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Homeostatic system of sheep against the background of combined effects of pollutants and the use of therapeutic and preventive agents

Konstantin Kh. Papunidi,* Ilnur R. Kadikov, Vadim R. Saitov, Eduard I. Semenov, Dinard Kh. Gataullin, Andrey A. Korchemkin, Anna M. Tremasova

ABSTRACT

Nowadays, increasing harmful effects of environmental factors and their intensifying influence go beyond biological adaptability of ecosystems to the changes in the environment and pose a direct threat to the life and health of animals and humans.

In general, xenobiotics, especially dioxins, enter the environment in small amounts but possessing material and functional cumulation they can cause considerable harm to the body.

Analysis of our study shows that the xenobiotics entering the body of animals even at low concentrations cause functional changes in the body. Thus, the administration of 2,3,7,8-TCDD at a dose of 1/400 LD50 (0.5 μg/kg of body weight) and T-2 toxin at a dose of 200 μg/kg of fodder weight into the body of sheep causes erythropenia, leukopenia, hemoglobinemia, and a decrease in the number of T- and B-lymphocytes. The experiments show that xenobiotics is affecting the liver tissue cause lysis of hepatocytes releasing liver enzymes such as alanine aminotransferase and aspartate aminotransferase into the blood. The increase in these enzymes in serum reflects not only the liver injuries but also the dysfunction of other organs. In addition, dioxin and T-2 toxin cause an increase in the level of malondialdehyde, which results from lipid peroxidation.

The application of DAS-2 at a dose of 3 ml/h and bentonite at a dose of 2% of dry matter, succinic acid at a dose of 25 mg/kg of body weight, and bentonite at the above-mentioned dose has a positive effect on sheep in combined dioxin and T-2 toxin poisoning and is characterized by the normalization of hematological, biochemical indicators, the content of T- and B-lymphocytes, and a decrease in the malondialdehyde content.

Keywords: dioxin, T-2 toxin, tissue stimulant, succinic acid, liver enzymes, peroxidation

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Toxicology laboratory, Federal Center of Toxicological, Biological and Radiation Safety, Scientific Town-2, Kazan City, 420075, Russia

*Correspondence to: Konstantin Kh. Papunidi, Toxicology laboratory, Federal Center of Toxicological, Radiation and Biological Safety, Scientific Town-2, Kazan City, 420075, Russia
vnivi@mail.ru

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INTRODUCTION

One of the problems caused by scientific and technological progress in the last century is growing environmental pollution with toxic chemical compounds, the most dangerous of which are persistent organic pollutants (POPs), including dioxins. These substances were isolated in a separate group because of their high toxicity and significant effect on functional systems of animals. Their acceptable daily dose is measured in picograms.

The degree of toxicity of dioxins and dioxin-like compounds to laboratory animals varies widely and is determined by the number of substituted hydrogen atoms in the molecules by halogens and isomeric composition of the substance. The highest value of this indicator is observed in tetrachloropropane compounds; the most toxic of which is 2,3,7,8-TCDD.[3,5,7,10,13,17]

In addition to dioxins, mycotoxins being biogenic toxic substances also have a significant harmful effect on the environment, the most toxic of which is T-2 toxin.[4,11,12,16] This pollutant adversely affects health, conservation, the intensity of growth, and productivity of animals and birds causing destructive changes both at the organ and cell levels. Almost all functional systems of the body are affected by the mycotoxin.[6,8,15,18]

The nature of the individual biological effect of dioxin and the T-2 toxin is widely studied, and appropriate therapeutic and preventive means have been developed; however, there isn't enough information about combined toxicosis.[9,14] Meanwhile, it is quite possible for agricultural lands to get severely affected if they are close to the sources of dioxin emissions.

MATERIALS AND METHODS

The study was carried out in 12 sheep, 2,3,7,8-TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) and crystalline T-2 toxin with a purity of about 98.9% were used.

Tissue stimulant—DAS-2, adaptogen—succinic acid, and sorbent—bentonite were used as test preparations.

DAS-2 (Dorogov's antiseptic stimulant) is a product of dry distillation of raw materials of animal origin which contains compounds with an

active sulfhydryl group, aliphatic amine derivatives, carboxylic acids, aliphatic and cyclic hydrocarbons, amide derivatives, and water. It belongs to biogenic stimulants.

Succinic acid is a white, odorless crystalline powder; its boiling point is 185–189°C and solubility in water is 1g/10ml at 50°C. Molecular weight is 118,09 (HOOC(CH2)2 –COOH). It is a universal participant in the metabolism of a living cell, a natural endogenous substrate of oxidation-reduction reaction in living organisms.

Bentonite clay powder from Biklyanskoe deposit in the Republic of Tatarstan (Russia) is fine-pore clay composed mainly of the montmorillonite Al2[Si4010](OH)2·nH2O minerals. It has shape-selective properties, so it is a good adsorbent for many organic and inorganic substances.

To study the effects of therapeutic and preventive agents in the case of combined dioxin and T-2 toxin poisoning, four groups of sheep were formed, with three animals in each group. The first group was a biological control. The second group received dioxin at a dose of 1/400 LD50 (0.5 μg/kg of body weight) in combination with T-2 toxin at a dose of 200 μg/kg of fodder weight. The third group of experimental animals was given succinic acid at a dose of 25 mg/kg of body weight and enterosorbent-bentonite at a dose of 2% of the dry matter along with the toxicants. The fourth group received the toxicants with tissue stimulant DAS-2 (3 ml/h) and bentonite at the above-mentioned dose. On the 20th, 40th, and 60th days blood samples were taken from the jugular vein for carrying out hematological and biochemical analyses.

Hematological parameters (erythrocytes, hemoglobin, leukocytes) were studied with the Mythic 18 hematology analyzer.

The activity of enzymes, carbohydrate content, and products of protein and lipid metabolisms were determined with the Microlab 300 biochemical analyzer.

Products of lipid peroxidation were determined by M.S. Goncharenko and A.M. Latinova's method.[1]

The level of T-lymphocytes in peripheral blood was determined by the spontaneous rosette test with heterogeneous red blood cells (E-rosetting). Identification of B-lymphocytes was carried out by EAC-rosetting by G. Frimel's method.[2]

RESULTS AND DISCUSSION

On the 60th day of the study, in animals which were given dioxin and T-2 toxin, there was a decrease of 20%, 26%, and 14% in the content of erythrocytes, hemoglobin, and leukocytes, respectively. In the third group of animals, there was a decrease in those indicators, excluding the level of white blood cells. The red blood cells and hemoglobin decreased by 18% and 20% by the end of the experiment. In the fourth group where animals were given the tissue stimulant and the sorbent there was a decrease of 15% in the level of hemoglobin on the 60th day.

As can be seen from Table 1, in the serum of sheep which received only the toxicants, the concentration of AST on the control days increased by 1.1, 1.6, and 2 times; ALT—by 1.7, 1.9, and 1.9 times; LDH—by 1.2, 1.4, and 1.8 times; urea—by 1.6, 2, and 2.5 times. The level of glucose on the control days decreased by 27%, 30%, and 30%. The level of creatinine remained close to the background value. On the 40th and 60th days, the level of total bilirubin increased by 1.2 and 1.3 times and the level of cholesterol increased by 1.3 and 1.7 times.

In the third group, the number of liver enzymes increased—AST by 1.3 times and ALT by 1.1 times—by the end of the study. The level of urea increased by 1.6, 2, and 1.9 times, and the concentration of total bilirubin increased by 32%, 32%, and 30% on the 20th, 40th, and 60th days, respectively.

In animals, which received DAS-2 and bentonite along with pollutants, the concentration of urea increased by 1.4, 1.3, and 1.6 times. The level of total bilirubin increased by 1.2 times on the 40th and 60th days. Such indicators as ALT, AST, LDH, glucose, creatinine, and cholesterol did not change and remained about basal values.

Determination of the concentration of lipid oxidation products (LOPs) can give information about the extent of injury and severity of the pathological process. In this regard, estimation of biochemical processes by lipid peroxidation activity in the blood is of special significance.

In our study, the nature of the change in lipid peroxidation in the case of combined dioxin and T-2 toxin poisoning was estimated by the content of its final product—malondialdehyde in plasma and hemolysate of red blood cells.

In the group of the sheep which received dioxin in combination with the T-2 toxin, the concentration of malondialdehyde in the hemolysate increased by 30%, 45%, and 67%, and in the plasma group it increased by 36%, 40%, and 57% on 20th, 40th, and 60th days.

In the third group of the sheep, the indicator in the blood plasma increased by 15% on the 60th day of the study.

In the animals of the fourth group which was given DAS-2 and bentonite along with toxicants, there were no changes in the level of the product of lipid peroxidation, and the indicators remained at the level of background value (Table 2).

Table 1 **Biochemical indicators of blood serum of the sheep in the case of combined dioxin and T-2 toxin poisoning and the application of agents**

Indicator	The day of the study, group			
	Background	20	40	60
1	2	3	4	5
Biological control				
AST, IU/L	114.17 ± 11.42	108.33 ± 1.10	106.10 ± 4.10	105.10 ± 2.00
ALT, IU/L	17.80 ± 0.89	17.82 ± 0.24	18.11 ± 0.12	18.10 ± 0.19
Glucose, mmol/l	3.83 ± 0.24	4.30 ± 0.23	4.10 ± 0.13	3.83 ± 0.16
Creatinine, μmol/l	85.33 ± 0.81	86.80 ± 0.33	88.12 ± 0.35	85.13 ± 0.86
LDH, IU/L	186.10 ± 1.24	185.30 ± 2.57	188.10 ± 0.81	185.10 ± 1.33
Urea, mmol/l	2.11 ± 0.46	1.53 ± 0.40	1.91 ± 0.16	1.82 ± 0.55
Total bilirubin, μmol/l	14.29 ± 0.11	13.66 ± 0.55	13.19 ± 0.22	12.50 ± 0.15
Cholesterol, mmol/l	1.39 ± 0.16	1.39 ± 0.97	1.55 ± 0.16	1.50 ± 0.19
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC				
1	2	3	4	5
AST, IU/L	105.04 ± 13.50	115.13 ± 6.07	177.01 ± 6.88	207.33 ± 1.81
ALT, IU/L	24.10 ± 0.43	40.44 ± 0.88	44.51 ± 0.31*	46.94 ± 0.68*
Glucose, mmol/l	3.00 ± 0.83	2.17 ± 0.29	2.00 ± 0.15	2.00 ± 0.16
Creatinine, μmol/l	86.34 ± 0.14	85.82 ± 0.13	100.13 ± 0.95*	104.71 ± 0.68
LDH, IU/L	212.00 ± 2.34	265.10 ± 7.23	300.00 ± 8.45	390.00 ± 2.46*
Urea, mmol/l	1.60 ± 0.47	2.55 ± 0.27*	3.20 ± 0.60*	4.00 ± 0.80
Total bilirubin, μmol/l	15.11 ± 0.34	17.11 ± 0.16	19.09 ± 0.12	19.43 ± 0.16*
Cholesterol, mmol/l	1.41 ± 0.16	1.65 ± 0.14	1.86 ± 0.79	2.47 ± 0.19*
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC + succinic acid + bentonite				
AST, IU/L	105.18 ± 25.61	105.88 ± 8.17	117.10 ± 8.10	139.00 ± 4.31
ALT, IU/L	26.40 ± 0.21	28.44 ± 0.10	28.51 ± 0.13	30.10 ± 0.11*
Glucose, mmol/l	3.10 ± 0.12	3.17 ± 0.33	3.18 ± 1.46	2.99 ± 0.51
Creatinine, μmol/l	84.44 ± 0.31	83.30 ± 0.13	85.84 ± 0.15*	85.13 ± 0.17*
LDH, IU/L	210.11 ± 2.44	261.11 ± 5.21	260.00 ± 1.40	240.01 ± 2.77*
Urea, mmol/l	1.61 ± 0.51	2.66 ± 0.77*	3.20 ± 0.61*	2.99 ± 0.10
Total bilirubin, μmol/l	14.11 ± 0.29	18.71 ± 0.16*	18.10 ± 0.18*	17.08 ± 0.18
Cholesterol, mmol/l	1.31 ± 0.61	1.31 ± 0.05	1.45 ± 0.10*	1.18 ± 0.11
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC + DAS-2+ bentonite				
AST, IU/L	101.18 ± 25.10	105.11 ± 4.87	107.10 ± 4.11	108.10 ± 4.33
ALT, IU/L	26.11 ± 0.18	28.11 ± 0.90	28.11 ± 0.14*	28.90 ± 0.12*
Glucose, mmol/l	3.10 ± 0.11	3.87 ± 0.13	3.18 ± 0.17	3.82 ± 0.19*
Creatinine, μmol/l	80.14 ± 0.18	84.11 ± 0.13	84.13 ± 0.11	85.13 ± 0.77
LDH, IU/L	210.11 ± 1.14	205.11 ± 4.28	205.11 ± 1.41	201.31 ± 1.77
Urea, mmol/l	1.61 ± 0.18	2.36 ± 0.77*	2.16 ± 0.11*	2.61 ± 1.70
Total bilirubin, μmol/l	14.20 ± 0.38	14.14 ± 0.18	17.50 ± 0.19*	17.28 ± 1.16
Cholesterol, mmol/l	1.31 ± 0.66	1.31 ± 0.15	1.35 ± 0.10*	1.38 ± 0.38*

*indicates differences between the value and the control value are reliable with accuracy $p \leq 0.05$

Data on the influence of various toxicants on the immune system are currently very extensive and controversial. Analysis of immunotoxic effects of xenobiotics is due to the need to identify the most sensitive parameters of the immune system to certain poisons and to justify the

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Table 2 The content of malondialdehyde in the blood of the sheep in the case of combined dioxin and T-2 toxin poisoning and the application of agents

Indicator	Background	The day of the study, group			
		20	40	60	
Biological control					
Malondialdehyde, μmol/ml	Hemolysate	1.87 ± 0.04	1.73 ± 0.04	1.53 ± 0.04*	1.57 ± 0.04*
	Plasma	2.30 ± 0.07	2.53 ± 0.04*	2.23 ± 0.04	2.17 ± 0.04
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC					
Malondialdehyde, μmol/ml	Hemolysate	1.53 ± 0.04	1.97 ± 0.04*	2.23 ± 0.04*	2.57 ± 0.04*
	Plasma	2.13 ± 0.08	2.90 ± 0.07*	3.03 ± 0.04*	3.30 ± 0.12*
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC + succinic acid + bentonite					
Malondialdehyde, μmol/ml	Hemolysate	1.60 ± 0.07	1.53 ± 0.04	1.53 ± 0.04	1.57 ± 0.04
	Plasma	2.27 ± 0.11	2.17 ± 0.08	2.17 ± 0.15	2.63 ± 0.08*
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC + DAS-2+ bentonite					
Malondialdehyde, μmol/ml	Hemolysate	1.50 ± 0.07	1.57 ± 0.04	1.63 ± 0.04*	1.63 ± 0.04*
	Plasma	1.97 ± 0.04	2.00 ± 0.07*	2.13 ± 0.11	2.10 ± 0.07*

*indicates differences between the value and the control value are reliable.

Table 3 Immunobiological indicators in the sheep in the case of combined dioxin and T-2 toxin poisoning and the application of agents

Indicator	The day of the study, group			
	Background	20	40	60
Biological control				
T-lymphocytes, %	41.33 ± 1.08	41.00 ± 0.71	42.33 ± 0.71	40.67 ± 1.47
B-lymphocytes, %	19.67 ± 0.41	19.33 ± 0.41	19.67 ± 0.41	18.67 ± 0.41
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC				
T-lymphocytes, %	41.00 ± 0.71	40.67 ± 0.41	36.00 ± 0.41*	33.33 ± 1.08*
B-lymphocytes, %	16.33 ± 0.41	16.67 ± 0.41	14.67 ± 0.41*	13.33 ± 1.08
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC + succinic acid + bentonite				
T-lymphocytes, %	41.00 ± 1.22	41.67 ± 0.41	38.67 ± 0.41	38.67 ± 0.41
B-lymphocytes, %	16.33 ± 1.08	15.67 ± 0.41	15.67 ± 0.41	15.00 ± 0.71*
Dioxin at a dose of 1/400 LD50 + T-2 toxin at 2MPC + DAS-2+ bentonite				
T-lymphocytes, %	43.33 ± 0.82	45.00 ± 0.71	43.00 ± 0.71	42.33 ± 0.82
B-lymphocytes, %	16.00 ± 0.71	16.67 ± 0.41	17.00 ± 0.71	18.33 ± 0.41*

*indicates differences between the value and the control value are reliable with accuracy $p \leq 0.05$

application of medications adequate to the nature of immune homeostasis disorders caused by various toxicants.

It is seen from Table 3 that on 40th and 60th days in the group of animals which received only the toxicants, the content of T-lymphocytes decreased by 12% and 18% and the content of B-lymphocytes increased by 10% and 19%, respectively.

In the group of animals which were given succinic acid and sorbent, the number of T- and B-cells did not change.

In the sheep which received DAS-2 and bentonite, only the content of B-lymphocytes changed, with an increase in number of 13% on the 60th day.

CONCLUSION

Thus, the application of the developed schemes of treatment using succinic acid (25 mg/kg of body weight) in combination with bentonite (2% of dry matter) and DAS-2 (3 ml/h) in combination with bentonite protects animals from the adverse effects of dioxin and T-2 toxin and reduces the toxic load on the body and normalizes blood hematological and biochemical indicators, free radical oxidation, and immunobiological reactivity.

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