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EFFECTS OF NATURAL AND RECOMBINANT INTERFERON ON CARBON TETRACHLORIDE-INDUCED LIVER CIRRHOSIS

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ABSTRACT

Carbon tetrachloride (CCl_4) is a well-known hepatotoxicant that is used to develop a model of liver cirrhosis in rats. The present study was conducted to investigate the effects of chronic treatment with natural or recombinant interferon (IFN), Ismafron or Roferon, respectively, on CCl_4-induced liver cirrhosis in rats. Seventy male Sprague-Dawley rats were used in the current study; animals were divided into three major groups, Group I, non-treated animals; Group II, normal animals treated with Ismafron, Roferon or liquid paraffin (the vehicle of CCl_4); Group III, cirrhotic animals, the cirrhotic rats were divided into 3 subgroups, cirrhotic animals treated with Ismafron, cirrhotic animals treated with Roferon, and untreated cirrhotic animals. Liver cirrhosis was induced in rats after 10 weeks of treatment with CCl_4 at a dose of 0.3 ml/100 g body weight twice a week.

Two weeks of treatment with Ismafron (10,000 IU/rat/day) or Roferon (50,000 IU/rat/day) could decrease the serum activities of the enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), and suppress the serum level of procollagen III. Furthermore, treatment with Ismafron or Roferon decreased fibrosis and inflammation of cirrhotic livers. It is concluded that treatment with Ismafron or Roferon could normalize the above serum markers of liver cirrhosis and enhance the histological picture of the liver in CCl_4-treated rats.

INTRODUCTION

Cirrhosis is a chronic liver disease that has received a great attention throught decades. Liver cirrhosis is a chronic, diffuse, degenerative disease in which normal liver cells are damaged and replaced by a scar tissue. Several etiologies may lead to liver cirrhosis including excessive alcohol intake, types B, C and D of chronic viral hepatitis, inherited or congenital diseases,e.g., Hemochromatosis and Wilson's disease, alpha 1-antitrypsin deficiency, glycogen storage disease, autoimmune hepatitis, and prolonged obstruction of the bile ducts[1].

Several animal models have been developed to study liver cirrhosis. Liver cirrhosis could be developed in Sprague-Dawley rats 3 weeks after common bile duct ligature[2,3]. Rats infected with Helminth Capillaria Hepatica regularly developed septal hepatic fibrosis and concluded by cirrhosis 40 days after inoculation[4]. Different doses of thioacetamide (0.05%, 0.1% and 0.15%) were used to induce liver cirrhosis in Wistar rats[5-7]. Experimental hepatic fibrosis could also be produced by dimethylnitrosamine in rats[8]. Hepatic fibrosis in rats could be sufficiently induced by repetitive intraperitoneal injections of allylalcohol[9].

Carbon tetrachloride (CCl_4) is a well known hepatotoxicant. To develop a reproducible model of liver cirrhosis in rats, CCl_4 and phenobarbital were administered to male Wistar rats for 9 weeks[10,11] and Sprague-Dawley rats for 8 weeks[12]. In another design, experimental cirrhosis was produced in rats after a 6-month period of exposure to CCl_4 [13]. Liver cirrhosis was also induced in rats by a weekly intragastric administration of CCl_4 for 16 weeks[14,15]. Furthermore, fibrosis was seen in rats after weekly administration of CCl_4 for 4 weeks, and cirrhosis was evident after 8 weeks of treatment[16].

Chronic hepatitis B virus infection remains a significant worldwide cause of liver cirrhosis and hepatocellular carcinoma[17]. Hepatitis C virus infection often progresses to chronic hepatitis, cirrhosis, and possibly hepatocellular carcinoma[18-20]. Interferon (IFN)-alpha is used among the antiviral therapy in humans mainly for the treatment of viral hepatitis B and C. Biochemical and virological responses to IFN therapy are associated with an improvement in liver histology during and shortly after treatment[21]. An antifibrotic effect of IFN has been postulated even in the absence of anti-viral response, which suggests that IFN directly inhibits fibrogenesis[22]. IFN-gamma elicits antiproliferative and antifibrogenic activity in a variety of mesenchymal cells, including hepatic satellite cells. Furthermore, IFN-gamma inhibits collagen synthesis including type I and III collagen[23].

Several synthetic interferons have been developed. Although comparative studies have not yet been conducted, clinical studies suggest that there may be significant therapeutic differences between the use of natural and synthetic interferons[24]. Studies have found that treatment with synthetic INF in certain cases may cause an immunological response (the production of neutralizing and/or binding antibodies by the human immune system) that reduces the effectiveness of the treatment or which may cause adverse side effects. It is believed that the production of neutralizing and/or binding antibodies is virtually non-existent in patients treated with natural IFN. Furthermore, primarily due to biological differences, the side effects of treatment with natural IFN, in certain instances, may be less severe than those with synthetic IFN[25].

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The current study was conducted to study the effects of the natural and recombinant IFN, ISMAFRON® and ROFERON®-A, respectively, on liver cirrhosis, induced by subcutaneous chronic injections of CCl_4, in male Sprague-Dawley rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180±20 g, obtained from the National Center of Drug Control and Research, were used in these studies. Animals were kept at 25°C on a 12-hr light-dark cycle in a room with 35-70% relative humidity. Three animals were kept per cage, and food and water were allowed ad libitum. The rats were housed in stainless steel cages with mesh floor.

Animals were randomly divided into three groups, Group I, non-treated animals, this group served as a general control; Group II, this group was subdivided into subgroup 1, normal animals treated with natural IFN, subgroup 2, normal animals treated with synthetic IFN, and subgroup 3, normal animals treated with liquid paraffin (the vehicle of CCl_4); Group III, cirrhotic animals.

After induction of cirrhosis with CCl_4, as evident by the histopathology of randomly selected samples of rats' livers, animals were divided into three subgroups, subgroup 1, cirrhotic animals treated with natural IFN, subgroup 2, cirrhotic animals treated with synthetic IFN, and subgroup 3, non-treated cirrhotic animals. Each working group was composed of 10 animals, according to the sample size equations.

Induction of cirrhosis: Rats were treated with subcutaneous injections of CCl_4 in liquid parafin at a dose of 0.3ml/100g body weight twice a weak for 10 weeks. Control rats received an equal volume of liquid paraffin. Rats were treated daily with interferon for 2 weeks, 10,000 IU/rat/day Ismafron® (natural IFN) or 50,000 IU/rat/day Roferon-A® (synthetic IFN, provided by ACAPI). A summary of the studied groups is shown in Table 1.

Collection of samples: At the end of the experiment, blood samples were collected from the retro-orbital venous plexus using capillary tubes[26]. Rats were sacrificed after blood collection and liver tissues were collected. Blood samples were centrifuged at 4000 rpm for 5 min. The serum was removed, frozen immediately on dry ice, and stored at - 80°C to be used for the determination of liver function tests. All groups were examined for liver function tests, serum collagen level, and histopathology as follow:

1. Analysis of liver enzyme activities: serum enzyme activities of alanine aminotransferase (ALT)[27], alkaline phosphatase (ALP)[28] and gamma-glutamyl transpeptidase (GGT)[29] were measured using specific relevant kits (bioMerieux).
2. Estimation of serum procollagen III (PCIII) using radioimmunoassay technique[30].
3. Liver preparation for histopathology: Animals were sacrificed to obtain liver tissues. Samples of liver tissues for histopathology were fixed by immersion in 10% neutral buffered formalin for 24 hr. Slices (4mm thickness) of fixed tissue were processed, embedded in paraffin, sectioned to a thickness of 5 µm, mounted on glass slides, and stained with Hematoxylin and Eosin (HE), and Masson Trichrome for histopathological evaluation.

Statistical analysis: Data were collected, tabulated, and analyzed by one way analysis of variance (ANOVA) followed by Bonferroni test for contineous variables (liver enzyme activities). Chi-square test of significance was used for the analysis of discrete variables (histopathology).

Table 1: Summary of the studied groups.

	Normal			Cirrhotic Animals		
Control	Ismafron (natural IFN)	Roferon (synthetic IFN)	Liquid paraffin	Non-Treated	Ismafron (natural IFN)	Roferon (synthetic IFN)

RESULTS

The serum activity of ALT was significantly different among the seven groups as indicated by ANOVA ($p \leq 0.005$). ALT serum activity in cirrhotic animals treated with Ismafron was significantly higher than that in cirrhotic animals treated with Roferon and the normal group treated with Ismafron ($p \leq 0.05$), Table 2.

GGT data analysis showed significant ANOVA results ($p \leq 0.01$); that was, apparently, due to the elevated activity of GGT in the cirrhotic untreated group as compared to the control ($p \leq 0.005$). Other groups exhibited no significant difference, Table 2.

Although ANOVA results of ALP were non-significant among all groups, Table 2 shows that ALP serum activities tended to decrease in the groups treated with Ismafron and Roferon as compared to the cirrhotic untreated group.

Comparing the procollagen III (PIIINP) serum levels among the seven groups, ANOVA analysis showed significant effect ($p \leq 0.001$); as indicated by Bonferroni test, this significant effect was as follow:

- A significantly higher level of PIIINP in the untreated cirrhotic group than the level in the cirrhotic group treated with Isamfron or Roferon ($p \leq 0.01$).
- A significantly lower level of PIIINP in the control group than its level in the cirrhotic group treated with Ismafron or Roferon ($p \leq 0.001$).
- The serum level of PIIINP in the untreated cirrhotic group was significantly higher than its level in the control and in both normal groups treated with either Ismafron or Roferon ($p \leq 0.001$).

The death rate was 40% in the untreated cirrhotic group, whereas the death rate was 20% in the cirrhotic animals treated with Ismafron or Roferon. Although

Chi-square analysis of the scores of fibrosis, inflammation, or fatty change showed no significant results (p=0.48, p=0.13, and p=0.95, respectively), histopathological changes could be detected. Degree IV of fibrosis was detected only in the untreated cirrhotic group (Table 3). Further, the latter exhibited degree III of fibrosis in three animals out of six, whereas the cirrhotic animals treated with Ismafron or Roferon exhibited the same degree of fibrosis in only two and one animal out of eight, respectively. The latter groups showed the lower degree of fibrosis in five animals each.

Similarly, degree IV of inflammation was detected in four animals of the untreated cirrhotic group, in contrast to one animal or non in the cirrhotic groups treated with Ismafron or Roferon, respectively. Further, the cirrhotic groups treated with Ismafron or Roferon showed degree I of inflammation in two and five animals, respectively, as compared to non in the untreated cirrhotic group. No specific trend was observed in the fatty change parameter among various groups. Figure 1 shows the histological changes that took place in the liver.

Table 2: The serum activities of ALT, GGT, ALP, and PIIINP in all studied groups of rats, untreated control, normal animals treated with Ismfron, Roferon, or liquid paraffin, and the cirrhotic groups, untreated and treated with Ismafron or Roferon.

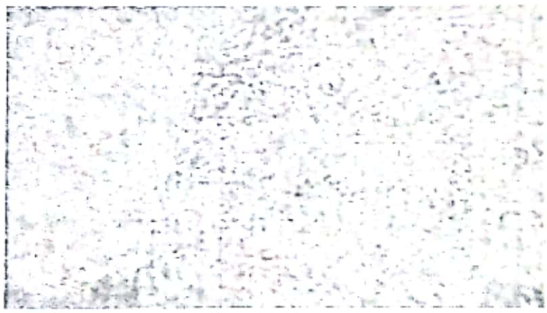
Group \ Parameter		ALT (U/L)	GGT (U/L)	ALP (U/L)	PIIINP (µg/L)
Control		82.7±4.36	1.2±0.13 (1)	831±30.16	7.26±0.008 (1,2,3)
Normal	Ismafron	67.2±2.58 (1)	2.1±0.35	775.7±49.13	7.48±0.181 (4)
	Roferon	79.3 ±2.97	2.5 ± 0.48	847.6±25.28	7.51±0.154 (5)
	Liquid paraffin	72.7±4.55	1.6±0.27 (2)	831.2±55.31	7.30±0.008 (7,9,10)
Cirrhotic	Ismafron	87.6±3.27 (1,2)	2.5±0.42	630±43.81	7.988±0.109 (1,6,7)
	Roferon	64.4±6.64 (2)	2.8±0.31	662.38±24.68	7.962±0.164 (2,8,9)
	Untreated	76.2±6.42	3.3±0.42 (1,2)	793.67±119.37	8.800±0.159 (3,4,5,6,8,10)

- Values are expressed as the mean ± the standard error of the mean.
- All data were analyzed using one way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparison of means.
- Significantly different groups are indicated by the same italic number.

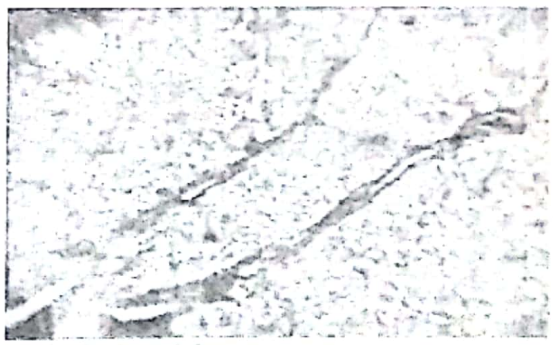
Table 3: The histological profile of the liver, including fibrosis, inflammation, and fatty change, in treated and untreated cirrhotic rats.

Parameter/Degree \ Group		Cirrhotic+ natural IFN (n=8)	Cirrhotic+ synthetic IFN (n=8)	Cirrrhotic (n=6)
Fibrosis	I	1	2	0
	II	5	5	1
	III	2	1	3
	IV	0	0	2
Inflammation	I	2	5	0
	II	0	2	0
	III	2	0	0
	IV	1	0	4
	portal	3	1	2
Fatty change	Steatosis	4	6	4
	No steatosis	4	2	2

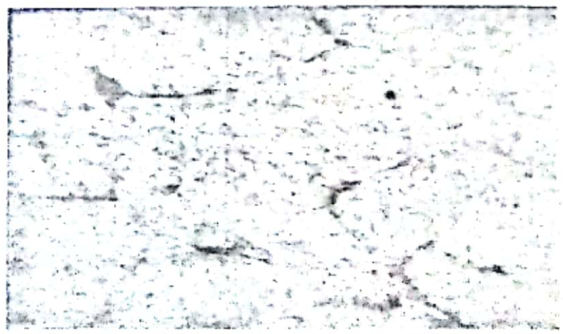
Figure 1: Show normal liver cells (A), cirrhotic nodules (B, C, and E), inflammation (F) as well as steatosis (D) in hepatocytes.



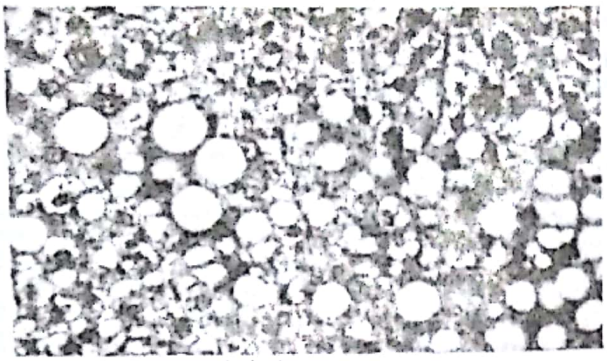
A- Normal control liver.



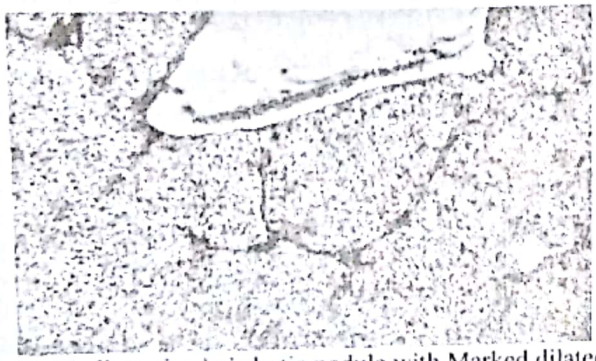
B- Cirrhotic nodule



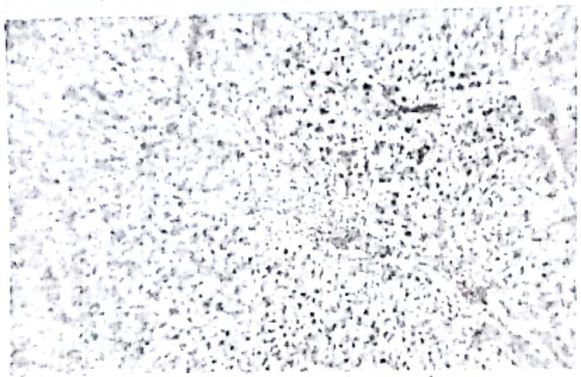
C- Hepatocytes show hydropic degeneration, a band of fibrous tissue surrounding a group of hepatocytes forming cirrhotic nodule.



D- Marked steatosis in hepatocytes.



E- Medium sized cirrhotic nodule with Marked dilated blood capillary and RBCs in the middle.



F- Early fibrosis, inflammation in portal tract.

DISCUSSION

In the present study, two weeks of treatment with IFN produced beneficial effects on the serum activity of ALT; this effect was evident with the synthetic IFN, Roferon, as compared to the natural IFN, Ismafron. Further, IFN treatment improved the histological picture of the liver in CCl_4-treated rats. In agreement with these results, several investigators have reported improvement of histological appearance in patients with normalized ALT levels after IFN therapy[31]. The protection by IFN from CCl_4-induced damage was explained on the basis of inhibition of cytochrome P_{450} 2E1 activity[32]. This would prevent the bioactivation of CCl_4 to the highly reactive intermediate $CCl_3^{\cdot}$. Indeed, in vitro experiments with IFN have shown that it suppressed cytochrome P_{450} 2E1[33,34]. An interaction between IFN and CCl_4 metabolism cannot be ruled out.

Although IFN exhibits protective effects against liver damage, it has its own toxic effects. Hepatocyte necrosis has been reported in mice receiving high doses of interferon[35]. IFN induces a decrease in protein synthesis by inhibiting several enzymes[36]. This could lead to hepatocyte injury[37]. In the current study, the serum activities of ALT, GGT, ALP, and PIINP tended to increase after Roferon, and sometime Ismafron, treatment as compared to animals treated with the vehicle.

It has been shown that serum level of PIIINP is a marker of fibrogenesis as well as inflammation in the liver[38]. Because liver fibrosis is characterized by an increase in the hepatic extracellular matrix formed by collagens, the best-known marker of hepatic fibrosis is the terminal propeptide of type III procollagen (PIIINP), a cleavage product of collagen precursor[39]. Therefore, PIIINP was used in the current study as a marker of fibrosis. Ismafron or Roferon treatment had a significant decreasing effect on the serum level of PIIINP. In agreement with these results, IFN has been shown to suppress collagen synthesis and decrease procollagen mRNA levels in vitro and in vivo[40,41].

The reduction in serum markers was strongly correlated with an improvement in the histological profile, suggesting that the effect of IFN was due to, at least in part, anti-inflammatory properties. The present results prove anti-inflammatory effects of both Ismafron and Roferon in cirrhotic rats. These results confirm in vitro data that demonstrated that IFN inhibits proliferation, activation as well as collagen synthesis of human myofibroblast-like cells[42].

Because the severity of hepatic inflammation seems to be a major factor leading to cirrhosis, any reduction in hepatic inflammation may potentially delay fibrosis progression. It has been suggested that IFN may play a role in regulating collagen production, particularly under pathologic conditions characterized by accumulation of inflammatory cells[43]. Abnormalities in collagen regulation may be responsible for fibrosis characteristic of certain diseases, such as liver cirrhosis[44].

It was reported that IFNα treatment preserved hepatocyte and erythrocyte plasma membrane function and composition as well as the normal structure of liver parenchyma in CCl_4-cirrhotic rats. the authors suggested antifibrogenic actions of IFN[45]. The present study showed that Ismafron or Roferon treatment alleviated fibrosis in addition to inflammation.

It is generally accepted that once fibrosis is established, it is irreversible[46]. Only few observations in human[47,48] and studies with animals have led to the hypothesis that hepatic fibrosis is a reversible process, after discontinuation of the causative factors[49]. In human, fibrosis requires years to become established, thus long term treatments with IFN are required to evaluate its antifibrogenic effects in chronic liver diseases. Plasma membrane alterations in hepatocytes

and erythrocytes produced by CCl_4-cirrhosis revert spontaneously after 4 weeks of CCl_4 withdrawal[50].

This discrepancy may arise from the difference in scoring systems used for evaluating fibrosis. It was suggested that the histological improvement observed in a fraction of non-responder patients was not due to a therapeutic effect of IFNα, but rather to either a random sampling fluctuation or change in the natural course of chronic hepatitis[39].

It is concluded that chronic treatment with Ismafron or Roferon has beneficial effects in the treatment of CCl_4-induced liver cirrhosis. Both drugs could normalize the serum markers of liver cirrhosis and improve the histological profile of the liver in CCl_4-treated rats.

REFERENCES

1- Kulkarni, A.P. and Byczkowski, J.Z., Hepatotoxicity in: Introduction to Biochemical Toxicology, 2nd ed., Appleton & Lange (1994).

2- Park, E.J.; Nan, J.X.; Park, E.J.; Kang, H.C.; Park, P.H.; Kim, J.Y.; Ko G. and Sohn, D.H., Effect of Tetrandrine on experimental hepatic fibrosis induced by bile duct ligation and scission in rats, **Pharmacol. Toxicol.**, 87(6), 261-8 (2000).

3- Prost, M.; Hartner, A.; Krause, H.; Hilgers, K.F. and Veelken, R., Inducible nitric oxide synthase and glomerular hemodynamics in rats with liver cirrhosis, **Am. J. Physiol. Renal. Physiol.**, 281(2), 293-9 (2001).

4- de Souza, M.M.; Silva, L.M. and Barbosa, A.A., Jr, de Oliveira I.R, Parana, R. and Andrade, Z.A., Hepatic capillariasis in rats: a new model for testing antifibrotic drugs, **Braz. J. Med. Biol. Res.**, 33(11), 1329-34 (2000).

5- Al-Bader, A.; Mathew, T.C.; Abdul, H.; Al-Sayer, H.; Singal, P.K. and Dashti, H.M., Cholangiocarcinoma and liver cirrhosis in relation to changes due to thioacetamide, **Mol. Cell. Biochem.**, 208(1-2), 1-10 (2000).

6- Sato, M.; Kakubari, M.; Kawamura, M.; Sugimoto, J.; Matsumoto, K. and Ishii, T., The decrease in total collagen fibers in the liver by hepatocyte growth factor after formation of cirrhosis induced by thioacetamide., **Biochem. Pharmacol.**, 59(6), 681-90 (2000).

7- Wardi, J.; Reifen, R.; Aeed, H.; Zadel, L.; Avni, Y. and Bruck, R., Beta-carotene attenuates experimentally induced liver cirrhosis in rats., **Isr. Med. Assoc. J.**, 3(2), 151-4 (2001).

8- Tada, S.; Nakamuta, M.; Enjoji, M.; Sugimoto, R.; Iwamoto, H.; Kato, M.; Nakashima, Y. and Nawata, H., Perfenidone inhibits dimethylnitrosamine-induced hepatic fibrosis in rats., **Clin. Exp. Pharmacol. Physiol.**, 28(7) 522-7 (2001).

9- Jung, S.A.; Chung, Y.H.; Park, N.H.; Lee, SS.; Kim, J.A.; Yang, S.H.; Song, I.H.; Lee, Y.S.; Suh, D.J. and Moon, I.H., Experimental model of hepatic fibrosis following repeated periportal necrosis induced by allylalcohol., **Scand. J. Gastroenterol.**, 35(9), 969-75 (2000).

10- Rivera, C.A.; Bradford, B.U.; Hunt, K.J.; Adachi, Y.; Schrum, L.W.; Koop, D.R; Burchardt, E.R.; Rippe, R.A. and Thurman, R.g., Attenuation of CCl_4-induced hepatic fibrosis by gadolinium chloride ($GdCl_3$) treatment or dietry glycine., **Am. J. Physiol. Gastrointest. Liver. Physiol.**, 281(1), 200-7 (2001).

11- Sakadamis, A.K.; Ballas, K.D.; Tzioufa-Asimakopolou, V. and Alatsakis, M.B., A rat model of liver cirrhosis and esophageal varices., **Res. Exp. Med. (Berl)**, 200(3), 137-54 (2001).

12- Mucke, I.; Richter, S.; Menger, M.D. and Vollmar, B., Significance of hepatic arterial responsiveness for adequate tissue oxygenation upon portal vein occlusion in cirrhotic livers., **Int. J. Colorectal. Dis.**, 15(5-6), 335-41 (2000).

13- Kudriavtseva, M.V.; Bezborodkina, N.N. and Kudryavtsev, B.N., Glycogen-forming function of hepatocytes in cirrhotically altered rat liver after treatment with chorionic gonadotropin., **Exp. Toxicol. Pathol.**, 53(1), 57-63 (2001).

14- Kudriavtseva, M.V.; Bezborodkina, N.N.; Sek, E.N.; Shapiro, I.I.; Baranovskii, A.I. and Kudriavtsev, B.N., Effect of "vilon" on cirrhotically changed rat liver. Liver regeneration, and status of glycogen-forming funcyion of hepatocytes, **Tsitologiia**, 42(8), 758-64 (2000).

15- Masson, S.; Scotte, M.; Garnier, S.; Francois, A.; Hiron, M.; Teniere, P.; Fallu, J.; Salier, J.P. and Daveau, M., differential expression of apoptosis-associated genes post-hepatectomy in cirrhotic vs. normal rats, **Apoptosis**, 5(2), 173-9 (2000).

16- Hamasaki, K.; Nakashima, M.; Naito, S.; Akiyama, Y.; Ohtsuru, A.; Hamanaka, Y.; Hsu, C.T.; Ito, M. and Sekine, I., The sympathetic nervous system promotes CCl_4-induced liver cirrhosis in rats by suppressing apoptosis and enhancing the growth kinetics of regenerating hepatocytes., **J. Gastroenterol**, 36(2), 111-20 (2001).

17- Walsh, K. and Alexander, G.J., Update on chronic viral hepatitis, **Postgrad. Med. J.**, 77(910), 498-505 (2001).

18- Collier, J. and Chapman, R., Combination therapy with interferon-alpha and ribavirin for hapatitis C: practical treatment issues, **Biodrugs**, 15(4), 225-38 (2001).

19- Zeuzem, S., What is (cost) effective in patients with chronic hepatitis C virus infection, **Eur. J. Gastroenterol Hepatol**, 13(5), 473-6 (2001).

20- Sobesky, R. and Buffet C., Management of patients infected with hepatitis C virus, **Presse Med.**, 30(14), 667-72 (2001).

21- Omata, M. and Shiratori, Y., Long-term effects of interferon therapy on histology and development of hepatocellular carcinoma in hepatitis C., **J. Gastroenterol Hepatol**, 15 Suppl, 134-40 (2000).

22- Souza, M.M.; Silva, L.M.; Barbosa, A.A., Jr, Oliveira IR, Parana, R. and Andrade, Z.A., Effect of interferon-alpha on experimental septal fibrosis of the liver-study with a new model., **Mem. Inst. Oswaldo. Cruz.**, 96(3), 343-8 (2001).

23- Zhang, L.; Mi, J.; Yu, Y.; Yao, H.; Chen, H.; Li, M. and Cao, X., IFN-gamma gene therapy by intrasplenic hepatocyte transplantation: a novel strategy for reversing hepatic fibrosis in Schistosoma japonicum-infected mice., **Parasite Immunol**, 23(1), 11-7 (2001).

24- Morozov, V.G. and Khavinson, V.K., Natural and synthetic peptides as therapeutics for immune dysfunction. **Int. J. Immunopharmacol.**, 19, 501-5 (1997).

25- Smith, J.R. and Herrera, J.L., Further evolution of hepatitis C treatment: Individual therapy. **Am. J. Gastroenterol**, 95, 2383 (2000).

26- Van Herck, H.; Baumans, V.; Brandt, C.J.; Boere, H.A.; Hesp, A.P.; Van Lith, H.A.; Schurink, M. and Bevnen, A.C., Blood sampling from the retro-orbitalplexus, the saphenous vein and the tail vein in rats: comparative effects on selected behavioral and blood variables. Lab. Anim., 35, pp. 131-9 (2001).

27- White, J.S. and White, D.C., Source book of enzymes. CRC press LLC, p. 216 (1997).

28- White, J.S. and White, D.C., Source book of enzymes. CRC press LLC, pp. 330, 657 (1997).

29- White, J.S. and White, D.C., Source book of enzymes. CRC press LLC, p. 194 (1997).

30- Cinz, P.; Buyan, N.; Gokcora, N.; Elbe, S. and Hasanoglu, E., The changes in serum type I and III procollagen, insulin-like growth factor binding protein-3 levels in children with chronic renal failure. **J. Trop. Pediatr.**, 42, 271-5 (1996).

31- Camps, J.; Castilla, A.; Ruiz, J.; Civeira, M.P. and Prieto, J., Randomized trial of lymphoblastoid α-interferon in chronic hepatitis C. **J. Hepatol.**, 17, 390-6 (1993).

32- Fort, J.; Pilette, C.; Veal, N.; Obert, F.; Gallois, Y.; Douay, O.; Rosenbaum, J. and Cales, P., Effects of long term administration of interferon α in two models of liver fibrosis in rats, **J. Hepatol.**, 29, 263-270 (1998).

33- Sakai, H.; Olamoto, T. and Kikkawa, Y., Suppression of hepatic drug metabolism by the interferon inducer, polyriboinosinic acid:polyribocitidylic acid. **J. Pharmacol. Exp. Ther.**, 363, 381-6 (1992).

34- Abdel-Razzak, Z.; Loyer, P.; Fautel, A.; Gautier, J.C.; Corcos, L. and Turlin, B., Cytokines down regulate expression of major cytochrome P450 enzymes in adult human hepatocytes in primary culture. **Mol. Pharmacol.**, 44, 707-15 (1993).

35- Gresser, I.; Tovey, M.G.; Maury, C. and Chouroulinkov, I., Lethality of interferon preparations for new born mice. **Nature**, 258, 76-8 (1975).

36- Baglioni, C., Interferon induced enzyme activities and their role in antiviral state. **Cell.**, 17, 255-64 (1979).

37- Cervoni, J-P.; Degos, F.; Marcillin, P. and Erlinger, Acute hepatitis induced by α-interferon with viral clearance, in chronic hepatitis C, **J. Hepatol.**, 27, 1113-1116 (1997).

38- Guessot, J.; Poupon, R.E.; Giral, P.; Balkau, B.; Giboudeau, J. and Poupon, R., Relationship between procollagen III aminoterminal propeptide and hyaluronan serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis C. **J. Hepatol.**, 20, 388-393 (1994).

39- Leroy, V.; Traversay, C.D.; Barnoud, R.; Hartmann, J.D.; Baud, M., Ouzan, D. and Zarski, J.P., Changes in histological lesions and serum fibrogenesis markers in chronic hepatitis C patients non-responders to interferon alpha, **J. Hepatol.**, 35, 120-126 (2001).

40- Omata, M., Ito, Y., Yokosuka, O., Imazeki, F., Uchiumi, K. and Takano, S., Histological changes of the liverby treatment of chronic non-A, non-B hepatitis with recombinant leukocyte interferon alpha. **Dig. Dis. Sci.**, 34, 330-7 (1989).

41- Berman, B. and Duncan, M.R., Short term keloid treatment in vivo with human interferon alpha -2b results in a selective and persistent normalization of keloid fibroblast collagen, glycosaminoglycan, and collagenase production in vitro. **J. Am. Acad. Dermatol.**, 21, 694-702 (1989).

42- Mallat, A.; Preaux, A-M., Blazejewski, S.; Rosenbaum, J.; Dhumeax, D. and Mavier, P., Interferon α inhibit proliferation and collagen synthesis of human Ito cells in culture. **Hepatology**, 21, 1003-10 (1995).

43- Muriel, P., Alpha-interferon prevents liver collagen deposition and damage induced by prolonged bile duct obstruction in the rat. **J. Hepatol.**, 24, 614-621 (1996).

44- Aycock, R.S. and Seyer, J.M., Collagens of normal and cirrhotic human liver. **Connect. Tiss. Res.**, 23, 19-31 (1989).

45- Muriel, P.; Bolanos, J.; Barral, J.M. and Torres, G., Effect of alpha-interferon on erythrocyte and hepatocyte plasma membranes derived from cirrhotic rats. **Pharmacology**, 48, 63-8 (1994).

46- Popper, H. and Kent, G., Fibrosis in chronic liver diseases, **Clin. Gastroenterol**, 4, 315 (1975).

47- Williams, R.; Smith, P.; Spicer, E.; Barry, M. and Sherlock, S., venesection therapy in idiopathic haemochromatosis. **Q. J. Med.**, 38, 1-16 (1969).

48- Yeong, M.L., Nicholson, G.I. and Lee, S.P., Regression of biliary cirrhosis following choledochal cyst drainage. **Gasrtoenterology**, 82, 332-5 (1982).

49- Abdel-Aziz, G.; Leveau, G.; Rescan, P.Y.; Clement, B.; Sieel, M.; Deugnier, Y.; Campion, J.P. and Guillouzo, A., Reversibility of hepatic fibrosis in experimentally induced cholestasis in the rat, **Am. J. Pathol.**, 137, 1333-42 (1990).

50- Muriel, P.; Favari, L. and Soto, C., Erythrocyte alterations correlate with CCl_4 and biliary obstruction-induced liver damage in the rat, **Life Sci.**, 52, 647-55 (1993).

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تأثيرات الأنترفيرون الطبيعى والمصنع على التليف الكبدى المصاحب للتعرض لرابع كلوريد الكربون

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يستخدم رابع كلوريد الكربون "المعروف بسميته للكبد" فى عمل نموذج حيوانى لتليف الكبد ، وقد استخدم هذا النموذج الحيوانى فى دراسة تأثير العلاج المزمن لكل من الأنترفيرون الطبيعى "اسما فيرون" والصناعى "روفيرون" على الجرذان. استخدم فى هذه الدراسة سبعون جرذاً قسموا إلى ثلاث مجموعات رئيسية ، المجموعة الأولى غير معالجة ، والمجموعة الثانية هى حيوانات طبيعية معالجة باسما فيرون أو روفيرون ، أو سائل البرافين "مذيب رابع كلوريد الكربون" ، المجموعة الثالثة هى حيوانات مصابة بتليف الكبد ، قسمت هذه المجموعة الأخيرة إلى ثلاث مجموعات : حيوانات معالجة باسما فيرون وأخرى بروفيرون وحيوانات غير معالجة (مجموعة ضابطة). ظهر تليف الكبد فى حيوانات التجارب بعد عشرة أسابيع من التعرض لرابع كلوريد الكربون بجرعة قدرها ٠,٣ملل / ١٠٠جم من وزن الجسم مرتين أسبوعياً.

لوحظ نقص فى نشاط الأنزيمات الكبدية ALT ، GGT ، ALP بعد أسبوعين من العلاج باسما فيرون ١٠٠٠٠ وحدة / حيوان/ يوم أو روفيرون ٥٠٠٠٠٠ وحدة / حيوان / يوم ، مع تراجع مستوى البروكولاجين-٣ ، هذا بالإضافة إلى أن العلاج باسمافيرون وروفيرون قد أثمر عن تراجع مستوى التليف والالتهاب الكبدى. تخلص الدراسة إلى أن العلاج باسمافيرون أو روفيرون أثمر عنه تعديل مستوى الأنزيمات الكبدية وتحسين صورة الكبد فى الحيوانات المعرضة لرابع كلوريد الكربون.