

Phase I Trial of Rosiglitazone in FSGS: I. Report of the FONT Study Group

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Background and objectives: Patients with primary focal segmental glomerulosclerosis (FSGS) who are resistant to standard therapy are at high risk for progressive chronic kidney disease. Prevention of renal fibrosis represents a promising strategy to slow or halt kidney function decline. This paper presents the results of a Phase I clinical trial of rosiglitazone, a thiazolidinedione, that exerts antifibrotic effects in animal models of FSGS. The primary goal was assessment of safety, tolerability, and pharmacokinetics (PK) of rosiglitazone.

Design, setting, participants, & measurements; Eleven patients, including eight boys/men and three girls/women, with mean age 15 ± 6 yr and estimated GFR 131 ± 62 ml/min/1.73 m², received rosiglitazone, 3 mg/m²/d for 16 wk. PK was assessed twice, after the initial dose and after attaining steady state, in a General Clinical Research Center.

Results: There were no serious adverse events or cardiovascular complications. Rosiglitazone was well tolerated by all patients, as judged by the Treatment Satisfaction Questionnaire for Medication. The PK studies indicated that the area under the curve was decreased by 40 to 50% and oral clearance of rosiglitazone was increased by 250 to 300% in patients with resistant FSGS compared with healthy controls and patients with nonproteinuric stage 2 chronic kidney disease.

Conclusions: Rosiglitazone therapy was safe and well tolerated. PK assessment of potential novel therapies for resistant FSGS is necessary to define appropriate dosing regimens. There is rationale to evaluate the efficacy of rosiglitazone as an antifibrotic agent for resistant FSGS in Phase II/III clinical trials.

Clin J Am Soc Nephrol ●●: ●●●–●●●, ●●●●. doi: 10.2215/CJN.02310508

Rosiglitazone is an oral peroxisome proliferator-activated receptor- γ agonist that increases insulin sensitivity. It is used as a hypoglycemic agent in patients with type 2 diabetes mellitus. The thiazolidinedione class of drugs also has direct effects on kidney structure and function. Administration of rosiglitazone (5 mg/kg for 20 d) to pregnant diabetic rats leads to inhibition of mesangial cell proliferation, downregulation of apoptosis, and reduced responsiveness to angiotensin II (1). Pioglitazone, another peroxisome proliferator-activated receptor- γ agonist, exerts antifibrotic effects at 10 μ M in renal tubular cells exposed to high glucose (2) and reduces extracellular matrix production at 1 to 3 μ M after

incubation with LDL (3). In puromycin aminonucleoside nephropathy, there is a decrease in podocyte injury, glomerulosclerosis, infiltrating glomerular macrophages, and plasminogen activator inhibitor-1 mRNA expression after treatment with pioglitazone, 10 mg/kg/d for 6 to 12 wk (4). In patients with diabetic nephropathy, short-term treatment with rosiglitazone reduces albuminuria by up to 42% (5–7). Rosiglitazone has an antiproteinuric effect in patients with other forms of chronic glomerulonephritis (8).

Despite the potential benefits of rosiglitazone treatment in primary and secondary glomerular disorders, there are no data to guide dosage recommendations in these diseases. This shortcoming is relevant because several factors might lead to altered pharmacokinetics (PK) under these circumstances. First, rosiglitazone is highly protein bound (approximately 99%) (9). Hypoalbuminemia as part of the nephrotic syndrome may increase the amount of unbound drug available for conversion to the primary metabolite *N*-desmethylrosiglitazone, which has minimal activity. Alternatively, hypoalbuminemia may in-

Received May 12, 2008. Accepted September 16, 2008.

Published online ahead of print. Publication date available at www.cjasn.org.

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crease the availability of unbound, biologically active drug and enhance efficacy or side effects (hypoglycemia, edema). Second, the kidneys generally do not eliminate protein-bound drugs, but this has not been rigorously assessed in proteinuric individuals. Third, alterations in extracellular fluid volume status in nephrotic syndrome could change the volume of distribution of rosiglitazone, and an expanded extracellular fluid volume could result in increased tissue distribution. Fourth, alterations in metabolism have been documented for several enzymes in patients with kidney disease (10–12). Because rosiglitazone is metabolized to *N*-desmethylrosiglitazone for elimination via the kidneys, decreased metabolism may enhance exposure to pharmacologically active drug. Finally, the effect of nephrotic syndrome on bioavailability of drugs has not been systematically studied. Alterations in one or more of these factors could significantly alter PK of rosiglitazone, necessitating adjustments in dosage to achieve plasma levels that maximize safety and efficacy in patients with proteinuric glomerular disease.

The primary purpose of the first portion of the Novel Therapies for Resistant Focal Segmental Glomerulosclerosis (FSGS) (FONT) study was to evaluate safety, tolerability, and PK characteristics of pharmacologic agents that hold promise as novel antifibrotic therapies for the treatment of FSGS. The first two agents selected for testing were rosiglitazone and adalimumab. In this report, we summarize the Phase I evaluation of rosiglitazone in patients with resistant primary FSGS. Secondary objectives were to determine the effects of clinical parameters (urinary protein excretion, serum albumin, GFR, and demographic variables [age, pubertal status, body surface area (BSA)] on rosiglitazone PK.

Materials and Methods

Patients

Patients, 2 to 41 yr of age, with biopsy-confirmed primary FSGS and calculated GFR > 40 ml/min/1.73 m², were eligible to participate in the FONT study. They were resistant to a standard course of glucocorticoids and had either been treated unsuccessfully with mycophenolate mofetil, azathioprine, cyclosporine, or tacrolimus in the past or failed to respond to 6 mo of therapy (cyclosporine or the combination of dexamethasone and mycophenolate mofetil) in the FSGS clinical trial. The institutional review board at each site approved the protocol and patient (and/or parent/guardian) consent was obtained before enrollment.

Participants were off all immunosuppressive medications for at least 4 wk before enrollment. Therapy with angiotensin converting enzyme inhibitors and/or angiotensin receptor blocker drugs was permitted, provided dosages were maintained for the duration of the study. Patients were then assigned to receive either rosiglitazone or adalimumab. This report summarizes the results in the patients who were given rosiglitazone.

The total rosiglitazone dose was 3 mg/m²/day given orally twice a day, every 12 h, with a maximum daily dosage of 8 mg, in accord with prior reports and to prevent hypoglycemia. Rosiglitazone was given for 16 wk and patients were evaluated at week 1, 2, 4, 8, 12, and 16. The following clinical and laboratory data were measured at each assessment and at the time of both PK studies: vital signs, height, weight, edema, serum creatinine, estimated GFR (eGFR; Cockcroft–Gault equation if ≥18 yr and Schwartz formula for <18 yr), urinary protein: creatinine ratio (UP:Cr) in a first morning specimen, serum albumin,

and blood glucose. Ten out of the 11 patients enrolled in the trial completed the 4-mo treatment period and laboratory evaluation.

All adverse effects were tabulated and patient satisfaction with therapy was assessed at the completion of the study using the Treatment Satisfaction Questionnaire for Medications (TSQM) instrument (13).

PK Study

Patients underwent a comprehensive PK assessment twice during the 16-wk treatment period. The initial PK evaluation was conducted with the first dose of rosiglitazone and the second (steady-state) PK evaluation was conducted after patients had received rosiglitazone for 16 wk.

Patients were admitted to the General Clinical Research Center (GCRC) for a 48-h PK analysis. Patients were fasting at the start of each PK study and then fed a standard diet throughout the procedure. After obtaining a baseline blood sample, patients took their morning oral dose of rosiglitazone. This was the only dose administered during the initial (single dose) PK study. Rosiglitazone was given twice daily, every 12 h, during the steady-state PK study. Additional EDTA-anticoagulated plasma samples were obtained at 0.5, 1, 2, 4, 6, 8, 12, 18, 30, and 42 h. A total of 27.5 ml of blood was drawn during each PK study. Urine was collected from 0 to 2, 2 to 12, 12 to 24, 24 to 36, and 36 to 48 h for measurement of protein excretion. Blood samples were immediately centrifuged for 10 min at 4°C, and plasma was transferred to plastic tubes and stored at –80 °C.

Plasma was processed by protein precipitation with acetonitrile. Samples were assayed by HPLC-MS/MS (PRA International–Early Development Services, Zuidlaren, Netherlands), using an ammonium acetate buffer (10 mM, pH 2.5): methanol (65:35, vol/vol) at a flow rate of 0.2 ml/min, with pioglitazone as the internal standard. For MS/MS, turbo ion spray (45°C) in positive ion mode was used. Rosiglitazone and *N*-desmethylrosiglitazone concentrations were reported in ng/ml. The lower limit of quantification was 1.0 ng/ml, and the assay was linear in the validation range of 1.0 to 1000 ng/ml for both compounds.

PK Analyses

Noncompartmental PK analysis of rosiglitazone and *N*-desmethylrosiglitazone was conducted using WinNonlin v.4.1 (Pharsight, Mountain View, California) using linear up-log down for area under the curve (AUC) determination. The following parameters were analyzed: concentration maximum (C_{max}), time to maximum concentration (T_{max}), area under the plasma concentration time curve from zero to infinity (AUC_{0 to ∞}), area under the plasma concentration time curve from 0 to 12 h (AUC_{0 to 12}), oral clearance (Cl/F), oral volume of distribution (V_z/F), mean residence time (MRT), and half-life (T_{1/2}). C_{max} parameters were adjusted to a dose of 2 mg. AUC data were adjusted to a 2 mg dose and 70 kg weight. Cl/F data were scaled to a weight of 70 kg and then raised to a power of 0.75 to minimize differences related to body size (14).

Statistical Methods

Data are presented as mean ± SD. Descriptive analyses for PK parameters, demographic variables, and laboratories include mean, SD, and median as appropriate. Differences between groups were assessed with an ANOVA followed by paired comparisons using a *t* test with a Bonferroni correction. Spearman correlations were determined for key clinical characteristics (serum albumin, UP:Cr, GFR, glucose, age, BSA) versus each PK parameter of interest (AUC, Cl/F, V_z/F, T_{1/2}, MRT). The correlations and resultant *P* values from the univariate assessments were analyzed for possible inclusion into a multiple regression model for prediction of AUC_{0 to ∞} and AUC_{0 to 12} of rosiglitazone. Data that did not follow a normal distribution were transformed before testing in the

models. Model building for analysis of determinants of rosiglitazone AUC consisted of multiple regression analysis with backward elimination of one clinical variable at a time, with selection of the variable based on level of contribution to the model. The final model was selected based on significance of each clinical variable as a predictor of the rosiglitazone AUC in the model as well as the overall R^2 .

Results

Patients

Eleven patients with biopsy-confirmed resistant FSGS participated in the Phase I trial and underwent PK studies for rosiglitazone and *N*-desmethylrosiglitazone. Patients were 15.4 ± 6.2 yr (range: 4 to 28 yr), 45% were prepubertal, 27% female, 64% nonwhite, and BSA was 1.6 ± 0.5 m² (Table 1). Concomitant medications included hepatic hydroxymethyl glutaryl-CoA reductase inhibitors (64%), angiotensin converting enzyme inhibitors/angiotensin receptor blocking drugs (100%), and diuretics (55%).

Key laboratory results at baseline were: serum creatinine 0.9 ± 0.6 mg/dl, UP:Cr 5.5 ± 2.6 , GFR_e 131 ± 62 ml/min/1.73m², serum albumin 2.3 ± 1.0 g/dl, and serum glucose 84 ± 26 mg/dl. At 16 wk, serum creatinine was 1.2 ± 0.7 mg/dl, UP:Cr 7.2 ± 4.5 , GFR_e 117 ± 67 ml/min/1.73m², serum albumin 2.2 ± 1.0 g/dl, and serum glucose 76 ± 11 mg/dl (Figure 1, a through d). None of these changes were significant. Among the ten patients who completed the treatment period, two had at least a 40% reduction in proteinuria.

Safety

Adverse events were generally mild (Table 2). Six patients contributed a total of 12 events, with three events (hives, penile swelling, dizziness) deemed possibly related to rosiglitazone

therapy. The child who experienced hives discontinued rosiglitazone after 6 wk and was withdrawn from the study after 8 wk. Other adverse effects that were not coded as drug-related included muscle cramps, headaches, diarrhea, gastroenteritis, cellulitis, transient acute renal failure with hyperkalemia, and a viral illness.

No changes in weight, blood pressure, glucose concentration (Figure 1d), liver function tests, or hematocrit were documented (Table 3). In particular, plasma glucose concentration and body weight demonstrated 8 ± 25 mg/dl reduction and 3.3 ± 4.1 kg increase, respectively, after 16 wk of therapy. One patient had a rise in serum glucose concentration but the baseline and final levels were less than 126 mg/dl. Four patients experienced >10% increase in weight, of whom one had a concomitant decline in hematocrit. Patients who achieved a dose normalized AUC that was similar to healthy subjects exhibited a reduction in blood glucose of 38 ± 21 mg/dl and an increase in body weight of 5.6 ± 4.2 kg, whereas patients who obtained a lower AUC showed an increase of blood glucose of 1 ± 21 mg/dl and weight of 3.1 ± 4.4 kg.

Tolerability

Patient tolerance of rosiglitazone was assessed by the TSQM, which provides data in four domains (maximal score of 100 in each category). The mean scores were: effectiveness 65, side effects 97, convenience 76, and global satisfaction 65. These data show acceptable tolerability of rosiglitazone in patients with FSGS.

PK Findings

PK data were available for ten patients at each evaluation (*i.e.*, initial dose and steady state). The initial dose sample set

Table 1. Patient baseline demographics

ID	Age (yr)	Pubertal Status ^a	Gender ^b	Race ^c	Weight (kg)	Height (cm)	UP/C ^d ratio (mg/mg)	Creatinine (mg/dl)	eGFR ^d (ml/min/1.73m ²)
001	20	Post	M	A	54	163	4.3	1.4	71
002	16	Pre	M	H	52	144	4.5	0.4	167
003	17	Post	F	A	48	160	8.1	0.5	176
004	16	Post	M	A	94	172	6.3	0.9	133
005	18	Post	M	W	67	172	4.6	1.1	110
006	4	Pre	M	W	15	97	9.2	0.2	265
007	9	Pre	M	W	28	127	2.0	0.7	100
008	16	Post	F	W	55	167	4.2	0.6	153
009	10	Pre	F	A	32	134	6.9	0.5	147
010	15	Pre	M	M	54	149	8.7	2.0	52
011	28	Post	M	A	123	193	1.4	2.1	63
Mean ± SD	15.4 ± 6.2	Post: 54% Pre: 45%	M: 72% F: 27%	A: 45% W: 36% H: 9% M: 9%	56.5 ± 30.3	152.5 ± 26.4	5.5 ± 2.6	0.9 ± 0.6	131 ± 62

^aPubertal status: Pre = prepubertal, Post = postpubertal.

^bGender: M = Male, F = Female.

^cRace: W = Caucasian-non-Hispanic, A = African-American, M = multiracial, H = Hispanic.

^dUP/C, urine protein/creatinine ratio; eGFR, estimated GFR.

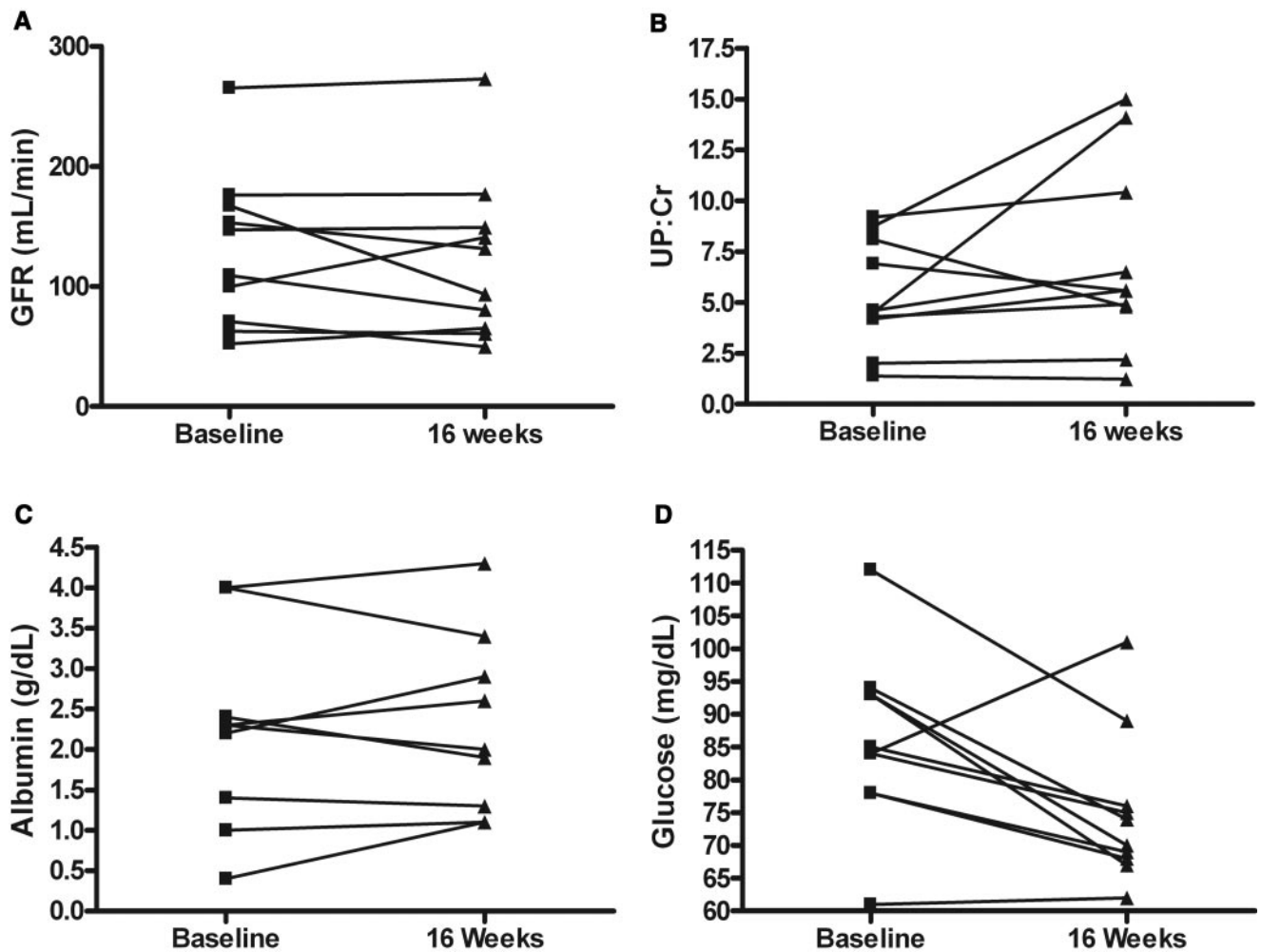


Figure 1. Graphic illustration of the baseline and 16-wk values for each specific laboratory test. Each data point represents an individual patient who participated in the Novel Therapies for Resistant Focal Segmental Glomerulosclerosis (FSGS) (FONT) study. The mean \pm SD values at baseline and 16 wk, respectively, for each figure are: (a) GFR 131 ± 62 and 117 ± 67 mL/min/ 1.7m^2 ; (b) urinary protein:creatinine ratio (UP:Cr) 5.5 ± 2.6 and 7.2 ± 4.5 , based on the ten patients who completed the 16-wk treatment period; (c) albumin 2.3 ± 1.0 and 2.2 ± 1.1 g/dl; and (d) glucose 84 ± 26 and 76 ± 11 mg/dl.

was incomplete in one patient and the steady-state PK study was not performed in the child withdrawn because of a possible allergic reaction.

The rosiglitazone dose at the initial PK evaluation was 2.6 ± 0.8 mg and the steady-state daily dose was 5.4 ± 1.2 mg. A concentration *versus* time profile for single-dose rosiglitazone and *N*-desmethylrosiglitazone is presented in Figure 2.

The main rosiglitazone PK parameters for the patients with FSGS and published data from healthy, normal individuals and patients with stage 2 and stage 4/5 chronic kidney disease (CKD) (15) are provided in Table 4. To eliminate differences secondary to body weight and rosiglitazone dose, the $\text{AUC}_{0\text{ to }\infty}$ data for the FONT patients was adjusted to 70 kg body weight and a rosiglitazone dose of 2 mg. Cl/F values in the FSGS patients were also adjusted by a scaling method to 70 kg that used a power of 0.75 to eliminate differences secondary to body size (14). The published results for Cl/F and $\text{AUC}_{0\text{ to }\infty}$ values could not be adjusted to a 70 kg weight without access to the raw data. However, mean weight in the report by Chapelsky

et al. (15) was approximately 75 kg, similar to the normalization weight used in this study. Initial dose and steady-state PK parameters from the FONT patients were compared and were found to be similar. The single dose PK parameters T_{max} , C_{max} (dose-corrected) and V_z/F in the FSGS group were comparable to data for healthy controls and all stages of CKD. Review of the $T_{1/2}$ results demonstrated that one patient was an outlier with a $T_{1/2}$ of 11 h and this contributed to a mean of 3.4 ± 2.8 h. When this value was removed, the $T_{1/2}$ was 2.6 ± 0.7 h, compared with 4.1 h reported by others (15).

Differences between the four groups were noted for single dose $\text{AUC}_{0\text{ to }\infty}$ and Cl/F . Cl/F was enhanced in the FSGS patients, with resultant and predictable decreases in $\text{AUC}_{0\text{ to }\infty}$ compared with healthy subjects and stage 2 CKD patients. Direct statistical comparison was done between the FSGS patients and those with stage 2 CKD because they had comparable lower limit values of GFR. The Cl/F was increased approximately threefold ($P < 0.05$), whereas $\text{AUC}_{0\text{ to }\infty}$ in FSGS

Table 2. Adverse events^a

Patient ID	Gender	Adverse Event	Level of Severity	Possibility of Being Caused by Drug	History of Similar Event	Action Taken	Resolution	Cause of Study Exit?
002	Male	Muscle cramps	Mild	Unlikely	No	None	Spontaneously resolved, drug not stopped	N
		Headache	Mild	Unlikely	Yes	None	Spontaneously resolved, drug not stopped, reappears	N
		Diarrhea	Mild	Unlikely	Yes	None	Spontaneously resolved, drug not stopped	N
		Diarrhea	Mild	Unlikely	Yes	None	Spontaneously resolved, drug not stopped	N
		Gastroenteritis	Moderate	No	Yes	Study drug stopped for 4 wk.	Resolved with ceftriaxone and hydrocortisone, drug resumed	N
		Cellulitis–leg	Moderate	Unlikely	No	Rosiglitazone held until antibiotic stopped.	Resolved with antibiotics	N
004	Male	Muscle cramps	Mild	No	Unknown	None	Spontaneously resolved, drug not stopped	N
005	Male	Dizziness	Mild	Possibly	Yes	None	Spontaneously resolved	N
006	Male	Penile swelling	Mild	Possibly	No	Study drug held	Resolved spontaneously. Held drug for 3 days	N
		Hives	Mild	Possibly	No	Study drug stopped at 6 wk	Hives resolved with benadryl, drug not resumed	Y
010	Male	ARF high-K med, noncompliant ^b	Severe	No	Yes	Psych evaluation for med noncompliance	Resumed meds. Psych evaluation. Creatinine before event: 1.8, creatinine after event: 1.6	N
011	Male	Viral illness	Moderate	Unlikely	Yes	Rosiglitazone and lisinopril held until vomiting subsided	Resolved in 48 h	N

^aFive patients—001, 003, 007, 008, and 009—did not have adverse effects.^bARF, acute renal failure.

Table 3. Serial assessment of vital signs and laboratory testing

ID	Weight (kg)		Blood Pressure (mmHg)		Glucose (mg/dl)	Hematocrit (vol%)	ALT ^c (U/ml)		AST ^c (U/ml)	
	0	16	0	16			0	16	0	16
001	54	60	114/69	101/64	93	35	15	15	22	23
002 ^a	52	52	112/54	110/70	78	29	12	9	14	24
003	48	49	109/64	108/69	61	43	12	14	21	21
004	94	105	148/90	157/92	83	38	10	7	15	22
005	67	74	91/59	136/76	84	37	16	18	31	39
006 ^b	15	16	143/96	98/71	85	39	29	13	55	32
007	28	37	120/62	86/51	84	43	33	14	85	28
008	55	56	114/68	103/56	112	32	18	19	15	39
009	32	33	99/63	89/59	93	32	21	23	21	28
010	54	53	122/65	140/77	78	33	13	25	20	22
011	120	123	131/86	141/85	126	41	36	34	47	33
Mean	56 ± 30	60 ± 31	118 ± 17/70 ± 12	115 ± 24/70 ± 12	84 ± 26	37 ± 5	20 ± 9	17 ± 8	31 ± 22	28 ± 7
± SD										

^aPatient 002 screening values were used for week 0.

^bPatient 006 exited the FONT study at week 12 and values at week 12 are shown for week 16.

^cALT, alanine aminotransferase; AST, aspartate aminotransferase.

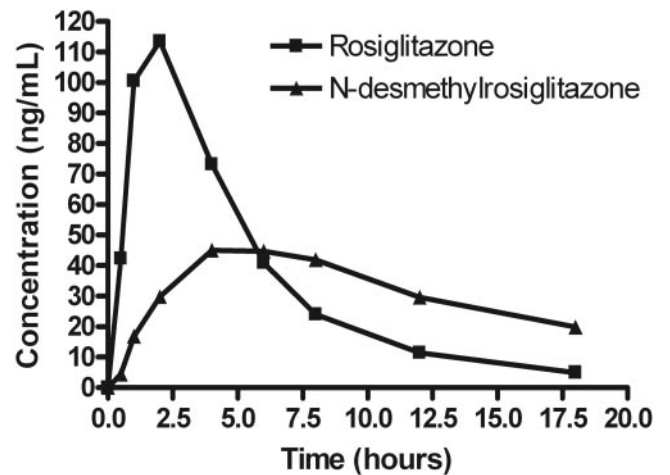


Figure 2. A representative single-dose plasma concentration versus time curve for rosiglitazone and *N*-desmethylrosiglitazone.

patients was approximately 50% lower ($P < 0.01$). The data from patients with stages 4/5 CKD indicate that the alterations in rosiglitazone PK in the FSGS patients were not a consequence of a low GFR *per se*.

In a preliminary attempt to evaluate AUC in relationship to clinical response in the FONT patient cohort, the group was divided into those with a “therapeutic” AUC (500 to 900 ng·h/ml), defined as the expected AUC values in normal patients, versus those who had AUC values less than a therapeutic AUC. Patients in the therapeutic range had changes in GFR (23 ± 25 ml/min/1.73 m²) and UP:Cr (-0.035 ± 0.33) that were favorable, whereas those with lower AUC values had less favorable changes in clinical parameters (GFR -36 ± 56 ml/min/1.73m² and UP:Cr 2.2 ± 4.1). Toxicity was not enhanced in the therapeutic group, suggesting that increased unbound concentrations were not present; however, this was not directly evaluated.

N-desmethylrosiglitazone is the primary metabolite of rosiglitazone and has 1/100th the potency of the parent drug (16). Whereas rosiglitazone is not appreciably eliminated in the urine as unchanged drug, *N*-desmethylrosiglitazone is eliminated mainly in the urine (64%) (9) by an unspecified mechanism (filtration versus tubular secretion). C_{max} (dose-adjusted) of 63.4 ± 41 ng/ml was reached at 2.3 ± 1.3 h. The AUC_{0-12} at steady state was 443 ± 300 ng · h/ml. The plasma AUC ratio of *N*-desmethylrosiglitazone to rosiglitazone was 1.5 ± 0.3 in the FONT patients. This suggests a greater contribution of the metabolite to systemic concentrations secondary to increased access of rosiglitazone to metabolism and enhanced CI/F. All subjects had ratios of between 1.2 and 1.7 except for two subjects (002 and 004) who exhibited ratios of approximately 1.0.

Correlations between Clinical Variables and PK Parameters

Each clinical variable of interest (UP:Cr, GFR, albumin, glucose, age, and BSA) was plotted against the key PK variables (CI/F, AUC, MRT, and V_z/F) and assessed by univariate analysis to evaluate the likelihood of predicting PK responses. In developing a multiple regression model, the main focus was on

Table 4. Rosiglitazone pharmacokinetics (PK) parameters^a

Parameter	FSGS (n = 11)	Healthy Subjects (n = 12) (15)	CKD Stage 2 (n = 15) (15)	CKD Stage 4/5 (n = 12) (15)
Single-dose				
T _{max} (h)	1.8 ± 0.9	2.0 (1.0 to 6.0)	2.0 (1.0 to 4.0)	2.0 (1.5 to 4.0)
C _{max} (ng/ml)	112 ± 88 ^b	115 ± 22 ^b	114 ± 27 ^b	90 ± 26 ^b
Lambda (h ⁻¹)	0.26 ± 0.1	NR	NR	NR
T _{1/2} (h)	2.6 ± 0.7	4.1 ± 1.1	4.5 ± 1.9	4.1 ± 1.0
MRT (h)	5.1 ± 1.4	NR	NR	NR
AUC _{0 to ∞} (ng · hr/ml)	341 ± 210 ^{c,e}	710 ± 195 ^b	782 ± 310 ^b	572 ± 147 ^b
V _z /F (L)	24 ± 19	20 ± 3	19.3 ± 4.1	26 ± 7
Cl/F (ml/min)	159 ± 170 ^{df}	51 ± 17	48.0 ± 16	62 ± 18
Steady-state				
T _{max} (h)	1.1 ± 0.5			
C _{max} (ng/ml)	100 ± 58 ^b			
Lambda (h ⁻¹)	0.27 ± 0.05			
T _{1/2} (h)	2.7 ± 0.9			
MRT (h)	4.6 ± 2.7			
AUC _{0 to 12} (ng · hr/ml)	301 ± 216 ^{ce}			
V _z /F (L)	31 ± 31			
Cl/F (ml/min)	149 ± 78 ^{dg}			

^aData shown as mean ± SD. Lambda, decay constant; MRT, mean residence time; AUC, area under curve; V_z/F, apparent volume of distribution; Cl/F, apparent volume of distribution.

^bDose-corrected to 2 mg.

^cDose-corrected to 2 mg and weight corrected to 70 kg.

^dWeight-corrected to 70 kg.

^eP < 0.01 versus healthy subjects and stage 2 CKD.

^fP < 0.05 versus healthy subjects and stage 2 CKD.

^gP < 0.001 versus healthy subjects and stage 2 CKD.

AUC because it reflects overall exposure to rosiglitazone and is related inversely to Cl/F. Because dose linearity has been established between 1.0 and 1000 ng/ml, if attainment of AUCs that have been reported for non-nephrotic patients is the goal for FSGS patients, a proportional dosage adjustment would be reasonable as long as the concentrations were maintained within the linear range. As shown in Table 4, non-nephrotic patients who were dose normalized to receive a 2 mg dose of rosiglitazone demonstrated AUC_{0 to ∞} values in the range of 500 to 1000 ng · hr/ml, whereas the FONT patients had dose-normalized AUC_{0 to ∞} values in the range of 120 to 500 ng · hr/ml. Prediction of single-dose AUC_{0 to ∞} and steady-state AUC_{0 to 12} were evaluated in the tested models. A limit of two variables per model was permitted secondary to the small number of subjects. Serum albumin had the most significant effect on predicting both AUC variables and remained highly significant in all models evaluated. GFR also was an important variable in predicting AUC_{0 to ∞} of rosiglitazone in both the single-dose and steady-state models. UP:Cr, pubertal status, age, and BSA did not predict either of the regression models. BSA may have been partially accounted for by adjustments to a 2-mg dose and a 70-kg weight. Multicollinearity was not relevant in either final model. The final models were defined by:

single-dose AUC: AUC_{0 to ∞} = 156.99 + 150.16 (albumin) – 1.14 (GFR)

R² = 95.53%, P < 0.0001 (components of model: albumin, P < 0.0001; GFR, P = 0.0018)

steady-state AUC: log AUC_{0 to 12} = 2.310 + 0.2262 (albumin) – 0.0039 (GFR)

R² = 73.52%, P = 0.0096 (components of model: albumin, P = 0.0034; GFR, P = 0.0188)

Discussion

In this Phase I trial, we demonstrate that rosiglitazone was generally safe and well tolerated in patients with nephrotic syndrome due to primary FSGS that was resistant to treatment with steroids and other immunosuppressive agents. Because of the inherent limitations of Phase I trials, we are reluctant to make any unsubstantiated assertions about efficacy to reduce proteinuria or act as a renoprotective agent. There were significant alterations in the PK of rosiglitazone, a highly protein-bound drug, in this patient cohort. In particular, increases in Cl/F and reductions in AUC were demonstrated compared with healthy subjects and those with all stages of CKD (15). Patients in the FONT study had normal renal function, except for three who were classified as stage 2 (n = 2) and stage 3 (n = 1) CKD at the baseline evaluation. Furthermore, because rosiglitazone is only eliminated via the kidneys after metabolism to N-desmethylrosiglitazone and does not undergo enterohepatic recycling, one would predict a minimal effect of GFR on

rosiglitazone clearance with regular dosing. This was not the case in the AUC regression models for single dose or steady state. GFR was an important contributor to $AUC_{0\text{ to }\infty}$ and $AUC_{0\text{ to }12}$. In patients with diminished GFR, decreased hepatic metabolism secondary to CKD itself (17) or the effect of CKD on the accumulation of endogenous substrates (18,19), may result in an increased AUC of rosiglitazone. In subjects with both decreased albumin and decreased GFR, the AUC lowering effect of hypoalbuminemia may be balanced by an increased AUC effect of a reduced GFR. Limitations in the PK studies were lack of measurements of urinary loss of protein-bound drug, degree of protein binding, and bioavailability.

Reduction in serum albumin was the predominant factor responsible for altering rosiglitazone PK characteristics in resistant FSGS. This suggests an effect of increasing unbound fraction on the measured clearance of a low-extraction-ratio drug. Univariate and multivariate assessments of the relationship between serum albumin and AUC support the notion that serum albumin is a primary variable responsible for altering total drug exposure (AUC) after the initial single dose as well as multiple doses of rosiglitazone. The UP:Cr was only significantly predictive in univariate analyses. Attainment of a therapeutic AUC after single dose and multiple doses was predicted by a positive relationship with serum albumin and a negative relationship with GFR.

The two patients in the therapeutic AUC group had relatively preserved serum albumin levels. They differed with regard to age (28 and 9 yr) and GFR (older patient had a lower GFR at follow-up, 61 ml/min/1.73m² versus 141 ml/min/1.73m² in the child). Rosiglitazone is primarily metabolized by CYP450 2C8. A dual amino acid modification in CYP450 2C8 (Arg139Lys and Lys399Arg), the *3 variant, results in higher *in vivo* metabolic capacity for rosiglitazone (20). One patient, who had considerably lower AUC values than the other patients in the nontherapeutic AUC group, could be heterozygous or homozygous for the *3 allele of CYP 450 2C8. Further studies are needed to clarify the effects of genotype and proteinuria on the PK of rosiglitazone in patients with glomerulopathies.

Although the cardiovascular safety of rosiglitazone is the subject of considerable debate in adults (21,22), the drug was well tolerated in this small, mostly pediatric (8 of 11) patient group. No cardiovascular side effects were reported. One patient discontinued therapy secondary to a presumed allergic reaction. Half of the adverse effects occurred in one patient who had the lowest AUC value, suggesting that side effects are not directly related to drug concentration. Four of the 10 patients who completed the treatment period had at least a 10% increase in body weight. This may complicate management of patients with refractory nephrotic syndrome and edema. However, addition of a thiazide diuretic may alleviate rosiglitazone-induced fluid retention and weight gain (23). The scores on the TSQM were comparable to other marketed drugs that are used in practice. On the basis of the safety and tolerability profile of rosiglitazone in our cohort of resistant FSGS patients, thiazolidinediones may be a viable treatment option. However, the duration of treatment was only 16 wk. Long-term follow-up is required to verify the overall safety of thiazolidinedione ther-

apy and the cardiovascular risk before it is introduced as an adjunctive antifibrotic and renoprotective therapy in patients with glomerular diseases such as FSGS.

Data from animal models of glomerulosclerosis suggest that pioglitazone and rosiglitazone at concentrations of 1 to 10 μM reduce expression of profibrotic molecules as well as pathologic measures of sclerosis. (1–4) Only one patient in the FONT study, who had the highest therapeutic AUC and favorable changes in clinical parameters, achieved this plasma concentration. This suggests that total concentration may be more important than unbound concentration to achieve benefit in FSGS. It is plausible that because of the profound effect of reductions in serum albumin concentration on AUC and Cl/F, many of our patients were underdosed. The lack of relation of AUC to adverse effects in the FONT patients suggests that altered unbound concentrations are not a concern because unbound concentrations are readily metabolized to *N*-desmethylrosiglitazone. The increased metabolite-to-rosiglitazone ratio supports this contention. To detect a clinical benefit of rosiglitazone in FSGS will require pre-emptive dosage modifications (on the basis of serum albumin and GFR) to achieve the therapeutic AUC in longer-term evaluations powered to detect changes in clinical parameters. On the basis of multiple regression models, we anticipate that raising the daily rosiglitazone dose to 4 mg/m² in Phase II/III studies will increase the AUC and therapeutic efficacy without compromising patient safety or tolerability. Any altered dosing scheme will need to be applied with caution and monitoring of side effects.

Steady-state AUC values that are predicted from the patient's serum albumin concentration and GFR could be used for dosage modifications on the basis of dose linearity. This may be necessitated by changes in clinical parameters to maintain efficacy and prevent adverse events. Routine use of our model will require validation with more subjects, more diverse groups of patients, and prospective assessment of dosing and achieved AUC. Assessment of free drug fraction and predicted unbound concentrations (C_{max} and AUC) would provide the information to assess the relevance of altered albumin on "pharmacologically active" drug. In light of the limited therapeutic options available for diseases such as FSGS, assessment of the effects of clinical features that may reduce efficacy is crucial to avoid mislabeling novel therapies as ineffective and abandoning potentially useful drugs.

Conclusions

Rosiglitazone was safe and well tolerated in children and young adults with resistant FSGS. The drug displays an altered PK profile—reduced AUC and increased Cl/F—compared with healthy controls and patients with nonproteinuric CKD. Changes in serum albumin and GFR were the main factors accounting for the differences in rosiglitazone PK. Modified dosing schemes for highly protein-bound drugs secondary to reductions in serum, albumin, and nephrotic-range proteinuria, and altered may be required when novel therapies are used in patients with glomerular diseases such as primary FSGS.

Acknowledgments

This work was supported by a grant from the National Institutes of Health (NIH)–National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (R21-DK70341) to Howard Trachtman and funds from the NIDDK Intramural Research Program to Jeffrey B. Kopp. The authors thank Jose Groenboom for his technical assistance in measuring the plasma rosiglitazone and *N*-desmethylrosiglitazone levels and Dr. Philip C. Smith, Associate Professor, School of Pharmacy, University of North Carolina (UNC), for his critical comments on this manuscript.

Disclosures

This work was supported by grants from the NIH–NIDDK (5R21-DK070341), and the GCRC program of the Division of Research Resources, NIH RR00046 (UNC) and NIH RR018535 (North Shore Long Island Jewish Health System). This work was presented in part at the Annual Meeting of the American Society of Nephrology, November 2007, San Francisco, California.

None of the authors had any competing financial interests to report.

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