Morphology of fetal liver under the influence of silver and gold citrates on a background of lead intoxication in rats

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Abstract

Background: Pregnant women and children are especially sensitive to lead exposure. Natural trace elements with bio antagonistic properties against lead are known. However, liver organogenesis under combined influence of silver and gold citrates on a background of lead intoxication isn’t studied enough.

Methods: the aim of present study was to identify morphological state of fetal liver under combined influence of silver, gold and lead acetate.

Results: combined influence of silver citrate and lead acetate caused increase of fetal liver weight and number of hepatocytes, while the relative area of blood vessels and number of hematopoietic cells were reduced.

Conclusions: morphological state of fetal liver was improved by injection of silver citrate on a background of lead intoxication; oxidative stress was decreased; processes of maturation and differentiation of liver cells were improved.

Keywords: Fetal liver, silver citrate, gold citrate, lead acetate.

1. Introduction

Lead is a natural toxic metal, which is a part of the crust. This metal is widely used in industry that has led to global environmental pollution and disorders of public health in many countries of the world. There are a lot of metabolic disorders of various organs of the human body, which occurs as a result of lead intoxication. Lead accumulates in bones, causes the damage and increases risk of osteoporosis [1]. Pregnant women and children are especially sensitive to lead exposure. Influence of high level of lead during pregnancy causes miscarriage, stillbirth, premature birth, low birth weight and anomalies in the different organs of fetus [2]. Nowadays liver disorders associated with the influence of toxic substances, appear more often, notably during prenatal ontogenesis. Particularly, hepatic beams and blood vessels are damaged on a background of lead influence [3, 4]. It is known, that liver performs hematopoietic function during embryonic development. Lead causes a violation of hematopoiesis, disorganization of parenchyma and vascular components, dilatation of central vein, elevation of enzyme level [5, 6]. Currently natural trace elements with bio antagonistic properties against lead are known. Studies show, that zinc, engaging in competitive interaction with lead, minimizes damaging effects of lead [7, 8]. Selenium is a known bio antagonist of lead. It improves overall health and reduces lead-induced oxidative stress [9]. Gold and silver are occupy a special place among trace elements. Nowadays gold is used in therapy of oncological diseases, diagnostics and treatment of rheumatic diseases [10]. Silver has a wide antibacterial and antifungal effects and stimulates the blood-forming organs [11, 12]. Scientists of Dnipropetrovsk medical academy identified modifying effect of gold and silver on a background of lead intoxication during embryogenesis [13]. However, liver organogenesis under combined influence of silver and gold citrates on a background of lead intoxication isn’t studied enough. Thus, the aim of our experiment was to identify morphological state of fetal liver under combined influence of silver, gold and lead acetate.

2. Materials and methods

2.1. Experimental Animals

In an experimental model 24 healthy female albino rats (95-110 days old) weighing 180-200 g were used. They were housed in regular cages at constant temperature (22±2 °C) with 12 h light/dark cycle and standard water-food diet.

Experiments were conducted according to European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose (Strasbourg, 1986);
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Directive 2010/63/EU on the protection of animals used for scientific purposes, and were approved by the Institutional Animal Care Committee.

2.2. Chemicals and Reagents
Lead acetate was obtained from Closed Joint-Stock Company “Research Center of Pharmacotherapy” (St. Petersburg, Russia). Solutions of silver and gold citrates were obtained using aquananotanotechnology from Nanotechnology Research Institute (Kiev, Ukraine).

2.3. Study design
The animals were randomly assigned into 4 experimental groups of eight each, as given below:
1) Group K: intact rats (control), used standard water-food diet;
2) Group Pb: rats, injected by lead acetate (0.05 mg/kg/day);
3) Group Pb+Ag: animals, injected by combination of lead acetate (0.05 mg/kg/day) and silver citrate (2 mcg/kg/day);
4) Group Pb+Au: animals, injected by combination of lead acetate (0.05 mg/kg/day) and gold citrate (1.5 mcg/kg/day).

Animals pairing was by natural way. The first day of pregnancy identified since the moment of detection of sperm in vaginal smears. The metal solutions were administered to females enterally every day at the same time from the 1st to the 19th day of pregnancy. Rat fetuses were removed from the uterus on 20th day of pregnancy. They were checked with the help of "live-dead" test, weighed, photographed, and fixed in 10% formalin solution. Weight, size, color and consistency were estimated. The hepatofetal index (HFI) was calculated using the formula:

$$HFI = \frac{LW}{FW}$$

Where LW – liver weight, FW – fetus weight.

2.4. Morphometric study
Liver was removed from the fetuses after 24-hours fixation in 10% formalin solution. Weight, size, color and consistency were estimated. The hepatofetal index (HFI) was calculated using the formula:

$$HFI = \frac{LW}{FW}$$

Where LW – liver weight, FW – fetus weight.

2.5. Histological study
Histological specimens were stained by Mallory and hematoxilin/eosin technology. To found differences between control and experimental groups the microscope Zeiss Primo Star was used. Photo of specimens were gotten using the camera Axiocam ERc 5s. Relative area of liver vessels, hematopoietic cells and hepatocytes was calculated using of accurate counting method \[14\] with the help of Photoshop CS Program and calculated by the formula corresponding to GG Avtandilov \[15\]:

$$V_v = \frac{P}{P_t}$$

where $V_v$ – the relative area of the structure;
$P_t$ – number of test points that fall on the structure;
$P_t$ – the total number of points of the test system.

2.6. Statistical Analysis
The results were analyzed with the help of Microsoft Excel 2010 Program and Atestat Program. Data were expressed as means ± S.E.M. (n = 8). The statistical significance of differences (p) was evaluated by Student's t-test. The differences between the group were significant at p < 0.05.

3. Results
3.1. Effects of lead acetate, silver and gold citrates to morphometric indicators.
The average liver weight was 0.1 ± 0.01 g under the influence of lead acetate, that was 60% less compared to control group (p < 0.05). The average liver weight was 0.29 ± 0.01 g under lead acetate and silver citrate combination, that was higher by 14% in comparison to control group and higher by 65% (p < 0.05) in comparison to group of lead intoxication. The average liver weight was 0.15 ± 0.01 g under the combined influence of lead acetate and gold citrate, that was less than control group by 40%, but more by 34% in comparison to group of lead intoxication (p < 0.05).

Calculation of HFI showed that in lead intoxication group it was 0.078±0.003, that was 33% less compared to control group (p < 0.05). In group of combined influence of lead acetate and silver citrate the HFI was 0.098±0.002, that was less by 12% compared to control group, but more by 20% compared to lead intoxication group (p < 0.05). Under the combined influence of lead acetate and gold citrate the HFI was 0.08±0.02, that was 22% less compared to control group (p < 0.05) and more by 2% in comparison to lead intoxication group (Fig.1).

![Fig 1: Weight of fetal liver and HFI in control and experimental groups.](image)

3.2. Effects of lead acetate, silver and gold citrates to relative area of blood vessels in rat fetal liver.
The relative area of blood vessels in the group of lead poisoning was considerably higher by 42% (p < 0.05) compared to control group. Under the combined influence of lead acetate and silver citrate the relative area of vessels was smaller by 32% compared to control group, but 43% less (p < 0.05) compared to group of lead intoxication. The relative area of vessels was increased by 44% in comparison to control group (p < 0.05) under the combined influence of lead acetate and gold citrate. This indicator was increased by 4% compared to lead intoxication group. (Fig.2, Fig.3).
Fig 2: Blood vessels of fetal liver in rats. A – Group K, B – Group Pb, C – Group Pb+Ag, D – Group Pb+Au; 1 - liver parenchyma, 2 – blood vessels of fetal liver. Malory stain. x100.

* - statistically significant difference to Group K (p<0.05);
** - statistically significant difference to Group Pb (p<0.05).

Fig 3: Relative area of blood vessels of fetal liver in rats, [%].

3.3. Effects of lead acetate, silver and gold citrates to relative area of hematopoietic cells and hepatocytes of rat fetal liver.
The relative area of hematopoietic cells in group Pb was considerably higher by 39% compared to control group (p<0.05). The relative area of hematopoietic cells was higher in Group Pb+Ag by 9% in comparison to control and less by 33% (p<0.05) in comparison to lead intoxication group. Combination of gold citrate and lead acetate caused increasing of the relative area of hematopoietic cells by 43% in comparison to control (p<0.05) and increasing of this indicator by 3% compared to Group Pb.
The relative area of hepatocytes in Group Pb was much less by 41% in comparison to control group (p<0.05). The relative area of hepatocytes was higher by 2% in group Pb+Ag compared to control group and higher by 42% (p<0.05) compared to Group Pb. The relative area of hepatocytes was reduced by 35% in Group Pb+Au in comparison to control group (p<0.05) and slightly increased in comparison to Group Pb (Fig.4, Fig.5).

Fig 4: Hematopoietic cells and hepatocytes of fetal liver in rats. A – Group K, B – Group Pb, C – Group Pb+Ag, D – Group Pb+Au; 1 - hematopoietic cells, 2 – hepatocyte, 3 – reticular stroma. Hematoxilin/eosin stain. x400.

* - statistically significant difference to Group K (p<0.05);
** - statistically significant difference to Group Pb (p<0.05); RAHPC – relative area of hematopoietic cells of fetal liver in rats; RAH – relative area of hepatocytes of fetal liver in rats.

Fig 5: Relative area of hematopoietic cells and hepatocytes of fetal liver in rats.

4. Discussion
The oxidative stress of fetal liver under the influence of lead was confirmed, because liver weight and HFI were decreased. Lead acetate, even at low concentrations, has a toxic effect on liver development, causes decreasing of liver weight and reducing of HFI. Oxidative stress occurs because of increasing of relative area of blood vessels during liver organogenesis. Our results confirmed data of other scientists [4, 5, 6]. The fact of violations of the processes of maturation and differentiation during hepatogenesis was confirmed in our experiments because of increasing the number of hematopoietic cells and the decreasing number of hepatocytes.

Influence of silver citrate on a background of lead intoxication caused increasing of liver weight and HFI. Also the relative area of blood vessels was reduced, that indicates that hypoxia was decreased. The combined effect of lead acetate and silver citrate caused decrease the number of hematopoietic cells in comparison to the lead exposure group and increase number of hepatocytes that indicates the improvement of processes of maturation and differentiation of cells during hepatogenesis. We suggested that silver citrate had antagonistic properties in relation to the lead during embryogenesis.

From the literature review we found that gold is a stronger antagonist of lead because of increasing of corpora lutea
number during pregnancy, live fetuses number and their weight [13]. However, we found that gold had extremely toxic effect on morphology of fetal liver during embryogenesis. Gold citrate on a background of lead exposure caused oxidative stress in fetal liver and processes of differentiation and maturation of cells were violated.

5. Conclusions
Silver citrate improved morphological state of fetal liver on a background of lead intoxication during embryogenesis. Oxidative stress was decreased under the combined influence of silver citrate and lead acetate. Also silver citrate improved processes of maturation and differentiation of cells during hepatogenesis.

6. References
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