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• BRIEF REPORTS •

Effects of heparin on liver fibrosis in patients with chronic hepatitis B

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Abstract

AIM: To evaluate the effects of heparin on liver fibrosis in patients with chronic hepatitis B.

METHODS: Fifty-two cases under study were divided into two groups, group A and group B. The two groups were given regular treatment and heparin/low molecular weight heparin (LMWH) treatment respectively. Hepatic functions, serum hyaluronic acid (HA) and type IV collagen levels were measured before and after the treatment, and six cases were taken liver biopsy twice.

RESULTS: After treatment, hepatic functions became significantly better in both groups. Serum HA and type IV collagen levels in group B compared with group A, decreased significantly after treatment. Collagen proliferation also decreased in group B after treatment.

CONCLUSION: Heparin/LMWH can inhibit collagen proliferation in liver tissues with hepatitis B.

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INTRODUCTION

The treatment of liver cirrhosis is always a problem in the clinical practice. To control and stop liver fibrosis towards liver cirrhosis is of utmost importance. A recent trial indicated that heparin could inhibit the growth of Ito cells effectively *in vitro*[1], which suggested that heparin might act as an antifibrosis drug. In this study, we aimed to seek a safe and effective antifibrosis drug in 52 patients with chronic hepatitis B.

MATERIALS AND METHODS

Materials

Fifty-two patients were treated in Shandong Provincial Hospital from 1999 to 2002. There were 39 males and 13 females, age ranged from 14 to 70 years, diagnosis was made by clinical manifestations and serum hepatitis B viral markers.

Experimental design

These 52 cases were divided into two groups randomly. The treatment regime of each group is listed in Table 1.

Table 1 Treatment regimes in group A and B

Group	*n*	Treatment regime
A	18	regular treatment(GIK,diamnonium glycyrrhizinate injection,potassium magnesium aspartate,et al)
B	34	regular treatment and heparin(25mg,iv,bid) or low molecular weight heparin(6400IU,iH,qd)

Note: In group B, 18 cases were treated with heparin and 16 with low molecular weight heparin (LMWH). The LMWH was FLUX manufactured by ALFA WASSERMANN S.P.A (Italy).

All cases were treated for a course of 3 weeks. Serum alanine transaminase (ALT), prothrombin time (PT), total bilirubin (TBIL), hyaluronic acid (HA) and type IV collagen (IV-C) were measured before and after treatment. The liver tissue specimens were obtained by percutaneous needle biopsy. Ten cases in group A and sixteen in group B had liver biopsies before treatment. Six cases in group B had a second biopsy at 30-60 days after treatment.

Determination of serum HA and IV-C level

Serum HA and IV-C level was determined with radioimmunoassay. The procedures were strictly in accordance with the instructions.

Light microscopic examination

Part of the liver tissues were fixed in 10 % formalin, embedded in paraffin, and then cut into slices. The sections were stained with hematoxylin and eosin for histological study and Masson trichrome for collagen stained green.

Electron microscopic examination

Small liver blocks were fixed in 2.5 % glutaraldehyde, postfixed in 1 % OsO_4, dehydrated with ethanol, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with H-800 transmitted electron microscope (Tokyo, Japan).

RESULTS

Changes of serum/plasma indexes before and after treatment

As shown in Table 2, the levels of ALT and TBIL decreased significantly after treatment, while the level of PT changed slightly only. The level of HA and IV-C in group B decreased markedly, while those in group A were elevated.

Table 2 Changes of the serum / plasma indexes before and after treatment in group A and B ($\bar{x}\pm s$)

	A		B	
	Before	After	Before	After
ALT	136.45±103.46	69.88±43.58[a]	185.58±138.54	84.93±57.14[a]
PT	17.84±3.22	15.98±2.67	18.45±4.25	18.62±3.67
TBIL	64.65±21.35	38.42±14.38[a]	69.54±26.53	31.25±17.84[a]
HA	254.43±116.37	309.48±214.03	579.59±191.45	286.45±136.54[a]
IV-C	237.5±104.44	259.3±137.65	349.56±112.43	189.8±79.63[a]

[a]$P<0.05$, *vs* before treatment.

Histologic changes before and after treatment with heparin/ LMWH

Hematoxylin and eosin staining Hepatocytes swelled and appeared balloon-like before treatment. Inflammatory cells penetrated into the interstitium. Red blood cells congregated in the sinusoids. After treatment with heparin/LMWH, the swollen hepatocytes alleviated , and the sinusoids became clearly seen (Figure 1,2).

Masson trichrome staining Collagens could be seen evidently before treatment. Some sinusoids had been compressed by collagens. After treatment with heparin/LMWH, the collagen fibers decreased significantly (Figure 3,4).

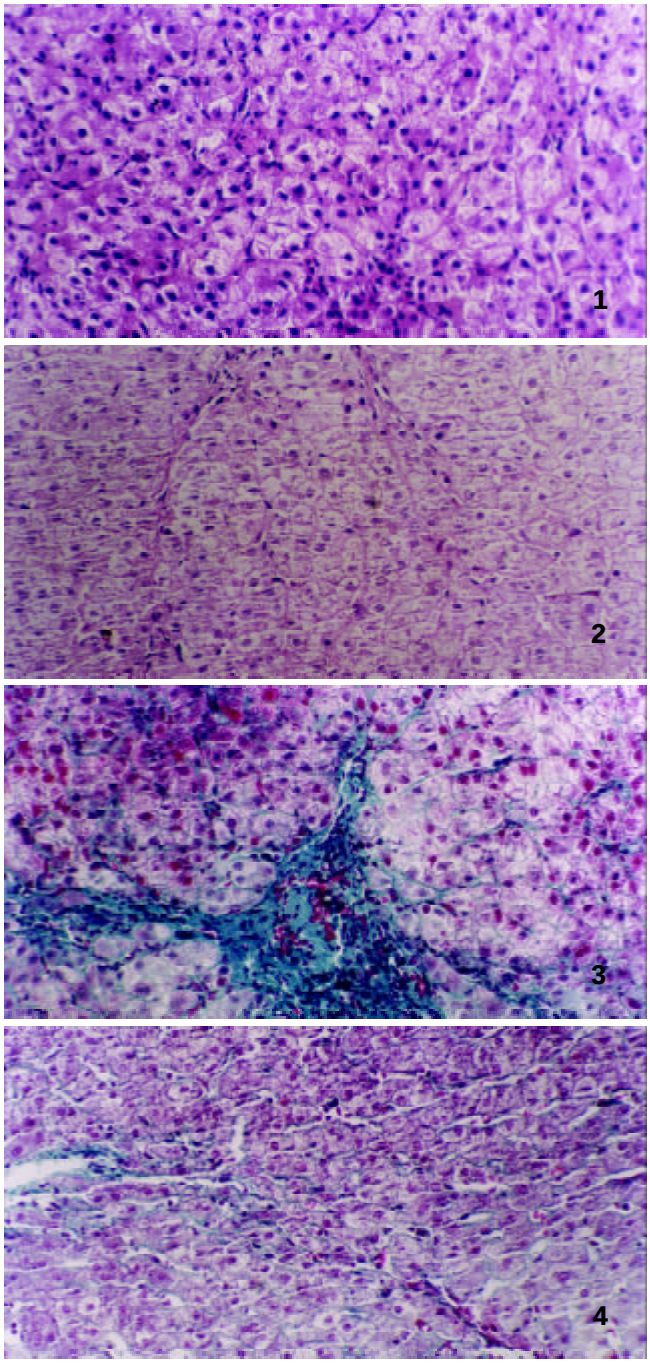

Figure 1 The liver tissue before treatment with heparin. H&E staining. ×200.
Figure 2 The liver tissue after treatment with heparin. H&E staining. ×200.
Figure 3 The liver tissue before treatment with heparin. Masson staining. ×200.
Figure 4 The liver tissue after treatment with heparin. Masson staining. ×200.

Electron microscopic observation Before treatment, hepatocytes were enlarged and cytoplasm appeared dissolved with swollen mitochondria. Base membrane was seen under the hepatic sinusoidal endothelial cells with collagen deposited in the Disse' s space. The Ito cells simulated fibroblasts. The edge of membrane looked uneven, saw-like in severe cases. The number of fat drops decreased markedly. There was microfilament-like structure in the cytoplasm, fibrils were seen around the Ito cells. After treatment, the swollen hepatocytes decreased , so did the base membrane and the depositioning collagen in the Disse' s space. The edge of Ito cells turned smooth. Several fat drops could be seen in the cytoplasm of Ito cells (Figure 5-8).

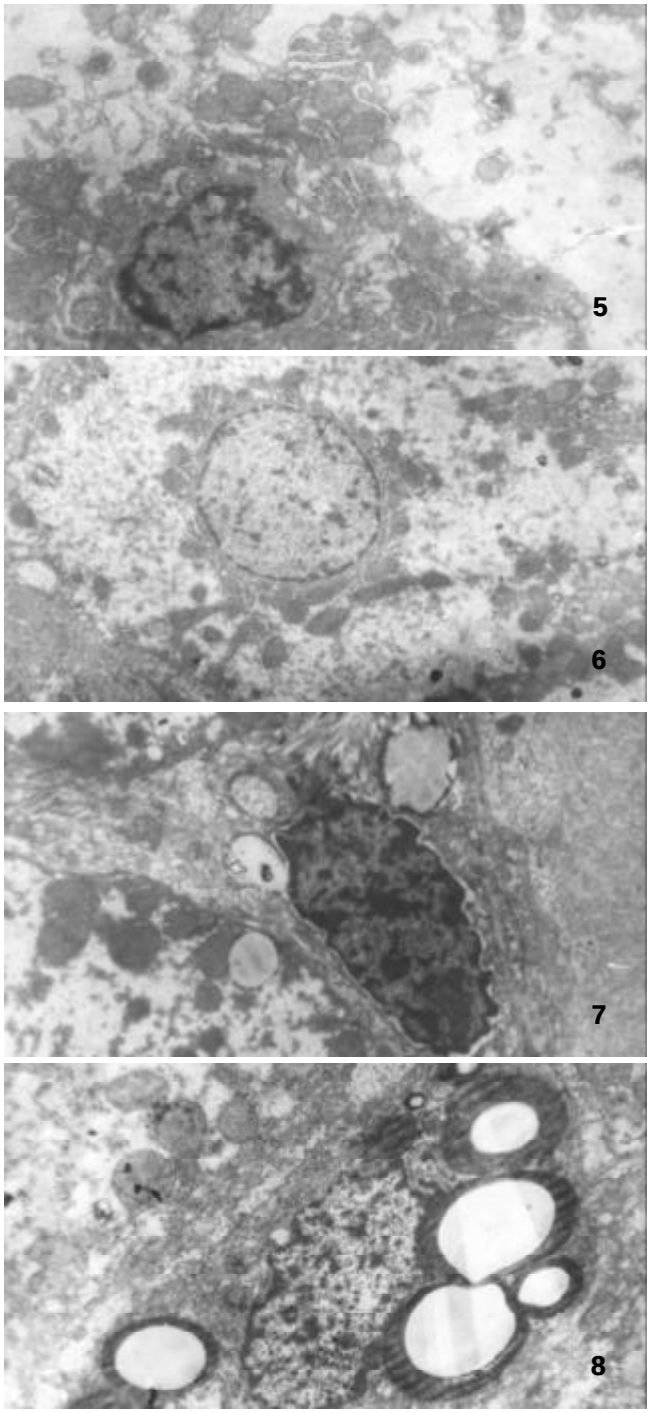

Figure 5 The hepatocyte before treatment heparin. ×6000.
Figure 6 The hepatocyte after treatment with with heparin. ×3500.
Figure 7 The Ito cell before treatment with heparin. ×5000.
Figure 8 The Ito cell after treatment with with heparin. ×5000.

DISCUSSION

Liver fibrosis is caused by the deposition of extracellular matrix (ECM)[2-3]. All cells in the liver can synthesize and secrete ECM, which regulates the proliferation, differentiation and metabolism of liver cells. The abnormal metabolism and deposition of ECM lead to liver fibrosis. It has been recognized that Ito cells have intimate relationships with liver fibrosis[4], which have been postulated to play critical roles in the development of fibrosis of the liver from viral infection, alcohol and many drugs[5,6].Ito cells are relatively inactive fibroblasts in the liver lobules. During liver fibrogenesis, cytokines such as TGF-β_1, PDGF can activate Ito cells[7-9] to acquire a myofibroblast-like phenotype characterized by increased proliferation and synthesis of ECM component[10-27].

It has been proved in animal studies that heparin can inhibit the growth of Ito cells and the expression of α-actin, types I and IV procollagen *in vitro*[1]. Our studies showed that heparin/LMWH could decrease serum HA and IV-C levels in patients with chronic hepatitis B. After treatment, the collagen fibrils in the liver tissues decreased significantly and Ito cells turned oval and fatty drops reappeared in the cytoplasm. The above results indicate that heparin/LMWH act on Ito cells.

The liver functions were improved in both group A and B after treatment. HA and IV-C levels decreased significantly in group B, in contrast, they were elevated in group A. These results suggest that the routine liver function tests could not reflect the fibrosis completely. Kopke-Aguiar *et al*[28] also proved that serum hyaluronic acid was a good marker for hepatic fibrosis at the initial phase.

Wanless *et al*[29] have studied hepatic veins of medium size (0.2 to 3 mm in diameter) in 61 cirrhotic livers. Intimal fibrosis with at least 10 % luminal narrowing was found in 70 % of cirrhotic livers. They considered that multiple layers of intimal fibrosis in some livers suggested the presence of recurrent thrombosis. In other words, thrombosis was related to intimal fibrosis and even caused obstruction of the veins. Our previous studies[30] also showed that as an anticoagulant agent, heparin could improve hepatic microcirculation significantly and lessen sinusoidal capillarization. IV-C is considered an important marker of the development of hepatic sinusoidal capillarization and may appear basal-like membrane[31,32]. Therefore, decrease of the IV-C concentration can not only reflect the improvement of hepatic microcirculation, but also inhibit the fibrosis. It can be used as antifibrosis drug together with antiviral drugs.

Heparin is cheap and safe. LMWH has a weaker effect on thrombin than heparin, but has stronger effect on Xa. 90 % of LMWH can be absorbed hypodermically and its anti-Xa effect can last for 24 hours and therefore, LMWH can be used once a day. One needs not measure the activated coagulation time (ACT) during the procedure[33]. As to the mechanisms of its antifibrosis effect ,further studies are necessary.

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