PROTECTIVE EFFECT OF VITAMINS AND EPICATECHIN ON CADMIUM TOXICITY IN RATS

Roquia Ibrahim Rizk[1, 2], Samir Ismail[2], Mohamed Ali Rawash[3, 4]
Tatjana Juzsakova[1]

[1] University of Pannonia, Institute of Environmental Engineering, Veszprém 8200, Hungary
[2] Cairo University, Faculty of Agriculture, Giza, 12613 Egypt
[3] University of Pannonia, Georgikon Faculty, Keszthely H-8360, Hungary
[4] Regional Center for Feed and Food, Agricultural Research Centre, 12619, Egypt
[1]roquia.rizk@mk.uni-pannon.hu

Abstract

Cadmium is one of the most toxic heavy metals. It causes serious environmental and occupational contamination which may represent a health hazard to both humans and animals. Cd is efficiently retained in the human body, in which it accumulates throughout life. This heavy metal is a potential carcinogenic environmental pollutant. Antioxidants such as vitamin C, vitamin E and epicatechin (green tea extract) are important for preventing the damage caused by reactive oxygen species or Cd toxicity. The present study aimed to investigate the protective effects of vitamins C, E, and epicatechin on cadmium induced toxicity in public health. Rats exposed on cadmium 5 times per week for 21 days exhibited kidney failure and lipid peroxidation measured by using new sensitive biomarkers. Vitamin C, vitamin E and epicatechin treatments restituted in some extent the lowered level of alanine-amino-peptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG) caused by Cd exposure. The oxidative stress biomarkers such as serum glutathione peroxidase (GPx) activity decreased by the effect of cadmium and those improved by the protective factors in the present study, while the elevated

level of malonaldehyde (MDA) by Cd was ameliorated by antioxidants supplements. Our results suggest that vitamin C, vitamin E and epicatechin have symbiotic protective effect against cadmium-induced toxicity.

Key words: cadmium toxicity, N-acetyl-β-D-glucoaminidase, alanine-amino-peptidase, epicatechin, vitamins C, E

Összefoglalás

A kadmium az egyik legmérgezőbb nehézfém. Jelentős környezeti és foglalkozási kontaminációja egészségügyi kockázatot jelent mind az emberi mind az állati szervezetekre. A kadmium az emberi szervezetben felhalmozódik, és potenciális rákkeltő hatása van. Az antioxidánsok, mint a C-vitamin, E-vitamin és epikatechin (zöld tea kivonat) fontos szereppel bírnak a kadmiummérgezés során jelentkező oxidatív stressz okozta károsodások megelőzésében. Vizsgálatunkban a C-vitamin, E-vitamin és az epikatechin védőhatását tanulmányoztuk a kadmium indukálta oxidatív stresszre. Patkányokon végeztük el a vizsgálatot, amelyeket hetente 5 napon át, 21 napig kezeltünk kadmiummal. Biomarkerek segítségével mértük a kadmiummérgezés hatására jelentkező veseelégtelenséget és a lipid peroxidációt. A C-vitamin, az E-vitamin és epikatechin javította a kadmiumos kezelés hatására lecsökkent szérum alanin-amino-peptidáz (AAP) és a N-acetil-β-D-glukozaminidaz (NAG) szinteket. Az oxidatív stressz biomarkerek közül a szérum glutathion peroxidáz (GPx) szintet a kadmiumos kezelés lecsökkentette, de az alkalmazott antioxidánsok a vizsgált enzim aktivitásának mértékét fokozták. Az eredményeink azt támasztották alá, hogy a C-vitamin, E-vitamin és epikatechin (zöld tea kivonat) bizonyos fokú védelmet biztosítanak a kadmiummérgezés okozta oxidatív stresszel szemben.

Kulcsszavak: kadmiummérgezés, N-acetil-β-D-glukozaminidáz, alanin-amino-peptidáz, C-vitamin, E-vitamin, epikatechin

Introduction

Cadmium metal has specific properties that make it suitable for a wide variety of industrial applications (ATSDR, 2008). In the earth's crust, cadmium appears mainly in association with ores containing zinc, lead and copper in the form of complex oxides, sulfides and carbonates (UNEP, 2008). Exposure to cadmium occurs through intake of contaminated food or water, or by inhalation of polluted air (Järup et al., 1998). Occupational exposures are found in many industries such as electroplating, welding, smelting, pigment production, cement production and battery manufacturing. Other respiratory exposure to cadmium can occur through inhalation of cigarette, car and fuel oil smoke (Rzigalinski and Strobl, 2009) especially in big cities. Cadmium concentration in tested Nile Delta ground water samples from industrial areas, Hilwan and El-Tebbin, Egypt was considerably high (0.010-0.038 mg/L) (Hefny, 1984) compared with the permissible cadmium content in drinking water standard (0.003 mg/L) (WHO, 2000). Several health organizations in the world have established values for cadmium in drinking water. The U.S. EPA has set a maximum contaminant Cd level of 0.005 mg/L. The Australian National Health and Medical Research Council has established a guideline Cd value of 0.002 mg/L. The World Health Organization has published a drinking-water quality guideline of 0.003 mg/L, and the European Union directive includes a parametric value of 0.005 mg/L for cadmium in drinking water.

The cadmium level in Cairo City air ranged between 0.01-2 $\mu g/m^3$ with an average value of 0.05 $\mu g/m^3$ (EEAA, 1992). Celery, parsley and spearmint, cultivated in Egypt Nile valley, contain the highest mean level of cadmium (2.44 µg/g). In addition, barely grain, potato, spinach, green bean and pea contain 0.012, 0.32, 3.9, 0.4 and 0.043 mg Cd per kg plant dry weight respectively (Abdel-Sabour and Rabie, 2000). The average concentrations of cadmium in most of foodstuffs must be less than 0.02 µg/g (WHO, 2011).

Cadmium is a potential carcinogenic environmental pollutant that has been lastly linked to breast cancer (El-Harouny et al., 2010), pancreatic cancer (Kriegel et al., 2006), carcinomas in the lung (Hartwig, 2012), sarcomas and testicular tumours (Hartwig, 2012). Northeast Nile Delta region exhibits a high incidence of early onset

of pancreatic cancer. It is well documented that this region has one of the highest levels of cadmium pollution in Egypt (Kriegel et al., 2006). Cadmium has been reported to induce nephrotoxicity (Prozialeck and Edwards, 2012), hepatotoxicity (Arroyo et al., 2012), cytotoxicity (Krichah et al., 2003), mortality (Nawrot et al., 2010), teratogenesis (Messaoudi et al., 2009), foetal toxicity (Abshire, 1996), testicular toxicity (Kini et al., 2009), metabolic bone diseases such as osteoporosis and osteomalacia (Brzóska et al., 2007), oxidative stress (Newairy et al., 2007), and disturbances in lipid metabolism (Rogalska et al., 2009).

Cadmium has an indirect role in the generation of various free radicals (Bolkent et al., 2008). Non-enzymatic antioxidants such as vitamin C (El-Refaiy and Eissa, 2013), vitamin E (Kara et al., 2008) and epicatechin, green tea extract, (El-Shahat et al., 2009) are important for preventing the damage caused by reactive oxygen species induced by cadmium (Bolkent et al., 2008). Disorders of homeostasis leading to increased stationary concentrations of reactive forms of oxygen are referred to as oxidative stress. Oxidative stress induced by heavy metals can reduce the capacity of the antioxidant defence system. The antioxidant effect of epicatechin is based on its reducing properties, including direct inactivation of reactive oxygen and nitrogen species and decreasing the production of reactive oxygen species, as well as an indirect effect being the regeneration of other antioxidants such as α-tocopherol or β-carotene and chelating transitional metals (Kim et al. 2014).

Thus, our objective in this study was to investigate the protective effect of vitamins C, E and epicatechin on cadmium–induced toxicity in the kidney and oxidative stress.

Materials and Methods

Chemicals

All chemical, solvents and reagents were of analytical grade purity were purchased from Sigma, St. Louis, Mo, USA.

Animals

A total of 30 male albino rats (Spraque Dawely strain) had a body weight (bw) from 100 to 120 g were supplied by Helwan Station for Experimental Animals, Helwan, Cairo, Egypt. The animals were housed in stainless steel cages and raised in the animal house of Biochemistry Department, Faculty of Agriculture, Cairo University. The rats were kept under normal healthy laboratory conditions. Temperature was adjusted at 22 ± 2 °C with humidity of 50 ± 10% in July and 12 h of light and 12 h of dark cycles. The animals were adapted on free access of redistilled water and fed basal diet for two weeks before the initiation of the experiments. The protocol conforms to the guidelines of the National Institute of Health (NIH).

Diets

All animals had standard diet protocol (Ulloa et al., 1988) containing vitamins and salt mixtures recommended by Campbell (1961) and Hegsted et al. (1941).

Experimental procedure

Thirty rats were divided into five groups (six animals in each group) and were subcutaneously injected with cadmium chloride solution (1.23 mg Cd/kg bw) concentration corresponding to 13% of LD_{50} (15.2 mg $CdCl_2$/kg of albino rats), five times a week according to method as reported (AFDOAQ, 1951). Each group received subcutaneous injection with cadmium chloride and at the same time the rats were differently orally administered (1) 100 mg/kg bw of vitamin C; (2) 40 mg/kg bw of vitamin E (as α-tocopherol acetate); (3) 4.5 mg/kg bw of epicatechine, (4) saline solution (Cd effect control), no antioxidants treatment was applied. The total volume injected or orally administered by gavage for each rat did not exceed 0.5 mL once a day. Control group (6 rats) was also monitored at the same time (neither Cd exposure nor antioxidants treatment was applied). The experiment continued for 21

days and at the end of experiment, the animals were fed-deprived for 12 h. Rats were killed by decapitation, and the blood sample of each rat was collected in dry clean centrifuge glass tube, and was centrifuged at 3000 rpm for 15 min to separate the serum. The clear non haemolyzed serum was pipetted into epindorff tubes and stored at –20 ºC until biochemical determination of urea by (Foster and Hochhlozer, 1971), creatinine by (Schirmeister et al., 1964), N-acetyl-β-D-glucosaminidase activity (NAG) by (Numata et al., 1997), alanine-amino-peptidase activity (AAP) by (Jung and Scholz, 1980), malondialdehyde (MDA) (Satoh, 1978) and glutathione peroxidase (GPx) by (Paglia and Valentine, 1970) procedures.

Statistical analysis

All the data summarized in Table 1 were expressed as mean ± standard error (SE). Differences among the experimental groups were assessed by one way analysis of variance (ANOVA) followed by protected least significant difference. The grey shaded boxes of Table 1 indicates the best results achieved by antioxidants treatment.

Results and Discussion

Table 1 shows the effect of Cd and some antioxidants on serum urea levels. These data illustrate that the urea concentrations in serum were significantly increased by 47.6% due to Cd injection (saline solution). Administration of vitamins C and epictechin slightly improved the renal tubular filtration. However, the vitamin E was able to decrease the serum level of urea almost to the control level of 31.12 mg/dL.

Table 1. Effect of oral administration of vitamins C, E and epicatechin for 21 days on some serum parameters of $CdCl_2$ treated rats.

Parameter	Control	Cd (1.23 mg/kg bw)			
		Saline solution	Vitamin C 100 mg/kg bw	Vitamin E 40 mg/kg bw	Epicatechine 4.5 mg/kg bw
Urea (mg/dL)	31.12 ± 1.86 [c]	45.92 ± 2.75 [a]	42.77 ± 1.73 [a]	30.43 ± 0.17 [c]	39.73 ± 4.8 [b]
Creatinine (mg/dL)	0.74 ± 0.20 [c]	2.21 ± 0.16 [a]	2.31 ± 0.32 [a]	2.17 ± 0.25 [ab]	1.73 ± 0.28 [b]
NAG (U/L)	9.85 ± 0.21 [a]	5.17 ± 0.2 [c]	6.32 ± 0.46 [b]	4.87 ± 0.03 [bc]	6.56 ± 0.47 [b]
AAP (U/L)	58 ±7.36 [a]	20.5 ± 0.64 [c]	30.2 ± 0.02 [bc]	45 ± 0.5 [b]	49 ± 0.08 [ab]
MDA (µmol/L)	2.23 ± 0.12 [b]	3.11 ± 0.12 [a]	2.09 ± 0.14 [c]	3.07 ± 0.20 [a]	2.54 ± 0.16 [ab]
GPx (U/L)	342.12 ± 8.68 [b]	164.48 ± 7.48 [d]	353.3 ± 5.28 [a]	304.14 ± 6.88 [c]	358.61 ± 7.76 [a]

[a, b, c] means within a row show the difference between the results

In addition, injection of Cd into experimental rats resulted three-fold increase serum creatinine values and those slightly decreased after administration of vitamin E and epicatechin in higher extent. Vitamin C did not influence the creatinine level.

Data given in Table 1 show also that cadmium administration significantly decreased both serum NAG activity in rats by 47.5% due to the severe damage of nephrons. Similar trend was observed in alanine-amino-peptidase activity in serum of rats. The activity of AAP was drastically decreased by 64.6%. Antioxidants are used to restore the enzymes activity in serum to the normal level. In our study the applied antioxidants treatments failed to readjust the NAG and AAP values to control level, 9.85 and 58 U/L respectively. The best results regarding the renal tubular filtration of AAP (49 U/L) and NAG (6.56 U/L) activities restoration was achieved by epicatechin treatment.

Exposure to cadmium may affect on the antioxidant defense system of red blood cells and lipid peroxides concentration in blood. Therefore the oxidative stresses of cadmium as well as the possible protective roles of vitamins C, E and

epicatechin were studied. Cadmium increased serum malonaldehyde (MDA), as a measurement of lipid peroxidation, from 2.43 to 3.11 μmol/L. Antioxidants had a protective role against cadmium induced lipid peroxidation and were able to slightly adjust the serum malonaldehyde level toward the normal value. The best improvement in MDA value was observed after vitamin C treatment (2.09 μmol/L).

From the present data, it was shown that exposure to cadmium induced a significant decrease in glutathione peroxidase (GPx) activities in blood from 342.12 to 164.48 U/L. Vitamins C, E and epicatechin had beneficial effects on Cd induced decrease in GPx activities. While the vitamin E and epicatechin produced better results than the control level.

The kidney is highly susceptible to chemical injury because of its high blood flow. Chemicals and minerals also concentrate in the tubular fluid due to reabsorption and transcellular renal transport effects. Elements have the potential to accumulate in the tubular cells (Price et al., 2009). In addition, the kidney is a major metabolic organ, where the chemicals may be bio-transformed into more toxic derivatives (Price, 1992). Currently, urea and creatinine clearance are used as benchmarks for renal damage, but these tests are insensitive because some of kidney function is lost before changes occur in the value of each test (Babaknejad et al., 2015). Therefore, new early and sensitive biomarkers of nephrotoxicity have to be found. Many circulating high molecular weight lysosomal proteins such as N-acetyl-β-D-glucosaminidase and proximal tubular enzymes (alanine-amino-peptidase) can be used as urinary early sensitive biomarkers of site-specific renal insult as was shown in this work. The AAP is also proper early sensitive biomarker in serum of renal damage.

Some evidences indicated that metallothionein biosynthesis (a thiol-rich low molecular weight protein involved in the detoxification of heavy metals) was increased in the presence of Cd (Yoshida et al., 1992). The increase in metallothionein biosynthesis may decrease the glutathione (GSH) biosynthesis because of consumption of SH groups in metallothionein biosynthesis (Jemai et al., 2010) and induce the oxidative stress (Dzobo and Naik, 2013). It is also well known that GSH level is a good indicator of oxidative stress (Messaoudi et al., 2009). Cadmium may also react with active SH group of GSH. Therefore, GSH may also

be consumed in the scavenging of free radicals generated by Cd. Finally, the glutathione reduced (GSH)/oxidized (GSSG) ratio may be decreased resulting in great changes in the redox state (Jihen et al., 2010).

Decreasing of the GSH level and GSH/GSSG ratio could decrease the GPx activity and increase the lipid peroxidation measured as malonaldehyde (MDA) (Messaoudi et al., 2009). This observation is in harmony with present results. Vitamins C, E and epicatechin are good acceptors of free radical produced by high rate of lipid peroxidation and oxidation stress (Karabulut-Bulan et al., 2008). Therefore, administration of these antioxidants decreased GPx activity, as shown in our results.

Conclusions

Cadmium provoked oxidative stress by increasing the levels of free radicals and by decreasing antioxidants level. This oxidative stress could be the primary cause of Cd induced nephrotoxicity following by renal failure. Antioxidants ameliorated the elevated blood levels of kidney biomarkers due to their protective role against cadmium-induced lipid peroxidation and restored the lowered values of glutathione peroxidase activity in blood.

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