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INFLUENCE OF HEROIN ON THE HEPATOCYTE GLYCOGEN CONTENT

SUMMARY

Direct action of i.v. administered heroin causes activation of opioid brain receptors, which results in an increase of hepatic glycogen lysis and reduction of hepatocyte glycogen content. However, that reduction is more significant due to associated morphologic findings, especially in the cases with diffuse fatty changes, and in the cases with chronic active hepatitis and cirrhosis, glycogen depletion was proportional to the degree of degenerative-necrotic and regenerative hepatocyte changes. Glycogen preservation is most significant in acinar zone 1, and the degree of reduction of glycogen depositions is proportional to the duration of i.v. heroin abuse.

Key words: **heroin, glycogen, liver damage**

INTRODUCTION

Hepatocyte is the main locus of biotransformational systems which through the action of their enzymes enable the metabolites of these compounds to be excreted from organism. During these processes toxic liver damage occurs, too, and intravenous heroin administration leads also to liver tissue infections (hepatitis, AIDS), ultrastructural hepatocyte changes occur etc., so that the effects of heroin intake are most marked and characteristic in the liver. Morphologic changes in the liver tissue are associated with its function disturbances, which results in altered metabolism of heroin and other toxins taken simultaneously (alcohol, drugs), so the effects of abuse of these substances are altered and often surprising (1, 2).

There is glycogen in the hepatocyte cytoplasm, since an important function of the liver is its synthesis (from glycose, milk or pyruvic acid or from glycerol), storing and decomposition with consequental release into the circulation (3).

Hepatocytes can store glycogen in the amount of 5-8% of the liver cell. Glycogen molecules can be polimerized 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 (4).

Depletion of stored glycogen in ischemic conditions is evident (5). It is most marked in the peripheral acinar zones where the physiologic glycagon reserves are poor. Grana (6) suggests in 1968 that glycogen store depletion can be observed after 30-45 minute 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.

Depletion of glycogen depositions leads to energy reserve deficiency, cellular metabolic reactions cannot be supported and cell death occurs (7).

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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 (3).

Hashiguchi et al. (8) studied the effects of intracerebroventricular cannula (ICV) (implanted for the application of morphin-sulphate (MOR) and its metabolite morphin-6-glucuronide (M_6G)) on the content of glycogen in the brain, liver and muscles. ICV resulted in almost 30% reduction of brain glycogen, and ICV-MOR in 36% reduction of hepatic glycogen, compared to simultaneous controls, but had no effect on glycogen content in the brain or muscles.

ICV M_6G demonstrated significant reduction of 50% of liver glycogen, but without effects on brain and muscular glycogen. Neither ICV-MOR nor ICV-M_6G did produce significant alteration of tissue glycogen content. The results indicate that stress associated with neurosurgical intervention (especially with ICV cannule placement) was accompanied by brain glycogen reduction. Activation of opioid receptors in the brain results in the increase of hepatic glycogenolysis, but without any further effect on the glycogen content in the brain.

AIMS

Micromorphologic, histochemical and ultrastructural investigations of the liver, as an organ most commonly seriously affected in heroin abuse, should enable precise insight into the type and degree of liver injury induced by i.v. heroin abuse, and whether the severity of these lesions depends on the duration of i.v. heroin application.

MATERIAL AND METHODS

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

Autopsy served as a proof of the status of i.v. heroin abuser (fresh and old injection scars), as well as chemical-toxicologic demonstration of heroin in the blood and organs, evidence from the Registry at the Department for Addictions, Mental Health Centre in Nis, and information from close relatives before autopsy at the Centre of Forensic Medicine in Nis. In the similar way, data was obtained on the duration of i.v. heroin abuse, frequency of heroin abuse, possible abstinency phases, alcohol intake and/or sedatives (benzodiazepine etc.).

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

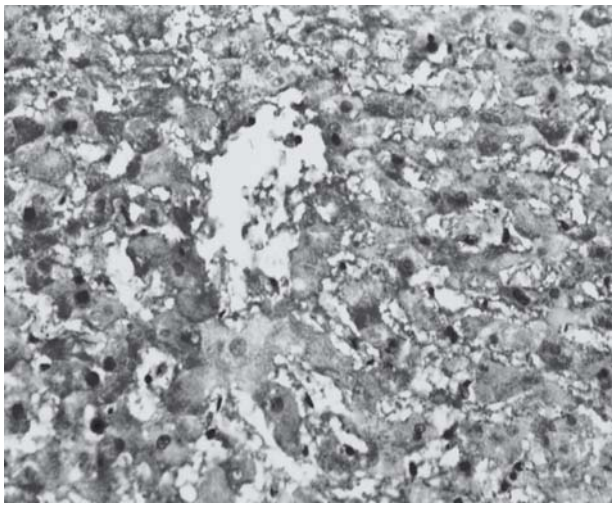
During autopsies livers were sampled (3-5 samples per autopsy), fixated in 10% formaldehyde solution, processed in autotechnicon. Paraffin sections, 5 μm thick, were stained by PAS method for deposited glycogen staining. Glycogen content determination was performed semiquantitatively: its normal amount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

Cellular organelles, collagen, macrophages and other structural changes were ultrastructurally studied. The investigation here was performed on transmission electron microscope JEM 100 CX JEOL.

RESULTS

Glycogen in the form of dense, PAS positive granules can be found in all hepatocytes *(Figure 1)*. Its amount is reduced proportionally to the severity and distribution of degenerative and necrotic hepatocytic lesions.

Figure 1. Preserved glycogen depositions in the form of purple-red granules inside hepatocytes. PAS x 300



In early stages of heroin-induced damage glycogen is reduced in zone 2 *(Figure 2)*, and later its depletion extends 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%) *(Figure 3)*.

Figure 2. Severe glycogen depletion in acinar zones 2 and 3. PAS x 200

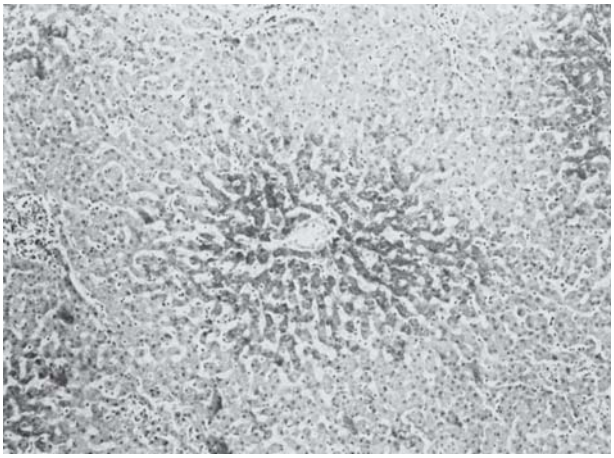
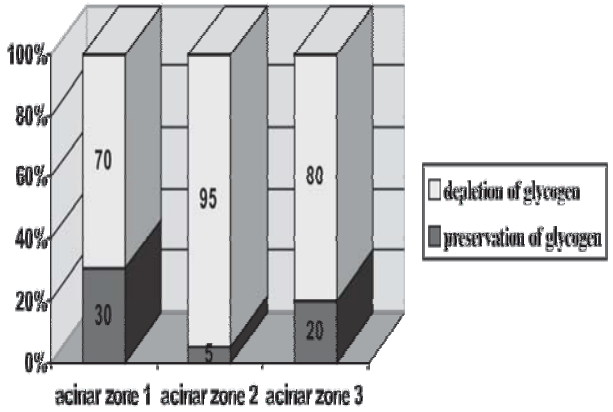


Figure 3. Preservation of glycogen in particular acinar zones



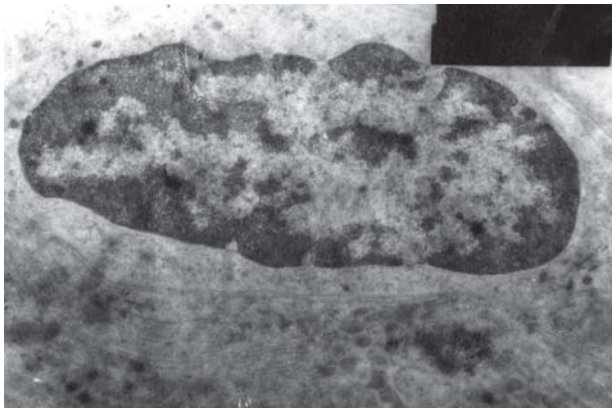
Evident glycogen depletion was found in macrodroplet-like phase of fatty change, since it was found only in the perinuclear sickle halo of the cytoplasm, which is moved by fatty vacuole to the periphery together with the nucleus. Out of 11 cases with diffuse fatty change, in 6 cases glycogen was completely 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.

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

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

Figure 4. Chromatin condensation at the nucleus periphery, rare glycogen granules, dense mitochondrial matrix, reduced partially degranulated RER. EM x 10.000



In the group of i.v. heroin abusers 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%.

DISCUSSION

Direct action of i.v. administered heroin causes activation of opioid brain receptors, which results in an increase of hepatic glycogen lysis and reduction of hepatocyte glycogen content, but that reduction was more significant due to associated morphologic 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.

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 (9).

With duration of i.v. heroin abuse glycogen depositions are being reduced. This agrees with the previously mentioned information 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 i.v. heroin abuse.

Heroin action induced glycogen metabolism reduction (10), especially if hepatocytes were exposed to alcohol, too. Therefore most evident glycogen reduction was one accompanying dominant, diffuse, fatty hepatocyte changes, which was confirmed with electromicroscopy.

Reduction of glycogen depositions leads to depletion of energy reserves, insufficient to support cellular metabolic reactions with ensuing cell death (7).

CONCLUSION

Intravenously administered heroin directly influences glycogen reduction in the hepatocytes, and the effect is potentiated by morphologic changes in the liver due to i.v. 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 i.v. heroin abusers. The degree of reduction of glycogen depositions is proportional to the duration of i.v. heroin abuse.

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UTICAJ HEROINA NA SADRŽAJ GLIKOGENA U HEPATOCITIMA

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SAŽETAK

Direktno delovanje intravenski unetog heroina uzrokuje aktivaciju opioidnih receptora u mozgu, što rezultuje povećanjem hepatične glikogenolize i smanjenjem glikogena u hepatocitima, ali je to smanjenje izraženo u znatno većoj meri, zbog pratećih morfoloških nalaza, naročito u slučajevima sa difuznim masnim promenama, dok je u slučajevima sa hroničnim aktivnim hepatitisom i cirozom deplecija glikogena proporcionalna stepenu degenerativno-nekrotičnih i regenerativnih promena na hepatocitima. Najveća očuvanost glikogena je u zoni 1 acinusa, a sa porastom dužine staža intravenske aplikacije heroina smanjuje se i količina deponovanog glikogena.

Ključne reči: **heroin, glikogen, oštećenje jetre**