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ORIGINAL ARTICLE

Effect of antibiotics, hormones and anthelmintic on high molecular weight protein fractions in the common carp

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We assumed that effect of sulfanilamide, chlortetracycline, nandrolone, and albendazole on the carp fishes depends on substance concentration in the water and associated with changes in the fractional composition of plasma protein. We found that sulfanilamide in concentration of 0.015 mg/dm^3 in water practically had no effect, while at concentrations of 0.15 and 0.30 mg/dm^3 it changed the protein spectrum of carp blood plasma, reduced the level of proteins with molecular weights of 260 and 140 kDa. Chlortetracycline in the concentration of 1.10 mg/dm^3 did not affect the protein content of macromolecular fractions in the blood plasma of fish, and at the doses of 3.15 and 6.30 mg/dm^3 it reduced the content of proteins with molecular weights of 450, 340, 260 and 140 kDa. Nandrolone in the concentration of 1.10 mg/dm^3 increased the content of individual proteins in carp plasma compared to the control, and in the concentration of 0.10 and 0.50 mg/dm^3 it did not affect the fractional composition of proteins with high molecular weight. Albendazole in the concentrations of 0.2, 0.5, and 1.00 mg/dm^2 reduced the protein content of macromolecular fractions in carp plasma. Such xenobiotics, like sulfanilamide, chlortetracycline, and albendazole in different concentrations did not affect the total protein content of fish plasma, while the nandrolone increased it. The number of respiratory movements, behavior, body surface, and pathomorphological parameters of the main internal organs in the fish from the experimental groups did not differ from the control. Our results indicated the important role of proteins of high molecular weight fractions in carp blood plasm as these proteins determine the fish adaptation to the water xenobiotics.
Keywords: carp, blood plasma, proteins, sulfanilamide, chlortetracycline, nandrolone, albendazole.

Introduction

Chlortetracycline is one of the toxicants found in effluents from livestock enterprises and in water (Ivanova & Zakharenko, 2010; Kurbatova et al., 2008). It belongs to the tetracycline group and this is a non-degradable synthetic compound. The action of chlortetracycline on the body is associated with inhibition of protein biosynthesis by blocking the RNA polymerase enzyme and binding to template DNA on ribosome's (White et al., 1981). It is likely that the accumulation of chlortetracycline in ponds, as the most commonly used antibiotic in animal husbandry except the impact on the fish development in different stages of ontogeny, will change the biosynthesis of proteins in the liver and muscles, as well as the fractional composition of blood plasma proteins.

In the ponds of the sulfonamide group except the sulfanilamide, there are sulfaguanidine, sulfadimesine, sulfamerazine, and sulfamethoxazole, whose content in water varies significantly (Bukreeva et al., 2011). Xenobiotics are capable to change microbiocenosis reservoirs, to destroy the food chains, and to disturb the natural state of aquatic ecosystems (Horzheyev et al., 2013; Kurbatova & Tsedyk, 2012). Sulfanilamide is one of the widespread groundwater pollutants and its bacteriostatic properties are associated with deceleration of enzymatic reactions in the cell (Horzheyev et al., 2013). Due to its high solubility in water and organic solvents, sulfanilamide is able to easily penetrate through cell membranes, affect the growth of animals, exhibit mutational properties (Ivanova & Zakharenko, 2013). At the same time, the sulfanilamide does not affect the activity of cholinesterase, endocrine glands and does not have a neurotoxic effect. However, albendazole is an inhibitor of acetylcholinesterase and has a neurotoxic effect in animals. It is partially metabolized in the liver with the formation of sulfoxidalbendazole and sulfonalbendazole (Tsudevich et al., 2012; Prikhodko, 2000), cause allergic reactions in the body. Acute and chronic toxicity of sulfanilamide to fish, aquatic organisms and aquatic plants have not been established, whereas for mammals its LD_{50} is 3700 mg/kg of body weight (Lukyanenko, 1987).

Since sulfanilamide affects the processes of protein synthesis in the animal tissues, in particular by altering the activity of key enzymes of the process, it is possible to predict its ability to influence the composition of fish blood plasma proteins. Estrogens and androgens and their conjugates in rivers are a real problem (Fine et al., 2003; Takuma & Kurunthacha, 2006), which include

19-nortestosterone (nandrolone) (Ivanova & Zakharenko, 2013; Bradley et al., 2009). A synthetic steroid is widely used as a therapeutic agent and stimulant for animal performance. It is a member of the progesterone group and it influences digestive processes and stimulates metabolic processes in animal tissues (Ivanova, 2014; Kurbatova et al., 2014). Nandrolone degradation products 19-noradrostosterone, 19-norethicholanolone and 5-dihydro-19-nortestosterone (dihydronandrolone) have also been found in effluents, which also have hormonal activity in the body (Kurbatova et al., 2014; Huang & Sedlak, 2001).

Effluents also contain the anthelmintic albendazole, a major anti-parasitic drug that belongs to the benzimidazole group (Ivanova, 2014). Albendazole causes degenerative changes in cell membranes in parasites by the decelerating the polymerization process of tubulin (Kurbatova et al., 2008). This leads to the disappearance of the microtubules of the cytoplasm of the parasite cells and its death (Tafiychuk, 2008; Prikhodko, 2000). In addition, albendazole is an acetylcholinesterase inhibitor and has neurotoxic effects in the body of animals. It is partially metabolized in the liver to form sulfoxidalbendazole and sulfonbendazole (Prikhodko, 2000). Since sulfanilamide, chlortetracycline, nandrolone, and albendazole have been found in the wastewater of livestock enterprises, and their destabilizing effect on physiological processes in fish has not been sufficiently studied, studies of proteins of high-molecular fractions of fish blood plasma are relevant, which will make it possible to deepen understanding of the mechanisms of their adaptation to the action of xenobiotics. The aim of the research is to determine the effect of sulfanilamide, chlortetracycline, nandrolone and albendazole on the protein content of high-molecular fractions of carp blood plasma (*Cuprinus carpio* L.).

Material and methods

The study was performed at the Department of Animal Biology, National University of Life and Environmental Sciences of Ukraine. We used 16 two-years-old carp (*Cuprinus carpio* L.) with an average live weight of 450-500 g. Our fish were kept in aquariums with a volume of 40 liters in tap water for 72 hours, two individuals in each aquarium. During the experiment, the fish were not fed, the water temperature of 18-20 °C, oxygen content of 7-8 mg/l, and pH value of 7.85-8.14 were maintained. Before submerging them in water, the sulfanilamide (4-aminobenzenesulfonamide, Sigma-Aldrich, Germany) was added to the water at concentration of 0.015 mg/dm^3 in first group, 0.15 in second group, and 0.30 in the third group. Alternatively, we added chlortetracycline (chlortetracycline hydrochloride, "Sigma Aldrich", Germany) with concentration of 1.10 mg/dm^3 in the first group, 3.15 mg/dm^3 in the second and 6.30 mg/dm^3 in the third group. In third variant we added nandrolone (Sigma-Aldrich, Germany) at the concentration of 0.10 mg/dm^3 in the first group, 0.50 – in the second and 1.00 mg/dm^3 in the third group. In fourth variant we added albendazole (Termopharm LLC) in the concentration of 0.2 mg/dm^3 – in the first group, 0.5 – in the second and 1.0 mg/dm^3 in third group. Chlortetracycline, sulfanilamide, nandrolone and albendazole were not added to the fish of the control groups.

We monitored the behavior of the fish, controlled the number of respiratory movements and motor activity. At the end of the experiment, we collected the blood samples from the fish and determined the total content and level of proteins of the high molecular weight fractions. A method based on different mobility of proteins in polyacrylamide gel (PAGE) in a concentration gradient (7-18%) was used to separate the blood plasma proteins into the fractions. The gels after electrophoresis were fixed with a mixture of methanol, formaldehyde and water in a ratio of 6: 1: 7. Protein zones on the gels were detected by staining them with 0.1% of Kumasi R-250 solution ("Serva", Sweden). Standard protein markers Thermo Bioscience (England) determined the molecular weight of individual fractions of blood plasma proteins. For quantitative characterization of gel protein regions, a gel scanner Hewlett-Packard HPS-5500C (USA) with DensitoAnalyse software (Gulkowska et al., 2008) were used. Data in the tables presented like means and standard deviations.

Results and discussion

Carp blood plasma, like warm-blooded animals, contains a significant amount of proteins whose molecular mass (MM) varies from 25 to 450 kDa or more (Ivanova & Zakharenko, 2013). Holding carp during 72 hours in an aquarium water with a sulfanilamide concentration of 0.015, 0.15 and 0.30 mg/dm^3 did not affect the total protein content of the blood plasma of the fish. This indicator in the fish of the experimental groups and in the control was within the values of the optimal level of carbohydrate plasma protein (Table 1).

Table 1. Carp plasma proteins under sulfanilamide treatment

Protein fractions	MM, kDa	Control	Experimental groups (sulfanilamide, mg/dm^3)		
			0.015	0.15	0.30
A	>450	3.24 ± 0.45	3.02 ± 0.73	3.68 ± 0.28	3.68 ± 0.39
B	340	2.44 ± 0.24	1.96 ± 0.60	2.59 ± 0.28	2.70 ± 0.15
C	260	10.43 ± 0.66	7.54 ± 0.97 *	6.75 ± 0.37 *	7.32 ± 0.61 *
D	140	2.64 ± 0.30	2.63 ± 0.38	1.37 ± 0.24 *	1.56 ± 0.37 *
Total protein, g/l	–	40.25 ±1.78	42.50 ±0.45	48.00 ±5. 34	43.00 ±0.38

*$p \leq 0.05$ compared to control

It should be noted that different concentration and short action of sulfanilamide in water does not affect the number of respiratory movements, behavior, body surface, pathomorphological parameters of the main internal organs in the fish of the experimental groups compared with the control. Studies have shown that sulfanilamide affects the protein content of

macromolecular fractions in carp blood plasma, with increasing levels of xenobiotics in water largely (Table 1). With the concentration of sulfanilamide in water 0.015 mg/dm^3, the protein content of some high molecular weight fractions in the carp plasma did not change, except for proteins with a molecular mass of 260 kDa located in zone C, whose level decreased by 28% compared to the control (Table 1). The change in protein content in fish blood plasma probably depends on the ability of the sulfanilamide to influence the charge of the protein molecule and their electrophoretic mobility, which is related to the presence of amino and sulfo groups in the xenobiotic molecule.

Along with the increasing the concentration of sulfanilamide in water to 0.15 mg/dm^3, the protein content of macromolecular fractions in carp blood plasma was significantly more variable compared to the control. This is evidenced by a decrease in the plasma of fish in the protein content with a molecular weight of 260 kDa (fraction C) and 140 kDa (fraction D), respectively, by 35 and 48% compared with similar indicators in the fish of the control group (Table 1). Protein content with molecular weights of 340 and 450 kDa or more in the blood plasma of fish under the action of sulfonamide did not change. Studies have also found that the protein content of blood plasma of fish, which were kept in water with a sulfonamide concentration of 0.30 mg/dm^3, with a molecular weight of 260 kDa (fraction C) is reduced by 30 %, and 140 kDa (fraction D) - by 41 % compared to control. In the fish of the third experimental group, the protein content of the high molecular weight fractions (zones A and B) did not change, which is also consistent with the data in the fish of the second experimental group.

Therefore, we established that sulfanilamide at a concentration more than 0.015 mg/dm^3 in water changes the protein spectrum of blood plasm by reducing the proteins in high molecular weight fractions. Chlortetracycline added to aquariums at concentration of 1.10, 3.15 and 6.30 mg/dm^3 did not affect the protein content of blood plasma in the fish exposed for 72 hrs. compared to controls (Table 2).

Table 2. Carp plasma proteins under chlortetracycline treatment

Protein fractions	MM, kDa	Control	Experimental groups (sulfanilamide, mg/dm^3)		
			1.10	3.15	6.30
A	>450	9.14±1.63	10.18±1.24	5.25±0.14*,**	5.21±0.49*,**
B	340	7.26±1.02	8.42±0.25	4.39±0.23*,**	3.84±0.55*,**
C	260	11.73±1.15	12.37±0.77	8.73±1.30*,**	8.34±1.09**
D	140	4.26±0.56	4.20±0.13	2.77±0.10*,**	3.71±1.79
Total protein, g/l	–	40.25 ±3.50	42.50 ±3.00	48.00 ±4.10	43.00 ±2.7

*p ≤ 0.05 compared to control, **p ≤ 0.05 compared to the first group

Protein content in carp blood plasma remained within the physiological norm in the fish of the experimental groups and in the control (Muradova & Rabadanova, 2012). The obtained results indicate that despite the ability of chlortetracycline to inhibit the protein synthesis of bacterial cells at the studied concentrations in water and short exposure, its effect on the processes of protein biosynthesis in fish tissues is less evident. However, as determined by further studies, the antibiotic chlortetracycline added to water altered the protein content of macromolecular fractions in fish blood, especially at concentrations of 3.15 and 6.30 mg/dm^3. Analysis of the composition of macromolecular protein fractions in the blood plasma of fish in the test and control groups showed that chlortetracycline alters the protein content in the test zones A, B, C, corresponding to molecular masses of 140, 260, 340 and 450 kDa or more. The protein content of macromolecular fractions in the blood plasma of fish at a concentration of chlortetracycline 1.10 mg/dm^3 did not changed compared to the control (Table 2).

Keeping fish in water with 3.15 mg/dm^3 of chlortetracycline for three days caused a significant decrease in the protein content of high molecular weight fractions in the carp plasma. This values were higher by 1.7 times in zone A, by 1.6 times in zone B, by 1.3 times in zone C, and by 1.5 times - in zone D compared to the control (Table 2). This is probably caused by the effect of chlortetracycline on the protein synthesis of fish hepatopancreas, which produces a significant amount of blood plasma proteins. Protein content with a molecular weight of 450 kDa or more (zone A) in the blood plasma of the fish from second group decreased by 1.94 times compared to the first group with a molecular weight of 340 kDa (zone B) – 1.92 times, with a molecular weight of 260 kDa (Zone C) – 1.42 times, with a molecular weight of 140 kDa (Zone D) – 1.52 times. Because these protein fractions contain immunoglobulins, it is likely that high concentrations of chlortetracycline in the water reduced the fish immune capacity. Our data indicated a significant effect of chlortetracycline on the protein content of high molecular weight fractions in carp plasma, which probably also alters the electrophoretic mobility of individual proteins. This conclusion is confirmed by studies of the protein content of high molecular weight fractions in the blood of carp in the third group with a concentration of chlortetracycline of 6.30 mg/dm^3 (Table 2). The content of proteins in the blood plasma of the fish from the third group with a molecular weight (MM) of 450 kDa or more decreased by 1.7 times (zone A), with MM of 340 kDa decreased by 1.9 times (zone B), with MM of 260 kDa decreased by 1.4 times (zone C), and 1with MM of 40 kDa (zone D) it did not change compared with the control (Table 2).

The level of proteins with a molecular weight of 450 kDa or more (zone A) in the blood plasma of carp of the third experimental group decreased by 1.75 times, with MM of 340 kDa (zone B) - by 1.8 times, with MM of 260 kDa (zone C) - by 1.4 times compared with the fish in the first experimental group. The decrease in the protein content of high molecular weight fractions in carp blood plasma probably indicates the effect of this xenobiotic on the immune defense in fish. Therefore, we concluded that at a dose of 1.0 mg/dm^3 and with short exposure, chlortetracycline does not affect the protein content of high molecular weight fractions in carp plasma, and at doses of 3.15 and 6.30 mg/dm^3 reduces the level of proteins with a molecular weight of 140-450 kDa. The nandrolone at concentrations of 0.10, 0.50 and 1.00 mg/dm^3 changes in total protein content in blood plasma in

carps after keeping them for 72 hours. In fish from the first group, this indicator increased by 53%, in the third - by 49%, whereas in second group it did not change compared to the control (Table 3)

Table 3. Carp plasma proteins under nandrolone treatment

Protein fractions	MM, kDa	Control	Experimental groups (sulfanilamide, mg/dm^3)		
			0.10	0.50	1.00
A	>450	2.90 ± 0.89	2.67 ± 0.80	4.63 ± 0.64	4.01 ± 0.60
B	340	4.41 ± 0.05	4.33 ± 0.60	4.36 ± 0.27	5.29 ± 0.26 *
C	260	4.34 ± 2.00	4.42 ± 2.84	4.90 ± 0.96	6.28 ± 0.71
D	140	6.09 ± 0.43	6.20 ± 0.94	5.98 ± 0.23	7.38 ± 0.68
Total protein, g/l	–	22.80± 2.12	34.83 ± 2.07*	26.65±2.04	34.00 ± 1.19*

*$p \leq 0.05$ compared to control

In the experimental groups, nandrolone had different effects on the plasma protein content of macromolecular fractions. Thus, the level of proteins with a molecular mass of 140–450 kDa (zones A, B, C, D) in the blood plasma of fish from the first group did not changed compared to the control (Table 3). Nandrolone at concentration up to 0.5 mg/dm^3 did not affect the carbohydrate protein content of carp in the second group with a molecular weight of 450 kDa and higher (zone A), as well as proteins located in zones B, C, and D compared to control. A much greater effect of nandrolone on the protein content of high molecular weight fractions was recorded in third groups with it concentration of 1.0 mg/dm^3. Thus, the level of proteins with a molecular weight of 340 kDa and higher in the blood plasma of fish from third group (zone B) increased by 1.20 times compared to control (Table 3). The data obtained suggest a stimulating effect of nandrolone at a concentration of 1.10 mg/dm^3, even with short-term effects on biosynthetic processes in tissues. Keeping the carps for 72 hours in the water with albendazole concentrations of 0.20, 0.50 and 1.00 mg/dm^3 did not affect the total protein content in the fish plasma compared to the control (Table 4).

Table 4. Carp plasma proteins under albendazole treatment

Protein fractions	MM, kDa	Control	Experimental groups (sulfanilamide, mg/dm^3)		
			0.20	0.50	1.00
A	>450	4.06 ± 0.98	3.63 ± 0.25	3.52 ± 0.52	3.66 ± 0.76
B	340	4.71 ± 0.56	3.55 ± 0.56	3.69 ± 0.10	3.95 ± 0.22
C	260	4.25 ± 0.92	3.16 ± 0.34	3.76 ± 0.18	3.09 ± 0.48
D	140	4.75 ± 1.14	3.70 ± 0.25	3.76 ± 0.06	3.13 ± 0.45
Total protein, g/l	–	20.28 ± 2.29	20.95 ±2.29	17.05 ±1.28	23.20 ± 1.49

We registered that the protein content with molecular weight exceeding 450 kDa in zone A, 340 kDa in zone B, 260 kDa in zone C, and 140 kDa in zone D did not changed compared with control under concentration of albendazole of 0.2 mg/dm^3. Increasing the concentration of albendazole to 0.5 mg/dm^3 also did not affect the plasma protein content of fish located in zones A, B, C, and D with the molecular weight of 450, 340, 260, and 140 kDa, respectively. More higher concentration of albendazole up to 1.0 mg/dm^3 also did not change the protein content of the high molecular weight fractions in the blood plasma of the fish from third group, located in zones A, B, C and D with molecular weight of 450, 340, 260, and 140 kDa respectively. We assumed that probable short-term effect of albendazole could explain these zero changes in the protein content in the high molecular weight fractions of carp plasma under conditions of albendazole concentrations from 0.2 to 1.0 mg/dm^3.

Conclusions

We found that the influence of sulfanilamide on the protein content of macromolecular fractions in carp blood plasma was characterized by the decrease in the level of proteins with molecular weights 340 and 350 kDa into 140 and 260 kDa respectively. Moreover, we registered that the chlortetracycline at the concentrations of 3.15 and 6.30 mg/dm^3 reduced the plasma protein content of fish with a molecular weight of 140-450 kDa. We established slight stimulating effect of nandrolone action on the biosynthetic function of hepatopancreas, caused the increase in the protein content of individual fractions in carp blood plasma. Albendazole in discussed concentrations did not affect the total content and protein level of the high molecular weight fractions in the blood plasma of the fish.

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