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STRESS IN FISH – HEMATOLOGICAL AND IMMUNOLOGICAL EFFECTS OF HEAVY METALS

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Abstract: The aim of present study was to evaluate whether short-term exposures to high concentrations of heavy metals may induce stress symptoms in fish blood. Common carp were subjected for 3 hours to 10, 5, 10 or 20 mg/l of lead, copper, cadmium or zinc, respectively, and subsequently transferred to clean water. Blood was sampled immediately after the end of exposures, and then daily until 96 hours post exposures. In red blood cell system transient changes were observed such as an increase in hematocrit value without a substantial changes in red blood cell count (which indicates swelling of the cells), and an increase in erythropoietic rate (indicated by an increase in percentage of immature cells in circulation). In the white blood cell system – a decrease in total leukocyte count took place caused by a considerable drop in lymphocyte count. In some cases (particularly in Pb and Cd-intoxicated fish) percentage of neutrophils increased. However, metal exposures (Cd, Zn) resulted in a reduced ability of intracellular killing by these cells (a decrease in reactive oxygen radical production). The changes gradually developed with time after the end of exposure, and no recovery was observed until 96 hours from transfer of fish to clean water. The obtained data show that a short-term exposures to high levels heavy metals induced stress reactions in fish. In the red blood cell system adaptive changes prevailed, preparing the organism to an increased energy expense, while the changes observed in the white blood cell system indicate a considerable immunosuppressive effect of stress. Therefore, even a short-term exposure to heavy metals induces a persistent stress in fish which may render them more susceptible to diseases.

Key words: carp, stress, heavy metals, blood

Introduction

Stress is a general, and non-specific response to any factor disturbing homeostasis. Neural and hormonal control of stress reaction involves activation of sympathetic neural system, and head kidney secretory regions that release stress hormones: epinephrine and cortisol (Wendelaar Bonga 1997, Svoboda 2001). In case of light stress the inner balance is usually restored but under severe or prolonged stress conditions compensatory abilities of the organism may be exhausted which results in physiological disturbances or even death.

Stress in fish may be induced by various abiotic environmental factors (changes in water temperature, pH, O_2 concentration, pollution), biotic interactions (predator pressure, parasitic invasions or strong competition), and by human activities related to fish rearing and harvesting (manipulation, transport, crowding).

Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms. These changes include: osmotic disturbances, increase in energetic substrate concentrations (glucose, fatty acids), increase in activity of certain enzymes (lactate dehydrogenase, transaminases, in toxic stress also cytochrome P-450 and glutatione

transferase), increase in stress protein level (HSP, ubiquitin, metallothionein), and a decrease in humoral immune factors (lysozyme, antibodies) (Wendelaar Bonga 1997, Svoboda 2001).

Stress induces also changes in blood cell numbers and activities. In the red cell system – increase in hematocrit, cell count and volume, and hemoglobin level usually take place (Fletcher 1975, Houston et al. 1996). Stress-induced swelling of fish erythrocytes is a result of osmotic disturbances, and uptake of electrolytes and water into the cells, accompanied by an acidification of plasma, and alkalization of erythrocyte cytoplasm (Nikinmaa and Huestis 1984). Rapid increase in erythrocyte number (causing even 25% increase in hematocrit) is a result of spleen contraction (Caldwell and Hinshaw 1994), and 90% of new cells may be released within several minutes (Houston et al. 1996). Sometimes division of circulating cells may be observed (especially in case of hypoxic stress) (Murad et al. 1993). These are adaptive changes enabling the organism higher energy production.

The data obtained by various authors (review: Jezierska and Witeska 2001, Witeska 2003) show that intoxication of fish with heavy metals may sometimes cause symptoms similar to the stress reaction. Usually, red blood cell system of fish reacts to heavy metal intoxication with anemia but in some cases, particularly after short exposures, red blood parameters (Ht, RBC, MCV, Hb) may increase (Christensen et al. 1972, Pravda et al. 1989, Svobodova et al. 1994, Vosyliene 1996, Witeska 1998, Dethloff et al. 1999, Witeska and Jezierska 1999).

In the white cell system – a decrease in cell count, especially of lymphocytes usually occurs in fish subjected to stress (Elsaesser and Clem 1986, Siwicki and Studnicka 1992). The level of phagocytes sometimes increases. This is accompanied by a decrease in activity of all cell types: lymphocytes (Elsaesser and Clem 1986), and phagocytes (Jeney et al. 1997). Thus, stress considerably impairs immune mechanisms in fish. The count of thrombocytes may increase (Casillas and Smith 1977), and blood clotting is usually accelerated proportionally to the degree of stress (Ruis and Bayne 1997).

Heavy metal intoxication also almost always reduces count of white blood cells, particularly lymphocytes (review: Jezierska and Witeska 2001, Witeska 2003). It is usually accompanied by impairment of their activities (Viale and Calamari 1984, Khangarot and Tripathi 1991, Dunier and Siwicki 1994, Siwicki et al. 1994, Viola et al. 1996). On the other hand, neutrophilia is often observed (Murad and Houston 1988, Vosyliene 1996, Dethloff and Bailey 1998, Witeska 1998, Dethloff et al. 1999, Witeska 1999). Thus, metal-induced changes in fish blood are very similar to stress reaction. According to Donaldson and Dye (1975), heavy metal exposure causes in fish an increase in cortisol level which is responsible for a decrease in WBC, particularly in count of lymphocytes and their activity.

The aim of present study was to evaluate the effects of toxic stress caused by acute heavy metal exposures on selected blood parameters of common carp.

Materials and Methods

Common carp of 12-18 cm obtained from the fish ponds and acclimated for 3 weeks to the laboratory conditions were subjected to 3 hour exposures to high concentrations of heavy metals: Pb – 10 mg/l, Cu – 5 mg/l, Cd – 10 mg/l, or Zn – 20 mg/l. After the exposures fish were transferred to metal-free water. Control fish were exposed the same way as experimental ones, in clean water. Tap water was used of the temperature 17-18ºC, and total hardness 133.7-235.5 mg dm^{-3} as $CaCO_3$. The blood was collected from live fish by heart puncture, immediately after the end of exposures (Pb0, Cu0, Cd0, Zn0), and after 24, 48 and 96 hours (Pb24, Cu24, Cd24, Zn24, Pb48, Cu48, Cd48, Zn48, Cu96, Cd96, Zn96), from 10 fish of each group. The blood of control fish was sampled immediately after the end of 3

hour exposure. Each fish was used for blood sampling only once, to avoid the effect of bleeding on blood parameters.

The following parameters were evaluated: hematocrit (Ht), red and white blood cell counts (RBC, WBC), and number of erythroblasts, lymphocytes, neutrophils and thrombocytes (calculated basing on the blood smear analysis), and the reactive oxygen radical production by phagocytes (as NBT reduction test). The results were subjected to U test, and shown as percentage of the control values.

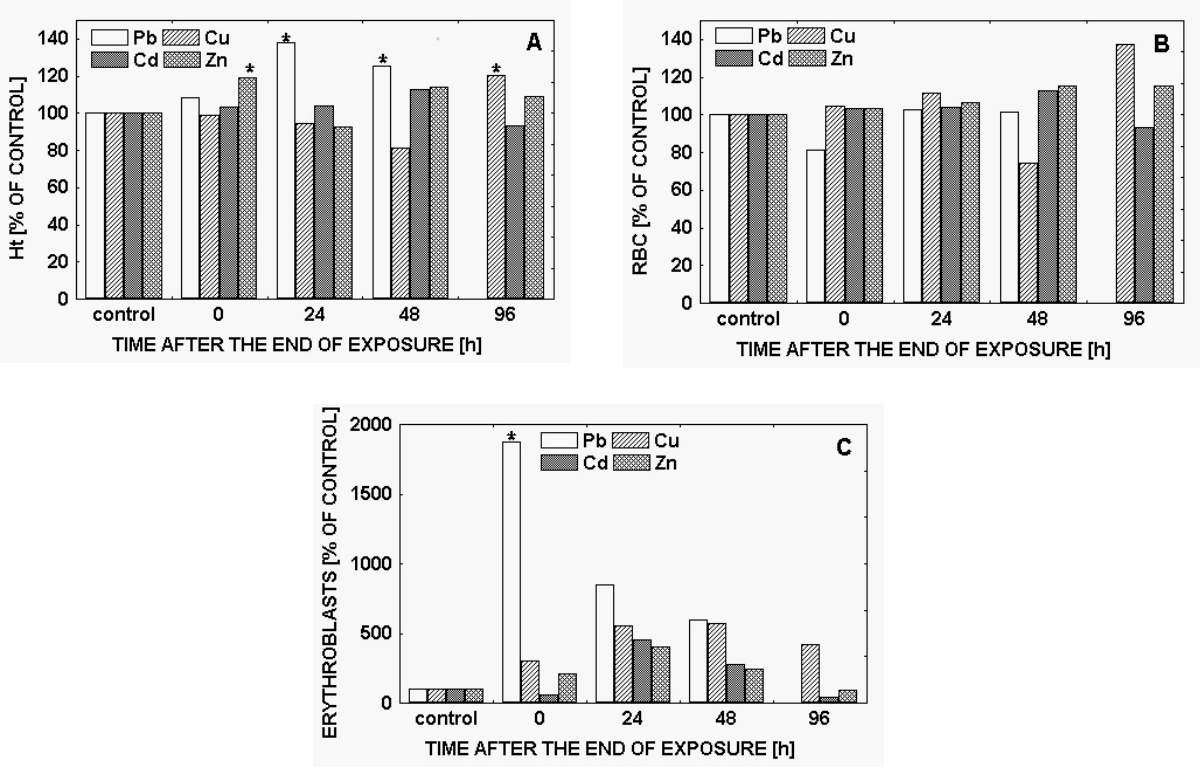

Figure. 1. The effects of metals on red blood parameters (A – hematocrit, B – red blood cell count, C – erythroblast frequency), * - significantly different from the control ($p<0.05$).

Results and Discussion

All metal exposures induced changes in fish blood indicating stress. In the red blood cell system only transient changes in Ht values occurred without significant changes in RBC which indicates temporary erythrocyte swelling (Figure 1 A, B). According to Nikinmaa and Huestis (1984), stress-induced red blood cell swelling is caused by osmotic imbalance, acidification of plasma and alkalization of erythrocyte cytoplasm. This promotes Na^+ and K^+ transport into the cells, and subsequent water uptake. According to Vosyliene (1996), an increase in hematocrit in metal-exposed fish is an "alarm reaction", and subsequent decrease indicates adaptation.

All metals induced an increase in erythroblast frequency which was particularly pronounced in Pb-exposed fish (Figure 1 C). This indicates stress-related, catecholamine-induced contraction of spleen which is a blood cell storage site, and within a short time may release new erythrocytes to the blood stream (Caldwell and Hinshaw 1994, Houston et al. 1996). No increase of RBC value indicates accelerated destruction of the cells. According to Vosyliene (1999), the quantitative red blood parameters are rather stable and little sensitive to environmental factors, due to considerable compensatory abilities of fish organism.

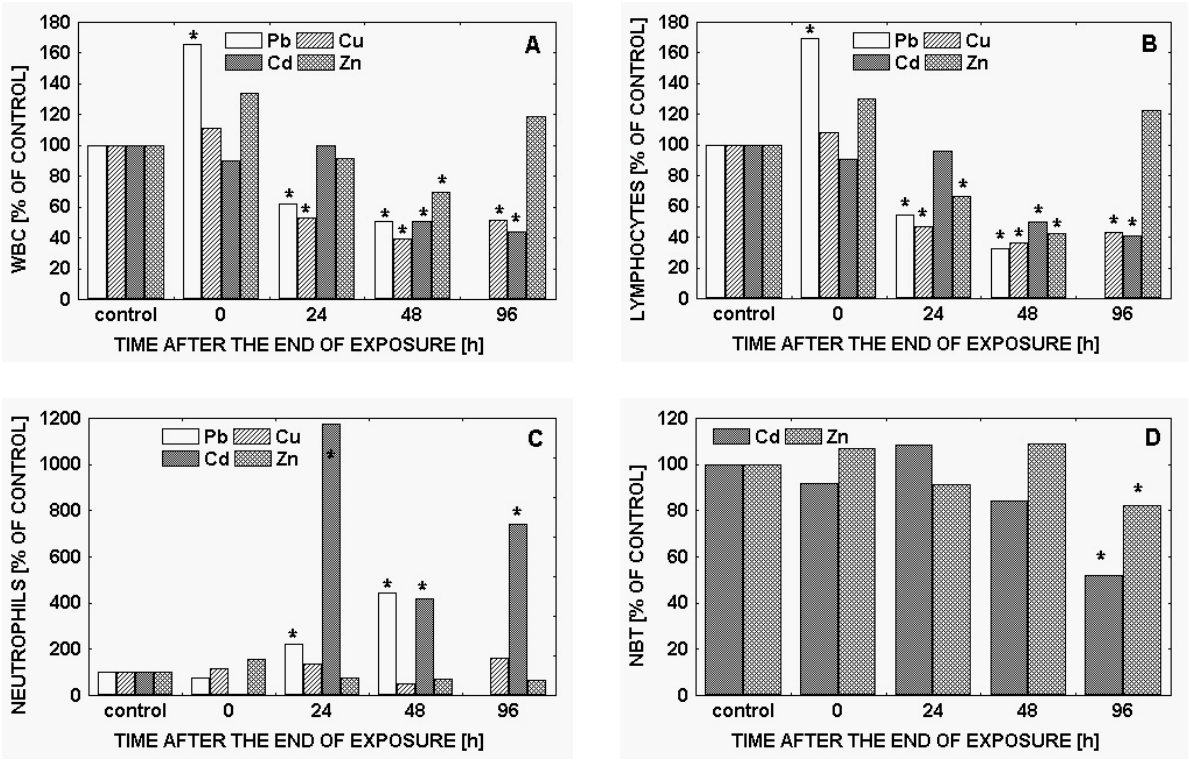

Figure. 2. The effects of metals on white blood parameters (A – white blood cell count, B – lymphocyte proportion, C – neutrophil proportion, D – phagocyte metabolic activity) * - significantly different from the control (p<0.05).

Distinct changes took place in the white blood cell system. An initial increase in WBC was observed in Pb0 and Zn0 groups, but subsequently this parameter decreased, and in 48 hours it was lower comparing to the control in all metal-exposed groups (Figure 2 A). White blood cell count remained reduced until the end of the experiment except for Zn96 group where a recovery took place. Very similar changes were observed in lymphocyte counts (Fig. 2 B). It is known that cortisol secreted during stress reaction shortens the life span of lymphocytes and promotes their apoptosis (Wyets et al. 1998, Verburg van Kemenade 1999), and reduces their proliferation (Espelid et al. 1996), so a decrease in lymphocyte count, as well as in their activity are often observed effects of stress, irrespectively of the stressing agent. Siwicki and Studnicka (1992) observed a 20% drop in WBC of common carp subjected to a 30 minute chemical stress with 1% solution of trichlorfon, while Elsaesser and Clem (1986) reported a 50% decrease in WBC and a considerable drop in lymphocyte percentage in *Ictalurus punctatus* after a 15 minute transport. Leukopenia is also a common reaction of fish to metal exposure. According to Dick and Dixon (1985) and Vosyliene (1996), a decrease in leukocyte count following an acute metal exposure is rather a non-specific stress reaction caused by a metal-induced stimulation of kidney chromaffine cells and cortisol secretion than a specific toxic actio of metals upon the cells. However, the results of the in vitro exposure of lymphocytes indicates that both reactions may be involved (Dunier and Siwicki 1994, Siwicki et al.1994, Viola et al. 1996, Dethloff and Bailey 1998). An increase in neutrophil count occurred in Pb, Zn, and particularly in Cd-exposed fish (Figure 2 C) which was probably also cortisol-induced since this hormone prevents neutrophil migration

into the tissues (inhibiting inflammative response) and extends their life span by inhibition of apoptosis (Wyets et al. 1998). A four-fold increase in neutrophil percentage in fish subjected to transport was observed by Elsaesser and Clem (1986), which was about a two-fold increase in their number. However, the intracellular killing activity of phagocytes (Figure 2 D) was significantly reduced in Cd96 and Zn96 groups. This was also typical for stress conditions, under which all non-specific immune functions become suppressed. According to Jeney et al. (1997), a 2 hour transport of rainbow trout resulted in a considerable impairment of phagocyte functions: a 50% reduction of particle ingestion ability, a 25% decrease in respiratory burst, and a 20% decrease in lysozyme level. Siwicki and Studnicka (1992) reported a 30% drop in lysozyme level, a 35% decrease in the percentage of active (NBT-positive) phagocytes, and a 20% decrease in myeloperoxidase activity in common carp subjected to a chemical stress. Neutrophilia is an often observed result of metal exposure (Garofano and Hirshfield 1982, Ruparelia et al. 1990, Svobodova et al. 1994, Vosyliene 1996, Dethloff and Bailey 1998, Dethloff et al. 1999). This reaction is also explained with stress-induced increase in cortisol level (Ellis 1977, Vosyliene 1996).

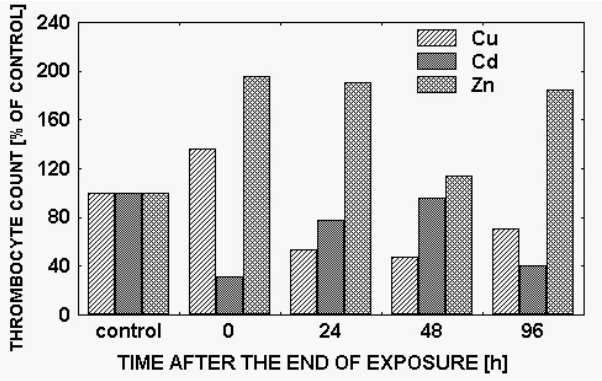

Figure 3 The effect of metals on thrombocyte count.

No distinct tendencies were observed in thrombocyte count changes: their number either decreased, comparing to the control (Cd), or increased (Zn) (Figure 3). Under stress conditions blood clotting is often accelerated but it is not always accompanied by significant increase in thrombocyte count. According to Al-Akel and Shamsi (1996), cortisol may affect fish thrombocytes in a similar way as lymphocytes, reducing their number.

The presented results indicate that a short-term exposure to high levels of heavy metals induced stress reaction in fish. The changes in red blood cell system were minor, and reflected a transient stress-induced osmotic imbalance. However, deep changes observed in the white blood cell system show that stress reduced the immune potential of fish. This reduced immunological status persisted for at least several days after removal of stressing agents from the water. Thus, it seems that even an incidental toxic stress may result in a considerable increase in susceptibility of fish to infections.

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