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Effect of combinations of four trace elements on cadmium bioaccumulation in a few tissues of male albino rats

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Abstract: The present study is designed to investigate the accumulation of cadmium (Cd) in selected tissues such as liver, kidney, testis and small intestine of male albino rats treated for Cd and after combined supplementation with Zinc (Zn) + Iron (Fe) and Selenium (Se) + Copper (Cu). The wistar albino rats were treated with cadmium chloride($CdCl_2$)at a dose of $1/10^{th}$ LD_{50} i.e. 22.5 mg / kg body weight for 7, 15 and 30 days(d) time intervals. After 15 d to Cd treatment, the rats were then supplemented with the above combination of two trace elements and then observed for accumulation of Cd at specific time intervals. These trace elements at a dosage of 1 mg/ kg body weight of Se, 16mg/kg body weight of Cu, 12 mg / kg body weight of Zn and 40 mg / kg body weight of Fe were given as supplements. There was significant Cd accumulation in liver and kidney among the selected tissues before to supplementation and there was significant decrease in the Cd accumulation levels in all the tissues after trace element supplementation. Moreover the 30d Zn + Fe supplemented rat kidney showed maximum decrease in Cd accumulation (8.327µg/g wet wt. of the tissue).

Keywords: Trace element supplements, Cadmium bioaccumulation, Rats

INTRODUCTION

As the civilization advances, growing environmental pollution brings the effect of various xenobiotics, including heavy metals, on the functioning of the living organisms (Moniuszko, 1999). Cadmium (Cd) is an environmental pollutant that has serious toxicity in humans and animals and causes Itai-Itai disease (Nad *et al.*, 2005) and also induces the onset of anemia, decreases red blood cell count and hemoglobin concentration (Ognjanovic *et al.*, 2003). Metals can not be destroyed metabolically. However, toxic metals may bind to organic molecules or compete with physiological metals, which may result in a metal-specific toxic effect. For example the Cd effect on Metallothionein (MT). Cd is an industrial and environmental pollutant arising primarily from battery, electroplating, pigment, plastics, fertilizer industries and cigarette smoking. The accumulation of Cd is consistently increased when a certain amount is ingested continuously (Shibutani *et al.*, 2001). When Cd enters the body, it reaches the liver within the first 6h and binds to metallothionein, which is a protein with a low molecular weight (6000-10,000 Da) and rich in cystein (Haki Kara *et al.*, 2005 and Chan and Cherian, 1993). The Cd-MT complex generated in liver was reported to mainly to be distributed to the kidney and other tissues and hence it causes damage in the tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis (Ognjanovic *et al.*, 2008; Haki Kara *et al.*, 2005; Sirja Basha and Usha Rani, 2003; Usha Rani, 2000 and Liu *et al.*, 1996). Cd has an extremely long half-life (20-30 Years) in the human body (Flora *et al.*, 2008) and is highly cumulative, especially in the liver and kidney (Tim *et al.*, 2008; Nordberg *et al.*, 2007; Mahtap and Ethem, 2006 and Hijova and Nistiar, 2005). The kidney is considered as the critical organ in long term low level exposure to Cd (Trzcinka *et al.*, 2004). Chronic exposure to Cd caused kidney damage and renal tubular dysfunction (Horiguchi *et al.*, 2005). It is shown that Cd is an inhibitor of enzymes with sulfhydral (-SH) groups and disrupts the pathways for the oxidative metabolism. Because of its carcinogenic properties, Cd has been classified as a#1 category human carcinogen by the International Agency for Research on Cancer of USA (IARC, 1993).

The complex inter-relationships between Cd and some essential trace elements have not been elucidated. Several essential trace elements like Selenium (Se), Copper (Cu), Zinc (Zn) and Iron (Fe) participate in controlling various metabolic and signaling pathways. In vitro studies suggests there is competition for transport mechanism between Cd and some essential trace elements like Zn and Cu (Hakan *et al.*, 2001), Se (Hijova and Nistiar, 2005) and Fe (Rodriguez- Matas *et al.*, 1998) in rats and Zn and Se in Japanese quails(Nad *et al.*, 2005) and calcium in suckling rats (Saric *et al.*, 2002). Se is involved in the metabolism of glutathione (GSH) which is related to reduce

Table 1. Cd accumulation (µg / g wet weight of the tissue) levels in the tissues of rats before and after supplementation.

Tissues	Control	Concentrations								
		Cd			Se and Cu			Zn and Fe		
		7	15	30	7	15	30	7	15	30
Liver	0.015 ±0.002	8.649 ±0.272	16.019 ±0.085	28.487 ±0.125	14.306 ±0.411	13.298 ±0.317	11.567 ±0.473	13.378 ±0.454	13.378 ±0.454	13.378 ±0.454
Kidney	0.023 ±0.0029	11.7785 ±0.094	19.326 ±0.062	31.578 ±0.499	17.298 ±0.398	16.152 ±0.212	14.345 ±0.502	15.319 ±0.362	15.319 ±0.362	15.319 ±0.362
Testis	0.006 ±0.0018	4.966 ±0.248	10.798 ±0.601	17.328 ±0.459	7.820 ±0.254	6.845 ±0.183	4.585 ±0.429	8.018 ±0.184	8.018 ±0.184	8.018 ±0.184
Small Intes -tine	0.012 ±0.0021	2.593 ±0.220	6.764 ±0.392	9.673 ±0.508	5.027 ±0.138	3.740 ±0.268	2.807 ±0.186	4.953 ±0.051	4.953 ±0.051	4.953 ±0.051

All values are expressed as Mean ± SD of 6 individual samples; Mean difference is significant at 0.05 levels.

toxicity of chemical carcinogen and several heavy metals. The ability of Cu to alternate between its oxidized (Cu II) and reduced (Cu I) forms is critical for its functions as a redox co-factor and this same property has the propensity to generate damaging intracellular free radicals. Consequently the organism must also tightly regulate Cu acquisition, utilization, detoxification and storage. Zn occurs in all living cells as a constituent of metallo enzymes involved in major metabolic pathways. Zn controls several enzymes of intermediatary metabolism, DNA and RNA synthesis, gene expression, immunocompetence and plays a significant role in homeostasis of hormones (Brandao *et al.*, 1995). Fe plays an essential role in biological processes. It is also a vital component of other enzymes and proteins. Fe supplementation reduces cadmium retention and cadmium-induced anemia during fast growth in young rats (Schumann, 1996).

However, there is not much available information on the combined effect of trace element sequences as supplements on Cd accumulation. So, we made an attempt to investigate the effect of four trace elements Se, Cu, Zn and Fe in combination sequence of Se + Cu and Zn + Fe and their possible effect on accumulation of Cd in select tissues such as liver, kidney, testis and small intestine of male albino rats.

MATERIALS AND METHODS

Cd as cadmium chloride ($CdCl_2$), Se as Sodium selenite, Cu as Copper sulphate, Zn as Zinc chloride and Fe as ferric chloride were purchased from Merck (Dormstadt, Germany). The chemicals used were of the highest purity. Three months old Wistar strain male albino rats weighing 180±20g were chosen for the present study. The animals were obtained from Sri Venkateswara Traders, Bangalore, Karnataka, India and were kept in stainless-steel mesh cages, housed under standard laboratory conditions (23 ± 2^0C, 50±20% relative humidity, 12h light- dark cycle) with rat diet and drinking water *ad libitum*. The rats were acclimatized to the laboratory conditions for 10 days. After acclimatization the animals were treated with Cd as $CdCl_2$ at the dose of $1/10^{th}$ LD_{50} i.e. 22.5 mg /kg body weight for a period of 7, 15 and 30d time intervals. Then the 15d Cd treated rats were divided into two groups. Group I was supplemented with the combination of Se (1mg/kg body weight) and Cu (16 mg/kg body weight) and group II received the combination of Zn (12 mg/kg body weight) and Fe(40 mg/ kg body weight) as supplements for a period of 7d, 15d and 30d duration. After specific time intervals, the rats were decapitated and tissues like liver, kidney, testis and small intestine were isolated. Then the tissues were washed with saline and 50mg of each tissue was digested in acid mixture of Nitric acid : Perchloric acid (3:2) for overnight. The acid mixture was then subjected to evaporation and the residue obtained was dissolved in 5ml of double distilled water. From this, 1ml was withdrawn and analysed for Cd concentrations by using Atomic Absorption Spectrophotometer (Schimadzu AA, 6300).

Data analysis: Statistical analysis was performed with SPSS software. The statistical analysis of data was performed by one way ANOVA test and P value less than 0.05 was carried to be statistically significant.

RESULTS AND DISCUSSION

The mean Cd levels were found to be significantly increased in all the tissues of Cd treated rats when compared with control rats (Table 1). Kidney, liver, lung, testes, small intestine and heart are the target organs following Cd exposure, with the severity of their intoxication depending on the route, dose, and duration of the exposure to the metal (Ognjanovic, 2008). The accumulation of Cd in the tissues were as follows: kidney > liver > testis > small intestine. Several authors reported that the organ of higher accumulation of Cd is kidney and also it is a detoxifying organ (Nad *et al.*, 2005; Linde *et al.*, 2004 and Massanyi *et al.*, 2003). In our study also revealed high accumulation of Cd was observed in 30d Cd treated rat kidney (31.578±0.499 µg/g) and low level of Cd concentration was observed in small intestine

(9.673±0.508 µg/g).

The principle role of Se is associated with the control of lipid peroxidation, because this trace element is a component of Selenoenzymes contributing to the antioxidant system. Thus, a number of studies have been carried out to determine the protective effects of Se in different biological models of injury (Agay *et al*., 2005, Combs and Gray, 1998).This protection includes the capability of Se to alter the distribution of Cd in tissues and to induce binding of the Cd-Se complexes to proteins, which are similar to metallothioneins. Shuenn-Jiun Yiin (1999) reported that Se is decreased in 'Cd alone' than Cd + Se group, the reason might be that Cd will decrease the bioavailability of Se. May be due to bioavailability of Se as supplement in our study the accumulation of Cd is reduced in the select tissues with major decrement in 30d Se +Cu supplemented rats. Further this suggests Cd accumulation is reduced by Se supplementation along with Cu in the tissues of rats. This might be the reason for decreased Cd levels in 30d rat kidney (11.567±0.473 µg/g). Many of the Cd induced changes in tissue Cu and Zn distribution appear to result from increased incorporation of other metals in MT. Cd, Se and Cu can all apparently induce synthesis of MT, displacement of one metal by another and competition for binding sites on the protein may also occur. Thus toxicity of Cd may result from disturbances in Zn metabolism, leading to the description of Cd as an antimetabolite of Zn. The most compelling reason for the protective effects of Zn against Cd toxicity is that Zn induces the protection of the metal binding protein metallothionein (MT) (Salem *et al*., 2008, Bonda *et al*., 2004). Hence, this may be the cause for lowering the concentration of Cd in the select tissues primarily in 30d kidney (8.327±0.450 µg/g). It has also been suggested that higher gastrointestinal absorption of Cd is due to lower body Fe stores as measured by the concentrations of serum ferritins (Vahter *et al*., 1996). Petring *et al*., (1977) and Casalino *et al*., (1997) reported that Fe supplementation corrects the anemia caused by Cd exposure in rats.

Therefore it is proposed that combination of trace elements in the sequence of Se + Cu and Zn + Fe are effective in reducing the Cd accumulation and thereby providing some therapeutic measures in Cd induced toxicity in rats. Among the combinations of four trace elements used as supplements, it is the Zn and Fe combination which is more effective to Se + Cu in bringing down the Cd levels of the rat tissues in the long sojourn to Cd exposures.

REFERENCES

Agay, D., Sandre, C., Ducros, V., Faure, H., Cruz, C., Alonso, A., Roussel, A.M. and Chancerelle, Y.(2005). Optimization of selenium status by a single intraperitoneal injection of Se in Se deficient rat: Possible application to burned patient treatment, *Free Radic Biol Med*, 39: 762-768.

Bonda, E., Wlostowki, T. and Krasowska, T. (2004). Testicular toxicity induced by dietary cadmium is associated with decreased testicular zinc and increased hepatic and renal metallothionein and zinc in the ban vole (Clethrionomys glareolus), *Biometals*, 17: 615-624.

Casalino, E., Sesare Sblano and Clemente Landriscina. (1997). Enzyme activity alteration by cadmium administration to rats: The possibility of iron involvement in lipid peroxidation. Archives of Biochemistry and Biophysics, 346(2):171-179.

Chan, H.M. and Cherian, M.G.(1993). Mobiliziation of hepatic cadmium in pregnant rats, *Toxicol. Appl. Pharmacol*., 120: 308-314.

Combs, G. and Gray, W.P.(1998). Chemopreventive agents: selenium, *Pharmacol Ther*, 79: 179-192.

Flora, S.J.S., Megha Mittal and Ashish Mehta (2008). Heavy metal induced oxidative stress and its possible reversal by chelation therapy, *Indian J Med Res*, 128: 501-523.

Hakan Aydin, H., Canan Coker and Biltan Ersoz (2001). In vivo interaction between cadmium and essential trace elements copper and zinc in rats, *Turk J Med Sci*, 31: 127-129.

Haki Kara, Fikret Karatas, Halit Canatan (2005). Effect of Single Dose Cadmium Chloride Administration on Oxidative Stress in Male and Female Rats, *Turk J Vet Anim Sci*, 29: 37-42.

Hijova, E. and Nistiar, F.(2005). Plasma antioxidant changes after acute cadmium intoxication in rats, *Acta Vet Brno*, 74: 565-568.

Horiguchi, H., Oguma, E., Sasaki, S., Mimyamoto, K., Akeda, Y., Machida, M. and Kayama, F.(2005). Environmental exposure to Cadmium at a level insufficient to induce renal tubular dysfunction does not affect Bone density among female Japanese farmers, *Environ. Res*., 97: 83-92.

IARC, International Agency for Research on Cancer, Beryllium, cadmium, mercury and exposures in the glass manufacturing industry, (1993). In: International agency for research risks to humans, Lyon: IARC Scientific Publications, 58: 119-237.

Linde, A.R., Sanchez-Galan, S. and Garcia-Vazquez, E. (2004). Heavy metal contamination of European eel *(Anguilla anguilla)* and brown trout *(Salmo trutta)* caught in wild ecosystems in Spain, *J. Food Prot.*, 67: 2332-2336.

Massanyi, P., Tataruch, F., Slamecka, J., Toman, R. and Jurcik, R.(2003). Accumulation of lead, cadmium, and mercury in liver and kidney of the *brown hare (Lepus europaeus)* in relation to the season, age, and sex in the West Slovakian Lowland, *J.Environ. Sci. Health*, A39: 1299-1309.

Mahtap Kocak and Ethem Akcil (2006). The effects of chronic cadmium toxicity on the hemostatic system, *Pathophysiol Haemost Thromb*, 35: 411-416.

Moniusko-Jakoniuk, J. and Brzoska, M.M. (1999). Environment pollution and Health, *lek Wojsk*, 75(7-8): 419-426.

Nad, P., Massanyi, P., Skalicka, M., Korenekova, B. and Cigankova, V. (2005). The effect of cadmium in combination with Zinc and selenium on ovarian structure in Japanese quails, *Rizilove factory Potravoveho Refazca*, V: 241-247.

Nordberg, G.F., Bigawam, K., Nordberg, M. and Friedmann, J.M. (2007). Cadmium. In: Hand book on the toxicology of Metals, (Nordberg GF, Fowler BA, Nordberg M, Friberg L, eds). Amsterdam: Elsevier, 445-486.

Ognjanovic, B.I., Markovic, S.D., Pavlovic, S.Z., Zikic, R.V.,

Stajn, A.S. and Saicic, Z.S. (2008). Effect of Chronic Cadmium exposure on Antioxidant defense system in some tissues of rats: Protective effect of Selenium, *Physiol. Res*., 57: 403-411.

Ognjanovic, B.I., Pavlovic,S.Z., Maletic,S.D., Zikic, R.V., Stajn, A.S., Radojicic R.M., Saicic, Z.S. and Petrovic,V.M.(2003). Protective influence of Vitamin E on Antioxidant Defence system in the blood of rats treated with Cadmium, *Physiol. Res*., 52: 563-570.

Petering, H.G., Murthy, L. and Cerklewski, F.L. (1977). Role of nutrition in heavy metal toxicity. In: Biochemical Effects on Environmental Pollutants (Lee SD ed). Ann Arbor, MI: Science Publishers.

Rodriguez-Matas, M.C., Lisboma, F., Gomez-Ayala, A.E., Lopez-Aliaga, I. and Campos, M.S. (1998). Influence of nutritional iron deficiency development on some aspects of iron, copper and zinc metabolism, *Laboratory Animals Ltd. Laboratory Animals*, 32: 298-306.

Salem Amara, Hafedh Abdelmelek, Catherine Garrel, Pascale Guirud, Thierry Douki, Jean-Luc Ravant, Alian Favier, Mohsen Sakly and Khemais Ben Rhouma (2008). Preventive effect of Zinc Against Cadmium-induced oxidative stress in the rat testis, *Journal of Reproduction and Development*, 54(2): 129-134.

Saric, M.M., Blanusa, M., Piasek, M., Varnai, V.M., Juresa, D. and Kostial, K. (2002). Effect of dietary cadmium on cadmium absorption and retention in suckling rats, *Biometals*, 15(2): 175-182.

Schumann, K., Friebel, P., Schmolke, G., Elsenhans, B.(1996). State of iron repletion and cadmium exposure. *Arch. Environ. Contam. Toxicol.*, 31(4): 483-487.

Shuenn-Jiun Yiin, Chi-Liang Chern, Jenn-Yuan Sheu and Te-Hsien Lin. (1999). Cadmium induced lipid peroxidation in rat testes and protection by Selenium, *BioMetals*, 12, 353–359.

Shibutani, Makoto, Kunitoshi Mitsumori, Shin-ichi Satoh, Hideaki Hiratsuka, Masahiko Satoh, Masami Sumiyoshi, MotohiroNishijima, Yasutaka Katsuki, Jin Sujuki, Jun-ichi Nkagawa, Takumi Akagi, Takayoshi Imazawa and Masanori Ando (2001). Relationship between toxicity and cadmium accumulation rats given low amounts of Cadmium chloride or cadmium – polluted rice for 22 months, *The Journal of Toxicological Sciences*, 26(5): 337-358.

Siraj Basha, P. and Usha Rani, A. (2003). Cadmium induced antioxidant defense mechanism in fresh water teleost *Oreochromls mossambicus* (Tilapia), *Ecotoxicol. Environ. Safety*, 56: 218-221.

Tim S. Nawrot, Etienne Van Hecke, Lutgarde Thijs, Tom Richert, Tatiana Kuznestsova, Yu Jin, Jaco Vangronsveld, Harry A Roels and Jan A. Staessen (2008). Cadmium- Related mortality and long-Term Secular Trends in the Cadmium body burden of an Environmentally Exposed Population,*Environmental Health Perspectives*, 116(12): 1620-1628.

Trzcinka-ochocka, M., Zakubowski, M., Razniewska, G., Halatek, T. and Gazewaski, A. (2004). The effects of environmental Cadmium exposure on kidney function: the possible influence of age, *Env. Res*.**,** 95: 143-150.

Usha Rani, A. (2000). Cadmium induced bioaccumulation in the tissues of fresh water teleost *Oreochromis mossambicus.*, *The Annals. N. Y. Acad. Sci.*, 919(1): 318 – 320.

Vahter, M., Berglund, M., Nermell, B. and Akesson, A.(1996). Bioavailability of cadmium from shellfish and mixed diet in women, *Toxicol. Appl. Pharmacol.*, 136: 332-341.