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Influence of Copper, Iron, Lead and Zinc Ions on Cadmium Ion Nephrotoxicity in Wistar Albino Rats

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Abstract

The influence of copper, iron, lead and zinc ions on cadmium-induced nephrotoxicity has been examined in the rat (Wistar strain). Six groups of five (05) rats each were used for the study. Group A, the control rats were not exposed to any of the metal ions. Group B rats were exposed to Cd alone (42.86 ml of 0.229 mg Cd ion /L solution/kg bw);group C to cadmium and copper (42.86 ml of same amount of Cd and 0.189 mg Cu ion/L solution/kg bw); group D to cadmium and iron (42.86 ml of same amount of Cd and 1.90 mg Fe(II) ion/L solution /kg bw), group E to cadmium and lead (42.86 ml of same amount of Cd and 1.08 mg Pb ion/L solution/kg bw) while those in group F were administered cadmium and zinc (42.86 ml of same amount of Cd and 0.505 mg Zn ion /L solution /kg bw). Relative to the control, serum chloride and potassium ion levels (Cl^- = 80.32 ± 11.50 mEq /L ; K^+ = 5.38 ± 1.65 mEq / L) were significantly (p≤0.05) reduced in the cadmium only group (Cl^- = 52.13 ± 2.13 mEq /L ; K^+ = 3.82 ± 0.98 mEq/L) but when it was combined with copper and iron separately, the control serum levels of both analytes were reduced to levels that were not significantly (p> 0.05) different from the control group values. Serum creatinine level was increased significantly (p≤ 0.05) in the cadmium only group (control 0.81± 0.26 mg /dL while that of Cd only was 1.26 ± 0.07 mg /dL), as well as in the groups exposed to its binary with copper, iron and lead respectively, relative to control. However creatinine was reduced to the control level (0.81 ± 0.21 mg /dL control – 1.00 ±0.07mg/dL for the Cd plus Zn group) when cadmium was combined with zinc.Compared to control Cd alone and its binaries with Fe and Pb significantly (p≤ 0.05) decreased kidney malondialdehyde level but was significantly increased by its binary with zinc.Evidently Cd induced hypercreatinineamia was enhanced by Cu, Fe and Pb while the hypochloremia and hypokalemia associated with its toxicity were ameliorated by Cu,Fe,Pb, and Zn.

Keywords: Nephrotoxicity, Copper, Iron, Lead, Zinc Creatinine, Chloride, Potassium, Sodium

Introduction

Cadmium (Cd) is one of the thirty metallic elements of the periodic table described as heavy metals. It is an established toxic heavy metal. Reports abound on its toxic effects in humans (1,2.3), rodents (4,5,6,7,8,9,10) and different species of fish (11,12,13,14,15,16).

Land (17,18 ,19, 20,) and bodies of water (21,22,23,24) are reportedly contaminated by cadmium. Such lands and waters are sources from which plants like tobacco, lettuce, carrot, spinach and many others (20,25) and fishes (21,26,27,28) are exposed to this toxicant resulting in bioaccumulation in plants and intoxicationof fishes (29).

Biochemical investigations have revealed the fact that Cd in humans and rodents can be distributed to the kidneys and liver (30), bone (31), brain (32), and testes (33). It has the capacity to cause tissue injury but the mechanism by which it produces tissues injury appears to be different from that of the other transition metals of the periodic table to which it belongs. For instance it can effect it's toxicity via free radical-induced damage but it is incapable of direct production of free radicals (34) unlike copper and iron. It is currently believed to be capable of reducing tissue levels of reduced glutathione (35) which leaves the tissue and cell membrane unprotected and readily vulnerable to reactive oxygen species (ROS) damage by way of lipoperoxidation. It is also presently believed, based on studies involving MCF-7 cells, that nitric oxide synthase (NOS) plays a crucial role in Cd-induced ROS production (34).

The general effects of Cd toxicity are increased blood L-alanine aminotransferase (L-ALT), L-aspartate aminotransferase (L-AST) and alkaline phosphatase (ALP) activities (8,36), which are indices of liver damage. Other biomolecules that can also be elevated in the blood are urea and creatinine (8) both of which are indices of nephrotoxicity.

In most laboratory studies on cadmium toxicity, this element is usually considered in isolation. The same is true of the other toxic metals. However, in the polluted air, water and soil, these toxic metals, like most other toxicants that pollute ecosystems, do not exist alone. On land (17,37 ,38) and water (39,40,41) they occur along

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with other co-pollutants. In the event that a polluted body of water is consumed as "table water" or the aquatic animals that have bioaccumulated the pollutants are caught and consumed as sources of animal protein, a given toxic heavy metal will not be ingested alone. The co-polluting metallic elements will be ingested simultaneously.

Elements tend to influence the tissue concentration and action of each other in an organism, be it a beneficial or a non-beneficial action. Selenium can replace sulphur and Cd can replace Zn when the concentration of the replaceable entity in the body is overwhelmed by that of the replacing entity (42,43,44). In view of the fact that specific toxic elements like Cd exists with co-pollutants and the co-pollutants may possess the capacity to influence its biological action(s), it is conceivable that the toxic effects of Cd that were observed when it was examined in isolation may be enhanced or impaired in the presence of the co-pollutants. The Warri river Cd concentration which was in the range of 0.229 - 0.291 mg/L (23) provides a fairly reasonable condition for investigating these possibilities. The same water sample contained copper (Cu^{+}) at a concentration of 0.189 mg/L, iron (Fe^{2+}) 1.90 mg/L, lead (Pb^{2+}) 1.08 mg/L and zinc (Zn^{2+}) 0.505 mg/L. A number of studies have been done (7,45,46) on Cd alone using the range of Cd concentration in Warri river/creek waters between 1986-1991 as reported by Egborge (23).

The aim of the present study was therefore to examine the nephrotoxic effect of aquatic Cd at a concentration of 0.229 mg/L in the presence of each of the other co-polluting metal ions separately, using their 1986 -1991 Warri river/creek water concentrations. This will provide some insight into what will really happen to the kidney when an individual is exposed to a "cocktail" of metallic pollutants containing Cd instead of just Cd per se. The 'cocktail' being a reasonable reflection of what is naturally ingested by way of water and/or food.

Materials and Methods

Materials

Animals

Thirty rats (Wistar strain) were used for this study. They were obtained from the Animal Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria and distributed into experimental cages of five rats each. They were left in the new condition to acclimatize for 2 weeks with 12 hours of day/night cycle, allowed access to chow (Grand Cereals Ltd, subsidiary of UAC Nigeria) and water *ad libitum*, before the commencement of the experiment.

Chemicals and Reagents

The chemicals used were cadmium chloride ($CdCl_2$. 2½ H_2O – Kermel China), anhydrous copper (II) sulphate ($Cu(II)SO_4$–Kermel China),ferrous sulphate ($FeSO_4$. $7H_2O$ – Kermel, China), zinc sulphate septahydrate ($ZnSO_4$. $7H_2O$-Kermel, China), lead sulphate ($PbSO_4$–Kermel, China), 2-thiobabituric acid (Kem Light Lab, PVT Ltd, India), trichloroacetic acid (JHD, China) and chloroform (BDH, Poole, England).

Reagents used were obtained in form of biochemical assay kits. They include creatinine and urea assay kits (Randox, UK), potassium, sodium and chloride ion as well as bicarbonate assay kits (Teco Diagnostics, Netherland).

Methods

Preparation of cadmium, copper, iron, lead and zinc ion tainted waters

Cadmium ion tainted water was prepared by dissolving cadmium chloride ($CdCl_2$. 2½ H_2O), 0.465mg, in distilled water and making it up to a litre. This solution contained the equivalent of 0.229mg Cd ion/L.

The mixture of Cd and each of the other metal ions 0.465 mg cadmium chloride hemidihydrate with either 0.469 mg anhydrous copper (II) sulphate (0.189 mg Cu ion), 9.50 mg ferrous sulphate (1.90mg Fe(II) ion), 1.59 mg lead sulphate (1.08 mg Pb ion) or 2.23 mg zinc sulphate septahydrate (0.505 mg Zn ion) per litre of distilled water were also prepared.

Treatment of Animals

The rats were divided into six experimental groups of 5 rats each. Group A rats served as control and were maintained on rat chow and water *ad libitum*. Each rat in this group received the equivalent of 42.86ml of water kg^{-1} body weight (b w) by gavage daily besides being allowed free access to water. The amount of water given by gavage was predicated on the fact that on the average a 70kg man or woman consumes about 3.5 litres of water daily. Group B rats were given Cd tainted water by gavage - 42.86 ml/ kg bw. Group C rats were given the Cd and Cu tainted water by gavage- 42.86ml/kg bw. Groups D, E and F received Cd and Fe, Cd and Pb and Cd and Zn tainted water respectively- 42.86 ml/ kg bw.The treatments were given to each group of rats, twice a day, seven days a week for four weeks.After each treatment , rats in each group just like the control were allowed free access to chow and their respective tainted water via drinking bottles.

Animal Sacrifice

At the end of four weeks, on the morning of day 29, all the rats were sacrificed. Each was anaesthetized as described previously (6,47). While under anaesthesia the thoracic and abdominal regions were opened. Blood was obtained by heart puncture and put in appropriately labelled anti-coagulant-free sample bottles and left standing on ice. The kidneys were excised and put in labelled sample bottles and left standing on ice as well.

Preparation of sera samples

The blood samples, now clotted, were centrifuged at 3500 rpm for 15 minutes. The sera samples were separated and stored at -20^{o}C until they were required for the analyses of the biochemical parameters.

Preparation of Kidney Homogenates

The kidneys of each rat were homogenized in ice-cold saline solution (1:9 w/v) using mortar and pestle. Each homogenate was centrifuged at 3500 rpm for 15 minutes and the separated supernatant stored at -20^{o}C until required for the analyses of selected biochemical parameters.

Biochemical Assays

Creatinine level in the serum was estimated based on the alkaline picrate method (48) while urea level was determined by the urease method (49). Sodium and potassium ion concentrations in the serum were assayed using the methods described by Maruna (50) and Trinder (51) for sodium and the method described in Teco Diagnostic leaflet for potassium assay (52). Chloride ion concentration was quantified by the method of Skeggs and Hochestrasser (53) while bicarbonate was estimated by the method described by Forrester *et al.* (54). Kidney malondialdehyde level was determined using the method of Buege and Aust (55).

Statistical Analysis:

Results are shown as means ±SEM. Data were analyzed using one way analysis of variance (ANOVA) and posthoc least square difference (LSD) test by SPSS software version 23 for window (SPSS, IBM Chicago, IL, USA) with $p \leq 0.05$ considered as statistically significant.

Results

Serum Creatinine and Urea levels

The effects of Cd alone and its binaries with Cu, Fe, Pb and Zn on serum creatinine and urea levels are presented in Table 1. The treatments except that of the binary of Cd and Zn, significantly ($p \leq 0.05$) increased serum creatinine level when compared with the control. Compared with the Cd only group, the binaries of Cd and Cu and that of Cd and Fe produced significant ($p \leq 0.05$) increases in serum creatinine. Cadmium associated increase in serum creatinine was reduced to value that was not significantly ($p>0.05$) different from the control, in the group exposed to Cd and Zn.

Table 1: Effects of Cd and its Binaries with Cu, Fe,Pb and Zn on serum creatinine and urea concentrations

Experimental groups	Treatments	Creatinine concentration Mean ± SD(n) (mg/dL)	%Difference Relative to group A	Urea concentration Mean ± SD(n) (mg/dL)	%Difference Relative to group A
A	Control (-Cd, -Cu, -Fe, -Pb, -Zn)*	0.81 ± 0.26(4)	–	7.92 ± 5.26(4)	
B	+Cd	1.26 ± 0.07(4)^{a} **	55.56↑	14.44 ± 8.72(5)	82.32↑
C	+Cd + Cu	1.61 ± 0.09(3)$^{a, b}$	98.77↑	10.14 ± 5.46(5)	28.03↑
D	+Cd + Fe	1.32 ± 0.40(3)^{a}	62.96↑	7.86 ± 6.61(5)	0.07↓
E	+Cd + Pb	1.64 ± 0.16(3)$^{a, b}$	102.47↑	10.40 ± 4.97(5)	31.31↑
F	+Cd + Zn	1.00 ± 0.07(3)^{b}	11.11↑	6.08 ± 3.31(5)^{b}	23.23↓

*Cd= Cadmium; Cu= Copper; Fe= iron; Pb= lead; Zn = Zinc

**Values with superscripts a, b, c, d, e or f within the same column are significantly different ($p \leq 0.05$) relative to the value of the group bearing the corresponding uppercase letter A, B, C, D, E or F.

(n) = Values in parentheses represents the number of data in the group used for computation.

↑ = Percentage increase. ↓ = Percentage decrease.

Serum HCO_3^- , CL^- , K^+ and Na^+ levels

The effects of Cd alone and the combines, Cd + Cu, Cd + Fe, Cd + Pb and Cd + Zn, on serum HCO_3^-, Cl^-, $K^+$and Na^+ ion levels are presented in Table 2. Cadmium alone and the combine, Cd + Pb significantly ($p \leq 0.05$) reduced serum chloride ion level relative to the control. Compared with Cd only group, the groups exposed to Cd and Cu, Cd and Fe, Cd and Pb as well as Cd and Zn had significantly ($p \leq 0.05$) increased serum chloride ion status. Compared with the control, Cd alone significantly ($p \leq 0.05$) reduced the level of K^+ ion in the serum but the combine, Cd + Fe, increased it. When compared with the Cd only group, the combines, Cd + Cu and Cd + Fe significantly ($p \leq 0.05$) increased serum K^+level. Relative to the value of the control, Cd only did not cause significant ($p>0.05$) change in serum Na^+ ion concentration but the combine, Cd + Fe, caused a significant ($p \leq$

0.05) reduction. However, relative to the Cd only group, the combines, Cd + Cu and Cd + Fe, caused significant ($p \leq 0.05$) reduction in serum Na^+ ion levels.

Table 2: Effects of Cd and its Binaries with Cu , Fe ,Pb ,and Zn on Serum bicarbonate, chloride, potassium and sodium ion concentrations

Experimental groups	Treatments	HCO_3^- concentration Mean ± SD (n) (mmol/L)	CL^- concentration Mean ± SD (n) (mEq/L)	K^+ concentration Mean ± SD(n) (mEq/L)	Na^+ concentration Mean ± SD(n) (mEq/L)
A	Control (-Cd, -Cu, -Fe, -Pb, -Zn)*	30.65±0.71(5)	80.32±11.5(4)	5.38±1.65(5)	134.17±5.84(4)
B	+Cd	31.08±0.80(5)	52.13±2.13(3)[a]	3.82±0.98(5)[a]	139.63±4.50(3)
C	+Cd + Cu	31.50±0.19(5)	71.96±4.75(5)[b]	7.78±0.78(4)[b]	126.90±5.64(5)[b]
D	+Cd + Fe	31.28±0.58(5)	78.54±6.75(5)[b]	5.91±0.74(5)[a,b]	123.57±6.18(4)[a, b]
E	+Cd + Pb	31.45±0.23(5)	65.52±7.05(3)[a, b]	5.77±0.53(3)	139.38±6.54(5)
F	+Cd + Zn	31.11±0.49(5)	77.49±3.63(3)[b]	5.27±0.85(5)	139.16±9.30(3)

*Cd= Cadmium; Cu= Copper; Fe= iron; Pb= lead; Zn = Zinc
**Values with superscripts a, b, c, d, e or f within the same column are significantly different ($p \leq 0.05$) relative to the value of the group bearing the corresponding uppercase letter A, B, C, D, E or F.
(n) = Values in parentheses represents the number of data in the group used for computation.

Table 3: Effects of Cd and its Binaries with Cu ,Fe ,Pb and Zn on kidney malondialdehyde level

Experimental groups	Treatments	Malondialdehyde level Mean ± SD (n) (MDA Units/mg tissue)	% Differences relative to groups A and B (A)	(B)
A	Control (-Cd, -Cu, -Fe, -Pb, -Zn)	9.06 ± 3.31(5)	–	40.29↑
B	+Cd	6.28 ± 0.95(5)[a]	30.68↓	–
C	+Cd + Cu	7.78 ± 1.45 (5)	14.13↓	23.89↑
D	+Cd + Fe	6.43 ± 0.81(5)[a]	27.92↓	2.39↑
E	+Cd + Pb	6.00 ± 0.65(5)[a]	33.77↓	4.46
F	+Cd + Zn	13.02 ± 1.74(5)[a, b]	43.71↑	107.32↑

*Cd= Cadmium; Cu= Copper; Fe= iron; Pb= lead; Zn = Zinc
**Values with superscripts a, b, c, d, e or f within the same column are significantly different ($p \leq 0.05$) relative to the value of the group bearing the corresponding uppercase letter A, B, C, D, E or F.
(n) = Values in parentheses represents the number of data in the group used for computation.
↑ = Percentage increase. ↓ = Percentage decrease

Kidney Malondialdehyde level

Kidney malondialdehyde level was significantly ($p \leq 0.05$) decreased in the Cd only group compared with the control. The combines, Cd + Fe and Cd + Pb caused similar effect (Table 3). However, relative to control and the Cd only group, the combine, Cd + Zn caused significant ($p \leq 0.05$) increase in kidney malondialdehyde level.

Discussion

This study was designed to examine what role, if any, other metallic co-pollutants would have on cadmium associated kidney toxicity in the rat. The level of creatinine in the serum increased by 55.56 % in the group of rats exposed to Cd only (0.81±0.26 mg/dL in the control to 1.26±0.07mg/dL in the Cd only group). Cu, Fe and

Pb when co-consumed separately with Cd increased serum creatinine status further by 98.77%, 62.96% and 102.47% respectively relative to the control value.

These findings indicate that Cd likely caused a reduction in glomerular filtration rate (GFR) in the exposed rats (56, 57), an established toxic effect of cadmium in the kidney. The fact that Cu, Fe and Pb each in conjunction with Cd increased serum creatinine level suggests that when they exist in bodies of water as co-pollutants they are likely to contribute to impaired GFR when such body(ies) of water are consumed as "table water". In this study, serum urea was not affected by the treatments. Increase in serum levels of urea and creatinine are accepted as indirect markers of poor GFR (58). Hence, we attribute the observed increase in serum creatinine in this study to altered GFR with caution. The fact that chemically induced increases in blood urea nitrogen and/or serum creatinine may not by themselves indicate poor GFR, but a reflection of dehydration, hypovolemia and/or protein catabolism (58) in some cases, make the cautious interpretation of the current findings imperative.

In a study in which catfish were exposed to Cd alone, chloride ion, potassium ion and sodium ion levels were significantly altered compared with the control group and the changes were attributed to altered osmoregulation (59). In this study, the same pattern was observed with regardsto chloride, potassium and sodium. These disturbances may likely be associated with Cd –induced changes in tissues involved with regulation of the levels of these ions *in vivo*.

Copper (Cu), Fe, Pb and Zn prevented Cd from decreasing serum chloride status (Table 2) but of the four chemical entities Pb was the least effective as Cd antagonist in this respect. As in the case of chloride, Cd reduced serum potassium ion level (Table 2) but when combined with Cu and Fe separately, this ability of Cd was lost. Both Cu and Fe antagonized Cd so profoundly that they caused an elevation rather than a decrease in serum K^+ ion level (Table 2). However, unlike Cu and Fe, the antagonism ofPb and Zn to Cd in the groups where they were separately combined with it prevented an increase in K^+ ion status relative to the control . With respect to serum Na^+ ion status, Cd caused an elevation which was reduced remarkably by Cu and Fe, to values that were below that of the control group (Table 2). In terms of their influence on serum Na^+ ion level, the actions of Cu and Fe were the reverse of the actions exhibited toward serum K^+ ion level whereas Pb and Zn retained the same pattern of behavior vis-à-vis Na^+ and K^+ ions.

The behavior of Cu and Fe in the results presented in Table 2, is one of reversal of the actions of Cd. This is likely due to the fact that absorption of Cd in the gastrointestinal tract (GIT) is affected by GIT status of Zn, Cu and Fe (60). Since the route of administration in this study is oral, more Cd was likely absorbed when the concentration of the others were low in the GIT, which was the case in group A. Evidently, low absorption of Cd meant reduced potency toward the specific biochemical processes it deranged and a tendency to return to the control value (Table 2).

It does appear that any drug or agent that affects blood Na^+ ion levels has the capacity to cause a change in chloride ion status as well, since chloride ion levels normally change in direct relationship to sodium (61). However, the findings in this particular study do not agree with what is apparently the norm. Cd reduced serum chloride concentration but increased the level of sodium ions (Table 2) , an apparently compensatory effect. Why this is so remains largely unclear presently. Elevated serum Na^+ ion level is believed to be due to the ability of Cd to damage the filtering capacity of the renal tubules and by so doing triggers sodium retention probably like the situation that arises in certain types of renal disease (62).

The antagonistic or protective roles played by the metallic ions co-administered with Cd ion in this study are predicated on different mechanisms of action. Copper can minimize Cd toxicity by reducing the amount of Cd absorbed in the GIT since it competes with Cd for absorption just as it has been reported in other organisms and situations (63). Iron at sufficiently high concentration does the same (64) and the Cd: Fe mole ratio of 3:25 used in this study offered iron a competitive advantage which is reflected in the results (Table 2).

As is evident from the results in Table 3, Cd caused significant reduction in kidney level of malondialdehyde compared with the control. Presently, there are sufficient pieces of evidence in the literature which show that Cd can deplete the level of reduced glutathione and other thiol groups in the tissue as an indirect way of generating ROS (35, 65). The consequence of this is heightened lipid peroxidation characterized by increased level of malondialdehyde (65). The finding in this study does not agree with earlier findings with regard to the MDA level of the Cd only group of fishes (66) and rats (36).Fe and Pb in separate binary association with Cd also produced reduced kidney MDA relative to the control group (Table 3, group D and E). The extent to which Cd lowered kidney MDA level was not profoundly different from that produced by its binary with Fe as well as its binary with Pb which were 27.92 and 33.77% respectively compared with the control (Table 3). It is therefore obvious that neither of them impressively enhanced or impaired cadmium's ability to lower kidney MDA level in this study. Kidney MDA level produced by the binary of Cd and Cu was not significantly different from the control. The capacity of Cd to impair MDA production was evidently diminished but not completely obliterated by Cu.

The reason why the findings in this study on kidney MDA status in the presence of Cd and its co-pollutants, do not accord with what appears to be its established effect remains largely unclear. MDA, though, is the lipoperoxidation product of membrane polyunsaturated fatty acid (PUFA), normally released by phospholipase

A_2 activity (PLA_2) (67). Therefore it is likely that impaired release of arachidonate due to low phospholipase activity made the arachidonate less readily available for lipoperoxidation pathway and consequently low tissue MDA level. It has been demonstrated that cadmium ions (Cd^{2+}) cause a reductionin the rate of liberation of arachidonate from alveolar macrophages, and it was attributed to the documented findings that Cd^{2+} inhibits PLA_2 activity (68). This remains the only plausible interpretation of our current finding on Cd-associated low MDA production in rat kidney. However, whether this pattern of alveolar macrophage response to Cd exposure plays out in the kidney remain to be investigated.

Zinc acted in a unique way in its binary with Cd when compared to the other binaries. It restored to the control serum values, the level of creatinine, as well as those of chloride and potassium ions that were significantly altered in the Cd only group (Tables 1 and 2). The only exception was observed in the kidney MDA level that was significantly reduced in the Cd only group but increased in the Cd and Zn binaryrelative to the control. Here, the Cd plus Zn binary enhanced the level of MDA beyond the control and Cd only values respectively (Table 3).

For parameters not altered in the Cd only group, the Zn + Cd group values were not markedly different from the appropriate control values. The parameters not altered were urea, bicarbonate ion, and sodium ion (Tables 1 and 2). Evidently, these parameters appear not to be neither profoundly affected by Cd alone nor by its binary with Cu, Fe, Pb and Zn in Wistar rats.

The results presented in this report evidently gives credence to our hypothesis that the toxicological findings obtained when Cd and possibly any other heavy metal is examined in isolation may not be the same when investigated in the presence of other metallic co-pollutants since the co-pollutants may impair or enhance the adverse effects that are produced by the toxic heavy metal when investigated in isolation.

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