Activated Liver Stellate Cells in Chronic Viral C Hepatitis: Histopathological and Immunohistochemical Study

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Abstract

Background. There is positive correlation between the number of activated hepatic stellate cells and necroinflammatory activity and/or the stage of liver fibrosis in viral hepatitis. No study has investigated such a relationship with regard to the activated hepatic cells within specified zones of liver tissue in chronic C hepatitis. The aim of the present study was to correlate the level of activated hepatic stellate cells within perivenular, intermediate, periportal, and portal tracts area and fibrous septa with stages of liver fibrosis and necroinflammatory activity in patients with chronic C hepatitis. Methods. This retrospective study included 20 liver biopsy samples from patients with chronic C hepatitis and 10 normal liver biopsies. Biopsy specimens were processed routinely and stained with haematoxylin–eosin, periodic acid–Schiff, Masson’s trichrome, aldehyde fuchsin, reticulin and iron (Pearls). Activated hepatic stellate cells were identified immunohistochemically using antibody to α-smooth muscle actin. Assesment of immunoreactivity was performed using a semiquantitative method. Results. In chronic C hepatitis, a positive correlation between the stage of fibrosis and the number of activated hepatic stellate cells within portal spaces and fibrous septa was found. These cells were increased in number in other areas of liver tissue as well, but without statistical significance. There was no correlation between either the stage of fibrosis and necroinflammatory activity or the number of activated hepatic stellate cells and necroinflammatory activity. Conclusion. An increased number of activated hepatic stellate cells within portal spaces and fibrous septa may be a useful prognostic marker for the development of advanced fibrosis and cirrhosis in chronic C hepatitis.

Key words

Hepatic stellate cells – hepatitis C – liver fibrosis – α-SMA immunoreactivity

Introduction

Approximately 3% of world population suffers from chronic C hepatitis (CCH). C viral proteins seem to modulate apoptosis and steatosis, ultimately leading to hepatic stellate cells (HSCs) activation, fibrosis and hepatocellular carcinoma. The immune system initially attempts to eradicate the virus, but in the setting of chronic infection, it probably promotes hepatocyte damage and fibrosis through direct cellular toxicity and the release of inflammatory cytokines [1, 2]. Some patients with CCH will develop cirrhosis in a short period of time (“fast fibrosers”), some will have very slow progression of the disease (“slow fibrosers”) and the rest belong to a category named as “intermediate fibrosers” [3]. It is well known that HSCs play an important role in development of fibrosis and its progression to cirrhosis [4-7]. Under conditions of stress and injury, such as in CCH, HSCs are activated and acquire myofibroblastic phenotype, contributing to excessive extracellular matrix deposition [7-10]. Activated HSCs loose lipid droplets stored in their cytoplasm, proliferate and gain the abundance of microfilaments that consist mainly of α-smooth muscle actin (α-SMA). Accordingly, α-SMA has been established as a reliable marker of activated HSCs, although these cells are immunoreactive for various antibodies, as well [6, 11-13]. Only a few series analyzed the association between activated HSCs and CCH [3, 14, 15] with results showing positive correlation between an increased number of activated HSCs and necroinflammatory activity and/or the stage of liver fibrosis. However, there have been no data as to in which areas of liver tissue HSCs are most numerous along the course of CCH. The aim of the present study was to establish a correlation between the activity of HSCs within fibrous septa, perivenular, intermediate, periportal and portal tract area of liver tissue and the stages of liver fibrosis and necroinflammatory activity in patients with CCH.

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Methods

Patients

The retrospective study included liver biopsy samples from patients infected with hepatitis C virus (HCV), with the presence of anti-HCV antibodies and HCV RNA in the serum (n = 20). Liver tissue samples were obtained at the Institute of Infectious and Tropical Diseases, School of Medicine, Belgrade, during a two year period as a part of a routine clinical evaluation, prior to interferon therapy. Chronic liver disease was established using histopathological evaluation at the Institute of Pathology, combined with virological and biochemical tests, and according to the established criteria: active virus replication (HCV RNA positivity), presence of anti-HCV antibody, and elevated serum ALT activities observed for a period longer than six months. Five patients were female (mean age 43 years), 15 patients were male (mean age 38 years). The control group included 10 patients with normal liver biopsy samples. The age of patients in the control group was similar to the group of patients with chronic viral C hepatitis.

Liver biopsy specimens and histopathological examination

Liver biopsy specimens were obtained percutaneously, using 18-G needle. Only samples longer than 20 mm with more than eight portal tracts were included in this study. Tissue samples were fixed in buffered formalin, embedded in paraffin and sliced into 5 µm sections. Sections were stained with hematoxilin-eosin, periodic acid-Schiff (PAS), Masson’s trichrome, aldechide-fuchsin, reticulin and iron (Pearls). Biopsy samples were evaluated according to the Ishak’s scoring system [16].

Immunohistochemical analysis

Slices set aside for immunohistochemical evaluation after deparaffinisation and endogenous peroxidase blocking (3% solution of H2O2 during 15 minutes) were submitted to a microwave treatment (20 minutes on 620 W in 0.01M citrate buffer, pH 6.0). Antibody for α-SMA, dilution 1:100 (DAKO, Carpenteria, Ca, USA) was applied during 60 minutes on room temperature. Immunohistochemical staining was performed by streptavidin-biotin method using LSAB+ kit (DAKO, Carpenteria, Ca, USA) with dianimobensidine as a chromogen and Meyer’s hematoxilin for contrasting. Muscle cells of small arterial blood vessels in portal tracts served as a positive internal control. Negative control was performed by leaving out the primary antibody during the staining procedure.

Assessment of immunoreactivity was performed using a semiquantitative method by determining the percentage of positive cells on a x100 magnification (Olympus BX50F4 microscope). Counting was performed in specified areas of liver biopsy samples: perivenular area, intermediate area, periportal area, and the area of portal tracts and fibrous septa. The percentage of immunoreactive cells was grouped as follows: negative: up to 3% (0), slight: 3 - 33% (1), moderate: 34 - 66% (2) and strong: more than 66% (3) of cells in the examined area.

Statistical analysis

Statistical analysis of the data was conducted using the Analysis ToolPak of Microsoft Excel programme. Correlation between necro-inflammatory activity, degree of fibrosis and number of α-SMA positive stellate cells was evaluated using a Pearson’s correlation test. A p value <0.05 was considered significant.

Results

Control group

HSCs immunopositive for α-SMA were identified in all specified areas of liver tissue in the control group. In portal areas, α-SMA immunoreactivity was designated as variable, since in some cases it was negative (2 samples) and in some, moderate (3 samples), slight in 4 samples (Fig.1a) and negative in 1 case. Immunoreactivity in intermediate areas of liver tissue was slight and regular in all examined samples. Both in perivenular (Fig. 1b) and periportal areas, α-SMA immunoreactivity was slight in 6 and negative in 4 samples.

Chronic C viral hepatitis group

The evaluation of the liver biopsy according to Ishak’s scoring system showed that 3 patients were in a stage without fibrosis, 8 patients had slight fibrosis, 3 patients moderate fibrosis and 3 patients had severe fibrosis. Cirrhosis was
diagnosed in 3 patients. Minimal necroinflammatory activity was found in 17 cases, accompanied by slight and moderate fibrosis in two patients and one patient, respectively.

In patients without hepatic fibrosis, α-SMA expression of activated HSCs was slight in perivenular and portal spaces area, but in the intermediate and periportal zone it was negative in 2 patients and slight in 1 patient.

In the group with slight fibrosis, α-SMA expression within the perivenular and intermediate zone was moderate and slight in 2 and 6 patients, respectively. Periportal area showed negative α-SMA expression in 4 patients, slight in 2 patients and moderate in 1 patient. Area of fibrous septa showed slight and moderate α-SMA expression in 6 and 2 patients, respectively (Fig. 2).

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Patients with moderate fibrosis had slight α-SMA expression in perivenular and intermediate area, whilst in periportal area, 1 patient had moderate α-SMA expression. In portal spaces and fibrous septa, α-SMA expression was moderate in all cases (Figs. 3, 4).

In severe hepatic fibrosis, α-SMA expression within portal spaces and fibrous septa was strong in all three cases (Fig. 5). α-SMA expression within perivenular and intermediate zone was slight and moderate in 2 patients and 1 patient, respectively. α-SMA expression in periportal zone in this group was moderate in 2 patients.

All cases with hepatic cirrhosis exhibited strong α-SMA
expression within portal spaces and fibrous septa (Fig. 6) and slight – in perivenular area. In this group, intermediate area contained moderate and slight α-SMA expression in 1 patient and 2 patients, respectively, while periportal zone displayed slight immunoreactivity in 2 patients and negative in 1 patient.

We found a statistically significant positive correlation (p<0.05) between the stage of fibrosis and α-SMA expression in portal spaces (p=0.002) and fibrous septa (p=0.004). We found no correlation (p>0.05) between the grade of necroinflammatory activity and α-SMA expression in any of examined areas of liver tissue; also there was no correlation between the stage of fibrosis and grade of necroinflammatory activity.

Discussion

Chronic viral C hepatitis is a common disease that frequently causes severe liver damage. Liver lesions in CCH are commonly attributed to interaction of several pathways in the host immune response, disruption of pathogen-associated pattern recognition pathways and interference with cellular immunoregulation and subversion of NK cell activity [16, 17]. Extracellular stimuli can activate numerous intracellular signaling cascades during HSCs activation, leading to cellular responses including increased proliferation and changes in gene transcription. Among those, TGF-β, a multifunctional growth factor, is the most potent fibrogenic cytokine described [7, 17, 18]. Liver damage in chronic hepatitis eventually leads to fibrosis and subsequently to cirrhosis. Liver biopsy is mandatory for management of these patients, especially for staging fibrosis [19]. α-SMA is a reliable marker of hepatic stellate cell activation which precedes fibrous tissue deposition, and it can be used for identification of the earliest stages of hepatic fibrosis and for monitoring the efficacy of the therapy [20].

In normal liver tissue, activated HSCs can be found in portal areas around hepatic artery branches [21], within the perivenular zone [22], or rarely, in liver parenchyma [23].

In the control group, we identified activated HSCs in portal areas around hepatic artery branches, within the perivenular zone and, rarely, in liver parenchyma, similarly to the findings in previously reported series [21-23]. In the area of portal spaces, α-SMA expression in some cases was designated as moderate, but in some cases it was negative.

Perivenular and intermediate area of normal liver tissue exhibited regular, slight α-SMA immunoreactivity. Findings in our study are comparable with previously published studies, since we found α-SMA expression in all examined areas of liver tissue. Different findings of α-SMA expression in normal liver tissue can be explained by the differences in laboratory techniques that were used for immunodetection [24], or by presumption that increased α-SMA expression reflects activation of quiescent HSCs, rather than their proliferation [25]. Activation of HSCs may be present without clear evidence of liver tissue damage or inflammation, being the consequence of action of mediators that are not associated with cell necrosis.

In chronic liver disease, activated HSCs are grouped among Kupffer cells, inflammatory cells and damaged hepatocytes. Patients with CCH may have expression of α-SMA without an obvious correlation with the severity of liver injury [24,26]. However, among responders to interferon therapy, a reduction in hepatic necroinflammatory activity is associated with a trend towards a decreasing in number of activated HSCs [27]. A study performed on a higher number of patients showed significant correlation between α-SMA expression of activated liver stellate cells and the stage of fibrosis or necroinflammatory activity [28].

In the literature, the correlation between the number of activated HSCs and the stage of fibrosis or necroinflammatory activity is somewhat controversial, being positive in some series [28] or negative in others [29, 30]. Moderate or severe hepatic fibrosis was independently associated with the severity of necroinflammation [31].

In our study, we also found no correlation between necroinflammatory activity and the stage of fibrosis, or between necroinflammatory activity and α-SMA expression of activated HSCs, especially with regard to the specified area of liver tissue. Schultze-Krebs et al [29] and Delic et al [30] also noted progression of liver fibrosis despite the low level of inflammation due to direct induction of profibrogenic mediators by hepatitis C virus.

First studies that connected stage of fibrosis with increased number of α-SMA positive HSCs reported negative correlation between the stage of fibrosis and α-SMA expression in perivenular zone and portal area zone [21, 23, 32]. This negative correlation could be explained by the loss of activated HSCs by apoptosis, as these cells probably transform into quiescent phenotype during evolution of chronic hepatitis [33, 35]. It has been recently shown that there is highly significant correlation between fibrosis scores and periportal, pericentral and perisinusoidal α-SMA expression in patients with chronic viral hepatitis [35]. An increased number of α-SMA positive HSCs in portal spaces and fibrous septa was also described in CCH complicated by hepatic fibrosis [21, 22, 32]. Similarly, we have found a statistically significant correlation between α-SMA expression of activated HSCs within portal spaces and fibrous septa and stage of fibrosis. HSCs activity was more impressive, although statistically insignificant, within perportal areas in moderate or severe hepatic fibrosis in CCH.

Conclusion

In chronic C hepatitis, the number of activated HSCs in portal tracts and fibrous septa correlates positively with the stage of liver fibrosis. The histological analysis of HSCs activity within specified zones of liver tissue is justified as this specified activity can serve as a prognostic marker of severe hepatic fibrosis and cirrhosis in patients with chronic C hepatitis.

Conflicts of interest

None to declare.
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References


