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Estimation of absorbed cadmium in tissues of male and female albino rats through different routes of administration

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Summary: The resultant effects of cadmium exposure are seen in almost all the systems of the body, however, this study is designed to quantify its accumulation in tissues of animals exposed to cadmium. The rats were divided into two distinct groups of males and females, which were then divided into three groups, each for the monitoring of exposure. Group 1 served as control male and female and received normal rat chow and tap water. Group 2 males and females were treated with 5 mg/kg body weight of cadmium chloride (Cd) intraperitoneally for eight days while Group 3 males and females rats received 100 ppm of Cd in drinking water for 18 days. The concentrations of cadmium were analyzed in tissues (lung, stomach, kidney, heart, spleen, blood) by AAS. There were significant ($P<0.05$) increase in Cd (ppm) accumulation in males compared with females lungs (2.253 ± 1.47 vs 0.317 ± 0.001), stomach (0.187 ± 0.094 vs 0.045 ± 0.032) and blood (0.070 ± 0.001 vs 0.001±0.001) when Cd was administered intraperitoneally. Following oral administration, there were significant ($P<0.05$) difference in Cd (ppm) content between males and females (kidney (0.506 ± 0.074 vs 0.748 ± 0.147), stomach (0.045 ± 0.020 vs 0.001±0.001) and blood (1.126 ± 0.001 vs 0.114 ±0.001). Our results suggest that Cd accumulation in the various organs was sex and route of exposure-dependent in rats.

Keywords*:* Cadmium, Heavy metals, Organs, Route of administration, Sex

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INTRODUCTION

Humans are exposed to cadmium (Cd) primarily through the ingestion of contaminated food or water and the inhalation of cigarette smoke (Oberdorster, 1992, ATSDR, 2003). Major sources of dietary Cd are fish, liver, grains, leafy vegetables, potatoes, and other root vegetables. Exposure to Cd on a chronic basis can cause adverse effects in the kidneys, liver, lung, pancreas, testis, placenta, and bone (ATSDR, 2003; Bhattacharyya *et al*, 2000; Jarup *et al*, 1998, Liu *et al*, 2000).

Following oral exposure, Cd is absorbed by the intestines and subsequently delivered to the liver by portal blood. In the liver, Cd is taken up avidly from sinusoidal blood by hepatocytes. Cd is also taken up preferentially by the liver following parenteral exposure (Zalups, 2000, Liu *et al*, 2001, ATSDR, 2003). Gastrointestinal absorption of cadmium is of the order of 2-8% (Friberg *et al*, 1986) and physiological and nutritional factors e.g. GSH or cysteine will play major roles in Cd uptake (Zalups, 2000). Cd is extracted from the blood very rapidly by the liver and other organs and tissues (Zalups, 2000). Of the Cd remaining in the blood, approximately 50% is distributed among the cellular components of blood, with the majority being present in erythrocytes. It has been suggested that the absorption of Cd by erythrocytes may be mediated by an anion exchanger (Dawson and Ballatori, 1995). Within hepatocytes, a significant amount of Cd is bound to metallothionein (MT). Cd is delivered to the kidneys, where it is filtered by the glomeruli and is then reabsorbed by the epithelial cells of the proximal tubule (Dudley *et al*, 1985), some fraction of the Cd that enters into hepatocytes is secreted into the bile, and is subsequently delivered to the duodenum for excretion in the feces (Leslie *et al*, 2001).

Cd may gain entry into cells through cation channel (e.g. Ca^{2+} channels) in isolated cells from

other organs, including liver and intestine (Blazka and Shaikh, 1991a, Friedman and Gesek, 1994), or even through the process of endocytosis (Zalups and Ahmad, 2003). Other means of Cd entrance into cells may be by mimicking of estrogen (estradiol) at the site of the estrogen receptor (Martin et al., 2003; Stoica et al., 2000). As such, Cd can activate the estrogen receptor and change the conformation of the receptor to that created by the binding of estradiol (Martin et al., 2003; Stoica et al., 2000). Diet and nutritional status can also influence the absorption and distribution of this metal (Zalups and Ahmad, 2003).

There has been conflicting reports on the sex differences in the accumulation of cadmium in various organs of the body, for instance, early reports shows that cadmium accumulation / uptake levels in tissues were higher in females than in males (Mirranda *et al*, 2000; Massanyi *et al*, 2003). Whereas Beltrame *et al* (2009) and Rautio *et al* (2010) had suggested that sex had no significant effect on concentrations of cadmium accumulation. Blazka and Shaika, (1991b) and Franklin *et al* (2005) have also reported that Cadmium accumulation and uptake by tissues could be influenced by route of administration or exposure. We had earlier reported (Nwokocha *et al*, 2011) that the tissue accumulation of lead (Pb) is affected by the sex and the routes of exposure. The aim of the study was then to investigate and compare the accumulation of cadmium in the various tissues following exposures through the oral and intraperitoneal routes in male and female rats.

MATERIALS AND METHODS

Animal monitoring and feeding

Healthy Wistar Albino rats of both sexes, weighing between 150 – 200g were randomly picked and grouped male and female matched controls as follows.

Group 1, Males and female (control) fed with normal rat chow and distilled water for 18 days.

Group 2, Males and female administered with cadmium (5mg/Kg b.w) intraperitoneally daily for eight days.

Group 3, Males and female administered with cadmium (100ppm) in drinking water for 18days.

All animal experiments were in conformity with the ethical guidelines of the faculty.

Sample collection and analysis

After the exposure, the animals were sacrificed and the tissues (lung, stomach, kidney, heart, spleen, blood) were (1 g) were removed and placed in polypropylene vials. Tissues were ground and homogenized in 5 ml of normal saline before acid digestion with 60% hydrochloric acid and 10 ml of 70% nitric acid (Merck). The digest was allowed to cool and then filtered through a Whatman's filter paper, leaving a whitish residue. The filtrate was then made up to 50 ml using distilled water and kept for further analysis. The quantity/ concentration of cadmium were analyzed using an Atomic Absorption Spectrophotometer (AAS).

Statistical analysis

The results are expressed as mean ± SEM. The data obtained was analyzed using the students't-test. A p value of 0.05 was considered statistically significant.

RESULTS

Organ distribution of Cadmium following intraperitoneal route of administration:

The measured values for cadmium accumulation were all significantly raised for males and females when compared to the control in animals exposed to cadmium via the ip route; this is as shown in table 1. Cadmium level were also lower for the female values when compared with the male values except for the cadmium values found in the spleen, though this was not significant. The measured cadmium concentrations observed in the stomach, blood and lungs were all significantly ($P<0.05$) higher in males when compared with female values. The measured concentrations in the heart and Kidney though lower for the female rats were not statistically significant.

Organ distribution of cadmium following oral route of administration:

The measured values for cadmium accumulation were all significantly raised for males and females when compared to the control in animals exposed to cadmium via the ip route; this is as shown in table 2. Cadmium level were also lower for the female values when compared with the male values except for the cadmium values found in the kidney, this value was significant ($P<0.05$). The measured cadmium concentrations observed in the stomach and blood were all significantly ($P<0.05$) higher in males when compared with female values. The measured concentrations in the heart though lower for the female rats were not statistically significant.

A comparison of the cadmium accumulation in the tissues/ organ for both groups exposed through the oral and i.p routes showed a significant ($P<0.05$) low level accumulation through the oral routes for both the males and female groups for all tissues. Cadmium concentration in the Lungs, blood and stomach were

Table 1:
Cadmium accumulation in organs following interperitoneal route of administration

Cadmium (ppm)	Heart	Lungs	Kidney	Spleen	Blood	Stomach
Control	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001± 0.001	0.001 ± 0.001	0.001 ± 0.001
Male (ip)	0.313 ± 0.215	2.253 ± 1.470	12.690± 0.146	0.788± 0.032	0.070 ± 0.001	0.187 ± 0.094
Female (ip)	0.242 ± 0.132	0.317 ± 0.001*	10.940± 4.670	0.843 ± 0.108	<0.001±0.001*	0.045± 0.032*

Data are presented as means ± S.E. of tissue Cadmium composition in ppm * P< 0.05, n = 6

Table 2:
Cadmium accumulation in organs following oral route of administration

Cadmium ppm	Heart	Lungs	Kidney	Spleen	Blood	Stomach
Control	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.00 ± 0.001	0.001 ± 0.001	0.001 ± 0.001
Male (oral)	0.124 ± 0.192	0.001 ± 0.001	0.506 ± 0.074	0.001 ± 0.001	1.126 ± 0.001	0.045 ± 0.020
Female (oral)	0.075 ± 0.037	0.001 ± 0.001	0.748 ± 0.147*	0.001 ± 0.001	0.114 ±0.001 *	<0.001±0.001*

Data are presented as means ± S.E. of tissue cadmium composition in ppm * P< 0.05, n = 6

significantly (P<0.05) higher than the values observed in the female tissues exposed through the ip route, but for the values observed in the groups exposed through the oral route, the heart, blood and stomach were significantly elevated in the male tissues, we observed that only the tissues of the stomach and blood showed significant consistent elevation when tissues of both the males and females were compared.

On the other hand, values of cadmium concentration were higher in female tissues of the spleen (through i.p routes) and the kidney (for oral route) (P<0.05). The order of cadmium accumulation in different tissues of Wistar rats following cadmium chloride treatment through i.p. was kidney > lungs > spleen > heart > stomach > blood for the male rats, while for the female rats it was in the order of kidney > spleen > lungs > heart > stomach > blood. With oral administration the order for males was Blood> Kidney> heart> stomach>spleen and lung tissue, in the female rats the order were kidney> blood> heart> stomach, lungs and spleen.

DISCUSSION

In this study, we sought to find the effects of sex and routes of administration on cadmium distribution in some selected organs using rat as the experimental model, the tissues used were heart, lungs, kidney, spleen, blood and stomach of Wistar rats exposed using two routes of administration: oral and intraperitoneal injections. Different routes of administration have been used, since they imply different absorption and tissue distribution of the metal. Thus, by oral administration cadmium goes on to the gastrointestinal tract, from which it is distributed, and it is mainly eliminated by faeces, this may also contribute to its low accumulation / uptake. In the case of intraperitoneal administration, the metal goes on initially to the peritoneal cavity and later to the blood. Our results show that accumulation / uptake of cadmium was different among the sexes and with different routes of administration. The order of cadmium accumulation in different tissues of Wistar rats following cadmium chloride treatment through i.p. was kidney > lungs > spleen > heart > stomach > blood for the male rats, while for the female rats it was in the order of kidney > spleen > lungs > heart > stomach > blood. The distribution of this metal from blood might suggest its heavier accumulation but it was not so in our results as it accumulated least in blood among all tissues studied. With oral administration the order for males was Blood> Kidney> heart> stomach>spleen and lung tissue, in the female rats the order were kidney> blood> heart> stomach, lungs and spleen. The higher accumulation of cadmium in kidney may be attributed to the higher metabolic activity of these organs and their role in detoxification of xenobiotics, while its low accumulation / uptake could be due to the availability or non availability of cadmium binding proteins (CdBPs) which participates in the accumulation and distribution of cadmium (Sato & Takizawa, 1982). Blood and tissue pharmacokinetics can also play major roles in the distribution of this metal because of their unusually sensitive to fat and other tissue storage (Andersen *et al*., 2001).

These differences between the male and female rats were significant in the lungs, blood and stomach tissues when the exposure was through intraperitoneal routes, and also significant in the kidney, blood and stomach tissues when exposure was through the oral routes. Early workers had reported that cadmium accumulation / uptake levels in tissues were higher in females than in males (Mirranda et al; 2000; Zalups 2000; Oishi et al, 2000; Massanyi et al, 2003) irrespective of routes of

administration, while Beltrame et al; (2009) and Rautio et al; (2010) had reported no sex difference in the accumulation of cadmium in various organs/ tissues. Our results rather showed that uptake and accumulation of this metal were more in the males when compared with the female rats except for spleen (through i.p routes) and the kidney (for oral route), this still gives evidence that accumulation / uptake of this metal exhibits some sex difference. Our results were similar to those of Shimada *et al* (1997) and Lanszik et al (2009) who had also reported that lead and cadmium concentrations were sex dependent but more in males when compared with females in samples of Eurasian otters. We also found that the tissue uptake of this metal showed some slight differences when values were compared between the males and female rats with highest accumulation in the kidney for both but lungs in the next order for males while spleen in the next order for females following i.p. administration. Oral administration showed similar order of accumulation / uptake of this metal with the only alteration being that we observed more accumulation in blood for males while in females it was the kidney that had the highest accumulation / uptake.

Blazka & Shaika, (1991) and Hoffer et al (2009) had earlier reported that cadmium accumulation and metallothionein induction were noticable after s.c. but not i.v. administration, Kasprzak & Poirier (1985) also reported that the route of administration might play major roles in its accumulation / tissue uptake. We observed that concentration of cadmium was higher in the animals exposed through the i.p. route when compared with the oral route, this could be explained by the doses administered, and also of the fact that metabolism may reduce or eliminate some through feces. Other factors that may contribute to the lower values observed in the tissues from the oral exposure group could be due to the fact that the gut wall forms an important protective barrier reducing Cd accumulation into internal tissues (Franklin et al 2005), this may also contribute to its low accumulation / uptake but the order of accumulation and uptake were different when values between the oral and i.p. routes administrations were compared with values higher in blood, kidney and the heart tissues but negligible in the stomach, spleen and lung tissues in both sexes. However, the pharmacokinetics of heavy metals, following oral and i.p. exposure, could be sensitive to the mode of entry into the blood compartment. As heavy metal (cadmium) delivered by oral or i.p. routes appears to enter the blood compartment in a form different from that for the inhalation and dermal routes, which are diffusion-controlled processes. The present results suggest that the there is more uptake of cadmium ion in some tissues of males than in females. Route of administration plays a great role in the levels of uptake or accumulation by various tissues / organs and also the bioavailability of this metal since tissue accumulation was greater in intraperitoneal than oral route of administration, this is consistent with the works of (Jarup *et al* 1998). This is very important as many of the toxicological manifestations of cadmium poisoning are related, in part, to its pattern of tissue distribution. The different pathways of deposition after oral vs. i.v. exposure may in part explain why acute parenteral cadmium exposure causes liver toxicity, but chronic oral exposure causes renal toxicity.

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