Comparative Study between the Effect of Atorvastatin and Naltrexone on Hepatic Fibrosis Induced by Bile Duct Ligation in Rats

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Abstract: Hepatic fibrosis is one of the common pathological features of chronic liver disease. Atorvastatin and naltrexone previously showed a beneficial effect on hepatic fibrosis. In the present study, we compared the effect of atorvastatin and naltrexone on a rat model of hepatic fibrosis induced by BDL. Methods: This study was carried out on 4 groups each of 10 albino rats; Group 1: Sham operated group, Group 2: BDL group + No treatment, Group 3: BDL + Atorvastatin orally for 4 weeks and Group 4: BDL + Naltrexone SC for 4 weeks. Serum bilirubin, ALT, AST, ALP and hyaluronic acid and hepatic hydroxyproline content, GSH, GSH/GSSG ratio and TNF-α level were measured. Histopathological examination of the liver tissue was performed. Results: Treatment with either atorvastatin or naltrexone showed significant increase in hepatic GSH and hepatic GSH/GSSG ratio with significant decrease in serum hyaluronic acid and hepatic hydroxyproline content and TNF-α and improvement of histopathological picture of hepatic tissue. Also, naltrexone produced significant decrease in serum total bilirubin, AST, ALT and ALP while atorvastatin showed significant increase in their levels. Moreover, treatment with naltrexone showed significant reduction in serum hyaluronic acid level and more improvement in the histopathological picture of hepatic tissue than atorvastatin. Conclusion: Naltrexone is more effective than atorvastatin in attenuation of BDL induced hepatic fibrosis and both could be of beneficial effects in treatment of liver fibrosis in clinical practice.
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Abbreviations: HSCs (hepatic stellate cells); BDL (bile duct ligated); SC (subcutaneous); ALT (alanine aminotransferase); AST (aspartate aminotransferase); ALP (alkaline phosphatase); GSH (glutathione); GSSG (oxidized glutathione); TNF-α (tumour necrosis factor alpha).

1. Introduction

Hepatic fibrosis is a common pathological feature of progressive chronic liver disease that is characterized by excessive accumulation of extracellular matrix proteins in which hepatic stellate cells (HSCs) play a key role. Injury of hepatic parenchymal cells leads to enlargement, proliferation and transformation of HSCs into myofibroblast-like cells or fully activated hepatic stellate cells which are responsible for secretion of excessive amount of extracellular matrix proteins leading to fibrosis which may progress to cirrhosis [1-3]. Inhibition of this differentiation may inhibit the accumulation of extracellular matrix proteins and decrease the amount of hepatic fibrosis [4].

Atorvastatin is one of statins which inhibits HMG-CoA reductase enzyme responsible for cholesterol synthesis and widely used in clinical practice to reduce plasma cholesterol level. Many studies on statins have demonstrated that they may have other biological effects such as reducing oxidative stress, enhancing nitric oxide production and down-regulating the expression of angiotensin Π receptors in the smooth muscle cells [2,5&6]. Also, it was suggested that atorvastatin attenuates hepatic fibrosis by decreasing turnover of HSCs and inhibiting inflammatory reactions in the liver [3].

In the course of cholangiopathies, the biliary epithelium acquires neuroendocrine features that are not present in normal liver [7]. Recent data showed that cholestasis is associated with increased opioidergic neurotransmission [8]. The plasma level and activity of opioid peptides were markedly increased in both human and experimental cholestasis [9]. Naltrexone is an opioid receptor antagonist with strong affinity to both mu and kappa subtypes. It is used primarily in management of alcohol and opioid dependence and proposed as treatment of pruritus in liver cirrhosis [10]. Recent studies showed a beneficial role for naltrexone in biliary cirrhosis [7&11].

The aim of the present work is to compare the effect of atorvastatin and naltrexone on a rat model of hepatic fibrosis induced by bile duct ligation (BDL) with regarding to serum total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and hyaluronic acid and hepatic hydroxyproline content, glutathione (GSH) level and GSH/ GSSG (oxidized glutathione) ratio, and tumour necrosis factor alpha (TNF-α) and histopathological examination of livers tissue.

2. Materials and Methods

2.1. Animals:

Forty albino rats of local strain from Tanta University animal house weighing 150-200 grams were used. They were housed at ordinary room temperature, exposed to natural daily light-dark cycles, fed with standard laboratory diet and given water ad libitum. Rats were divided into 4 groups each of 10 rats; Group 1: (sham operated group) in which rats underwent laparotomy with manipulation of bile duct but without ligation and served as the normal control group. Group 2: Bile duct ligated rats for induction of hepatic fibrosis and received no treatment. Group 3: Bile duct ligated rats, received oral atorvastatin (Lipitor, Pfizer, Egypt) dissolved in saline-10% ethanol, in a dose of 15 mg/kg/day immediately following BDL for 4 weeks [3]. Group 4: Bile duct ligated rats, received subcutaneous naltrexone HCL (Sigma, St. Louis, MO, USA) dissolved in distilled water, in a dose of 20 mg/kg/day immediately following BDL for 4 weeks [7]. All experiments were carried out following the guideline for the care and use of experimental animals in Tanta Faculty of Medicine with an approval of Research Ethics Committee of the Faculty.

2.2. Induction of Hepatic Fibrosis by BDL:

Hepatic fibrosis was induced as described previously by Ebrahimkhani et al. [12]. Briefly, laparotomy was performed under general anesthesia (Ketamine HCl 50 mg/kg and Xylocaine HCl 10 mg/kg i.p.). The bile duct was exposed and triple ligated, then cut between the second and third distal ligations.

2.3. Specimens Collection and Experimental Procedures:

Twenty four hours after the last treatment, rats were decapitated and blood samples were collected. Serum was separated immediately by centrifugatfor 5 minutes at 4°C and stored at −30°C for estimation of serum total bilirubin [13], ALT, AST, ALP [14] and hyaluronic acid [15]. Liver was removed, liver samples were taken for determination of hepatic hydroxyproline content [16], GSH level, GSH/ GSSG ratio[17] and TNF-α (enzyme-linked immunosorbent assay kits, RayBiotech, Inc., Norcross, USA) then hepatic tissue was immediately fixed in 10% buffered formalin. Then, hepatic tissues embedded in paraffin and sliced to 5 µm tissue sections which were stained with hematoxylin and eosin (H & E) and Masson's trichrome (MT) light green stains.

2.4. Statistical Analysis:

Data were presented as mean ± SEM. Data were analyzed with two-tailed Student's t-test or Mann-Whitney's U-test after evaluation with F-test. Differences between the means of different groups were considered significant at a level of $P < 0.05$.

3. Results

3.1. Effect of Treatment on Serum AST, ALT, ALP and Total Bilirubin:

The treatment with naltrexone was significantly reduce ($P < 0.05$) the serum levels of AST, ALT, ALP and total bilirubin. However, treatment with atorvastatin was significantly increase ($P < 0.05$) their serum levels when compared with BDL group which showed significant increase ($P < 0.05$) in their levels when compared with the sham operated group (Figure 1).

3.2. Effect of Treatment on Serum Hyaluronic Acid, Hepatic Hydroxyproline Content and Hepatic TNF-α:

The treatment with atorvastatin and naltrexone was significantly reduce ($P < 0.05$) the serum hyaluronic acid level and the hepatic hydroxyproline content when compared with BDL group which showed significant increase ($P < 0.05$) in their level when compared with the sham operated group. Also, they significantly reduce ($P < 0.01$) the hepatic TNF-α when compared with BDL group which showed significant increase ($P < 0.01$) in their level when compared with the sham operated group. Moreover, treatment with naltrexone showed more significant reduction in serum hyaluronic acid level than atorvastatin ($P < 0.01$) (Figure 2).

3.3. Effect of Treatment on Hepatic GSH Level and GSH/GSSG Ratio:

The treatment with atorvastatin and naltrexone was significantly increase ($P < 0.05$) the GSH level and GSH/ GSSG ratio when compared with BDL group which showed significant decrease ($P < 0.05$) in their levels when compared with the sham operated group (Figure 3).

3.4. Effect of Treatment on Liver Tissue Histopathological Picture:

Hepatic tissue of BDL group showed sever destructive changes in hepatocytes around central vein and in the portal area (Piece-meal necrosis) with chronic inflammatory reaction, massive proliferation of small bile ducts around central vein and in the portal area, massive dilatation of blood sinusoids with proliferation of Kupffer cells and marked deposition of collagen fibers around the proliferated bile ducts leading to loss of hepatic lobular architecture (Biliary cirrhosis). These changes were greatly ameliorated by the treatment with both atorvastatin and naltrexone with restoration of normal hepatic lobular architecture. However, the treatment with naltrexone apparently showed more improvement in the histopathological picture of hepatic tissue than atorvastatin treatment manifested by very little inflammatory cells centered mainly around the portal area, no bile duct proliferation and minimal collagen fibers deposition around central vein and in the portal area (Figures 4, 5).

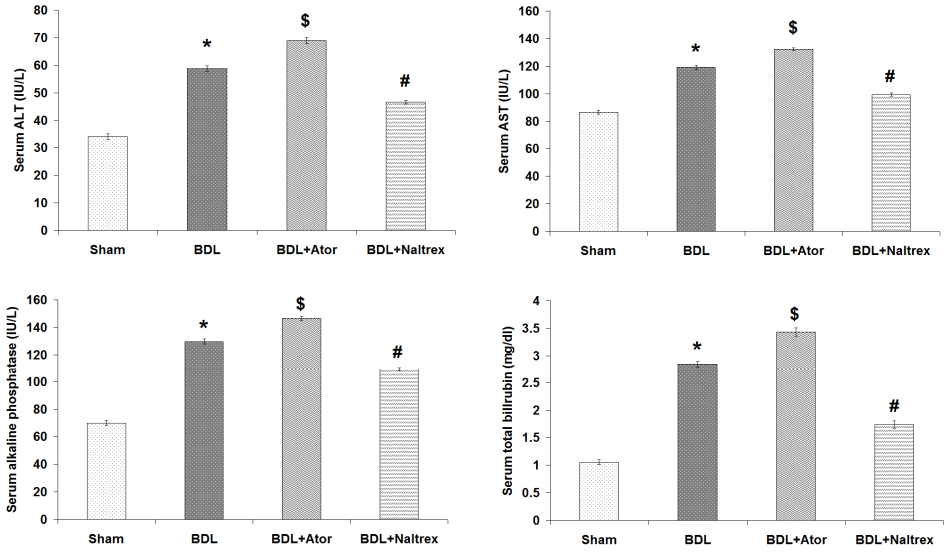

Figure (1): Effect of atorvastatin and naltrexone treatment on serum ALT, AST, ALP and total billrubin in a rat model of BDL induced hepatic fibrosis expressed as mean ± SEM. Sham: normal control group. BDL: Bile duct ligated group received no treatment. BDL+Ator: Bile duct ligated group received oral atorvastatin. BDL+Naltrex: Bile duct ligated group received subcutaneous naltrexone HCL. * P < 0.05(Vs. sham group); $ P < 0.05(Vs. BDL group); # P < 0.05(Vs. BDL group).

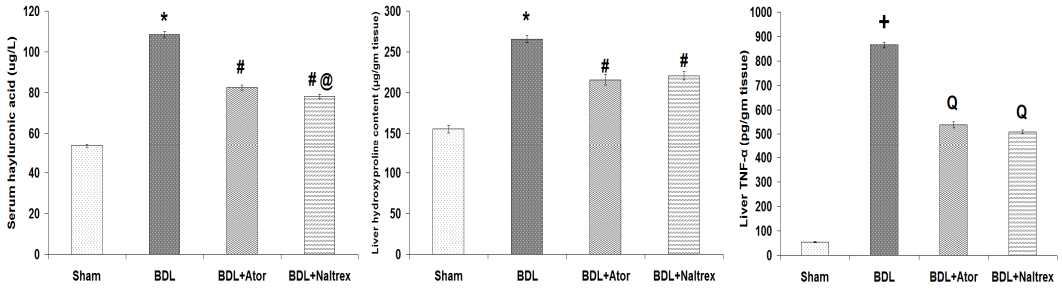

Figure (2): Effect of atorvastatin and naltrexone treatment on serum hyaluronic acid, liver hydroxyproline content and liver TNF-α level in a rat model of BDL induced hepatic fibrosis expressed as mean ± SEM. Sham: normal control group. BDL: Bile duct ligated group received no treatment. BDL+Ator: Bile duct ligated group received oral atorvastatin. BDL+Naltrex: Bile duct ligated group received subcutaneous naltrexone HCL. * P < 0.05(Vs. sham group); # P < 0.05 (Vs. BDL group); @ P < 0.01(Vs. BDL+Ator group); + P < 0.01(Vs. sham group); Q P < 0.01(Vs. BDL group).

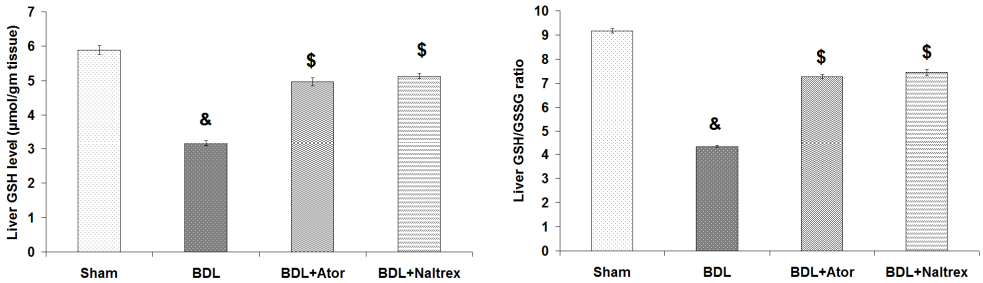

Figure (3): Effect of atorvastatin and naltrexone treatment on liver GSH level and liver GSH/GSSG ratio in a rat model of BDL induced hepatic fibrosis expressed as mean ± SEM. Sham: normal control group. BDL: Bile duct ligated group received no treatment. BDL+Ator: Bile duct ligated group received oral atorvastatin. BDL+Naltrex: Bile duct ligated group received subcutaneous naltrexone HCL. & P < 0.05 (Vs. sham group); $ P < 0.05 (Vs. BDL group).

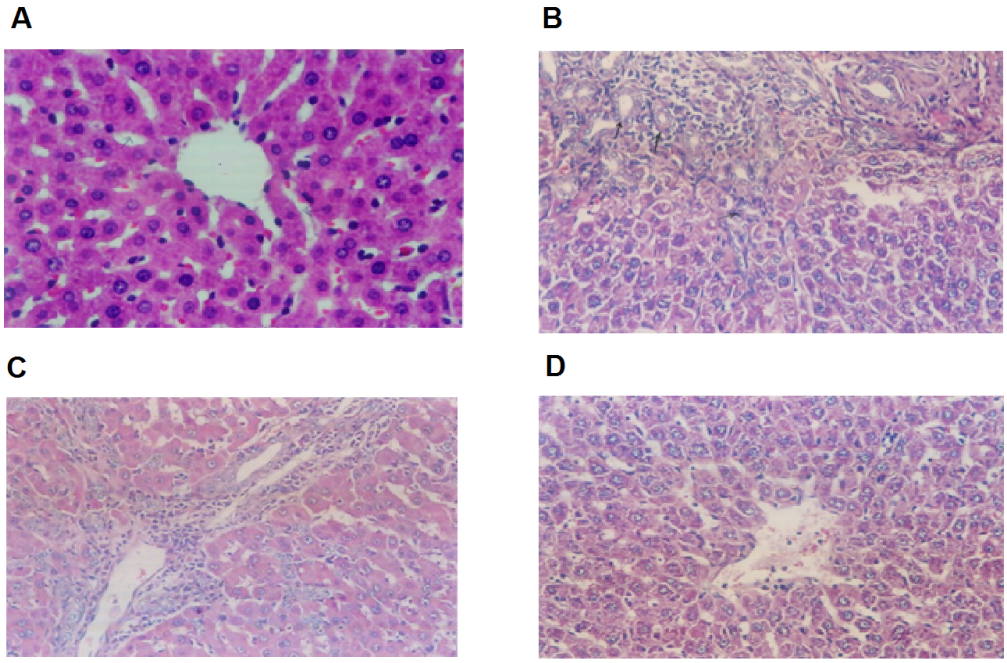

Figure (4): Effect of atorvastatin and naltrexone treatment on histopathological picture of liver tissues in a rat model of BDL induced hepatic fibrosis. A: sham operated group. B: Bile duct ligated group received no treatment. C: Bile duct ligated group received oral atorvastatin. D: Bile duct ligated group received subcutaneous naltrexone HCL. (H&E X 400 & 200).

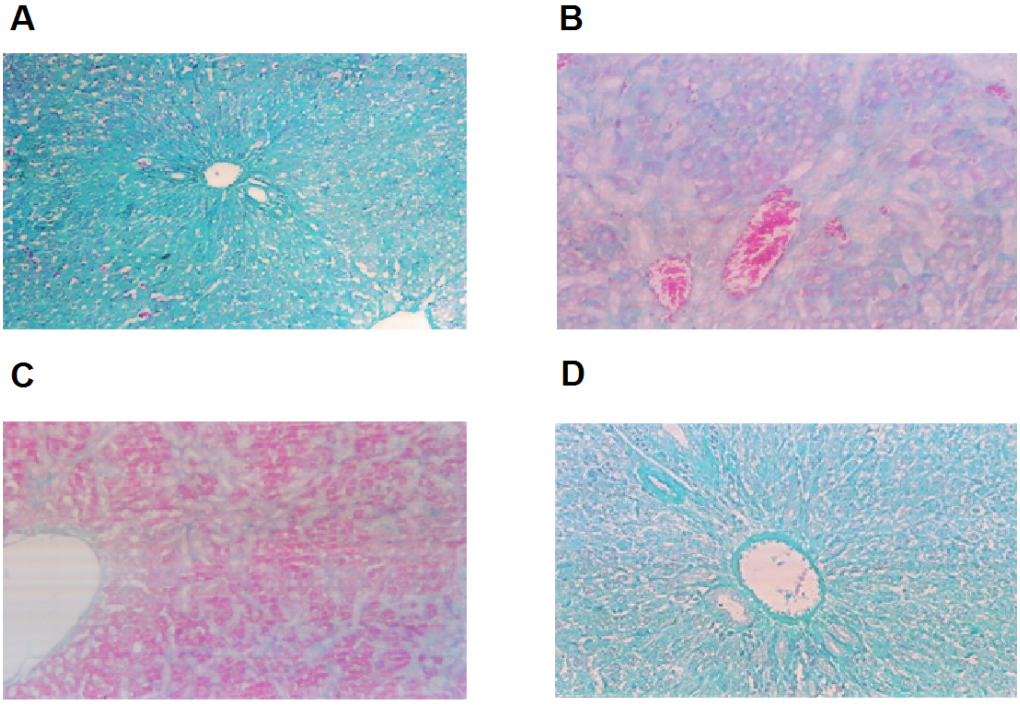

Figure (5): Effect of atorvastatin and naltrexone treatment on Histopathological picture of liver tissues in a rat model of BDL induced hepatic fibrosis. A: sham operated group. B: Bile duct ligated group received no treatment. C: Bile duct ligated group received oral atorvastatin. D: Bile duct ligated group received subcutaneous naltrexone HCL. (MT light green X 100).

4. Discussion

In the present study, we compared the effect of atorvastatin and naltrexone on a rat model of hepatic fibrosis induced by BDL with regarding to serum total bilirubin, ALT, AST, ALP and hyaluronic acid and hepatic hydroxyproline content, GSH level and GSH/

GSSG ratio, TNF-α and histopathological examination of liver tissue. Our results showed that treatment with both atorvastatin and naltrexone showed significant increase in hepatic GSH and hepatic GSH/GSSG ratio with significant decrease in serum hyaluronic acid and hepatic hydroxyproline content and improvement of histopathological picture of hepatic tissue with restoration of normal hepatic lobular architecture. However, treatment with naltrexone showed significant decrease in serum total bilirubin, AST, ALT and ALP while atorvastatin treatment showed significant increase in their levels. Moreover, treatment with naltrexone showed more significant reduction in serum hyaluronic acid level and apparently more improvement in the histopathological picture of hepatic tissue than atorvastatin treatment.

Trebicka et al. [3] stated that BDL produces oxidative stress and injury of biliary epithelium followed by inflammatory response which leads to hepatic fibrosis. The underlying basis of oxidative stress in cholestatic liver disease is complex involving generation of reactive oxygen species, possibly mediated by bile acids, endotoxins, leucocytes and activated HSCs [1,3]. HSCs play a pivotal role in the hepatic wound healing in response to injury and are considered when activated as a source of free radicals generation during liver fibrogenesis which result into amplification of the inflammatory response to injury. Moreover, oxidative stress itself is involved in the activation of HSCs into fibrogenic myofibroblasts that result into viscious circle [5&18]. Also, Hepatic metalloproteinase-2 (MMP-2) is secreted by activated HSCs in the injured liver and is responsible for degrading type IV collagen, which is a major constituent of the basement membrane-like matrix of the perisinusoidal space of Disse. Replacement of perisinusoidal type IV collagen with type I/III collagen is promoted by HSCs-derived MMP-2 and is a key event in the fibrogenic process [19]. The activity of MMPs in the transformed fibroblasts is markedly inhibited by GSH [20].

Yang et al. [21] demonstrated that hepatic concentration of TNF-α increases following BDL in mice. The primary role of TNF-α in the inflammatory liver disease has been proposed to be the initiation of HSCs activation. Previous data showed that TNF-α knockout mice possessed lower hepatic collagen expression and decreased fibrosis compared to the wild-type mice in hepatic fibrosis murine model [22].

Our result showed attenuation of BDL induced hepatic fibrosis by treatment with atorvastatin and naltrexone. This attenuation is due to their inhibitory effect on oxidative stress and reduction of hepatic TNF-α level. Atorvastatin reduced oxidative stress in the liver and thus inhibiting hepatic inflammation and fibrosis [3]. Liao [23] explained the increase in the hepatic GSH and hepatic GSH/GSSG ratio by that statins have antioxidant properties. Wassmann et al. [24] reported that inhibition of the small GTP-binding proteins, including Rac1, plays an important role in mediating the antioxidant effects of statins. Membrane translocation of Rac1, which is required for the activation of NAD(P)H oxidase, is inhibited by atorvastatin in liver and other organs. Chronic administration of naltrexone was reported with Faramarzi et al. [11] to significantly improve the amount of liver GSH concentration and therefore, has protective effects against oxidative damage in the cholestatic rat liver by blocking the opioid receptors. Also, Day et al. [25] reported that naltrexone and JKB-119, a morphinan analogue of naltrexone, suppresses TNF-α production and increased the level of S-adenosyl-L-methionine which is the precursor of glutathione. This can inhibit oxidative stress and transforming growth factor beta (TGF-β) signaling thus decreasing collagen expression in activated HSCs while the levels of GSH were increased suggesting reduced oxidative stress as a possible mechanism.

Moreover, Atorvastatin reduces the expression of proinflammatory cytokines which promote recruitment of inflammatory cells [5], attenuates the expression of procollagen-α 1, reduces the accumulation of activated HSCs [3] and is able to block the activated HSCs in the G_2 phase of the cell cycle and induce their apoptosis in vitro [26]. However, the inability of atorvastatin to reduce the elevated liver enzymes is explained by Trebicka et al. [3] who stated that atorvastatin lacks inhibitory effect on the inflammation of this model because cytokines at this model are produced by inflammatory cells and macrophages but not by quiescent HSCs. Naltrexone in addition; suppresses TLR4-mediated up-regulation of NFκB [25], inhibits hepatic polymorphnuclear infiltration [22] and has antifibrogenic effect by inhibiting δ opioid receptors thus suppresses tissue MMP-1 and procollagen I expression [17]. All these factors with inhibition of oxidative stress and reduction of TNF-α level are responsible for the ability of naltrexone to decrease hepatic tissue damage and reduce the elevated liver enzymes.

In conclusion, the present study showed that naltrexone is more effective than atorvastatin in attenuation of BDL induced hepatic fibrosis and both could be of beneficial effects in treatment of liver fibrosis in clinical practice.

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