SOHAG MEDICAL JOURNAL
Vol. 21 No. 3 october 2017

Quantitative Analysis of Reticular Fibers in CCl_4-Induced Liver Cirrhosis in Mice.

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Abstract

Background

Cirrhosis is the final stage of chronic liver disease. It is characterized by diffuse fibrosis and disruption of hepatic lobular architectureinto abnormal cirrhotic nodules.

Aim of the work

This study was conducted to investigate the extent of reticular fibersdeposition in induced liver cirrhosis and their resolution after cessation of the insult.

Materials and methods

Three groups of animals were used; control, treated and recovery groups. The treatedgroup injected subcutaneously with CCl4 for 16 weeks for induction of cirrhosis, and the recovery group were kept for two weeks without injection after the 16 weeks of CCl4 treatment.

Results

Our results indicated thatreticular fibers increased in liver cirrhosis and decreased with fibers resolution in mice.

Conclusion

Reticular fibers areinvolved in the process of fibrogenesis in mice and decreased in the resolution of fibers in the recovery of the model which can be consideredin newstudies for the treatment of liver cirrhosis.

Keywords: liver, cirrhosis, fibrosis, CCl_4.

Abbreviations; CCl_4: carbon tetrachloride, ECM: extracellular matrix, HSCs: hepatic stellate cells.

Introduction

Liver cirrhosis is the final outcome of chronic liver diseases. Hepatic fibrosis is a wound-healing response which leads to accumulation of fibers in the extracellular matrix (ECM).Activation of hepatic stellate cells (HSCs); main source of ECM is key event in the development of hepatic fibrosis (***Wang et al., 2011***).

Studying the pathogenesis of liver cirrhosis can help more effective treatment options to develop. Animal models are used to study fibrogenesis and different strategies for the treatment of liver cirrhosis (***Jang et al., 2008).***

Evidence that fibrosis and even cirrhosis are reversible has intensified interest in understanding fibrosis resolution, and the type of fibers.This can be applied in new therapiesto reverse liver cirrhosis ***(Iredale et al., 1998, and Domitrović et al., 2009).***

Materials and methods

Animals:

60 adult male Balb/c mice; about 2 months old with average weight 35 gm,

were purchased from Assuit Experimental Animal Facility, Assuit University. Animals were housed in Sohag University Animal House with free access to water and chow. They were acclimatized to this environment for 5 days prior to the experiment. All procedures used in this experiment were approved with the local Ethics Committee of Sohag University, Faculty of Medicine.Animals were randomly divided into four groups:

Group I (control): 5 animals were injectedsubcutaneously with the vehicle; sunflower oiltwice weekly for 16 weeks, and another 5 animals werekept without treatment for 16 weeks

Group II: 25 animals were subcutaneously injected with 20% CCl_4(SigmaAldrich Company, Germany) in a dose of 1 ml /Kg twice weekly for 16 weeks for induction of cirrhosis(***Vanheule et al., 2008***).

Group III: 25 animals were treated with CCl_4 for 16 weeks in the same dose of the previous groupfor induction of cirrhosis , then kept without for additional two weeks.

Methods:

Histological studies:

Liver samples were taken for processing of paraffin sections .Formalin fixed paraffin embedded section were used for detection of reticular fibers by silver stain according to ***Bancroft et al.(2013).***

Morphometric studies

The light microscope Leica ICC50 Wetzlar (Germany) at the Histology Department, Faculty of Medicine, Sohag University was used, ten high power fields (x400) for each case in all groups were examinedand analysis of each field was doneusing Image J software (version 1.46r) to detect the percentage areas of reticular fibers.

Statistical analysis

Paired sample Student *t*-test was used to analyze the data by using SPSSprogram (version 16.00; SPSS Inc., Chicago, Illinois, USA) with a statistical significance of $P<0.05$. Data were expressed as mean ± standard error (SEM).

Results

The reticular fibers in the control groupappeared around the central veins, in the portal areas and in between the hepatic plates surrounding the sinusoids. In group II, reticular fibers significantly increased as compared to that in group I with formation of fibrous septa completely surrounding the hepatic lobules and the cirrhotic nodules. In group III, reticular fibers significantly decreased compared to that in group II; with fibrous septa partially surrounding the hepatic lobules (Fig. 1).

The mean percentage areas of reticularin different groups of the experiment were summarized in table (1), and Fig. (2).

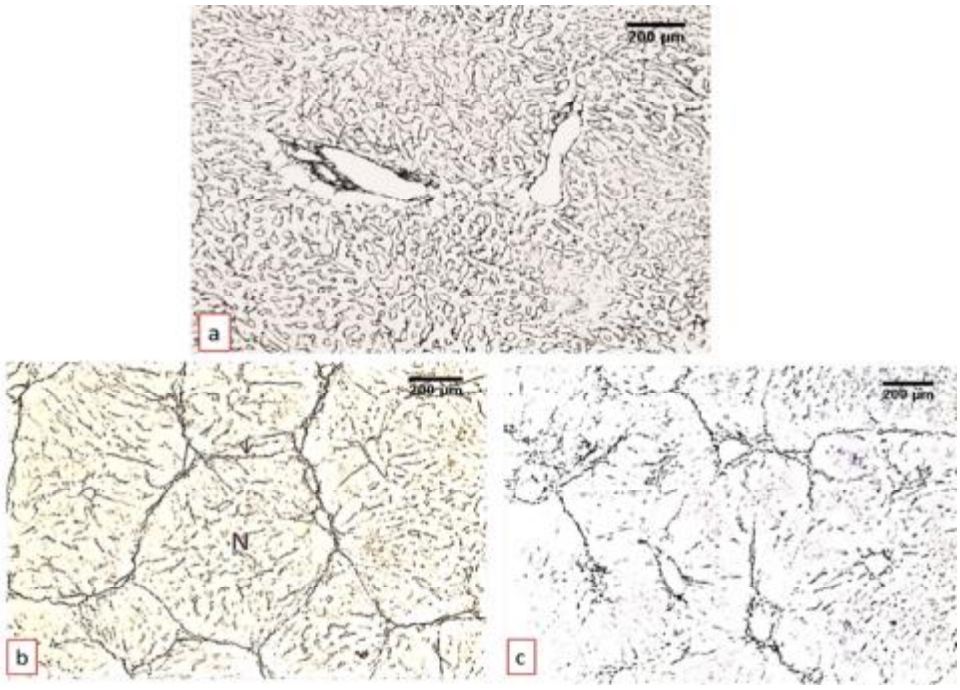

Fig. (1): Photomicrographs of liver sections from animals of; (a) group I showing reticular framework surrounding the central vein, in the portal area and around hepatic plates of hepatocytes.(b)group II showing numerous reticular fibers with complete septa surrounds the hepatic lobules and the cirrhotic nodules(N) and collapse of the fine fibers inbetween the parenchymal cells.(c) groupIII showing few reticular fibers around the central veins and in the portal areas with incomplete septae and inbetween the parenchymal cells (Silver stain).

Table (1): The mean percentage area of reticular fibers

Group	Reticular fibers percentagearea. mean(±SEM)
GroupI	10.92%(±0.79)
Group II	14.26%(±0.64) *
GroupIII	6.63%(±0.60)*#

* Significant as compared to group IA (P value<0.05), # significant as compared to group II (P value<0.05)

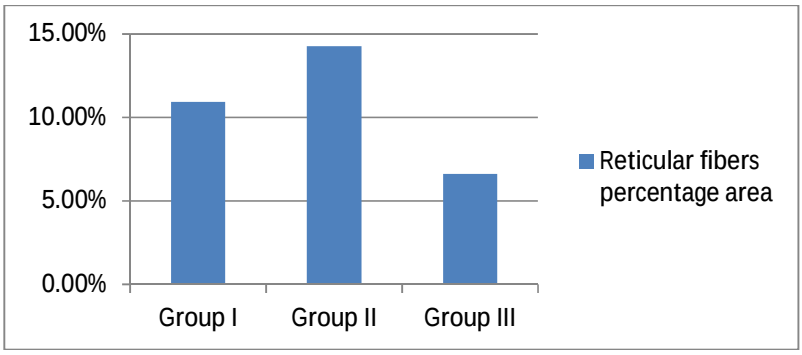

Fig.(2): The mean percentage area of reticular fibers.

Discussion

.Hepatic fibrosis is caused by increased synthesis of ECM, produced by HSCs. (***Wang et al., 2011***). In our study, reticular fibrous tissue significantly increased with disturbed architecture and formation of cirrhotic nodules. Similar findings were observed in previous studies ***Zaki et al. (2011)*** and ***Li et al. (2016***) due to increased synthesis of reticular fibers by activatedHSCs.

In our studies, we found that, 2 weeks after cessation of CCl_4 administration;there was significant reduction of reticular fibers; being demonstrated only in incomplete septaeand inbetween the parenchymal cells. The reticular fibers in this group were less than the controldue to the change of the type and characters of the extracellular matrix with collagen type I replacing the reticular fibers***Zaki et al. (2011) .***

Our results were confirmed by previous reports. They proved that the key events in the resolution of liver fibrosis include decreased HSCs activation and increased degradation of fibers (***Woessner, 1991***). Other reports reported that hepatic myofibroblasts derived from HSCs undergo apoptosis during the spontaneous regression of liver fibrosis induced by CCl_4***(Iredale et al., 1998).*** In constrast to our results, ***Domitrović et al.,(2009)*** showed persistence of fibrosis in their model 2 weeks after withdrawal of CCl_4 . ***Kang et al., (2005)*** also reported persistent fibrosis in their model after withdrawal of the insult with change of the localization of myofibroblast; being demonstrated only around the cirrhotic nodules.

In conclusion ;reticular fibers deposition increase in a CCl_4 induced model of liver cirrhosis in mice which can be applied in new strategies for the treatment of liver cirrhosis.

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