CADMIUM INTERACTION WITH IRON METABOLISM, IN VITRO AND IN VIVO STUDIES

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SUMMARY: In vitro and in vivo experiments were carried out to investigate the effects of Cadmium on iron metabolism. Iron as a complex with citric acid (1:20) binds to human serum apo-transferrin (apo-tf). Addition of varying concentrations of Cadmium to the reaction mixtures reduces iron uptake by approximately 30 percent. Daily administration (i. p.) of Cadmium as $CdCl_2$ (2 mg/kg) for 10 days reduced serum levels of hemoglobin (Hb), iron, total iron binding capacity (TIBC) and ferritin by 26, 39, 20 and 47 percent respectively.

Administration of Cadmium as $CdCl_2$ (200 µg/kg) daily for 60 days reduced serum Hb, iron, TIBC and ferritin concentrations by 17, 26, 15 and 29 percent respectively in comparison to control findings.

Inspite of reduction in hemoglobin level in Cadmium treated rat no inhibition effect of Cadmium on mitochondrial ferrochelatase activity was seen. A possible mechanism for hematological disorders following Cadmium intoxication is considered.

Key Words: Cadmium, transferrin, iron, deutroporphyrine.

INTRODUCTION

Cadmium (Cd) is a widespread industrial pollutant for acute and chronic poisoning in human and animals. It is known to be one of the most toxic heavy metals (1). This toxic element can enter the metabolic pathways of some essential trace elements including copper, zinc, iron, manganese, selenium and calcium by competing for ligands in biological systems (13). Cadmium toxicity in man causes a number of pathophysiological disturbances including proximal tubular dysfunction characterized by proteinuria, aminoaciduria and glucosuria, bone disease (Itai-Itai) (4), pulmonary emphysema (5) and liver damage (6). Exposure of man through contaminated air, food, water, manufactured goods and occupational hazards (7) might cause these disturbances. Cigarettes made from tobacco grown in soil containing cadmium is another major source of cadmium intoxication (8).

In the blood, cadmium is mainly found in cells and damages the erythrocytes (9). Approximately 60 % of the cadmium in cells is probably bound with metallothionine, a metal binding protein of low molecular weight (10). Animal experiments have shown that hypochromic microcytic anemia might be developed in rats with cadmium intoxication following exposure to dietary

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Table 1: Rats were injected i.p. with indicated dose of Cadmium. Controls were injected NaCl (0.9/100). They were killed by decapitation and sera were collected for serum Cadmium determination as described in Materials and Methods. All data appears as mean ± SD. Serum Cadmium was expressed asμμg/1. Each figure represents as the mean of five separate experiments.

Cd mg/kg group	**None**	**0.25**	**0.5**	**1**
30 days	2.4±0.25	3.93±0.11	6.22±0.17	11.17±0.17
60 days	3.13±0.52	1.76±0.14	2.64±0.26	10.66±0.55

cadmium (11). The exact mechanism of iron disturbances following cadmium intoxication however has not yet been determined. This project therefore was undertaken to investigate the possible mechanisms by which cadmium interferes with iron metabolism and causes anemia by using *in vitro* and *in vivo* experiments.

MATERIALS AND METHODS

All chemicals used in this study were reagent grade and obtained from Sigma Chemical Company (Germany). Human apo-tf (5 mg/ml) was dissolved in Earle's medium, pH 7.4 (12), placed in prewashed visking sacs, and dialyzed twice against 100 vol of 50 mM acetate buffer, pH 5.2, first for 6 h and then

Table 2: Rats were injected i. p. with indicated doses of Cadmium as $CdCl_2$ daily for 10 days. Animals were killed by decapitation and sera were collected for Hb, Fe, TIBC and Ferritin determinations. Data are expressed as mean ± SD. Each figure is the mean of five separate experiments.

Treatment	Hb (g/dl)	Fe (μg/dl) 1	TIBC (μg/dl)	Ferritin (ng/dl)
None	15.4±0.5	122.5±3.5	388.4±7.3	38.6±1.5
1mg/kg BW	12.8±1.5 (-16%) (P<0.079)	87.4±5.2 (-28%) (P<0.002)	336.5±8.2 (-13%) (P<0.003)	23.1±2.1 (-40%) (P<0.002)
2 mg/kg	11.4±1.3 (-26%) (P<0.03)	74.2±2.5 (-39.4%) (P<0.001)	309.8±7.1 (-20%) (P<0.001)	20.3±2 (-47%) (P<0.001)

Table 3: Rats were injected i. p. with indicated doses of Cadmium daily for 30 days. They were treated as described in ligand for Table 2.

Treatment	Hb (g/dl)	Fe (μg/dl) 1	TIBC (μg/dl)	Ferritin (ng/dl)
None	15.6 ± 0.9	122.6 ± 2.4	386.7 ± 8.4	38.3 ± 1.3
1mg/kg BW	12.8 ± 1.6 (-17%) (P<0.09)	85.4 ± 5.5 (-30%) (P<0.005)	307.9 ± 13.5 (-20%) (P<0.004)	22.4 ± 2.6 (-40%) 41.2 (P<0.002)
0.5 mg/kg	13.6 ± 1.7 (-12%) (P<0.2)	86.4 ± 5.1 (-29%) (P<0.003)	311.4 ± 2.8 (-19%) (P<0.001)	25.4 ± 1.6 (-33%) (P<0.02)
0.25 mg/kg	13.7 ± 1.9 (-12%) (P<0.3)	91.4 ± 4.6 (-25%) (P<0.003)	333.3 ± 10.9 (-13%) (P<0.006)	28.4 ± 1.7 (-26%) (P<0.005)

16 h. Protein solutions were then successfully dialyzed against 100 vol of 0.15 mM NaCl. 0.02 m $NaHCO_3$ in 0.15 M NaCl, and finally Earle's medium. The homogeneity of prepared apo-tf was checked by polyacrylamide gel electrophoresis.

Preparation of iron and cadmium citrate complexes

Separate stock standard solutions of ferric nitrate (3.75 mM) and CdCl (3.75 mM) were prepared in deionized water containing 0.1 M HNO3 and mixed with equal vol of 75 mM citric acid, the solutions are adjusted to pH 7.4 with 1 M NaOH and made up to final concentration of 1.5 mM iron and cadmium.

Spectrophotometric titration technique of metal binding to apo-transferrin

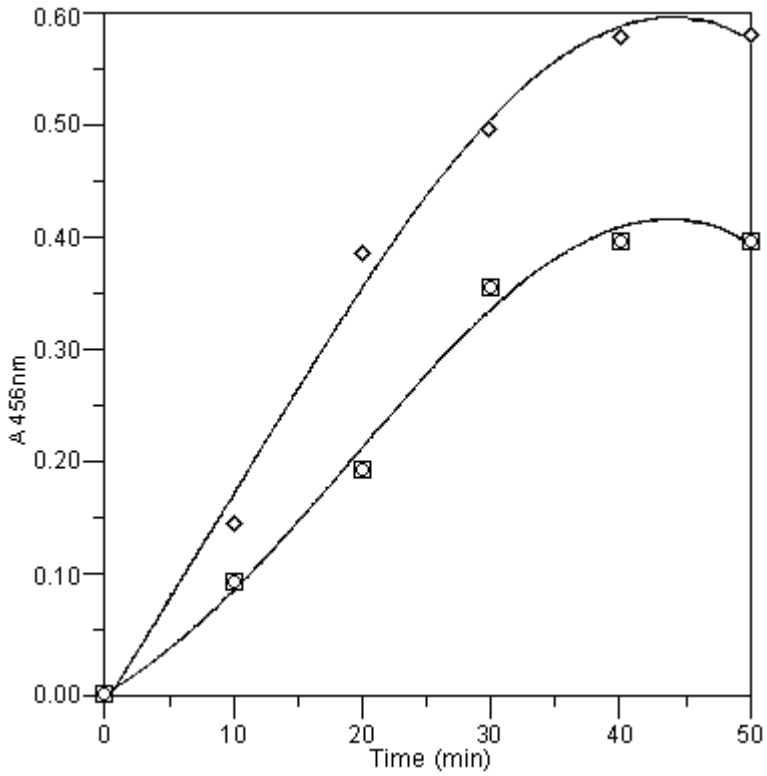
Approximately 1.5 mL of prepared apo-tf in Earle's medium was added to a standard 1 cm pre-acid washed glass cuvets. To these cuvets were added aliquots (0-100 μgl) of 1.5 mM metal ion with or without citric acid. The cuvets were covered with parafilm, mixed thoroughly, by vortexing, and left up for to 2 h at room temperature. The absorbance of the cuvets was measured at appropriate wavelength.

Animals

Male Wistar rats were purchased from Pasteur Institute (Tehran, Iran) at 150-175 g and maintained on purine chow and water ad libitum, until use at indicated weight (220-250 g).

Animals were treated with daily i. p. doses of cadmium as $CdCl_2$ dissolved in normal saline for up to 2 months. Animals were killed by decapitation and sera were collected for determination of Cd, Hb, Fe, TIBC, ferritin and protein concentrations.

Figure 1: Time course for birding for iron to human transferrin. Effect of Cadmium (◊ Fe-tf, o Fe-tf and Cadmium.)



Hemoglobin was determined by the method suggested by Fairbanks (13), and serum iron and TIBC were determined using phenanthroline as the chromogen (14). Ferritin was measured by immunoradiometric technique using laboratory kits provided by Amersham Amerlex laboratory kits (U.K.). Protein determination was carried out by the method of Lowry (15).

Livers from cadmium treated and also from controls were removed and immediately trimmed, chilled and homogenized in 1 vol of 0.25 M sucrose. This and all other preparatory steps were carried out at 0-4°C. Rat liver sub-cellular fractionations were prepared by the method of Fleischer and Kervina (16).

Cadmium determination

Cadmium determinations were carried out using a Schimadzu flameless atomic absorption spectrophotometer (670 G) with the cadmium hollow cathode lamp. The serum Cd determinations were carried out according to the method of Claeys (17).

RESULTS

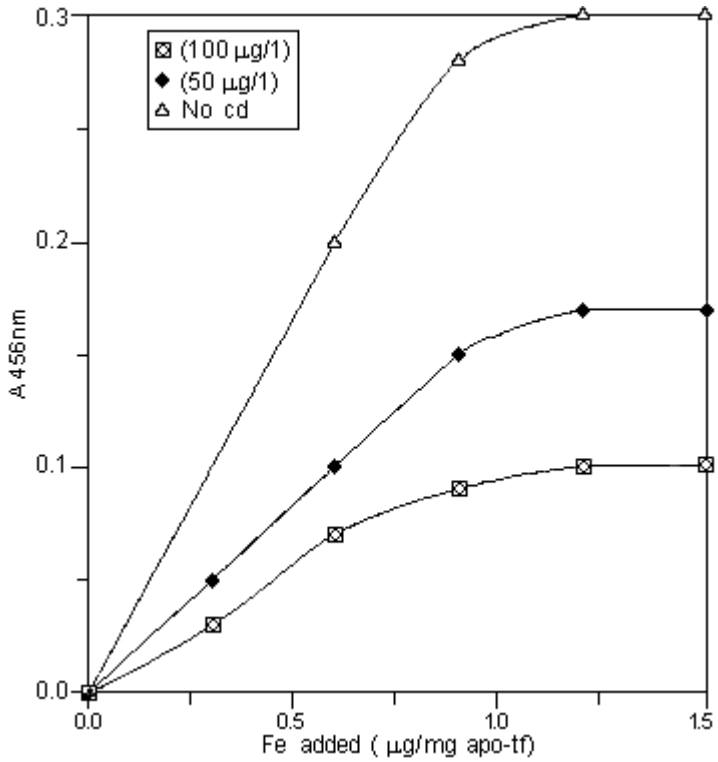
In vitro studies

Preminary experiments were established to study binding of iron to serum human apo-transferrin. To approach this, the absorption spectrum of Fe-transferrin complex was measured first. Aliquots of iron-citrate complex (1:20) was added to a solution of apo-transferrin and treated as described in the methods. The results obtained showed a broad peak at 465-470 nm.

Table 4: Rats were injected i.p daily with indicated doses of Cadmium as $CdCl_2$ for 60 days. Animals were treated as described in ligand for Table 2.

Treatment	Hb (g/dl)	Fe (μg/dl) 1	TIBC (μg/dl)	Ferritin (ng/dl)
None 0.5 mg/kg	15.6±0.87 12.6±0.98 (-197%) (P<0.03)	122.5 ± 2.4 80.8 ± 3.4 (-34%) (P<0.001)	386.7 ± 8.4 313.1 ± 7.9 (-19%) (P<0.001)	38.3 ± 1.3 24.2 ± 1.0 (-37%) 41.2 (P<0.001)
0.2 mg/kg	12.9±0.26 (-17%) (P<0.4)	90.1 ± 4.2 (-26%) (P<0.002)	326.9 ± 15 (-15%) (P<0.001)	27.0 ± 1.62.1 (-29%) (P<0.08)
0.1 mg/kg	14.4 ± 1.4 (-8%) (P<0.2)	100.4 ± 45.6 (-18%) (P<0.002)	347.7 ± 7.0 (-10%) (P<0.008)	30.4 ± 2.2 (-20%) (P<0.02)

Figure 2: Spectrophotometric titration of human apo-transferrin with iron. Effect of Cadmium (△ Fe-tf, ¿ o Fe-tf and Cadmium.)



The effects of time and also increasing concentrations of iron as a complex with citric acid on apo-transferrin binding were studied next. The iron uptake by apo-transferrin was linear up to 40 min of incubation (Figure 1) and it was not changed there after. Figure 2 shows that maximum binding occurred at 1.4-1.45 µg/mg apo-transferrin. The effect of Cadmium as Cadmium Chloride (50 µg/l) on iron binding apo-transferrin was studied and the results are presented in Figures 1 and 2. Indicating approximately 40 percent reduction when more Cadmium (100 µg/l) was added to reaction mixtures, more reduction in iron uptake was seen (Figure 2)

It should be noted that in the absence of citric acid no iron binding to apo-transferrin was observed.

In vivo studies

In these series of experiments, the serum concentration of cadmium in normal controls and in cadmium treated animals was determined first.

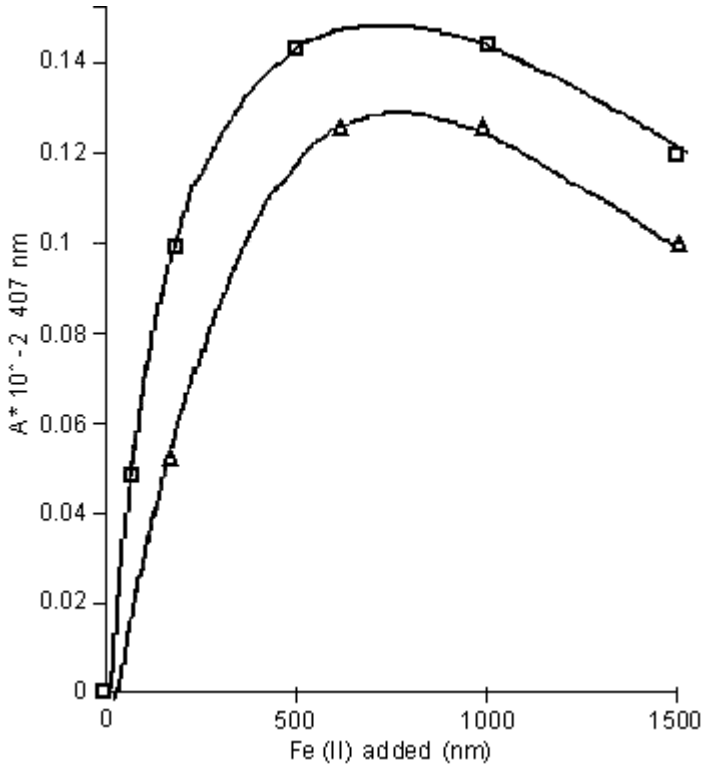
Data obtained are presented in Table 1 show significant elevation of serum cadmium in comparison to untreated controls.

The effects of cadmium on Serum Hb, iron, TIBC and ferritin in relation to iron metabolism was studied next.

Daily administration of cadmium (1 and 2 mg Cd/kg BW) for 10 days resulted in reduction of Hb (17 and 26%), serum iron (28 and 39%), serum TIBC (13 and 20%) and finally serum ferritin (40 and 47% respectively) (Table 2). The long term effects of cadmium on the same serum parameters was also investigated and results are presented in Tables 3 and 4. The percentage of reduction and p values all are appeared in Tables 3 and 4.

In order to study the probable mechanism of hemoglobin reduction in cadmium treated animals, the effect of cadmium on mitochondrial ferrochelatase activity was studied next. Rat liver mitochondrial fraction was

Figure 3: Iron incorporation to deuteroporphyrine (£) and effect of cadmium (△).



obtained as described in the methods. They were solublized in tween 20. To solutions containing deuteroporphyrine IX and varying amounts of iron (0-1500 nmole/l) as ferrous ammonium sulfate were added aliquots of mitochondrial extract. After 1h at 37°C trichloroacetic acid was added to the mixtures followed by centrifugation. The A 407nm (18) of the supernatant was measured giving the results shown in Figure 4. When 10 µg/l 148 cadmium as CdCl2 was added to the incubation mixtures. There was no significant difference in the results obtained.

DISCUSSION

Hematological disturbances following cadmium intoxication has been reported by a number of researchers including Brozowska *et. al.* (19), Schafer and Forth (11) and others (3). The probable mechanism by which cadmium Causes anemia in animal with cadmium overload has not been clearly reported. Small intestinal mucosal cells is the major site of iron absorption and serum transferrin is the major iron carrier protein (20).

Iron-transferrin complex bound to the transferrin receptor at plasma membrane (21) and internalized to the cells, where it is used for heme synthesis. Ceruloplasmin, a copper containing protein plays an important role in oxidation of Fe (II) to Fe (III) in the cells. Cadmium may interfere with one of the steps of iron metabolism and cause anemia.

Data which has been presented in this manuscript shows that addition of cadmium to reaction mixtures causes significant reduction in iron uptake by apotransferrin (Figures 2 and 3). These two metal ions may compete for the same binding sites on the transferrin molecules. It might, therefore be hypothesized that reduction in hemoglobin level following i. p. administration of cadmium could be due to the interaction of cadmium with iron in the plasma. Data from other

laboratories, says, anemia is caused by reduction in iron absorption as a result of the competition between iron and cadmium ions in mucosal cells (22).

There is also another suggestion that cadmium is responsible for the inhibition of heme synthesis (23). Following internalization of Fe-transferrin complex, iron is taken up by mitochondrial fraction for heme synthesis. Ferrochelatase plays an important role in heme synthesis (24). Using deuteroporphyrine and rat liver mitochondrial extract, no inhibition in the activity of enzyme was seen following cadmium addition to the reaction mixture. We may therefore reach to this conclusion that damage to heme system following exposure to cadmium may be due to interaction of this metal with iron uptake by transferrin.

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