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THE EFFECTS OF LAPROL-604 EXPOSURE ON PREGNANT RATS AND THEIR POSTERITY

Keywords: *Laprol-604, surfactant, rat progeny, reproductive toxicity, gestation day.*

Abstract. *This study was carried out to understand unfavourable reproduc-tive effect associated with Laprol-604. Laprol-604 showed dosage dependent tox-ic action on young rats. Statistically significant decrease of a number of young rats and body weight has been found. Disorders of the liver metabolic parameters correlating with an increase of the rats, received Laprol-604, have been revealed.*

Introduction

Surfactants are widespread in several human activities because of a series of excellent performances like wetting and emulsifying. A large number of surfactant containing wastewater are discharged into the environment, resulting in harming aquatic life, polluting the water and endangering human health [8, 9].

The organism is especially sensitive to the negative effects of xenobiotics in the early periods of ontogenesis.

While most researches of surfactants have been focused on toxicological and carcinogenic endpoints, reproductive effects have received recent attention [7]. Although the effects of surfactants exposures in adulthood animals are known, but only a few data of researches of nonionic surfactants impact on animal fetal development are presented [3, 4, 6, 10]. So the studying effects of nonionic surfactant exposures during sensitive windows in fetal development, perinatal life, childhood and puberty require careful scrutiny.

The goal of study is to examine liver metabolic parameters and development effect of Laprol-604 in rat offspring exposed during perinatal life.

Materials and methods

All the procedures were performed in the Kharkiv Medical Academy Postgraduate Education, according to Ukrainian and International guidelines for the use of animals in research (Law of Ukraine of 21.02.2006 № 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.

One hundred pregnant Wistar rats (body weight, 180±30 g at study start) bred within a 4-h period in the afternoon and overnight. Those animals with spermatozoa in a vaginal smear were considered to be at gestation day (GD) 0. They were randomly divided into four groups (25 animals in each group). Laprol-604 was administered to 75 pregnant dams once daily by gavage at doses of 0,125; 1,25 and 12,5 mg/kg, respectively is the 1-st; 2-nd and 3-rd group from GD 2 until GD 20. The 4-th group (controls) consisted of 25 intact animals without Laprol-604 administration. Pregnant rats have been monitored at hourly intervals, during the 22 GD and later. After parturition, twenty five pups of both genders were randomly chosen from several litters (one sample per litter), were weighed, and sacrificed by decapitation. Trunk blood was collected and serum samples were prepared and stored at -20°C. During the necropsy, the livers were removed, weighed and immediately frozen on dry ice and stored at - 80°C for investigation. Liver weights were recorded. The activity of sorbitol dehydrogenase was determined by spectrophotometric method in serum of blood. Separation of proteins and determination of molecular weight were performed by electrophoresis [5]. The glycogen concentration was determined by spectrophotometric methods in live homogenates [5]. The activity of aminotransferases, alkaline phosphatase (ALP), the levels of total proteins, lipids in liver homogenates were determined with the help of reagent kits of the firm "Filisit Diagnostika" (Dnipro, Ukraine). Aldolase activity was determined using reagent kits "Olvex" (Russian Federation).

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 $p<0,05$.

Discussion of results

Laprol-604 showed dosage dependent repro-

ductive toxicity when the pregnant rats were exposed. It led to reduced statistically significant litter size in the 3-rd group (5,48±0,21), 2nd (7,07±0,15) and 1-rst (8,20±0,12) groups compared with controls (9,31±0,23) ($p<0,01$). Laprol-604 administration decreased of body weight of newborn rats, such in the 1,25 mg/kg and higher in the 12,5 mg/kg dosage groups body weight significantly lagged behind those of the controls (Table). The body weight deficits were taken into consideration. At the same time, liver weights of the Laprol-604-exposed pups were higher and appreciably differenced from controls. It should be noted the relative liver weight of 2-nd and 3-th Laprol-604 dosage groups was significantly increased (Table).

There was a tendency, the body weight of the 1st group's progeny was lower and liver weight was higher then controls.

The sorbitol dehydrogenase activity in the serum of newborn rats' 3-rd group (4,76± 0,18) nmol/(s·L)* appeared to reach the highest concentration compared with control (1,24 ± 0,07), 2-nd (3,28 ± 0,16) nmol/(s·L) and 1-rst groups (1,96 ± 0,18) nmol/(s·L). Sorbitol dehydrogenase is a liver-specific enzyme, which enters the bloodstream when hepatocytes are destroyed. Therefore, a significant increase in the activity of sorbitol dehydrogenase in the blood indicates a disturbance of the liver morpho-functional state.

The exposure of Laprol-604 to pregnant rats led

Table 1

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

Postnatal day	Groups of animals			
	Control group (n=25)	First group 0.125 mg/kg (n=25)	Second group 1.25 mg/kg (n=25)	Third group 12.5 mg/kg (n=25)
	Body weight (g)			
0	5.8±0.1	5.4±0.1	4.9±0.1*	4.7±0.1*
0	Liver weight (mg)			
	310.3±4.3	330.4±5.2	352.3±6.9	377.2±6.4*
	Liver/body weight (mg/g)			
	53.5±1.8	61.1±2.6	71.8±2.4*	80.2±2.8*

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

to significant increase the levels of aminotransferases and alkaline phosphates (ALP), in the liver homogenates of progeny. Thus, activity of alanine aminotransferase (ALT, mmol/min/g protein) was 52,18±3,14 (controls), 55,24±4,08, 61,25±2,44* and 67,45±4,22* (1rst, 2nd and 3rd group, respectively); activity of aspartate aminotransferase (AST, mmol/min/g protein) was 13,06 ± 1,43 (controls), 16,32 ± 1,16, 19,24 ± 1,58* and 22,76 ± 1,48* (1rst, 2nd and 3rd group, respectively). The activity of alkaline phosphates (ALP mmol/s/ g protein) was determined 1,58 ± 0,06 (controls), 1,76 ± 0,14, 2,43 ± 0,18* and 2,76 ± 0,12* (1rst, 2nd and 3rd group, respectively). The increase of activities ALT and ALP in groups of exposure to Laprol-604 may be associated with elevation of catabolism of proteins in liver cells. During the study reported here, there was a significant reduction in total protein level and increase of total lipid level in liver homogenates of Laprol-604 administration rats when compared to the control group. Thus, the total protein concentration (mg/g tissue) was determined 215,44 ± 14,31 (controls), 204,28 ± 18,16, 184,33 ± 12,05* 171,22 ± 15,0* (1rst, 2nd and 3rd group, respectively); the total lipid level (mg/g tissue) was found 65,43 ± 3,17 (controls), 68,24± 4,06, 81,39 ± 4,18* and 88,16 ± 5,32* (1rst, 2nd and 3rd group, respectively).

Nonionic surfactants are known to catalyze oxidative stress as activation of protein catabolism which is probably the reason for the decrease in content protein in liver homogenates [18; 19; 24]. It should be noted, oral administration of Laprol-604 for 20 continuous days of pregnancy significantly decrease level of glycogen in liver homogenates of their progeny. Thus, the level of glycogen (mg/g tissue) 81,12 ± 4,41 (controls), 79,63 ± 3,89, 74,15 ± 4,45* and 70,26 ± 4,52* (1rst, 2nd and 3rd group, respectively).

The Laprol-604-induced reductions of the litter size and body weights rat pups. In all groups, the severity of the Laprol-604 adverse effects were dose-dependent. The lag in body weight was particularly pronounced in the 3-rd group (4,7±0,1 g). Moreover, the progeny of rats Laprol-604-exposed had significantly higher liver weights and disturbing liver metabolic parameters compared with controls. A somewhat similar finding was obtained in another study. An increase of liver weight is generally observed in rodents during pregnancy (by about 24% in rat) [1]. Generally, hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably ALT, AST and ALP, sorbitol dehydrogenase and study with Laprol-604 has shown the hepatotoxic effects on liver.

The liver is such a frequent target organ in toxicity studies (in fact, the most common) that a discussion of some of the more common lesions that occurred in the pregnant rats seems warranted. All these facts underline metabolic relations between mother and fetus during pregnancy.

Conclusion

1.Laprol-604 administration reduced litter size in the 3-rd group (5,48±0,21), 2-nd (7,07±0,15) and 1-rst (8,20±012) groups compared with controls 9,31±0,23) ($p<0.01$).

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

3.The liver-specific enzyme is the sorbitol dehydrogenase has been increased significant in serum of blood rat pups exposed to Laprol-604.

4.The adverse effect of Laprol-604 administration was determined as liver enlargement was associated with biochemical disturbances, such as the reduction total protein concentration, the decreased of glycogen, the elevation of total lipid level, increasing alanine aminotransferase activity.

Acknowledgement If Laprol-604 is intended for long-term use, it will be needed to study the offspring be examined for possible adverse effects on later development, behavior and reproductive capacity.

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ВЛИЯНИЕ ЛАПРОЛА-604 НА БЕРЕМЕННЫХ КРЫС И ИХ ПОТОМСТВО

Т.М. Попова

Резюме. Данное исследование было проведено, для понимания неблагоприятного репродуктивного эффекта Лапрола-604. Лапрол-604 оказал дозо-зависимое токсическое действие на потомство крыс. Установлено статистически значимое уменьшение численности и веса тела потомства крыс. Обнаружены нарушения метаболических параметров печени, коррелирующие с увеличением массы печени у потомства крыс, получавших Лапрол-604.

Ключевые слова: Лапрол-604, сурфактант, потомство крыс, репродуктивная токсичность, гестационный день.

ВПЛИВ ЛАПРОЛУ-604 НА ВАГІТНИХ ЩУРІВ ТА ЇХ ПОТОМСТВО

Т.М. Попова

Резюме. Дане дослідження проведено, для розуміння несприятливого репродуктивного ефекту Лапролу-604. Лапрол-604 проявив дозозалежну токсичну дію на потомство щурів. Встановлено статистично значиме зменшення чисельності і маси тіла потомства щурів. Виявлено порушення метаболічних параметрів печінки, що корелює зі збільшенням маси печінки у потомства щурів, які отримували Лапрол-604.

Ключові слова: Лапрол-604, сурфактант, потомство щурів, репродуктивна токсичність, гестаційний день.

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