VETERINARSKI ARHIV 71 (6), 337-344, 2001

Effect of sublethal lead concentrations in feed on δ–aminolevulinic acid dehydratase activity in young carp

Emil Srebočan*, Jelena Pompe-Gotal, Andreja Prevendar-Crnić, and Zvjezdana Špacir

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

SREBOČAN, E., J. POMPE-GOTAL, A. PREVENDAR-CRNIĆ, Z. ŠPACIR: Effect of sublethal lead concentrations in feed on δ–aminolevulinic acid dehydratase activity in young carp. Vet. arhiv 71, 337-344, 2001.

ABSTRACT

The δ–aminolevulinic acid dehydratase (ALA-D) activity was examined in two groups of carp (*Cyprinus carpio L.*) exposed to sublethal lead concentrations (1.3 and 2.6 ppm) for 60 days. There was no statistically significant change in ALA-D activity at one-month lead exposure. After two months, ALA-D activity was decreased in both experimental groups of fish compared with the control group. The decrease in ALA-D activity reached statistical significance ($P<0.05$) in the group of fish administered 2.6 ppm lead in feed. ALA-D is probably the best indicator of fish exposure to lead. Feed is an important source of ground-fish lead contamination that can compromise fish health and reduce their productivity.

Key words: lead, δ–aminolevulinic acid dehydratase (ALA-D), sublethal toxicity, carp

Introduction

The enzyme δ–aminolevulinic acid dehydratase (ALA-D) catalyzes the formation of porphobilinogen, a haemoglobin precursor, from two molecules of δ–aminolevulinic acid. ALA-D is one of the best indicators

* Contact address:
Prof. Dr. Emil Srebočan, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 162; Fax: +385 1 2441 390; E-mail: emil@vef.hr

ISSN 0372-5480
Printed in Croatia

of lead exposure and lead poisoning in humans and animals, including fish. In fish red blood cells, the activity of this enzyme is inhibited even at a very low lead concentration in water, which has been experimentally confirmed (HODSON et al., 1977; SCHMITT et al., 1984). This biochemical parameter is a valuable indicator also in field studies, as it readily differentiates lead contaminated from non-lead contaminated water areas (DWYER et al., 1988).

Feed is an important source of lead contamination in fish (PATRICK and LOUTIT, 1978; SEGNER and BACK, 1985; DALLINGER et al., 1987), especially ground-fish such as carp that take feed mostly from the bottom. Due to the fact that in Croatia lead birdshot is still used in water-fowl kill over fish-farms, the sediments of these fishponds contain high lead concentrations (SREBOČAN et al., 1995). Benthos organisms present in the sediment, a major constituent of carp feed, also contain elevated lead concentrations (WOODWARD et al., 1995). Although the phenomenon of biomagnification has not been demonstrated and the factor of bioconcentration is low for lead, these amounts may prove adequate to provoke unfavourable effects in fish (DALLINGER et al., 1987).

The aim of the study was to determine whether sublethal feed concentrations of lead influenced the activity of ALA-D.

Material and methods

Common carp (*Cyprinus carpio L.*), mean mass 106±20 g, were used in the study. The fish were transported to the laboratory from the Novi Marof hatchery. Prior to the experiment the fish were adapted to laboratory conditions by keeping them in 70 L open-flow glass aquariums for 14 days. The water was dechlorinated, and water temperature (16-17 °C), pH (pH=7) and flow (60 L/h) were constant. Other water quality parameters were determined on several occasions by chemical testing. The total ammonia (NH_4-N) concentration of 0.02-0.16 mg/L was below the noxious concentration for carp at the above temperature and pH. Other parameters were also within the optimal range for carp (free Cl_2 0.03-0.05 mg/L; O_2 5.9-10.5 mg/L; and total hardness 17.08-19.95 °dH.

During the adaptation period the fish were fed on a daily basis with cakes prepared from a commercial trout feed and rough wheat flour (50%:50%) to make a meal containing an adequate amount of protein for carp kept at the above temperature. The following parameters were chemically determined in feed: humidity 36.40%; protein 20.44%; calcium 1.10%; phosphorus 0.61%, sodium 3010 mg/kg; and NaCl 0.76%. The fish were fed once a day at approximately the same time of the day, the amount of feed administered being 4.2% of fish body mass. The fish regularly consumed the feed within two hours.

After 14 days the fish were divided into three groups of 20 carp each: one control group and two experimental groups, I and II. Each of the groups of fish was placed in a separate aquarium. Control fish were fed only the above mentioned cake. For experimental groups, lead acetate ($Pb(C_2H_3O_2)_2$ $3H_2O$ purissumum containing 54.6% Pb) was manually admixed to the cake in the amount required to obtain a lead concentration of 1.3 ppm (group I) and 2.6 ppm (group II). The study lasted for 2 months, during which time the fish were fed and observed on a daily basis. Half of the fish from each group were sacrificed on day 30, and the other half on day 60 of the study. Blood samples were obtained by tail venipuncture. Dissection was performed.

The activity of ALA-D was determined by 50 mL blood reaction with aminolevulinic acid in PBS buffer at 25 °C for 1 hour. Upon protein precipitation the concentration of porphobilinogen was measured at 553 nm by use of Ehrlich's reagent as a chromogen (HODSON et al., 1977; NAKAGAWA et al., 1995). Enzyme activity was expressed as nm of porphobilinogen per ml of blood during 60 min.

Lead concentration in feed was determined by atomic absorption spectrophotometry. Homogenized samples were dried overnight at 460 °C with 15% H_2SO_4. The ash thus obtained was dissolved with diluted HNO_3, evaporated to dryness, and dried again at 300 °C. The ash was then re-dissolved with diluted HNO_3, and ammonium pyrrolidine dithiocarbamate (APDC) was added. Chelated lead was extracted with methylisobutyl ketone (MIBK) and the MIBK solution was taken for measurement. All samples were treated in duplicate, in parallel with three standard lead

concentrations (0.5, 1.0 and 1.5 mg/ml), with two blind tests. Measurements were performed on a Pye Unicam atomic absorption spectrophotometer, model SP 192 supplied with a deuterium lamp for non-specific absorbance. Lead concentration was expressed in mg/g wet mass.

Statistical analysis was done by calculation of mean value, standard deviation, and t-test.

Results

No changes in fish behavioural pattern or lethal outcomes were recorded during the study. Dissection also yielded negative results.

Mean values of ALA-D activities in carp fry red blood cells on days 30 and 60 of the experiment, and lead concentration (per wet mass) in feed samples are shown in Table 1. On day 30, there was no significant difference in the activity of ALA-D between either of the two experimental groups and control group of fish. However, on day 60, the activity of ALA-D was decreased in the experimental groups of fish. The difference was statistically significant in the group II of fish, receiving the higher lead concentration (Table 1).

Table 1. Mean ± sd values of δ–aminolevulinic acid dehydratase (ALA-D) activity (nMPBG/mL RBC)/h) in carp red blood cells on days 30 and 60 of the experiment, and lead concentration (per wet mass) in feed samples

Fish group	Day 30 (nMPBG/mL RBC)/h	Day 60 (nMPBG/mL RBC)/h	Day 1 feed ppm	Day 30 feed ppm
Control	922.7 ± 131.8 (9)	1078.3 ± 99.4 (7)	0.17	0.24
Experiment I	1037.1 ± 82.3 (10)	1002.8 ± 130.2 (9)	1.34	1.39
Experiment II	989.9 ± 69.5 (10)	912.8 ± 144.3 (7)*	2.72	2.86

( ) values in parentheses denote number of blood samples

* statistically significant ($P<0.05$)

Discussion

As well as scientists and those engaged in environmental protection, the awareness of pollution and of the potential environmental risks associated with pollution has gradually developed also in politicians, which has resulted in numerous decisions imposing strict standards for environmental control, regulating emission and sewage treatment, etc. These legal provisions have led to modifications in the bioavailability of heavy metals in water systems. A minor part of heavy metals are now found in the form of water-transferred metal ions, whereas a major part are found in the form of particles, thus increasing the concentration of metals in the bodies of benthos animals and plants, as well as in the water sediment (McINTOSH et al., 1978; TESSIER et al., 1984).

Considering the above and being aware that lead birdshot in the water system sediment is not inert but undergoes gradual lead dissolution due to various chemical processes, followed by its scatter in the surrounding water, in sediment formation consisting of lead oxide, carbonate or other lead compounds (SEVER, 1993), and deposition in benthos organisms that serve as fish feed, efforts were made to perform an experiment as close as possible to natural conditions. Study fish were exposed to a concentration of 1.3 and 2.6 ppm lead administered in feed. The former concentration corresponded to the lead concentration measured in a Croatian fish farm benthos with a long history of mallard kill with lead birdshot (data not published). A concentration of 55.68 ppm lead per dry mass was measured in this fish-pond sediment sample (data not published). Lead can enter the fish body via the alimentary canal with natural or artificial feed, as well as accidentally with water sediment and suspended substances. We therefore decided to include another, higher, lead concentration in feed (2.6 ppm).

In their field studies conducted in the area of rivers contaminated with sewage waters from mines, and lead, zinc and copper smelting plants in Missouri, U.S.A., SCHMITT et al. (1984, 1993) and DWYER et al. (1988) found a statistically significant negative correlation between ALA-D activity and lead concentration in fish blood. They consider ALA-D activity a useful bioindicator of sublethal lead poisoning of the fish from lead-contaminated water systems. In experimental studies in which fish were exposed to a

sublethal lead concentration in water, a significant inhibition of ALA-D activity was also observed. Water lead concentrations greater than 0.013 ppm caused significant inhibition of ALA-D activity in rainbow trout (*Salmo gairdneri*) after only a 4-week exposure (HODSON, 1976). Upon exposure of rainbow trout, brown trout, goldfish and gold sunfish to a concentration of 0.010, 0.090, 0.470 and 0.090 ppm lead, respectively, ALA-D inhibition occurred at 2 weeks. Other metals (Cd, Cu, Zn and Hg) did not induce inhibition of this enzyme, indicating that ALA-D inhibition is lead-specific (HODSON et al., 1977). NAKAGAWA et al. (1995) measured red blood cell activity in carp at various levels of lead exposure. A two-week exposure to a lead concentration of 0.010-1.0 ppm in water resulted in strong inhibition of ALA-D activity. When the carp were exposed to a lead concentration of 0.1 ppm, inhibition of ALA-D activity could be observed as early as day 2, with further ALA-D activity decrease with prolonged exposure. Interestingly enough, although not directly related to the latter study, PATRICK and LOUTIT (1978) fed tropical fish (*Hyphessobrycon serpae*) with tubificid worms contaminated with various metals (Cr, Cu, Mn, Fe, Pb), and found only lead concentration to increase in experimental fish as early as day 2 of exposure.

Experimental studies attempting to assess the effect of lead on ALA-D activity generally investigated the impact of this metal in the water, whereas there were few data on the effect of lead in the feed. Our study demonstrated that very low concentrations of lead in feed also had an unfavourable effect on this enzyme. This effect was manifested after a longer exposure. Such delay was anticipated, knowing that lead resorption is far lower from the digestive system than from the gills. It should be emphasized that lead resorption through the gills occurred only when lead was present in the ionic form, and that many experimental studies used relatively high lead concentrations normally not occurring under natural conditions. Also, both running and stagnant waters have recently become progressively less contaminated with lead. However, the sediment and its organisms still contain considerable amounts of lead.

Our results showed ALA-D activity to be a useful indicator that could be readily modified by a low concentration of lead. These changes can, in the long run, exert unfavourable effects on haematopoiesis and lead to

anaemia in fish, which in turn may result in immunologic, reproductive and productivity disturbances. Therefore, studies should be conducted in natural conditions, their duration being consistent with the natural life expectancy of the fish under study.

References

DALLINGER, R., F. PROSI, H. SEGNER, H. BACK (1987): Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia (Berlin) 73, 91-98.

DWYER, F. J., C. J. SCHMITT, S. E. FINGER, P. M. MEHRLE (1988): Biochemical changes in longear sunfish, *Lepomis megalotis*, associated with lead, cadmium and zinc from mine tailings. J. Fish. Biol. 33, 307-317.

HODSON, P. V. (1976): d-Aminolevulinic acid dehydratase activity in fish blood as an indicator of a harmful exposure to lead. J. Fish. Res. Board Can. 33, 268-271.

HODSON, P. V., B. R. BLUNT, D. J. SPRY, K. AUSTIN (1977): Evaluation of erythrocyte d-aminolevulinic acid dehydratase activity as a short-term indicator in fish of a harmful exposure to lead. J. Fish. Res. Board Can. 34, 501-508.

McINTOSH, A. W., B. K. SHEPHARD, R. A. MAYES, G. J. ATCHINSON, D. W. NELSON (1978): Some aspects of sediment distribution and macrophyte cycling of heavy metals in a contaminated lake. J. Environm. Qual. 7, 301-305.

NAKAGAWA, H., K. NAKAGAWA, T. SATO (1995): Evaluation of erithrocyte d-aminolevulinic acid dehydratase in the blood of carp *Cyprinus carpio* as an indicator in fish with water lead pollution. Fish. Sci. 61, 91-95

PATRICK, F. M., M. W. LOUTIT (1978): Passage of metals to freswater fish from their food. Wat. Res. 12, 395-398.

SCHMIT, C. J., F. J. DWYER, S. E. FINGER (1984): Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte d-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). Can. J. Fish. Aquat. Sci. 41, 1030-1040.

SCHMITT, C. J., M. L WILDHABER, J. B. HUNN, T. NASH, M. N. TIEGER, B. L. STEADMAN (1993): Biomonitoring of lead-contaminated Misouri streams with an assay for erythrocyte d-aminolevulinic acid dehydratase activity in fish blood. Arch. Environ. Contam. Toxicol. 25, 464-475.

SEGNER, H., H. BACK (1985): Importance of contaminated food for the uptake of heavy metals in the rainbow trout, *Salmo gairdneri*. Naturwissenschaften 72, S. 379-380.

SEVER, C. (1993): Lead and outdoor ranges. In: National shooting range symposium proccedings. Salt Lake City, Utah. October 17-19, pp. 87-94.

SREBOČAN, E., J. POMPE- GOTAL, V. SREBOČAN, A. PREVENDAR-CRNIĆ (1995): Monitoring of mercury, lead and cadmium concentrations in animals in the Republic of Croatia. II. Sources and magnitude of lead contamination in freshwater fish. Vet. arhiv 65, 93-100.

TESSIER, A., P. G. C. CAMPBELL, J. C. AUCLAIR, M. BISSON (1984): Relationships between the partitioning of trace metals in sediments and accumulation in the tissues of the freswater mollusc *Ellipitio complanta* in a mining area. Can. J. Fish. Aquat. Sci. 41, 1463-1472.

WOODWARD, D. F., A. M. FARAG, H. L. BERGMAN, A. J. DELONAY, E. E. LITTLE, C. E. SMITH, F. T. BARROWS (1995): Metals-contaminated benthic invertebrates in the Clark Fork Rive, Montana: effects on age-0 brown trout and rainbow trout. Can. J. Fish. Aquat. Sci. 52, 1994-2004.

Received: 24 September 2001
Accepted: 20 December 2001

SREBOČAN, E., J. POMPE-GOTAL, A. PREVENDAR-CRNIĆ, Z. ŠPACIR: Učinak subletalnih koncentracija olova u hrani na aktivnost dehidrataze δ-aminolevulinske kiseline u šaranske mlađi *(Cyprinus carpio L.)*. Vet. arhiv 71, 337-344, 2001.

SAŽETAK

U našem istraživanju izlagali smo šarane subletalnim koncentracijama olova u hrani (1,3 i 2,6 ppm) tijekom 60 dana. Nakon jednomjesečne izloženosti olovu aktivnost ALA-D u pokusnih skupina nije se značajno mijenjala u odnosu na kontrolnu. Nakon dvomjesečnog izlaganja došlo je do pada aktivnosti ALA-D u obje pokusne skupine riba u odnosu na kontrolnu, s time da je u drugoj pokusnoj skupini, koja je dobila 2,6 ppm olova u hrani, taj pad statistički značajan ($P<0,05$). ALA-D vjerojatno je najbolji pokazatelj izloženosti olovu u riba. Hrana je važan izvor kontaminacije olovom pridnenih riba, što može negativno utjecati na zdravlje tih životinja i smanjiti proizvodnju.

Ključne riječi: olovo, dehidrataza δ-aminolevulinske kiseline, subletalna toksičnost, šaran