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Research Article

Morphological Features of Liver Reactivity After Intranatal Dexamethazone Influence

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Abstract

Introduction: Morphological peculiarities of liver after dexamethazonne influence on the mother-placenta-fetus system is not well studied.

Materials and Methods: The study included the livers of 144 white laboratory rats from 1 to 90 days of life. All animals were conditionally divided into 3 groups: Group I - intact animals, which were born from healthy rats without any dexamethazone administration during pregnancy; group II - control animals, which were exposed to antenatal intrafetal administration of 0.05 ml saline solution on the 18th day of prenatal development; group III - experimental animals, which were exposed to antenatal dexamethazone administration at 18th day of prenatal development. The absolute and relative mass of the liver was calculated. For light microscopy, sections were stained with hematoxylin and eosin. Detection of β-D-galactose carbohydrate residues was performed using a Lectin from Ricinus communis Agglutinin (RCA) by standard methods, using standard set of reagents "LectinTest" (Lviv). Quantitative and qualitative morphological characteristics of the liver were studied. Analysis of the obtained results was conducted by means of statistical methods with the use of computer license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J).

Results: It was found that in rats after intrauterine administration of dexamethasone morphological changes in the liver are determined within three months after birth, which are manifested by changes in absolute and relative liver mass, as well as the rate of increase in absolute organ mass. The relations between hepatic membranes, vessels, connective tissue, cells of hematopoietic centers change. Changes in the cellular composition of the liver lobes are detected. The introduction of dexamethasone leads to changes in the formation of connective tissue in the liver, namely an increase in the amount of connective tissue in the long-term follow-up.

Conclusions: In rats after intrauterine administration of dexamethasone from the 14th to the 90th day there is an increase in the relative area occupied by connective tissue in the liver, reaching statistical significance on the 30th day. In animals after intrauterine administration of dexamethasone on the 1st day of life increases the absolute number of mononuclear hepatocytes in the central, and in the peripheral zone is detected. In the group of experimental animals, after intrauterine administration of dexamethasone there is a decrease in the relative area occupied by PAS-positive structures in the central zone from the 7th to the 21st day. In the peripheral zone, the decrease is more pronounced and is observed during the first three weeks after birth.

Keywords: Liver; Intranatal Dexamethazone Influence; Lymphocytes; PAS-Reaction

Introduction

The liver is one of the largest glands in the human body. It plays an important role in the metabolism of proteins, fats, carbohydrates, cholesterol, hormones, etc. [31]. The liver is usually perceived as a non-immunological organ, but among its many functions both in utero and after birth, it plays an important role in maintaining homeostasis [4,17,18,32]. It is a site of complex immunological activity mediated by a diverse composition of immune

cells [3,4,24,26,29,38,39]. In the foetal period, the liver plays the role of the main transient immune organ [11,22,24,34]. Due to its location on the path of blood from mother to foetus, it becomes most vulnerable to disorders in the mother-placenta-foetus system [2].

There is a number of investigations concerning the effects of glucocorticoids on the body. Glucocorticoids exert their effects through glucocorticoid nuclear receptors located in cells of various organs and tissues [9,19]. A significant number of nuclear glucocorticoid receptors are located in embryonic tissues. Glucocorticoids mimic the pharmacological signal of stress, with a single or multiple injections [24]. Regardless of how glucocorticoids enter the foetus, through the placenta or when injected directly into the foetus, they show similar potency. The placenta may partially metabolize natural glucocorticoids, but synthetic glucocorticoids such as betamethasone and dexamethasone may resist this process and enter the foetus completely. Dexamethasone stimulates cytotrophoblast terminal villi to form syncytium when administered in the first trimester of pregnancy by increasing aberration and deposition of periplacental fibrin, which may be complicated by preeclampsia and foetal growth retardation [10]. The teratogenic potential of glucocorticoids has a wide variety. For example, mice often have cleft palate due to blockage of mesenchymal cell proliferation and epithelial cell differentiation [13]; rabbits often have heart abnormalities. Rats and monkeys are more tolerant of glucocorticoids during pregnancy, but foetal growth retardation may occur. In human studies, a teratogenic effect may occur with the use of glucocorticoids, especially in the first trimester of pregnancy. The least side effects were observed with the use of inhaled forms of glucocorticoids, but it is impossible to completely rule out their harmful effect on foetal development. In the liver, glucocorticoids activate the processes of gluconeogenesis, also participate in the formation of liver enzyme systems - reduce glucokinase activity [13].

Some studies suggest that prednisolone administration in the first trimester of pregnancy (4 to 13 weeks) did not cause a teratogenic effect on the fetus, but doubled the number of preterm births and reduced body weight at birth [27]. On the other hand, there is evidence of an increased risk of infectious complications in mothers and an increased risk of antenatal foetal death. Thus, for every 1,000 women who receive glucocorticoids during pregnancy, there are 3-5 neonatal deaths [7].

Betamethasone and dexamethasone are the glucocorticoids of choice for reaching foetal organs, both are more closely related to glucocorticoid receptors than cortisol and are not inactivated by 11β-HSD2 and therefore pass freely through the placenta. There are conflicting data on behavioral changes that are observed by parents at later stages of child development [24]. There is also a lack of data on metabolic, vascular and immunological effects [26,37].

A number of experimental models of prenatal stress and glucocorticoid excess have pointed to later effects such as high blood pressure in adults, glucose and insulin levels, and behavioral changes resembling anxiety and depression. All of these effects have been found in many animal species, including rats, mice, guinea pigs, sheep, and pigs. Similar effects have been found in studies in primates with low-dose glucocorticoids [16].

Unfortunately, despite the large number of studies on the effects of glucocorticoids on the foetus, there is almost no data on the antenatal effects of glucocorticoids on the morphogenesis of the liver, the ratio of its cellular composition, as well as studies of the lymphoid component of the liver.

The aim

To study morphological features of liver reactivity after intranatal dexamethazone influence.

Materials and Methods

To solve the problem we investigate he livers of 144 white laboratory rats of the first three months of life. All animals have been provided by the vivarium of PE "Biomodelservice" Kyiv, veterinary certificates KI - 33 №042560 dated 17.09.2014, KI - 33 №049566 dated 23.05.2016, KI - 33 № 054439 dated 15.09.2016. The keeping of rats was conducted kept in standard conditions of vivarium in acrylic cages with a volume of 300 cm^3 for 4-5 animals each and free access to water according to "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 18.03.86 G.) and the Law of Ukraine № 1759-VI (15.12.2009).

In order to define the peculiarities of liver reactivity we divide animals into three groups: the first one included intact animals, which were born from healthy rats; the second group included control animals, which were exposed to antenatal intrafetal ad-

ministration of 0.05 ml saline solution on the 18th day of prenatal development. Experimental animals composed the third group, the rats of this group were exposed to antenatal dexamethazone administration at 18th day of prenatal development by analogy with the control group in volume of 0.05 mL in delution of 1:40. After operation of intranatal injection of solutions of different nature, the animals were kept in separate cages up to parturition and 30 days after it. In a month the female was separated from the offspring. The liver was examined at days 1, 3, 7, 14, 21, 30, 60 and 90 after birth.

We weighed animals on pharmacy scales, livers up to 1 gram we weighted on Torsion Scales, and livers exceeded 1 gram we weighted on Pharmacy Scales. We calculated the absolute and relative mass of the liver. 10% neutral formalin solution was used for fixation of the liver samples of 2 mm in thickness. The fixation lasted for 48 to 72 hours. Standard histological methods were used for analyzes of histological samples. For light microscopy, we stained We with hematoxylin and eosin. To detect the complete complex of glycoproteins in the liver parenchyma used PAS-reaction. To differentiate glycoproteins, histological samples were pre-treated with amylase. Phenylhydrazine (10% solution) was used to control and block 1,2-glycol groups. The evaluation of the obtained results was performed semi-quantitatively in points. 0 points - no reaction, 1 point - pale pink color, 2 points - pink-red color, 3 - red color, 4 points - burgundy-red color. The calculation of the relative area occupied by PAS-positive structures was done using the electronic program ImageJ with masking. To detect the content of glycogen in hepatocytes used the color of sections with carmine according to Best.

To study the content and distribution of connective tissue in the structures of the liver it was used staining of sections by the method of Van Gizon. To determine the relative amount of type III collagen fibers, a Leidlow histochemical "silver impregnation" reaction was used, followed by processing of the results using the ImageJ program with the application of masks. We detected residues of β-D-galactose using a Lectin from Ricinus communis Agglutinin (RCA) by lectinhistochemical method, using standard set of reagents "LectinTest" (Lviv).

For electron microscopy, 1x1 mm thick pieces of liver were fixed immediately after extraction in 2.5% glutaraldehyde solution, followed by treatment in 1% osmium tetraoxide solution. Subsequently, the pieces were carried out on an ascending battery of alcohols up to 100% alcohol, acetone with additional contrast for two hours in 2.5% uranyl acetate at 700C, pouring into the unit was carried out by gradual impregnation of tissue with acetone oxide with epon (3:1; 1:1; 1:3) and poured into a clean epon. The polymerization of the resins was performed in two stages at 36^0C (12 h) and 56^0C (24 h). Semi-thin (1-2 μm) and ultra-thin (55-65 nm) sections were obtained on a Boeckeler RMC PowerTome. Semi-thin sections were stained with methylene blue. Ultrathin sections were contrasted with Reynolds lead citrate for 25 minutes at room temperature. Ultrathin sections were studied in an electron microscope PEM-100-01 at an accelerating voltage of 75 kV.

We studied quantitative and qualitative structural characteristics of the liver including analysis of cell composition and content of connective tissue substances in central and peripheral areas of the classical lobule, according to the International Anatomical Nomenclature (2005).

The relative area of the liver structures was calculated on a unit area of 10,000 μm^2. We examined the relative area occupied by hepatic plates, sinusoidal capillaries, central veins, interparticle veins, arteries and bile ducts, connective tissue and foci of hematopoiesis in two areas of the liver zones: central and peripheral. With an oil immersion technique we calculated the number of liver cells: mononuclear and multinucleated hepatocytes, hepatocytes with signs of mitosis, endothelial cells of sinusoidal capillaries, Kupffer cells, lymphocytes, hematopoietic cells.

We conducted analysis of the data by statistical methods (license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J)). The significance of the differences between the experimental groups was assessed using the Student's criterion t, considering the differences to be reliable at $p < 0.05$, that is generally accepted for biological and medical researches. The numerical data of the obtained results are presented as M ± m (arithmetic mean ± standard error of the mean).

Ethnical approval

Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

Results and Discussion

Earlier it was shown the dynamics of absolute and relevant mass of the rats' liver in early postnatal period in normal conditions and after intranatal antigen injection [14].

In the experimental group of animals injected with dexamethasone in utero, the absolute mass of the liver on the first day of life is 197.17 ± 13.84 mg, which is lower than in the intact and control groups. The decrease in absolute mass is more pronounced and becomes statistically significant on the 7th, 21st and 30th day of life. In the future, there is an increase in the absolute mass of the liver, which exceeds the indicators in the intact and control groups on the 60th and 90th day of life. Indicators of increase in absolute mass of the liver are uneven and lower than in the control group until the 60th day of observation, but on the 90th day of life there is an increase compared to control, the increase in absolute mass of the liver almost twice. The dynamics of the relative mass of the liver is also wavy. The indicators are lower than those in the intact and control groups and have significant differences on the 1st, 3rd and 60th day of observation. The obtained data coincide with the data of [20] on the study of the effects of glucocorticoids during pregnancy and their impact on the development of pathological conditions in the postnatal period.

In experimental animals, which were injected with dexamethasone in utero, as in the control, most of the parenchymal elements of the liver are hepatocytes, which in contact with each other form liver plates (or liver transplants). In these animals there is a gradual increase in the relative area occupied by hepatic plates during the first week of observation from 58.67 ± 2.84% on the 1st day to 76.19 ± 2.08% on the 7th day, which is almost indistinguishable from indexes in intact and control groups. On the first day of life, there is a prevalence of mononuclear hepatocytes in both zones of the liver lobes (63.65 ± 1.74 cells per unit area in the central and 60.70 ± 1.74 cells per unit area in the peripheral zone). On the 21st day, there was a statistically significant decrease in the relative area occupied by hepatic plates by 6.13% compared with the same index in the control group (74.12 ± 2.38% in the experimental group and 80.25 ± 1.99% in control group), which is accompanied by a gradual decrease in the number of mononuclear hepatocytes on the 14th day (51.83 ± 1.22 cells per unit area in the experiment and 66.61 ± 1.39 cells per unit area - in control) in the central zone, in both zones on the 21st and 30th day of life. From the 30th to the 90th day of life there is a significant decrease in the number of both mononuclear and multinucleated hepatocytes in the peripheral zone of the lobules.

Sinusoids (Vas sinusoideum) are small (9-12 μm in diameter) vessels. They are formed by an intermittent endothelium in which there is no basement membrane. The mucopolysaccharide subendothelial extracellular matrix is a special type of basement membrane with which sinusoidal cells and hepatocytes come into contact. It consists of collagen IV, VI, XIV types, glycoproteins such as fibronectin, laminin, hyaluronic acid and others, as well as proteoglycans such as heparan, chondroitin sulfate and others [16]. The relative area of sinusoids tends to gradually decrease during the first week from 17.33 ± 2.19% on the first day to 9.05 ± 1.40% on the seventh day. On the 14th day there is an increase in the relative area of sinusoids, which becomes statistically significant on the 21st day, exceeding the indicators in the control and intact groups by 4.94%. In the future, there is a gradual decrease in the relative area of the, which does not differ from the intact and control groups. The number of sinusoidal endothelial cells is decreased on the 1st day in the central zone of the hepatic lobules compared with the control group (4.52 ± 0.87 cells per unit area - in experimental animals and 7.48 ± 0.87 cells per unit area in control). Subsequently, there is a gradual increase in number of endothelial cells, which reaches maximum values on the 14th day (14.96 ± 1.39 cells per unit area in the central and 11.13 ± 1.22 cells per unit area) in the peripheral zone of the lobes.

Intrahepatic connective tissue is formed mainly by type III collagen fibers and extracellular hepatic matrix. Type III collagen fibers are located in the form of a network in the sinusoidal space on the basal surface of the liver plates and provides mechanical support to the sinusoids, as well as involved in the regeneration of hepatocytes. The connective tissue of the liver is mainly represented by collagen fibers, which are located around the interparticle vessels, form a framework of sinusoids and are located in the capsule of the liver. Due to the dynamics of the relative area occupied by connective tissue in rats treated with dexamethasone solution in utero, there is a gradual increase in this indicator from the first day to the end of the second week of life. On the 21st day, this indicator is almost twice as high as in the control group (4.71 ± 1.15% - in the experimental group and 2.25 ± 0.74% - in the control group). On the 30th day, the excess reaches statistical significance (5.78 ± 1.10% in the experimental group and 2.56 ± 0.80% in the control group). The relative area occupied by connective tissue on the 60th and 90th days of life in the experimental group continues to exceed similar indicators in the control group by 2.6% and 1.48%, respectively, but such an increase is not statistically significant. The dynamics of the relative area occupied by the interparticle arteries

tends to increase gradually and slightly exceeds the control group with 7th to 14th days and 30th to 90th days of life, but such changes have no statistical significance. The dynamics of the relative area of the interparticle veins is wavy. Indicators during the first week of life are slightly lower than those of the control group, gradually increasing from the 14th to the 21st day. But such changes do not acquire statistical significance.

Stellar macrophages (Kupffer cells) - belong to the specialized resident macrophages of the liver and are part of the reticulendothelial system of the body. [6,11,30,33,35,36]. They are located inside the sinusoids, occupying a third of the total volume of sinusoidal cells, which is about 20% of non-hepatocyte cells [7,15,17,40]. They have an irregular shape, 9 μm thick in diameter. Plasmalemma has a large number of condyles, which gives them the shape of stars and pyramids. With their outgrowths, they can penetrate through the fenestrae in the endothelium into the surrounding sinusoidal space in direct contact with hepatocytes and surrounding sinusoidal cells, but they do not form specific connections with these cells. In the experimental group of animals injected with dexamethasone in both areas of observation, the minimum number of stellate macrophages is detected on the 1st day of life: 2.26 ± 0.69 (8.52 ± 1.04 - in the control) in the central zone of the lobules and 5.04 ± 0.86 (9.57 ± 1.21 - in the control) - in the peripheral zone of the liver lobes. On the 14th day after birth in both zones of the liver lobes there is a statistically significant increase in number of Kupffer cells: 14.61 ± 1.39 (10.61 ± 1.39 - in the control) in the central zone and 13.91 ± 1.39 (9, 56 ± 1,22 - in control) - in the peripheral zone of the lobes.

Intra-tissue lymphocytes are constantly present in the liver, which are located in all areas of the lobules. The dynamics of the number of lymphocytes in the group of rats after intrafetal injection of dexamethasone is more uniform and does not differ from the intact and control groups in the peripheral zone of the lobules. However, in the central zone there is a significant increase in the number of lymphocytes on the 14th day of life (3.48 ± 0.69 cells per unit area in the experimental group and 1.57 ± 0.52 cells per unit area). - in the control group). On the 30th day, the number of lymphocytes in the central zone of the lobules has a statistically significant decrease reaching 0.70 ± 0.35 cells per unit area.

The cytoplasmic membrane, intracytoplasmic inclusions and nuclear membrane of the liver dendritic cells contain β-D-Gal carbohydrate residues' receptors, which specifically bind with Castor lectin (RCA). In animals of experimental group after intrafoetal dexamethasone injection in the central zone of the lobules the number of dendritic cells during the first two weeks decreases with minimal indexes on the first and the14th day. In the peripheral zone of the liver lobules of experimental animals during the first week of life there is a statistically significant decrease in the number of dendritic cells per unit area.

The dynamics of the distribution of glycoproteins in the central zone of the liver lobes in the intact and control groups is wavy in nature with a gradual increase of almost 2 times the relative area of PSA-positive structures from 1 to 14 days of life from 10.71 ± 0.10% to 19, 22 ± 0.25%, respectively. Subsequently, by the 30th day, there is a gradual decrease in the relative area occupied by PAS-positive structures in the central zone of the lobes, reaching 12.69 ± 0.14% on the 30th day after birth. After that, there is a second wave of increase in the relative area of PAS-positive structures in the central zone of the particles during the 90th day of observation, reaching values of 19.83 ± 0.20%. In the peripheral zone of the lobes, the relative area of PAS-positive structures also gradually increases from the first to the seventh day, increasing by 13.69% on the 7th day compared to the first day of life.

Subsequently, there is a slight decrease in this index by the 14th and up to 30th days of life, and a re-increase by the 60th and 90th days, increasing on the 90th day by 12.47% compared to the first day. Probably, the second wave of increase in the content of PAS-positive structures in the liver, a significant number of which is represented by glycogen, which is confirmed by the control reaction with amylase, is associated with the accumulation of this substance. In the group of experimental animals after the introduction of dexamethasone in the central zone of the liver lobes on the 7th day there is an increase in the relative area occupied by PAS-positive structures by 3.39% compared to the control. Subsequently, from the 7th to the 60th day, the relative area of PAS-positive structures in the central zone of the lobes decreases. In the peripheral zone of the lobes there is a decrease in the content of PAS-positive structures, starting from the first and up to the 21st day of life. Glucocorticoids are able to affect gluconeogenesis by increasing it.

Smoothing of crypts in hepatocyte mitochondria of experimental rats was observed on the 3rd and 7th days of observation during electron microscopy investigation (Figure 1 and 2). It may be a manifestation of oxidative stress in cells.

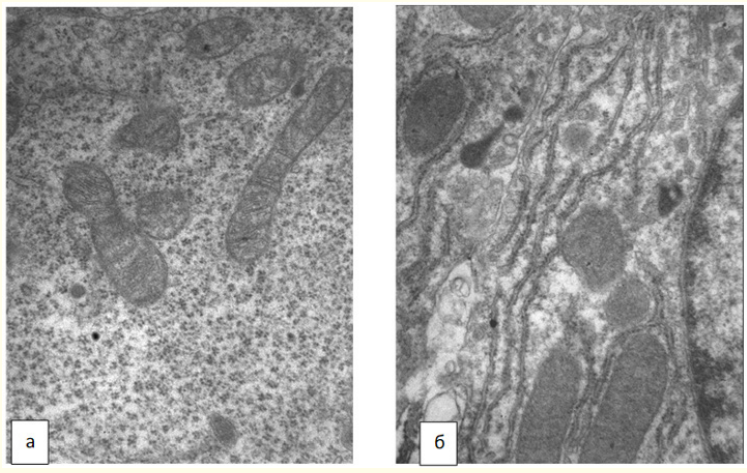

Figure 1: Rat liver 3 days of life. Electronogram. x 15000.
a: Intact group; b: Experimental group, after the injection of dexamethasone.

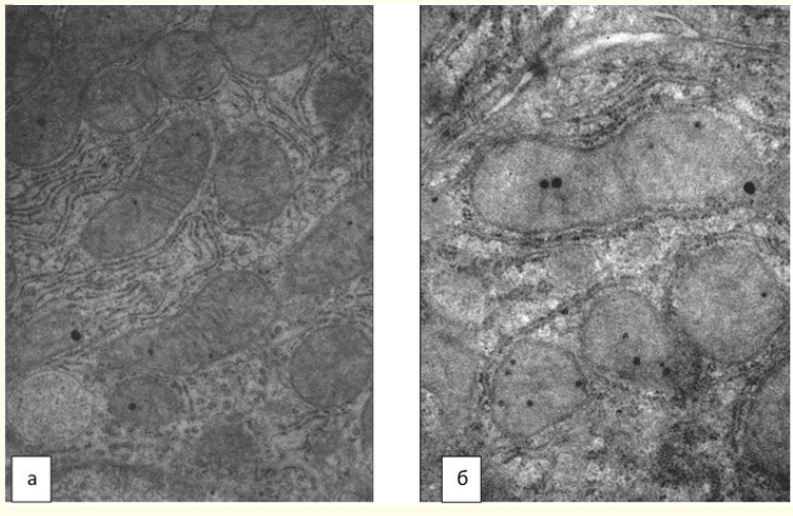

Figure 2: Rat liver at the 7th of life. Electronogram. a: Intact group, x11000; b: Experimental group, after injection of dexamethasone in fetal period, x 15000.

Thus, it was found that in rats after intrafetal injection of dexamethasone structural changes in the liver were determined within three months after birth, they were mainly represented by absolute and relative liver mass changes (increase of absolute organ mass index). The relations between hepatic membranes, vessels, connective tissue, cells of hematopoietic centers change. Changes in the cellular composition of the liver lobes are detected. The influence of dexamethasone in fetal period leads to changes in the formation of connective tissue in the liver, namely an increase in the amount of connective tissue in the long-term follow-up.

Conclusions

- In rats after intrafetal injection of dexamethasone from the 14th to the 90th day there is an increase in the relative area occupied by connective tissue in the liver, reaching statistical significance on the 30th day (5.78% ± 1.10 in the experimental group and 2.56% ± 0.80 - in the control group). An increase in the content of collagen fibers of type III from the 14th to the 90th day, with a peak on the 30th day of observation (2.95% ± 0.34 - in the experimental group and 1.94% ± 0.38 - in the control group).

- In animals after intrafetal injection of dexamethasone on the 1st day of life increases the absolute number of mononuclear hepatocytes in the central (63.65 ± 1.74 cells per unit area (in the experiment) and 51.83 ± 1.22 cells per unit area (in control), and in the peripheral zone (60.70 ± 1.74 cells per unit area in the experiment and 50.96 ± 1.04 cells per unit area - in control).
- In the group of experimental animals, after injection of dexamethasone in fetal period there is a decrease in the relative area occupied by PAS-positive structures in the central zone from the 7th to the 21st day. In the peripheral zone, the decrease is more pronounced and is observed during the first three weeks after birth.
- In animals after dexamethasone injection during fetal period the number of dendritic cells in the central zone of the lobules decreases throughout the first two weeks with minimum indexes on the 1st day of life. In the peripheral zone of the lobes in experimental animals during the first week of life there is a statistically significant decrease in the number of dendritic cells per unit area.

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Conflicts of Interest

Authors have no conflict of interest to declare.

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