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Influence of dietary iron deficiency on acute metal intoxication

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The influence of dietary iron deficiency on acute nickel, lead or cadmium toxicity as reflected by the induction of hepatic, renal and intestinal metallothionein (MT), disposition of the metals, and alterations in hematological parameters was investigated in rats. The administration of cadmium induced the hepatic, renal and intestinal MT while that of nickel or lead induced hepatic MT only. However, dietary iron deficiency did not influence the cadmium induced tissue MT but enhanced the ability of nickel or lead to restore the normal synthesis of renal and intestinal MT lowered under the influence of reduced body iron status. The accumulation of lead in liver and kidney and that of cadmium enhanced in liver only, while tissue deposition of nickel remained unaffected by iron deficiency. The induction of hepatic MT by three metals appears related to the concomitant rise in the hepatic zinc, calcium and iron levels in normal rats. However, dietary iron deficiency increased the hepatic zinc in response to nickel or cadmium and that of heptic calcium in response to lead.

Keywords: biochemical alterations, cadmium, dietary iron deficiency, lead, metallothionein, metals, nickel, rat, tissue

Introduction

The toxic manifestations of heavy metals are governed among others by dietary constituents, particularly the essential trace elements (Schafer & Forth 1985, Elsenhans *et al.* 1987). Thus, while dietary iron deficiency increases metal absorption (Ragan 1977, Hashmi *et al.* 1989a, Schafer *et al.* 1990) its excess decreases their absorption (Schafer & Forth 1985, Clark *et al.* 1988). The dietary iron status, therefore, seems to influence the disposition and effects of toxic metals following exposure.

Dietary constituents, particularly metals, markedly influence the induction of metallothionein (MT) (Cousins 1985, Bremner 1987). Considering the fact that MT is induced in response to various stresses (Hidalgo *et al.* 1988, Bremner & Beattie 1990), including the administration of heavy metals such as cadmium or mercury, the specific genes coding for this protein may be expressed as an acute-phase response of the body. Like synthesis of other acute-phase proteins, synthesis of MT under such circumstances is probably designed to impart stress resistance or tolerance to cells and to maintain homeostasis. In addition to zinc and cadmium, both of which bind to MT and are potent MT inducers, parenteral iron loading caused a marked accumulation of hepatic Zn–MT (McCormick 1984). The MT induction in the intestinal mucosa and brain in anemic rats led Ribas *et al.* (1987) to propose the participation of MT in both the iron absorption by the intestinal mucosa and the involvement of brain in iron homeostasis. Recently, Robertson *et al.* (1989) reported an increase in MT-I concentration in blood cells and bone marrow, a reduction in MT level in kidney and no change in hepatic MT as a consequence of iron deficiency.

In view of the current lack of knowledge of the biochemical mechanism of metal interactions, attempts were made to study the influence of dietary iron deficiency on acute toxicity of nickel, lead or cadmium as evaluated by tissue MT induction, metal disposition and certain related hematological alterations in young rats.

Materials and methods

Animals and treatment

Forty-eight male rats (70 ± 10 g), bred in the Industrial Toxicology Research Centre's colony, were equally divided into two batches. One batch was fed an iron sufficient

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and the other an iron deficient synthetic diet for 6 weeks (Table 1). The animals of the iron deficient group were subjected to orbital plexus puncturing twice a week for the first 2 weeks to ascertain if they were in an iron deficient state. Thereafter, the animals were sub-divided into four groups in each batch. Three groups in each batch were administered a single dose of 120 μmol kg^{-1} nickel as $NiCl_2 \cdot 6H_2O$, 400 μmol kg^{-1} lead as $Pb(CH_3COO)_2 \cdot 3H_2O$ or 20 μmol kg^{-1} cadmium as $CdCl_2 \cdot H_2O$, intraperitoneally, dissolved in distilled water (2 ml kg^{-1}). The solution of lead acetate was prepared in boiled water and bubbled with nitrogen immediately prior to administration. The remaining group in each batch was given an equal volume of water and served as a control. The metals were given at equitoxic doses [i.e. maximum tolerated dose (MTD)] previously determined and defined as the maximum dose which produced death in less than 10% of the exposed population (Eaton *et al.* 1980). The use of the MTD provides information on the maximal effectiveness of the metal to induce MT at the time point selected. The animals were sacrificed 24 h post metal treatment and blood drawn by cardiac puncture into heparinized vials. An aliquot of blood was centrifuged to separate the plasma. The liver and kidney were collected, and the small intestine (about 10 cm from the pylorus) excised, cut open and washed with saline.

Biochemical estimations

The liver and kidney were homogenized in 4 volumes and the intestine in 10 volumes of 0.01 M Tris–HCl buffer (pH 7.4). The homogenates were centrifuged at 10 000 × *g* for 30 min and the heat stable fraction obtained by placing the post-mitochondrial supernatant in a boiling water bath for 5 min. The MT was estimated in this fraction using the Cd/Hb affinity method (Eaton & Toal 1982). Standard procedures were employed for the estimation of blood hemoglobin (Clegg & King 1942)—packed cell volume (Keele & Neil 1966) and plasma cholesterol (Zlatkis *et al.* 1953)—total iron and iron binding capacity (Peters *et al.* 1956).

Table 1. Chemical composition of diet

Ingredients	g kg^{-1}
Casein	220
Corn starch	370
Sucrose	300
Groundnut oil	50
Mineral mixture (modified AIN-76 mineral mixture)	45
Vitamin mixture (AIN-76 vitamin mixture)	10
Choline chloride	1.5
DL-methionine	2.0
$FeSO_4 \cdot 7H_2O$ in sucrose[a]	1.5

[a]Analytical grade $FeSO_4 \cdot 7H_2O$ triturated in powdered sucrose (1.5 g) to supply 35 or 5 mg Fe kg^{-1} diet for an iron sufficient or iron deficient diet, respectively.

Estimation of metals

The weighed hepatic and renal samples were mineralized in nitric and perchloric acids (6:0.5). The residue was dissolved in 5% HNO_3 and the metals were determined on a flame absorption spectrometer (Perkin-Elmer 5000). The readings were recorded for copper (324.7 nm), zinc (213.9 nm), calcium (422.7 nm), iron (248.3 nm), nickel (232.0 nm), lead (283.3 nm) and cadmium (228.8 nm) against suitable standards processed identically. The standard stock solutions (1 mg ml^{-1}, as an element) were prepared by dissolving Analytical Reagent grade $CuSO_4 \cdot 5H_2O$; $NiSO_4 \cdot 6H_2O$; $CdCl_2 \cdot H_2O$ (Merck, Darmstadt, Germany), $ZnSO_4 \cdot 7H_2O$; $CaCO_3$ (Merck, India) and $FeSO_4 \cdot 7H_2O$; $Pb(CH_3COO)_2 \cdot 3H_2O$ (BDH, Poole, UK) in 5% HNO_3 using double glass distilled water. Six working standard solutions within the linear range for each element were prepared by dilutions of the stock.

Statistical analysis

A 2 × 2 randomized block factorial design was used to analyze various parameters using six replicates in each group (normal diet, normal diet plus nickel, iron deficient diet and iron deficient diet plus nickel). Prior to this analysis, normality assumption of the data and homogenity of variance between the treatment groups was ascertained. The means of the treatment groups from the controls were compared separately using Dunett's test (Zar 1984). The same analysis was performed for lead and cadmium.

Results

Dietary iron deficiency decreased plasma iron levels but increased plasma cholesterol and iron binding capacity. The administration of lead but not nickel or cadmium caused similar effects in animals fed a normal diet. The influence of lead was more prominent in animals maintained on an iron deficient diet (Table 2).

Dietary iron deficiency caused a significant decrease in renal and intestinal MT levels; the hepatic MT remained unaffected. Cadmium was highly effective in inducing MT synthesis in all three tissues examined, an effect which remained uninfluenced by dietary iron deficiency. Nickel and lead were successful in inducing MT in liver only but failed to induce the same in iron deficient rats. However, these metals could restore the iron deficiency-led lowering in renal and intestinal MT levels (Table 3).

Dietary iron deficiency enhanced the accumulation of lead in both liver and kidney and that of cadmium in liver only. However, it had no effect on the tissue deposition of nickel (Figure 1).

Table 2. Influence of dietary iron deficiency on metal induced biochemical alterations in rat blood

Treatment	Blood			Plasma	
	Hb ($g\,100\,ml^{-1}$)	PCV (%)	total cholesterol ($mg\,1/00\,ml^{-1}$)	total iron	iron binding capacity ($megui\,100\,ml)^{-1}$
Normal diet	11.8 ± 0.50	40.8 ± 3.38	98.5 ± 6.03	229.3 ± 9.20	447.8 ± 37.26
Nickel	9.6 ± 2.63	32.2 ± 6.44	104.2 ± 2.02	235.4 ± 19.42	490.7 ± 56.10
Lead	12.8 ± 1.62	45.8 ± 5.55	140.9 ± 5.79[a]	170.1 ± 26.34[a]	516.75 ± 24.85[a]
Cadmium	13.4 ± 1.61[c]	42.2 ± 1.86	109.6 ± 12.14	191.5 ± 34.75	466.6 ± 64.01
Iron deficient diet	9.3 ± 0.58	34.4 ± 3.77	114.2 ± 15.92[c]	177.3 ± 19.50[b]	637.9 ± 15.0[a]
Iron deficient + nickel	10.5 ± 1.81	31.6 ± 3.72	92.9 ± 5.18[y]	181.3 ± 11.1	556.5 ± 18.01[y]
Iron deficient + lead	10.5 ± 1.94	29.2 ± 4.41	145.3 ± 5.63[x]	140.2 ± 14.94[y]	592.6 ± 23.84[z]
Iron deficient + cadmium	10.2 ± 1.11	35.3 ± 4.68	106.7 ± 9.43	167.4 ± 20.34	637.7 ± 16.59

Each figure is mean ± SD (6): [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ versus normal diet; [x] $P < 0.001$, [y] $P < 0.01$, [z] $P < 0.05$ versus iron deficient diet.

Table 3. Influence of dietary iron deficiency on the induction of tissue MT by metals in rat

Treatment	Liver	Kidney ($\mu g\,g^{-1}$, fresh tissue)	Intestine
Normal diet	4.5 ± 1.69	12.8 ± 1.51	5.4 ± 0.71
Nickel	14.4 ± 4.32[a]	13.5 ± 1.69	4.8 ± 1.02
Lead	6.5 ± 1.74[b]	14.4 ± 0.78	4.6 ± 0.81
Cadmium	46.2 ± 6.27[a]	31.0 ± 4.57[a]	18.4 ± 3.09[a]
Iron deficient diet	5.4 ± 1.03	5.6 ± 1.30[a]	2.7 ± 0.67[a]
Iron deficient + nickel	5.8 ± 1.35	15.1 ± 2.03[x]	4.4 ± 0.74[y]
Iron deficient + lead	4.7 ± 0.73	13.2 ± 1.82[x]	4.6 ± 0.70[y]
Iron deficient + cadmium	63.0 ± 10.98[x]	32.0 ± 4.01[x]	13.9 ± 2.13[x]

Each figure is mean ± SD (6): [a] $P < 0.001$, [b] $P < 0.01$ versus normal diet; [x] $P < 0.001$; [y] $P < 0.01$ versus iron deficient diet.

The administration of all the three metals, i.e. nickel, lead and cadmium, significantly increased hepatic zinc, calcium and iron levels of rats maintained on a normal diet. The iron deficient diet, however, decreased the zinc and iron contents of the liver. The administration of nickel caused an increase in hepatic zinc content while that of cadmium produced a significant increase in hepatic zinc as well as iron levels in animals fed an iron deficient diet (Table 4).

The administration of cadmium significantly enhanced renal zinc, copper and calcium, and the dietary iron deficiency maintained such an influence of cadmium. However, the injection of lead decreased the renal level of copper in normal animals which remained so under dietary iron deficiency. Interestingly, the administration of lead significantly elevated the lowered renal level of iron in dietary iron deficient animals (Table 5).

Discussion

Hypochromic microcytic anemia as a consequence of iron deficiency results in the loss of the normal conservation of iron. The hematopoitic alterations under iron deficiency are generally akin to lead or cadmium exposure (Hashmi *et al.* 1989b, Baynes and Bothwell 1990, Schafer *et al.* 1990), but in the present study only lead seems to support this observation and nickel appears to reduce the influence of iron deficiency. The stimulating effect of metals on the hepatic synthesis of cholesterol is believed to result in increased blood cholesterol, phospholipid or triglycerides in experimental animals (Yoshikawa *et al.* 1974, Mathur & Tandon 1979, Hashmi *et al.* 1989b). This proposition has been supported by lead only, irrespective of the dietary status of animals in the present study. The higher retention of cadmium or lead in liver and

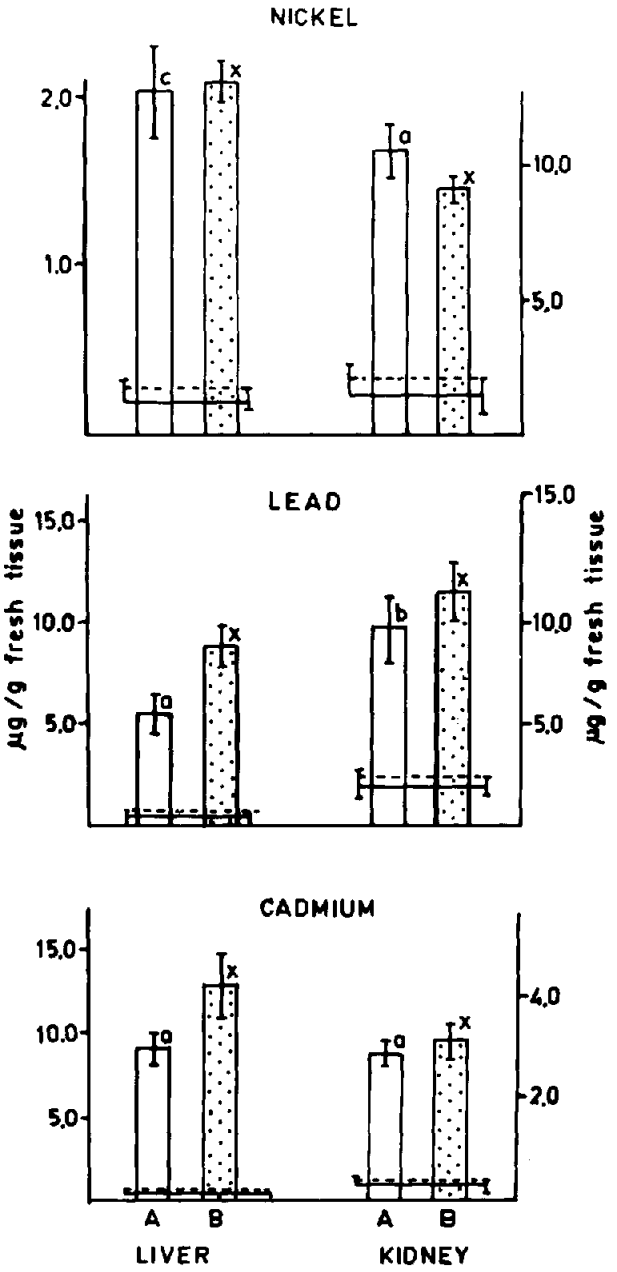

Figure 1. Influence of dietary iron deficiency on the levels of nickel, lead or cadmium in rat liver and kidney. Each bar is mean ± SD (6): $^{a}P < 0.001$, $^{b}P < 0.01$, $^{c}P < 0.05$ versus normal diet (——); $^{x}P < 0.001$ versus iron deficient diet (– – –) alone. □, normal + metal; ▨, iron deficient + metal.

kidney of rats maintained on an iron deficient diet is in agreement with the earlier observations and may be attributed to the greater availability of the binding sites for these metals under the iron deficiency status of the tissues (Ragan 1977, Schafer *et al.* 1990). The results, however, indicate that the body status of iron has no influence on the retention of nickel in the intestinal mucosa.

The fact that dietary iron deficiency decreased the native levels of renal and intestinal but not hepatic MT indicates the selective influence of dietary iron status on MT in different tissues. However, the enhanced MT synthesis by cadmium in all the three organs examined irrespective of body iron status shows that it is the relative ability of various metals to induce MT rather than the selective response of the tissues towards such an induction. On the other hand, nickel and lead could biosynthesize the hepatic MT only, and that to a far lesser extent than cadmium. Whereas, cadmium is a well known strong tissue MT inducer (Waalkes & Klaassen 1985), nickel (Khandelwal *et al.* 1990) and lead (Maitani *et al.* 1986) have occasionally been shown to be hepatic and/or renal MT inducers. It is interesting to note that nickel or lead were able to increase the biosynthesis of hepatic MT in normal-diet fed animals but failed to do so in iron deficient-diet fed animals, and further that the administration of these two metals significantly raised the renal and intestinal MT levels which were lowered under iron deficiency. It appears that nickel and lead are capable of inducing MT in liver only and that the significance observed (between control and experimental groups) in kidney and intestinal MT, in response to nickel or lead administration in animals fed an iron deficient diet, is actually due to the decrease in MT levels attributable to the body iron deficiency. In other words, nickel or lead might have compensated for the decreasing influence of iron deficiency alone on the tissue MT induction.

The increase in hepatic zinc in response to nickel, lead or cadmium administration in normal-diet fed animals, in response to cadmium in iron deficient-diet fed animals and the increase in renal zinc and copper in response to cadmium injection both in normal-diet fed as well as in iron deficient-diet fed animals could be attributed to the induction of tissue MT by these toxic metals, capable of strongly binding additional zinc and copper. The increase in hepatic calcium and iron levels following nickel, lead or cadmium administration, particularly in normal-diet fed animals, may be the result of their increased uptake or retention upon heavy metal exposure. However, the extent of cadmium induced hepatic and renal MT and lead or nickel induced hepatic MT appears related to the intracellular calcium levels in the present study. A simultaneous rise in calcium and MT has also been shown by Yamane *et al.* (1990) in rats at 12 h after the subcutaneous administration of $CdCl_2$.

Table 4. Influence of dietary iron deficiency on metal induced alterations in essential elements in rat liver

Treatment	Zinc ($\mu g\,g^{-1}$, fresh tissue)	Copper ($\mu g\,g^{-1}$, fresh tissue)	Calcium ($\mu g\,g^{-1}$, fresh tissue)	Iron ($\mu g\,g^{-1}$, fresh tissue)
Normal diet	32.5 ± 4.44	3.4 ± 1.15	17.1 ± 1.52	182.6 ± 25.86
Nickel	41.4 ± 3.37[b]	4.2 ± 0.36	20.7 ± 2.46[b]	246.9 ± 22.29[a]
Lead	41.1 ± 4.57[b]	3.6 ± 0.38	22.2 ± 2.95[c]	239.6 ± 28.77[b]
Cadmium	45.1 ± 3.19[a]	4.0 ± 0.46	21.1 ± 2.56[c]	207.0 ± 33.98
Iron deficient diet	25.7 ± 2.14[b]	4.4 ± 0.76	18.0 ± 2.02	84.6 ± 15.26[a]
Iron deficient + nickel	33.8 ± 2.35[y]	3.9 ± 0.38	19.0 ± 0.61	71.8 ± 9.79
Iron deficient + iron	26.1 ± 4.11	3.5 ± 0.28	35.8 ± 4.93[x]	63.4 ± 9.75
Iron deficient + cadmium	52.57 ± 3.96[x]	4.2 ± 0.61	19.7 ± 2.52	119.3 ± 4.65[z]

Each figure is mean ± SD (6): [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ versus normal diet; [x] $P < 0.001$, [y] $P < 0.01$, [z] $P < 0.05$ versus deficient diet.

Table 5. Influence of dietary iron deficiency on metal induced alterations in essential elements in rat kidney

Treatment	Zinc ($\mu g\,g^{-1}$, fresh tissue)	Copper ($\mu g\,g^{-1}$, fresh tissue)	Calcium ($\mu g\,g^{-1}$, fresh tissue)	Iron ($\mu g\,g^{-1}$, fresh tissue)
Normal diet	34.6 ± 3.48	7.1 ± 0.42	7.5 ± 2.40	120.2 ± 5.15
Nickel	35.5 ± 3.53	6.9 ± 1.60	7.8 ± 1.46	89.2 ± 14.71[a]
Lead	35.8 ± 3.59	5.5 ± 0.99[b]	6.9 ± 2.01	118.9 ± 5.37
Cadmium	49.6 ± 5.81[c]	8.6 ± 1.22[c]	10.9 ± 2.22[b]	122.6 ± 13.19
Iron deficient diet	37.2 ± 4.01	7.3 ± 1.19	7.6 ± 1.28	51.4 ± 15.1[a]
Iron deficient + nickel	34.8 ± 3.30	6.2 ± 1.11	7.1 ± 0.96	75.1 ± 9.40
Iron deficient + lead	30.1 ± 2.53[z]	5.4 ± 0.62[x]	6.1 ± 0.61	87.0 ± 7.16[x]
Iron deficient + cadmium	47.6 ± 2.94[y]	9.0 ± 1.63[z]	9.1 ± 1.09	50.2 ± 10.61

Each figure is mean ± SD (6): [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ versus normal diet; [x] $P < 0.001$, [y] $P < 0.01$, [z] $P < 0.05$ versus iron deficient diet.

Conclusion

The results suggest that the disadvantageous effects of dietary iron deficiency in response to nickel, lead or cadmium were variable and metal–metal interaction may be influenced by the nature of the toxic metals.

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