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RESEARCH ARTICLE

Effect of deferiprone, an oral iron chelator, in diabetic and non-diabetic glomerular disease

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Abstract

Compelling experimental evidence exists for the role of oxidants and iron in glomerular disease. In preliminary studies, we confirmed increased urinary catalytic iron in patients with glomerulonephritis and diabetic nephropathy. We conducted two separate single-center, prospective, single-armed, open-labeled, proof-of-concept studies to evaluate the safety and efficacy of an oral iron chelator in patients with glomerulonephritis and diabetic nephropathy. Study 1 comprised 15 patients with biopsy-proven glomerulonephritis who had persistent proteinuria despite treatment with steroids and/or cyclophosphamides. Study 2 comprised 38 adult patients with diabetic nephropathy. Patients in Study 1 were treated with deferiprone (50 mg/kg/day) in three divided doses for 6 months and Study 2 patients were treated for 9 months. In Study 1, two patients had severe gastrointestinal intolerance and withdrew from the study after one dose and are not included in the results. There was a significant reduction (47 ± 9% mean) in 24-h urinary protein (4.01 ± 1.61 to 2.21 ± 1.62 [$p = 0.009$]), with no significant changes in serum creatinine. In Study 2, treatment with deferiprone resulted in a marked, persistent drop in the mean albumin/creatinine ratio (187 ± 47 at baseline to 25 ± 7 mg/g, [$p = 0.01$]) and stable renal function over a 9-month period. No clinically significant adverse events were observed in either study. Although these are small, open-labeled, and non-randomized studies, our results suggest that future randomized, double-blind trials examining the utility of deferiprone to treat glomerular diseases appear warranted.

Keywords: Glomerulonephritis, diabetic nephropathy, oral iron chelator, deferiprone, proteinuria

Introduction

Chronic kidney disease is a worldwide public health problem that affects approximately 10% of the adult population (Coresh et al. 2007) and is associated with a high prevalence of cardiovascular disease (Sarnak et al. 2003) and high economic cost (Xue et al. 2001; Szczech & Lazar 2004). Despite the use of drugs that block the renin-angiotensin system, diabetic nephropathy and other glomerular diseases continue to be major causes of chronic kidney disease worldwide.

A large body of experimental evidence exists for the role of oxidants and iron in various glomerulopathies (Shah et al. 2007). The ease with which iron is reversibly oxidized or reduced, while essential for its metabolic functions, also makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species and/or highly reactive iron-oxygen complexes such as ferryl or perferryl species (Halliwell & Gutteridge 1990). Animal studies have provided considerable evidence for the role of iron in glomerular disease (Boyce & Holdsworth 1986; Thakur et al. 1988; Shah 1988; Ueda et al. 1996; Baliga et al. 1996; Shah et al. 2007). In a model of minimal-change disease, we have shown that labile iron capable of catalyzing free-radical reactions is markedly increased in glomeruli (Ueda et al. 1996) and that an iron chelator prevented an increase in catalytic iron in glomeruli and

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provided protection against proteinuria (Thakur et al. 1988; Ueda et al. 1996). Passive Heymann nephritis, induced by a single intravenous injection of anti-Fx1A, is a complement-dependent and neutrophil-independent model of glomerular disease that resembles membranous nephropathy in humans. The protective effect of an iron chelator (Shah 1988) as well as an iron-deficient diet (Baliga et al. 1996) suggests a role of iron in membranous nephropathy. Iron chelation has also been effective in an immune model of proliferative glomerulonephritis, anti-GBM antibody disease (Boyce & Holdsworth 1986). Iron-deficient diets in rats have shown to reduce proteinuria in a model of spontaneously-developed glomerulosclerosis (Remuzzi et al. 1991).

There is a large body of evidence indicating that diabetes is a state of increased oxidative stress (Nishikawa et al. 2000; Hartnett et al. 2000; Matteucci & Giampietro 2001; Shin et al. 2001; Pennathur et al. 2001; Hinokio et al. 2002; Nishikawa et al. 2003; Shah et al. 2007). Iron content in the kidney has been shown to be increased in an animal model of diabetes (Johnson & Evans 1984) and urinary iron excretion is increased early in the course of diabetic renal disease in humans (Howard et al. 1991).

There is limited information on the role of iron in human disease. In preliminary studies, using the bleomycin-detectable iron assay to detect iron capable of catalyzing free-radical reaction (Shah et al. 2007), we have shown a marked increase in catalytic iron in patients with biopsy-proven glomerulonephritis and diabetic nephropathy (Shah et al. 2007). We conducted two prospective, open-labeled, proof-of-concept studies to evaluate the effect of an oral iron chelator, deferiprone, in patients with biopsy-proven glomerulonephritis (Study 1) and diabetic nephropathy (Study 2).

Experimental analysis

Study 1 design

We conducted a single-center, prospective, single-armed, open-labeled, proof-of-concept study at the Muljibhai Patel Urological Hospital in Nadiad, Gujarat, India to evaluate the safety and efficacy of an oral iron chelator, deferiprone, in patients with biopsy-proven glomerulonephritis. The institutional review board and ethical committee approved the protocol and all patients gave informed consent. The medical records of patients were reviewed including renal biopsy reports, treatment history with steroids/cyclophosphamide, co-morbidities, blood pressure measurements, and laboratory data during the 6 months prior to enrollment. The baseline laboratory tests included hemoglobin, complete blood count and differential count, chemistry profile, hepatic panel, lipid profile, hepatitis B and C and HIV serologies, ANA, ANCA, and urine microscopy, culture, and 24-h collections for protein. All patients were treated with deferiprone (50 mg/kg/day) in three divided doses for 6 months.

The inclusion criteria for this study were biopsy-proven glomerulonephritis and persistent proteinuria (>1 g/24 h) in patients who had received steroids and/or cyclophosphamides. The exclusion criteria were clinical or laboratory evidence of secondary glomerular disease; diabetic kidney disease; evidence of significant hepatic disease, as indicated by serum bilirubin and serum transaminases more than twice the upper limit of normal; and patients having hemoglobin levels <10 g/dL.

We enrolled 15 patients with glomerulonephritis who had persistent proteinuria despite treatment with steroids and/or cyclophosphamides. Two patients complained of severe nausea and vomiting after administration of the first dose of the drug and did not participate in the study. Compliance was assessed with pill counting. Patients were instructed to supplement a missed dose at the time of the subsequent dose (recorded as a delay but not as noncompliance) but not to supplement more than one missed dose. All of the results are presented as mean ± SEM. Comparison of the parameters during baseline and at the end of 6 months was compared using a paired *t*-test. A p value of less than 0.05 was considered to be statistically significant.

Study 2 design

A single-center (Baroda Medical College, Gujarat, India), single-armed, open-labeled, proof-of-concept study was conducted to evaluate the safety and efficacy of oral deferiprone in reducing albuminuria in patients with diabetic nephropathy. Adult patients with a diagnosis of diabetes mellitus and a urinary albumin/creatinine ratio >30 μg/mg on two different occasions were included in the study. A commercial radioimmunoassay kit (Immunotech, France) with monoclonal anti-albumin antibody-coated tubes was used for the detection and quantification of urine albumin. Inclusion criteria were the presence of albuminuria (urinary albumin/creatinine ratio >30 μg/mg) on two occasions more than 3 months apart and serum creatinine levels <1.4 mg/dL. Exclusion criteria were patients with a hematocrit of <30% and iron saturation of <–10%, WBC of <3000/mm^3, and presence of active urinary tract infection or urinary sediment. The endpoints monitored were change in urinary albumin/creatinine ratio (primary endpoint), change in serum creatinine, mean arterial blood pressure, and HbA_{1c}. The safety endpoints studied were liver function tests and WBC. In addition, we evaluated the effect of deferiprone on hemoglobin and serum iron.

Patients received standard of care and enalapril, which was kept constant throughout the study. During the study the recommended treatment goals were for blood pressure <130/80 mm Hg and hemoglobin A_{1c} <7%. Deferiprone was administered in a daily dose of ~50 mg/kg in three divided doses for 9 months. The protocol was approved by the institutional review board and all participants gave informed consent. Statistical analysis was performed using SPSS14®. For continuous variables a Student's *t*-test was used and for categorical variables, χ^2 and Fisher's exact tests were used as appropriate. For repeated measurements over time, repeated-measures

analysis of variance (ANOVA) was applied and p values were adjusted for repeated measurements. All test results reported were two-tailed and a p value <0.05 was considered significant.

Study 1 results

Of the 15 patients enrolled in Study 1, two patients withdrew due to gastrointestinal intolerance after the first day. Thirteen patients (six males and seven females) who completed the study were considered for analysis. The age at study entry was 26.8 ± 4 years; four were pediatric subjects. Among the 13 patients, four had membranous nephropathy, three had focal segmental glomerulosclerosis (FSGS), three had diffuse proliferative glomerulonephritis, two had mesangial proliferation, and one had chronic sclerosing glomerulonephritis. These patients had persistent proteinuria despite receiving steroids (seven subjects) or steroids and cyclophosphamides (six subjects) (Table 1).

Deferiprone therapy reduced the proteinuria from 4.05 ± 0.46 g/day (range 1.2–6.5) to 2.21 ± 1.61 g/day by 6 months, an overall reduction of 47 ± 9% (Figure 1). Seven of 13 subjects had >50% improvement in proteinuria and three had <600 mg/day proteinuria after therapy (Figure 1, Table 1).

The mean baseline serum creatinine at enrollment was 1.05 ± 0.14 mg/dL. After deferiprone therapy, four subjects had an improvement (>0.3 mg/dL reduction) in serum creatinine, eight subjects had no significant change, and one subject had worsening (>0.3 mg/dL rise) in serum creatinine (Figure 1). This increase from 0.9 mg/dL to 2.0 mg/dL started after the 5th month of therapy and did not improve after stopping deferiprone. A repeat biopsy performed after 2 months of stopping deferiprone showed the presence of crescents.

All patients were monitored clinically and with laboratory evaluation for evidence of development of adverse effects of deferiprone. Two patients complained of severe nausea and vomiting after administration of the first dose of the drug and withdrew from the study. Three patients noticed dark coloration of urine during administration of the medication. No patients developed arthritis. No patients developed agranulocytosis. Bilirubin and Serum glutamic oxaloacetic transaminases (SGOT) and serum glutamic pyruvic transaminases (SGPT) were not significantly different from baseline at the end of the study (Table 2).

Study 2 results

A total of 38 subjects were enrolled in the study. One patient discontinued treatment within the first two days because of nausea and vomiting and is not included in the analysis. The mean age of the 37 patients (21 males and 16 females) was 51.3 (±1.7) years and the mean weight was 63.2 ± 1.9 kg. The primary endpoints, evaluated using repeated-measures ANOVA, were changes in urinary albumin/creatinine ratio over 9 months. p values were adjusted for repeated measurements. The mean albumin/creatinine ratio decreased significantly from 187 ± 47 at baseline to 25 ± 7 mg/g, (p = 0.01) at 9 months (Figure 2). There was a small increase in serum creatinine between baseline (0.85 ± 0.282) and 3 months (0.98 ± 0.2 mg/dL), but it remained stable at 6 months (0.98 ± 0.2 mg/dL; p = 0.86) and 9 months 0.96 ± 0.3 mg/dL; p = 0.56) (Table 3).

Table 1. Study 1: Results and clinical information.

					Weight		Serum creatinine		24-h urinary protein	
S. No.	Age	Gender	Histology	Previous treatment	Initial	6 months	Initial	6 months	Initial	6 months
1	52	F	MPGN	Steroids	60	59.4	1.3	0.8	2.6	1.2
2	40	F	Membranous GN	Steroids & cyclophosphamides	40	41.5	1.4	0.9	2.8	0.6
3	35	M	Membranous GN	Steroids & cyclophosphamides	50	52	0.8	0.7	5.3	4.8
4	12	F	MPGN	Steroids	22	22.7	0.9	2	4.5	3.8
5	32	M	FSGS	Steroids & cyclophosphamides	62	63	2.1	0.9	4.6	4.5
6	29	F	Chronic GN	Steroids	42	40	1	0.9	3.4	1.5
7	28	M	FSGS	Steroids	40	45	1.6	1.2	2.8	1.6
8	33	M	Membranous GN	Steroids & cyclophosphamides	64	64	0.8	0.9	6.5	4
9	40	F	Membranous GN	Steroids & cyclophosphamides	64	64	0.8	0.9	6.5	4
10	3	M	Diffuse mesangial proliferation	Steroids	14	11	0.7	0.5	5.8	2.8
11	14	M	Diffuse mesangial proliferation	Steroids	37	37	0.6	0.5	6	0.5
12	18	F	FSGS	Steroids	36	34	0.7	0.6	4.2	1.6
13	12	F	MPGN	Steroids & cyclophosphamides	32	30	0.4	0.5	2.5	2.1
				Mean ± SEM	44.2 ± 5.0	44.2 ± 5.2	1.05 ± 0.13	0.90 ± 0.12	4.05 ± 0.46	2.21 ± 0.47

FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; MPGN, membranoproliferative glomerulonephritis.

Results

	24-hr Urinary Protein (g/day)	Serum Creatinine (mg/dL)	Mean Arterial Pressure
Baseline	4.01±1.61	1.05±0.13	91±3
After 6 Months	2.21±1.62	0.9±0.12	91±3
P-value	0.009*	0.28	0.49
% Reduction	**47±9**		

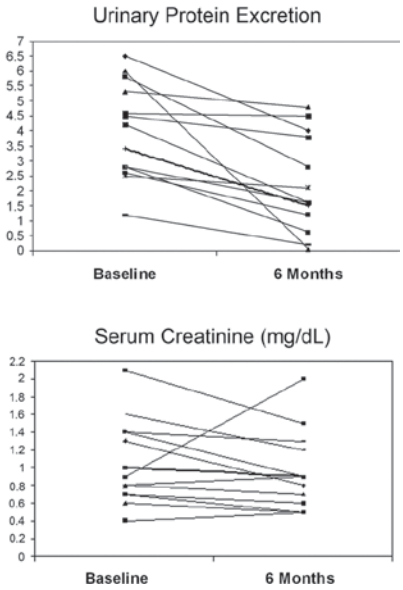

Figure 1. Study 1: Urinary protein and serum creatinine before and after treatment with deferiprone.

Table 2. Study 1: Patient safety data.

Parameter	Baseline	After 6 months	*p* value
Hb (g/dL)	10.56 ± 0.56	10.7 ± 1.31	0.42
Total WBC (103)	9595 ± 2998	9776.9 ± 2249	0.43
Total bilirubin	0.93 ± 0.3	0.86 ± 0.4	0.31
SGOT (IU/L)	30 ± 7	35 ± 17.3	0.16
SGPT (IU/L)	20 ± 12	21 ± 12	0.46

Hb, hemoglobin; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cells.

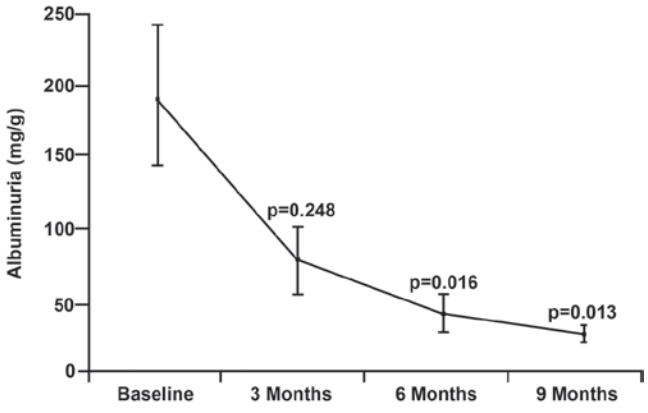

Figure 2. Study 2: Urinary albumin/creatinine ratio. Urinary albumin/creatinine ratio (mg/g) at baseline, 3, 6, and 9 months. The primary endpoints, evaluated using repeated-measures ANOVA, were changes in urinary albumin/creatinine ratio over 9 months. *p* values were adjusted for repeated measurements. All test results reported are two-tailed and a *p* value of less than 0.05 is considered significant.

The blood glucose control measured by HbA_{1c} was not significantly different between the baseline (7.44 ± 0.18 and final visits (8.04 ± 0.29), (p = 0.614). The calculated mean arterial blood pressure (MAP) declined from a baseline value of 100.76 ± 1.2 to 97 ± 0.6 at 3 months (p = 0.002) but remained relatively stable from then on to 6 months and 9 months (Table 3).

Although up to 15% of patients on deferiprone have been reported to have arthralgia (Kontoghiorghes et al. 2000), none of the patients in this study reported this side effect. Iron studies, liver enzymes, and complete blood counts were checked every 3 months. As expected, iron stores declined from a mean (SEM) iron level of 116 ± 15 µg/dL at baseline to 81 ± 5 µg/dL (p = 0.019) and serum ferritin from 144 ± 30 to 59 ± 7 ng/mL (p = 0.01) at 9 months. These changes in iron stores were associated with a small but statistically significant drop in hemoglobin (Table 4). There were no clinically significant differences in the mean WBC counts at baseline and final visit (Table 4). No episode of neutropenia or agranulocytosis was noted. None of the subjects developed abnormalities of liver enzymes or liver function tests, which is defined as a > two-fold increase in the test result compared to baseline. Alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphate, and total bilirubin levels remained stable throughout the study period (Table 4).

Discussion

Oxidants and iron have been implicated in proteinuria and progressive kidney disease in a wide variety of glomerular diseases (Shah et al. 2007). Using the urinary catalytic iron assay, we have shown a marked increase in patients with biopsy-proven glomerulonephritis. In Study 1, we demonstrated that 6 months of deferiprone therapy reduced proteinuria by 47 ± 9%. This study has some significant limitations. This was a single-armed, non-randomized pilot study with a small number of subjects. In addition, the short duration of the study does not permit any conclusions about the effect of deferiprone on the rate of progression of kidney disease.

Table 3. Study 2: Efficacy and iron parameters over 9 months of deferiprone treatment.

Parameter	Baseline	3 months	6 months	9 months
MAP (mmHg)	102 ± 1	97 ± 0.6 ($p = 0.002$)	96 ± 0.7 ($p = 0.002$)	95 ± 0.7 ($p < 0.001$)
Serum iron (μg/dL)	116 ± 15	—	81 ± 5 ($p = 0.137$)	72 ± 5 ($p = 0.019$)
Serum ferritin (ng/mL)	144 ± 30	139 ± 26 ($p = 1.0$)	72 ± 21 ($p = 0.41$)	59 ± 7 ($p = 0.061$)
Serum creatinine (mg/dL)	0.86 ± 0.03	0.98 ± 0.02 ($p = 0.02$)	0.98 ± 0.02 ($p = 0.002$)	0.95 ± 0.03 ($p = 0.190$)
Albuminuria (mg/g)	188 ± 47	80 ± 23 ($p = 0.248$)	35 ± 14 ($p = 0.016$)	25 ± 7 ($p = 0.013$)

p values are compared to baseline.
MAP, mean arterial pressure.

Table 4. Study 2: Safety parameters following 9 months of deferiprone treatment.

	Mean value ± SEM ($N = 37$)	
Parameter	Baseline	9 months
Hematology		
Hb (g/dL)	12.7 ± 0.09	11.87 ± 0.13 ($p < 0.01$)
WBC (103/mL)	7338 ± 170	6937 ± 155
Platelets (10^3/mL)	2.8 ± 0.08	2.85 ± 0.07
Liver function		
Total bilirubin (mg/dL)	0.9 ± 0.01	0.94 ± 0.02
AST (U/L)	20 ± 0.6	24 ± 1
ALT (U/L)	20 ± 0.7	23 ± 1
Alkaline phosphatase (U/L)	100 ± 1.4	104 ± 1

AST, aspartate aminotransferase; ALT, alanine aminotransferase; Hb, hemoglobin; WBC, white blood cells.

Study 2 is the first human study which demonstrates that the oral iron chelator deferiprone decreases albuminuria in patients with diabetes. In addition, after the initial increase, renal function remained stable throughout the remainder of the study period. The changes in blood pressure at 3 months are similar to those reported in other studies and may among other reasons reflect observational bias. It should be noted that, despite blood pressure remaining stable between 3 months and 9 months, there was a marked reduction in urinary albumin. Nonetheless, considerable caution is warranted in interpreting these results because of the known effect of angiotensin-converting-enzyme (ACE) inhibitors and blood-pressure control on proteinuria in patients with diabetic nephropathy.

Support for mechanisms in which metals may play a role in progression comes from the effect of a metal chelator on progressive kidney disease. Lin et al. have shown that chelation therapy with ethylenediaminetetraacetic acid (EDTA) in patients with chronic renal insufficiency results in a reduced rate of decline in the glomerular filtration rate (Lin et al. 2003). The authors attribute the beneficial effect to the chelation of lead, which also participates in the Fenton reaction. However, given the affinity constants for iron and lead, the large experimental evidence for the role of iron in kidney disease, and the demonstrated efficacy of EDTA in enhancing excretion of urinary iron, we believe that the beneficial effects are more likely to be explained by chelation of iron rather than lead (Owda et al. 2003). The data supporting the role of iron in models of progressive renal disease consist of a demonstration of increased iron in the kidney in models of progressive kidney disease (Nankivell et al. 1992; Harris et al. 1994, evidence for enhanced generation of oxidants, which provides a mechanism by which iron can be mobilized, and demonstrating more directly the beneficial effect of iron-deficient diets and iron chelators (Alfrey et al. 1989; Alfrey 1992, 1994).

Deferiprone (1,2-dimethyl-3-hydroxypyridin-4-1, also known as L1) is the most extensively studied oral iron chelator (Cohen et al. 2003; Hoffbrand 2005; Kontoghiorghes et al. 2009) and is approved for treatment of iron overload states in Europe and India. In addition to its suitability for long-term treatment (because of oral administration), it has better tissue penetration compared to deferoxamine. Deferiprone is rapidly absorbed and has a peak plasma level usually within 45–60 min of ingestion. Deferiprone forms a 3:1 chelator/iron complex that is excreted together with free drug in the urine. The major adverse effects reported so far in over 7500 patients receiving deferiprone for periods of up to 14 years and at doses of 50–150 mg/kg/day are transient agranulocytosis in 0.6% of patients, neutropenia in about 6% of patients, transient musculoskeletal and joint pains in about 15% of patients, gastric intolerance in about 6% of patients, and zinc deficiency in about 1% of patients. All of the adverse effects of deferiprone are considered reversible, controllable, and manageable (Kontoghiorghes et al. 2003).

Although these are small, open-labeled, non-randomized studies, our results suggest that examining the utility of oral iron chelators to treat diabetic and non-diabetic glomerulopathies appears warranted. Future randomized, double-blind trials, with careful monitoring of safety issues and use of deferiprone or any newly-developed, non-nephrotoxic oral iron chelator (Cohen 2006) may lead to the use of a new class of agents for the treatment of glomerular diseases.

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Declaration of interest

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