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A comparative evaluation of the biological effects of environmental cadmium-contaminated control diet and laboratory-cadmium supplemented test diet

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Abstract

This study was designed to examine the effects of a diet contaminated by environmental cadmium on organ/body weight ratio and selected toxicological indices. It was also designed to permit a comparative analysis of the effects of the diet contaminated by environmental cadmium and that of cadmium supplemented diet based on the same parameters. Our results show that even though the cadmium content of the environmental cadmium contaminated diet was 300% less than that of the cadmium supplemented diet, the former caused statistically significant changes in Superoxide dismutase (SOD) activity ($P < 0.025$), Na^+/K^+ ATPase activity ($P < 0.005$) and Malondialdehyde (MDA) level ($P < 0.025$) when values at the end of 1 month exposure were compared to the values at the end of 3 months exposure. These parameters were altered in the same manner by the cadmium content of the supplemented diet in addition to significant reduction in liver/body weight ratio($P < 0.005$) within the exposure periods examined. By virtue of the very close nature of the values of these parameters in rats exposed to the two different diets, it appears that the background cadmium (cadmium from the environment) content of the diets is largely responsible for the observed changes, except in the case of liver/body weight ratio.

Introduction

Due to atmospheric, aquatic, and terrestrial cadmium, agricultural products and water consumed by man and animals get contaminated with cadmium (Wolnik *et al.* 1985; Lopez *et al.* 1994). Phosphate fertilizers are reportedly another source of cadmium in arable land (Jackson & Alloway 1991, McLaughlin *et al.* 1996).

Cadmium has been implicated in the development of 'itai–itai' disease (Kasuya *et al.* 1992; Yasuda *et al.* 1995; Ishihara *et al.* 2001). In order to provide a clear understanding of the biochemical changes that precipitates the disease and other associated effects controlled laboratory experiments have been done (Horiguchi *et al.* 1994, 1996). In such experiments, the control animals are supposedly maintained on cadmium-free water and diet, while the test animals are maintained on cadmium supplemented water and/or diet. However, in view of the possible presence of cadmium in agricultural products and water with which these diets are compounded, the question remains as to whether the control and the test animals are ever at zero cadmium load and zero cadmium-induced biological effects before the commencement of an experiment involving cadmium.

The discovery of cadmium in the control diet in a study designed to examine the role of food chain in cadmium-induced tissue toxicity provided us the opportunity to address the above question. The purpose of this study was therefore to ascertain whether any biochemical change can take place in rats exposed to control diet found to be Cd

contaminated and to compare such changes (if any) with those caused in rats by test diet deliberately supplemented with cadmium-tainted catfish as source of protein. We focused attention on liver weight and selected biochemical parameters which are known indicators of Cd toxicity.

Materials and methods

Twenty adult male albino rats (Wistar strain) mean weight 186 ± 2 g were used for this study. Two diets (control and test diet) that differed in terms of the nature of the protein were formulated. The test diet contained milled cadmium exposed catfish as the source of protein. The control diet contained milled non-exposed catfish as protein source. Other components of the diets were cornstarch (Livestock Feed, Plc, Lagos), ABC multivitamin/minerals mix (Vitamin World, New York, USA), palm oil and peanut husk (obtained locally in Benin City, Nigeria).The following chemicals/reagents were used: Magnesium chloride hexahydrate, ATP (disodium salt), 2-thiobabituric acid, hydrogen peroxide (BDH, Poole, England), epinephrine, $3CdSO_4{\cdot}8H20$ (May and Baker, Dagenham, England).

The rats were divided into four experimental groups of five rats each and housed singly in metabolic cages. Rats in cages I and II were maintained for 1 month on the control and test diet respectively. Those in cages III and IV were maintained for 3 months on the control and test diet respectively. They were allowed free access to water but the appropriate control diet and test diet groups were weight matched and pair-fed. All these animal treatments were carried out in accordance with the principles of laboratory animal care (NIH publication no. 85–93, revised 1985). At the end of the specific periods of exposure all rats were weighed and anaesthesized (in chloroform saturated chamber) and while under anaesthesia the kidney and liver samples were quickly excised placed on ice and subsequently weighed. Portions of the liver and kidney were homogenized to give a 20% homogenate and centrifuged at $10{,}000 \times g$ for 15 min as described by Aksnes & Njaa (1981) to obtain clear supernatants as the source of superoxide dismutase (SOD) and catalase (CAT), respectively.

SOD activity was assayed by the method of Misra & Fridovich (1972) and the activity computed and expressed as described by Baum & Scandalios (1981) in which one unit represents the amount of the enzyme required for 50% inhibition of epinephrine during 1 min. CAT activity was assayed by using the method of Cohen *et al.* (1970). Each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as $K\ min^{-1}$. The homogenate for ATPase assay was prepared as described by Adam-Vizi & Seregi (1982) and the method described in the same report was used for Na^+/K^+ATPase assay (Adam-Vizi & Seregi 1982).The Na^+K^+ATPase activity was taken as the difference between total ATPase and Mg^{2+}ATPase activities. The specific activity of the ATPase is expressed in standard units of micromoles inorganic phosphate released per minute per milligram protein. The inorganic phosphate released was assayed by the method of Annino & Giese (1976), while the protein content of the samples were estimated by the method of Lowry *et al.* (1951). The amount of thiobarbituric acid reactive substances (TBARS) which are indicators of lipid peroxidation was assayed by the method of Gutteridge & Wilkins (1982). Values for TBARS are quantitated using a molar extinction coefficient of 1.56×10^5 M/cm and expressed in terms of malondialdehyde (MDA) units per gram tissue. Each unit represents one micromole of MDA.

Digestion of samples. one gram of liver or feed was put into a beaker containing 20 ml of acid mixture ($HNO_3/HCLO_4$; 4:1 v/v) followed by heating at 100 °C to facilitate digestion. The digests were allowed to cool and thereafter diluted with deionised water to give a final volume of 100 ml.

Cadmium analysis. The cadmium concentrations in the liver and feed were measured using a Varian AA 1475 atomic absorption Spectrophotometer with deuterium background subtraction and a detection limit of 10 μg/l. Impact bead was utilized to improve the sensitivity. The test metal was dissolved in deionised water and used as standards. Calibrations were performed with the standards in the range of 0.5–2.0 mg Cd/l and a correlation coefficient for the calibration curve of 0.98 was obtained. An International Atomic Energy Agency (IAEA) reference biological sample V-10 (Hays) with a certified value of 0.029 ±

Table 1. Composition of control and test Diets.[a]

Ingredients	Control diet[a] (%)	Test diet[a] (%)
Milled Cd-exposed catfish	0.00	22.00
Milled 'Cd-free' catfish	22.00	0.00
Corn starch	53.00	53.00
Sugar	05.00	05.00
Palm oil	07.00	07.00
Dried peanut husk	08.00	08.00
ABC multivitamin/minerals	05.00	05.00
Total	100.00	100.00

[a]AAS analysis revealed that the Cd contents of the control and test diets were 0.10 and 0.40 mg/kg feed respectively.

0.004 mg Cd/Kg dry weight was used for the evaluation of the accuracy and precision of the analysis. The cadmium concentration obtained for the reference sample was in agreement with the certified value. In all the determinations, blanks were prepared to determine the effect of reagents purity on the metal levels.

The data are presented as means ± SD. The mean values of the control and test groups were compared using student's *t*-test, one tail analysis (Elzey 1971).

Results and discussion

The composition of the control and test diets and their respective cadmium content are presented in Table 1. At the end of the exposure periods, the groups that were maintained on the control diet were considered together and the same was done for the groups kept on the test diet. The Cd load, organ/body weight ratio, SOD, CAT, Na^+/K^+ ATPase activities and MDA level of the liver of rats at the end of 1 and 3 months exposure to the control diet are presented in Table 2. Similar results for rats exposed to the Cd supplemented diet are presented in Table 3. The data at the end of 3 months exposure to both diets are presented in Table 4.

Numerous controlled studies on the biochemical effects of cadmium using different models have been conducted (Gupta *et al.* 1991; Lind *et al.* 1995; Liu *et al.* 1995; Gur *et al.*1995; Horiguchi *et al.* 1996; Sidhu *et al.*1997). In this study, control rats maintained on the control diet were part of the experimental design. Ideally, the control rats and control diet should be Cd-free but this criterion was not satisfied in our study since the control diet contained 0.10 Cd/kg (Table 1) while the liver, between the end of 1 and 3 months exposure accumulated 4.40×10^{-2} μg Cd from the control diet (Table 2). This criterion was also not satisfied in the studies conducted by others. Cd was detected in control rainbow trout (Olsson *et al.* 1996), female wistar rats (Horiguchi *et al.* 1996) and feed (Lind *et al.* 1995).

The net Cd load accumulated by the liver from the control diet between the end of 1 and 3 months exposure (4.40×10^{-2} μg Cd/g tissue) had no effect on the organ/body weight ratio and CAT activity but caused significant decrease in SOD activity and significant increase in Na^+/K^+ ATPase activity and MDA level (Table 2). When compared to the data in Table 3 due to Cd-supplemented test diet, the liver Cd load between the end of 1 and 3 months exposure, 11.80×10^{-2} μg Cd/g was

Table 2. Status of rat liver Cd load, organ/body weight ratio, SOD, CAT, Na^+K^+ATPase activities and MDA level at the end of 1 and 3 months exposure to control diet.

Parameter	1 Month	3 Months			
	Mean ± SD (n)	Mean ± SD (n)	*P*-value	Difference + % difference[a]	
Cd load (μg/g tissue) $\times 10^{-2}$	3.00 ± 0.50 (3)	7.40 ± 1.10 (3)	< 0.005	+4.40	+146.7
Organ/body weight ratio (w/w %)	3.40 ± 0.48 (5)	3.30 ± 0.21 (5)	NS	–	–
SOD (Unit/g tissue)	33.19 ± 13.61 (5)	15.81 ± 2.24 (5)	< 0.025	−17.38	−52.4
CAT (K/min) $\times 10^{-1}$	0.19 ± 0.04 (5)	0.20 ± 0.02 (5)	NS	–	–
Na^+/K^+ATPase (μmole pi/min/mgprotein)	1.30 ± 0.40 (5)	2.08 ± 0.06 (5)	< 0.005	+0.78	+60.0
MDA (Unit/g tissue) $\times 10^2$	5.37 ± 1.10 (5)	6.97 ± 1.19 (5)	< 0.025	+1.60	+29.8

[a]Relative to 1 month value.

Table 3. Status of rat liver Cd load, organ/body weight ratio, SOD, CAT, Na^+K^+ATPase activities and MDA level at the end of 1 and 3 months exposure to test diet.

Parameter	1 Month	3 Months			
	Mean ± SD (n)	Mean ± SD (n)	*P*-value	Difference +	% difference[a]
Cd load (μg/g tissue) $\times 10^{-2}$	4.20 ± 0.40 (3)	16.00 ± 0.70 (3)	<0.050	+11.80	+ 81.0
Organ/body weight ratio (w/w %)	3.80 ± 0.09 (5)	3.10 ± 0.80 (5)	< 0.005	−0.70	−18.4
SOD (unit/g tissue)	26.14 ± 10.19 (5)	15.20 ± 3.69 (5)	<0.05	−10.94	−41.9
CAT (K/min) $\times 10^{-1}$	0.21 ± 0.06 (5)	0.21 ± 0.08 (5)	NS	–	–
Na^+/K^+ATPase (μmole pi/min/mg protein) $\times 10^{-1}$	1.50 ± 0.30 (5)	2.30 ± 0.06 (5)	<0.005	+0.80	+53.3
MDA (Unit/g tissue) $\times 10^2$	3.62 ± 0.42 (5)	6.14 ± 1.03 (5)	<0.005	+2.52	+69.6

[a]Relative to 1 month value.

Table 4. Status of rat liver Cd load, organ/body weight ratio, SOD, CAT, Na^+/K^+ATPase activities and MDA level at the end of 3 months exposure to control and test diets.

Parameter	3 Months control diet values	3 Months test diet values			
	Mean ± SD (n)	Mean ± SD (n)	*P*-value	Difference +	% difference[a]
Cd load (μg/g tissue) $\times 10^{-2}$	7.40 ± 1.10 (3)	16.00 ± 0.70 (3)	<0.005	+8.60	+116.2
Organ/body weight ratio (w/w %)	3.30 ± 0.21 (5)	3.10 ± 0.08 (5)	NS	–	–
SOD (Unit/g tissue)	15.81 ± 2.24 (5)	15.20 ± 3.69 (5)	NS	–	–
CAT (K/min) $\times 10^{-1}$	0.20 ± 0.02 (5)	0.21 ± 0.08 (5)	NS	–	–
Na^+/K^+ATPase (μmole pi/min/mg protein)	2.08 ± 0.06 (5)	2.30 ± 0.06 (5)	<0.005	+0.22	+10.6
MDA (unit/g tissue) $\times 10^2$	6.97 ± 1.19 (5)	6.14 ± 1.03 (5)	NS	–	–

[a]Relative to control diet values.

168.2% greater than that accumulated from the control diet. (See Tables 2 and 3). Despite the increase in Cd load though, the only unique effect attributable to the increase, is a significant decrease in organ/body weight ratio. SOD and Na^+/K^+ATPase activities and MDA level were apparently altered by the Cd load occasioned by the test diet exactly in the same manner as the low Cd load occasioned by the control diet (Table 3).

The Cd present in the control and test diets appears to be in dissolvable complexes in view of its bioavailability in vivo. This is evident in Tables 2 and 3 which shows significant increase in liver Cd loads of 146.7 and 281% at the end of 3 months exposure when compared to the Cd load at the end of 1 month exposure. However, bioavailability of Cd from ingested feed does not always occur with ease. For instance, the bioavailability of Cd from crab hepatopancreas is slightly lower than that from mushroom (Lind *et al.* 1995). With particular reference to the control diet and control rat liver, the evidence here indicates that they were not free of Cd. Since the pretreatment cadmium load and status of the various biochemical parameters of the liver were not determined, the Cd load and status of the parameters at the end of 1 month exposure to the control diet were used as reference values. Against this post-one month control diet exposure data, the 3 months post-exposure data were compared (Table 2). In order to find out whether any biochemical alterations were effected by the test-diet-associated increased liver Cd load after 3 months exposure the data generated there from were compared to the corresponding data for 3 months (Table 4). The data in Table 4 show that liver Cd load due to the test diet increased significantly by 116.2% relative to the control diet value but it produced alteration in Na^+/K^+ATPase activity only. Evidently, changes in the parameters at the end of 3 months were predominantly effected by the Cd present in the control diet. And this to a large extent got into the components of diet from the environment. The almost insignificant role of

the test diet Cd is not seen when data are analyzed as in Tables 2 and 3 only but becomes evident when analyzed as in Table 4.

Direct change in organ weight (Wilson *et al.* 1941, Der *et al.* 1976; Horiguchhi *et al.* 1996) or organ/body weight ratio (Ikatsu *et al.* 1998) has been used as an index of toxicity. The latter method was used in this study. Liver/body weight ratio was significantly decreased when the 3 months ratio was compared to the 1 month ratio in rats exposed to the test diet (Table 3). However, our finding is not in agreement with that of Horiguchi *et al.* (1996) who demonstrated significant increase in liver weight after 6 and 9 months exposure to $CdCl_2$ solution subcutaneously. It is important to add though, that Horiguchi *et al.* (1996) considered the weight of liver directly and not in relation to the body weight as we did. Using direct organ weight as toxicity index has at times been questioned since organ/tissue weight is almost invariably influenced by the body weight of the animal.

Lipid peroxidation is known to occur in biological membranes with potential injurious consequences. Cd is known to produce a dose-and time-dependent formation of thiobarbituric acid (TBA) reactants which are indicative of lipid peroxidation (Stacey *et al.* 1980). Among the known TBA reactants is malondialdehyde (MDA). We used this parameter as an index of Cd toxicity and our results show significant increase in MDA after maintaining rats on the control and test diet respectively (Table 2 and 3). Increased lipid peroxidation in rat heart, kidney and liver due to Cd intoxication has been demonstrated by others (Sarkar *et al.* 1995). However, the mechanism by which Cd initiates lipid peroxidation besides the fact that it causes oxidative stress is yet to be completely clear. Closely associated with Cd intoxication is the level of antioxidant enzymes such as SOD and CAT (Gupta *et al.*1991, Sarkar *et al.* 1995). Early effects of cadmium intoxication is the inhibition of antioxidant enzymes such as SOD (Gupta *et al.* 1991) but its activity has also been known to rise in the heart, kidney, and liver within 24 h of Cd intoxication (Sarkar *et al.*1995). However, there is an inverse relationship between tissue SOD activity and the level of TBA reactive substances (Gupta *et al.* 1991). This is reflected in our results. Tables 2 and 3 shows that decreased SOD activity was accompanied by significantly increased MDA level at the termination of the control and test diet treatments. The activity of the other antioxidant enzyme considered in this study, CAT was not altered by either diet. This is in general agreement with earlier report by Sarkar *et al.* (1995) who found increased CAT in the heart but no significant change in kidney and liver tissues following Cd intoxication.

Cadmium has been reported as inhibitory to renal adenosine triphosphatase, ATPase (Nechay & Saunders 1978) and lymphocyte ATPase (Gaworski & Sharma 1978). Inhibition of liver ATPase has been reported by Abe and Itokawa (1973). The inhibition has been attributed to the binding of Cd to the cysteine residues of Na^+/K^+ ATPase, a property it shares with other heavy metals (Zichitella *et al.* 2000). These earlier reports are, however, not in consonance with our present finding which suggests that both diets enhanced the activity of liver Na^+/K^+ ATPase activity between the end of 1 month and the end of 3 months exposure. The reason for the differences in our finding is not certain. All the same it is known that ATPase inhibition by Cd is dose-dependent. For instance, it failed to inhibit ATPase from liver cell membrane when used in the region of 50–400 μM (Stacey *et al.* 1979). The likelihood is that the level of Cd in the diets is stimulatory rather than inhibitory. A situation that can be observed if ATPase has a dual but opposite response to Cd intoxication as is the case of alkaline phosphatase, cytochrome oxidase and glucose-6-phosphate dehydrogenase in response to lead intoxication (Vallee & Ulmer 1972).

We set out to examine the possibility that even without Cd supplementation, environmentally acquired Cd present in the feed can accumulate in the tissues and cause discernable biochemical changes therein. Our results indicate that the presence of Cd in the control diet (theoretically Cd-free) due to environmental contamination at whatever point altered the status of most biochemical indicators of its toxicity. And it does appear that once the alteration has occurred incoming Cd from the supplement may not contribute significantly to further changes in most parameters. This will be particularly so if the Cd from the environment (free or in the diet) has built up in the tissue to the point of initiating and sustaining the changes until a plateau is attained. Once a plateau has been attained, additional Cd from the supplemented diet (in the context of the present study) becomes relatively

inconsequential in terms of Cd-induced biochemical changes. Therefore, in the context of this study the liver from rats maintained on the control diet had neither zero Cd load (absence of detectable cadmium deposit in the tissue) nor zero cadmium-induced biochemical effects (absence of any biochemical effect attributable to cadmium even when it is present in the tissue), at least not at the end of 3 months exposure to the control diet. It is conceivable that a cadmium-free diet control group would have added credence to the findings presented in this study. This though, may have necessitated the provision of a synthetic cadmium-free diet but was omitted in the present study. We intend to incorporate it in our future experimental design.

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