Kidney International, Vol. 45 (1994), pp. 76–84

Urinary and biliary excretion of aluminoxamine and ferrioxamine in dogs with various renal function

PATRICK C. D'HAESE, LUDWIG V. LAMBERTS, GERT A. VERPOOTEN, WOUTER VANEERDEWEG, ANGELIKA JURGENS, SAHAK ARAKELIAN, ARA BABLOYAN, PANAGIOTIS DIGENIS, WIEBREN TJALMA, and MARC E. DE BROE

Department of Nephrology-Hypertension, University of Antwerp, Antwerp, Belgium; Department of Pediatric Urology, Erevan, Armenia; and Renal Unit, Sismanoglion General Hospital, Athens, Greece

Urinary and biliary excretion of aluminoxamine and ferrioxamine in dogs with various renal function. We assessed the pharmacokinetics of aluminoxamine and ferrioxamine in dogs with sustained intermittent bile duct ligation and either normal renal function or stable chronic renal failure. A first group of male beagle dogs were given aluminoxamine and ferrioxamine, while a second group received desferrioxamine after loading them with iron and aluminum. Only minute amounts of ferrioxamine and aluminoxamine were found in the bile after administration of these compounds. The distribution volume of aluminoxamine and ferrioxamine appeared to be confined to the extracellular space and their renal excretion correlated with renal function. Administration of desferrioxamine to iron and aluminum-loaded dogs resulted in an increased biliary ferrioxamine but negligible aluminoxamine excretion. Renal clearance of the *in vivo* formed ferrioxamine and aluminoxamine in this group strongly correlated with renal function. Our observations indicate that biliary excretion of intravenously administered ferrioxamine and aluminoxamine is negligible even in renal failure. The data presented in this study provide indirect evidence that desferrioxamine administration to iron- and aluminum-loaded dogs results in the intra-hepatic formation of ferrioxamine which is partly excreted in the bile. Biliary excretion of aluminoxamine after desferrioxamine administration remained negligible.

Desferrioxamine (DESFERAL®), a trihydroxamic acid obtained from isolates of an actinomycete, Streptomyces pilosus, effectively chelates trivalent ions such as iron (stability constant; Ks = 10^{31}). Therefore, it was introduced in 1963 as an iron chelating agent in patients with chronic iron overload. Although it has been known for some time that desferrioxamine forms a complex with aluminum (Ks = 10^{22}) also, it is only since 1980, following the observation of Ackrill et al [1] that the chelator has been used in the therapy of aluminum overload in dialysis patients.

Desferrioxamine displays rather complicated physicochemical characteristics [2]. Unchelated desferrioxamine is a straight chained lipophilic molecule penetrating plasma membranes and undergoing metabolic breakdown. In contact with either iron or aluminum, it twines itself around the metal to form stable hydrophilic complexes.

Minor and major side effects have been observed during desferrioxamine treatment, particularly in dialysis patients. These include allergic skin reactions [3], ocular and auditory neurotoxicity [4], hypotension [5], thrombocytopenia [6], acute changes in renal function [7] and gastrointestinal disturbances [5]. Exacerbation of aluminum encephalopathy has been reported during the early stages of desferrioxamine treatment [8, 9]. The iron-desferrioxamine complex (ferrioxamine) both provides a source of iron and stimulates the growth of certain microorganisms of the Rhizopus class, and there is a strong correlation between treatment with desferrioxamine and the development of fatal mucormycosis [10, 11].

Only scarce information dealing with the kinetics of desferrioxamine and its chelated compounds is found in the literature. This is primarily due to the lack until recently [12] of accurate and sensitive methods for assaying desferrioxamine and its iron and aluminum-chelated substances.

In a previous study [13] we examined the kinetics of the chelator and its chelated compounds, ferrioxamine and aluminoxamine in dialysis patients. We found that the serum half-life ($t_{1/2}$) of ferrioxamine was increased in dialysis patients, particularly those with iron overload combined with liver disease. Moreover, as have others [14, 15], we noted that whereas serum aluminoxamine remained stable during the inter-dialytic period indicating the unique role of the kidney in the elimination of this compound, ferrioxamine levels decreased during this time interval. These observations prompted us to suggest that in contrast to aluminoxamine, the liver plays a role in the elimination of ferrioxamine. To test this hypothesis, kinetics of aluminoxamine and ferrioxamine were studied in dogs with external biliary derivation and either normal renal function or reduced renal mass [16]; the dogs received either (i) aluminoxamine and ferrioxamine or (ii) desferrioxamine after iron and aluminum loading.

Methods

Pharmacokinetics of desferrioxamine, ferrioxamine and aluminoxamine were studied in 16 adult male beagle dogs. The set-up of the study is schematically presented in Figure 1. Group I dogs (N = 8) received (i.v.) aluminoxamine and

Received for publication March 25, 1993
and in revised form July 28, 1993
Accepted for publication July 29, 1993

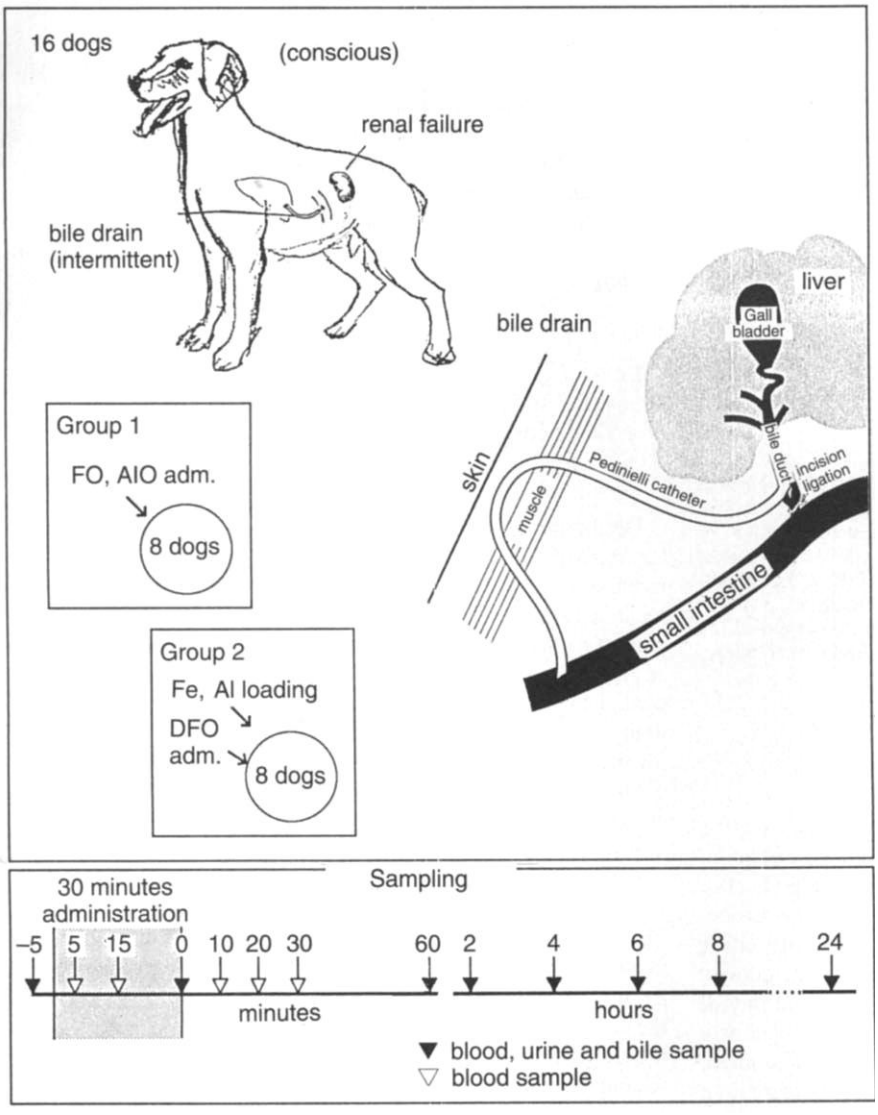

Fig. 1. *Study set-up and sampling points (▽ and ▼).* A reduction in renal mass was induced in 4 dogs of Group I and 5 dogs of Group II. The inset shows the bile duct ligation in the dog allowing sustained intermittent bile collection during at least 3 weeks. Abbreviations are: AlO, aluminoxamine; FO, ferrioxamine; DFO, desferrioxamine.

ferrioxamine. Group II dogs ($N = 8$) were pre-loaded with iron and aluminum and then received desferrioxamine (i.v.). Renal function in both groups varied from normal (Group I: $N = 4$; Group II: $N = 2$) to severe chronic renal failure (Table 1). In dogs with normal renal function the GFR as determined by measurement of the Cr-EDTA clearance was 71.9 ± 13.1 ml/min. Dogs were fed a standard chow (Doko, Ghent, Belgium) with free access to tap water before and during the experimental period. Time intervals for sample collection are presented in Figure 1. Venipuncture, sampling of bile and urine and storage of these specimen were done following the procedures described elsewhere [17]. Venipuncture was done using a winged infusion set (Viggo-Spectramed, JMS, Singapore). Blood and bile samples were collected and stored in 10-ml sterile syringes (Monovette, Sarstedt, Nümbrecht, Germany). No anticoagulant was used to reduce the risk for iron and/or aluminum contamination on the one hand, and to prevent interference with the chelated products on the other. Urine samples were collected by catheterization of the urinary bladder and stored in stoppered polystyrene containers. Storage of samples was done at −20 or −80°C.

Canine remnant kidney model

A stable chronic renal failure was induced in four dogs of group I (E-H; Table 1) and in five dogs of group II (D-H; Table 1) according to the method described by Vaneerdeweg et al [16]. In summary, with this procedure 50 to 60% of the cortex of the left kidney is resected followed by nephrectomy of the right kidney one week later. This surgical intervention was performed under anesthesia induced by FENTANYL® (Janssen Pharmaceutica, Beerse, Belgium) and 20 mg/kg Pentobarbital (NEMBUTAL®; Abbott, Chicago, Illinois, USA). After intubation of the trachea, lungs were normoventilated with an O_2/N_2O mixture. Anesthesia was maintained by 0.5 to 1.0% Enflurane (ETHRANE®; Abott). A stable chronic renal failure was obtained two months after surgery, and was maintained during a 9 to 12 month time period.

Table 1. Individual serum creatinine and mean ± SD body weight and biochemical blood values of dogs of Group I and Group II

	Dog	Group I	Group II
Serum creatinine *mg/dl*	A	0.50[a]	0.60[a]
	B	0.70[a]	0.90[a]
	C	0.83[a]	1.71[b]
	D	0.95[a]	2.79
	E	2.18	2.90
	F	2.20	2.90
	G	4.10	3.00
	H	6.30	9.62
Body weight *kg*		12.8 ± 3.0	13.1 ± 2.9
Calcium *mg/dl*		9.7 ± 1.1	10.3 ± 1.3
Phosphorous *mg/dl*		6.8 ± 3.0	7.0 ± 2.6
Bilirubin total/direct *mg/dl*		0.1/0.01	0.1/0.01
Alkaline phosphatase *U/liter*		68 ± 65	97 ± 92

Data of group I versus group II are not significantly different. Normal values as determined from ongoing screenings in 1783 normal Beagle dogs (Janssen Pharmaceutica, Beerse, Belgium) are: creatinine 0.82 ± 0.09 mg/dl; calcium 10.9 ± 0.5 mg/dl; phosphorous 5.7 ± 1.1 mg/dl; alkaline phosphatase 125 ± 50 U/liter; total bilirubin 0.12 ± 0.04 mg/dl.

[a] Dogs that are considered to have normal renal function

[b] This dog did not undergo kidney tissue resection, however, at the onset of the experiment appeared to have developed mild renal failure, the etiology of which we were not able to define

Bile collection

Four days prior to sampling, dogs with normal renal function as well as those with renal failure underwent an operation allowing sustained and intermittent bile sampling (Fig. 1) [18]. Dogs were anesthetized as described above. With this procedure the common bile duct was isolated and distally ligated. A Pedinielli PVC catheter (Porges, Sarlat, France) was introduced and fixed into the bile duct, taking care not to obstruct any of the hepatic ducts. The catheter was brought subcutaneously under the skin incision and repositioned into the abdomen, where it was fixed into the duodenum. At the time of sampling, the skin incision was reopened under local anesthesia. The catheter was cut in order to collect the bile flowing from the bile duct. At the end of the experiment, a silicon tube was slipped over the catheter to reconnect both ends and the skin was closed again.

Aluminoxamine and ferrioxamine administration

Aluminoxamine and ferrioxamine were freshly prepared by mixing equivalent amounts of desferrioxamine with aluminum or iron. Theoretically, 100 g of desferrioxamine binds 4.12 g of aluminum or 8.54 g of iron. As such, 20.6 mg of aluminum (corresponding with 184.3 mg of $AlCl_3 \cdot 6H_2O$) or 42.7 mg of iron (corresponding with 206.2 mg of $FeCl_3 \cdot 6H_2O$) were each solubilized in 5 ml of a 5% glucose solution. The corresponding solutions were then transferred to a flacon containing 500 mg of desferrioxamine. Solutions were mixed for one hour on a rotary mixer to allow complete formation of aluminoxamine and ferrioxamine, respectively. Six hundred and seventy μl of the aluminoxamine solution and 640 μl of the ferrioxamine solution were then added to 30 ml of a 5% glucose solution in water. This solution thus contained 60 mg of both compounds and was administered i.v. during 30 minutes to dogs of group I.

Iron and aluminum loading

Iron loading was performed in dogs of group II by i.v. administration of 150 mg iron per day as dextran iron (Fisons Pharmaceuticals, Leicesterhire, UK). Aluminum loading was done by i.v. administration of 30 mg aluminum per day as $AlCl_3 \cdot 6H_2O$. Both compounds were solubilized in 50 ml of 0.85% saline and administered simultaneously every two days for 10 days (that is, 5 injections). The kinetic study protocol was then started three days after completion of aluminum and iron loading.

Desferrioxamine administration

Dogs of group II received 200 mg (305 μmol) of desferrioxamine (DESFERAL®; Ciba-Geigy, Basle, Switzerland) in 30 ml of a 5% glucose solution in water by a 30 minute i.v. infusion.

Chemical analysis

Desferrioxamine, aluminoxamine and ferrioxamine were determined by Zeeman atomic absorption spectrometry using a method we previously described [12]. With this method the mean ± SD recoveries of aluminoxamine and ferrioxamine after extraction were 98.3 ± 2.7% and 101 ± 3.7%, respectively. The sensitivity was 45 pg/0.0044 Abs · sec for aluminum and 10 pg/0.0044 Abs · sec for iron, corresponding with detection limits of 0.22 μmol/liter for aluminoxamine and 0.06 μmol/liter for desferrioxamine and ferrioxamine, respectively.

Measurement of aluminum and iron in tissues was done by electrothermal atomic absorption spectrometry using our previously described methods [17, 19].

In addition, the following blood parameters were determined: serum creatinine (kinetic Jaffé method; assay kit; J.T. Baker, The Netherlands), direct and total bilirubin [dichlorophenyldiazonium (DPD) method; T-BiL assay kit; Boehringer Mannheim, Germany], calcium (colorimetric method; Ca-assay kit; bio Mérieux, France), inorganic phosphate (enzymatic colorimetric method; PHOS assay kit; Boehringer Mannheim) and alkaline phosphatase (enzymatic method [20]).

Data analysis

The rate constant of elimination (β) for ferrioxamine and aluminoxamine was determined by least squares non-linear regression analysis of the post-distribution phase of the serum concentration-time curve, using the BMDP Statistical Software [21]. The corresponding serum t½ was calculated as $0.693/\beta$. The area under the concentration-time curve (AUC) was determined with the trapezoidal rule. Renal clearances were calculated by dividing the cumulative amount of the respective compounds in the urine by the corresponding AUC values. Data are expressed as mean ± SD, except the serum t½ values for which the harmonic mean (range) is reported.

The paired *t*-test was used for comparison of distribution volumes, renal clearance and cumulative renal and biliary excretion of aluminoxamine versus ferrioxamine. We used the unpaired *t*-test to compare data on body weight, calcium, phosphorus, alkaline phosphatase and bilirubin concentrations, peak serum level of aluminoxamine and ferrioxamine (C_{max}) and biliary excretion of these compounds of group I versus group II. Because of the limited sample size, the Mann-Whitney U-test was used to compare serum t½ and renal clearance of

Table 2. Comparison of pharmacokinetic data of aluminoxamine versus ferrioxamine after aluminoxamine/ferrioxamine administration (Group I)

Parameter	Aluminoxamine	Ferrioxamine	*P*
C_{max}[a] *μmol/liter*	24.7 ± 8.0	29.3 ± 10.1	NS
Serum t½ vs. serum creatinine	Y = 126.7X −93.4	Y = 102.6X −42.1	NS
	r = 0.9483	*r* = 0.9891	
AUC vs. serum creatinine	Y = 4.06X − 1.91	Y = 4.64X − 1.05	NS
	r = 0.9395	*r* = 0.9566	
t½[b] *min*	33.7 (29.5 − 37.8)	37.4 (23.9 − 51.5)	NS
Relative distribution volume *liter/kg*	0.34 ± 0.14	0.29 ± 0.14	NS
Cumulative renal excretion *μmol/0–24 hr*	52.4 ± 16.7	57.0 ± 21.9	NS
Cumulative renal excretion/0–24 hr/total dose %	51.3 ± 16.4	58.2 ± 22.0	NS
Renal clearance vs. 1/serum creatinine	Y = 37.69X − 4.49	Y = 33.95X − 7.27	NS
	r = 0.9541	*r* = 0.9652	
Renal clearance[c] *ml/min*	41.0 ± 18.8	34.4 ± 22.6	NS
Cumulative biliary excretion *μmol/0–24 hours*	0.19 ± 0.20	0.36 ± 0.32	NS
Cumulative biliary excretion/0–24 hr/total dose %	0.19 ± 0.20	0.37 ± 0.33	NS

[a] Peak serum level of ferrioxamine and aluminoxamine
[b] Harmonic mean
[c] Dogs with normal function (*N* = 4)

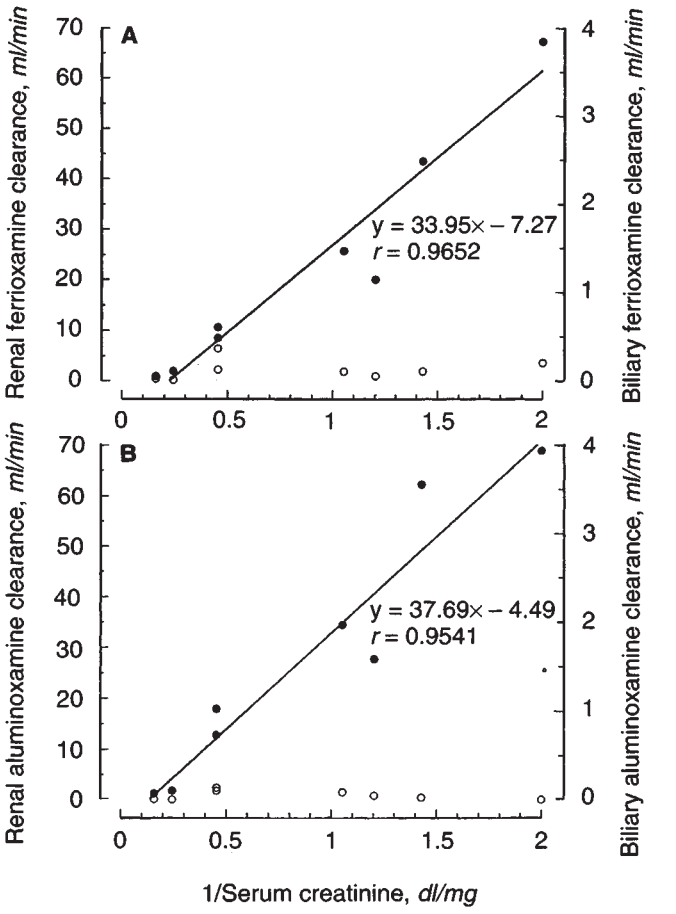

Fig. 2. *Renal (●—●) and hepatic (○) clearance of ferrioxamine (A) and aluminoxamine (B) in dogs with various renal functions after i.v. administration of the compounds.*

ferrioxamine versus aluminoxamine in dogs with normal renal function, and C_{max} levels of these compounds in dogs with normal renal function versus those with renal failure. Comparison of slopes and intercepts of linear regression curves was done by analysis of variance of regression coefficients over groups. A P value < 0.05 was considered to be significant at a two-tailed level.

Results

Table 1 summarizes the biochemical characteristics of dogs of groups I and II. Data do not differ significantly between groups I and II and show the presence and characteristics of a chronic renal failure, the absence of metabolic bone disease, and impairment of renal function. In contrast to Henry et al [22], hypercalcemia was not noted after aluminum loading.

Aluminoxamine/ferrioxamine administration

In group I (Table 2) peak serum levels of aluminoxamine and ferrioxamine were 24.7 ± 8.0 μmol/liter and 29.3 ± 10.1 μmol/liter (NS), respectively. Peak levels of both compounds were not significantly different in dogs with normal renal function as compared to those with renal failure [23.4 ± 5.8 μmol/liter vs. 26.1 ± 10.5 μmol/liter (NS) for aluminoxamine and 26.2 ± 13.5 μmol/liter vs. 32.0 ± 13.5 μmol/liter (NS) for ferrioxamine].

A linear correlation existed between the serum creatinine concentration and the serum t½ of aluminoxamine (r = 0.9483) and ferrioxamine (r = 0.9891), as well as between the serum creatinine concentration and the AUC of aluminoxamine (r = 0.9395) and ferrioxamine (r = 0.9566; Table 2). In dogs with normal renal function (serum creatinine < 1.2 mg/dl) serum t½ of aluminoxamine was 33.7 (29.5 to 37.8) minutes, compared to 37.4 (23.9 to 51.5) minutes (NS) for ferrioxamine. Relative distribution volumes were not significantly different, being 0.34 ± 0.14 liter/kg for aluminoxamine versus 0.29 ± 0.14 liter/kg for ferrioxamine.

Respectively, 51.3 ± 16.4% and 58.2 ± 22.0% (NS) of the chelates were recovered in the urine within 24 hours following administration of 60 mg of aluminoxamine and ferrioxamine. The cumulative renal excretion of aluminoxamine and ferrioxamine over 24 hours did not correlate significantly with the serum creatinine level (r = −0.65; P = 0.081 and r = −0.52; P = 0.186, respectively).

A linear correlation existed between the renal clearance of aluminoxamine (r = 0.9541) and ferrioxamine (r = 0.9652) and the reciprocal value of serum creatinine (Table 2). As indicated by the slopes of the equations, renal clearance of aluminoxamine and ferrioxamine did not differ significantly (Fig. 2). Mean renal clearance of aluminoxamine and ferrioxamine in dogs with

Table 3. Hepatic and bone iron and aluminum concentrations in aluminum- and iron-loaded dogs (Group II) versus unloaded dogs (Group I)

	Group I (N = 8)	Group II (N = 8)
Liver iron	227 ± 33	2084 ± 1044
μg/g wet wt	(203–250)	(901–3958)
Liver aluminum	2.5 ± 2.7	267 ± 287
μg/g wet wt	(0.6–4.4)	(50–780)
Bone iron	96.8 ± 61.0	301 ± 55
μg/g wet wt	(42–150)	(227–361)
Bone aluminum	1.4 ± 0.14	22 ± 9.6
μg/g wet wt	(1.2–1.5)	(12.3–33.0)

Data are means ± SD (range).

Table 4. Comparison of pharmacokinetic data of aluminoxamine versus ferrioxamine after desferrioxamine administration (Group II)

Parameter	Aluminoxamine	Ferrioxamine	P
C_{max}[a] *μmol/liter*	10.8 ± 5.2	11.8 ± 4.5	NS
Serum t½ vs. serum creatinine	Y = 27.56X + 102	Y = 26.99X + 196	NS
	r = 0.7721	r = 0.6246	
AUC vs. serum creatinine	Y = 0.50X + 1.61	Y = 0.59X + 0.37	NS
	r = 0.8156	r = 0.9216	
Renal clearance vs. 1/serum creatinine	Y = 11.23X + 3.25	Y = 12.29X + 1.47	NS
	r = 0.9353	r = 0.9486	
Cumulative biliary excretion μmol/0–24 hours	0.21 ± 0.13	4.89 ± 3.12	0.0022

[a] Peak serum level of ferrioxamine and aluminoxamine

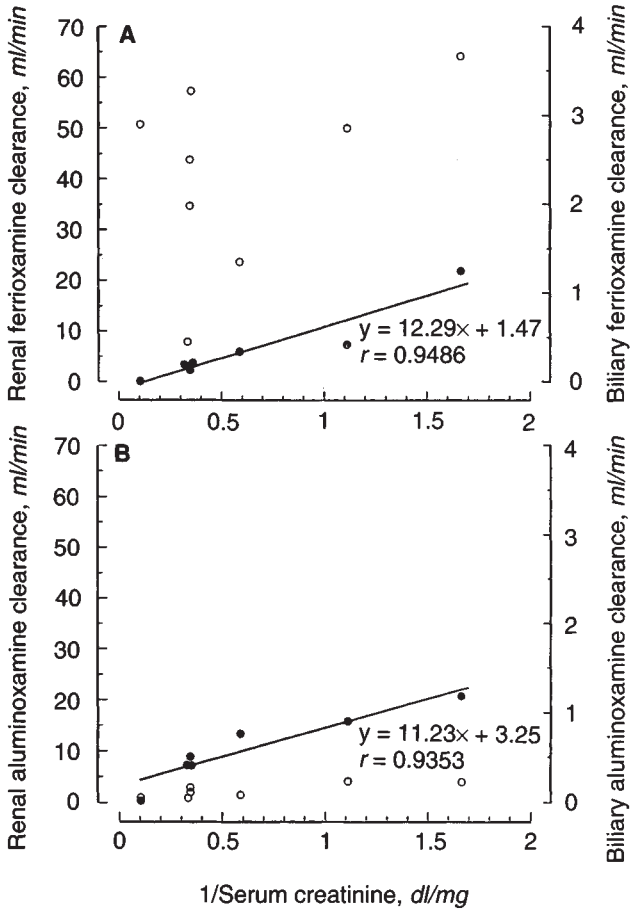

Fig. 3. *Renal (●——●) and hepatic (○) clearance of ferrioxamine (A) and aluminoxamine (B) in dogs with various renal functions after i.v. administration of desferrioxamine.*

normal renal function was 41.0 ± 18.8 and 34.4 ± 22.6 ml/min (NS), respectively.

When compared to their renal excretion, the biliary elimination of aluminoxamine and ferrioxamine was negligible and accounted for only 0.19 ± 0.20% and 0.37 ± 0.33% (NS) of the total administered dose of aluminoxamine and ferrioxamine, respectively. As shown in Figure 2 biliary elimination of the chelates was not influenced by the level of renal function.

Desferrioxamine administration

When compared to the unloaded dogs (that is, dogs of group I), in group II dogs the efficiency of the i.v. iron and aluminum loading was demonstrated by a 9.2-fold increase in the hepatic iron content and a 107- and 15.7-fold increase in the hepatic and bone aluminum content, respectively (Table 3). Body weight was not affected by iron and aluminum loading [mean weight ± SD of iron- and aluminum-loaded dogs 13.1 ± 2.9 kg vs. 12.8 ± 3.0 kg (NS) for the unloaded animals (Table 1)]. The results of iron and aluminum loading were not related to the level of renal function as indicated by the lack of a significant correlation between the serum creatinine concentration and the (i) liver aluminum (r = −0.2634, NS), (ii) bone aluminum (r = −0.1073, NS), and (iii) liver iron (r = −0.1265, NS).

Peak serum ferrioxamine and aluminoxamine levels did not differ significantly, that is, 11.8 ± 4.5 versus 10.8 ± 5.2 μmol/liter. When iron- and aluminum-loaded dogs received desferrioxamine, a striking linear correlation was found between the serum t½, AUC and the serum creatinine and between the renal clearance of the *in vivo* formed aluminoxamine and ferrioxamine and the reciprocal value of serum creatinine (Table 4, Fig. 3). As indicated by the slopes of the linear regression curves, serum t½, AUC and renal clearance of aluminoxamine and ferrioxamine after desferrioxamine administration did not differ significantly. The cumulative biliary excretion of aluminoxamine after administration of desferrioxamine was unchanged when compared to that of dogs of group I which received the aluminoxamine/ferrioxamine mixture (0.21 ± 0.13 μmol/0 to 24 hr vs. 0.19 ± 0.21 μmol/0 to 24 hr). However, the biliary elimination of ferrioxamine significantly increased (P = 0.0022; Fig. 4) and, in the renal failure group of dogs (IIC-H; N = 6), approached or even exceeded the cumulative renal excretion [mean ± SD (range) cumulative biliary excretion 5.4 ± 3.4 (1.2 to 9.0) μmol/0 to 24 hr vs. cumulative urinary excretion 9.8 ± 4.8 (0.5 to 13.1) μmol/0 to 24 hr]. Hepatic clearance of the *in vivo* formed ferrioxamine and aluminoxamine was not affected by renal insufficiency (Fig. 3A, B). After i.v. administration of a dose used in clinical medicine, that is, 200 mg (15.3 ± 3.5 mg/kg) of desferrioxamine, 13 ± 16% of the administered desferrioxamine dose was recovered unchelated in the urine 24 hours after administration of the compound, while only 1.1 ± 0.7% of the initial desferrioxamine

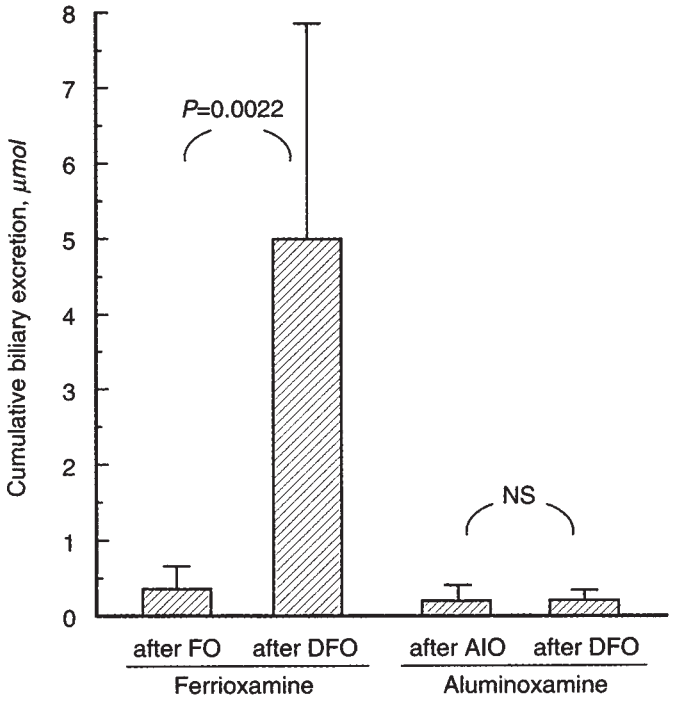

Fig. 4. *Comparison of the cumulative biliary excretion over 24 hours of ferrioxamine and aluminoxamine after i.v. administration of the compounds and after desferrioxamine.* Data are the mean ± SD of all dogs included in Groups I ($N = 8$) and II ($N = 8$).

dose appeared in the bile. Between 2.7 and 11.0% and between 2.9 and 4.3% of the initial desferrioxamine dose was excreted in the urine as aluminoxamine and ferrioxamine, respectively.

The C_{max} level of aluminoxamine was correlated with both the bone aluminum ($r = 0.8215$; $P = 0.0204$; $N = 8$) and the liver aluminum content ($r = 0.8337$; $P = 0.0182$; $N = 8$; Fig. 5). Slopes of these linear regression curves differed significantly ($P = 0.0006$). The C_{max} of ferrioxamine correlated with the liver iron content ($r = 0.7410$; $P = 0.0358$; $N = 8$). No relationship was noted between the C_{max} of ferrioxamine and the bone iron content (Fig. 5).

Discussion

Desferrioxamine has recently been introduced as a non-invasive diagnostic tool and the only available therapeutic agent for aluminum overload/toxicity in dialysis patients. In these subjects, however, the potential for desferrioxamine, presumably when chelated to iron, to predispose to infections or aggravate their severity has repeatedly been reported [10, 11, 23, 24]. On the other hand, it has been speculated that the aluminum chelated compound, that is, aluminoxamine by its ability to cross the blood/brain barrier might precipitate or exacerbate aluminum-related encephalopathy [8, 9, 25].

Prevention of these side effects during treatment of aluminum and iron overload requires a solid knowledge of elimination routes, distribution and sites of action of the chelator. In a previous study in dialysis patients, we noted an inter-dialytic decline in the serum ferrioxamine levels whereas the serum aluminoxamine concentrations remained stable after desferrioxamine administration. Moreover, when compared to dialysis patients with normal iron status we found that for ferrioxamine both the serum t½ and the AUC were increased in hemosiderotic dialysis patients, particularly when iron overload was combined with liver disease. Since this phenomenon could not be attributed to differences in residual renal function, the contribution of a extrarenal (biliary ?) route for ferrioxamine elimination was suggested.

To confirm our previous data and to determine the role of the liver in the elimination of ferrioxamine and aluminoxamine in the presence of either renal failure or intact renal function, the pharmacokinetics of these compounds were studied in dogs with normal renal function as well as in animals with various degrees of renal insufficiency. Studies in the literature dealing with these topics in humans [13, 14, 26, 27] and in animals [28–31] are scarce. They have mostly been carried out using methodology showing poor analytical performance [26, 32, 33] and were incapable of determining aluminoxamine.

Data in the present study are based on a validated indirect atomic absorption spectrometric method having great accuracy and precision [12]. Its low detection limits and high sensitivity were appropriate for the purpose of this study. The method was found to be superior [12] to these described using high performance liquid chromatography [34, 35], particularly in view of complexity and risk for contamination.

A reduction in renal mass was induced in dogs according to the method described by Vaneerdeweg et al [16]. All dogs underwent a ligation and catheterization of the bile duct allowing intermittent bile drainages during a three week period under sterile conditions. Serum alkaline phosphatase and total and direct bilirubin determinations together with the histological examination of liver biopsies [16, 18] revealed that the liver function was kept intact after ligation of the common bile duct. A comparable technique for bile collection in rats with normal renal function has been used by La Russo and Fowler [36] and Pippard, Johnson and Finch [28]. In these animals, however, repeated bile, urine and blood sampling is rather complex and sample volumes are limited being inadequate for use in longitudinal studies. The main advantages of the proposed dog model include the induction of a stable chronic renal failure allowing protracted biliary ligation/sampling in the absence of any interference with the renal and hepatic functions. It is of note that pharmacokinetic data reported in the present paper were obtained in the conscious animal, keeping metabolic functions intact.

After aluminoxamine and ferrioxamine administration, elimination of these compounds was mainly related to the renal function as indicated by the good correlation between the reciprocal value of the serum creatinine concentration and the renal clearance of these compounds.

Our results on the cumulative renal excretion of ferrioxamine agree with those noted by others in humans [37–39] reporting that approximately 50% of the ^{59}iron-labeled ferrioxamine was found in the urine 24 hours after i.v. administration of this compound. These data are in contrast to those of Keberle [31], who reported that dogs excreted 94% of ^{59}iron-labeled ferrioxamine in five hours, however, after i.v. injection of a very large dose (100 mg/kg vs. 4.7 ± 1.1 mg/kg in the present study). The above contradictory results must be interpreted keeping in mind the data of Allain et al [40] reporting the renal clearance of ferrioxamine to be greater in subjects with hemochromatosis as

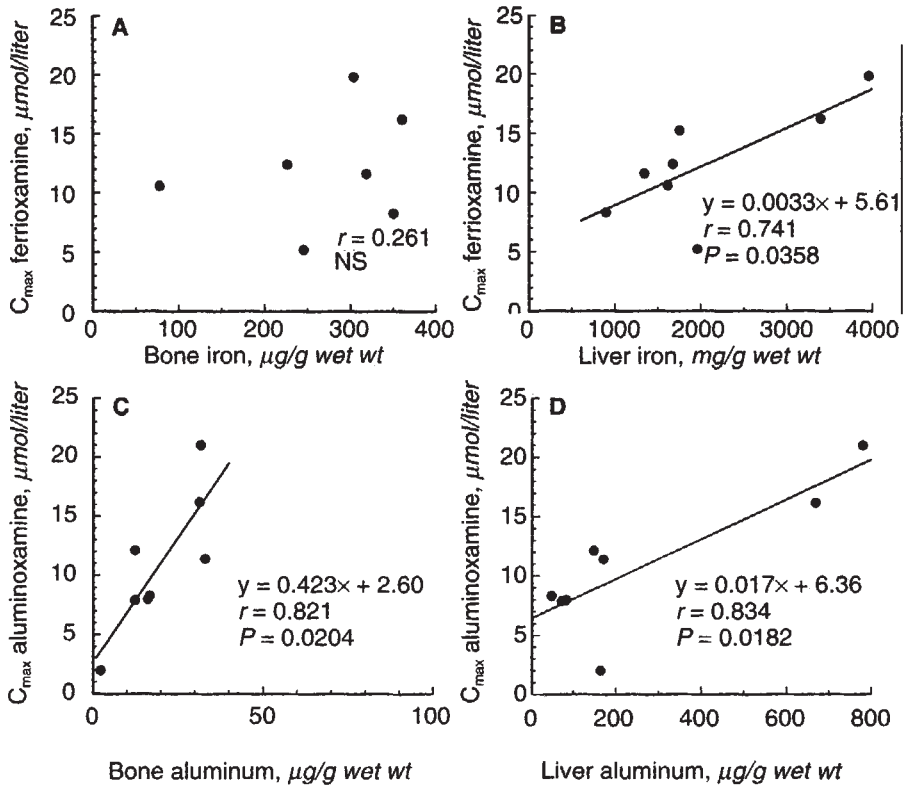

Fig. 5. *Correlation of the C_{max} of aluminoxamine and ferrioxamine with the bone and liver aluminum/iron concentrations after desferrioxamine administration to dogs.* Note that scales of X-Y plots differ.

compared to those with normal iron status, possibly representing a saturation effect of the renal tubular reabsorption mechanism of ferrioxamine that is known to occur at low concentrations of the compound in animals [29, 41]. Our results showing the renal clearance of the non-protein bound drugs aluminoxamine and ferrioxamine in dogs with normal renal function to be lower than the GFR, and the observation that the renal clearance was lower at lower C_{max} levels as noted in dogs of group II versus those of group I, suggest the possibility of a saturable tubular reabsorption mechanism that, however, needs further investigation.

No data are available in the literature which deal with the renal excretion of aluminoxamine. Our data on the cumulative renal excretion and the renal clearance of this compound indicate that, with respect to renal elimination and other pharmacokinetic parameters (Table 2), aluminoxamine and ferrioxamine behave in a similar fashion.

Only minute amounts of aluminoxamine and ferrioxamine substances injected as such were eliminated via the bile, demonstrating the negligible role of the liver in the elimination of these compounds. Furthermore, we noted that the hepatic clearance of aluminoxamine and ferrioxamine did not increase in dogs with reduced renal mass as compared to those with normal renal function.

A different situation exists when desferrioxamine was given to dogs preloaded with iron and aluminum. Whereas the biliary elimination of aluminoxamine did not change, ferrioxamine concentrations significantly increased in the bile after desferrioxamine as compared to when the chelated compound itself was administered. This is in agreement with data provided by Hershko [42], and supports the concept that desferrioxamine being a lipophilic molecule penetrates the liver cells resulting in intrahepatic chelation of iron followed by biliary excretion of ferrioxamine.

Although the liver represents an important storage site for aluminum, intrahepatic chelation of the element by desferrioxamine also appears to be limited. This suggests that chelation of aluminum by desferrioxamine takes place at more accessible sites such as the extracellular aluminum deposited at the osteoid calcified bone boundary. The fact that the value of the slope of the linear regression curve correlating C_{max} of aluminoxamine with the bone aluminum content is more than one order of magnitude greater than that noted for the liver aluminum concentration supports this interpretation. Moreover, it agrees with our unpublished observations in a dialysis patient given a weekly desferrioxamine dose for six months. In this subject, a remarkable decrease of the bone aluminum content was noted with only a slight decrease in the hepatic aluminum content.

Despite observations obtained *in vitro* which indicate that the affinity of desferrioxamine for iron is nine orders of magnitude greater than that for aluminum, the present data and other observations [13, 26] show little difference in their affinity for chelation by desferrioxamine *in vivo* based on the C_{max} of ferrioxamine/C_{max} of aluminoxamine ratio varying only between 0.8 and 1.7.

Our kinetic findings on the negligible biliary elimination of aluminoxamine and ferrioxamine after administration of these compounds are corroborated by the hydrophilicity of these molecules which makes them unlikely to cross the hepatocyte membrane. This is supported by our data showing that the

relative distribution volumes of both aluminoxamine and ferrioxamine are confined to the extracellular fluid. These observations confirm the inter-dialytic stability of the serum aluminoxamine levels [13]; however, they do not to any extent explain the steady inter-dialytic decline of serum ferrioxamine levels after desferrioxamine administration previously noted in dialysis patients, most of whom were without any renal function [13]. The fact that the biliary excretion of ferrioxamine after administration of the compound as such was negligible suggests the presence of an alternative mechanism in addition to the renal elimination. Possibly, cellular assimilation of ferrioxamine via an oxido/reductase mechanism by a specific ferrisiderophore receptor in close proximity of that described for transferrin [43–45] might be responsible for this phenomenon. Once transferrin, or in the actual situation ferrioxamine, is captured by its receptor, iron release from the siderophore at the level of the hepatocyte plasma membrane is thought to happen via reduction of ferric iron to its ferrous state, resulting in destabilization of the siderophore iron bond, liberating iron for cellular uptake or release into the serum [44], and involving a decrease of the serum ferrioxamine levels. The proposed mechanism is based on enzymatic activity and thus is saturable. This might explain the increased serum t½ of ferrioxamine in hemosiderotic dialysis patients as compared to those with normal iron status [13] and in nephrectomized dogs having received an increased ferrioxamine dose [29]. The hypothesis of a reductive mechanism for the release of iron from ferrioxamine is also supported by the *in vitro* observations of Emery [46] demonstrating that under reducing conditions and in the presence of an iron (2+) trapping agent the element can be displaced from ferrioxamine. Aluminum (3+) cannot be reduced to a lower valency state. Therefore, aluminoxamine, being chemically similar to ferrioxamine, is not subject to reductive degradation, which explains the stable inter-dialytic aluminoxamine levels in dialysis patients.

Overall, the results of our study indicate that the dog is a valuable model to study the renal and biliary elimination of desferrioxamine and its chelated compounds. Renal clearance of ferrioxamine and aluminoxamine is related to the renal function. Both compounds are confined to the extracellular space. Furthermore, biliary excretion of intravenously administered ferrioxamine and aluminoxamine is negligible even in renal failure. Our findings further provide indirect evidence that desferrioxamine administration to iron- and aluminum-loaded dogs results in the intrahepatic formation of ferrioxamine which is partly excreted in the bile. Biliary excretion of aluminoxamine after desferrioxamine administration however also is negligible.

Acknowledgments

This work was supported by a grant from the Ciba-Geigy Company, Basel, Switzerland. The authors are indebted to Erik Snelders and Eddy Van Hout for in-house editing and to Dirk De Weerdt for his excellent drawings. We greatly appreciate the advice on statistics of Guy Nuyts and Monique Elseviers. The advisory directions of Prof. Emeritus N. Buyssens were greatly appreciated. Furthermore, we are grateful to M. Nysten and R. Neirinckx for their technical assistance.

Reprint requests to Marc E. De Broe, M.D., Ph.D., Department of Nephrology-Hypertension, University of Antwerp, p/a University Hospital Antwerp, Wilrijkstraat 10, B-2650 Edegem/Antwerpen, Belgium.

References

1. Ackrill P, Ralston AJ, Day JP, Hodge KC: Successful removal of aluminum from a patient with dialysis encephalopathy. *Lancet* 2:692–693, 1980
2. Moeschlin S, Schnider U: Treatment of primary and secondary hemochromatosis and acute iron poisoning with a new, potent iron-eliminating agent (desferrioxamine-B). *N Engl J Med* 269:57–66, 1963
3. Nebeker HG, Milliner DS, Ott SM, Sherrard DJ, Alfrey AC, Abuelo JG, Wasserstein A, Coburn JW: Aluminum-related osteomalacia: Clinical response to desferrioxamine. (abstract) *Kidney Int* 25:173, 1984
4. Gallant T, Boyden MA, Gallant LA, Carley H, Freedman MH: Serial studies of auditory neurotoxicity in patients receiving desferrioxamine therapy. *Am J Med* 83:1085–1090, 1987
5. Simon P: Detoxification in hemosiderosis. *Blood Purif* 3:75–88, 1985
6. Walker JA, Sherman RA, Eisinger RP: Thrombocytopenia associated with intravenous desferrioxamine. *Am J Kidney Dis* 6:254–256, 1985
7. Koren G, Bentur Y, Strong D, Harvey E, Klein J, Baumal R, Spielberg SP, Freedman MH: Acute changes in renal function associated with deferoxamine therapy. *Am J Dis Child* 143:1077–1080, 1989
8. Mccauley J, Sorkin MI: Exacerbation of aluminum encephalopathy after treatment with desferrioxamine. *Nephrol Dial Transplant* 4:110–114, 1989
9. Yokel RA, Lidums V, Ungerstedt U: Aluminum mobilization by desferrioxamine assessed by microdialysis of the blood, liver and brain. *Toxicology* 66:313–324, 1991
10. Daly AL, Velazquez LA, Bradley SF, Kauffman CA: Mucormycosis: Association with deferoxamine therapy. *Am J Med* 87:468–471, 1989
11. Van Cutsem JA, Boelaert JR: Effects of deferoxamine, feroxamine and iron on experimental mucormycosis (zygomycosis). *Kidney Int* 36:1061–1068, 1989
12. D'haese PC, Lamberts LV, De Broe ME: Indirect measurement of desferrioxamine and its chelated compounds aluminoxamine and ferrioxamine by Zeeman atomic absorption spectrometry. *Clin Chem* 35:884–887, 1989
13. Verpooten GA, D'haese PC, Boelaert JR, Becaus I, Lamberts LV, De Broe ME: Pharmacokinetics of aluminoxamine and ferrioxamine and dose finding of desferrioxamine in haemodialysis patients. *Nephrol Dial Transplant* 7:931–938, 1992
14. Allain P, Chaleil D, Mauras Y, Beaudeau G, Varin MD, Poignet JL, Ciancioni C, Ang KS, Cam G, Simon P: Pharmacokinetics of desferrioxamine and of its iron and aluminium chelates in patients on haemodialysis. *Clin Chim Acta* 170:331–338, 1987
15. Canavese C, Gurioli L, D'amicone M, Cardelli R, Caligaris F, Bongiorno P, Arnaud A, Matiello G, Marchiori M: Kinetics of aluminoxamine and feroxamine chelates in dialysis patients. *Nephron* 60:411–417, 1992
16. Vaneerdeweg W, Buyssens N, De Winne T, Sebrechts M, Babloyan A, Arakelian S, De Broe ME: A standardized surgical technique to obtain a stable and reproducible chronic renal failure model in dogs. *Eur Surg Res* 24:273–282, 1992
17. D'haese PC, Van De Vyver FL, de Wolff FA, De Broe ME: Measurement of aluminum in blood, urine and tissues of chronic hemodialyzed patients by use of electrothermal atomic absorption spectrometry. *Clin Chem* 31:24–29, 1985
18. Vaneerdeweg W: A reproducible renal failure model in combination with a bile duct catheterisation model in the dog. (PhD Thesis) Antwerp, 1992
19. Lian L, D'haese PC, Lamberts LV, De Broe ME: Direct determination of iron in urine and serum using graphite furnace atomic absorption spectrometry. *Analyst* 114:143–147, 1989
20. Scandinavian Society for Clinical Chemistry and Clinical Physiology: Recommended methods for the determination of four enzymes in blood. *Scand J Lab Invest* 33:291–303, 1974
21. Dixon WJ: *BMDP Statistical Sofware*. Berkeley, University of California Press, 1989

22. Henry DA, Goodman WG, Nudelman RK, Didomenico NC, Alfrey AC, Slatopolsky E, Stanley TM, Coburn JW: Parenteral aluminum administration in the dog: I. Plasma kinetics, tissue levels, calcium metabolism and parathyroid hormone. *Kidney Int* 25:362–369, 1984
23. Boelaert JR, Fenvez AZ, Coburn JW: Deferoxamine therapy and mucormycosis in dialysis patients: Report of an international registry. *Am J Kidney Dis* 18:660–667, 1991
24. Abe F, Inaba H, Katoki T, Hotchi M: Effects of iron and desferrioxamine on Rhizopus infection. *Mycopathologia* 110:87–91, 1990
25. De Broe ME, D'haese PC, Van De Vyver FL: Aluminum toxicity, in *Handbook of Dialysis*, edited by Daugirdas JT, Ing TS, Boston, Little Brown, 1988, pp. 397–408
26. Stivelman J, Schulman G, Fosburg M, Lazarus JM, Hakim RM: Kinetics and efficacy of deferoxamine in iron-overloaded hemodialysis patients. *Kidney Int* 36:1125–1132, 1989
27. Pippard MJ, Callender ST, Finch CA: Ferrioxamine excretion in iron-loaded man. *Blood* 60:288–294, 1982
28. Pippard MJ, Johnson DK, Finch CA: Hepatocyte iron kinetics in the rat explored with an iron chelator. *Br J Haematol* 52:211–224, 1982
29. Peters G, Keberle H, Schmid K, Brunner H: Distribution and renal excretion of desferrioxamine and ferrioxamine in the dog and in the rat. *Biochem Pharmacol* 15:93–109, 1966
30. Lipschitz DA, Dugard J, Simon MO, Bothwell TH, Charlton RW: The site of action of desferrioxamine. *Br J Haematol* 20:395–404, 1971
31. Keberle H: The biochemistry of desferrioxamine and its relation to iron metabolism. *Ann NY Acad Sci* 119:758–768, 1964
32. Summers MR, Jacobs A, Tudway D, Perera P, Ricketts C: Studies in desferrioxamine and ferrioxamine metabolism in normal and iron-loaded subjects. *Br J Haematol* 42:547–555, 1979
33. Gillan AH, Lewis AO, Anderson RJ: Quantitative determination of hydroxamic acids. *Anal Chem* 53:841–844, 1981
34. Mattiello G, Rizzo F, Zoccolan R, Andriani M: High pressure liquid chromatography determination of low levels of free desferrioxamine and its iron and aluminum chelates. *Proc Eur Dial Transplant Assoc* 3:241–245, 1986
35. Kruck TPA, Kalow W: Determination of desferrioxamine and a major metabolite by high-performance liquid chromatography. Application to the treatment of aluminum-related disorders. *J Chromatogr* 341:123–130, 1985
36. La Russo NF, Fowler S: Coordinate secretion of acid hydrolases in rat biles. Hepatocyte exocytosis of lysosomal protein. *J Clin Invest* 64:948–954, 1979
37. Bannerman RM, Callender ST, Williams DL: The effect of desferrioxamine and DTPA in iron overload. *Br Med J* 2:1573–1577, 1962
38. Keller MM: Experience to date with desferrioxamine in the treatment of diseases involving a pathological depostion of iron in the organism, in *A Report on a Meeting of Clinical Investigators*, compliled by Imhof P, Basle, 1961
39. Hedenberg L: Studies on iron metabolism with desferrioxamine in man. *Scand J Haematol* 6:5–86, 1969
40. Allain P, Mauras Y, Chaleil D, Simon P, Ang KS, Cam G, Le Mignon L, Simon M: Pharmacokinetics and renal elimination of desferrioxamine and ferrioxamine in healthy subjects and patients with haemochromatosis. *Br J Clin Pharmacol* 24:207–212, 1987
41. Pippard MJ: Desferrioxamine-induced iron excretion in humans. *Clin Hematol* 2:323–343, 1990
42. Hershko C: Determination of fecal urinary iron excretion in desferrioxamine-treated rats. *Blood* 51:415–423, 1978
43. Crichton RR, Charloteaux-Wauters M: Iron transport and storage. *Eur J Biochem* 164:485–506, 1987
44. Thorstensen K, Romslo I: The role of transferrin in the mechanism of cellular iron uptake. *Biochem J* 271:1–10, 1990
45. Sun IL, Navas P, Crane FL, Morré DJ, Low H: NADH diferric transferrin reductase in liver plasma membrane. *J Biol Chem* 262:15915–15921, 1987
46. Emery T: Exchange of iron by gallium in siderophores. *Biochemistry* 25:4629–4633, 1986