Original article

The effect of nalbuphine administration on the rat liver ultrastructure

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Abstract

Introduction. Opioid analgesics remains irreplaceable in a pain control therapy. On another hand, side-effects including not only potential addiction and withdrawal but morphological changes in different organs, which can lead to their dysfunction. The current study aimed to evaluate the long-term effect of nalbuphine hydrochloride on rat liver ultrastructure. Materials and methods. The experiment was carried out on white male rats 130-210 gr. body weight. Animals were injected by nalbuphine hydrochloride every day during the 6-weeks experiment with weekly dose increasing. Liver samples for TEM were taken at the end of every week. Conclusion. These finding indicated that long-term nalbuphine administration causes changes in hepatocytes, liver sinusoids and their components and organelles. These changes affect hepatocytes mitochondria, first of all, and becomes well-defined at the 3-rd week of experiment with the later development of the deep changes of hepatocytes and liver sinusoids.

Key words: opioid, liver, nalbuphine

Introduction:

Nalbuphine hydrochloride is a nonscheduled potential analgesic, which belongs to the group of agonist-antagonist opioid receptors, widely used in different branches of medicine (1). The increasing popularity of nalbuphine in our country is due not only to its efficiency as a pain-killer and accessibility as a nonscheduled drug but because of using with non-medical purpose too (2). The data about an abuse potential of nalbuphine and social and medical problems, related to these questions, were frequently raised up in the literature (3-5).

On another hand, numerous data demonstrated different side-effects, caused by a long-term nalbuphine administration, including morphological changes of different organs (6-10). Liver, kidney, and heart are first organs, aimed by opioids in a case of a long-term administration (11-13). In particular, well-expressed changes in rats liver microstructure under the routine light microscopy, as well as changes of biochemical parameters were revealed in our previous studies (14, 15). With the purpose of better understanding the key mechanism of these changes, the transmission electron microscopy was planned as the next stage of our project. The disturbance of microcirculation and hepatocytes dystrophy were marked previously as are the key moments in liver pathology as consequences of nalbuphine administration. But pathophysiology of these changes remains uncertain. Thus, the current study aimed to evaluate the long-term effect of nalbuphine hydrochloride on rats liver ultrastructure, in particular, to obtain a new data about liver sinusoids endothelioocytes and hepatocytes changes, using electron microscopy.

Material and methods:

The experiment was carried out on 53 male white rats 130-210 gr. body weight. The animals were divided into the 3 groups – experimental group (30 animals), control group (18 animals) and 5 animals to study the norms before the experiment. The experiment was conducted in accordance with the provision of the European Convention for the protection of the vertebrate animals used for the experimental and another scientific purpose from 24.11.1986 and the approved by Ethical Committee or Institutional Animal Care and Use Committee Approval, protocol №2 from 20.02.2012. The animals of the experimental group were daily injected by nalbuphine hydrochloride according to scheme what were proposed by Onysko and co-authors (16) with weekly dose increasing from 8 mg/kg body weight to 35 mg/kg body weight. Control group animals were injected daily by 0,5 ml saline. Liver samples (less than 1 mm in each direction) were taken at the end of every week after the intraperitoneal injection of sodium
thiopental (25 mg per 1 kg body weight). After immediate fixation in 2.5 % buffered glutaraldehyde, the samples were prepared for the electron microscopy following standard procedure \(^{(17,18)}\). For studying and photographing of the samples a microscope UEMV – 100K was used at an accelerating voltage of 75 kV and magnification range x2000-x15000.

**Results:**
After the 1-st week of the experiment there no significant changes were found in the experimental group, compared to control group. Rarely occurs swelling of mitochondria with decreasing they cristae in numbers. After the 2-nd week of experiment ultrastructural changes becoming better expressed. In particular, glycogen depletion, swelling, and vacuolization of hepatocytes and endotheliocytes cytoplasm with small peroxisomes and lysosomes were revealed. Swelling of mitochondria and their cristae with focal cristolysis occurs more frequently, moreover, regularly occurs elongated mitochondria with an irregular shape.

Revealed uneven nuclei of parenchymal cells with swelling of nucleoli and chromatin fragmentation. In some endotheliocytes nuclear heterochromatin adjacent to their external border, their cytoplasm fragments are swollen and local interruption of endothelial lining occurs, which leads to direct contact with parenchymal cells to a sinusoidal lumen (larger in size, than normal fenestrations of sinusoidal lining).

Bile canaliculi remain unchanged with well-expressed microvilli. Microvilli of lumenal surface of parenchymal cells with swelling of cytoplasm are fragmented or pure-developed and space of Disse, adjacent to swollen fragments of cellular cytoplasm, are dilated.

![Image](image.png)

**Pic.1. Hepatocytes after the 2-rd week of the experiment.** 1 – mitochondria with focal cristolysis, 2 – swelling of cytoplasm, 3 - smooth endoplasmic reticulum, 4 - rough endoplasmic reticulum, 5 – lysosomes with dense body inside, 6 – nuclei, 7 – nucleoli, 8 – junction between hepatocytes.

After 3-rd and 4-th weeks of experiment enhanced of previously observed changes was found. Revealed a lot of hepatocytes with enlightened, partial and total swelling of cytoplasm, numerous small and large peroxisomes and lysosomes with varying density and diameter, autophagosomes with parts of organelles, swelling and cristolysis of mitochondria with enlightened matrix, fragmentation and denudation of rough endoplasmic reticulum. The cisterns of smooth endoplasmic reticulum are dilated and fragmented. Indicated a lot of mid-size and small fat-storing vacuoles. Some of the mitochondria are with flocculated matrix and dense deposits inside. Apoptotic bodies occur with
the fragments of cytoplasmic organelles and nuclei. Uneven nuclei of parenchymal cells with clumping of chromatin and swelling of nucleoli (or without nucleoli) also observed. Dense deposits indicated within the bile connection between neighboring parenchymal cells, some of the bile canaliculi are occluded by dense deposits (microvilli usually are absent inside them, or are small and fragmented), some of them are narrowed. Endothelial lining of the sinusoidal wall has local thickening because of swelling of cytoplasm. Besides, the cytoplasm of endothelial cells (usually, in peripheral processes) included numerous vacuoles and lysosomes, various in size and density. The central part of endothelial cells remains unchanged, but the nuclei usually irregular in shape with chromatin condensation closer to the nuclear envelope. The red blood cells and the platelets are observed in the sinusoidal lumen with their adhesion to the luminal membrane of the endoteliocytes. Because of these, the sinusoidal lumen is often narrowed or even occluded, moreover, in some sinusoidal capillary, the blood clots were observed with total sinusoidal congestion.

Pic.2. Hepatocytes after the 3 weeks of the experiment. 1 – swelling of cytoplasm, 2 – junction between hepatocytes with dense deposits, 3 – mitochondria, 4 – Golgi apparatus.
After the 5-th and 6-th week of the experiment, in addition to above-mentioned changes, some new changes were observed. The mitochondria are various in size, shape, and density, sometimes with dense deposits. There were numerous worm-like, hook-like and amoebian-in-shape mitochondria. Their cristae are various in size and direction, and a lot of the mitochondria with cristolysis or even without cristae. In the hepatocytes cytoplasm numerous small and large vacuoles, with varying density and diameter, short profiles of rough endoplasmic reticulum, numerous fat-storing vacuoles and complete absence of glycogen granules were revealed. The nuclei of parenchymal cells are irregular in shape with chromatin fragmentation, thickening of the nuclear envelope and dense deposits between the two layers of membrane. Often there are nuclei with pyknosis or even karyorrhexis. Most remarkable differences as compared to the previous terms are occasional thick bundles of collagen fibers within the space of Disse, numerous blood clots, platelets and erythrocytes congestion and their adhesion to the luminal surface of endoteliocytes. The sinusoidal diameter becomes remarkable smaller, and in addition to previously mentioned changes, these lead to total occlusion of some sinusoids. The endoteliocytes have a different thickness – in some place, the endothelial lining is thinner, in another – thicker because of swelling, with cytoplasmic processes faced into the sinusoidal lumen. A lot of area of the sinusoidal walls with fragmented or even without the endothelial lining, with a big area of direct contact between the parenchymal cells and the sinusoidal lumen. The space of Disse is uneven - narrowed often, with the fragments of basal lamina-like material in these areas and the lack of membranous microvilli of parenchymal cells. On another hand – a lot of area with dilated space of Disse and numerous cytoplasmic processes, which are varying in diameter, direction, and shape.
Pic. 4. Occlusion of the liver sinusoid with platelets adhesion to the wall of sinusoid. 1 – swelling of central part of endotheliocytes, 2 – sinusoidal lumen, 3 – platelets adhesion, 4 – peripheral part of endotheliocytes, 5 – hepatocytes, 6 – narrowed space of Disse with collagen fibers.

Pic. 5. Big and elongated mitochondria with cristolysis (1) of hepatocytes after 6 weeks of experiment. 2 – lysosomes, 3 – junction between hepatocytes.

Pic.7. Big and elongated mitochondria with cristolysis (1) of hepatocytes after 6 weeks of experiment.

Discussion:
Thus, the pathophysiology of these changes, caused in the rat’s liver by the nalbuphine administration, can best be described as a mitochondrial and microcirculatory distress syndrome with parenchymal cells apoptosis development. The key point in the pathophysiology is the changes in mitochondria caused by POL system disturbance because of opioid administration. Opioid group’s agents caused different changes in the POL system and the mechanism of these processes depending on the chemical structure of the
particular drug (19–21). A critical level of mitochondria changes because of the POL system disturbance can initiate parenchymal cells apoptosis, which was revealed as the changes in hepatocytes nuclei morphology. Most researched compound from opioid group is the morphine. But the experimental morphine administration more often caused rat’s liver lipid dystrophy, as one of the most prominent signs, whereas nalbuphine caused swelling and the pattern of hydropic dystrophy. The local fat-storing vacuoles also observed at the ultrastructural level in a case of nalbuphine administration, but it does not lead to the well-expressed lipid dystrophy (steatosis), as in a case of morphine administration (12). Thus these indicate that they have a different mechanism of POL system disturbance and pathology development.

Moreover, we can suggest, that nalbuphine has an ability to block the peroxide because of “scavenger” syndrome, similar to that was found in morphine in-vitro. It is only one way to explain why the increasing of nalbuphine’s doses does not lead to dramatic changes no at the microstructural, neither at the ultrastructural level.

**Conclusion:**
The nalbuphine administration causes well-expressed changes on the ultra-structure of the rat liver. First of all, it’s manifested as changes in the mitochondria shape, size and cristae design and numbers. Besides, there were changes in the nuclei of parenchymal cells – they becoming uneven in shape, with chromatin margination and fragmentation, what can indicate the beginning of apoptosis. Moreover, changes in the structure of microvascular flow occurs – changes in the endothelial lining of hepatic sinusoids, occlusion of the sinusoidal lumen, erythrocytes and platelets aggregation to the luminal surface of endoteliciocytes.

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