ЕКСПЕРИМЕНТАЛЬНА МЕДИЦИНА

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THE REPRODUCTIVE INFLUENCE OF LAPROL-604 ON WISTAR RATS*

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У статті представлені результати досліджень впливу Лапрола-604 на пренатальний та ранній постнатальний розвиток щурів лінії Вістар. Високі обсяги виробництва і широке застосування поверхнево-активних речовин викликало стурбованість екологів з початку 1960-х років. Поверхнево-активні речовини використовуються в промисловості і сільському господарстві, а також представлені в продукції для побутового призначення та особистої гігієни. Присутність поверхнево-активних речовин і продуктів їх розпаду в навколишньому середовищу може викликати негативний вплив на біоту [13]. Токсична дія поверхнево-активних речовин на живі організми опублікована у науковій літературі. Відомі дані з окремих досліджень впливу поверхнево-активних речовин на репродуктивну функцию тварин. Це потрібно мати на увазі, щоб уникнути віддалених негативних наслідків на майбутні покоління людей та навколишнє середовище. Вагітність - це один з найважливіших біологічних періодів життя людини і тварин. Ембріон і плід мають незрілий гематоенцефалічний бар'єр, печінкову детоксикацію, метаболізм і тільки рудиментарні механізми репарації ДНК [17]. Таким чином, ембріон і плід більш уразливі, ніж дорослі до несприятливого впливу ксенобіотиків, таких як поверхнево-активні речовини. Дослідження проведено з метою поглиблення розуміння негативних репродуктивних ефектів Лапролу- 604 під час ембріонального, фетального і раннього постнатального розвитку. Вагітним щурам (3 групи по 25 тварин у кожній) вводили Лапрол-604 у дозі 0,125; 1,25 і 12,5 мг/кг один раз на добу за допомогою шлункового зонду з другого до двадцятого дня вагітності. Контрольною групою були 25 вагітних щурів, які перебували на стандартному раціоні віварію без введення Лапролу-604. В постнатальний період кількість живих щурят підраховували, важили, їх стан обстежували. Від самок, що отримували Лапрол-604 у дозі 12,5 мг/кг/добу народитивні щурята, які померли протягом перших 48 годин після народження. Близько 50% щурят, із групи, якій вводили Лапрол-604 1,25 мг/кг/добу, померли протягом перших 10 днів після народження. Інші 50% тварин вижили і досягли статевої зрілості, але вони мали значне уповільнення зростання та розвитку. Щурята, що вижили мали значно більш високу вагу печінки в порівнянні з контрольними тваринами. Виживання щурят в постнатальний період було дозозалежним. Так, більше 80% щурят, мати яких отримували 0,125 мг/кг/добу Лапролу-604, народилися живими, залишалися активними протягом постнатальної життя і досягли статевої зрілості. Результати дослідження показали, що вплив Лапролу-604 на вагітних щурів зумовив зниження постнатального виживання новонароджених, уповільнене зростання та розвиток щурят, що вижили.

Ключові слова: Лапрол-604, поліоли, поверхнево-активна речовина, моделювання, щури, репродуктивна токсичність, негативний вплив на внутрішньоутробний розвиток, постнатальний день.

Introduction

Population exposure to toxic environmental emicals is ubiquitous and adverse health outcomes associated with exposure to such chemicals as surfactants are prevalent and on the rise [17; 24].

Surfactants have widely used all over world. A large number of surfactants containing wastewater are discharged into the environment, resulting in harming aquatic life, polluting the water and endangering human health [12;16;26].

Wildlife and humans are exposed to surfactants in several different ways. Air, water, soil, sediment and food are sources of polyols for living organisms. With regard of lack of toxicokinetic data, it is known that women may be exposed to lipophilic chemicals from various sources including air, water, food, occupational and household environments. Surfactants can also be transferred from the pregnant woman to the developing fetus through the placenta. The most sensitive time of exposure to surfactants during critical periods of development, such as during fetal development [21].

Laprol-604 is found among non-ionic group of surfactants, including Laproxide-303, methycellosolve, methylcarbitol et cetera [27]. Laprol-604 is a synthetic complex organic mixture of polyoxypropylene polyols. During Laprol-604 production a xylitol basis is used as the starting point for anionic polymerization propylene oxide. Laprol-604 has been produced industrially for several decades for use primarily as ingredients of manufacturing epoxide resin, enamels, varnishes, plastic, fiber, glues, emulsifiers et cetera [28].

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The potentially toxic effects of surfactants are presently being studied with increasing intensity [25]. The relevance of this topic is also clearly reflected by the number of publications that have appeared in recent years [1;2;18]. This increasing interest is the result of reports of toxic effects of different groups of surfactants in connection with the ubiquitous detection of these substances in the environment and in sundry matrices, bodies of water, wild animals, human blood, and breast milk samples, all of which have come to the attention of the public [9;11;15].

Nonionic surfactants have been most intensively studied from a toxicological standpoint [14;19;20;27]. Multiple reports, in a variety of animal models, paint a concerning picture of the potential for surfactants to contribute to negative health effects, including developmental toxicity [4;7;10], immunological suppression [3], in neonatal mortality [8]. Although the toxicity of Laprol-604 has been studied and the findings showed that Laprol-604 was moderately toxic, however the influence of Laprol-604 on reproductive system is still unknown.

Survival of the newborn rats, their body weight as well as their liver status have been examined with the goal of researching the reproductive and developmental toxicities of Laprol-604.

Materials and methods

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.

According to biologic characteristic of Wistar rat, the placenta is considerably more porous. This property may increase the chance of fetal exposure to an administered test material

One hundred pregnant Wistar rats (body weight, 180 ± 20 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 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 21. The 4-th group (controls) consisted of 25 intact animals without Laprol-604 administration. The pregnant dams were kept individually in polypropylene cages with heat-treated pine shavings for bedding and tap water ad libitum. Pelleted diets were presented to the rats in wide mouthed jars with lids. Animal facilities were controlled for temperature (20-22°C) and relative humidity (50-60%) and kept under a 12-hr light/ 12-hr dark cycle.

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 [5; 6].

Pregnant rats and their pups routinely monitored during study as an assessment of their general health and to effect of Laprol-604 administration.

Pregnant rats were twice daily clinical observed. They have been weighed on the 0, 6, 9, 12, 15, 18, 20-th days of gestation. Pregnant rats have been monitored at hourly intervals, during the 22 GD and later. Time of parturition for each animal, number of live pups and their conditions have been examined. All of live pups were daily counted and tabulated, and they have been weighed on 0, 2, 3, 7, 10, 13, 16, 19, 22, 28 and 35-th postnatal day (PD). The loss of neonates was within 2nd and 3rd groups. The surviving newborns were distributed randomly to lactating rats into the same group with a litter size of less than 8.

All young rats were weaned on 22-th PD and separated by gender.

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

Laprol-604 showed dosage dependent developmental toxicity when the pregnant rats were exposed. It led to reduced statistically significant 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) ($p<0.01$). Laprol-604 administration decreased of body weight of rat newborns and diminished the number of live pups and the viability of the progeny during the first ten days after birth. All live pups have been born by rats of 3-rd group was daily exposed to Laprol-604 12,5 mg/kg were pallid, inactive, became moribund and died within the first 48 hours after birth (Fig. 1). Approximately 50% of the pups of mother animals (2nd group) were daily administrated 1,25 mg/kg died during the first 10 days after birth.

Influence of prenatal exposure to Laprol-604 on postnatal survival in rats. Each data point represented by average of 9-12 pups. The 3-rd group (dose of 12,5 mg/kg) was varied significantly from 2-nd (dose of 1,25 mg/kg), 1-rst groups (dose of 0,125 mg/kg) and controls ($p<0.001$), whereas the 1-rst group was not significantly different from the controls.

Other 50% of these animals survived and reached puberty. However, offspring that survived showed delays in growth and opening of the eyes. The pups opened their eyes beginning on 14 postnatal day.

Survival improved with lower Laprol-604 exposure and over 80% pups of 1st group have been born alive, stayed active for postnatal life and reached puberty.

The progeny of rats of 4-th group (animals was not exposed by Laprol-604) showed their viability and all control pups survived for the duration of the study. In addition, the mortality was not different between pups of 1st and control groups for the duration of the study.

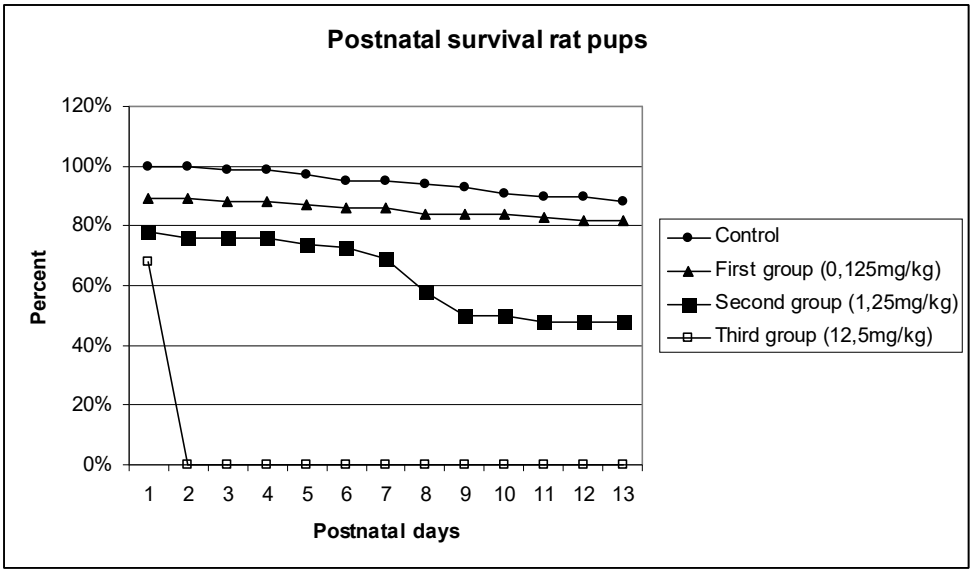

Fig. 1. Postnatal survival rat pups

Altered fertility parameters were found among animals of the 2nd and 3rd groups. The surviving rat pups of these groups were lagging behind in postnatal growth and development. Body weights of pups in the 12,5 mg/kg dosage 3rd group significantly stunted behind control pups. This process has persisted after weaning (Table 1).

No signs of maternal toxicity were observed in the animals at exposures to 0,125 mg/kg dosage (first group). However, there was a tendency, the body weight of the progeny was lower and liver weight was higher then controls.

Table 1
The effects of prenatal exposure to Laprol-604 on body weight of neonatal rat pups (M±m)

Postnatal day	Groups of animals			
	Control group	First group 0.125 mg/kg	Second group 1.25 mg/kg	Third group 12.5 mg/kg
	Body weight, g			
0	5.8±0.1	5.4±0.1	4.9±0.1*	4.7±0.1*
2	7.2±0.1	6.8±0.2	5.9±0.1*	4.9±0.1*
3	8.4±0.2	7.8±0.3	6.5±0.2*	–
7	14.8±0.4	14.1±0.5	10.6±0.2*	–
10	20.8±0.4	19.2±0.8	16.9±0.4*	–
13	23.3±0.8	22.2±0.4	18.8±0.4*	–
16	32.6±0.8	31.3±0.7	23.5±0.1*	–
19	39.2±0.9	38.6±0.9	30.1±0.7*	–
22	51.0±1.2	49.2±1.4	38.1±1.2*	–
28	78.6±2.4	76.4±2.2	68.6±1.4*	–
35	129.7±4.2	128.2±4.6	106.7±2.2*	–

Note. ** Significant differences (p < 0.01) from control values.*

Table 2
The effects of prenatal exposure to Laprol-604 on liver weight of neonatal rat pups (M±m)

Postnatal day	Groups of animals			
	Control group	First group 0.125 mg/kg	Second group 1.25 mg/kg	Third group 12.5 mg/kg
	Liver weight, g			
0	0.31±0.01	0.33±0.02	0.36±0.01	0.40±0.01*
2	0.36±0.01	0.34±0.01	0.37±0.01	0.39±0.02
7	0.49±0.02	0.48±0.03	0.51±0.02	–
13	0.92±0.06	0.89±0.03	0.96±0.02	–
22	1.89±0.13	1.93±0.03	2.14±0.05	–
28	3.75±0.13	3.98±0.25	3.99±0.11	–
35	5.97±0.31	6.03±0.06	6.55±0.13	–

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

Liver weights of the Laprol-604 -exposed pups, except 3-rd group, did not differ from controls appreciably. It

should be noted, while body weight was deficit, the relative liver weight of all Laprol-604 dosage groups was significantly increased.

According to litter size, all Laprol-604 dose groups were significantly different from controls.

The teratogenic effects such as skeletal abnormalities, craniofacial malformation (cleft palate), cardiac defects (ventricular septal defects, enlargement of the right atrium), and delayed ossification (sternebrae, phalanges) were not detected, during examination of rat pups. Reduced litter size, fetal losses were observed. These adverse outcomes were dose-dependent.

Discussion

This study coupled with increased exposure to daily use of surfactants. The importance of identifying and characterizing the reproductive risks of Laprol-604 intended for using by reproductive age population. These risks broadly divided into two categories, reproductive risks and developmental risks. The reproductive risks are related to impact on processes like fertility (male and female), giving birth and lactation. The developmental risks are related to the fetus and include mortality, alteration in growth and functional deficits.

On the one hand, the teratogenic effects were not detected in pups of pregnant rats exposed to Laprol-604, during gestation, on the other hand, the litter size was reduced in the all Laprol-604 administration group. Furthermore, a full neonatal mortality was observed in 3-rd group (with dosages 12,5 mg/kg) and a significant neonatal mortality was found in the offspring of rats treated with dosages 1,25 mg/kg**.**

These findings are consistent with the results of other studies of non-ionic surfactants employing a different dose-regimen of surfactant [23; 27]. The mortality of the new-born rats appeared to be related directly to dose-dependent administration. Indeed, although rats from all dosage groups were born alive, neonates exposed to the high dosages of Laprol-604 (12,5 mg/kg,) survived for two days.

Survival rate was better in the first and second groups (lower dosage groups), and the first 5–7 days of postnatal life were critical to the long-term survival of the neonatal rats.

The pathophysiological mechanisms underlying Laprol-604 -induced neonatal mortality are largely unknown at present. However, among the available data, [22] reported a similar pattern of neonatal death that was explained by numerous structural metabolic disorders, as well as possibility to condition remote consequences.

The profile of neonatal mortality induced by Laprol-604 can be explained of the developmental toxicity of Laproxide L-303, polyols P373-2-20 that triggered profound impairment of structural metabolic processes in endocrine system on all the levels of its structural functional organization: hypothalamus – hypophysis – adrenal and other glands of internal secretion, which can manifest in dysfunction of all kinds of metabolic exchange and energy.

In subchronic studies with adult rat and mice, liver enlargement and hepatic toxicity were associated with non-ionic surfactants exposure [23; 27]. Where as significant increases of relative liver weight were detected in rat pups. These findings suggest that the developing liver is another potential target for Laprol-604 action.

Conclusion

Although the results were limited to the first few days of life, several points can be made concerning the body burden of Laprol-604 in the neonatal rats:

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 postnatal survival.

3. Laprol-604 administration decreased of body weight of rat newborns and diminished the number of live pups and the viability of the progeny during the first ten days after birth, delayed developmental progress.

4. The relative liver weight of all Laprol-604 dosage groups was significantly increased.

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Summary

This article introduces the results of research the Laprol-604-induced effects on the prenatal and early postnatal development of the Wistar rats. The high production volumes and widespread use of surfactants have been an environmental concern since the early 1960s. Surfactants are used in industry and agriculture and are also found in household and personal care products. The presence of surfactants and their biodegradation products in different environmental compartments can invoke a negative effect on the biota [13]. The toxicity of surfactants to living organisms has been summarized in the scientific literature. Nevertheless, some information is still lacking in relation to the reproductive effects need to be understood in order to avoid unexpected adverse effects on future generations of people and the environment. Reproduction is a critical biological process of human and animal populations. It is known the embryo and fetus have immature blood–brain barrier, hepatic detoxifying, metabolizing properties and only rudimentary DNA repair mechanisms [17]. Therefore, the embryo and fetus are more vulnerable than adults to the adverse effects of xenobiotics such as surfactant exposures. This study has been performed to increase the understanding of the adverse reproductive effects associated with Laprol-604 animals exposed during embryonic, fetal, and early postnatal development. Pregnant Wistar rats were administered 0,125; 1,25 and 12,5 mg/kg Laprol-604 once daily by gavage from the second gestation day (GD) to the twenty first GD. Controls consisted of 25 intact pregnant Wistar rats without Laprol-604 administration. Time of parturition for each animal, number of live pups and their conditions have been examined. All of live pups were daily counted, tabulated and they have been weighed several times during postnatal period. All live pups have been born by rats exposed to Laprol-604 12,5 mg/kg were pallid, inactive and died within the first 48 hours after birth. Approximately 50% of the pups of pregnant rats administrated 1,25 mg/kg died during the first 10 days after birth. Other 50% of these animals survived and reached puberty, but they had significant growth retardation. The surviving young rats had significantly higher liver weight compared to the control animals. Survival has been improved by lower dosage Laprol-604 administration and over 80% pups exposed to 0,125 mg/kg Laprol-604 have been born alive, stayed active for postnatal life and reached puberty. The results of study indicated that exposure of Laprol-604 to the pregnant rats caused decreasing postnatal survival of neonates, stunted growth in the surviving rat pups.

Key words: Laprol- 604, polyols, surfactant, modeling, rats, reproductive toxicity, developmental toxicity, gestation day, postnatal day

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