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Kinetic Behaviour of Glucose 6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase in Different Tissues of Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Non-Lethal Concentrations of Cadmium

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Abstract

The effects of cadmium (Cd) on the enzymatic activities of glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) were investigated in the gill, liver and kidney tissues of rainbow trout (*Oncorhynchus mykiss*). Three test groups of fish were subjected to increasing concentrations (1, 3 and 5 mg/l) of cadmium (Cd) *in vivo*, respectively. The G6PD and 6PGD activities in the gill, liver, and kidney tissues of each group of fish were measured on days 1, 3, 5 and 7.

G6PD and 6PGD enzyme activities, measured in gill, liver and kidney homogenates, were stimulated by various concentrations (1, 3, and 5 mg/l) of cadmium. Although the dose-response pattern of G6PD enzyme activities in liver and kidney tissue was very similar, that in gill was different from both other tissues. The enzyme activity of G6PD enzyme was significantly stimulated after three days (Day 3) in liver and kidney tissues at a dose of 1 mg/l Cd ($p < 0.05$), whereas it was stimulated on the first day of experiment (Day 1) in gill, liver and kidney tissues at doses of 3 and 5 mg/l Cd ($p < 0.05$). However, the activity of 6PGD was stimulated after three days (Day 3) in the liver at a dose of 1 mg/l Cd ($p < 0.05$) and on the first day in gill, liver and kidney tissues at doses of 3 and 5 mg/l Cd ($p < 0.05$). The stimulation effect of the 5 mg/l dose of Cd on G6PD and 6PGD enzyme activities was significantly diminished after seven days (Day 7) in all tissues ($p < 0.05$). In contrast to the dose-response pattern at the dose of 5 mg/l Cd, G6PD and 6PGD enzyme activities were stimulated significantly ($p < 0.05$) in liver and kidney tissues at the doses of 3 and 1 mg/l Cd. The stimulation effect of cadmium on the three tissues studied was also calculated; for both of the enzymes (G6PD and 6PGD), the enzyme activity levels were stimulated by approximately 60% and 38% in gills, 68% and 44% in liver, and 67% and 41% in kidneys, respectively, over the base-line enzyme activity of the control groups during the seven-day experimental period. These findings indicate that tissue G6PD and 6PGD enzymes function to protect against cadmium toxicity.

Enzyme activity, in vivo, kidney, gill, G6PD, 6PGD

It is generally recognized that the cell has four major NADPH-production systems that correspond to the activities of four cytoplasmatic enzymes: glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) belong to the pentose phosphate pathway, malic enzyme (ME) and NADP-dependent isocitrate dehydrogenase (NADP-IDH).

Glucose 6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) is the first enzyme in the pentose phosphate pathway. It converts glucose-6-phosphate into 6-phosphoglucono-δ-lactone and is the rate-limiting enzyme of the pentose phosphate pathway (Kuo et al. 2000). 6-phosphogluconate dehydrogenase 6PGD (E.C.1.1.1.44) is the third enzyme of the pentose phosphate metabolic pathway; it catalyzes the conversion of 6-phosphogluconate to D-riboluse-5-phosphate in the presence of $NADP^+$ (Adams et al. 1983; Broedel and Wolf 1990). The main physiological function of these enzymes is to produce NADPH, which is essential for reductive biosynthesis and nucleic acid synthesis and protects the

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cell against oxidants by producing reduced glutathione (GSH) (Kuo et al. 2000; Bianchi et al. 2001).

Cadmium (Cd) is known to be extremely toxic to mammals, fish, and other fauna and flora. It is generally viewed along with mercury as a toxic element and environmental problem (Lionetto et al. 2000; Basha and Rani 2003). Sunda et al. (1978) and Sprague (1985) reported that cadmium is most available to aquatic organisms in the form of the free metal ion Cd^{2+}, whereas inorganic cadmium complexes appear not to be taken up by fish (Paert et al. 1985). The main uptake route in fresh water fish is from the water via the gills (Williams and Giesy 1978), whereas food is the major cadmium source for marine fish (Pentreath 1977; Dallinger et al. 1987). May and McKinney (1981), reported cadmium concentrations ranging from 0.01 to 1.04 mg/kg (wet weight), for freshwater fish (*Cyprinus carpio* or *Micropterus* spp.) Hardisty et al. (1974), sampled flounder (*Platichthyes flesus)* with mean cadmium concentrations of 3.4–7.3 mg/kg (dry weight).

Many chemicals at relatively low doses affect the metabolism of biota by altering healthy enzyme activity (Hochster et al. 1972). Like the other heavy metals such as mercury and lead, cadmium causes significant metabolic alterations, e.g. enzymatic activities and membrane transport mechanisms (Viarengo 1989) and injuries of biological systems at different levels (Pratap and Wendelaar-Bonga 1990).

It is hypothesized that elevated cadmium levels may cause some impairment to enzymatic processes in fresh water fish; therefore the investigation of the effects of cadmium on G6PD and 6PGD enzyme activities from rainbow trout gills, livers and kidneys is aimed in the present *in vivo* study to determine the effects of cadmium on these tissues.

Materials and Methods

Animals and experimental design

Rainbow trout weighing 200 ± 20 g were obtained from the Fisheries Department of Agricultural Faculty at Atatürk University in Erzurum. Fish were placed in tanks (12 fish per tank) where they acclimated for 15 days. Twelve (12) cylindrical fiberglass tanks, 1 m in depth (h) and 1 m in diameter ($D = 2r$) with a total volume of 0.785 m^3 ($V = \pi r^2 h$) per tank were used in the study. Fish in each of the three tanks were subjected to one dose of Cd [(0 mg/l (control), 1 mg/l, 3 mg/l and 5 mg/l)]. Recirculated, aerated, and dechlorinated tap water with a flow rate of 1.5 l/min was used to maintain a supply of fresh water; the flow rate was equivalent to one full exchange of water every 8.7 h. Other conditions of the water were maintained as follows: the average water temperature was monitored and maintained at 9 ± 1 °C; the concentration of dissolved oxygen was maintained at 9 mg/l; the pH was maintained at 7.8; total water hardness was measured to be 102 mg as $CaCO_3$.

Fish were fed *ad libitum* and divided into four groups. Fish in Group I served as controls. Fish in Groups II, III and IV were exposed to sublethal concentrations of cadmium at doses of 1, 3 and 5 mg/l of Cd, respectively. Six randomly selected fish from each group except for the control (the single control group (n = 2) and the three test groups (n = 18) in total) were sacrificed and enzyme levels in the target tissues were tested after 1, 3, 5 and 7 days of exposure to cadmium. Fish treatments were conducted according to Applied Research Ethics National Association (2002).

Before taking tissue samples, fish were killed by an overdose of an anaesthetic compound (MS-222) (Arashisar et al. 2004). Subsequent to death, their gills, livers and kidneys were removed.

Each tissue was homogenized in a Potter-Elvehjem homogenizer and put into homogenization medium (Vaglio and Landriscina 1999) and then centrifuged at 4 °C, 10,000 × g for 45 min (Lionetto et al. 2000). After centrifugation, the supernatant was removed from the centrifuge tubes and used for these *in vivo* studies. All of the tests were carried out in triplicate.

Biochemical analysis

Glucose 6-phosphate dehydrogenase (G6PD) activity was determined by monitoring NADPH production at 340 nm and 25 °C. The assay mixture contained 10 mM magnesium chloride, 0.2 mM $NADP^+$, and 0.6 mM G6PD in 100 mM Tris-hydrochlorid buffer solution at pH 8.0. Assays were carried out in triplicate and the activities were followed up for 60 s. One unit of activity (U) is defined as the amount of enzyme required to reduce 1 µmol/min of $NADP^+$ under the assay conditions. Specific activity is defined as units per gram of protein. Activity of 6-phosphogluconate dehydrogenase (6PGD) was measured in the same manner as previously described by substituting 0.6 mM 6PGD as the substrate in the assay mixture for G6PD (Beutler 1971).

Protein levels were determined spectrophotometrically (595 nm) according to the Bradford method (Bradford 1976), using bovine serum albumin (BSA) as the standard.

Statistical analysis

The effects of three different Cd concentrations on the G6PD and 6PGD activities obtained from the tissues of fish in the *in vivo* experiment were determined using one-way ANOVA and Dunnett's test for multiple comparisons of the individual concentration effect and that of controls (Winer et al. 1991). A significant difference in the enzyme activities was reported for *P* values less than 0.05.

Results

The specific enzyme activity of G6PD and 6PGD in kidney, liver, and gill tissue of rainbow trout exposed to three different concentrations of cadmium (1, 3, and 5 mg/l Cd) were measured on days 1, 3, 5 and 7. All of the specific activity values of the G6PD and 6PGD enzymes from the gills, erythrocytes, livers and kidney tissues are summarized in Fig. 1 and Fig. 2, respectively. The variations in the G6PD and 6PGD activities of the control groups over the course of the seven-day experiment were not significant ($p > 0.05$). At all concentrations (1, 3, and 5 mg/l) cadmium produced significant ($p < 0.05$), cumulative dose-dependent stimulations of G6PD and 6PGD enzyme activities in all the tissues investigated in this *in vivo* study.

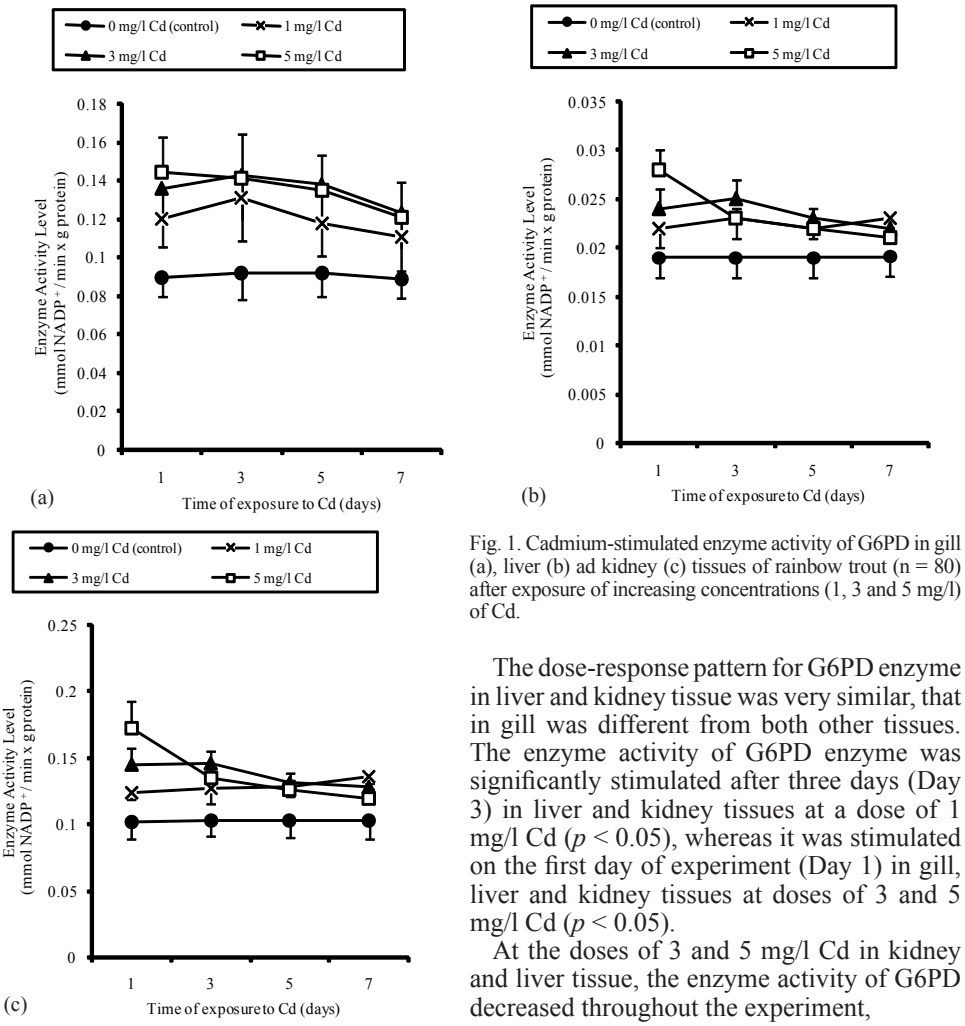

Fig. 1. Cadmium-stimulated enzyme activity of G6PD in gill (a), liver (b) ad kidney (c) tissues of rainbow trout (n = 80) after exposure of increasing concentrations (1, 3 and 5 mg/l) of Cd.

The dose-response pattern for G6PD enzyme in liver and kidney tissue was very similar, that in gill was different from both other tissues. The enzyme activity of G6PD enzyme was significantly stimulated after three days (Day 3) in liver and kidney tissues at a dose of 1 mg/l Cd ($p < 0.05$), whereas it was stimulated on the first day of experiment (Day 1) in gill, liver and kidney tissues at doses of 3 and 5 mg/l Cd ($p < 0.05$).

At the doses of 3 and 5 mg/l Cd in kidney and liver tissue, the enzyme activity of G6PD decreased throughout the experiment,

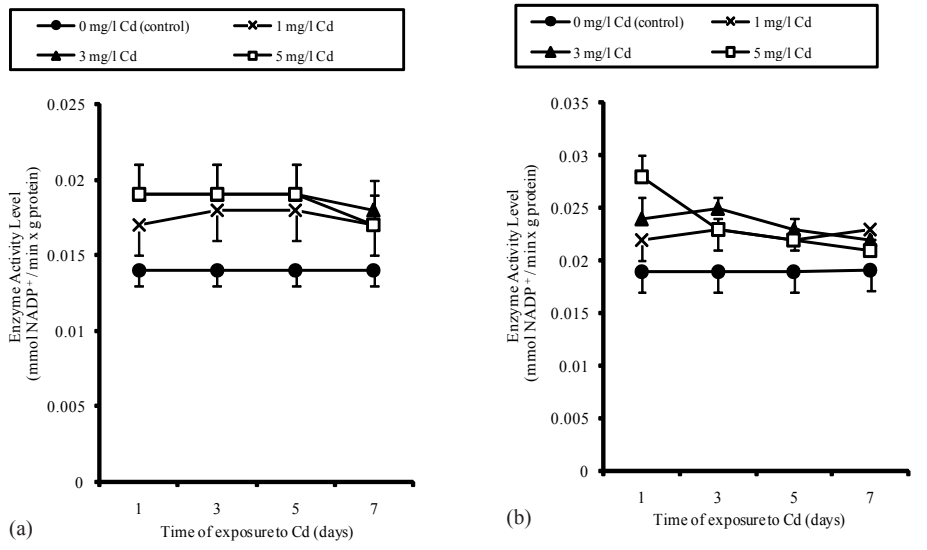

Fig. 2. Cadmium-stimulated enzyme activity of G6PD in gill (a), liver (b) ad kidney (c) tissues of rainbow trout (n = 80) after exposure of increasing concentrations (1, 3 and 5 mg/l) of Cd.

whereas it increased at 1 mg/l Cd. After 3 days of experiment, the slope of the enzyme activity was about the same for both 3 and 5 mg/l. However, a decrease in enzyme activity was observed at all three concentrations in the gills after the third day of the experiment. In the kidney and liver tissues after seven days the enzyme activity levels at 3 and 5 mg/l Cd were lower than the enzyme activity level at 1 mg/l Cd.

From the data presented in Fig. 2, it can be seen that the stimulation effect on 6PGD enzyme activities started immediately after the first day in the gills, liver and kidneys at all doses of Cd, except for the liver at 1 mg/l. On day 7, all concentrations of Cd did not stimulate 6PGD enzyme activity significantly ($p < 0.05$) in gill. Similarly, a 5 mg/l dose of Cd did not stimulate 6PGD enzyme activity starting day 7 in liver and kidney. In comparison to the stimulation effect of Cd among the three tissues studied, it was recorded that the G6PD and 6PGD enzymes were stimulated by approximately 60% and 38% in gills, 68% and 44% in liver, and 67% and 41% in kidneys, respectively, during the experimental period.

Discussion

In fish, as in mammals, endogenous antioxidants such as reduced glutathione (GSH), antioxidant enzymes and dietary antioxidants such as vitamin C and α-tocopherol protect cells against oxidative damage (Winston and Di Giulio 1991). The importance of G6PD and 6PGD in metabolism has been well known for many years. GSH is used by

antioxidant defense mechanisms and its production requires NADPH to be synthesized in the pentose phosphate metabolic pathway in which G6PD and 6PGD participate. For this reason, G6PD and 6PGD are considered as antioxidant enzymes (Reiter et al. 1997).

The results of this *in vivo* experiment indicate that an increase in the activities of G6PD and 6PGD enzymes in the rainbow trout gills, liver and kidneys were induced in the presence of Cd and that these increased enzyme activity levels ultimately served to protect fish from oxidative stress by increasing the level of GSH because the regeneration of GSH from glutathione disulfide (GSSG) depends on the presence of glutathione reductase (GR) and NADPH in the environment; high activity of the NADPH-generating enzymes, G6PD and 6PGD, leads to an increase in GSH levels (O'Brien et al. 2001).

Taken into consideration the results of the study; it can be also said that the response pattern for G6PD enzyme was almost identical in the kidney and liver tissue. For example, the stimulation in G6PD started immediately after the first day in gill at a 1 mg/l dose of Cd. However, the stimulation in G6PD started on the 3rd day in the liver and kidneys. It is well known that gill epithelium is the first interface of the organism exposed to the aquatic environment and for this reason a primary target for the action of environmental pollutants on fish (Lionetto et al. 2000). Therefore, it was thought that cadmium had entered the organs of the fish first through its gills; it then bonded to albumins and erythrocytes in the blood, and was transferred into organs (kidneys and liver) where it accumulated (Kim et al. 2006). Similarly, Kraal et al. (1995) noted that a substantial amount of accumulated Cd in the organism was accumulated in liver and kidneys.

G6PD enzyme showed significantly different increased activity in the tissues, with a decrease especially during a 5 mg/l Cd exposure of 7 days; this can be attributed to Cd producing a cumulative dose-dependent G6PD enzyme activation and adapting to oxidative conditions to which fish are exposed. Similarly, Lenártová et al. (1997) reported that the increased G6PD activity demonstrated increased production of NADPH for the detoxification process and Basha and Rani (2003) determined that the antioxidant enzymes showed significantly increased activity in the liver and kidney tissues, then a slight decrease during a chronic Cd exposure of 30 days, and this probably reflected an adaptation to oxidative conditions. In addition, an effect of Cd was also reported on carbonic anhydrase (CA) enzyme activity in rainbow trout erythrocytes, gill, liver and kidneys. Contrary to our experimental findings, an inhibitory effect of Cd was observed on the CA enzyme in some rainbow trout tissues. It was determined that the inhibitory effect started after day 3 in the erythrocytes, liver and kidneys at a dose of 1 mg/l Cd, whereas it started immediately after the first day in the gills and erythrocytes at 3 and 5 mg/l doses of Cd (Bektas et al. 2008).

The dose-response pattern for 6PGD was almost identical for all three concentrations of Cd in all three tissue samples. The data for 6PGD indicate that the enzyme activity levels reached a steady state on day 1 and stayed there throughout the experiment. All three concentrations responded in the same way of chemical reaction, but there was no significant difference in the enzyme activities of 6PGD at the different cadmium concentrations in the gill tissue. Moreover, it was determined that the behaviour of 6PGD in gill tissue is different than the behavior of G6PD. From these results, it can be reasoned that G6PD is the first enzyme in the pentose phosphate pathway and the increasing or decreasing of the 6PGD enzyme activity depends not only on Cd exposure but also on the G6PD enzyme activity.

In conclusion, it was determined that G6PD and 6PGD enzymes are potential targets for Cd and they are adaptive and demonstrate protective responses to exposure to Cd. Moreover, Cd produced a cumulative dose-dependent G6PD and 6PGD enzymes activation in the gill, liver and kidneys, and the degree of susceptibility to Cd-induced activation was also different among the trout tissues.

We suggest that further researches with an extended sample size should be conducted in order to evaluate the effects of other heavy metals such as lead, mercury and chromium in other fish species.

Kinetika glukózo-6-fosfátdehydrogenázy a 6-fosfoglukonátdehydrogenázy v tkáních pstruha duhového (*Oncorhynchus mykiss*) po vystavení subletálním koncentracím kadmia

V této studii byl sledován vliv kadmia (Cd) na enzymatickou aktivitu glukózo-6-fosfátdehydrogenázy (G6PD) a 6-fosfoglukonátdehydrogenázy (6PGD) v žábrách, játrech a ledvinách pstruha duhového (*Oncorhynchus mykiss*).

Do pokusu byly zahrnuty 3 skupiny ryb, které byly vystaveny různým koncentracím (1, 3 a 5 mg/l) kadmia *in vivo* a jimž byla 1., 3., 5., a 7. den pokusu měřena aktivita G6PD a 6PGD ve tkáni žaber, jater a ledvin. Enzymatická aktivita G6PD a 6PGD v homogenátech z výše uvedených tkání byla stimulována různými koncentracemi kadmia. Odezva G6PD na kadmium byla v játrech a ledvinách velmi podobná, avšak v žábrách se aktivita tohoto enzymu značně odlišovala. U skupiny vystavené 1 mg/l kadmia vzrostla po třech dnech (den 3) experimentu významně ($p < 0.05$) aktivita G6PD v játrech a ledvinách, zatímco u koncentrací 3 a 5 mg/l Cd signifikantně ($p < 0.05$) vzrostla aktivita tohoto enzymu v játrech, ledvinách i žábrách již první den (den 1) experimentu. Aktivita enzymu 6PGD významně ($p < 0.05$) vzrostla v játrech po třech dnech (den 3) expozice koncentraci 1 mg/l Cd a v žábrách, játrech a ledvinách první den (den 1) u dávek 3 a 5 mg/l Cd. Stimulační potenciál 5 mg/l Cd na aktivitu enzymů G6PD a 6PGD významně poklesl ($p < 0.05$) po sedmi dnech experimentu ve všech sledovaných tkáních. Narozdíl od pozitivní korelace mezi koncentrací Cd a aktivitou enzymů v případě 5 mg/l, byly významně ($p < 0.05$) zvýšené aktivity enzymů v případě jater a ledvin u koncentrací kadmia 3 a 1 mg/l. Stimulační vliv kadmia vzhledem k tkáni, v níž byly dané enzymy studovány, byl taktéž vypočítán a pro oba enzymy (G6PD a 6PGD) byl zaznamenán vzestup aktivity během 7 dnů experimentu a to: o 60% a 38% v žábrách, 68% a 44% v játrech, a 67% a 41% v ledvinách oproti bazální úrovni enzymatické aktivity pozorované u zvířat z kontrolní skupiny. Tyto výsledné hodnoty signalizují, že funkcí zvýšené aktivity sledovaných enzymů, 6-fosfátdehydrogenázy a 6-fosfoglukonátdehydrogenázy, je chránit tyto orgány před toxickými účinky kadmia.

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