DOI: 10.2478/10004-1254-62-2011-2082

Scientific Paper

ANTIOXIDATIVE EFFECTS OF *N*-ACETYLCYSTEINE, LIPOIC ACID, TAURINE, AND CURCUMIN IN THE MUSCLE OF *CYPRINUS CARPIO* L. EXPOSED TO CADMIUM

Yusuf SEVGILER[1], Sahire KARAYTUG[2], and Fahri KARAYAKAR[2]

Adiyaman University, Faculty of Science and Letters, Department of Biology, Adiyaman[1], University of Mersin, Faculty of Aquaculture, Yenişehir Campus, Mersin[2], Turkey

Received in October 2010
CrossChecked in October 2010
Accepted in February 2011

We investigated the muscle tissue of a teleost *Cyprinus carpio* L. to find out whether *N*-acetylcysteine (NAC), *alpha*-lipoic acid (LA), taurine (TAU), and curcumin (CUR) were able to counteract oxidative stress induced by acute exposure to cadmium (Cd). The muscle tissue was dissected 96 h after a single intraperitoneal injection of Cd (5 mg kg^{-1}) and of antioxidant substances (50 mg kg^{-1}). Using spectrophotometry, we determined the glutathione redox status, lipid peroxidation levels and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione disulphide reductase (GR). Accumulation of Cd in the muscle was analysed using inductively coupled plasma - optical emission spectrometry (ICP-OES).

All substances lowered Cd levels in the following order of efficiency; LA=NAC>TAU=CUR. Cadmium increased SOD activity, but CAT activity declined, regardless of antioxidant treatment. Treatment with CUR induced GPx activity. Treatment with TAU lowered Cd due to higher total glutathione (tGSH). The most effective substances on lipid peroxidation were LA and NAC due to a greater Cd-lowering potential. It seems that the protective role of TAU, LA, and NAC is not necessarily associated with antioxidant enzymes, but rather with their own activity.

KEY WORDS: *accumulation, antioxidants, fish, muscle, oxidative stress*

Studies of the protective role of exogenous antioxidants against Cd toxicity in living organisms have mostly been limited to mammals and have seldom included aquatic organisms. Yet, aquatic organisms are more sensitive to Cd exposure and toxicity than mammals and may provide a good experimental model for the evaluation of subtle effects of oxidative stress and other adverse effects of pollutants (1). Fish are often not directly exposed to Cd, because they are often at the top of aquatic food chains (2).

Cadmium is a heavy metal; about 18,800 t were produced worldwide for nickel-Cd batteries, pigments, chemical stabilizers, metal coatings and alloys in 2009 alone (3). The oxidative potential of Cd in animals is well-known; it depletes glutathione (GSH) and induces or inhibits antioxidant enzymes and lipid peroxidation (4-6).

A useful protection against its oxidative effects could be supplementation with antioxidants. *N*-acetylcysteine (IUPAC name: (*R*)-2-acetamido-3-sulfanylpropanoic acid; CAS number: 696-91-1; NAC) has widely been used to protect against the toxic effects of a number of chemicals. It is a free-radical scavenger, a precursor of GSH, and it can form stable

water-soluble complexes with mercury and other metals (7).

Alpha-lipoic acid (thioctic acid; IUPAC name: (*R*)-5-(1,2-dithiolan-3-yl) pentanoic acid; CAS number: 1200-22-2; LA) is a disulphide compound and a natural coenzyme of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Lipoic acid and its reduced form dihydrolipoic acid are present in all kinds of microbial and eukaryotic cells and act as antioxidants not only through free-radical quenching, but also indirectly through recycling other cellular antioxidants (8). Its potential as a chelating agent against heavy metal poisoning was also evaluated by some laboratories (9, 10).

Taurine (IUPAC name: 2-aminoethanesulfonic acid; CAS number: 107-35-7; TAU), a semi-essential amino acid, is known to have antioxidant, membrane-stabilising properties, as it inhibits lipid peroxides (11). Contradictory data were reported for the kidney of a teleost *Clarias batrachus* and mice treated with TAU. It lowered Cd content in the fish and mice, but lipid peroxidation decreased only in mice (12, 13).

Curcumin [diferuoyl methane, IUPAC name: (1*E*,6*E*)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; CAS number: 458-37-7; CUR] from the rhizomes of turmeric (*Curcuma longa* L.) is a phenolic and natural yellow pigment. Its biological activity has been studied by many. Daniel et al. (14) reported that CUR chelated Cd and lead in solutions and decreased lipid peroxidation induced by these metals in rat brain homogenate. Curcumin possesses antioxidative and anti-inflammatory properties, and Eybl et al. (15, 16) associated it with a drop in Cd content *in vivo* in rats and mice.

The muscle tissue was chosen in this study because of its usage as food supply by humans as well as high levels of oxygen consumption which results in higher concentrations of ROS compared to other tissues (17). The goal of the present study was to investigate the effect of antioxidant compounds NAC, LA, CUR and TAU on oxidative damage induced by Cd by whether their antioxidant and/or Cd-decreasing potential in the muscle of carp (*Cyprinus carpio L.)* used as a model organism.

MATERIALS AND METHODS

Young carps (*C. carpio*) [mean weight: (54.39± 3.11) g; mean length: (16.02±0.47) cm] were obtained from the State Hydraulic Works fish culture pools (Adana, Turkey) and transferred to our laboratory. The animals were placed in glass tanks for a month before the experiments to get acclimatised to laboratory conditions. The experimental tanks were filled with 120 L of dechlorinated and gently aerated tap water with the following physicochemical properties: total hardness (318.60±4.74) mg L^{-1} $CaCO_3$; pH (8.65±0.04), dissolved oxygen (7.49±0.09) mg L^{-1} and temperature (22.6±0.6) °C. Light and dark cycles exchanged every 12 h. The fish were fed with commercial food pellets (Camli Feed Co., Izmir, Turkey) once a day, receiving about 2 % of their body weight per meal.

The experiments followed the American Public Health Association (APHA) standard methods (18). The fish were divided into seven experimental tanks; each tank accommodating four fish. All experimental chemicals were given in a single intraperitoneal (*i.p.*) dose injected after anaesthetising the fish with ice-containing water because of the interference of chemical anaesthetics with GSH metabolism (19). All experimental doses were determined with pre-experimental results according to the effects of chemicals on lipid peroxidation.

Curcumin was dissolved in ethyl oleate (14), while $CdCl_2$, NAC, LA, and TAU were dissolved in saline (0.59 % NaCl for freshwater fish). Antioxidants NAC, LA, TAU, and CUR were injected immediately after Cd. The fish then were returned into experimental tanks for 96 h and experimental water was not changed during experiments.

Experimental design

Fish in the control group received 150 μL of saline. Oleate control fish received 150 μL of ethyl oleate. Fish in the Cd group received 5 mg kg^{-1} Cd alone. Fish in the Cd+NAC group received 50 mg kg^{-1} NAC immediately after receiving 5 mg kg^{-1} Cd. Fish in the Cd+LA group received 50 mg kg^{-1} LA immediately after receiving 5 mg kg^{-1} Cd. Fish in the Cd+TAU group received 50 mg kg^{-1} TAU immediately after receiving 5 mg kg^{-1} Cd. The last Cd+CUR group of fish received 50 mg kg^{-1} CUR immediately after receiving 5 mg kg^{-1} Cd.

No fish died during the experiments. Ninety-six hours after injection the fish were removed from the tanks and killed by decapitation. Their muscle tissue was dissected out on an ice-cold plate, washed with saline, blotted dry, weighed, and stored at -80 °C until analysis.

A part of muscle tissue from each fish was washed with saline and analysed for Cd using the method of Muramoto (20). Oven-dried muscle samples were digested in a mixture of nitric and perchloric acid (2:1). Cadmium was analysed with a Perkin-Elmer 5300 DV ICP-OES spectrometer (USA). The instrument was calibrated and the standard curve prepared using custom standards supplied by Inorganic Ventures Inc. (USA), with a detection limit of 0.02 mg L^{-1}.

Muscle tissues were homogenised in a teflon homogeniser (Daihan, WiseStir, HS30E, Korea) with 0.25 mol L^{-1} sucrose (Fluka, Switzerland) containing a 50 mmol L^{-1}, pH 7.4 phosphate buffer. Homogenates were centrifuged at 9500 x *g* (Hettich Universal 320R, Germany). The whole homogenisation process was carried out at 4 °C. Supernatants were used to determine tGSH, oxidised glutathione (GSSG), thiobarbituric acid reactive substances (TBARS), protein content, and SOD, CAT, GPx, and GR activities.

The activity of SOD was measured using the method described by McCord and Fridovich (21). The inhibition of iodo-*p*-nitro tetrazolium violet reduction by superoxide anion radical (O_2^-) generated by xanthine-xanthine oxidase was monitored at 505 nm (Shimadzu UV-Mini 1208, Japan) for 3 min at 37 °C. A standard graphic formed by RANSOD kit (Randox Laboratories Ltd., UK) was used to evaluate enzyme activity.

Catalase activity was determined according to Beutler (22) as decrease in absorbance of 10 mmol L^{-1} H_2O_2 at 37 °C for 2.5 min. (ε=0.71 L mol^{-1} cm^{-1}) in Tris-HCl buffer (pH 8.0). The reaction was initiated by adding 20 µL of the sample. The rate of degradation of H_2O_2 by CAT was measured spectrophotometrically at 230 nm.

Glutathione peroxidase-specific activity was analysed by monitoring the consumption of NADPH by GR at 340 nm at 37 °C. For substrate we used *t*-butylhydroperoxide (Sigma-Aldrich, USA) (22). The reaction medium contained 0.1 mol L^{-1} GSH, 10 units of GR, 2 mmol L^{-1} NADPH, and 7 mmol L^{-1} of *t*-butylhydroperoxide.

Glutathione disulphide reductase-specific activity was assayed by monitoring oxidation of NADPH with GSSG at 37 °C and at 340 nm (23). The reaction medium included 100 mmol L^{-1} phosphate buffer (pH 8), 0.12 mmol L^{-1} NADPH, and 1 mmol L^{-1} GSSG.

Before tGSH and GSSG analysis, homogenates were mixed with 10 % sulphosalicylic acid (Sigma-Aldrich, USA) at a ratio of 1:0.5 (v:v), further homogenised, and then centrifuged at 9500 x *g* for 5 min to precipitate the proteins. Total glutathione content in the resulting supernatant was analysed according to Anderson (24). The reaction medium contained 143 mmol L^{-1} sodium-potassium buffer (containing 6.3 mmol L^{-1} EDTA, pH 7.5), 0.3 mmol L^{-1} NADPH, 6 mmol L^{-1} DTNB, and 50 units of GR. The total volume was adjusted to 1 mL with distilled water. Absorbance was monitored at 412 nm at 30 °C and converted to concentration using the standard curve prepared with GSH. Oxidised glutathione content was measured after trapping the reduced fraction with 2-vinylpyridine (25). The derivatised samples were analysed using the same method used for tGSH. Glutathione concentrations were expressed as micromoles of GSH equivalents per miligrame of protein. GSH was calculated by subtracting GSSG levels from tGSH levels (tGSH=GSH+2xGSSG). The GSH/GSSG ratio was calculated using the following formula (26):

$$\frac{GSH}{GSSG} = \frac{tGSH - GSSG}{GSSG/2}$$

Muscle tissue TBARS as a marker of lipid peroxidation was determined according to Ohkawa et al. (27). Homogenates were treated with thiobarbituric acid (TBA) (Sigma-Aldrich, USA) at pH 3.4 and 95 °C for 30 min, and the absorbance of developing pink colour was measured at 532 nm. The reaction mixture contained 8.1 % of SDS, 20 % of acetic acid, pH 3.4, 0.8 % of TBA, and a mixture of *n*-butanol and pyridine (14:1). The concentration of TBARS was determined using the standard curve prepared with 1,1',3,3' tetramethoxypropan.

Protein concentration was measured using a Folin-phenol reagent according to Lowry et al. (28). Absorbance was measured at 750 nm and converted to concentration using bovine serum albumin as a standard.

All parameters were expressed as mean ± standard error (SE). Equality of variances was tested with Levene's test. Statistical differences between the treated and control groups were determined with the analysis of variance (ANOVA), using SPSS 17.0 statistical package. For homogenous subsets we used Duncan's multiple comparison test and for non-homogeneous subsets Tamhane's T2 test. The level of significance was set at 5 %. Pearson correlation analysis was used to determine whether a decrease in Cd level was related to other studied parameters.

RESULTS

Muscle Cd levels

Ninety-six hours after treatment we observed a significant accumulation of Cd in carp muscle (Table 1). All antioxidants lowered its tissue content ($P<0.05$) in the following order (from most effective to least): LA=NAC>TAU=CUR. Lipoic acid and NAC lowered the Cd content by more than 65 %, and TAU and CUR by 40 %.

Effects of antioxidants on antioxidant enzyme activities

All tested antioxidants and Cd alone stimulated SOD activity in the muscle tissue, but the increase was significant only in the Cd+LA and Cd+CUR groups (Table 2). Cadmium treatment lowered CAT activity by more than 60 %. Antioxidants had no effect on Cd-lowered CAT activity. Curcumin increased GPx activity by 20 % compared to the oleate control group. Cadmium did not change GR activity.

Table 1 *Carp muscle accumulation of cadmium (Cd) 96 h after* i.p. *injection of 5 mg kg^{-1} Cd and 50 mg kg^{-1} of antioxidant*

Groups	Cd / µg g^{-1} d.w.
Control	<d.l.
Cd	0.48±0.05[a]
Cd+TAU	0.25±0.03[b]
Cd+LA	0.15±0.03[b]
Cd+CUR	0.28±0.03[b]
Cd+NAC	0.15±0.03[b]

N = 4 fish per group
d.w. – dry weight
TAU – taurine, LA – α-lipoic acid, CUR – curcumin, NAC – N-acetylcysteine; given immediately after Cd injection
[a] *significantly different from control (P<0.05)*
[b] *significantly different from the Cd group (P<0.05)*
d.l. = detection limit (0.02 mg L^{-1})

Effects of antioxidants on GSH redox status

Neither Cd treatment nor co-treatment with antioxidants affected muscle GSH redox status (Table 3). An exception is TAU, which increased tGSH by more than 60 % compared to fish treated with Cd alone. This increase was related to TAU lowering the Cd level, r^2=-0.708, $P<0.05$ (Figure 1).

Effects of antioxidants on lipid peroxidation

Treatment with Cd alone increased TBARS content in carp muscle, but not significantly (Table 4). Lipid peroxidation dropped 60 % with LA and NAC compared to the Cd group and correlated with a decrease in Cd accumulation caused by LA and NAC co-treatments (r^2=0.840, P<0.01 and r^2=0.762, P<0.05, respectively) (Figure 2). No antioxidative effect was observed in the Cd+CUR group, while other compounds were effective in the following order: LA=NAC>TAU.

Neither Cd nor any of the antioxidants significantly changed tissue protein content (Table 4).

DISCUSSION

This study has shown that co-treatment with antioxidants, LA and NAC in particular, plays an important role in preventing Cd^{2+} accumulation and its oxidative effects in carp muscle tissue. Lipoic acid

Table 2 *Carp muscle antioxidant enzyme activities 96 h after* i.p. *injection of 5 mg kg^{-1} Cd and 50 mg kg^{-1} of antioxidant*

Groups	ENZYME ACTIVITIES / U mg^{-1} protein			
	SOD	CAT	GPx	GR
Control	2.51±0.57	90.22±16.09	0.78±0.07	0.029±0.004
Oleate control	3.56±0.48	80.87±11.45	0.76±0.02	0.020±0.001
Cd	8.01±1.07	27.73±3.54[a]	0.80±0.03	0.030±0.004
Cd+TAU	10.02±1.60	45.67±5.01[a]	0.75±0.02	0.028±0.004
Cd+LA	9.89±0.61[a]	48.70±7.86[a]	0.76±0.02	0.036±0.003
Cd+CUR	11.73±0.42[a]	30.96±9.01[a]	0.90±0.01[a]	0.035±0.004
Cd+NAC	10.58±2.71	24.95±1.73[a]	0.57±0.07	0.027±0.001

N = 4 fish per group
SOD – superoxide dismutase, CAT – catalase, GPx – glutathione peroxidase, GR – glutathione disulfide reductase
TAU – taurine, LA – α-lipoic acid, CUR – curcumin, NAC – N-acetylcysteine; given immediately after Cd injection
[a] *significantly different from corresponding control group (the control for Cd+CUR was the oleate group) (P<0.05)*

Table 3 *Carp muscle GSH redox status 96 h after* i.p. *injection of 5 mg kg^{-1} Cd and 50 mg kg^{-1} of antioxidant*

	Glutathione / µmol mg^{-1} protein			
Groups	**tGSH**	**GSH**	**GSSG**	**GSH/GSSG**
Control	11.74±1.40	8.90±1.01	1.61±0.09	11.66±0.62
Oleate control	8.96±1.30	7.51±1.11	1.86±0.05	10.33±0.37
Cd	8.56±0.68	7.25±0.65	1.27±0.02	11.82±0.72
Cd+TAU	14.34±1.50[b]	8.19±1.18	2.27±0.15	11.00±0.30
Cd+LA	8.03±1.11	6.78±1.04	1.38±0.09	12.70±0.37
Cd+CUR	8.15±1.52	6.71±1.37	2.15±0.25	11.36±0.38
Cd+NAC	9.61±0.88	7.15±0.86	1.01±0.09	11.13±0.95

N = 4 fish per group
GSH – glutathione; tGSH – total glutathione, GSSG – oxidised glutathione
TAU – taurine, LA – α-lipoic acid, CUR – curcumin, NAC – N*-acetylcysteine; given immediately after Cd injection*
[b]*significantly different from the Cd group (P<0.05)*

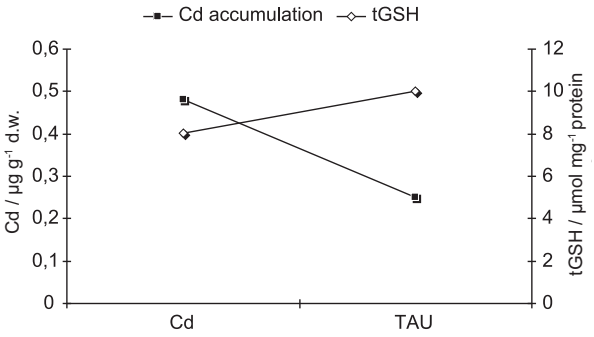

Figure 1 *Relationship between cadmium (Cd) accumulation and total glutathione (tGSH) content in the muscle of carp treated with Cd+TAU.*
TAU – taurine; given i.p. *(50 mg kg^{-1}) immediately after Cd injection*
d.w. – dry weight

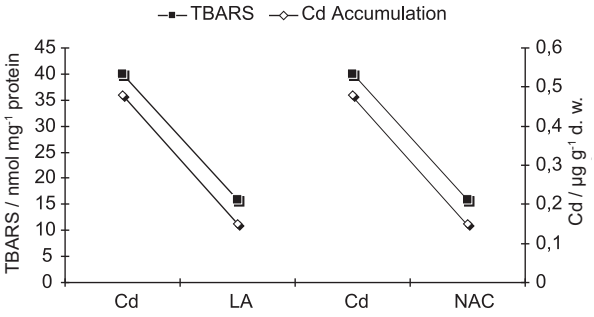

Figure 2 *Relationship between cadmium (Cd) accumulation and lipid peroxidation levels in carp treated with Cd+LA and Cd+NAC, respectively*
TBARS – thiobarbituric acid reactive substances
LA – α-lipoic acid, NAC – N-acetylcysteine were given i.p. *(50 mg kg^{-1}) immediately after Cd injection*

was more effective than TAU and CUR. It is a potent chelator, especially of divalent redox-active metal ions such as Cu^{2+}, Zn^{2+}, and Pb^{2+} (29). In contrast, an acute toxicity study (30) showed that LA injected with Cd

Table 4 *Carp muscle TBARS and protein content 96 h after* i.p. *injection of 5 mg kg^{-1} Cd and 50 mg kg^{-1} of antioxidant*

Groups	**TBARS / nmol mg^{-1} protein**	**Protein / mg mL^{-1} homogenate**
Control	25.50±2.50	0.19±0.01
Oleate control	23.30±2.21	0.17±0.01
Cd	39.89±10.55	0.19±0.02
Cd+TAU	28.98±3.30	0.17±0.03
Cd+LA	15.82±1.50[b]	0.24±0.02
Cd+CUR	40.40±7.95	0.19±0.03
Cd+NAC	15.85±4.03[b]	0.24±0.02

N = 4 specimens per group
TBARS – thiobarbituric acid reactive substances
TAU – taurine, LA – α-lipoic acid, CUR – curcumin, NAC – N*-acetylcysteine; given immediately after Cd injection*
[b]*significantly different from Cd group (P<0.05)*

had no effect on Cd accumulation in mice liver, kidney, brain, and testes. In our study, NAC co-injection decreased Cd content in the muscle, which confirms the findings of Tandon et al. (31) for the liver, kidney and blood tissues of female rats. They have proposed that Cd detoxification may require a sulphur and an oxygen atom, which LA and NAC can provide. As far as we know, we are the first to report about the detoxifying effects of LA and NAC in a metal-treated teleost, and further *in vivo* metal-ligand interaction studies with fish species are needed to understand this interaction.

In several acute and sub-acute treatment studies, TAU acted as a Cd detoxifier in the blood, liver, and kidney tissues of mice (12, 32, 33). It possesses one amino ($-NH_2$) and one sulphonate ($-SO_3H$) group, which may be responsible for its detoxifying action.

Similar to our findings, TAU exhibited lowered sub-chronic Cd accumulation in the liver and kidney of *Clarias batrachus* (13).

Curcumin owes its chelating property to the *β*-diketone moiety (34, 35). Infra-red spectral analysis has shown that CUR with its hydroxyl groups and *β*-diketone moiety binds directly or indirectly to toxic metals, including Cd, through strong metal-ligand interactions (14). In contrast to our results, however, acute CUR pre-treatment by gastric gavage had no effect on acute Cd accumulation in the liver, kidney, brain, and testes of mice and rats (15, 16). We too found that liver Cd was not affected by CUR co-treatment in carp 96 h after *i.p.* injection (unpublished data). We are the first to report about decreasing efficiency of CUR co-treatment in muscle tissue of a teleost and we thought that this effect may be owed to tissue-specific action.

Superoxide dismutase, CAT, and GPx enzymes provide first-line cell defence against toxic ROS action. In our study, SOD activity in carp muscle revealed higher Cd-induced O_2^- production. According to Kono and Fridovich (36) excessive O_2^- accumulation inhibits CAT activity and SOD prevents this inhibition but does not reverse it *in vitro*. They reported that this synergism may be important *in vivo*, but we could not confirm it. In another study, sub-chronic Cd treatment induced SOD and decreased CAT activity in rat kidney and the authors have suggested that Cd can reduce enzyme activities which have a Fe ion in their active sites such as CAT by decreasing liver and kidney Fe levels (37). In a study by Kumar et al. (13) Cd exposure increased SOD and CAT activity in the liver and kidney of *C. batrachus*, while TAU had no effect on SOD activity. Bludovská et al. (30) reported that LA altered Cd-induced CAT inhibition in the liver of mice. In contrast, CUR did not affect liver CAT activity lowered by Cd treatment in mice (16), but improved SOD and CAT activities in the liver of sodium arsenite-exposed rats in another study by El-Demerdash et al. (38). The authors concluded that the protective role of antioxidants against Cd-induced oxidative stress is not necessarily associated with antioxidant enzyme activities.

Eybl et al. (39) found that neither Cd nor Cd+CUR treatments affected liver GPx activity in mice. In our study, CUR may have caused H_2O_2 accumulation in carp muscle, reflected by higher SOD and lower CAT activity, which in turn may have increased GPx activity. In another study (40), curcumin caused a sudden rise in ROS levels in mice L929 fibroblast cells, and this effect was blocked by antioxidants NAC and GSH. Induced GPx activity could also be related to higher lipid peroxide levels in Cd+CUR group. A more detailed investigation should be able to answer if ROS accumulation in Cd-exposed teleost species is related to CUR.

Thiol-based intracellular antioxidant system is considered to be the second line of cellular defence against ROS-mediated oxidative damage (33). It is well-known that cellular GSH redox status is affected by Cd action (41, 42). This, however, is questioned by the results of our and other studies in which Cd had no effect on cellular GSH redox status (43, 44). Thijssen et al. (44) reported that the treatment of mice with 100 mg L^{-1} Cd had no effect liver and kidney GSH and GSSG levels, and that cellular redox status was protected by elevated metallothionein levels, which are important in Cd detoxification. Non-enzymatic reaction between GSH and O_2^- radical is well-known (45). The rise in tGSH levels in the TAU co-treated group in our study may also be an adaptive response to O_2^- elevation, reflected by SOD activity. As there was no change in GSH and GSSG level, we believe that SOD activity is more important in protecting the cell against Cd-induced ROS effect than GSH.

In our study, GR did not vary significantly across groups, most probably because it is usually activated by a disturbance in the GSH/GSSG ratio (which reflects cellular thiol redox status), and this ratio was not disturbed.

Kumar et al. (13) have reported that Cd catalyses oxidation of biomolecules and that its destructive effects on cell components could be weakened by antioxidants. While studies disagree whether exogenous antioxidants can lower lipid peroxidation and Cd levels at the same time, the action of LA and NAC, as the most effective antioxidants in our study, was related to lowering Cd levels, and not as much to stimulating antioxidant enzyme activity. Müller and Menzel (9) reported that LA complexes with intra- and extracellular Cd in isolated rat hepatocytes protected rat liver cells from Cd-induced membrane destruction. While in the study by Buldovská et al. (30) LA did lower Cd-induced lipid peroxidation to control levels in mice liver, it did not change tissue Cd content. Neither did NAC, in the study by Jones et al. (45), change tissue Cd contents or improve enzymatic and non-enzymatic antioxidant levels in the kidney of Cd-treated rats; it decreased renal Cd toxicity by

decreasing Cd-induced lipid peroxidation (46). In our study and the study by Kumar et al. (13), TAU lowered both lipid peroxidation and Cd tissue content. Curcumin, however, was not effective against lipid peroxidation; its pro-oxidant properties have also been confirmed by Thayyullathil et al. (40).

CONCLUSION

To sum up, the antioxidants used in this study seem not only to counter the oxidative effects of Cd, but also to reduce its tissue content. The most efficient against Cd toxicity are LA and NAC, followed by TAU, and CUR. We have also observed that their protective role is not necessarily associated with antioxidant enzymes, but rather to their own activity.

Acknowledgement

Adiyaman University Scientific Research Commission is gratefully acknowledged for supporting the project FEFBAP2008-5. We declare no conflict of interest whatsoever.

REFERENCES

1. Lackner R. "Oxidative stress" in fish by environmental pollutants. In: Braunbeck T, Hinton DE, Streit B, editors. Fish Ecotoxicology. Basel: Birkhauser Verlag; 1998. p. 203-24.
2. Lushchak VI. Oxidative stress as a component of transition metal toxicity in fish. In: Svensson EP, editor. Aquatic Toxicology Research Focus. Hauppauge (NY): Nova Science Publishers Inc.; 2008. p. 1-29.
3. Tolcin AC. Cadmium. In: U.S. Geological Survey Mineral Commodity Summaries. Washington: United States Government Printing Office; 2010. p. 36-7.
4. Hwang DF, Wang LC. Effect of taurine on toxicity of cadmium in rats. Toxicology 2001;167:173-80.
5. Basha PS, Rani AU. Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). Ecotox Environ Saf 2003;56:218-21.
6. Soares SS, Martins H, Gutiérrez-Merino C, Aureliano M. Vanadium and cadmium *in vivo* effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. Comp Biochem Physiol C 2008;147:168-78.
7. Goyer RA, Clarkson TW. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's Toxicology: The Basic Science of Poisons. 6th ed. New York (NY): McGraw-Hill; 2001. p. 811-67.
8. Aly HAA, Lightfoot DA, El-Shemy HA. Modulatory role of lipoic acid on lipopolysaccharide-induced oxidative stress in adult rat Sertoli cells *in vitro*. Chem Biol Interact 2009;182: 112-8.
9. Müller L, Menzel H. Studies on the efficacy of lipoate and dihydrolipoate in the alteration of cadmium^{2+} toxicity in isolated hepatocytes. Biochim Biophys Acta 1990;1052: 386-91.
10. Lodge JK, Traber MG, Packer L. Thiol chelation of Cu^{2+} by dihydrolipoic acid prevents human low density lipoprotein peroxidation. Free Radic Biol Med 1998;25:287-97.
11. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 2006;11:114-27.
12. Manna P, Sinha M, Sil PC. Taurine plays a beneficial role against cadmium-induced oxidative renal dysfunction. Amino Acids 2009;36:417-28.
13. Kumar P, Prasad Y, Patra AK, Ranjan R, Swarup D, Patra RC, Pal S. Ascorbic acid, garlic extract and taurine alleviate cadmium-induced oxidative stress in freshwater catfish (*Clarias batrachus*). Sci Total Environ 2009;407:5024-30.
14. Daniel S, Limson JL, Dairam A, Watkins GM, Daya S. Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J Inorg Biochem 2004;98:266-75.
15. Eybl V, Kotyzová D, Bludovská M. The effect of curcumin on cadmium-induced oxidative damage and trace elements level in the liver of rats and mice. Toxicol Lett 2004;151: 79-85.
16. Eybl V, Kotyzová D, Lesetický L, Bludovská M, Koutensky J. The influence of curcumin and manganese complex of curcumin on cadmium-induced oxidative damage and trace elements status in tissues of mice. J Appl Toxicol 2006;26: 207-12.
17. Fulle S, Protasi F, Di Tano G, Pietrangelo T, Beltramin A, Boncompagni S, Vecchiet L, Fano G. The contribution of reactive oxygen species to sarcopenia and muscle ageing. Exp Gerontol 2004;39:17-24.
18. Clescerl LS, Greenberg AE, Eaton AD, editors. Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington (DC): American Public Health Association; 1999.
19. Peña-Llopis S, Ferrando MD, Peña JB. Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by *N*-acetylcysteine. Aquat Toxicol 2003;65:337-60.
20. Muramoto S. Elimination of copper from Cu-contaminated fish by long term exposure to EDTA and freshwater. J Environ Sci Health A 1983;18:455-61.
21. McCord JM, Fridovich I. Superoxide dismutase: An enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-55.
22. Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. New York (NY): Grune and Starton; 1984.
23. Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 1975;250:5475-80.
24. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113:548-55.
25. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980;106:207-12.

26. Peña-Llopis S, Peña JB, Sancho E, Fernández-Vega C, Ferrando MD. Glutathione-dependent resistance of the European eel *Anguilla anguilla* to the herbicide molinate. Chemosphere 2001;45:671-81.
27. Ohkawa H, Ohishi N, Tagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Chem 1979;95:351-8.
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951;193:265-75.
29. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. Biochim Biophys Acta 2009;1790:1149-60.
30. Bludovská M, Kotyzová D, Koutensky J, Eybl V. The influence of alpha-lipoic acid on the toxicity of cadmium. Gen Physiol Biophys 1999;18:28-32.
31. Tandon SK, Prasad S, Singh S. Chelation in metal intoxication: influence of cysteine or *N*-Acetyl cysteine on the efficacy of 2,3-dimercaptopropane-1-sulphonate in the treatment of cadmium toxicity. J Appl Toxicol 2002;22: 67-71.
32. Sinha M, Manna P, Sil PC. Cadmium-induced neurological disorders: prophylactic role of taurine. J Appl Toxicol 2008;28:974-86.
33. Sinha M, Manna P, Sil PC. Induction of necrosis in cadmium-induced hepatic oxidative stress and its prevention by the prophylactic properties of taurine. J Trace Elem Med Biol 2009;23:300-13.
34. Schwarz K, Mertz W. A physiological role of chromium (III) in glucose utilization (glucose tolerance factor). Fed Proc 1961;20:111-4.
35. Albert A. Design of chelating agents for selected biological activity. Fed Proc 1961;20:137-47.
36. Kono Y, Fridovich I. Superoxide radicals inhibit catalase. J Biol Chem 1982;257:5751-4.
37. Jurczuk M, Brzóska MM, Moniuszko-Jakoniuk J, Gałazyn-Sidorczuk M, Kulikowska-Karpińska E. Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. Food Chem Toxicol 2004;42:429-38.
38. El-Demerdash FM, Yousef MI, Radwan FME. Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs Food Chem Toxicol 2009;47:249-54.
39. Eybl V, Kotyzova D, Koutensky J. Comparative study of natural antioxidants - curcumin, resveratrol and melatonin - in cadmium-induced oxidative damage in mice. Toxicology 2006;225:150-6.
40. Thayyullathil F, Chathoth S, Hago A, Patel M, Galadari S. Rapid reactive oxygen species (ROS) generation induced by curcumin leads to caspase-dependent and -independent apoptosis in L929 cells. Free Radic Biol Med 2008;45: 1403-12.
41. Bertin G, Averbeck D. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie 2006;88:1549-59.
42. Franco R, Sánchez-Olea R, Reyes-Reyes EM, Panayiotidis MI. Environmental toxicity, oxidative stress and apoptosis: Ménage à Trois. Mutat Res 2009;674:3-22.
43. El-Maraghy SA, Gad MZ, Fahim AT, Hamdy MA. Effect of cadmium and aluminum intake on the antioxidant status and lipid peroxidation in rat tissues. J Biochem Mol Toxicol 2001;15:207-14.
44. Thijssen S, Cuypers A, Maringwa J, Smeets K, Horemans N, Lambrichts I, Van Kerkhove E. Low cadmium exposure triggers a biphasic oxidative stress response in mice kidneys. Toxicology 2007;236:29-41.
45. Jones CM, Lawrence A, Wardman P, Burkitt MJ. Kinetics of superoxide scavenging by glutathione: an evaluation of its role in the removal of mitochondrial superoxide. Biochem Soc Transact 2003;31:1337-9.
46. Wang L, Chen D, Cao J, Liu Z. Protective effect of *N*-acetylcysteine on experimental chronic cadmium nephrotoxicity in immature female rats. Hum Exp Toxicol 2009;28:221-9.

Sažetak

ANTIOKSIDATIVNI UČINCI *N*-ACETILCISTEINA, LIPOIČNE KISELINE, TAURINA I KURKUMINA U MIŠIĆNOM TKIVU ŠARANA (*CYPRINUS CARPIO* L.) TRETIRANIH KADMIJEM

Cilj istraživanja bio je utvrditi mogu li *N*-acetilcistein (NAC), *α*-lipoična kiselina (LA), taurin (TAU) i kurkumin (CUR) svojim antioksidativnim djelovanjem smanjiti razinu oksidativnog stresa u mišićnom tkivu šarana (*Cyprinus carpio* L.) akutno otrovanih kadmijem. Uzorci mišićnog tkiva skupljeni su 96 h nakon što su ribama intraperitonealno injicirani kadmij (5 mg kg^{-1}) i ispitivani antioksidansi (50 mg kg^{-1}). Primjenom spektrofotometrijskih metoda izmjereni su redoks status glutationa, razine lipidne peroksidacije te aktivnosti enzima superoksid dismutaze (SOD), katalaze (CAT), glutation peroksidaze (GPx) i glutation disulfid reduktaze (GR). Maseni udio kadmija u mišićnom tkivu izmjeren je s pomoću metode induktivno spregnute plazme – optičke emisijske spektrometrije (ICP-OES).
Ispitivani spojevi smanjili su nakupljanje kadmija u tkivu šarana sljedećim redoslijedom: LA=NAC>TAU=CUR. Tretman šarana kadmijem izazvao je porast aktivnosti SOD, ali se aktivnost CAT smanjila bez obzira na primjenu antioksidativnih spojeva. Dodatak CUR pojačao je aktivnost GPx. Dodatak TAU povećao je razinu ukupnoga glutationa te smanjio nakupljanje kadmija. Svi spojevi osim CUR smanjili su razinu lipidne peroksidacije te pretpostavljamo da su LA i NAC pridonijeli detoksifikaciji kadmija. Rezultati istraživanja upućuju na to da testirani spojevi, osim CUR, imaju antioksidativni učinak.

KLJUČNE RIJEČI: *akumulacija, antioksidansi, mišićno tkivo, oksidativni stres, riba*

CORRESPONDING AUTHOR:

Yusuf Sevgiler
Adiyaman University
Faculty of Science and Letters
Department of Biology
02040 Adiyaman, Turkey
E-mail: *ysevgiler@posta.adiyaman.edu.tr*