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Effect of Aluminum and Deferoxamine on Biliary Iron Elimination in the Rat (42762)

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Abstract. Iron (Fe) and aluminum (Al) eliminations in bile were studied in rats after intravenous administration of Fe, Al, deferoxamine mesylate (Desferal, Ciba) (DFA), feroxamine (FeA), and aluminoxamine (AlA) at the dose of 50 μmole/kg body weight. Bile was obtained from the bile duct of anesthetized rats, and the concentrations of Fe and Al in bile were measured by an inductively coupled plasma optical emission spectrometer. The results showed an increase of Fe elimination in bile, from 10 to more than 20 μmole/liter after Fe and also after Al administration; an increase to about 350 μmole/liter after DFA administration; to 250 μmole/liter after FeA administration; and to 100 μmole/liter after AlA administration. Aluminum elimination in bile was increased only after Al and particularly after AlA administration but not after Fe and FeA administration. In conclusion, Al and AlA were able to increase Fe elimination in bile. Thus Al overload observed in hemodialyzed patients may induce an excessive iron loss in bile and partly explain microcytic anemia.

The biliary excretion of xenobiotics is less known than their urinary elimination because the bile is relatively inaccessible in comparison to urine and because biliary excretion may be followed by digestive absorption. The biliary excretion of metals was reviewed some years ago by Klaassen (1). Recently, Thompson and Klaassen (2) have compared biliary excretion of a few metals after portal and systemic administration to rats. More data about biliary elimination of lead, mercury, zinc, copper, and manganese, for example, can be found in the literature than about iron (Fe) (3–5) and aluminum (6).

The effect of deferoxamine (DFA) on Fe elimination in bile was studied (7, 8), but the mechanisms and sites of action of DFA are not yet completely understood (9). DFA also increased the biliary and fecal eliminations of Al in patients (10).

We report here results of an experiment conducted in normal rats to study Fe and Al elimination in bile after systemic administration of metals (Fe and Al) and of DFA and its chelated forms, feroxamine (FeA) and aluminoxamine (AlA).

Materials and Methods. Male Wistar rats of about 200 g body weight, maintained on a standard laboratory diet, were fasted 16 hr before the cannulation of the bile duct. Rats were anesthetized with urethane (1.2 g/kg body weight), known to have little effect on the volume of bile excreted (11). The bile duct was surgically exposed by a midline incision and cannulated with polyethylene tubing. Rats were kept at a constant body temperature with a heat lamp. Bile was collected into preweighted polypropylene tubes every 15 min for 3 hr before and after intravenous administration of the products studied. Bile flow rate was 1.12 ± 0.16 ml/hr ($\bar{x} \pm$ SD, $n = 15$) and 1 ml/hr of saline was given via the jugular vein to compensate for the loss of fluid volume.

The drug used was deferoxamine mesylate (DFA) (Desferal, Ciba). FeA and AlA, the chelated forms of DFA, were obtained by mixing equimolecular quantities of DFA and Fe^{3+} and Al^{3+}, respectively. The substances used, Fe^{3+} ($Fe(NO_3)_3$, $9H_2O$) and Al^{3+} ($Al(NO_3)_3$, $9H_2O$), were obtained from Merck and Prolabo. The rats were injected iv with DFA, FeA, AlA, Fe^{3+}, and Al^{3+} at the dose of 50 μmole/kg body weight. A control group received an equimolecular solution of $Mg(NO_3)_2$. Four rats per group were used.

The concentrations of Al in bile, after a 10-fold dilution with demineralized water, were determined by inductively coupled plasma optical emission spectrometry, according to a method already described elsewhere (12, 13). Since we were using a JY 48 spectrometer, Fe as well as Na, K, Ca, Mg,

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Zn, and Cu were simultaneously determined.

All data are expressed as means ± standard deviations. Tests for statistical differences of means were carried out by the nonparametric Mann–Whitney test (14).

Results and Discussion. Since the quantity of bile secreted during 15 min was not modified by administration of Fe, Al, DFA, FeA, and AlA, the biliary elimination of Fe and Al is expressed as micromoles per liter of bile (Figs. 1, 2, 3). No modification of biliary elimination of Na, K, Ca, Mg, Zn, and Cu was observed in these experiments.

Figure 1 shows that administration of $Mg(NO_3)_2$ did not modify Fe elimination in bile. Fe administration slowly increased Fe biliary elimination to about 20 μmole/liter, 2 hr after administration. Al administration also gave an increase of Fe elimination in bile similar to that obtained after Fe administration.

Figure 2 shows that DFA and FeA gave a very considerable and rapid increase of the concentration of Fe in bile with a maximum of about 300 μmole/liter, 30 min after administration. AlA also gave a considerable increase of Fe elimination in bile with a maximum of about 100 μmole/liter.

The comparison of these results (Figs. 1 and 2) clearly shows that the concentration of Fe in bile increased slightly but continuously after Fe and Al administration, whereas after DFA, FeA, and AlA administrations it increased rapidly to reach high concentrations at 30 min and then decreased.

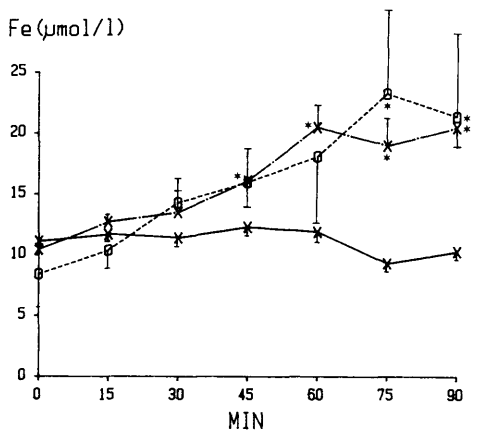

FIG. 1. Fe concentration in bile after intravenous administration of Mg $(NO_3)_2$ (× — ×), $Al(NO_3)_3$ (× — · — ×), and $Fe(NO_3)_3$ (□ --- □) at the same dose (50 μmole/kg). $\bar{x} \pm$ SD, $n = 4$. $^*P \leq 0.05$ (Mann–Whitney U test). Control group: Mg $(NO_3)_2$.

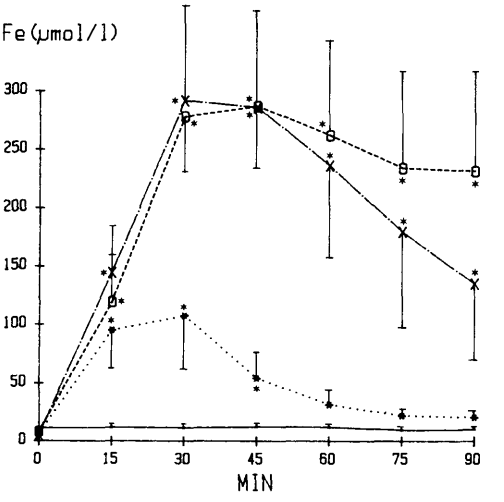

FIG. 2. Fe concentration in bile after intravenous administration of AlA (* --- *), FeA (× — · — ×), deferoxamine (DFA) (□ --- □), and Mg $(NO_3)_2$ (● — ●) at the same dose (50 μmole/kg). $\bar{x} \pm$ SD, $n = 4$. $^*P \leq 0.05$ (Mann–Whitney U test). Control group: Mg $(NO_3)_2$.

Figure 3 shows that after Al administration, Al concentration in bile increased slowly during 1 hr and then remained at the same level (about 10 μmole/liter). After AlA administration, Al elimination in bile increased to a maximum at 45 min and then slowly decreased. The biliary elimination of endogenous Al in control rats was very low and was not increased by $Mg(NO_3)_2$, DFA, or FeA administration.

Comparison of Fe elimination in bile after FeA administration (Fig. 2) and Al elimination after AlA administration at the same equimolecular dose (Fig. 3), shows that Fe elimination (250 μmole/liter) is about four time higher than Al elimination (60 μmole/liter).

These results confirm that the biliary elimination of endogenous Fe is considerably increased by DFA. This increase, in addition to the well-known increase in urinary elimination, may partly explain the clinical effect of DFA in Fe overloaded patients.

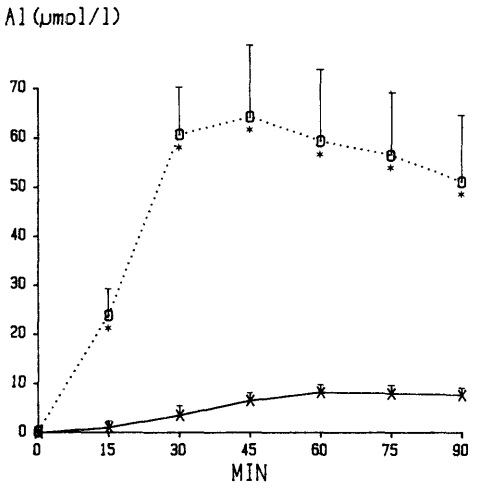

FIG. 3. Al concentration in bile after intravenous administration of Al (NO3) (× — ×) and AlA (□ --- □) at the same dose (50 µmole/kg). $\bar{x} \pm$ SD, $n = 4$. $^*P \leq 0.05$ (Mann–Whitney U test). Control group: Al $(NO_3)_3$.

The fact that the rates of Fe and Al elimination in bile were faster after DFA, FeA, and AlA administration than after Fe and Al administration suggests that the chelated forms can be directly eliminated in opposition to the free elements which probably need to be bound to endogenous ligands before elimination.

From a practical point of view, it is well known that Al may be responsible for microcytic anemia in hemodialyzed patients (13–19). Our results suggest that in these patients, besides erythropoietin deficiency, an excessive iron loss through biliary excretion induced by Al overload may be partly at the origin of this trouble.

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