Amyloidosis, change in macrophage number, and dysplasia in the liver of intravenous heroin users

Goran Ilic*, Radovan Karadzic, Lidiya Kostic-Banovic, Aleksandra Antovic

Abstract. Apart from the usual morphological changes in the liver of intravenous heroin users such as vesicular and fatty changes, various forms of viral hepatitis and development of cirrhosis, the onset of amyloidosis, the change in the number of hepatic sinusoidal macrophages, and dysplastic changes are of particular significance from the forensic point of view since they can indicate heroin abuse, probably associated with alcohol effects, and they can also be a factor for the occurrence of severe morphological liver damage with a possible lethal outcome.

A histopathologic and morphometric study of changes in the liver of heroin users aimed to show the presence of rarer morphologic changes in the liver as the indicators of heroin abuse, additional alcohol effects and viral infections.

The present study included 40 autopsies of intravenous heroin users and 10 control autopsies. In order to facilitate the investigation, all autopsies of intravenous heroin users were grouped according to the duration of intravenous heroin intake into 4 groups: up to 2 years, 2-5 years; 5-10 years; over 10 years. Paraffin sections 5 µm thick, were stained by hematoxylin method (HE), van Gieson and Congo red. Morphometric investigation of sinusoidal macrophages in the liver was done using the M42 test system. Liver tissue samples taken to be tested for ultrastructural changes were fixed in glutaraldehyde and epon-embedded, and the analysis was done using transmission electron microscopy JEM 100 CX JEOL.

Amyloidosis localized in portal tracts, mostly underneath the endothelium of the smaller branches of the hepatic artery was established in 9 cases (22.5%), out of which in 78% (in 7 of 9) the duration of intravenous intake lasted longer than 5 years. In 55.5% of cases (in 5 of 9), amyloidosis was associated with fatty alcohol changes. There was a statistically significant change in the number of sinusoidal macrophages in relation to the controls only in cases with chronic active hepatitis regardless of coexisting cirrhosis, whereas the other cases with established fatty alcohol changes showed no significantly higher number of these cells in relation to controls. Severe dysplastic changes were established in three cases with vesicular and fatty changes complicated with viral hepatitis, two of which were with coexisting cirrhosis; in all three cases (100%), the duration of intravenous heroin abuse lasted longer than 5 years.

Long duration of heroin abuse is a condition for the occurrence of liver amyloidosis and dysplastic changes in the liver. The high percentage of association of amyloidosis with fatty alcohol changes in the liver points to the conclusion that the inactivation of hepatic sinusoid macrophages and the reduction of their enzymatic capacities occur due to additive, immunosuppressive effect of alcohol which may enable the onset of liver amyloidosis.

Key words: heroin users, amyloidosis, sinusoidal macrophages, dysplasia

In addition to vesicular and fatty changes, various forms of viral hepatitis and development of cirrhosis as common morphologic changes in the liver of intravenous heroin users, the occurrence of amyloidosis, change in the number of sinusoidal macrophages, and dysplastic changes are very important, forensically viewed, since they may indicate heroin abuse and possible associated alcohol effects, and they may be implicated in the occurrence of severe, possibly fatal, morphologic damage of the liver.

Amyloidosis of the liver, associated with intravenous heroin use, belong to the forms of amyloidosis

* Corresponding author; Goran Ilic, Professor, Institute of Forensic Medicine, Medical Faculty of Nis, Zorana Djindjica 81, 18000 Nis +38118 4530 824, Email: gilke@medfak.ni
caused by repeated inflammatory processes (suppurative skin lesions), associated with long-lasting degradation of the cells. Abscesses of the injection sites in heroin, cocaine, and abuse of other drugs, are the most common infectious complication in this type of drug addicts [1]. It is a reactive amyloidosis consisting of AA (Associated Amyloid) fibrillar protein. It can be deposited in blood vessel walls (mostly in arterioles), in the perivascular connective tissue of the portal space, and in the parenchyma, i.e. in the space of Disse [2,3]. In the Disse’s space amyloid is deposited in a linear and globular fashion. Linear amyloid leads to progressive atrophy of the hepatocyte laminae (acinar zone 1 can be more severely affected). Globular amyloid is deposited in the Disse’s spaces, in various parts of the acinus. Globules may be phagocyted by the Kupffer cells [2]. In addition to amyloidosis, fibrosis of the portal spaces also develops, as well as inflammation in the portal spaces, irregularity of the border plate with ductular proliferation, and liver cirrhosis [4,5]. In chronic infections, amyloidosis has been identified after, on the average, 10 years, affecting mostly the kidney, liver, and gastrointestinal tract [6]. In intravenous heroin users systemic amyloidosis has been relatively rare, hepatomegaly has been a regular finding, hepatic amyloid distribution has been parenchymal and vascular, and cholestasis has appeared preterminally and in correlation with clinical status [7,8,9].

The wall of the liver sinusoids consists of a discontinuous basal membrane apposed by two types of cells: typical endothelial cells and fixed macrophages – Kupffer cells [10,11].

Stellate Kupffer cells are fixed hepatic macrophages, most numerous in the acinar zone 1. In addition to primarily phagocytic activity they also have a role in the secretion of endogenous pyrogens, collagenase, erythropoietin, cytokines, etc. [12]. They have a very high capacity for endocytosis, playing an important role in immune response [13,14].

Endothelial cells are flat, with a small, compact, elongated nucleus, plenty of micropinocytotic vesicles, few mitochondria, and even fewer rough endoplasmatic reticula. Endothelial cells are principally involved in pinocytosis, transcellular transport, and synthesis of extracellular matrix [13,15]. They have a very high capacity for endocytosis, being thus an important element in the mononuclear phagocytic system [13,14].

Studies have shown that the number of hepatic macrophages (Kupffer and endothelial cells) is reduced in alcohol-induced liver diseases and rises in chronic hepatitis cases [15,16].

Malignant neoplasms occurring under the action of chemicals are of hepatocellular type – these are rarely angiosarcomas originating in the sinusoidal endothelium [17]. Almost half of the cases of intravenous heroin users with hepatitis demonstrate abnormal liver function, indicating persistent infection, potentially contagious and apt to progress to cirrhosis, liver failure, and hepatocellular carcinoma [18].

Objective
Histologic and histochemical liver assays were implemented in this study in order to get a precise insight into the types of liver damage induced by heroin use, and by additive effects of alcohol and viral infections.

Methods
The study was performed on 40 autopsies of intravenous heroin users, and 10 control autopsies. There were 36 male and 4 female corpses aged 15-40 years, and among controls there were 8 male corpses and 2 female corpses aged 15-35 years. Heroin abuse was confirmed at autopsy (fresh and old injection marks) and by way of chemical-toxicologic assays of the organs and blood. The information obtained from the registry of the Department for Addictive Disorders, Center for Mental Health, were used, as well as the information obtained from close relatives before obduction. The information on the duration of intravenous heroin abuse, frequency of intravenous application, possible abstinence phases, intake of alcohol and/or psychopharmaceuticals were thus collected.

All autopsy cases were grouped according to the duration of heroin abuse into 4 groups: up to 2 years, 2-5 years, 5-10 years, over 10 years. During the morphometric analysis of sinusoidal hepatic macrophages, autopsy cases were grouped according to the predominant type of morphologic change identified with light microscopy analysis.
At autopsy, 3-5 liver samples were taken and fixed in 10% buffered formalin and processed to paraffin blocks in the automatic tissue processor. Paraffin tissue blocks 5μm thick were stained using the traditional hematoxylin-eosin (HE) method, Van Gieson, and Congo red.

Morphometric investigation of sinusoidal liver macrophages was done using the M 42 test system – an objective micrometer calibrated to the magnification at which the cells were counted (objective 40x; eyepiece 10x). The surface of the test system at that magnification was At= 0.058mm², and „chessboard“ method was used to count the cells (count in 10 fields). Out of the number obtained, the average number of sinusoidal liver macrophages per 1 mm² was calculated.

The ISPP statistical system was utilized for statistical analysis.

Liver tissue samples taken to be tested for ultrastructural changes were fixed in glutaraldehyde and epon-embedded. The analysis of sinusoidal macrophages, hepartocytic organelles, and collagen was done using the transmission electron microscope JEM 100 CX JEOL.

Research on the human cadavers was approved by Internal Ethic Committee, and conducted at the Institute of Forensic Medicine of Medical Faculty of Niš, Serbia.

Results

The presence of amyloid in this study was established in 9 cases (22.5%) (Figure A), situated in the portal spaces, mostly underneath the endothelium of smaller branches of the hepatic artery. In 2 cases, amyloid was found in the group of intravenous heroin addicts using heroin for 2-5 years, in 5 cases in those using heroin for 5-10 years, and in 2 cases in those with more than 10 years of heroin use (Figure B). In the present study, in 55.5% (5 out of 9) cases amyloidosis was associated with skin scarring in the cubital pits, as the consequence of skin infections occurring at the sites of heroin injection. Macroscopically, their livers were enlarged, pale, and tough.

Microscopically, applying a specific, histochemical method of amyloid staining (Congo red), amyloid deposits appeared orange. They were localized in portal spaces, predominantly underneath the endothelium of smaller branches of the hepatic artery, leading to the narrowing of their lumen (Figure 1). At the same time, amyloid deposits were also present in the stroma of the portal spaces in the form of homogenous, lumpy swellings, orange colored too. Intralobular localization of amyloids, along the connective tissue network, induced compressive atrophy of hepatocytes.

By way of micromorphometric investigation liver tissue lesions were identified, autopsy cases were grouped according to the type of identified morphologic changes, Kupffer and endothelial cells were counted, as hepatic sinusoidal macrophages, and the obtained results were statistically processed (Table 1).
Table 1. Number of hepatic sinusoidal macrophages related to hepatic morphologic changes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chronic active hepatitis</th>
<th>Chronic active hepatitis and cirrhosis</th>
<th>Chronic active hepatitis, cirrhosis and diffuse fatty change</th>
<th>Diffuse fatty change and cirrhosis</th>
<th>Other degenerative changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>–</td>
<td>332</td>
<td>439</td>
<td>458</td>
<td>337</td>
<td>334</td>
<td>350</td>
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<tr>
<td>±SD</td>
<td>13.75</td>
<td>75.58</td>
<td>40.50</td>
<td>49.26</td>
<td>38.09</td>
<td>42.61</td>
</tr>
<tr>
<td>t</td>
<td>3.95028</td>
<td>7.38226</td>
<td>0.2138</td>
<td>0.08702</td>
<td>1.5917</td>
<td></td>
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<tr>
<td>p</td>
<td>p&lt;0.01</td>
<td>p&lt;0.001</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
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</tr>
</tbody>
</table>

The results showed that the number of these macrophages was significantly higher compared to controls only in the groups of chronic hepatitis cases, regardless of the possible presence of concomitant cirrhosis, while other groups, in which alcoholic fatty changes were identified, did not demonstrate significantly higher numbers of these cells compared to controls. Figures 2, 3, and 4 illustrate hyperplasia and hypertrophy of hepatic sinusoidal macrophages.

In the three cases with vesicular and fatty changes complicated with viral hepatitis, out of which in two cases with cirrhosis too, there were severe dysplastic changes. These were localized in the middle acinar zone or in the border plate, and were characterized by a chaotic hepatocyte distribution and presence of polymorphic, enlarged, and hyperchromatic nuclei (Figure 5). Out of these three cases of dysplastic changes, two belonged to the group of intravenous heroin addicts with long-term (5-10 years) heroin use, and one of them used heroin intravenously for more than 10 years.

**Discussion**

In our study, amyloid deposits were identified in 9 cases (22.5%) in the hepatic artery branches in portal spaces, and they were associated with cubital pit scars as the consequence of skin infections at the sites of heroin application. Hepatic deposits of amyloid were absent only in the group of intravenous heroin users with duration of use below 2 years, and in 78% of cases (7 out of 9) the duration of intravenous heroin abuse was beyond 5 years. This finding further corroborated the findings of other studies [2,4,6,7]. Abscesses at the site of injection of heroin, cocain, and other drugs are the most common complication in this toxicomania type.
Authors have established that the infections are predominantly caused by anaerobic bacteria and, facultatively, gram-positive cocci [1].

In reactive systemic amyloidosis, long-term tissue destruction and inflammation is associated with SAA (Serum Associated Amyloid) elevation in the serum [1,10]. SAA is synthesized in the liver under the action of cytokines, primarily IL-6 and IL-1 [9]. One of the explanations of the onset of amyloidosis, in some individuals in the presence of inflammation, is perhaps an enzymatic defect disturbing the degradation of total SAA, due to which insoluble AA molecules are created [12].

In the present study, amyloidosis was associated with alcoholic fatty changes in 55.5% of cases, suggesting the possibility of reduced capacity of enzymatic systems in the presence of alcohol. Immune deficiency, macrophage number reduction, and infections account for the association of amyloidosis with more severe morphologic forms of liver damage and long-term heroin and alcohol abuse in 89% of cases, further contributing to already disturbed function of the liver.

In the studied groups with alcoholic fatty changes (three of the autopsy groups) there were no significant differences in macrophage number compared to controls. The suggestion has been made that the action of alcohol could preclude the activation of sinusoidal macrophages, confirming its immunosuppressive action [14]. By contrast, macrophages are activated in the cases with active hepatitis.

Dysplastic changes of hepatocytes were proven by the autopsies of the corpses of individuals who had a history of heroin abuse for more than 5 years, and in one case for more than 10 years. Since dysplasia precedes the onset of carcinoma [18], our results confirm the notion of the possibility of neoplasm in drug abuse. In a consideration of dysplastic changes additive actions (alcohol, viral infection) are more important, while the role of heroin is probably most prominent in the domain of its immunosuppressive action.

**Conclusion**

Long-term intravenous heroin abuse is a precondition for the occurrence of hepatic amyloidosis and dysplastic changes in the liver.

A statistically significant higher number of hepatic sinusoidal macrophages was found in the groups of cases with chronic active hepatitis, regardless of the possible concomitant cirrhosis, while other groups, in which alcoholic fatty changes were identified, did not show significantly higher numbers of these cells compared to controls.

In the studied autopsies, a high degree of association of amyloidosis with alcoholic fatty changes in the liver may indicate that the inactivation of hepatic sinusoidal macrophages and reduction of their enzymatic capacities in intravenous heroin abusers, due to additive, immunosuppressive action of alcohol, can be related to the disturbed SAA degradation and creation of insoluble AA fibrillar protein, making possible liver amyloidosis to occur.

Hepatocytic dysplasia is the sequel of more severe morphologic lesions of the liver in the form of vesicular and fatty changes, viral hepatites, and cirrhosis, and the consequence of action of alcohol and viral infections, together with immunosuppressive heroin eff.
References