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Usage of Sorbents, Hepatoprotectors and Antioxidants for Reduction in the Pathogenic Influence of Dioxin on the Body.

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ABSTRACT

Man, influencing the environment, causes deterioration in the state of biosphere, which affects all living beings of our planet. Currently, the rate of increase in the harmful effect of environmental factors and the intensity of their influence is already beyond the biological adaptability of ecosystems to changes in habitat and create a direct threat to the life and health of animals and humans. Such organics as extremely persistent dioxins and dioxin-like compounds refer to ecotoxicants, which have priority in terms of degree of danger to the health of animals and humans. Dioxin primarily affects the function of the immune system (decrease in phagocytic and lysozyme activity, decrease in the content of T- and B- lymphocytes), the liver, initiates lipid peroxidation (increase in the concentration of malondialdehyde), causes metabolic disorder of all kinds, and has a pronounced embryotoxic and teratogenic effect. The lack of specific and effective means of treatment for dioxin poisoning leads to the improvement of treatment and prevention regimens, including the combined use of medicines. In our studies antioxidants, hepatoprotector and sorbent were used to reduce the toxic load on the body. Selenium with vitamin E (tocopherol) and succinic acid were used as antioxidants, “Phosphogliv” - as hepatoprotector, and “Modibent” (modified bentonite) was used as sorbent. The findings lead to the conclusion that the usage of the tested drugs has a positive influence on the animal organism and reduces the teratogenic effect of dioxin. The positive effect of the studied drugs is related to the ability to trigger an antioxidant defense mechanism in the body, stimulate the immune system, restore cell membranes, and is it also related to the ability to remove xenobiotics from the body.

Keywords: dioxin, teratogenic effect, chronic poisoning

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INTRODUCTION

Pollution of the environment by xenobiotics causes a serious environmental problem connected with the input of so-called persistent organic pollutants. There are three groups of compounds in this list: pesticides, industrial products and undesirable by-products. The latter are particularly dangerous. These include dibenzodioxins and dibenzofurans, which have a polytropic effect, and affect practically all functional systems of the animal and human organism [1-5].

One of the factors that increase the danger of dioxins and dioxin-like substances is the long-term effects caused by these pollutants [6,7]. In recent years, many countries have registered a decrease in reproductive function and an adverse effect on the formation of the reproductive organs of the fetus in humans and animals. Dioxins play a significant role in this, however, there are no drugs that contribute to increase in persistence of the reproductive function of animals, and the problem of their finding is one of the most important problems in modern toxicology.

In this regard, the aim of our studies was to examine the effectiveness of hepatoprotectors, antioxidants and sorbents for the treatment and prevention of poisoning in animals caused by dioxins.

MATERIALS AND METHODS

The studies were carried out in two stages. At the first stage, therapeutic and preventive efficiency of drugs in subchronic poisoning with dioxin was studied. 2,3,7,8-TCDD was used for this purpose. The experiments were carried out on rabbits with a body weight of 2.5 kg. Animals were divided into 5 groups. The first group served as biological control. The second group was given dioxin in a dose of 1/100 LD_{50} (0,3 mcg/kg). The third group of animals was given the studied toxicant, the hepatoprotector “Phosphogliv” (phospholipids + glycyrrhizinic acid) in powder form in a dose of 100 mg/head and sorbent “Modibent” (modified bentonite) in a dose of 2 % of dry substance food; the fourth group was given dioxin, selenium with vitamin E (70 mcg of selenium and 15 mg of vitamin E) one pill and sorbent in the above-mentioned dose. The fifth group got succinic acid in a dose of 25 mg/kg of body weight and modified bentonite along with dioxin. The experiments were carried out for 60 days.

The purpose of the second stage was to study the efficiency of drugs to reduce teratogenic effects of dioxin. Experiments were carried out on females of white rats of breeding age with a body weight of 180-220 g. Preliminarily, the stage of the sexual cycle in rats was determined, namely proestrus or estrus stage. Then males were placed to females from the rate of 2 females per one male. A swab was taken once more the next day and, if sperm was found, it was considered the first day of pregnancy.

Animals were divided into 4 groups of 6 rats in each one. The first group served as biological control and got routine food. Rats of the second group were given with food an oily solution of 2,3,7,8-TCDD in a dose of 0,3 mcg/kg body weight (1/200 LD_{50}) from 1 to 17 days of pregnancy. Animals of the third group were given with toxicant succinic acid in a dose of 25 mg / kg of mass, the fourth group was given selenium with vitamin E in the form of tablets containing 70 of mcg of the element and 15 mg of tocopherol (before use it was ground in a mortar). Toxicants and preparations were given in the form of bread boluses.

Clinical state of animals, feed and water intake were evaluated in the course of the experiment. Complete blood count included determination of the content of erythrocytes, leukocytes and hemoglobin. These studies were implemented with the use of the hematology analyzer Mythic 18. The concentration of total protein of blood serum was examined refractometricly, protein fractions were examined by turbidimetric method using photoelectric photometer KFK-3, and the content of liver enzymes was studied on a biochemical analyzer Microlab 300. Products of lipid peroxidation were determined according to M. S. Goncharenko and A. M. Latinova.

The immune status of test animals was estimated by quantitative and functional indicators. The level of T-lymphocytes in peripheral blood was determined by the method of spontaneous rosette formation with heterogeneous erythrocytes (E-RFC), B-cells - by EAC-rosettes method, and neutrophil phagocytic rate was established according to S. A. Kost and M. I. Stenko. The processing of the obtained digital data was carried out by the method of variation statistics using the validation criterion of Microsoft Excel Student's t-test.

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The study of the dioxin's effect against the background of the use of drugs on the white rats' reproductive system was carried out according to the "Guidelines for the experimental (preclinical) study of new pharmacological agents".

RESULTS OF RESEARCH

In the second group which got dioxin only, clinical signs have appeared by the 27th day in the form of decrease in food and water intake. Body weight decreased by 20% by the end of the experiment. One rabbit has died by the 47th day (table 1). The content of erythrocytes and hemoglobin decreased on average by 20-30%. The number of leukocytes at the beginning of the experiment increased, and then a decrease of 20% was observed. There was a significant decrease in the amount of total protein by 15% by the 60th day. Along with this, there were changes in the ratio of protein fractions.

The activity of alanine aminotransferase and aspartate aminotransferase increased on average by 1.5-3.1 times in comparison with the control. During studying the indicators of phagocytic activity (phagocytic activity, phagocytic capacity, phagocytic index, phagocytic number), their increase has been observed on average by 20% by the 20-40th days , and it has been deceased by 35% by the 60th day. The content of T- and B-lymphocytes has decreased by 10-12%. The concentration of malondialdehyde in the blood of animals has increased on average by 1.5-1.8 times (table 2). The mechanism of increasing in this indicator in blood is connected with the fact that dioxins affect cell membranes, thereby triggering the activation of lipid peroxidation. Polyunsaturated fats are degraded by reactive oxygen species and the formation of this product, i.e. its release into the blood plasma, which is a sign of oxidative stress [8].

Table 1: Comparative effectiveness of therapeutic and preventive drugs in the poisoning of rabbits with dioxin

Name of animal groups	Number of animals			
	total	survived	died	% survival rate
Biological control	4	4	0	100
Dioxin	4	3	1	80
Dioxin + Phosphogliv + Modibent	4	4	0	100
Dioxin + selenium with vitamin E + Modibent	4	4	0	100
Dioxin + succinic acid + Modibent	4	4	0	100

Table 2: The content of malondialdehyde in the rabbits' blood with dioxin poisoning and in the use of therapeutic and preventive drugs (mcM/ml)

Duration of study (day) and the group			
Background	20	40	60
Biological control			
2,10±0,05	2,10±0,02*	2,21±0,05	2,15±0,03
Dioxin			
2,13±0,08	2,90±0,07*	3,10±0,04*	3,80±0,12*
Dioxin + Phosphogliv + Modibent			
1,98±0,05	2,05±0,06*	2,12±0,10	2,11±0,07*
Dioxin + selenium with vitamin E + Modibent			
2,17±0,10	2,17±0,08	2,19±0,15*	2,62±0,08*
Dioxin + succinic acid + Modibent			
2,11±0,06	2,11±0,02	2,19±0,04*	2,15±0,03

NB: * - differences with control are reliable

In rabbits treated with hepatoprotector and sorbent, clinical signs were absent except the decrease in body weight by 9%. Changes were observed on the part of phagocytosis indicators. Phagocytic activity of neutrophils decreased on average by 17-20% of the background value. The body weight remained normal in the fourth and fifth groups. Hematological, biochemical and immunobiological indicators did not change significantly and remained within background values.

Changes in the liver (densified, enlarged, granular) and in the spleen (densified, dark-brown colored) were visually found during autopsy of animals that had received dioxin. There were no changes in the macro-picture of the organs in rabbits that had received drugs.

The positive effect of drugs is connected with the peculiarity of their mechanism of action in dioxin poisoning, which is capable of violating the structures and functions of cell membranes [9,10,11].

The drug “Phosphogliv” (glycyrrhizinic acid+ phospholipids) has a hepatoprotective and immunostimulating effect. Phospholipids included in the preparation formula have membrane trophic activity, that is, they influence the state of the cell membrane. They also normalize the lipid and carbohydrate metabolism. Glycyrrhizic acid stimulates the immune system by enhancing the activity of immune system cells, such as natural killer cells and phagocytes. In addition, glycyrrhizic acid has an antioxidant and membrane-stabilizing effect. This component also potentiates the action of endogenous glucocorticosteroids, providing an anti-inflammatory effect in non-infectious liver damages.

The drug “Selenium with vitamin E” contains such active substances as sodium selenite and tocopherol acetate (vitamin E). Vitamin E regulates redox processes and influences carbohydrate-fat metabolism, enhances the effect of vitamins A и D_3, has an effect on the state of immunity and on the general resistance of the organism [12]. The biological role of selenium is connected with its antioxidant properties. It helps to remove toxic substances from the body, increase the immunity of animals [13].

Succinic acid has a strong stimulating effect on the body. The therapeutic effect is based on a modifying effect on the cellular metabolism - cellular respiration, transport of trace elements, production of proteins. It is an adaptogen (increases the body’s resistance to unfavorable environmental factors). As a result of stimulation of the liver and kidneys function, the body is more efficiently cleared of xenobiotics, toxic metabolites and other harmful agents [14].

Modibent (modified bentonite) is a sorbent for peroral use, intended for use by animals as an enterosorbent for gastrointestinal diseases and pathological conditions, accompanied by intoxication of the body.

Thus, the use of the above-listed treatment regimens for subchronic poisoning with dioxin has a positive effect on the body, however the usage of a selenium-containing drug with a sorbent and succinic acid with modified bentonite is the most effective.

High sensitivity of the reproductive function to the chemical factor is known [15,16,17,18]. Consequently, studies were conducted to develop new methods for protecting animals, taking into account the survival rate and long-term effects of dioxins exposure. Postnatal development of infant rats is indicated in the table 3.

Table 3: Offspring development indicators of white rats' females during dioxin poisoning and the use of antioxidants

Indicator		Groups		
	Biological control	Dioxin	Dioxin + succinic acid	Dioxin + selenium with vitamin E
Duration of pregnancy, day	23,30 ± 0,30	22,10 ± 0,20	23,00±0,18	23,30±0,10
The number of rats born per female	8,00±0,55	5,17±0,34	6,89±0,44	6,15±0,15
Dead birth, %	1,55±0,40	6,60±0,40	3,67±0,50	3,60±0,24

Body weight, g:				
newborns	5,23±0,02	5,02 ± 0,08	5,91±0,13	5,10±0,12
by the 3th day	7,43±0,05	6,90 ± 0,10	6,78±0,07	7,40±0,15
by the 5th day	9,78±0,09	8,10±0,02	9,80±0,10	9,80±0,19
by the 13th day	14,75±0,20	13,4 ± 0,2	16,12±0,25	14,85±0,10
by the 21th day	20,70±0,52	18,30±0,07	21,83±0,27	20,30±0,42
by the 28th day	29,57±0,18	26,7 ± 0,4	28,90±0,61	29,00±0,10
Time of pinna detachment, day	6,65 ± 0,62	6,40 ± 0,40	6,50 ± 0,30	6,60 ± 0,20
Time of downiness, day	8,30 ± 0,30	8,30 ± 0,20	8,10 ± 0,20	8,10 ± 0,30
Time of cutting teeth eruption, day	9,10 ± 0,32	9,30 ± 0,40	9,10 ± 0,20	9,30 ± 0,20
Time of vision formation, day	15,8 ± 0,50	15,8 ±0,60	15,80 ± 0,40	15,5 ± 0,30
Postnatal mortality by the 28th day, %	3,3 ± 0,2	17,0 ± 0,50	7,20 ± 0,40	7,00 ± 0,40

The table shows that the duration of pregnancy of females receiving dioxin has decreased by 5.1%, the number of born rats - by 35%, dead birth has increased by 4.2 times, and the average body weight of rats has been lower by 9.7 % in comparison with the biological control by the 28th day. In this group, the highest percentage of postnatal mortality of rats was observed by the 28th day of life, more than 5 times higher than this indicator of the biological control group.

The protective effect of the drugs was observed in the third and fourth groups. So the duration of pregnancy did not differ from biological control, the number of born rats was reduced in the third group by 13.7%, in the fourth - by 23%, dead birth increased on average by 2.3 and 2.3 times. The average body weight in the treated groups practically did not differ from biological control, the postnatal mortality increased by 2.1 times by the 28th day.

CONCLUSION

Thus, the usage of the tested drugs has a positive effect on the body of animals with dioxin poisoning, normalizes the clinical condition, hematologic, biochemical and immunobiological indicators.

The usage of succinic acid and selenium with vitamin E is characterized by an increase in the number of born offspring, a decrease in dead birth and postnatal mortality in contrast to the untreated group. In rats born from females poisoned with dioxin and receiving antioxidants, there is a more significant increase in body weight by the 28th day of life, mortality decreases.

The positive effect of the studied drugs is related to the ability to trigger the mechanism of antioxidant defense in the body, stimulate the immune system, restore cell membranes, and also the ability to remove xenobiotics from the body.

REFERENCES

[1] Mashkovcev NM. Materials of research and practical conference «Topical Issues of Animal Breeding and Veterinary Medicine» 1998; 221-222.
[2] Nigmatov DKh et al. Veterinarian 2005; 4: 9-11.
[3] Papunidi KK et al. Veterinarian 2000; 1: 32-34.
[4] Acira K et al. Environ pollut. 2013; 178: 300-305.
[5] Alonso K. Chemosphere 1998; 37 (9): 1873-1883.
[6] Budinsky R et al. Tocicol.Sci 2010; 118 (1);. 224-235.
[7] Bagshi D et al. Toxicology 2002; 175: 73-82.
[8] Crain DA et al. Fertility and Sterility 2008; 90 (4): 911-940.
[9] Chu I et al. Chemosphere 2001; 43 (4-7): 807-814.
[10] Courtney K, More J. Toxicol. appl. Pharmacol 1979; 20: 396 – 403.
[11] Haitian L. Cravfor Toxicol. Sci. 2010; 118, (1): 86-97.

[12] Katynski AL et al. Biochem. Physiol. 2004; 137 (1): 81-93.
[13] Nishimura N et al. Toxicol. Sci. 2005; 85 (1): 607-614.
[14] Papunidi KK et al. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2016; 7(4): 2214–2220.
[15] Shuhai L et al. Talanta. 2011; 85 (2), 1007-1012.
[16] Salnikova MM et al. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2017; 8(10): 1939-1946.
[17] Vyskocil A et al. Appl. Toxicol. 1995; 15 (4) 327 – 328.
[18] Webster WS. Teratol. 1990; 5: 255 - 282.