The Effect Of Interferon Alone Or Combined With Silymarin On Liver And Bone Parameters In Bile Duct Ligated Rats

O Abdel Salam, S Nofal, S El-Shenawy, N Shaffie

Citation

Abstract
Thirty-six rats with biliary obstruction induced by double ligation and section of the common bile duct were randomly and blindly assigned to receive subcutaneous injection of interferon alpha (INF-alpha at 6750 or 13500 IU/kg) three times weekly alone, a combination of INF-alpha and silymarin (25 mg/kg once a day orally) or saline, starting one day after surgery and continued for one month. At the end of the treatment period, rats were killed and analyzed for blood biochemistry, liver and bone histopathology. The administration of INF-alpha at 6750 IU/kg increased plasma aspartate aminotransferase by 60.6%. Serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities were markedly raised after INF-alpha 13500 U/kg by 74.6%, 89% and 109%, respectively. Such elevations in liver enzymes were not observed in rats treated with a combination of INF-alpha and silymarin. Serum bilirubin decreased by 30.8 and 36.1% after treatment with INF-alpha at 6750 and 13500 IU/kg and by 23.7 and 20.8% after treatment with INF-alpha at 6750 or 13500 IU/kg combined with silymarin, respectively. Calcium levels in plasma were not significantly altered by INF-alpha alone or combined with silymarin. On histology, INF-alpha at 6750 IU/kg failed to prevent fibrosis in liver of BDL rats, although many of the hepatocytes appeared normal, while the higher dose of resulted in some improvement in the degree of fibrosis, oedema and lymphocytic infiltration. The addition of silymarin to interferon did not result in further histological improvement. In contrast to observations in the liver, thickness of bone tissue at the diaphysis of tibia was reduced in BDL rats, but restored to normal values by treatment with INF-alpha alone or by the combination of INF-alpha and silymarin. The high dose of interferon either alone or accompanied with silymarin made much improvement in the bone changes that resulted from bile duct legation These results suggest that INF-alpha alone or co-administered with silymarin is of limited value in this model of cholestatic liver injury, but appear to prevent bone alterations in obstructive jaundice INF-alpha is likely to exert antifibrotic effects distinct from its antiviral properties. The study also indicates that bile duct ligation is a reliable and efficient model for producing osteoporosis in rats for the assessment of different drugs and pathophysiologic mechanisms involved.

INTRODUCTION
Interferon alpha (INF-alpha) alone or in combination with ribavirin is the standard treatment for chronic hepatitis C virus infection. The aim is to eradicate the virus, suppress the continuing necro-inflammatory process and consequently preventing the development of cirrhosis. The latter is the end result of fibrosis characterized by the excessive production of extracellular matrix proteins in the liver, including type I collagen. In human liver, fibrogenesis underlies development of hepatocellular carcinoma (HCC) in 90% of cases, and HCC is an ominous complication of cirrhosis in 30% of the patients. Despite the fact that therapy with interferon and ribavirin can eliminate the virus and prevent the progression of the disease, frequent side effects and relapses preclude the use of this form of therapy in a substantial proportion of patients. Such combination of interferon and ribavirin is associated with fatigue, influenza-like symptoms (headaches, fever, myalgia), hematologic abnormalities, and neuropsychiatric symptoms. From 10 to 14% of participants in the registration trials for combination therapy involving the peginterferons withdrew prematurely from therapy due to adverse events. This is in addition to the fact that sustained response is obtained in approximately 55% of patients with the combination of PEG-IFN and ribavirin. Whether INF-alpha possesses antifibrotic or hepatoprotective properties distinct from its antiviral effect are not clear. There is evidence to suggest that interferon-alpha might possess antifibrogenic properties. The present study was thus designed to test whether lower doses of INF-alpha would exert beneficial effects upon hepatocellular injury and fibrosis in rats subjected to bile duct ligation. This model has resemblance to the situation of chronic obstructive jaundice occurring in man as a result of primary
biliary cirrhosis, stricture of bile duct etc.. We also aimed to examine the effect of INF-alpha on bone changes in this model of obstructive jaundice. In addition, the possible modulation of the action of INF-alpha by silymarin, a standardized extract, derived from the milk thistle plant and is used as a hepatoprotective agent worldwide, was examined.

MATERIALS AND METHODS

DRUGS

The following drugs were used: INF-alpha (Egyifern), silymarin (SEDICO, Cairo).

ANIMALS

Sprague–Dawley rats of either sex, weighing 130–150 g of body weight were used throughout the experiments. They were housed under standard laboratory conditions with free access to standard laboratory chow and water. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals.

SURGERY

Under light ether anaesthesia, midline laparotomy was performed, the common bile duct isolated and ligated with 4-0 silk suture in two places just above the duodenum anterior to the pancreas and posterior to the hilum of the liver. The bile duct was then cut between the two ligatures and the abdominal wall was closed in two layers by continuous suturing. Animals were given topical betadine as an antiseptic and 0.2 ml gentamicin intramuscularly to control post-surgical infection. The rats were then allowed to recover with free access to food and water after the surgery.

DESIGN OF EXPERIMENTS

Rats with ligation-section of the common bile duct were randomly and blindly assigned to administration of subcutaneous interferon alpha (INF-alpha) 6750 or 13500 IU/kg three times weekly, daily oral silymarin 25 mg/kg, a combination of INF-alpha and silymarin or saline, starting 1 day after surgery and continued for 4 weeks (n = 7 per group). In sham-ligated animals (n = 7), the duct was located, manipulated, and replaced. At the end of the treatment period, rats were killed and analyzed for blood biochemistry and liver pathology. The doses of INF-alpha employed were based upon the human dose used in therapy of hepatitis C virus after conversion to that of rat according to Paget and Barnes.

BIOCHEMICAL ASSESSMENT

At the end of the experiments, blood samples were obtained from the retro-orbital vein plexuses, under ether anaesthesia. ALT and AST activities in serum were measured according to Reitman-Frankel colorimetric transaminase procedure, whereas colorimetric determination of ALP activity was done according to the method of Belfield and Goldberg, using commercially available kits (BioMérieux, France).

HISTOLOGICAL STUDIES

After the end of the treatment period, rats were killed; livers and right tibia were excised and fixed in 10% formalin saline. Sections were prepared and stained with hematoxylin and eosin (H & E) for the histological investigations.

STATISTICAL ANALYSIS

All results are expressed as means ± SE. Multiple group comparisons were performed by ANOVA followed by Duncan test. P< 0.05 was considered statistically significant.

RESULTS

BIOCHEMICAL CHANGES

EFFECT OF INF-ALPHA ALONE OR WITH SYLIMARYN ON SERUM TRANSAMINASES, ALKALINE PHOSPHATASE AND BILIRUIN

Results are presented in table 1. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin levels were significantly higher in bile duct ligated rats compared with their Sham-treated counterparts. Serum ALT increased by 98% (33.0 ± 2.1 vs 65.3 ± 5.7 U/l), serum AST by 343.3% (81.6 ± 7.3 vs 361.7 ± 29.4 U/l), serum ALP by 234% (236 ± 20.1 vs 788 ± 81.36 U/l), respectively. Serum total bilirubin increased markedly in BDL compared to Sham operated rats (10.3 ± 1.19 mg/dl vs 0.2 ± 0.1 mg/dl, p < 0.05, n = 6 per group).

The administration of INF-alpha at 6750 or 13500 U/kg to bile duct ligated rats resulted in further increase in ALT by 26.6% & 89.1%, respectively (65.3 ± 5.7 to 82.7 ± 7.2 and 123.5 ± 21.3 U/l). These elevations in ALT were restored to their BDL control values by the addition of silymarin to either dose of INF-alpha. Similar increments in AST by 60.5 & 109.5% (361.7 ± 29.4 to 580.7 ± 53.6 and 757.8 ± 75.4 U/l) were also noted upon administration of INF-alpha, and these were restored to BDL control values by silymarin. Meanwhile, ALP levels were increased by 30.8 & 85.3% (788 ± 81.3 vs 1030.6 ± 86.9 and 1460.5 ± 153 U/l) after
INF-alpha at 6750 or 13500 U/kg. This increase in ALP was reduced by 18.8 & 31.7% by silymarin. In contrast, serum bilirubin decreased by 30.8 and 36.1% after treatment with INF-alpha at 6750 and 13500 IU/kg and by 23.7 and 20.8% after treatment with INF-alpha at 6750 or 13500 IU/kg combined with silymarin, respectively.

**Figure 1**

Table 1: Serum total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in bile duct-ligated (BDL) rats treated with INF-alpha (INF-α) alone or combined with silymarin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham</th>
<th>BDL</th>
<th>BDL + INF-α</th>
<th>BDL + INF-α</th>
<th>BDL + INF-α</th>
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<tr>
<td>(normal)</td>
<td>INF-α</td>
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<td>INF-α</td>
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<tr>
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<tr>
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<td>U/kg</td>
<td>+</td>
<td>U/kg</td>
<td>+</td>
<td>U/kg</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.2 ± 0.1</td>
<td>10.3 ± 7.13</td>
<td>6.6 ± 1.3**</td>
<td>7.9 ± 1.5**</td>
<td>7.2 ± 1.2*</td>
<td>(mg/dl) 1.19</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>33.0 ± 2.1</td>
<td>65.3 ± 23.7</td>
<td>123.5 ± 73.3 ± 7.2 ± 6.8*</td>
<td>67.2 ± 7.2**</td>
<td>21.3**</td>
<td>7.1**</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>81.6 ± 7.3</td>
<td>361.7 ± 50.8 ± 775.7 ± 442.6 ± 595.9 ± 23.9 ± 5.6**</td>
<td>75.4 ± 31.7**</td>
<td>23.2**</td>
<td></td>
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<tr>
<td>ALP (U/l)</td>
<td>236 ± 7.8</td>
<td>780 ± 100.6 ± 1460.5 ± 836.7 ± 598.9 ± 99.8 ± 8.9**</td>
<td>20.3 ± 8.3*</td>
<td>86.9**</td>
<td>157**</td>
<td>48.9**</td>
</tr>
</tbody>
</table>

*: p<0.05 vs Sham group; **: p<0.05 vs BDL control group; ***: p<0.05 vs INF-α treated group.

**HISTOLOGICAL RESULTS**

**EFFECT OF INF-ALPHA ALONE OR WITH SILYMARIN ON LIVER INJURY**

Examination of liver tissue sections from rats subjected to bile duct legation only showed severe dilatation and congestion in portal vein accompanied with marked thickening in the wall of bile ducts. Around the portal area many of the hepatocytes suffered from necrosis. Severe lymphocytic infiltration was observed within the parenchyma of the liver (Fig. 1A). In liver sections of rats treated with INF-alpha 6750 IU/kg, slight dilatation with congestion of portal and central veins was seen. Marked fibrosis extending from the portal areas and surrounding the hepatic lobule was observed. Many of the hepatocytes appeared normal, while a few hepatocytes showed pyknotic nuclei (Fig. 1B). Liver sections from BDL treated with INF-alpha 13500 IU/kg displayed normal-sized blood vessels in portal areas and central veins. No lymphocytic infiltration was seen and most of the hepatocytes appeared normal, only a few of them showed dense small nuclei (Fig. 1C). The addition of silymarin to interferon did not result in further histological improvement. Fig. 1D represents a photomicrograph of a section of liver tissue from a BDL rat treated with INF-alpha 6750 and silymarin showing disturbance of the normal architecture of liver tissue with slight dilatation of central vein. The hepatic lobule is completely surrounded with fibrosis, necrotic cells and areas of hemorrhage in between. Silymarin co-administered with the higher dose of INF-alpha (13500 IU/kg) failed to alter the histological changes in BDL rats. Complete distortion of the normal architecture of liver tissue was observed, while most of the hepatocytes suffered from fatty degeneration.
showing small dense nuclei. The portal areas showed fibrosis, congestion of portal vein, thickening of the wall of bile ducts and necrosis in the hepatocytes around (Figs. 1E & F).

Figure 1: (A) A photomicrograph of a liver tissue section from a rat subjected to bile duct legation only showing severe dilatation and congestion in portal vein (arrow) accompanied with marked thickening in the bile duct's wall resulting from the transformation of the lining epithelium from low cuboidal cells to tall columnar epithelial cells (B). Around the portal area many of the hepatocytes suffer from necrosis (N) as they appear swollen, losing their characteristic color of cytoplasm with karyolysis. Severe lymphocytic infiltration (L) is observed within the parenchyma of the liver, while very small areas of liver tissue appear normal in shape and color (Hx. & E. X 100). (B): A photomicrograph of a section of liver tissue from a rat subjected to bile duct legation and treated with interferon at 6750 IU/kg, showing slight dilatation with congestion of blood vessels (portal vein and central vein). Marked fibrosis extending from the portal areas and surrounding the hepatic lobule completely with spots of hemorrhage within (arrow). Around the fibrous tissue many of the hepatocytes show necrosis (N). The center of the hepatic lobule shows dilatation of blood sinusoids denoting edema (E). Many of the hepatocytes appear normal, while a few show pyknotic nuclei (Hx. & E. X 100). (C): A photomicrograph of a section of liver tissue from a rat subjected to bile duct legation and treated with interferon at 13500 IU/kg, showing normal-sized blood vessels (neither dilatation nor congestion) in portal areas and central veins. Dilatation of blood sinusoids and fibrosis at the periphery of the hepatic lobule are less marked in comparison with the previous section. Among the necrotic cells begin to appear some structures that take the shape of small follicles or ducts (arrow). No lymphocytic infiltration is seen and most of the hepatocytes appear normal, only a few show dense small nuclei (Hx. & E. X 100). (D): A photomicrograph of a section of liver tissue from a rat subjected to bile duct legation and treated with interferon at 6750 IU/kg combined with silymarin, showing disturbance of the normal architecture of liver tissue with slight dilatation of central vein. The hepatic lobule is completely surrounded with fibrosis, necrotic cells and areas of hemorrhage in between (arrow). The portal areas show neither dilatation of their vessels nor lymphocytic infiltration. Signs of oedema appear as dilated blood sinusoids mainly at the center of the hepatic lobule (Hx. & E. X 100). (E & F): A photomicrograph of a section of liver tissue from a rat subjected to bile duct legation and treated with interferon at 13500 IU/kg combined with silymarin, showing complete distortion of the normal architecture of liver tissue. Most of the hepatocytes suffer from fatty degeneration showing small dense nuclei. The portal areas show fibrosis, congestion of portal vein, thickening of the bile duct's wall (arrow) and necrosis in the hepatocytes around (Hx. & E. X 100 & X 200).
Figure 3

EFFECT OF INF-ALPHA ALONE OR WITH SILYMARIN ON BONE CHANGES

Figs. 2A-D show a photomicrograph of a section of bone tissue of a normal rat. Examination of sections of bone tissue taken from BDL rats showed marked thickening of periosteum, accompanied by an increase in number and size of the vascular canals. The inner surface of the diaphysis showed irregularities at many places with invasion of bone matrix at some of them. In sections of bone tissue taken from BDL rats treated with INF-alpha at 6750 U/kg, the vascular canals retained their normal rounded shape, although their number was still more than normal. The collagenous fibers that form the organic part of the matrix showed irregularities in arrangement in many places. Gaps within the matrix were still present (Figs. 3A, B). In rats treated with INF-alpha at 13500 U/kg, both of the outer and inner surfaces of the diaphysis become smooth with very slight irregularities. The thickness of the periosteum was markedly decreased. The number of osteocytes was increased and the vascular canals appeared normal in shape and number, although the gaps in matrix were still present (Figs. 3C, D). In BDL rats treated with INF-alpha at 6750 U/kg and silymarin, marked thickening of the periosteum, with many irregularities in the outer surface of the diaphysis was seen (Fig. 4A, B), while in sections of bone tissue from BD rats treated with INF-alpha at 13500 U/kg and silymarin, marked decrease in periosteal thickening was evident and vascular canals retained their normal shape and size. Many areas of calcified cartilage appeared in the middle of the shaft denoting regeneration of bone tissue (Fig. 4. C, D).

Figure 2: (A): A photomicrograph of a section of bone tissue of a normal control rat showing, the shaft (diaphysis) of a long bone (femur) that is covered from the outside by a layer of connective tissue (periosteum) (P). Another similar layer (endosteum) lines the inner surface, facing the bone marrow cavity (E). Both surfaces are smooth with no irregularities. Bone tissue is pervaded by vascular canals and spaces that become embedded in the bony matrix (arrow) (Hx. & E. X 100). (B): A magnified photomicrograph of the previous section showing, the bone cells, osteocytes, that are flattened oval bodies lie in slit-like lacunae embedded in the matrix. The have a faintly basophil cytoplasm and flattened dark nucleus. The young osteocytes are found to be in more or less rounded lacunae (arrow), while the old ones are in oval or lenticular lacunae (O). The bone matrix appears acidophilic in color as it is composed mainly of collagenous fibers (Hx. &E. X 200). (C): A photomicrograph of a section of bone tissue taken from a rat subjected to bile duct ligation showing, marked thickening of periosteum (arrow), accompanied with increased number and size of the vascular canals (V). The inner surface of the diaphysis show irregularities at many places with invasion of bone matrix at some of them (double arrow)(X. & E X 100). (D): A magnified photomicrograph of the previous section showing many vascular canals, some of them are markedly widened.
(arrow). Gaps within the matrix are also appeared (double arrow). The osteocytes are still normal in appearance, although most of them tend to be young of small size (O) (Hx. & E X 200).

**Figure 4**

Figure 5

Figure 4 (A): A photomicrograph of a section of bone tissue taken from a rat subjected to bile duct ligation and treated with interferon at 6750 IU/kg showing, the vascular canals are no more widened, they retain their normal rounded shape, although their number is still more than normal (arrow). The collagenous fibers that form the organic part of the matrix show irregularity in arrangement in many places (double arrow). Gaps within the matrix are still present (g) (Hx. & E X 100). (B): A magnified photomicrograph of the previous section showing, many vascular canals (V) around them osteocytes in lacunae are distributed. Most of the osteocytes are of the young type with rounded shape and small size (arrow) (Hx. & E X 200). (C): A photomicrograph of a section of bone tissue taken from a rat subjected to bile duct ligation and treated with interferon at 13500 IU/kg showing, both of the outer and inner surfaces of the diaphysis become smooth with very slight irregularities. The thickness of the periosteum is markedly decreased (double arrow). The number of osteocytes is increased and the vascular canals appear normal in shape and number, although the gaps in matrix are still present (arrow) (Hx. & E X 100). (D): A magnified photomicrograph of the previous section showing, areas of calcified cartilage appear in the middle of the shaft with its basophilic color denoting regeneration of bone tissue (arrow). Around these areas a big number of osteocytes is present, most of them are of the young type (Y), while farther osteocytes are of the old type (O) (Hx. & E X 200).

**Figure 5**

Figure 3 (A): A photomicrograph of a section of bone tissue taken from a rat subjected to bile duct ligation and treated with interferon at 6750 IU/kg showing, the vascular canals are no more widened, they retain their normal rounded shape, although their number is still more than normal (arrow). The collagenous fibers that form the organic part of the matrix show irregularity in arrangement in many places (double arrow). Gaps within the matrix are still present (g) (Hx. & E X 100). (B): A magnified photomicrograph of the previous section showing, many vascular canals (V) around them osteocytes in lacunae are distributed. Most of the osteocytes are of the young type with rounded shape and small size (arrow) (Hx. & E X 200). (C): A photomicrograph of a section of bone tissue taken from a rat subjected to bile duct ligation and treated with interferon at 13500 IU/kg showing, both of the outer and inner surfaces of the diaphysis become smooth with very slight irregularities. The thickness of the periosteum is markedly decreased (double arrow). The number of osteocytes is increased and the vascular canals appear normal in shape and number, although the gaps in matrix are still present (arrow) (Hx. & E X 100). (D): A magnified photomicrograph of the previous section showing, areas of calcified cartilage appear in the middle of the shaft with its basophilic color denoting regeneration of bone tissue (arrow). Around these areas a big number of osteocytes is present, most of them are of the young type (Y), while farther osteocytes are of the old type (O) (Hx. & E X 200).
The Effect Of Interferon Alone Or Combined With Silymarin On Liver And Bone Parameters In Bile Duct Ligated Rats

magnified photomicrograph for the previous section showing, many widened vascular canals lined with a continuous layer of cells that might be osteoblasts (double arrow). Around these canals, some osteocytes of the young type appear (arrow) (Hx. & E X 200). (C): A photomicrograph of a section of bone tissue taken from a rat subjected to bile duct ligation and treated with interferon at 13500 IU/kg and silymarin showing, marked decrease in periosteal thickening (arrow). Vascular canals retain their normal shape and size (V). Many areas of calcified cartilage appear in the middle of the shaft denoting regeneration of bone tissue (double arrow). (Hx. & E X 100). (D): A magnified photomicrograph of the previous section showing, many areas of calcified cartilage that appear basophilic in color (double arrow). Among them a lot of osteocytes of the young type are seen (arrow). Vascular canals appear normal although some of them are still widened. (Hx. & E X 200).

Figure 6

DISCUSSION

Liver fibrosis or the excessive accumulation of extracellular matrix proteins including collagen occurs in most types of chronic liver diseases. Liver fibrosis results from chronic damage to the liver and is considered a wound-healing response to chronic liver injury. When advanced fibrosis results in cirrhosis, liver failure, and portal hypertension ensues and ultimately there is a need for liver transplantation. In the rat, bile duct obstruction results in hepatic fibrosis and is a widely used model to study the pathogenetic mechanisms of fibrosis and the effect of different drugs on it. This model closely mimics chronic obstructive jaundice occurring in man e.g., as a result of primary biliary cirrhosis or stricture of bile duct. In the present study, the effect of INF-alpha was studied on liver injury and fibrosis in rats submitted to bile duct ligation for one month. Findings in the present study indicate that the administration of IFN-alpha at doses comparable to those used in therapy of chronic hepatitis C in man have minimal beneficial effect on the degree of histological damage or experimental fibrosis induced by biliary obstruction. Furthermore, plasma indices of liver injury and cholestasis i.e., ALT, AST and ALP were markedly raised after IFN-alpha.

Currently, interferon-based therapies are the only effective treatment for chronic hepatitis C virus infection. IFN-alpha monotherapy or more commonly IFN-alpha combined with ribavirin is the treatment standard for chronic hepatitis C virus infection, and has been shown to reduce serum ALT levels and to eliminate serum virus RNA in about 40% of patients. Studies have suggested antifibrotic effects of IFN-alpha distinct from its antiviral properties. Improved or stable fibrosis scores were demonstrated in 66.7% of patients with recurrent hepatitis C virus who did not respond to treatment with pegylated interferon and ribavirin in terms of virologic response. In hepatitis C virus-infected liver graft recipients after treatment with ribavirin plus INF-alpha, the sustained virologic response was associated with a deceleration of fibrosis progression. In post-transplant recurrent HCV patients retreated with pegylated interferon alfa-2b with ribavirin for 48 weeks, fibrosis score was improved in 65% of treated patients despite failure of HCV eradication. In vitro, hepatocytes stimulated by inflammatory cytokines appear to participate in the activation of HSCs via matrix metalloprotease-9 (MMP-9) production and such activation can be modulated by interferon.

In experimental animals, several authors observed a
beneficial effect for IFN-alpha upon liver fibrosis caused by the hepatotoxin CCl<sub>4</sub>. Thus, Inagaki et al. reported that IFN-alpha administered into transgenic mice harboring the alpha2 (I) collagen gene promoter sequence, significantly repressed promoter activation and prevented the progression of hepatic fibrosis induced by carbon tetrachloride (CCl<sub>4</sub>) injection. In rats treated for seven weeks with CCl<sub>4</sub> and INF-alpha, renal interstitial fibrosis was prevented, though the interstitial inflammation score was higher in the CCl<sub>4</sub> + INF group than the control group. In rats treated with CCl<sub>4</sub> for the 6 weeks, high doses of IFN-alpha reduced ALT and AST and decreased number of Ito cells in Disse's space. These effects of INF-alpha were not observed if given 3 weeks after CCl<sub>4</sub> administration. In rats treated with CCl<sub>4</sub>, INF-alpha 150,000 U/day reduced expression of hepatic stellate cell and transforming growth factor-b1 and a-smooth muscle actin<sub>a</sub>. The administration of PEG-IFN 1.5 microg/kg/week after cessation of 12 weeks treatment with CCl<sub>4</sub> was associated with improved CCl<sub>4</sub> induced rat liver fibrosis. In rats given CCl<sub>4</sub> for 8 weeks, co treatment with interferon-alpha and –gamma was reported to suppress collagen and transforming growth factor-betal and has an overall anti-fibrotic effect without exacerbating inflammation. In rats infected with the helminth Capillaria hepatica regularly develop diffuse septal fibrosis of the liver, which terminated in cirrhosis 40 days after inoculation, treatment with interferon-alpha (500,000 and 800,000 IU) for 60 days inhibited the development of fibrosis in this model. Dimethylnitrosamine-induced liver fibrosis was reduced in rats treated with rIFN-alpha decreased FN deposition, but not histology and oxidative parameters.

In rats with biliary fibrosis, Fort et al. observed no significant effects for INF-alpha (100,000 UI/day), despite the finding that it significantly decreased fibrosis in the CCl<sub>4</sub> model only. In contrast, in bile duct ligated rats, Bueno et al. reported a decrease in liver enzymes, fibrosis and decreased bile duct proliferation by IFNAlpha-2a (100,000 IU/rat, daily, s.c.). Muriel, using bile duct ligated rat model, reported that interferon-alpha 2b (IFN-alpha; 100,000 IU/rat) when administered subcutaneously, daily after surgery for one month resulted in significant preservation of ultrastructure, histology, inhibition of collagen accumulation and in a partial improvement of serum markers of cholestasis. In another study, administration of IFN alpha2b (50000 IU s.c.) 15 days after biliary obstruction and for a further 15 days, ameliorated all markers of liver damage studied (liver glycogen and collagen, bilirubin and enzyme activities in serum).

Although in studies where INF-alpha was reported to result in reduction of experimental fibrosis, the doses employed were definitely higher than that used in the present study, this do not explain the lack of an antifibrotic effect for INF for even INF- administration was associated with biochemical and morphological findings indicative of increased hepatic damage. It is more likely that in cholestatic liver injury caused by bile duct ligation INF-alpha exacerbates such injury. During cholestatic liver injury, accumulation of bile acids in the liver is thought to play a role in causing hepatocyte damage. Toxic hydrophobic bile acids disrupt cell membranes resulting in the release of intracellular constituents, directly activate the Fas death receptor<sub>35,36</sub> and results in apoptotic cell death. Furthermore, glycochenodeoxycholic acid induces a decrease in the mitochondrial membrane potential and cytochrome c release from mitochondria<sub>37,38</sub>. The production of IL-12 and tumor necrosis factor-alpha increased 3 days after bile duct ligation<sub>39</sub>. Other studies suggest that oxidative stress is involved in mitochondrial dysfunction and biochemical and molecular changes induced in hepatocytes by bile acids<sub>40</sub>. Preventing or minimizing the deleterious effects of bile acids is thus a potential therapeutic target for patients with obstructive jaundice.

Also, in the present study, the INF-alpha -induced increases in ALT, AST and ALP were restored to their BDL control values by the addition of silymarin to either dose of INF-alpha, which might suggest a beneficial effect of silymarin. The hepatoprotective effect of silymarin has been ascribed to membrane-stabilizing action, free radicals scavenging properties and inhibition of lipid peroxidation<sub>41,42</sub>. This improvement of serum enzymes, however, was not associated with an improvement reflected on the degree of histological hepatic injury or fibrosis observed on examination of liver sections. It might be that silymarin was not able to counteract the deleterious effect of INF-alpha on the liver in this model of hepatic injury. In accordance with our observations are those of Fort et al. who found no significant effects for Interferon alpha (100,000 UI/day) on fibrosis or hydroxyproline content of liver in rats with biliary fibrosis. Similarly, Tarcin et al. observed no beneficial effect of IFN-alpha (100 000 IU/3 days a week) on experimental fibrosis induced by biliary obstruction.

In the present study, examination of sections of bone tissue taken from BDL rats showed marked thickening of
periosteum, increased number and size of the vascular canals with irregularities of the inner surface of the diaphysis. Morphometry indicated reduced thickness of bone tissue at the diaphysis of tibia in BDL rats reduced bone density is not uncommon in prolonged cholestasis. Osteoporosis occurs in up to one third of patients with primary biliary cirrhosis, a disease characterized by intrahepatic cholestasis due to immune-mediated destruction of the intrahepatic bile ducts. In the present study treatment with INF-alpha was associated with histological improvement of bone alterations and restoration of the thickness of bone tissue at the diaphysis of tibia to normal values. In their study, Avnet et al., indicated that INF-alpha displayed ability in vitro to reduce bone resorption and to impair tumor-associated angiogenesis. The expression of bone tartrate resistant acid phosphatase type 5b as well as calcium-phosphate resorption activity and expression of pro-osteoclastic transcription factor c-Fos were reduced by INF-alpha in culture. Indeed INF-alpha based therapies have been considered for the treatment of patients affected by osteolytic metastases. In patients with severe mastocytosis leads to osteoporosis and numerous compressed vertebrae, treatment with interferon alpha (IFN) 3 million units (MU) three times a week and pamidronate led to a major increase in bone marrow density.

In summary, the present study in bile duct-ligated rats suggest that INF-alpha alone or co-administered with silymarin is of limited value, but appear to prevent bone alterations in this model of cholestatic liver injury. The study also indicates that bile duct ligation is a reliable and efficient model for producing osteoporosis in rats for the assessment of different drugs and pathophysiologic mechanisms involved.

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The Effect Of Interferon Alone Or Combined With Silymarin On Liver And Bone Parameters In Bile Duct Ligated Rats

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