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Ultrastructural changes in blood cells in the hematopoietic organs of Lake Baikal fish exposed to phenol

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ABSTRACT

Baikal Lake fish: stone sculpin (*Paracottus knerii*), yellowfin (*Cottocomephorus grewingkii*) and perch (*Perca fluviatilis*) were exposed for 14 days to phenol (6mg·L^{-1}). The reaction of blood cells in all studied species had a similar character, whilst, also revealing distinctions. Fast activation of immune processes was found in the pelagic yellowfin. These changes were slower at the coastal stone sculpin. Quantitative changes of blood cells were minor in the coastal nonendemic perch. After four days of exposure, the cells of fish showed signs of destructive processes: strong vacuolization of cytoplasm and partial destruction of the cell membrane. In the cell nucleus the chromatin was condensed, the perinuclear space had expanded and the nuclear membrane had been partly destroyed. In another experiment, stone sculpin and perch were first exposed to phenol for 30 days and then they were kept for another 30 days in water without phenol. In the kidneys and spleen of stone sculpin the processes of lymphopoiesis were observed, whereas in perch the processes of lymphopoiesis and myelopoiesis occurred.

INTRODUCTION

For the maintenance of homeostasis in multicellular organisms the immune system plays a key role (Galaktionov 2005). In fish, unlike in higher vertebrates, the same organ is responsible for hematopoietic and immune functions (Doggertt and Harris 1989; Ellis 1976, 1977; Ferguson 1976, 1989; Imagawa et al. 1989; Kondratiyeva et al. 2001). Thus, the cellular elements of the immune system directly enter the peripheral blood and the immune responses of fish involve these immunocompetent cells in both the hematopoietic organs and the peripheral blood (Galaktionov 2005; Kondratiyeva et al. 2001; Mikryakov 2001). The condition of these blood cells may serve as an indicator of the physiological state of the organism and the quality of its environment (Nazarova and Zabotkina 2009; Saha et al. 1999).

Different phenolic compounds play an important role in the regulation of biosynthetic processes in organisms (Abdel-Hameid 2007; Amit et al. 2013; Oksama and Kristofferson 1979; Saha et al. 1999; Valedskaya 2005; Wlasow 1985; Wlasow et al. 2010; Zhang et al. 2014). Phenols of anthropogenic origin negatively affect fish health; they bioaccumulate rapidly when fish are chronically exposed to them even at a small concentration (Sokolov and Kharlampovich 1980).

Phenol is a 2nd class danger and its maximum permissible concentration in fresh water is 0.001mg·L^{-1}. In drinking water taken from Lake Baikal, the maximum permissible concentration is 0.0005mg·L^{-1}. The water in Baikal Lake near Irkutsk was assessed as "conditionally pure" and "slightly polluted" in 2013 (Kravchuk 2014).

There is limited information on the response of Lake Baikal fish to phenols. In coastal nonendemic perch exposed

to different concentrations of phenol the immune response was fast, whereas this response was slow in omul and stone sculpin, which are coastal endemic species. In the olfactory organ and the peripheral blood of Baikal omul, exposure to phenol caused ultrastructural disorders in cells (Yakhnenko et al. 2010, 2013). In other studies, when fish were exposed to toxicants, both the rate of cell differentiation in hematopoietic organs and cellular composition in peripheral blood changed (Mikryakov 2001).

The aim of this study was to determine how the general composition and structure of immunocytes change when fish endemic and not endemic to Baikal Lake are exposed to phenol.

MATERIAL AND METHODS

Examined fish

The following fish were used in the experiment: non-endemic coastal perch (*Perca fluviatilis*) found in the littoral zone, endemic stone sculpin (*Paracottus knerii*) found in the coastal-slope zone (sublittoral) at a depth of 0-200m and endemic yellowfin (*Cottocomephorus grewigkii*) from the pelagic zone, at a depth of 0-200m.

Exposure to phenol

Experimental exposure of fish to phenol was carried out at a constant temperature and in stable hydrochemical conditions in the Freshwater Aquarium Complex (FAC).

For the exposure, a phenol concentration of 6mg·L^{-1} was chosen based on results obtained by Mikryakov (2001) and Yakhnenko et al. (2010, 2013). In those studies, blood cells responded similarly to phenol at concentrations of 3, 6, and 12mg·L^{-1}, but at 3mg·L^{-1} the response was less pronounced, and at 12mg·L^{-1} fish mortality was observed. Accordingly, phenol concentration of 6mg·L^{-1} was chosen for the experiment.

Unchlorinated tap water was constantly aerated and its temperature was maintained at 14±1°C. Analytical grade phenol (C_6H_5OH, 99% purity) at a concentration of 6mg·L^{-1} was used as a toxicant.

All experiments were conducted in accordance with international recommendations for conducting biomedical research with animals (Smith et al. 2012).

14-day exposure

Static bioassays were conducted in 20L glass aquaria. As a control, groups of fish were kept in aquaria with water containing no phenol.

30-day exposure

In addition, to test the recovery of fish from phenol exposure, perch and stone sculpin were investigated according to the following scheme: 30 days in water with a solution of phenol, then 30 days in water without phenol. Yellowfin were not used in this scheme because 30 days of exposure to phenol killed most of these fish.

Histological studies

The immune competent cells were examined in the hematopoietic organs of fish of the control group and in fish exposed to phenol.

Smears of peripheral blood stained with azure-eosine were examined under a light microscope equipped with a camera. The structure of blood cells was analysed under a Leo 906 E transmission electron microscope (at an accelerating voltage of 80 kV). Standard ultrastructural analyses were performed according to Uikly (1975).

The cells were identified as cells of the myeloid, lymphoid and erythroid complexes according to the accepted classification of morphological, ultrastructural and histochemical features (Blaxhall and Daisley 1973; Felip et al. 2009; Hartenstein 2006; Ivanova 1983; Prihirunkit et al. 2007; Samsuev and Kapitonova 2010; Srivastava 1968, 1969; Zapata et al. 2006).

To assess the significance in differences in the number of immune competent cells between fish, Student's t-test was used (Urbakh 1963; Zhivotovsky 1991).

RESULTS

Group composition of blood cells in hematopoietic organs

Blast and maturing blood cells were observed in hematopoietic organs of the studied fish. Differently mature cells of the lymphoid complex were found mainly in the thymus, in the lymphoid organ, in the head kidney, in the body kidney and in the spleen. Hematopoietic cells were also recorded in the gills, heart, caudal kidney, anterior, mid- and posterior gut in different proportions.

Experiments revealed different levels of response to phenol amongst blood cells. First of all, in stone sculpin content of phagocytes decreased in kidney and spleen whereas content of lymphocytes and plasmocytes increased after 14 days exposure to phenol (Table 1).

In yellowfin spleen, the percentage of myeloblasts increased and that of lymphocytes decreased after 1-day of exposure to phenol. Indicators of lymphopoiesis and myelopoiesis matched the values in control yellowfin after 4 and 14 days of the experiment. In perch kidneys, the group composition of blood cells did not differ significantly during the whole experiment. In perch spleen, however, the content of phagocytes in the spleen decreased and the content of lymphocytes increased after 14 days of exposure (Table 1).

In the 30-day exposure experiment, fish were exposed to phenol for 30 days (time of experiment "30" in Table 1) and then kept in the water without phenol ("30^1" in Table 1). In stone sculpin and perch, the indicators of myelo- and lymphopoiesis did not differ significantly from control values (Table 1). In the spleen of stone sculpin, the content of phagocytes decreased and the content of lymphocytes in the kidneys and spleen slightly increased. In perch, the content of phagocytes in the kidneys decreased and the content of lymphocytes in spleen decreased. In both species, there were no plasmocytes (Table 1).

Table 1. Influence of phenol (6mg·L^{-1}) exposure time on the percentage of blood cells (mean value±standard error) in hematopoietic organs in Baikal fish. Abbreviations: 1. phagocytes (total phagocytes: myelocytes, myeloblasts, monoblasts and promonocytes), 2. lymphocytes (total lymphoblasts and lymphocytes). Significance of differences in percentage of blood cells between experimental and control fish: *p<0.05, **p<0.01, ***p<0.001. In another experiment fish were exposed to phenol for 30 days (time of experiment "30") and then kept for another 30 days (time of experiment "30^1") in water without phenol.

Species	Time of experiment (days)	Hematopoietic organ						Number of examined fish
		Kidney			Spleen			
		1. phagocytes	2. lymphocytes	3. plasmocytes	1. phagocytes	2. lymphocytes	3. plasmocytes	
Yellowfin	**Control**	**45.9±13.0**	**53.1±11.0**	**0.9±0,1**	**52.5±14.0**	**47.5±12.0**	**0.0**	**26**
	1	53.6±8.2	42.8±9.0	3.6±0.8***	92.0±9.0**	8.0±1.0***	0.0	16
	4	50.4±13.0	45.5±8.0	4.2±0.9***	66.6±10.0	33.4±7.1	0.0	12
	14	46.5±14.0	51.1±10.0	3.4±0.8***	51.8±9.0	44.4±7.1	3.8±1.0**	9
Stone sculpin	**Control**	**51.9±9.6**	**45.6±11.0**	**2.5±0.6**	**55.8±12.0**	**44.2±8.0**	**0.0**	**30**
	1	31.8±5.7*	63.7±11.0	4.5±0.3**	60.2±7.2	37.6±0.9	3.2±0.1***	21
	4	35.9±8.7	64.2±10.0	0.0***	19.4±1.3**	80.6±15.0*	0.0	15
	14	15.9±1.0***	77.8±13.0	6.3±0.8***	45.2±8.7	51.5±9.0	3.3±0.8***	12
	30	41.1±8.0	51.8±12.0	7.1±0.7***	41.8±14.0	58.2±12.0	0.0	8
	30^1	36.7±8.9	63.3±7.6	0.0***	9.8±0.3***	91.2±21.0*	0.0	3
Perch	**Control**	**33.2±8.7**	**66.8±5.4**	**0.0**	**67.2±5.2**	**32.8±4.2**	**0.0**	**23**
	1	52.0±12.0	48.0±13.0	0.0	42.9±9.6	57.1±14.0	0.0	10
	4	—	—	—	—	—	—	—
	14	53.9±13.0	46.1±11.0	0.0	30.2±8.4***	69.8±15.0*	0.0	11
	30	42.4±8.1	54.6±11.0	3.0±0.4***	38.7±9.6*	58.1±15.0	3.2±0.5***	6
	30^1	3.8±0.1***	96.2±23.0	0.0	94.6±16.0	5.4±0.9***	0.0	4

— no data.

Histological observations

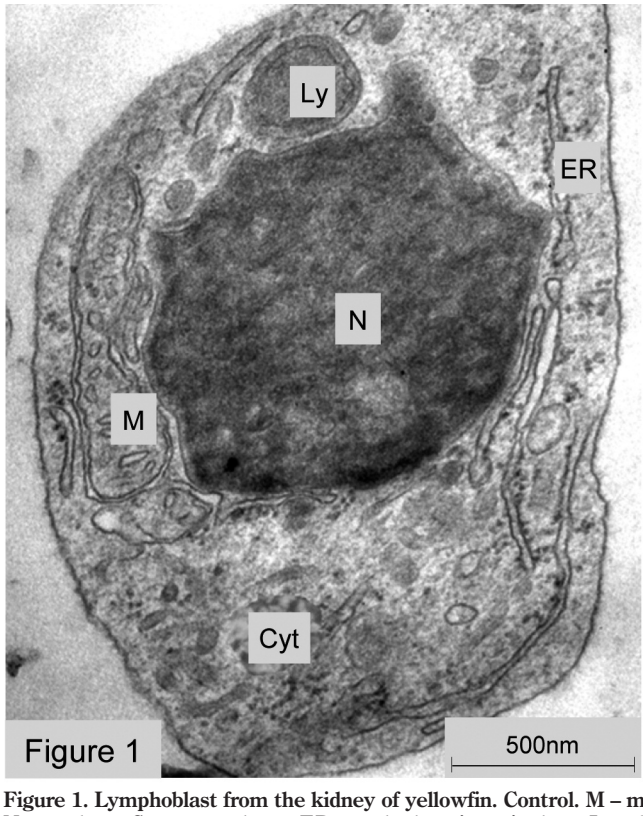


Figure 1. Lymphoblast from the kidney of yellowfin. Control. M – mitochondrion, N – nucleus, Cyt – cytoplasm, ER – endoplasmic reticulum, Ly – lysosome.

Normal oval mitochondria were observed in the cells of control fish. There were also channels of rough endoplasmic reticulum, dictyosome and vesicles of Golgi apparatus (Figure 1).

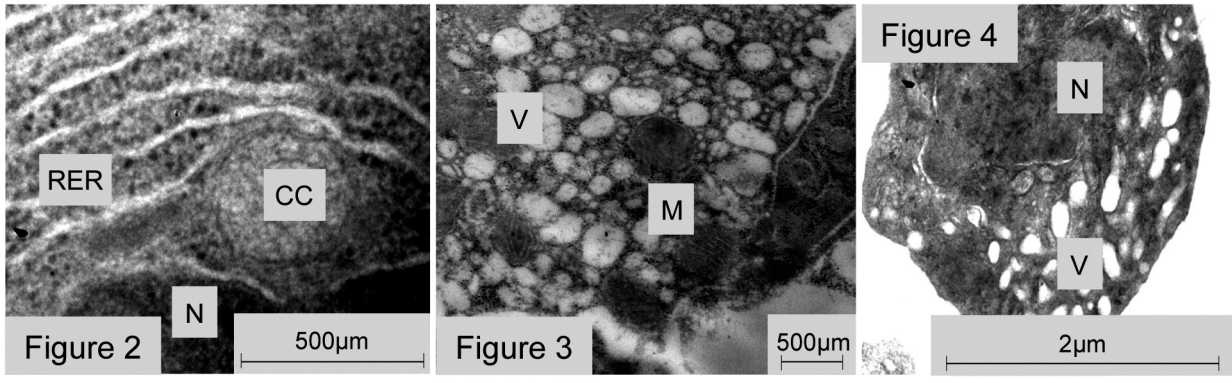


Figure 2. Myeloblast from the kidney of yellowfin exposed to phenol (6mg·L^{-1}) for 4 days. RER – rough endoplasmic reticulum, N – nucleus, CC – cisternal cavity.
Figure 3. Myeloblast from the spleen of perch exposed to phenol (6mg·L^{-1}) for 14 days. V – vacuole, M – mitochondrion.
Figure 4. Lymphocyte from the kidney of stone sculpin exposed to phenol (6mg·L^{-1}) for 30 days. V – vacuole, N – nucleus.

In all fish exposed to phenol similar ultrastructural disoerders were detected in the blood cells at all stags of the experiment. There was an increase in cell volume and in cisternal cavity of rough endoplasmic reticulum canals (Figure 2), vacuolization of different morpho-functional segments of Golgi apparatus, as well as complete or partial mitochondrial swelling with destructive changes of cristae (Figure 3). Large (1.0-1.3μm) vacuole-like formations with light content were observed in the cytoplasm of different blood cells (Figure 4).

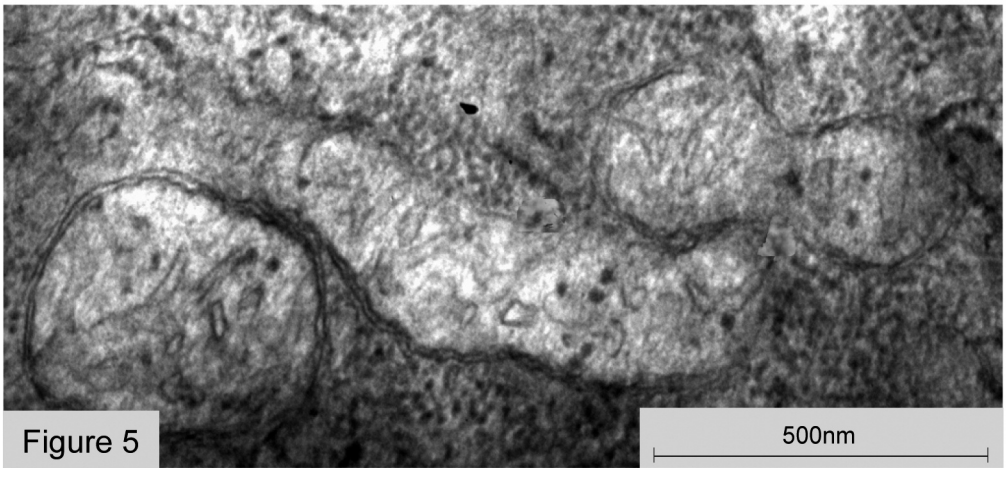
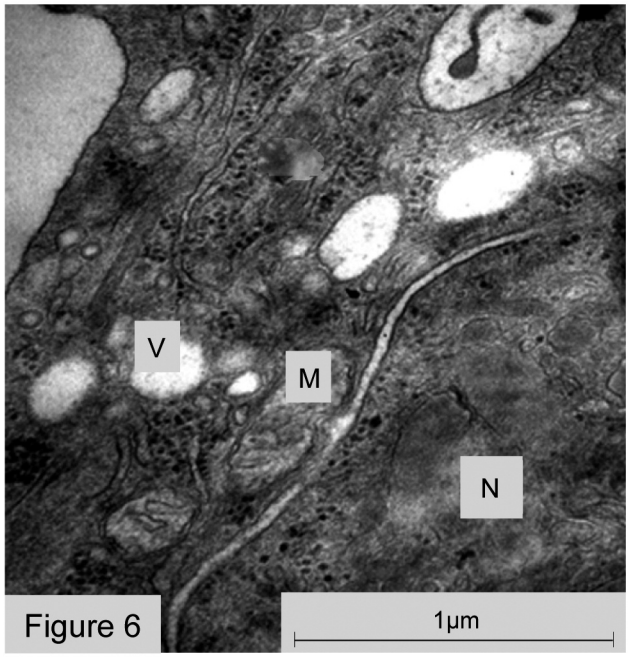


Figure 5. Enlightenment of mitochondrial matrix. Monoblast from kidney of yellowfin exposed to phenol (6mg·L^{-1}) for 14 days.

Enlightenment of mitochondrial matrix was revealed after 4 day phenol exposure. Cristae remained clear (Figure 5). The cells showed signs of strong vacuolization and damage of the cell membrane, remains of cytoplasm and of individual organelles (Figure 6). Chromatin condensation and expansion of the perinuclear space occurred in the nucleus, with partial destruction of the nuclear membrane in some places (Figure 7).

DISCUSSION

Our study showed that an exposure to phenol resulted in a variety of ultrastructural disorders in cell elements of the hematopoietic organs, which may affect the immunity and respiratory systems (Bras et al. 2005; O'Brien et al. 1998; Rahmatullina et al. 2009; Samsuev and Kapitonova 2010). In the case of irreversibility of disorders, the functioning of the immunocytes could be changed significantly, perhaps



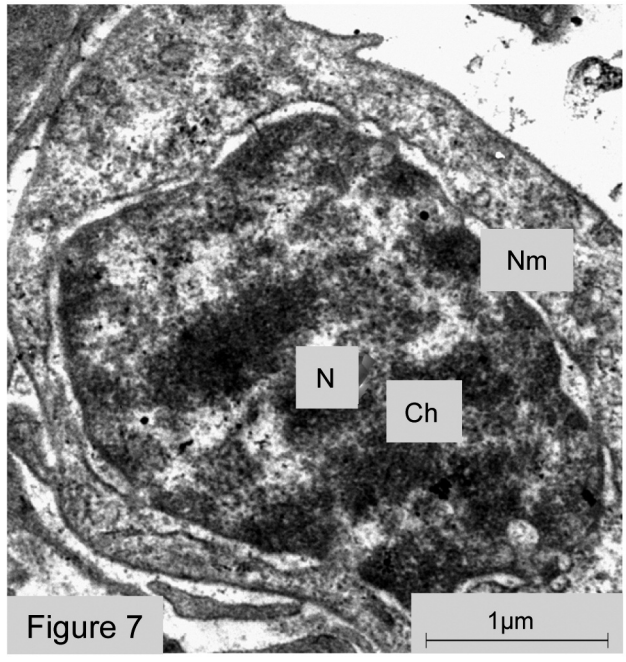


Figure 6. Lymphocyte from the spleen of stone sculpin exposed to phenol (6mg·L^{-1}) for 30 days. V – vacuole, M – mitochondrion, N – nucleus.
Figure 7. Lymphocyte from the spleen of perch exposed to phenol (6mg·L^{-1}) for 30 days. Ch – chromatin, Nm – nucleus membrane, N – nucleus.

influencing their adaptive behaviour. The possibility of reversibility is still uncertain. Questions which require attention include:

- Are receptor and supporting cells able to restore their initial morphology?
- Are phenol related structural changes of intracellular organelles irreversible?
- Do these changes cause cell apoptosis?

The conducted experiments revealed an identical cellular response to phenol amongst Lake Baikal fish, whilst also revealing distinctions (Yakhnenko et al. 2010, 2013). The similarities have been found in fish species from other water bodies (Antonyak 1999; Mikryakov 2001; Valedskaya 2005). Fast activation of immune processes in the first day of experimentation was found in the peripheral blood (Yakhnenko et al. 2010) and in the hematopoietic organs of pelagic yellowfin. These changes were slower in coastal stone sculpin. Quantitative changes of blood cells were minor in coastal nonendemic perch. However, with stone sculpin (30 days in the water with phenol then 30 days in the water without phenol) processes of lymphopoiesis were diagnosed in the kidneys and spleen. Perch (in the same experiment) displayed lymphopoiesis and myelopoiesis. Perhaps this may be due to the nature of the immune response of these fish species.

Baikal endemic cottoid fish are adapted to stable conditions in the pelagic zone (yellowfin) and sublittoral (stone sculpin), compared to perch which is adapted to more diverse conditions in the littoral zone (Sideleva and Kozlova 2010; Taliev 1955; Timofeyev et al. 2008; Timofeyev 2009; Zubin et al. 1994).

Phenols rapidly enter the blood, liver, kidneys and endocrine glands of fish (Amit et al. 2013; Bagirova et al. 2001; Sokolov and Kharlampovich 1980; Zhang et al. 2014). Phenol and its compounds influence the processes of oxidation and phosphorylation in the mitochondria, disturbing the transfer of hydrogen ions across the mitochondrial membrane (Koster 2011). It has been shown that phenol specifically increases the proton conductivity of mitochondria (Roussel et al. 2003), affecting levels of cytochrome C (Hegardt et al. 2003). It stops glycolysis, phosphorylation, ATP production, impairs ion homeostasis (Pokhilko et al. 2006) and promotes cell necrosis (Dzyubinskaya et al. 2006). The destruction of mitochondria suggests disturbances of cellular respiration, which cause the acidification of cytoplasm (Lejkina et al. 1990; Trofimova 2003) and the development of apoptosis (Saprunova et al. 2002). Apoptosis changes the size and shape of mitochondria, increases the number of cristae, causes mitochondria degradation and compacts their matrix (Savitskaya et al. 2009).

In this study we identified ultrastructural changes in the blood cells included swelling of mitochondria and of tubules in rough endoplasmic reticulum, expansion of perinuclear space, partial destruction of the plasma membrane and the fragments of different cytoplasmic organelles in extracellular space. These disorders are irreversible and will cause the cell death, regardless of the duration and frequency of exposure to phenol.

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