Asian Journal of Chemistry Vol. 21, No. 6 (2009), 4583-4592

Evaluation of Urinary Excretion and Renal Clearance of Deferiprone, Creatinine, Iron and Zinc in Human

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Iron chelators are used in medicine to protect patients from the consequences of iron overload and iron toxicity. Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one), which belongs to the family of α-ketohydroxypyridines, is an oral iron chelator that is used clinically, mainly in patients with thalassemia. The urinary excretion and renal clearance of deferiprone was investigated in 24 healthy male volunteers following oral administration of a single dose 500 mg. Concentration of deferiprone in plasma and urine was determined by high performance liquid chromatography, iron and zinc by atomic absorption spectrophotometer and the concentration of creatinine by chemistry analyzer. Total amount of deferiprone excreted in 24 h was 13.7 ± 1.24 mg. The renal clearance of endogenous creatinine was 0.90 ± 0.11 mL/min/Kg and deferiprone was 0.29 ± 0.04 mL/min/Kg being about one third of the filtration clearance. The renal excretory mechanisms involved glomerular filtration, excreting only 2.73 % of the oral dose of deferiprone through urine. Lower values of the deferiprone renal clearance than the GFR indicate that the excretion of the drug through kidneys involves glomerular filtration and extensive renal tubular back diffusion or reabsorption. The literature for excretion and renal clearance parameters of deferiprone is inadequate; however similarities and differences were both observed when the present findings were compared with the cited results.

Key Words: Deferiprone, Excretion, Renal clearance, Creatinine.

INTRODUCTION

Thalassemia (β-thalassemia) is one of the most common genetic defects seen in certain populations[1]. Transfusional iron overload is a major cause of morbidity and mortality in thalassemia, sickle cell disease and other chronic anemias. Regular transfusions deliver between 0.3 and 0.5 mg of iron per kg per day or nearly 10 g per year in a 70 kg man[2]. Iron chelation is needed to prevent damage to the heart, liver and endocrine glands from iron overload in patients with refractory anemia's who receive regular blood transfusions[3,4].

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The orally active iron-chelating agent, deferiprone (1,2-dimethyl-3-hydroxy-pyridin-4-one) has been used in human trials for the treatment of transfusional iron overload[5]. Deferiprone belongs to a group of bidentate chelators known as the α-keto-hydroxypyridones. The high activity of these compounds is probably related to their ability to quickly diffuse across cell membranes and chelate intracellular iron pools[6,7].

Deferiprone is metabolized primarily in the liver into its glucuronide conjugate that has no iron chelating properties[8]. Deferiprone is excreted in the urine in various forms, mainly as a glucuronide conjugate, in an unchanged form, bound to iron and to a lesser extent bound to other metals. Iron chelation precedes glucuronidation and that the major factor influencing iron mobilization is the availability of the chelatable iron pools and not the extent of glucuronidation of deferiprone[9]. Deferiprone and its metabolites are excreted in the urine and neither deferiprone nor their glucuronide metabolites have yet been identified in the faeces of patients nor was there an increase in faecal iron[10,11].

Environmental influences on glomerular filtration rate, blood composition, blood and urine pH in human significantly influenced the disposition and fate of drugs and were described by the original term geonetics; the geographical influences on genetics[12]. Geonetical influences create an awareness of the need for studying renal clearance, urinary excretion and fate of drugs in target species and environments. Deferiprone is used for the chelation of iron in human. Extensive literature was reviewed and found that no such work has been done previously that's why the present work was undertaken therefore, its renal clearance and urinary excretion were investigated in human.

EXPERIMENTAL

Subjects: Twenty four healthy male subjects ranging in age from 18 to 39 years (25.21 ± 1.49 years), in weight from 49 to 80 Kg (58.96 ± 2.03 Kg) and in height from 61.0 to 70.0 inches (66.17 ± 0.50 inches) were enrolled in this study. The study was conducted in accordance with good clinical practice guidelines. Subjects enrolled for this study were apprised in details about all aspects of the study. Those who agreed voluntarily and provided written 'informed consent' were registered. The study was designed and conducted according to principles of good clinical practice (GCP) keeping in view the national legal requirements, the ICH harmonized tripartite guideline for good clinical practice and the ethical principles laid down in the declaration of Helsinki[13].

Drug administration: The drug deferiprone commercially known Ferriprox® in the dosage form of oral tablets 500 mg each, manufactured by Apotex Europe Ltd., UK was used.

Sample collection: Before drugs administration, a control/blank urine sample was collected from each subject. Following drug administration, blood and urine samples were collected at pre-determined time intervals. The samples were kept frozen in deep freezer at < 20 °C until analysis.

Analysis: The concentration of deferiprone in blood and urine were determined by high performance liquid chromatography as described by Goddard and Kontoghiorghes[14], with some modifications. The development and validation of the method was performed on a Shimadzu high performance liquid chromatography system (LC-10A), Shimadzu Corporation, Kyoto, Japan. The analytical column used to achieve chromatographic separation was a stainless steel (C_{18}) column, Discovery Supelco (25 cm × 4.6 mm, 5 µm, Bellefonte, USA) protected by a guard column of the same material. The compounds were separated isocratically with a mobile phase consisting of (18:82) methanol:potassium phosphate buffer (50 mM) containing heptane sulfonic acid (5 mM). The pH was adjusted to 3.5 ± 0.1 using phosphoric acid. A constant flow rate of 1.2 mL/min was maintained. The effluent was monitored spectrophotometrically at a wavelength of 280 nm.

The concentration of iron and zinc were determined by atomic absorption spectrophotometer (AAS) on a Perkin-Elmer Analyst 300 using air-acetylene flame. The concentration of endogenous creatinine in the plasma and urine samples was determined by the method described by Bonsnes and Taussky[15]. Chemistry analyzer (Semar, Japan) was used for the determination of creatinine using a creatinine kit by Jaffe reaction.

Calculations: The HPLC acquisition software (Class LC-10) was used for the qualitative and quantitative determination of deferiprone. Other calculations and graphs were made using Microsoft Excel, 2006 software. The renal clearance of endogenous creatinine was used for the estimation of glomerular filtration rate (GFR). Renal clearance was calculated as given by Swenson[16]. Influence of urine pH, rate of urine flow and plasma drug concentration on the renal clearance of drug was examined by regression/correlation analysis.

Statistical analysis: The statistical calculations were carried out according to the standard method and results have been presented as mean ± SE[17].

Safety: The study was conducted in accordance with good clinical practice guidelines (GCPG). According to the protocol approved by Ethics Committee, the safety examination of volunteers was recorded on case record form by doctors. All volunteers completed the study without referring any abnormality.

RESULTS AND DISCUSSION

Urinary excretion

Deferiprone: Maximum concentration 42.6 ± 9.35 µg/mL of deferiprone was observed at 3 h after drug administration. The average amount of deferiprone (mg) excreted in urine at different time interval has been shown in Table-1. Maximum excretion (mg) of deferiprone was observed 3.67 ± 0.58 mg at 6 h and minimum amount of deferiprone excreted 0.75 ± 0.21 mg at 0.5 h. After 24 h of drug administration, the total drug excreted in urine was 2.73 ± 0.25 %. Average rate of excretion of deferiprone was 0.86 ± 0.16 µg/min/Kg at 1.0 h and 0.02 ± 0.004 µg/min/Kg at 24 h of drug administration in urine of healthy male subjects. There are limited literatures about excretion parameters in normal human volunteers.

TABLE-1
MEAN ± SE (n = 24) VALUES FOR AMOUNT EXCRETED OF DEFERIPRONE IN URINE OF NORMAL MALE VOLUNTEERS AFTER DRUG ADMINISTRATION OF 500 mg

Time (h)	Amount (mg)	Drug (%)	Cumulative amount (mg)	Cumulative (%)	Rate of excretion (µg/min/Kg)
0.5	0.75 ± 0.21	0.15 ± 0.04	0.75 ± 0.21	0.15 ± 0.04	0.45 ± 0.130
1.0	1.51 ± 0.30	0.30 ± 0.06	2.23 ± 0.44	0.45 ± 0.09	0.86 ± 0.160
2.0	2.79 ± 0.43	0.56 ± 0.09	4.93 ± 0.70	0.99 ± 0.14	0.82 ± 0.120
3.0	2.86 ± 0.27	0.57 ± 0.05	7.79 ± 0.86	1.56 ± 0.17	0.83 ± 0.090
6.0	3.67 ± 0.58	0.73 ± 0.12	11.5 ± 1.16	2.29 ± 0.23	0.34 ± 0.050
12.0	1.44 ± 0.15	0.29 ± 0.03	12.9 ± 1.21	2.58 ± 0.24	0.07 ± 0.010
24.0	0.79 ± 0.16	0.16 ± 0.03	13.7 ± 1.24	2.73 ± 0.25	0.02 ± 0.004

Iron: Maximum concentration of iron in urine 33.6 ± 7.62 µg/mL was observed at 6 h post dosing of deferiprone. The maximum urinary excretion for the iron was determined to be 4.41 ± 1.08 mg at 24 h post dosing of deferiprone. The minimum drug excretion was 0.87 ± 0.28 mg at 0.5 h after administration of deferiprone. The average rate of excretion of iron after 24 of drug administration in urine of healthy male subjects was 0.10 ± 0.02 µg/min/Kg presented in Table-2.

TABLE-2
MEAN ± SE (n = 24) VALUES FOR AMOUNT EXCRETED OF IRON IN URINE OF NORMAL MALE VOLUNTEERS AFTER DRUG ADMINISTRATION OF 500 mg

Iron **e**xcreted			
Time (h)	Amount (mg)	Cumulative amount (mg)	Rate of excretion (µg/min/Kg)
0.5	0.87 ± 0.28	0.87 ± 0.28	0.52 ± 0.18
1.0	1.04 ± 0.28	1.76 ± 0.52	0.62 ± 0.18
2.0	1.78 ± 0.37	3.47 ± 0.84	0.53 ± 0.11
3.0	3.58 ± 1.02	7.05 ± 1.43	0.93 ± 0.22
6.0	3.81 ± 0.90	10.9 ± 2.18	0.36 ± 0.08
12.0	4.07 ± 1.07	14.9 ± 3.03	0.18 ± 0.04
24.0	4.41 ± 1.08	18.8 ± 3.96	0.10 ± 0.02

Zinc: Maximum concentration of zinc in urine 1.52 ± 0.25 µg/mL was observed at 12 h post dosing of deferiprone. The average ± SE values for zinc excreted in urine at 0.5, 1.0, 2.0, 3.0, 6.0, 12 and 24 h after administration of drug to normal male subjects (Table-3). The maximum urinary excretion for the zinc was determined to be 0.46 ± 0.14 mg at 24 h post dosing of deferiprone. The minimum drug excretion was 0.04 ± 0.01 mg at 0.5 h after administration of deferiprone. The average ± SE cumulative amount of zinc excreted in urine samples of normal volunteers was 0.21 ± 0.03 mg after 2 h, 0.42 ± 0.05 mg after 6 h and 1.01 ± 0.15 mg after 24 h of

drug administration. After 3 h of drug administration, the average value for rate of excretion of zinc in 24 male volunteers was 0.03 ± 0.007 µg/min/Kg. The average value for rate of excretion of zinc after 24 h of drug administration in urine of healthy male subjects was 0.01 ± 0.003 µg/min/Kg.

TABLE-3
MEAN ± SE (n = 24) VALUES FOR AMOUNT EXCRETED OF ZINC IN URINE OF NORMAL MALE VOLUNTEERS AFTER DRUG ADMINISTRATION OF 500 mg

Zinc **e**xcreted			
Time (h)	Amount (mg)	Cumulative amount (mg)	Rate of excretion (µg/min/Kg)
0.5	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.006
1.0	0.07 ± 0.01	0.10 ± 0.02	0.04 ± 0.008
2.0	0.11 ± 0.02	0.21 ± 0.03	0.03 ± 0.006
3.0	0.13 ± 0.03	0.33 ± 0.04	0.03 ± 0.007
6.0	0.09 ± 0.02	0.42 ± 0.05	0.01 ± 0.002
12.0	0.25 ± 0.05	0.64 ± 0.09	0.01 ± 0.003
24.0	0.46 ± 0.14	1.01 ± 0.15	0.01 ± 0.003

Renal clearance: The renal clearance of endogenous creatinine, deferiprone, iron and zinc were investigated in four experimental periods in each experiment/ volunteer. The renal clearance of endogenous creatinine was measured as an index of glomerular filtration rate (GFR). The mean results showing diuresis, blood and urine pH, plasma and urine concentrations of endogenous creatinine, deferiprone, iron and zinc and its renal clearance have been presented in Table-4.

The renal clearance of endogenous creatinine was 0.90 ± 0.11 mL/min/Kg. The renal clearance of deferiprone was 0.29 ± 0.04 mL/min/Kg. The ratio between the clearance of deferiprone with the clearance of endogenous creatinine was 0.57 ± 0.11. Relationship of renal clearance of deferiprone to the urine pH, diuresis and plasma concentration of deferiprone are presented in Figs. 2-4. There was significant positive correlation between diuresis and deferiprone clearance (Fig. 2). As the rate of urine flow increases, rate of deferiprone clearance also increases. No correlation was observed between the urine pH and renal clearance of deferiprone as shown in Fig. 3. However, negative correlation was observed between the plasma concentrations of deferiprone with the renal clearance of deferiprone as shown in Fig. 4.

Mean values of 4 observations in 4 experimental periods for the concentration of iron and zinc in plasma were 1.01 ± 0.08 and 0.87 ± 0.02 µg/mL, respectively. While the mean ± SE concentrations of iron and zinc in urine was 23.4 ± 4.29 and 1.24 ± 0.15 µg/mL, respectively. The renal clearance of iron was 0.75 ± 0.17 mL/ min/Kg while the renal clearance of zinc was 0.034 ± 0.005 mL/min/Kg.

Deferiprone was found to undergo extensive phase II metabolism accounted for greater than 85 % of the dose administered in man and the unmetabolized deferiprone amounted to 4 % of the dose administered in man. A large portion of

TABLE-4
MEAN ± SE VALUES FOR THE RENAL CLEARANCE OF IRON, ZINC, ENDOGENOUS CREATININE AND DEFERIPRONE FOLLOWING ORAL ADMINISTRATION OF 500 mg DEFERIPRONE IN HUMAN MALE SUBJECTS

Parameters and Units	Mean ± SE
Diuresis mL/min/Kg	0.03 ± 0.004
pH	
Blood	7.4 ± 0.01
Urine	5.7 ± 0.12
Iron	
Plasma conc. µg/mL	1.01 ± 0.08
Urine conc. µg/mL	23.4 ± 4.29
Renal clearance mL/min/Kg	0.75 ± 0.17
Zinc	
Plasma conc. µg/mL	0.87 ± 0.02
Urine conc. µg/mL	1.24 ± 0.15
Renal clearance mL/min/Kg	0.034 ± 0.004
Creatinine	
Plasma conc. µg/mL	8.73 ± 0.28
Urine conc. µg/mL	340 ± 32.0
Renal clearance mL/min/Kg	0.90 ± 0.11
Deferiprone	
Plasma conc. µg/mL	3.23 ± 0.27
Urine conc. µg/mL	38.6 ± 6.82
Renal clearance mL/min/Kg	0.29 ± 0.04
Clearance ratio	
$Cl_{Deferiprone}/Cl_{Creatinine}$	0.57 ± 0.11

Correlation/Regression output: Y = Renal clearance of drug

When X =	Diuresis	Urine pH	Drug	Iron	Zinc
Intercept	0.16	0.73	0.42	0.24	0.45
Slope	3.93	-0.08	-0.04	0.04	-0.20
Correlation	0.35	-0.20	-0.36	0.07	-0.11
r^2	0.12	0.04	0.13	0.004	0.01

the dose is therefore probably eliminated *via* the bile[18,19]. In people, deferiprone is metabolized in the liver predominately (> 85 %) by glucuronidation to a conjugate that lacks chelating properties[20]. Deferiprone is inactivated (more than 85 %) by glucuronidation; the glucuronide derivative is also excreted in urine[21]. Another study demonstrated that only 4 % of a single oral dose of the drug is excreted bound to iron, even in heavily iron-loaded patients[7].

In the present study 2.73 ± 0.25 % dose of deferiprone is excreted in urine of 24 healthy subjects. It appears that only a small fraction of the administered dose of deferiprone appears in the urine unchanged within 24 h period while the remainder may be excreted as metabolite(s), through sweat, excreted in feces or is unabsorbed. Previous literature is deficient in urinary excretion parameters observed particularly in normal human. Some similarities were, however, observed when present values were compared with the values available in literature.

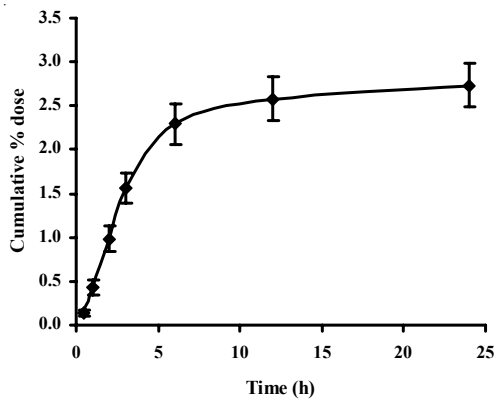

Fig. 1. Mean ± SE cumulative percentage of deferiprone excreted in the urine of 24 healthy male subjects at various time intervals following oral dose of 500 mg

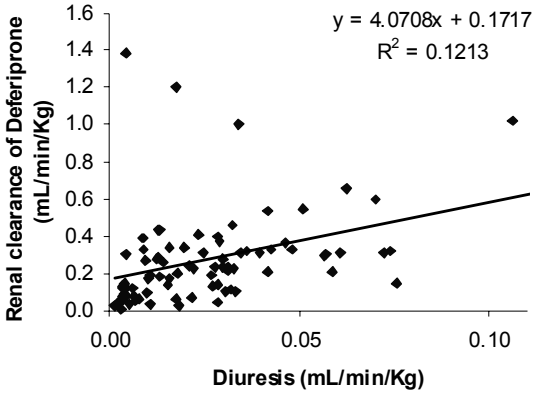

Fig. 2. Relationship between diuresis and renal clearance of deferiprone after oral dose of 500 mg tablet

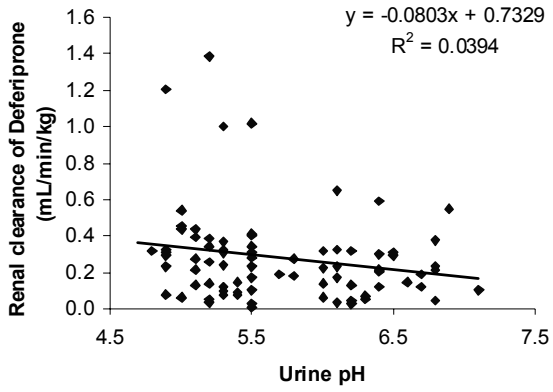

Fig. 3. Relationship between pH of urine and renal clearance of deferiprone after oral dose of 500 mg tablet

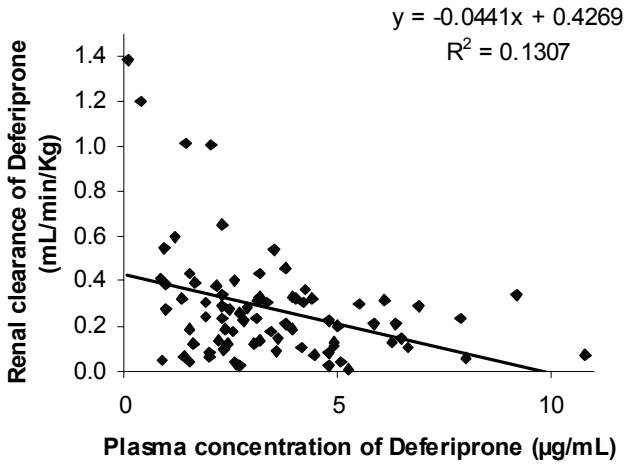

Fig. 4. Relationship between plasma concentration of drug and renal clearance of deferiprone after oral dose of 500 mg tablet

Urinary iron excretion (UIE) during 24 h in response to an iron chelator may be very useful in monitoring the iron chelation efficacy. Urinary iron excretion depends only in part on the level of iron overload[22]. Also the iron excretion increases with the dose of the drug[23,24]. Only a small fraction of body iron is available for chelation at any given time, as the majority of storage iron is not effectively chelated at clinically achievable chelator concentrations[25]. Urinary iron excretion increased with the dose and with the degree of iron loading of the patient. Giving 2 or 3 divided doses over 24 h resulted in higher urinary iron excretion than a single dose of the same amount over the same time[23]. Urinary iron excretion was proportional to the iron load but not to the serum or urine concentration of deferiprone[21].

Being a chelator of iron, it is possible that the drug may also chelate other metallic ions. Deferiprone also binds to aluminum, zinc and copper, but the stability of deferiprone complexes with these other cations is < 50 % of its binding stability with ferric iron, so the compound strongly favours interaction with iron over these other trace metals[19]. A few studies have indicated that deferiprone may, in addition to iron, chelate zinc as well. This is an important issue since zinc plays an important role in several physiological functions; protein synthesis, gene expression, immunity, wound healing and maintenance of integrity of intra-cellular organelles[26]. However, limited information is available regarding the effect of deferiprone administration on zinc status[27]. Hence, the effect of deferiprone therapy on urinary excretion of zinc was also determined in this study. The 24 h urinary excretion of zinc was significantly higher in children receiving deferiprone[27]. It was found that long-term use of deferiprone result in depletion of body zinc results in low zinc levels in the blood[27-30]. There is no significant relationship between dose of deferiprone and duration of chelation therapy and urinary zinc excretion[27]. Al-Refaie *et al.*[29] also did not find correlation between the dose of deferiprone and the extent of urinary zinc excretion.

The renal clearance of endogenous creatinine was 0.90 ± 0.11 mL/min/Kg and the average renal clearance of deferiprone was 0.29 ± 0.04 mL/min/Kg (0.017 ± 0.002 L/min) less than the renal clearance of deferiprone (0.038 ± 0.023) studied by Thuma *et al.*[30]. The ratio between the clearances of deferiprone with the clearance of endogenous creatinine was 0.57 ± 0.11. Lower values of the deferiprone renal clearance than the GFR indicate that the excretion of the drug through kidneys involves glomerular filtration and extensive renal tubular back diffusion or reabsorption[31]. There was significant positive correlation between diuresis and deferiprone clearance. No correlation was observed between urine pH and renal clearance of deferiprone in the present study. However, negative correlation was observed between the plasma concentrations of deferiprone with the renal clearance of deferiprone indicative of active tubular reabsorption.

In the present study, it was found that there was no correlation (r = 0.07) between renal clearance of deferiprone and concentration of iron. It was also observed no correlation between the concentrations of deferiprone and iron in the urines studied[9]. Similarly no correlation (r = -0.11) was observed between renal clearance of deferiprone and concentration of zinc in the present study. Also there was no correlation between the rate of urine flow, urine pH and zinc concentration with the renal clearance of zinc. The glomerular filtration rate also shows significant differences between the two, summer and winter seasons that affects the urinary excretion of drugs and has been attributed to hemoconcentration in summer[12]. Lower glomerular filtration rate under indigenous conditions warrants the exploration of role of kidneys in the maintenance of milieu interieur and excretion of endogenous and exogenous substances including drugs.

Conclusion

A significant positive correlation between diuresis and deferiprone clearance was observed in the present study. No correlation was observed between urine pH and renal clearance of deferiprone. However, negative correlation was observed between the plasma concentrations of deferiprone with the renal clearance of deferiprone indicative of active tubular reabsorption. Similarly, it was found that there was no correlation between renal clearance of deferiprone and concentration of iron and zinc. The literature for excretion and renal clearance parameters of deferiprone is inadequate. However, similarities and differences were both observed when the present findings were compared with the cited results.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the cooperation of Nuclear Institute for Agriculture and Biology (NIAB) using the laboratory facilities and also thankful to the Higher Education Commission (HEC), Islamabad, Government of Pakistan for financial support for this project.

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(*Received*: 21 July 2008; *Accepted*: 23 March 2009) AJC-7368