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CHANGES IN TYROSINE METABOLISM INDUCED BY XENOBYOTIC IN WISTAR RATS

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ИЗМЕНЕНИЯ В ОБМЕНЕ ТИРОЗИНА, ИНДУЦИРОВАННЫЕ КСЕНОБИОТИКОМ У КРЫС ЛИНИИ ВИСТАР

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ЗМІНИ В ОБМІНУ ТИРОЗИНУ, ЩО ІНДУКОВАНІ КСЕНОБІОТИКОМ У ЩУРІВ ЛІНІЇ ВІСТАР

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Summary (Резюме)

The impact of Laprol-604-associated changes in free radical oxidation of tyrosine has been investigated for the first time. The activity of monoamine oxidase, decarboxylases of ortho-tyrosine and meta-tyrosine was measured in heart, liver, kidney and brain of Wistar rats. The results of the study indicated an elevation of tissue levels of ortho-tyramine and meta-tyramine in animals exposed to Laprol-604. Ortho- and meta-tyramine was accumulated in rat organs exposed to Laprol-604, inhibiting monoamine oxidase. Significantly lower level of ortho- and meta- tyramine was detected without such an inhibitor as Laprol-604 in control animals. As the highest dose of Laprol-604 was 1/10 LD50, the amount of tyrosine metabolized via decarboxylation to tyramine in rats of the 1st group was more than in rats of the 2nd and 3rd group exposed to lower dose of Laprol-604 (1/100 and 1/1000 LD50 respectively).

These changes of meta-tyramine and ortho-tyramine levels were not observed in control animals not exposed to Laprol-604.

Keywords: *Laprol-604, surfactant, male Wistar rats, tyrosine, ortho-tyramine, meta-tyramine, monoamine oxidase*

Обнаружены изменения процесса декарбоксилирования тирозина, произошедшие под влиянием Лапрола-604. Лапрола-604 является представителем неионогенных поверхностно-активных веществ. Данный ксенобиотик широко используется в химической промышленности. Интоксикацию Лапролом-604 моделировали на взрослых крысах-самцах линии Вистар с массой тела 180 ± 20 г. Животных разделили на четыре группы, по 10 особей в каждой: первая, вторая, третья и контрольная группы. Крысам первой, второй и третьей групп ежедневно внутрижелудочно вводили водный раствор Лапрола-604 в дозах 1/10, 1/100 и 1/1000 LD50, соответственно, в течении 60 дней. Эвтаназию крыс выполнили под

тиопенталовым наркозом в дозе 20 мг/кг. Активность моноаминоксидазы, активность декарбоксилаз орто-тирозину и мета-тирозина определяли в ткани печени, почек, сердца и головного мозга крыс всех групп. Результаты исследования указывают на статистически достоверное повышение концентрации орто-тирамина и мета-тирамина в тканях животных, подвергшихся воздействию Лапрола-604 по сравнению с данными контрольной группы. Орто-тирамин и мета-тирамин накапливались в органах крыс первой, второй и третьей групп, на фоне значительного снижения активности моноаминоксидазы. Самая высокая концентрация орто- и мета-тирамина обнаружена в ткани печени крыс первой группы, получивших наибольшую дозу изучаемого ксенобиотика (1/10 LD50). Необходимо отметить, что вес печени крыс первой и второй групп был достоверно больше по сравнению с весом печени животных контрольной группы. Установлено, зависимое от дозы, негативное воздействие Лапрола-604 на печень, почки, сердце и головной мозг крыс экспериментальных групп.

Таким образом, длительное введение Лапрола-604 крысам привело к ингибированию моноаминоксидазы в тканях печени, почек, сердца и головного мозга животных, что в свою очередь, вызвало накопление атипичных продуктов декарбоксилирования тирозина в исследованных органах.

Ключевые слова: *Лапрол-604, поверхностно-активное вещество, крысы-самцы линии Вистар, тирозин, орто-тирамин, мета-тирамин, моноаминоксидаза.*

Виявлено зміни процесу декарбоксилювання тирозину, що відбулися під впливом Лапролу-604. Лапрол-604 є представником неіоногенних поверхнево-активних речовин. Даний ксенобіотик широко використовується в хімічній промисловості. Інтоксикацію Лапролом-604 моделювали на дорослих щурах-самцях з масою тіла 180 ± 20 г. Тварин розділили на чотири групи, по 10 щурів у кожній: перша, друга, третя і контрольна групи. Щурам першої, другої і третьої груп щодня внутрішньошлунково вводили водний розчин Лапролу-604 у дозах 1/10, 1/100 і 1/1000 LD_{50}, відповідно, протягом 60 днів. Евтаназію щурів виконали під тіопенталовим наркозом у дозі 20 мг/кг. Активність моноамінoксидази, активність декарбоксилаз орто-тирозину і мета-тирозину визначали в тканини печінки, нирок, серця і головного мозку щурів усіх груп. Результати дослідження вказують на статистично достовірне підвищення концентрації орто-тираміну і мета-тираміну в тканинах тварин, які зазнали впливу Лапролу-604 в порівнянні з даними контрольної групи. Орто-тирамін та мета-тирамін накопичувалися в органах щурів першої, другої і третьої груп, на тлі значного зниження активності моноамінoксидази. Найвища концентрація орто- та мета-тираміну виявлена в тканини печінки щурів першої групи, які отримали найбільшу дозу досліджуваного ксенобіотика (1/10 LD_{50}). Необхідно відзначити, що вага печінки щурів першої і другої груп була вірогідно більше в порівнянні з вагою печінки тварин контрольної групи. Виявлено залежний від дози негативний вплив Лапролу-604 на печінку, нирки, серце і головний мозок щурів експериментальних груп.

Таким чином, тривале введення Лапролу-604 щурам привело до пригнічення моноамінoксидази в тканинах печінки, нирок, серця і головного мозку тварин, що в свою чергу, викликало накопичення атипових продуктів декарбоксилювання тирозину у досліджених органах.

Ключові слова: *Лапрол-604, поверхнево-активна речовина, щури-самці лінії*

Вістар, тирозин, орто-тирамін, мета-тирамін, моноаміноксидаза.

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Introduction

Surfactants have been manufactured for more than 60 years. Due to specific chemical structure of nonionic surfactant molecules, they are applied in different areas of human activity. After use, compounds of nonionic surface active agents are emitted to various elements of the environment [1]. Surfactants can enter living organisms by different pathways, where they undergo numerous chemical processes [2]. Several studies reported elevation of the levels of intracellular reactive oxygen species triggered by the influence of surfactants. The metabolic products of surfactants are often more toxic than the original substances [3]. Reactive intermediates may play a key role in the pathological process induced by a surfactant. In spite of the considerable progress of our understanding of basic issues concerning the behavior of products transformed by surfactants in vivo, many issues remain unclear. These include the impact of Laprol-604 on metabolism of tyrosine, namely, the process of decarboxylation of tyrosine and activity of monoamine oxidase which is the major enzyme responsible for oxidative deamination of amines.

So, the research aim was to study the prolonged influence of subtoxic doses of Laprol-604 on tyrosine metabolism and mono amino oxidase activity in male Wistar rats.

Contingents

The study involved forty 100-days-old healthy and adult male Wistar rats. Their body weight was 180 ± 20g at the beginning of the study. The investigations were carried out according to the current principles of bioethics [4].

Object

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

Materials and methods

Male rats were randomly divided into four groups (10 animals in each group). Laprol-604 was administered to male rats once daily by gavage at doses of 1/10, 1/100 and 1/1000 LD_{50} -12.5g/kg, respectively in the 1st; 2nd and 3rd group for 60 days. The 4th group (controls) consisted of 10 intact animals without Laprol-604 administration. Animals were maintained at room temperature (20-22^{0}C) and relative humidity (50-60 %) and kept under a 14 hours light/ 10 hours dark cycle. Pelleted diets were presented to the rats in wide mouthed jars with lids and fresh water was provided ad-libitum throughout the study. At the end of the trial (on the 60th day), the male rats were weighed, sacrificed under thiopental (20 mg/kg) anesthesia, whole heart, liver, kidney and brain were removed and washed with 0.1 M Phosphate-Buffered Saline (PBS), pH 7,4. Isolation of mitochondria and estimation of monoamine oxidase were carried out by Oswald and Strittmatter [5]. The organs were homogenized in an ice-cold mixture of 0,25M sucrose and 0,001 M EDTA, at pH 7,0 using homogenizer. "Total particulate preparation", which essentially contained all the MAO activity of the homogenate, tyramine being used as substrate, was obtained on centrifugation of a 20 % homogenate at 100 g for 1 hour and subsequent single washing of the

sediment by its resuspension in the original volume of the sucrose-EDTA mixture, and recentrifugation. The sediment was resuspended in the sucrose-EDTA mixture and stored at — 10^0C.

Monoamine oxidase (MAO) activity was determined by Mc Caman, Mc Caman, Hunt and Smith (1965), modified by Jarrott (1970) [5].The enzyme activity was calculated from the known specific activity of the substrate and expressed as μ-mole/ hour/ mg protein.

The activity of decarboxylases of ortho-tyrosine and meta-tyrosine was measured by the method for the determination of amino acid decarboxylases suggested by V.E. Davis and J. Awapara [6]. Activity was found in heart, liver, kidney and brain of rats.

Quantification of total protein in liver tissue was determined by method proposed by M.M. Bradford [7].

The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test; $p < 0,05$ was considered significant. The means ± standard error of the mean (SEM) was calculated for all values.

Results and discussion

The present study shows that an increase in the dose of Laprol-604 was associated with elevation of tissue concentrations of ortho-tyramine and meta-tyramine and consequently, intensification of decarboxylation. Large amounts of tyramine were formed even in the lowest dose of Laprol-604 exposure to rats, compared with controls (table 1).

It should be noted that tyramine was found to accumulate in tissues following administration of Laprol-604 which inhibited monoamine oxidase. Without Laprol-604, tyramine was metabolized by monoamine oxidase. As the dose of La-

Table 1

The impact of Laprol-604 on ortho- and meta-tyramine levels of rat organs (M ± SEM)

Organs	Groups of animals			
	Control group (n = 10)	First group 1/10 LD_{50} (n = 10)	Second group 1/100 LD_{50} (n = 10)	Third group 1/1000 LD_{50} (n = 10)
Liver				
Ortho-tyramine (**μmoles/g/h**)	241,29 ± 4,97	384,49 ± 4,38*	316,20 ± 4,18*	276,46 ± 5,45*
Meta-tyramine (**μmoles/g/h**)	136,59 ± 3,33	297,27 ± 4,89*	224,41 ± 4,57*	184,68 ± 4,26*
Kidney				
Ortho-tyramine (**μmoles/g/h**)	257,34 ± 4,37	388,68 ± 4,55*	313,84 ± 5,36*	264,79 ± 5,81
Meta-tyramine (**μmoles/g/h**)	158,12 ± 4,38	249,34 ± 4,68*	196,23 ± 4,46*	187,44 ± 5,93
Heart				
Ortho-tyramine (**μmoles/g/h**)	3,13 ± 1,28	11,38 ± 0,66*	9,80 ± 0,27*	6,78 ± 0,30*
Meta-tyramine (**μmoles/g/h**)	0,70 ± 0,05	1,99 ± 0,09*	1,32 ± 0,04*	0,73 ± 0,05
Brain				
Ortho-tyramine (**μmoles/g/h**)	18,10 ± 3,12	31,18 ± 3,26*	25,34 ± 3,21*	18,67 ± 3,34
Meta-tyramine (**μmoles/g/h**)	9,28 ± 3,96	23,81 ± 3,46*	18,80 ± 3,16	11,51 ± 3,75

Note. * Significant differences ($p < 0,05$) from control values

prol-604 was increased from 1/1000 to 1/10 of LD 50, the amount of ortho-tyrosine and meta-tyrosine metabolized via decarboxylation to tyramine. By contrast, the concentrations of tyramine in tissues of control rats were about two orders of magnitude below this value in Laprol-604-exposed animals. Compared with controls, an elevated concentration of ortho-tyramine and meta-tyramine was found to be significantly elevated within the liver, kidney, heart and brain of Laprol-604-treated rats 1-st, 2-nd groups and some data in the 3rd group. It is known that tyramine is a potent vasoconstrictor. High concentrations of tyramine in the body can induce hypertension, migraines, brain hemorrhage, and heart failure [8, 9].

Normally, tyramine is broken down in mammals by MAO which catalyzes their oxidative deamination [8, 9]. In the present study MAO was measured in the liver, kidney, heart and brain of male Wistar rats exposed to Laprol-604 for 60 days as well as in control rats (table 2). In Laprol-604-exposed rats of the 1st and 2nd groups, the increase in liver mass was accompanied by a marked reduction in MAO concentration. The liver mass elevated by about 28 % and 14 % in the 1st and 2nd groups respectively, and MAO activity fell significantly per unit weight. These changes were also associated with an increase in the content of tyramine, and with a reduction in protein concentration in the liver. MAO activity was slightly reduced in the liver of the 3rd group rats.

The fall in monoamine oxidase activity in tissues of the 3rd group rats (with the lowest dose of Laprol-604 exposure) was significantly less than with higher dose of this surfactant. When monoamine oxidase activity was measured on the 60th day after administration of Laprol-604, there was

Table 2

The impact of Laprol-604 on monoamine oxidase activity (tyramine as substrate) of organs in rats (M ± SEM)

Organs	Groups of animals			
	Control group (n = 10)	First group 1/10 LD_{50} (n = 10)	Second group 1/100 LD_{50} (n = 10)	Third group 1/1000 LD_{50} (n = 10)
Liver				
Wet weight (mg)	1241 ± 47	1584 ± 43*	1416 ± 48*	1276 ± 45
Protein (μg/mg)	212,44 ± 15,31	158,37 ± 12,84*	176,41 ± 14,37*	187,23 ± 15,49
MAO activity (μmole/h/mg protein)	78,40 ± 12,3	35,3 ± 7,6*	47,21 ± 7,3*	69,15 ± 3,33
Kidney				
Wet weight (mg)	1554 ± 75	1417 ± 64	1463 ± 56	1526 ± 81
MAO activity (μmole/h/mg protein)	29,5 ± 1,6	14,33 ± 1,29*	19,14 ± 1,82*	23,42 ± 1,61
Heart				
Wet weight (mg)	1087 ± 25	1068 ± 26	1081 ± 27	1078 ± 31
MAO activity (μmole/h/mg protein)	7,75 ± 0,31	3,29 ± 0,8*	5,12 ± 0,44*	6,87 ± 0,53
Brain				
Wet weight (mg)	1887 ± 51	1894 ± 77	1923 ± 69*	1897 ± 75
MAO activity (μmole/h/mg protein)	19,78 ± 0,65	8,64 ± 0,12*	10,96 ± 0,16*	16,54 ± 0,77

Note * Significant differences (p < 0,05) from control values

a significant reduction in the enzyme activity of the 1st and 2nd groups. Significant difference was found in the enzyme activities between the 1st, 2nd groups and control group. No significant difference was found between the fall in monoamine oxidase activity in the 3rd and control groups.

In Laprol-604-exposed rats the heart mass and brain mass did not differ significantly from control animals. However, the reduction in concentration of monoamine oxidase was observed in these organs in animals of the 1st and 2nd groups.

Zhukov V.I. has reported similar falls in monoamine oxidase activity after exposure to 1/10 and 1/100 of LD50 dose of other surfactants [10]. The present results showed that the fall reached a maximum in tissues of the 1st and 2nd group rats and that the impact of Laprol-604 is most likely dose-dependent. The endogenous tyramine concentration in tissues of the 1st group rats was found to be higher than that of the 2nd group and this confirms the findings of MAO activity inhibition in tissues in the same animals. According to findings of some authors, the aromatic-L-amino-acid decarboxylase is enzyme presents in liver and kidney in a greater degree than in other organs [11, 12]. If tissue concentrations of tyrosine are increased, decarboxylation becomes predominant route of metabolism and large amounts of tyramine are formed [11]. It should be noted that tyramine accumulated in rats exposed to Laprol-604 by inhibiting monoamine oxidase. Without such an inhibitor as Laprol-604 the significant lower levels of ortho- and meta- tyramine were detected in control animals. As the highest dose of Laprol-604 was 1/10 LD50, the amount of tyrosine metabolized via decarboxylation to tyramine in the 1st group rats was more than in rats of the 2nd and 3rd group exposed to lower dose of Laprol-604 (1/100 and 1/1000 LD50, respectively).

A significant elevation of hepatic weight was found in the 1st and 2nd groups as much as 28 % and 14 %, respectively, as compared with the control group. Liver enlargement associated with tyramine accumulated and reduction MAO level is another feature seen after exposure to Laprol-604. This study with Laprol-604 has shown the hepatotoxic effects on liver.

The liver and kidney are such common target organs in toxicity studies that a discussion of some of the more common lesions that occurred in male Wistar rats seems warranted. Adverse effect on liver and kidney of male rats may be linked with intoxication caused by Laprol-604, which is a common feature of toxicity for the non-ionic surfactants.

The results reported here such as accumulation of ortho- and meta-tyramine in tissues may be indirect evidence of unnatural tyrosine isomers production. This stimulated further investigation of the effect of Laprol-604 on the amino acid phenylalanine metabolism.

Conclusion

1. An increase in endogenous ortho- and meta-tyramine concentration and fall in monoamine oxidase activity was dose-dependent for Laprol-604.
2. MAO activity inhibition in the liver, kidney, heart and brain of male rats related to the increase of ortho- and meta-tyramine in the same organs may be associated with changes in oxidation of phenylalanine.

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