The reduction of glycogen in the liver induced by chronic intravenous heroin abuse

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Abstract: Introduction. The liver plays a key role in the removal of lipophilic substances from the plasma, including both morphine and its derivative heroin. Intravenous heroin abuse leads to liver damages, so that the effects of heroin intake are the most marked and characteristic in the liver.

Objective. A histochemical and ultrastructural study of the liver, particularly hepatocyte glycogen content, should provide a precise insight into the type and degree of liver damage induced by intravenous heroin abuse.

Methods. The study included the analysis of 50 autopsies, 40 from the group of intravenous heroin abusers and 10 control autopsies. Paraffin sections, 5 µm thick, were stained by PAS method for deposited glycogen staining. The ultrastructural investigation was performed on transmission electron microscope.

Results. Glycogen amount was reduced proportionally to the severity and distribution of degenerative and necrotic hepatocytic lesions. Regarding deposited glycogen depletion in particular acinar zones, glycogen was most preserved in zone 1 (30% of studied cases), then in zone 3 (preserved in 25%), while the depletion was most significant in intermediary zone (preserved in 5%). In the intravenous heroin abusers group of up to 2 years glycogen was preserved in the acinar zones 1, 2 and 3 in 43%, 30% and 57%, respectively; in the group of over 10 years glycogen preservation in zone 1 was 25% and in other zones 0%.

Conclusion. Intravenously administered heroin directly influences glycogen reduction in the hepatocytes, and the effect is potentiated by morphologic changes in the liver due to intravenous heroin abuse. Glycogen depletion in the hepatocytes reduces energy reserves in these cells and causes cell death, which is an important segment of general liver injury in intravenous heroin abusers. The degree of reduction of glycogen depositions is proportional to the duration of intravenous heroin abuse.

Key Words: heroin, glycogen depletion, liver damage
consequential release into circulation. Hepatocytes can store glycogen in the amount of 5-8% of the liver cell. Glycogen molecules can be polymerized and the average molecular weight is around 5,000,000, and most of glycogen is precipitated in the form of solid granules. Transformation of monosaccharides into the high weight compound enables the storage of large amounts of carbohydrates in the cell, without significant change of the osmotic pressure of intracellular fluid [6].

Depletion of stored glycogen in ischemic conditions is evident. It is most marked in the peripheral acinar zones where the physiologic glycogen reserves are poor. The glycogen store depletion can be observed after 30-45 minutes ischemia, and it is conditioned by the characteristics of liver microcirculation. The difference in the degree of emptying of glycogen depositions is exclusively conditioned by the degree of metabolism of liver cells [7].

Depletion of glycogen depositions leads to energy reserve deficiency, cellular metabolic reactions cannot be supported and cell death occurs [8]. In fasting, glycogen is at first depleted from the acinar zone 1 and finally from the zone 3. When food is taken after fasting glycogen firstly appears in acinar zone 1 [6].

Hashiguchi et al. [9] studied the effects of intracerebroventricular cannula implanted for the application of morphine-sulphate and its metabolite morphin-6-glucuronide on the content of glycogen in the brain, liver and muscles. This application resulted in almost 36% reduction of hepatic glycogen, compared to simultaneous controls, but had no effect on glycogen content in the brain or muscles. Activation of opioid receptors in the brain results in the increase of hepatic glycogenolysis, but without any further effect on glycogen content in the brain.

**Objective**

A histochemical and ultrastructural study of the liver, particularly hepatocyte glycogen content, was to provide a precise insight into the type and degree of liver damage induced by IV heroin abuse, as well as to determine whether the degree of these lesions depends on the duration of intravenous heroin abuse.

**Material and methods**

The study included the analysis of 50 autopsies, 40 from the group of IV heroin abusers and 10 control autopsies (corpses of young and healthy individuals who died of mechanical traumas not involving the liver).

In order to facilitate the investigation all autopsies of IV heroin abusers were grouped into 4 groups according to the duration of IV heroin intake: up to 2 years, 2-5 years, 5-10 years, over 10 years.

During autopsies livers were sampled (3-5 samples per autopsy), fixed in 10% formaldehyde solution, processed in autotechnicon. Paraffin sections, 5 µm thick, were stained by PAS method for deposited glycogen staining. Glycogen content determination was performed semi quantitatively: its normal amount was marked (++), I degree reduction with (+), II degree reduction with (±), and total absence with (–).

Cellular organelles, collagen, macrophages and other structural changes were studied ultrastructurally. The liver extracts were fixed in glutar-aldehyde and the tissue was molded in epon. The investigation was performed on 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**

Glycogen in the form of dense, PAS positive granules was found in all hepatocytes (Figure 1). Its amount was reduced proportionally to the severity and distribution of degenerative and necrotic hepatocytic lesions. In early stages of heroin-induced damage glycogen was reduced in zone 2 (Figure 2), and later its depletion extended to both adjacent acinar zones – zone 1 and zone 3.

Regarding deposited glycogen depletion in particular acinar zones, glycogen was most preserved in zone 1 (30% of studied cases), then in zone 3 (preserved in 25%), while the depletion was most significant in intermediary zone (preserved in 5%) (Graph.1, Table 1).
**Figure 1.** Preserved glycogen depositions in the form of purple-red granules inside hepatocytes. PAS x 300

**Figure 2.** Severe glycogen depletion in acinar zone 2 and 3. PAS x 200
Evident glycogen depletion was found in macrodroplet-like phase of fatty change, for it was found only in perinuclear sickle halo of the cytoplasm, moved by fatty vacuole to the periphery together with the nucleus. Out of 11 cases with diffuse fatty change, in 6 cases glycogen was completely absent in one or two zones, in 3 cases there was II degree glycogen reduction in one or two zones, and in the remaining 2 cases there was I degree glycogen reduction in one or two zones.

Most serious glycogen depletion – up to its absence – is almost an ubiquitous finding in associated viral hepatitis. Glycogen absence was noted always in hepatocytes with viral hepatitis, if it occurred in the liver with already evident toxic-heroin induced and drug-alcohol induced changes.

<table>
<thead>
<tr>
<th>Drug abuse period</th>
<th>Acinar zones</th>
<th>Glycogen Preservation</th>
<th>I degree Glycogen Depletion</th>
<th>II degree Glycogen Depletion</th>
<th>III degree Glycogen Depletion</th>
</tr>
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<tbody>
<tr>
<td>Up to 2 yrs.</td>
<td>I</td>
<td>43 %</td>
<td>57 %</td>
<td>0 %</td>
<td>0 %</td>
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<tr>
<td></td>
<td>II</td>
<td>30 %</td>
<td>57 %</td>
<td>13 %</td>
<td>0 %</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>57 %</td>
<td>43 %</td>
<td>0 %</td>
<td>0 %</td>
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<tr>
<td>2-5 yrs.</td>
<td>I</td>
<td>19 %</td>
<td>50 %</td>
<td>31 %</td>
<td>0 %</td>
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<td></td>
<td>II</td>
<td>0 %</td>
<td>50 %</td>
<td>37 %</td>
<td>13 %</td>
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<tr>
<td></td>
<td>III</td>
<td>19 %</td>
<td>31 %</td>
<td>37 %</td>
<td>13 %</td>
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<tr>
<td>5-10 yrs.</td>
<td>I</td>
<td>38 %</td>
<td>8 %</td>
<td>31 %</td>
<td>23 %</td>
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<tr>
<td></td>
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<td>0 %</td>
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<td>38 %</td>
<td>31 %</td>
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<td>III</td>
<td>7.7 %</td>
<td>23 %</td>
<td>54 %</td>
<td>15.3 %</td>
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<tr>
<td>over 10 yrs.</td>
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<td>50 %</td>
<td>25 %</td>
<td>0 %</td>
</tr>
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<td>0 %</td>
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<tr>
<td></td>
<td>III</td>
<td>0 %</td>
<td>25 %</td>
<td>50 %</td>
<td>25 %</td>
</tr>
</tbody>
</table>

Table 1. The percentage of glycogen preservation and depletion in certain acinar zones in IV heroin abusers

Within the cirrhosis focus, glycogen depletion was proportional to the degree of degenerative-necrotic and regenerative hepatocytic changes.

In hepatocyte cytoplasm, glycogen depletion was observed ultrastructurally as well (Figure 3, 4).

In the IV heroin abusers group of up to 2 years, glycogen was preserved in the acinar zones 1, 2 and 3 in 43%, 30% and 57%, respectively; in the group of over 10 years glycogen preservation in zone 1 was 25% and in other zones 0% (Table 1).

**Discussion**

Direct action of IV administered heroin causes activation of opioid brain receptors, which results in an increase of hepatic glycogen lysis and reduction in hepatocyte glycogen content (9), but that reduction was more significant due to associated morphological findings, and in the cases with chronic active hepatitis and cirrhosis glycogen depletion was proportional to the degree of degenerative-necrotic and regenerative hepatocyte changes.
Figure 3. Chromatin condensation on the nuclear periphery, rare glycogen granules, dense mitochondrial matrix, reduced and partially degenerated RER. EM x 10 000

Figure 4. Evident depletion of glycogen or the glycogen’s absence in the hepatocyte cytoplasm. EM x 13 000
The highest degree of glycogen preservation in zone 1 can be explained by the closest contact of this zone with oxygen and nutrients from the blood [10].

With duration of IV heroin abuse glycogen depositions are being reduced. This agrees with the previously mentioned finding that glycogen reduction is proportional to the degree of morphologic hepatocyte changes (fatty changes, chronic active hepatitis, cirrhosis), the incidence of which was increased with longer IV heroin abuse [1, 2, 5].

Heroin action induced glycogen metabolism reduction [11], especially if hepatocytes were exposed to alcohol [12] as well. Therefore most evident glycogen reduction was one accompanying with dominant, diffuse, alcoholic, fatty hepatocyte changes, which was confirmed with electron microscope.

Reduction in glycogen depositions leads to depletion of energy reserves, making them insufficient to support cellular metabolic reactions with ensuing cell death [8].

Conclusion

Intravenously administered heroin directly influences glycogen reduction in the hepatocytes, and the effect is potentiated by morphologic changes in the liver due to IV heroin abuse. Glycogen depletion in the hepatocytes reduces energy reserves in these cells and causes cell death, which is an important segment of general liver injury in IV heroin abusers. The degree of reduction in glycogen depositions is proportional to the duration of IV heroin abuse.

References