Pathomorphology of fetal and mature liver under the lead intoxication and after the correction: the review of experimental data

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Lead is one of the most widespread pollutants which alters the mature liver and is especially harmful for the fetal liver. The typical alterations in the mature liver after the lead exposure are the hypertrophy and vacuolization of hepatocytes, circulatory disorders, mononuclear cellular infiltration. The morphological changes in the liver during prenatal development under the maternal lead treatment are inhibition of hematopoiesis, dystrophy of hepatocytes, disturbances in the liver architecture, its vessels and stroma with a gradient of pathological changes toward the peripheral parts of the organ. The manifestations of liver alteration after birth become deeper with age and develop to the necrosis, edema and inflammation. The biochemical disturbances in the liver are in the decrease in the activity of enzymes of energy metabolism, inhibition of protein and nucleic acids synthesis, imbalance of the lipid peroxide oxidation system, and the increasing of oxidative stress with the further alteration of the membranes of the endothelium, red blood cells, hepatocytes, as well as mitochondrial membrane.

The changes in expression of the immunohistochemical markers can differentiate the processes in the liver, which are relatively stable or sensitive to lead, especially in prenatal development, whereas the biochemical parameters are valuable for estimation of the liver damage in postnatal life. The immunohistochemical changes under the lead treatment reflect the inhibition in protein synthesis such as albumin and cytokeratins, as well as growth factors, nitric oxide synthases and matrix metalloproteinases expression; whereas the expression of apoptotic markers increases. The search for the natural products, dietary supplements and drugs with the protective properties is ongoing and covers the wide spectrum of agents, including vitamins, micro- and macroelements, antioxidants, chelating agents, natural extracts, proteins, sorbents and complex-producing drugs. The immunohistochemical markers as well as biochemical parameters can be used to prove the efficacy of protectants for chronic lead intoxication.

Key words: fetal liver, mature liver, pathology, heavy metal poisoning, therapies investigational.

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Патоморфологія плодової та зрілої печінки при інтоксикації свинцем і в умовах корекції: огляд експериментальних даних

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Головні слова: печінка, патологія, отруєння важкими металами, методи лікування.

Патоморфологія плодової та зрілої печінки при інтоксикації свинцем і в умовах корекції: обзор экспериментальных данных

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Использование иммuno histoхимических маркеров позволяет различать процессы в печени, которые относительно устойчивы или чувствительны к свинцу, особенно в пренатальном развитии, тогда как биохимические параметры более подходят для оценки повреждений печени в постнатальном периоде. Иммунологические изменения в печени под влиянием свинцовых соединений отражают ингибирование синтеза белков, таких как альбумин и цитокератин, а также экспрессию факторов роста, синтеза оксида азота и усиление экспрессии апоптотических маркеров. Поиск натуральных продуктов, пищевых добавок и препаратов с защитными свойствами продолжается и охватывает широкий спектр веществ, включая витамины, микро- и макроэлементы, антиоксиданты, хелатирующие агенты, нитраты и комплексные препараты. Иммунологические маркеры, как и биохимические показатели, могут быть использованы для доказательства эффективности протектантов, которые применяются при хронической интоксикации свинцом.
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Review

[3,10,25]. There was the intralobular necrosis of the liver tissue accompanied by the perportal macrophage-lymphocytic infiltration along the interlobular veins. The venous congestion, sludge of red blood cells, damage of the wall of the interstitial veins and arteries were observed. The necrosis of the hepatic parenchyma, which became multilobular, had been exacerbated by the end of the second month. The type of necrosis was coagulatory or colliquated. The vascular disorders included the disseminated erythrocyte sludge in sinusoid capillaries, stasis and hemolysis in central veins [10,25].

The deep tissue alterations like mentioned before has never been observed in the fetal liver [2,5]. The dystrophic phenomena as a change in the density of the cytoplasm of hepatocytes occurred from the 11th embryonic day in the lead treated embryos. The inhibition of the synthesis of acid glycosaminoglycans which are essential for the normal development of the connective tissue in the embryonic liver up to end of the prenatal period was also observed [5,7]. The earliest sign of the suppression of acid glycosaminoglycans synthesis was their total absence between the endothelium of sinusoids and hepatocytes. The acid glycosaminoglycans provide the processes of cell migration, and thus affect the formation of tissue architecture [6]. The further tissue decomposition in the fetal liver under the lead treatment might be due to decrease in acid glycosaminoglycans concentration. These matrix components are normally present subcapsularly in the liver and in the places where the hepatic parenchyma contacts with the mesenchyma of the body. The acid glycosaminoglycans are believed to be the conductor for cell migration of mesenchyme from the trunk and septum transversum to the liver [7]. The inhibition of the migration of these cells may have consequences in the misdevelopment of the liver vessels and the general disturbance in the tissue architecture.

The reduction of glycogen content in hepatocytes of the fetal liver was observed after maternal administration of the lead [2,25]. Lead was also described as an agent that stimulated the proliferation of stellate cells (Ito cells) in the rat fetal liver, which can be detected by the marker of α-smooth muscle actin (αSMA) and were mostly located along the sinusoids [6]. The concentration of these cells had increased since the 14th embryonic day till the end of fetal period and seemed to be maximal in the areas with the severe dystrophic alteration of the liver of lead treated fetuses [9]. The fibrotic changes in the liver of lead treated fetuses were accompanied by the accumulation of Ito cells. αSMA-positive cells were also observed in the walls of the blood vessels of the intact or altered areas [21].

Other immunohistochemical markers enable to give an additional piece of information about molecular changes in the liver of lead treated fetuses. On the 16th embryonic day, a part of parenchymal cells in the intact liver which were positive on albumin could belong to populations of bipotential cells [6], but later the further increase in the number of hepatocytes was observed, so after the 16th day this bipotential population produced cells of the liver parenchyma only [9]. The immunohistochemical researches showed that lead caused the inhibition of albumin synthesis in hepatocytes [9]. This protein is the main marker of maturation of the liver cells. The inhibition of albumin production indicates that lead impacts the most stable molecular processes in hepatocytes, and, perhaps, suppresses the differentiation of hepatocytes from bipotential cell population. The general degenerative phenomena in the liver parenchyma were correlated to the decrease of cytokeratins AE1/AE3 expression [21].

The lead impact on the vascular endothelial growth factor (VEGF) expression in the fetal liver resulted in the non-uniform pattern of immunostaining in the liver parenchyma, whereas the VEGF expression was intact in the stroma [21]. The increase in the number of the caspase-3-positive nuclei was observed in the liver epithelium under lead treatment. The alteration of the stroma was accompanied by the strong caspase-3 expression in the nuclei of stromal cells and the decrease in matrix metalloproteinases MMP-1 and MMP-9 expression [21]. The significant impact of the lead on the vascular endothelium was revealed in eNOS expression. Lead exposure stimulated the production of the inducible form of enzyme. After the maternal exposure before pregnancy and ongoing treatment in early prenatal development, the eNOS expression was weak in all the types of vessels and the suppression of enzyme activity was stable till the end of fetal period [9].

The decrease in the specific hepatocyte volume in the rat fetal under the maternal lead exposure is another possible cause of the disassembly of the liver tissue [5]. The most severe consequences of lead treatment were observed for the liver hematopoietic cells. The inhibition of the proliferation of hematopoietic cells was almost total. The rapidly proliferating population of blood cells may be the most vulnerable to the impact of toxicant due to hypoxia, which is a result of vascular disorders [2,14].

The tissue pathology in the rat fetal liver showed the clear gradient pattern with the increase of the morphological changes towards the peripheral parts of organ [5]. It can also be explained by the gradient of vascular damage in the liver. The dystrophic changes had rapidly transformed into partial necrosis and fibrosis of hepatic parenchyma up to the end of the fetal period. The vascular network was well developed in the areas with the replacement of the liver parenchyma by the fibrous tissue [5]. There was not the lymphohistiocytic infiltration in the liver parenchyma of lead treated fetuses, which was a regular finding in the liver of the lead exposed rat in postnatal life [24]. The lack of the immune reply in the fetal liver is a result of immaturity of the immune system of the fetus and its inhibition by the toxicant [14].

The dystrophic changes in hepatocytes under the lead treatment during the fetal period are dependent on terms of toxicant administration. There were much more pronounced morphological alterations in the liver if the maternal lead exposure had lasted a few weeks before pregnancy [5], than with only lead treatment after fertilization or later [10]. In this case the lead accumulation does not occur in the mother’s body. There is evidence that the lead administration before pregnancy can negatively
affect the development of embryo, because the pregnancy causes a release of lead ions from the maternal organs, which are depots of lead [10]. The largest amount of lead comes through the placental barrier when the placenta starts to form. When the lead is getting into the body of pregnant females, the significant morphological changes in the placenta are being observed, which is a reason for the further damage of the internal organs of the fetus, in particular the liver and kidneys [10]. Pregnancy, as a stress factor, increases the absorption of lead. Thus, the damage of the fetal liver in the experiments with lead administration before pregnancy is precisely caused by the previous accumulation of lead in the maternal organs and its further release from the depots during the early period of pregnancy [2,5].

The search for the drugs with the protective properties to lead has been carried out along with the studies of lead toxic effects. The antioxidants, chelating agents, sorbents and complex-producing drugs were proposed for the prevention and treatment of chronic lead intoxication. It is proved that the lead negative impact increases with low concentrations of minerals, micro- and macroelements in the diet [2]. The reducing of the lead absorption from the gastrointestinal tract in the presence of other metals or minerals can be explained by the competitive interaction. In comparative studies of complex-producing protectant as casein and sorbent as charcoal it was proved that the first had more positive effect for the level of liver enzymes [23].

The antioxidants compose the group of drugs which has been investigated the most. Selenium and α-tocopherol showed the positive effect for the level of aspartate and alanine transaminases, total protein, urea, creatinine, superoxide dismutase and glutathione, as well as the lipid peroxidation index [33]. Some drugs like morpholine salt of titanic acid with membrane stabilizing and antioxidant properties and sulfur-containing products improved hepatocytes morphology and biochemical parameters [1]. The use of α-tocopherol-containing natural products like cod liver oil for the correction of chronic lead intoxication caused the moderate membrane-protective effect in hepatocytes, the conservation of mitochondria and the maintenance of their proliferation [14]. The oxidative stress induced by lead caused the increase in reactive oxygen species, total protein carbonyl content and lipid peroxidation products in the rat liver, whereas the administration of Moringa oleifera seed powder restored all the parameters in the organ and the blood [12]. It has been proved that the caloric restriction prevented partly the harmful impact of lead and restored some parameters which depended on the liver function: the activity of glutathione peroxidase, superoxide dismutase, the concentration of malondialdehyde and tumor necrosis factor; the biochemical findings were supported by the histological studies which pointed to the attenuation of the inflammatory processes in the liver [13].

The positive shifts in the metabolic profile of lead treated rats occurred after addition of artichoke extract and vitamin C to the diet. The significant decrease of the serum lead, alanine and aspartate transaminases, alkaline phosphatase and malondialdehyde and improvement of the lipoprotein profile were observed [24]. Less pronounced degeneration of the liver parenchyma and reducing of vessels congestion were observed after the administration of protectants. The diminution of lymphocyte infiltration in the liver was the morphological evidence of the protective effect. It was proved that green tea extract [31] and extract of rosemary [32] had the positive effect for the level of the liver enzymes and serum protein as well as for the liver morphology. It has been found that the natural flavonoids prevented the tissue damage caused by lead due to maintaining of the activity of eNOS and inhibition of apoptosis [29]. According to the group of researchers [28], in the liver these agents can smooth the manifestations of oxidative stress and reduce hyperlipidemia in lead treated animals. The increase of 8-hydroxydeoxynucleosine as a marker of the DNA-damage induced by lead as well the caspase-3 activity in the rat liver was effectively suppressed by puerarin (flavonoid), so lead-induced apoptosis might be reversible [29].

The search for the natural products, dietary supplements and drugs, which can improve the state of liver in case of intoxication with heavy metals, is ongoing. Extract of tomatoes (Lyopersicon esculentum) [15, 21], and garlic (Allium sativum) [17], have significant hepatoprotective effect, but it was not the same for different metals. The highest hepatoprotective effect of extract of tomatoes was to cadmium and mercury and it showed the least protective property for lead [15]. The same tendency was revealed for the garlic extract and Garcinia kola extract [17,18]. The most toxic effect of lead for the liver among other heavy metal was also proved when the Ground Zingiber officinale or palm oil were used as the protectants and they were the least beneficial for lead compared to cadmium and mercury [16,19].

The supplementation of natural products like spirulina or dandelion during pregnancy diminished the signs of oxidative stress, restored the level of hepatic DNA, mRNA and protein, and eliminated the inhibition of the antioxidant enzymes in the liver induced by lead [20, 34]. The powder of wine yeast Saccharomyces vini administered before and during pregnancy showed the significant protective effect for the restoring of the blood cell population in the fetal liver; this result can be explained by its antioxidant effect. This protectant could also inhibit the absorption of lead and thus reduced the level of a toxicant in the maternal blood [5]. The microelements contained in powder of wine yeast Saccharomyces may also have a partial shielding effect, as it was shown in studies with the addition of calcium pectates in the diet; the concentration of lead in the blood of experimental animals was reduced [27]. Coenzyme Q10, which is capable to increase the cellular energy resources, exhibits antioxidant effects and supports cellular enzymatic systems; it had a protective effect for rat fetuses survival and liver parameters of lead treated fetuses [4,5]. The morphological and immunohistochemical findings proved that the coenzyme Q10 was less protective compared to wine yeast Saccharomyces vini, perhaps, because it did not affect the absorption of lead from the gastrointestinal tract [9]. Lycopene (pigment of tomato) as a protectant for lead treated fetuses restored partly the AE1/AE3 expression in the liver, and also caused the decrease in caspase-3 expression, but did not change VEGF, MMP-1
and MMP-9 expression. The positive effect was observed for the quantity and distribution of sSMa-positive cells [21]. All the manifestations of morphological pathologies were smoothed with the correction of wine yeast Sassharamyces vini, but not of coenzyme Q10. Both of protectors can partially mask the influence of lead and reduce the suppression of the activity of eNOS [9]. Some vessels of medium caliber saved the sufficient level of eNOS activity regardless of the degree of impairment of hepatic parenchyma, whereas specific hepatocyte volume did not change significantly after the addition of protectants [9].

Conclusions

1. The morphological changes in the liver under the lead exposure are different in prenatal and postnatal period. The main pathological features in the fetal liver are inhibition of hematopoiesis, dystrophy of hepatocytes, disturbances in the liver architecture, vessels and stroma, all the alterations become deeper after birth. The typical morphological changes in the mature liver after the lead exposure are the hypertrophy and vacuolization of hepatocytes, circulatory disorders, signs of inflammation; their expression is dependent on terms of toxicant administration.

2. The changes in expression of the immunohistochemical markers can differentiate the processes in the liver, which are relatively stable or sensitive to the lead, especially in prenatal development. The most vulnerable processes are the synthesis of the proteins, expression of markers of apoptosis, growth factors and enzymes.

3. The search for the natural products, dietary supplements and drugs with the protective properties is ongoing and covers the wide spectrum of agents. The immunohistochemical markers as well as morphological, histochemical or biochemical parameters can be used as testimonies to prove the efficacy of protectants.

Prospects for further research. The further development in this direction involves the search of new drugs with more protective effect, and broadening the range of the morphological, histochemical, biochemical and immunohistochemical methods to confirm the effectiveness of the agents with protective properties.

Conflicts of interest: authors have no conflict of interest to declare.

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