vein and portal spaces were also reduced (31.6% and 57.7%, respectively) after HF treatment. By contrast, rats in the S group showed a 42.7% increase in the IPC from around the central vein (Table 4), while the other areas showed no significant differences (P>.05) compared with those from the biopsies (Figure 1).

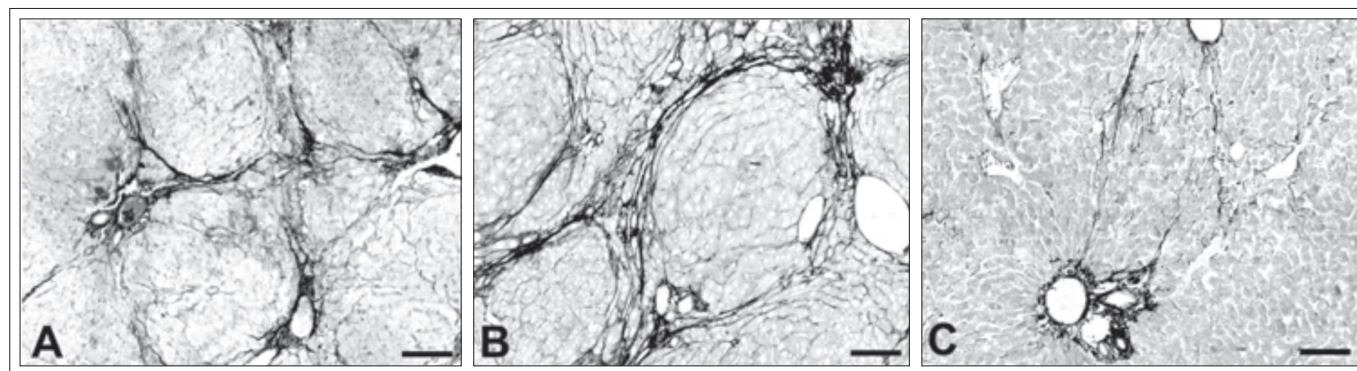
**TABLE 3.** Serum biochemical analysis of animals in the saline (S), hepatotropic factors (HF), and reference groups

	Albumin (g/dL)	Total protein (g/dl)	AST (U/L)	ALT (U/L)
S group	3.8 ± 0.1	6.1 ± 0.2	148.3 ± 21.7	86.9 ± 13.2
HF group	4.7 ± 0.2	7 ± 0.4	94.9 ± 16.5	44.1 ± 8.9
P	<0.05	<0.05	<0.05	<0.05
Reference group	3.3 ± 0.2	7.2 ± 0.5	111.3 ± 27.1	44.7 ± 14.8
Difference between S and HF groups (%)	+ 23.7	+ 14.7	- 36	- 49.2

Data are mean ± SD (n = 10). ANOVA test with Tukey post-test was used to analyze differences among the means

**DISCUSSION**

The rationale behind the use of liver-trophic substances is the assumption that liver size is regulated by the amount of blood arriving through the portal vein and by



**FIGURE 2.** Sections of rat liver stained with picrosirius. A. Fibrotic liver before treatment; B. fibrotic liver after treatment with saline solution; C. liver after treatment with hepatotropic factors. Collagen fibers appear black on a light-gray background. Note that in C the amount of collagen fibers is reduced after HF treatment and regenerative nodules are absent. Scale bar = 100 µm

**TABLE 4.** Collagen-fiber volumetric densities in perisinusoidal spaces and index of perivascular collagen around the portal and central veins before and after treatment

	VD (%)		P	IPC (%) Central vein		P	IPC (%) Portal Vein		P
	Biopsy	Treatment		Biopsy	Treatment		Biopsy	Treatment	
	S group	13.0 ± 1.7		13.8 ± 1.8	0.318		0.30 ± 0.03 (0.78)	0.44 ± 0.04 (0.72)	
HF group	12.3 ± 0.7	7.1 ± 0.9	< 0.001	0.26 ± 0.14 (0.68)	0.11 ± 0.06 (0.64)	<0.001	1.9 ± 0.3 (0.71)	1.3 ± 0.2 (0.77)	0.003
Collagen decrease (%)	- 42.3			- 57.7			- 31.6		

Data are mean ± SD. Numbers in parentheses are Pearson's coefficient of correlation. Student's t-test was used to test differences between means. (n = 10). R = Correlation coefficient between collagen area and area of the blood vessel lumen; VD = collagen-fiber volumetric densities in perisinusoidal spaces; IPC = index of perivascular collagen

the concentration of portal-blood components, as was suggested to explain liver growth in small-for-size liver transplants<sup>(12, 20, 23, 40)</sup>. An opposite effect is observed when the concentration of portal blood components is reduced by malnutrition, in which there is a marked decrease in the size of the liver<sup>(32)</sup>. Thus, increasing the concentration of hepatotrophic substances in the portal blood, such as by intraperitoneal administration of HF, is expected to result in liver enlargement. In our experiment, liver size indeed increased, confirming that hepatic growth can be modulated in fibrotic livers by the factors contained in the HF solution. In previous work, the total DNA content of healthy rat livers treated with HF increased by 60%, strongly suggesting that HF stimulates cell proliferation and that the liver grew through hyperplasia<sup>(32)</sup>.

Parra et al.<sup>(31, 32, 33)</sup> found that intraperitoneal administration of HF to healthy rats for 10 days promoted liver growth, with an increase in liver mass ranging from 34.5% to 149%. The same protocol was followed in treating animals with DMN-induced fibrosis and resulted in a 17.2% increase in liver mass. The effect was therefore less intense than observed in healthy livers. The fibrotic process causes a narrowing of the sinusoidal spaces and, consequently, impairs the exchange of nutrients between the blood and hepatocytes, which may account for the reduction of HF efficacy in the fibrotic liver<sup>(16, 24)</sup>.

Fibrosis is a predisposing component in the development of cirrhosis<sup>(25, 27, 39)</sup>, and different patterns of fibrosis are produced in response to injury. Liver biopsies showed microscopic lesions consistent with fibrosis, which indicated that extensive lesion formation was induced by the hepatotoxic action of DMN<sup>(2)</sup>. Bile-duct proliferation is a characteristic lesion that represents a functional deficit. The presence of an inflammatory process, with oval cells and hepatocyte megalocytosis, is pathognomonic for an immunological response to cellular injury<sup>(15, 31)</sup>. Animals from the HF group showed an improvement in this histopathological picture compared with the S group, indicating that treatment accelerated the recuperation of the liver parenchyma. The absence of neoplastic lesions indicated that the HF solution has no carcinogenic potential in rats with chronic hepatic disease.

The administration of HF to rats with hepatic fibrosis also promoted superior recovery of the liver microscopic architecture, stopping and reverting the fibrotic process. This was shown by the impressive reduction of the collagen volumetric density in the liver parenchyma of rats in the HF group. There was a larger reduction of the collagen content in the portal space than in the central vein region in HF-treated

rats. This was most likely resulted from the influx of a richer and more nutritious blood flow at the periportal region, due to its more favorable anatomical position.

The serum aminotransferases ALT and AST are reliable indicators of functional or structural alterations of hepatic cells, and can be useful in the diagnosis of hepatic diseases<sup>(29, 30)</sup>. The serum levels of these enzymes in the HF group returned to the values measured in rats of the reference group. The hepatocytes of HF-treated rats may thus have recovered their cellular integrity to a greater extent than those in the S group. In the latter, serum AST and ALT activities were higher than in the reference group. The persistently high levels of these enzymes indicated that hepatocellular lesions were still present 5 weeks after DMN treatment was interrupted. A similar, accentuated increase of these enzymes was noted in rats intoxicated by carbon tetrachloride<sup>(6)</sup>.

In hepatic fibrosis, hepatocellular protein secretion is reduced due to the loss of fenestrations on sinusoidal endothelial cells through continuous collagen deposition in perisinusoidal spaces<sup>(7)</sup>. Rats from the HF group had higher serum albumin and total protein concentration values than rats from the S group, suggesting greater hepatic secretion and reflecting the beneficial action of the HF solution on liver-cell function. It is possible that the HF-induced reduction of collagen-fiber deposition in perisinusoidal spaces allowed hepatic cells to gain easier access to blood and nutritional factors and improved secretion of cellular metabolic products<sup>(19, 24, 34)</sup>. Serum concentrations of aminotransferases and total protein were similar in rats from the HF group and the reference group.

The ability of HF to reduce the collagen content of fibrotic liver parenchyma, as demonstrated here, may have been the result of liver growth, which is associated with reorganization of the parenchymal architecture and, necessarily, with collagen rearrangement. A similar effect occurs during cell division after partial hepatectomy and results in both a loss of the liver typical histological architecture and a 50% reduction of the volumetric density of collagen<sup>(33)</sup>. We therefore postulate that the growing process triggered by HF stimulates a restructuring of the hepatic framework through the reabsorption and new synthesis of collagen. The removal of excess collagen around sinusoids and other vessels probably creates easier access by hepatocytes to the blood environment, which was enriched by the high concentration of HF. In turn, this promoted faster recovery of the liver from the DMN-induced lesions. Taken together, these results suggest the potential therapeutic use of HF solution to treat chronic liver diseases.

Cogliati B, Pereira HM, Dagli MLZ, Parra OM, Silva JRMC, Hernandez-Blazquez FJ. Fatores hepatotróficos reduzem a fibrose hepática em ratos. *Arq Gastroenterol.* 2010;47(1):79-85.

**RESUMO - Contexto** - A fibrose hepática ocorre em resposta a diversos agentes agressores e é um fator predisponente da cirrose. Fatores hepatotróficos são conhecidos por estimular o crescimento hepático e restaurar a arquitetura histológica do fígado. Promovem, também, melhora na função hepática e aceleram a reversão da fibrose antes de sua progressão para cirrose. **Objetivo** - Testar os efeitos de uma solução de fatores hepatotróficos, composta por aminoácidos, vitaminas, glicose, insulina, glugacon e triiodotironina na fibrose hepática em ratos. **Métodos** - No presente estudo, a fibrose foi induzida em ratos pela administração de dimetilnitrosamina (10 mg/kg) durante 5 semanas. Após a biopsia do fígado, os ratos receberam a solução de fatores hepatotróficos (40 mg/kg/dia) ou solução salina por injeção intraperitoneal, durante 10 dias. Amostras sanguíneas e fragmentos do fígado foram coletados para análise da função hepática, avaliação do critério histopatológico e quantificação morfométrica do colágeno. **Resultados** - Os ratos do grupo fatores hepatotróficos demonstraram diminuição dos componentes histopatológicos da fibrose e aumento de massa hepática (12,2%). Não houve o desenvolvimento de lesões neoplásicas em ambos os grupos. Comparado com o grupo de salina, no grupo fatores hepatotróficos também houve diminuição nos níveis dos marcadores sanguíneos de lesão hepática (AST e ALT), e diminuição da quantidade de colágeno nos espaços porta (31,6%) e espaços perissinusoidais (42,3%), assim como ao redor das veias terminais hepáticas (57,7%). Assim, a administração de fatores hepatotróficos no sangue portal promoveu resposta regenerativa hepática, com redução da densidade volumétrica de colágeno, melhora na função hepática e melhora geral nos aspectos histopatológicos da fibrose. **Conclusão** - Juntos, estes resultados sugerem o potencial uso terapêutico desta solução de fatores hepatotróficos para tratar doenças hepáticas crônicas.

**DESCRITORES** – Cirrose hepática experimental, quimioterapia. Ratos.

## REFERENCES

- Aherne WA, Dunnill MS. Morphometry. London: Edward Arnold; 1982.
- Ala-Kokko L, Stenback F, Ryhanen L. Preventive effect of malotilate on dimethylnitrosamine-induced liver fibrosis in the rat. *J Lab Clin Med.* 1989;113:177-83.
- Andiran F, Ayhan A, Tanyel C, Abbasoglu O, Sayek I. Regenerative capacities of normal and cirrhotic livers following 70% hepatectomy in rats and the effect of  $\alpha$ -Tocopherol on cirrhotic regeneration. *J Surg Res.* 2000;89:184-8.
- Asiyabolah B, Chang D, Gleisner AL, Nathan H, Choti MA, Schulick RD, Pawlik TM. Operative mortality after hepatic resection: are literature-based rates broadly applicable? *J Gastrointest Surg.* 2008;12:842-51.
- Chen JW, Chen DZ, Chen ZM. Unique pattern of fibrosing cholestatic hepatitis after liver transplantation. *Hepatobiliary Pancreat Dis Int.* 2002;1:33-4.
- Cornelius CE, Bishop J, Switzer J, Rhode EA. Serum and tissue transaminase activities in domestic animals. *Cornell Vet.* 1959;49:116-26.
- Crawford JM. The liver and biliary tract. In: Contran RS, Kumar V, Robbins SL, editors. Pathologic basis of disease. Philadelphia: Elsevier/Saunders; 2005. p.759-808.
- del Pozo JL. Update and actual trends on bacterial infections following liver transplantation. *World J Gastroenterol.* 2008;14:4977-83.
- Fallowfield JA, Iredale JP. Targeted treatment for cirrhosis. *Expert Opin Ther Targets.* 2004;8:423-35.
- Fan ST, Lo CM, Lai EC, Chu KM, Liu CL, Wong J. Perioperative nutritional support in patients undergoing hepatectomy for hepatocellular carcinoma. *N Engl J Med.* 1994;331:1547-52.
- Farkas SA, Schnitzbauer AA, Kirchner G, Obed A, Banas B, Schlitt HJ. Calcineurin inhibitor minimization protocols in liver transplantation. *Transpl Int.* 2009;22:49-60.
- Francavilla A, Zeng Q, Polimeno L, Carr BI, Sun D, Porter KA, Van Thiel DH, Starzl TE. Small-for-size liver transplanted into larger recipient: a model of hepatic regeneration. *Hepatology.* 1994;19:210-6.
- Friedman SL. Liver fibrosis-from bench to bedside. *J Hepatol.* 2003;38:38-53.
- Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology.* 2008;134:1655-69.
- Gertman PM, Madden J, Bevin AG, Smith HC. Hepatic collagen metabolism in dimethylnitrosamine induced cirrhosis in rats. *Surg Forum.* 1970;21:363-4.
- Goodman ZD, Ishak KG. Hepatic histopathology. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Schiff's diseases of the liver. 8th ed. Philadelphia: Lippincott/Williams & Wilkins; 1999. p.53-117.
- Hashimoto M, Watanabe G. Functional restoration of cirrhotic liver after partial hepatectomy in the rat. *Hepatogastroenterology.* 2005;52:897-902.
- Henderson NC, Forbes SJ. Hepatic fibrogenesis: from within and outwith. *Toxicology.* 2008;254:130-5.
- Holecck M. Nutritional modulation of liver regeneration by carbohydrates, lipids, and amino acids: a review. *Nutrition.* 1999;15:784-8.
- Ikegami T, Shimada M, Imura S, Arakawa Y, Nii A, Morine Y, Kanemura H. Current concept of small-for-size grafts in living donor liver transplantation. *Surg Today.* 2008;38:971-82.
- Institute of Laboratory Animal Resources (ILAR). Guide for care and use of laboratory animals. Washington: National Academy Press; 1996.
- Junqueira LCU, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J.* 1979;11:447-55.
- Kam I, Lynch S, Svanas G, Todo S, Polimeno L, Francavilla A, Penkrot RJ, Takaya S, Ericzon BG, Starzl TE. Evidence that host size determines liver size: studies in dogs receiving orthotopic liver transplants. *Hepatology.* 1986;7:362-6.
- Le Couteur DG, Fraser R, Hilmer S, Rivory LP, McLean AJ. The hepatic sinusoid in aging and cirrhosis: effects on hepatic substrate disposition and drug clearance. *Clin Pharmacokinet.* 2005;44:187-200.
- Lee MH, Ion S, Moon J-O. The flavonoid naringenin inhibits dimethylnitrosamine-induced liver damage in rats. *Biol Pharm Bull.* 2004;27:72-6.
- Li G, Ye QF, Xia SS, Chen ZS, Zeng FJ, Lin ZB, Gong NQ, Zhang WJ, Wen ZX, Sha P, Jiang JP. Acute respiratory distress syndrome after liver transplantation: etiology, prevention and management. *Hepatobiliary Pancreat Dis Int.* 2002;1:330-4.
- Maclachlan NJ, Cullen JM. Liver, biliary system and exocrine pancreas. In: McGavin MD, Zachary JF, editors. Pathologic basis of veterinary disease. Philadelphia: Mosby; 2006. p.395-6.
- Neubauer K, Saile B, Ramadori G. Liver fibrosis and altered matrix synthesis. *Can J Gastroenterol.* 2001;15:187-93.
- Noguchi A, Reis JMC, Dias CS, Epaminondas WA, Azevedo PSR, Brito MVH. Serum levels of aminotransferases, bilirubins and gamma-glutamyltranspeptidase after copaiba oil administration in rats. *Acta Cir Bras.* 2002;17:130-4.
- Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. *Toxicology.* 2008;245:194-205.
- Parra OM, Saad WA, Ferraz-Neto JBHE, Silva RAPS, Hernandez-Blazquez FJ, Peduto L, Ferraz-Neto B. Stimulation of intact rat liver by exogenous hepatotrophic factors with additional growth of its mass. *Acta Cir Bras.* 1994;9:7-11.
- Parra OM, Silva RAPS, Silva JRMC, Hernandez-Blazquez FJ, Peduto L, Saad WA, Saad Jr WA. Enhancement of liver size by stimulation of intact rat liver with exogenous hepatotrophic factors. *Sao Paulo Med J.* 1995;113:941-7.
- Parra OM, Hernandez-Blazquez FJ, Silva JRMC, Saad CA, Saad Jr WA. Behavior of collagen extracellular liver matrix during regenerative growth after partial hepatectomy or after stimulation by exogenous hepatotrophic factors: Study in rats. *Arq Gastroenterol.* 1996;33:212-6.
- Pinzani M, Rombouts K. Liver fibrosis: from the bench to clinical targets. *Dig Liver Dis.* 2004;36:231-42.
- Poon RTP, Fan ST. Perioperative nutritional support in liver surgery. *Nutrition.* 2000;16:75-6.
- Ramalho LNZ, Ramalho FS, Zucoloto S, Castro-e-Silva Jr O, Corrêa FMA, Elias Jr J, Magalhães JFG. Effect of losartan, an angiotensin II antagonist, on secondary biliary cirrhosis. *Hepatogastroenterology.* 2002;49:1499-502.

37. Rockey DC. Current and future anti-fibrotic therapies for chronic liver disease. *Clin Liver Dis.* 2008;12:939-62.
38. Schuppan D, Ruehl M, Somasundaram R, Han EG. Matrix as modulator of hepatic fibrinogenesis. *Semin Liver Dis.* 2001;21:351-72.
39. Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet.* 2008;371:838-51.
40. Van Thiel DH, Gavaler JS, Kam I, Francavilla A, Polimeno L, Schade RR, Smith J, Diven W, Penkrot RJ, Starzl TE. Rapid growth of an intact human liver transplanted into a recipient larger than the donor. *Gastroenterology.* 1987;93:1415-9.
41. Weibel ER. *Stereological methods: practical methods for biological morphometry.* London: Academic Press; 1989.
42. Wilasrusmee C, Sritheptawee S, Kanchanapanjapon S, Sopon P, Vanichanon C, Limphong W, Pongchailerks P, Lertsithichai P, Wilasrusmee S, Kittur DS. Ultrastructural change in cirrhotic and noncirrhotic patients due to hepatectomy. *J Hepatobiliary Pancreat Surg.* 2004;11:266-71.
43. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol.* 2008;214:199-210.
44. Zhang ZT, Jiang P, Wang Y, Li JS, Xue JG, Zhou YZ, Yuan Z. Effects of hepatotrophic factors on the liver after portacaval shunt in rats with portal hypertension. *Chin Med J.* 2006;119:1727-33.

Received 27/10/2008.

Accepted 3/4/2009.