UDC 615.07:612.64: 504.054

THE EFFECTS OF LAPROL-604 EXPOSURE OF PREGNANT RATS ON THE KIDNEYS OF THEIR PROGENY*

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The production of surfactants has been regarded as an important indicator of high-tech chemical technology industry all over the countries. It has become the world's chemical industry competitive focus. There is no doubt that the expanding of surfactants application field leads to the increase of its consumption. Eventually, the harm to the environment will be more serious. Therefore, the researches of affects of surfactants on living organisms are very important. The aim of this study was to study the influence of Laprol-604 on enzymatic system of kidney. Kidney's peculiarities of rat pups were analyzed. Laprol-604 was introduced during pregnancy of Wistar rats. It has been found that there is a statistically significant disturbance of metabolic parameters of kidneys. The exposure to Laprol-604 to pregnant rats led to the appearance of kidney-specific enzyme such as L-arginine: glycine amidinotransferase in blood serum of the offsprings. While the activities of alanine aminotransferase and aldolase were increased, the activity of isocitrate dehydrogenase was decreased in the kidney homogenates of progeny. These changes may be associated with mitochondrial dysfunction that is evidenced by decreasing concentration of adenosine triphosphate. The concentration of proteins was reduced in the kidney homogenates. With the help of chemiluminescent method it was found that such biochemical changes occurred during the activation of free-radical oxidation. The findings revealed the appearance of damages in the kidneys followed by an accelerated rate of free-radical oxidation.

Keywords: Laprol-604, surfactant, rat progeny, reproductive toxicity, gestation day, L-arginine: glycine amidinotransferase (AGAT).

Виробництво поверхнево-активних речовин є важливим показником індустрії хімічних речовин у всіх країнах. Існує конкуренція серед держав в хімічній промисловості. Немає сумнівів в тому, що розширення сфери застосування поверхнево-активних речовин призводить до збільшення їх виробництва та споживання. Виникли серйозні ризики шкідливого впливу поверхнево-активних речовин на навколишнє середовище. Тому дослідження дії поверхнево-активних речовин на живі організми важливі на сьогоднішній день. Метою цього дослідження було вивчення впливу Laprol-604 на ферментативну систему нирок потомства щурів. Laprol-604 вводили вагітним щурам Wistar протягом 20 гестаційних днів. Виявлено статистично значуще порушення метаболічних параметрів у нирках потомства щурів. Введення Laprol-604 вагітним щурам призвело до появи у сироватці крові потомства специфічного для нирок ферменту, такого як L-аргінін: гліцин-амідінотрансферази. Активності аланінамінотрансферази та альдолази підвищувалися, у той час як рівень ізоцитратдегідрогенази знижувався в гомогенатах нирок потомства. Ці зміни можуть бути пов'язані з мітохондріальної дисфункцією, про що свідчить зниження вмісту аденозинтрифосфату. Концентрація білків була знижена в гомогенатах нирок. За допомогою хемілюмінесцентного методу встановлено, що такі біохімічні зміни відбулися під час активації вільнорадикального окислення. Отримані дані виявили появу пошкоджень в нирках з підвищенням швидкості вільнорадикального окислення.

Ключові слова: Лапрол-604, поверхнево активна речовина, потомство щурів, репродуктивна токсичність, гестаційний день, L-аргінін: гліцин амідінотрансфераза.

Introduction

The production of surfactants has been regarded as an important indicator of high-tech chemical technology industry all over the countries. It has become the world's chemical industry competitive focus [7, 11]. There is no doubt that the expanding of surfactants application field leads to the increase of consumption. Eventually, the harm to the environment will be more serious. Therefore, the researches of affects of surfactants on living organisms are very important.

There are very few published studies assessing the safety of surfactants during animal pregnancies, therefore, data from animal reproductive studies are valuable [3, 5, 12].

The kidney, due to certain anatomical and functional features such as high blood flow intensity, participation in the elimination of a large number of endogenous metabolites and biotransformation products of xenobiotics from the body, is a vulnerable organ that is exposed to numerous chemical agents [8].

The present study was undertaken to see how prenatal Laprol-604 exposure affected the growth parameters in the kidney of rat offsprings.

Materials and methods

All the procedures were performed at Kharkiv Medical Academy of Postgraduate Education, according to Ukrainian and International guidelines for the use of animals in research (Law of Ukraine as of 21.02.2006 No. 3447-IV «On protection of animals from cruelty» // Supreme Council of Ukraine. 2006; 27:230 and European convention for the protection of vertebrate animals used for experimental and other scientific purposes [2].

Laprol-604 was provided from Science and Production Joint Stock Company "Sintez PAV" (Shebekino, Russian). Laprol-604 was reported to be 96% pure by the supplier. For all studies, Laprol-604 was diluted in deionized water and prepared fresh daily.

Forty pregnant Wistar rats (body weight, 180 ± 30 g at the beginning of the study) bred within a 4-h period in the afternoon and overnight. Those animals with spermato-

* *To cite this English version: Popova T.M. The effects of laprol-604 exposure of pregnant rats on the kidneys of their progeny // Problemy ekologii ta medytsyny. - 2017. - Vol 21, № 1-2. - P. 22-25.*

zoa in a vaginal smear were considered to be at gestation day (GD) 0. They were randomly divided into four groups (10 animals in each group). Laprol-604 was administered to 30 pregnant rats once daily by gavage at doses of 0.125; 1.25 and 12.5 mg/kg of body weight, respectively, the 1st; 2nd and 3rd group from GD 2 until GD 20. The 4th group (controls) consisted of 10 intact animals without Laprol-604 administration. Pregnant rats were monitored at hourly intervals, during the 22 GD and later. After parturition, 30 pups from each group of both genders were randomly chosen, weighed and euthanized by decapitation. Trunk blood was collected and serum samples were prepared and stored at -20°C. During the necropsy, both kidneys from each pup were dissected out and placed on blotting paper to make them free of surrounding fluid. The shape and colour of kidneys were observed. Detailed examination of external surface was performed. Each kidney was weighed on an electrical balance and weight was recorded. Relative Tissue Weight Index (RTWI) was calculated by the formula:

$$\text{RTWI} = \frac{\text{Average weight of kidney}}{\text{Average body weight}} \times 100$$

After that the kidneys were quickly frozen on dry ice and stored at -80°C for investigation. The activity of L-Arginine: glycine amidinotransferase (AGAT) was determined by uniform method with the help of spectrophotometer using the Sakaguchi test in serum of blood samples [9]. Separation of proteins and determination of molecular weight were performed by electrophoresis [6]. Isolation of mitochondria from kidney tissue was performed by the differential centrifugation method [4]. The mitochondrial NAD-isocitrate dehydrogenase (NAD-IDH) activity and adenosine triphosphate (ATP) content were determined by spectrophotometric method [6]. The activity of aminotransferases, the levels of total proteins, lipids in kidney homogenates were determined with the help of reagent kits of the firm "Filisit Diagnostika" (Dnipro, Ukraine). Aldolase activity was determined using the reagent kits "Olvex" (Russian Federation).

The chemiluminescence method was used to evaluate the free oxidation processes in kidney homogenates. Registration of the chemiluminescence (CML) was carried out on a luminescent spectrometer LS 50 B "PERKIN ELMER" according to the methodologies developed and proposed by A.V. Artyunyan and co-authors [1]. Spontaneous and Fe^{2+} – induced CML was determined by: S-sp - light-sum/ minute of spontaneous chemiluminescence, the maximum rate of flare (h) of induced CML, Sind-1 – the light-sum/ 2 minutes Fe^{2+} -induced CML. The free radicals generation rate is correlated with S-sp – light-sum/ minute of spontaneous CML. The content of lipid hydroperoxides is confirmed by the maximum rate of flare (h) of induced CML. Sind-1 – the light sum/ 2 minutes Fe^{2+}-induced CML is reflected by the intensity of peroxide radical's accumulation.

The kinetic of H_2O_2-initiated CML was analyzed with the presence of luminol by parameters: S-lum – the light-sum/ 1 minute luminol-dependent CML - the emission maximum (H), Sind-2 – light-sum for 2 minutes of H_2O_2-induced CML. S-lum – the light-sum/ minute luminol-dependent CML is directly dependent on the intensity of hydroxyl-radical production. Sind-2 – light-sum/ 2 minutes of H_2O_2-induced CML is inversely proportional to the activity of the antioxidant defense system. The intensity of CML was calculated per 1 mg of wet kidney tissue and expressed in relative units [10].

Statistical analysis of the data was performed using GraphPad Prism 5. Student's t test was used to detect differences between independent groups of normally distributed variables; difference between groups was considered statistically significant at $p<0.05$.

Results and discussion

Newborn rats were observed on the first postnatal day. Offsprings of all groups appeared normal. There was no gross malformation in any group. Laprol-604 administration led to the reduction of newborns' body weight in the experimental groups as compared with controls ($p<0.05$) (Table 1). The external kidney surfaces of pups in the control group were smooth and shiny. The colour of kidneys was whitish yellow. In the 1st and 2nd groups, the external kidney surfaces were smooth and glistening but sizes were decreased as compared to control group. In the 3rd group, kidneys were small and external surfaces were dim. The average kidney weights of newborn rats in the 1st and 2nd groups had tendency to decrease and the average kidney weight of pups in the 3rd group was significantly lower compared with controls. Relative tissue weight index (RTWI) of all groups is shown in Table 1.

Table 1

Body weight, absolute and relative kidney weights of newborn rats (M±m)

Postnatal day	Groups of animals			
	Control group (n=30)	First group 0.125 mg/kg (n=30)	Second group 1.25 mg/kg (n=30)	Third group 12.5 mg/kg (n=30)
	Body weight (g)			
1	6.34±0.12	5.7±0.13	4.9±0.1*	4.7±0.1*
1	Kidney weight (mg)			
	55.2±2.6	49.3±2.2	45.4±2.4*	40.6±2.5*
	RWTI			
	0.88	0.86	0.93	0.86

Note. ** Significant differences ($p < 0.05$) from control values.*

When mitochondria's renal tissue was studied, the isocitrate dehydrogenase activity decreased. At the same time, elevation of the aldolase activityy was found in the newborn rat cells. This is explained by the decrease of aerobic oxidation processes as well as the rise of glycolysis in the renal tissue. In this case, the cell energy was provided by glycolysis. Such changes may be associated with mitochondrial dysfunction. The ATP concentration was reduced in the kidney tissue homogenates which, apparently, is due to a disturbance of the ATP synthesis in the mitochondria (Table 2).

The L-arginine: glycine amidinotransferase activity in the serum of newborn rats of the 3rd group (17.84±0.12) mmol/(s·L)* appeared to reach the highest concentration

compared with the 2nd (8.64±0.16) mmol/(s·L), 1st groups (2.73±0.15) mmol/(s·L) and control group 0 mmol/(s·L). L-arginine: glycine amidinotransferase is a kidney-specific enzyme, which enters the bloodstream when nephrocytes are destroyed. Therefore, a significant increase in the activity of L-arginine is observed: glycine amidinotransferase in the blood indicates a disturbance of the kidney morpho-functional state.

Table 2

Biochemical indices in kidney homogenates of newborn rats (M±m)

Index	Groups of animals			
	Control group (n=30)	First group 0.125 mg/kg (n=30)	Second group 1.25 mg/kg (n=30)	Third group 12.5 mg/kg (n=30)
Isocitrate dehydrogenase activity, μmol/(min/g protein)	33.46±2.22	31.14±2.66	30.25±2.37	25.60±2.32*
Aldolase, μmol/(min/g protein)	4.18±0.31	5.17±0.24	6.47±0.42*	7.44±0.38*
ATP, μmol/g tissue	1.86±0,11	1.25±0,16	0.96±0.08*	0.78±0.05*

***Note.** * Significant differences (p < 0.05) from control values.*

The exposure of Laprol-604 to pregnant rats led to increase the levels of aminotransferases in the kidney homogenates of progeny. Thus, activity of alanine aminotransferase (ALT, mmol/min/g protein) was 5.48±0.46 (controls), 6.44±0.68,_7.25±0.44* and 9.62±0.84* (1rst, 2nd and 3rd group, respectively); activity of aspartate aminotransferase (AST, mmol/min/g protein) was 2.55±0.42 (controls), 3.12±0.76, 3.74±0.58 and 4.76±0.84 (1rst, 2nd and 3rd group, respectively). The increase of activities ALT in groups of exposure to Laprol-604 may be associated with elevation of catabolism of proteins in renal cells. During the study reported here, there was a reduction in total protein level in kidney homogenates of Laprol-604 administration rats when compared to the control group. Thus, the total protein concentration (mg/g tissue) was determined 148.24±10.31 (controls), 140.55±14.11, 136.43±16.05, 124.22±10.38 (1rst, 2nd and 3rd group, respectively).

The results of present research revealed that Laprol-604 induces disturbance in the function of kidney in rat pups whose mothers were exposed to Laprol-604 during pregnancy. Kidneys from experimental animals showed a dose-dependent decrease in weight as compared to their control. Decrease in weight was significantly marked in the 3rd group which was exposed to Laprol-604 in dose of 12.5 mg/kg during gestation. The decrease in weight can be explained by activation of oxidative stress in the renal tissue. It is known that nonionic surfactants catalyze oxidative stress as activation of protein catabolism which is probably the reason for the decrease in protein content in kidney homogenates [8; 11; 12].

All these facts underline metabolic relations between mother and fetus during pregnancy.

Analysis of chemiluminesce-indexes has been demonstrated in Table 3. The prenatal exposure to Laprol-604 led to increase of free radical oxidation (S-sp) processes intensity by 2,5, 4,2 and 5,3 times in the renal tissue of the 1st, 2nd and 3rd groups, respectively, compared with controls. The increase of the lipid hydroperoxides content has been registered, so the amplitude of h has been increased by 3,7, 5,4 and 6,1 times in the kidney of the 1st, 2nd and 3rd groups, respectively, compared with controls. While the formation of hydroperoxide radicals has been accelerated, the antiradical protection and resistance to peroxidation have been reduced.

Therefore, the prenatal exposure to Laprol-604 led to decompensated activation of free oxidation in the fetal kidney tissue. Probably, the biochemical disorders detected and reported here may be explained by the long-term free radical oxidative process which has damaged the fetal kidney tissue. The results of this study are consistent with those of the free radical nature of nephrotoxicity surfactants by direct exposure. At the same time, the nephrotoxic influence of Laprol-604 on fetal was detected for the first time. Similar facts were previously found in the study of the effects of Laprol-604 on the fetal liver.

Table 3

The influence of Laprol-604 on chemiluminescence indices of kidney rat pups (M±m)

Index		Groups of animals			
		Control group (n=10)	First group 0.125 mg/kg (n=10)	Second group 1.25 mg/kg (n=10)	Third group 12.5 mg/kg (n=10)
S-sp		0.068±0.006	0.169±0.008*	0.287±0.025*	0.364±0.022*
Fe^{2+}-induced CML	h	0.477±0.025	1.873±0.039*	2.566±0.132*	3.388±0.178*
	Sind-1	0.557±0.040	1.782±0.084*	2.340±0.149*	3.860±0.142*
luminol-dependent CML	H	0.634±0.030	1.326±0.080*	3.892±0.134*	5.682±0.287*
	Sind-2	1.295±0.051	2.274±0.248*	6.448±0.88*	8.186±0.616*

***Note.** * Significant differences (p < 0.05) from control values.*

Conclusion

1. Laprol-604 administration decreased the body weight of rat pups. The adverse effect was dose-dependent.

2. The kidney-specific enzyme is the L-arginine: glycine amidinotransferase has appeared in serum of blood rat pups exposed to Laprol-604. The level of L-arginine: glycine amidinotransferase was significantly higher in the blood serum of newborn rats of the 3rd group (17.84±0.12) mmol/(s·L)* than those in the 2nd (8.64±0.16) mmol/(s·L) and the 1st groups (2.73±0.15) mmol/(s·L).

3 The adverse effect of Laprol-604 administration was determined as kidney weight reduced which was associated with biochemical disturbances, such as the reduction the isocitrate dehydrogenase activity, ATP content and total protein concentration; at the same time, the in-

creasing aldolase and alanine aminotransferase activities were found.

4. Analysis of free radical oxidation showed that this state is accompanied by oxidative stress. Spontaneous and H_2O_2-induced luminol-dependent chemiluminescence in kidney homogenates was 1.8-6.3-fold higher compared to the reference group. The increase in free radical oxidation in the 3rd group of rat pups was more pronounced than this process in the 2nd, 1st and control groups. Our results suggest that oxidative stress is induced by Laprol-604 and products of its transformation.

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Матеріал надійшов до редакції 27.04.2017