# Level of Liver Fibrosis and Immune Status of Mice of Different Age after Heroin Treatment and Long Abstinence

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Young and middle-aged CBA mice were injected with "street" heroin in increasing doses for 14 days. Volume density of perisinusoid argirophilic fibers increased in both age groups (the increase being more pronounced in middle-aged mice), while the levels of spontaneous, LPS- and ConA-stimulated splenocyte proliferation decreased in young mice. Six months after heroin discontinuation further progress of liver fibrosis was observed in young mice.

**Key Words:** *heroin; liver fibrosis; age*

"Street" heroin, particularly poorly purified, produces complex effects on the organism because of the presence of various admixtures (including inorganic). Opioids are biotransformed in the liver [9], opioid receptors are present on lymphocytes [3], and hence, the liver and the immune system are the main targets for heroin. Opioids induce a transient increase (followed by long depression) in the production of IL-1β, IL-2, TNF-α, and IFN-γ by mouse splenocytes [7] and suppress humoral immune response [10]. Long-term use of opioids decreases the $CD4^+/CD8^+$ ratio and increases the risk of infections, the immune disorders persist for a long time after discontinuation of narcotics [3]. Morphological changes in the liver in children with heroin narcomania remain little studied.

## MATERIALS AND METHODS

Heroin addiction was modeled using "street" heroin selected at random from confiscated material from illegal turn-over, obtained in accordance with the decision of district court in Novosibirsk. The composition of the narcotic (according to conclusion of criminalistic expert evaluation of State Administration for Internal Affairs, Novosibirsk Region) was as follows: 19.2% diacetylmorphine (heroin), monoacetylmorphine; 3.6% acetylcodein and morphine; chloroquine, NaCl, and sugar. The preparation was water-soluble light-brown powder.

Young (6-7 weeks) and middle-aged (8-12 months) male CBA mice intraperitoneally received ascending doses of heroin (0.06 to 0.70 mg per animal) twice daily for 2 weeks (total dose 7.8 mg/mouse) in 0.5 ml distilled water (heroin addicts). Two groups of mice (young and middle-aged) received no treatment after the last heroin injection for 6 months (ex-addicts). Intact control group consisted of middle-aged mice.

Total volume density of argirophilic fibers (VDAF; morphometry, silver impregnation) in the liver was evaluated histologically. Splenic cells were cultured in round-bottom plates (Linbro) in a concentration of $300\times10^3$/well at 37°C at 5% $CO_2$ and 95% air in RPMI-1640 containing 10% FCS, 2 mM L-glutamine, and $4\times10^{-5}$ M 2-mercaptoethanol. The cells were stimulated with mitogens: ConA (2 μg/ml) and *E. coli* 055:B5 LPS (30 μg/ml; Sigma). Splenocyte proliferation was evaluated by $^3$H-thymidine

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incorporation into DNA. The results were statistically processed using Mann—Whitney test; the differences were considered statistically significant at $p<0.05$.

## RESULTS

Moderate fibrosis of the portal tract with the formation of short fibrous septae was observed after the end of treatment in mice of both age groups. Small solitary macrophagal granulomas and lymphoid infiltration with the minimum perifocal necrotic activity, focal hyperplasia of sinusoidal cells were seen in lobules. Hepatocytes were in a state of protein (moderate to pronounced) and small-droplet fatty degeneration. Silver impregnation showed perisinusoidal increase of VDAF, mainly in the pericentral zones; portal stroma looked rougher compared to that in intact animals. VDAF was 21% higher in middle-aged mice than in young animals (Table 1), which can reflect higher activity of enzyme systems metabolizing the narcotic and more pronounced immune response regulating collagenogenesis by the liver stellate cells. The latter fact is confirmed by the absence of significant immunosuppression in middle-aged mice treated with heroin, in comparison with young animals with spontaneous (by 43%) and LPS and ConA-stimulated splenocyte proliferation (by 35 and 15%, respectively) (Table 2).

Centrolobular fibrosis progresses in heroin addicts [2]; the volume of newly forming connective tissue was higher in ex-addicts than in addicts. Electron microscopy shows hypertrophic endotheliocytes and fibrosis of Disse spaces in heroin addicts [11]. The profibrogenic effect of addiction is mediated through the immune system: opioids inhibit production of the main antifibrotic cytokine (IFN-α) and suppress the response to it [4] and stimulate the production of profibrogenic TGF-1β cytokine by lymphocytes [8]. Long-term heroin abuse is associated with a decrease in mitochondrial content of glutathione (antioxidant), which augments hepatotoxicity of endotoxins, ethanol, TNF-α [7], promotes activation of Ito cells and production of collagen.

**TABLE 1.** Volume Density of Argirophilic Fibers (%) in the Liver of Mice with Modeled Heroin Addiction and Subsequent Abstinence Period

| Group | Control | Addicts | Ex-addicts |
|---|---|---|---|
| Intact | 9.1±4.3 | | |
| Young | | 16.7±3.6 | 24.8±6.7$^{+}$ |
| Middle-aged | | 20.2±5.6* | 16.8±5.1$^{**++}$ |

**Note.** *$p<0.001$, **$p<0.005$ compared to young animals of the same group; $^{+}p<0.001$, $^{++}p<0.005$ compared to addicts.

Unexpected changes in VDAF were detected in animals of different age groups after a long abstinence period: 17% decrease in middle-aged mice (which significantly surpassed the control; Table 1) and almost 50% increase after heroin discontinuation in young animals. Immunosuppression in young animals directly after discontinuation of heroin suggests more pronounced disorders in immune regulation of collagenogenesis in them with its persistent self-maintained activation in the liver in comparison with middle-aged animals with more mature immune system by the start of the experiment.

Clinical data indicate that the risk of cirrhosis of the liver is significantly higher in young people using ethanol in parallel with heroin, the majority (83%) of young patients with cirrhosis of the liver starting narcotic use in adolescence [6]. Our present experimental findings are in line with our previous data [1] indicating that heroin addiction in children with combined B+C viral hepatitis augmented portal and pericellular fibrosis in comparison with patients not using the narcotic, and that even short history of addiction (1-2 years) led to acceleration of fibrosis. The detected increase in the volume density of argirophilic fibers in young animals after discontinuation of narcotics can be due to inorganic admixtures in the "street" narcotics; these admixtures promote their phagocytosis and stable activation of liver macrophages and secretion of profibrogenic cytokines. Another possible mechanism of continuing fibrogenesis is narcotic-induced autosensitization by liver proteins: various autoantibo-

**TABLE 2.** Splenocyte Proliferation (cpm) in Mice with Modeled Heroin Addiction of Different Age ($M\pm m$)

| Group | Spontaneous | LPS-stimulated | ConA-stimulated |
|---|---|---|---|
| Intact ($n$=9) | 3000±800 | 24 300±2200 | 31 300±5200 |
| Young ($n$=18) | 1700±870* | 15 900±3800* | 26 600±4700* |
| Middle-aged ($n$=18) | 3600±590$^{+}$ | 19 600±1900$^{*,+}$ | 31 300±4400$^{++}$ |

**Note.** $n$: number of investigations. *$p<0.001$, **$p<0.02$ compared to intact animals, $^{+}p<0.001$, $^{++}p<0.005$ compared to young animals.

dies and systemic autoimmune manifestations are detected in heroin users [5].

These results indicate that intraperitoneal heroin narcomania in young mice leads to more severe consequences than in adult animals, activation of collagen-producing cells persisting even during complete long abstinence. Progressive perisinusoidal fibrosis leads to liver dysfunction, specifically detoxication dysfunction, which suggests (under conditions of more pronounced immunosuppression in young animals) more serious aftereffects of narcomania in childhood even after discontinuation of heroin use.

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