Pharmacokinetics of Zopolrestat, a Carboxylic Acid Aldose Reductase Inhibitor, in Normal and Diabetic Rats

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The pharmacokinetics of zopolrestat, a carboxylic acid aldose reductase inhibitor, were examined in normal male rats dosed intravenously at 2 mg/kg and in normal and streptozotocin-diabetic male rats after oral administration at 50 mg/kg. After oral dosing, C_{max} was 127 μg/ml for normal rats and 144 μg/ml for diabetic rats. AUC(0–∞), however, was lower for diabetic rats than for normal rats and plasma half-life was longer in normal rats (8.0 vs 6.6 hr). Half-lives of zopolrestat in nerve, kidney, and lens were longer than plasma half-life and were similar for both diabetic and normal rats. Less than 2% of the dose was excreted in the urine as unchanged zopolrestat during the 48-hr period following dosing by diabetic or normal rats. Protein binding of zopolrestat was less extensive in plasma from diabetic rats than in plasma from normal rats. Similar kinetics were observed in diabetic animals receiving five daily doses of zopolrestat at 50 mg/kg/day. There was no plasma or liver accumulation of zopolrestat at steady state, consistent with the observed half-lives. However, zopolrestat did accumulate in nerve, kidney, and lens to varying degrees during multiple dosing, reflecting the longer half-lives of zopolrestat in these tissues.

KEY WORDS: aldose reductase inhibitor; zopolrestat; rats; diabetes; pharmacokinetics; protein binding; target tissue levels.

INTRODUCTION

Increasing evidence indicates that inhibition of the polyol pathway in diabetic animals and man can ameliorate the complications of diabetes (1,2). In diabetic or galactosemic animals, aldose reductase inhibitors have been shown to normalize tissue sorbitol levels (3–5), reverse formation of diabetic cataracts (6), increase nerve conduction velocity (7–10), prevent basement membrane thickening (11–13), and reverse diabetes-induced increases in vascular permeability (14) and urinary albumin excretion (15). Several aldose reductase inhibitors have been shown to decrease red blood-cell sorbitol levels in human diabetics (16,17), and studies with sorbinil, an aldose reductase inhibitor in the hydantoin class, have shown effectiveness in treating diabetic neuropathy (18–20).

Recently, a novel carboxylic acid aldose reductase inhibitor, zopolrestat (CP-73, 850; Fig. 1), 3,4-dihydro-4-oxo-3[[5-(trifluoromethyl)-2-benzothiazolyl]-methyl]-1-phthalazineacetic acid (21), was shown to normalize sorbitol, fructose, and myo-inositol levels in sciatic nerve, retina, lens, and kidney in diabetic rats (22) and to normalize renal plasma flow in galactosemic rats (23). In this report we describe the pharmacokinetics of zopolrestat for normal and diabetic male rats.

MATERIALS AND METHODS

Drug Assay. All reagents used were obtained from J. T. Baker, American Burdick and Jackson, or Fisher and were HPLC grade or the equivalent. Zopolrestat was determined in plasma, plasma ultrafiltrate, urine, and tissues by a reverse-phase HPLC assay after extraction of acidified samples with ether. Homogenized tissue (approximately 50–100 mg) or aliquots (up to 300 μl) of plasma or urine were diluted with 200 volumes of water and 20–40 μl of 1 *N* HCl was added. Each sample was fortified with internal standard (CP-74, 725; Fig. 1) and extracted with 5 ml of diethylether. The ether was evaporated using a nitrogen stream, the residue was reconstituted in mobile phase, and aliquots were injected onto a C-18 column (Waters μBondapak or Beckman ODS) with a C-18 precolumn. Several similar mobile phase systems were used, but the one used most often for these studies consisted of acetonitrile/tetrahydrofuran/10 m*M* sodium phosphate buffer, pH 3 (45/10/45, v/v/v). With a flow rate of 1.2 ml/min, the retention times for zopolrestat and CP-74,725, monitored by ultraviolet absorption at 294 nm, on the Waters column were approximately 6 and 8 min, respectively. Lower limits of quantification were 0.2 μg/ml for plasma, 0.1 μg/ml for urine, 0.01 μg/ml for plasma ultrafiltrate, 0.2 μg/g for lens, 1 μg/g for sciatic nerve, 1.0 μg/g for kidney, and 0.5 μg/g for liver. Urine samples were incubated in 1.0 *N* NaOH for 1 hr at room temperature, a condition demonstrated to hydrolyze acylglucuronides of zopolrestat (data not presented), and reassayed for total zopolrestat concentration. A lack of increased concentration of zopolrestat after incubation was evidence that acylglucuronides (and other alkali labile conjugates) were not present.

Protein Binding Determinations. Freshly obtained plasma samples from normal and diabetic rats were fortified with zopolrestat at 20, 40, 60, 100, 150, and 200 μg/ml and incubated for 1 hr at 37°C. Aliquots (1 ml) were transferred in triplicate to warmed Centrifree micropartition tubes (Amicon, Danvers, MA) and centrifuged for 30 min at 37°C. Zopolrestat concentrations were determined in the ultrafiltrates and in three aliquots of the original fortified plasma samples by HPLC. A two-tailed paired *t* test was used to test the null hypothesis that the free fraction in diabetic rat plasma was equal to the free fraction in normal rat plasma.

Streptozotocin Treatment of Rats. Rats in the diabetic groups were administered a single 85-mg/kg iv dose of freshly prepared streptozotocin in saline via the tail vein. The diabetic condition in rats was confirmed by the observation of polyuria, lack of weight gain, and hyperglycemia (data not presented) at 7 days after streptozotocin treatment. Fed animals were dosed with zopolrestat starting at 7 days after streptozotocin treatment.

Intravenous Dose Study. Twelve fed male Sprague–Dawley rats (mean body weight of 153 ± 8 g) were dosed (2 mg/kg) via the tail vein with zopolrestat dissolved in distilled water with 30% propylene glycol. Blood samples were obtained from the orbital sinus at 0.5, 1, 2, and 4 hr after dosing

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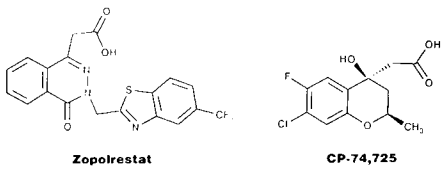

Fig. 1. Structures of zopolrestat and the analytical internal standard, CP-74,725.

from one group of four rats. Rats from the first group were sacrificed at 7 hr after dosing and rats from the second and third groups were sacrificed at 16 and 24 hr after dosing. Blood samples were obtained from all animals at sacrifice.

Single-Dose Study. All rats (mean body weight of 212 ± 6 g for normal and 222 ± 11 g for diabetic animals) were fed and dosed with zopolrestat (50 mg/kg) by gavage in the morning in groups of four. Blood and tissues from each group of four rats were collected after decapitation of the animals at 0.5, 1, 2, 4, 8, 12, 16, 24, 36, or 48 hr after dosing. Urine was collected from animals in the 24- and 48-hr groups prior to dosing and from 0 to 24 hr after dosing. Urine was also collected from animals in the 48-hr group from 24 to 48 hr after dosing. Dilute (2 *M*) phosphoric acid (1.5 ml for normal rats and 3.0 ml for diabetic rats) was included in the urine collection bottles to decrease urinary pH and thereby stabilize putative acyl glucuronides. Total volumes of urine were measured and recorded and transferred to glass storage bottles. Urine, plasma, and tissue samples were stored frozen until assayed for drug.

Multiple-Dose Study. All rats (mean body weight of 233 ± 13 g) were fed and dosed with zopolrestat (50 mg/kg/day) by gavage in the morning in groups of four. Some of the rats were sacrificed after a single dose while the rest were dosed on each of 5 sequential days. After the first or fifth dose, blood and tissues from each group of four rats were collected, after decapitation of the animals at 3, 12, 24, 48, and 72 hr after dosing. Additional samples were collected at 1 and 96 hr after dosing in the multiple-dose group. Plasma and tissue samples were stored frozen until assayed for drug.

Pharmacokinetic Calculations. Plasma elimination rate constants (λ_z) were calculated by linear regression of the logarithm of plasma concentration versus time over the terminal elimination phase as indicated for each study. Area under the concentration–time curve (AUC) was calculated using a linear trapezoidal approximation; zero was used as the concentration for any nonterminal sample below the quantitation limit of the assays. AUC(48–∞) was calculated by dividing the mean concentration at 48 hr after dosing by λ_z and AUC(0–∞) was the sum of AUC(0–48) + AUC(48–∞). Total-body clearance was calculated by dividing total intravenous dose by plasma AUC(0–∞). Volume of distribution was calculated by dividing total body clearance by λ_z. Renal clearance was calculated by dividing the total unchanged drug excreted in urine over the collection interval by the plasma AUC for the same interval.

RESULTS

Protein Binding

Zopolrestat was extensively bound to plasma proteins, and the free fraction in plasma from normal rats increased from 0.5 to 2.3 as the total plasma concentration of zopolrestat was increased from 20 to 200 μg/ml. Protein binding was more extensive ($P < 0.05$) in plasma from normal rats than in plasma from diabetic rats (where the free fraction increased from 1.2 to 6.5 as the total plasma concentration of zopolrestat was increased from 20 to 200 μg/ml). In a separate study, protein binding in plasma from diabetic rats obtained at 12 days after streptozotocin treatment was similar to protein binding in plasma from rats that had been diabetic for only 7 days (data not presented).

Intravenous Dose Kinetics

After a single intravenous dose of zopolrestat at 2 mg/kg, plasma concentrations of zopolrestat decreased with a half-life of 7.9 hr (Table I). Volume of distribution was 0.57 L/kg and total-body clearance was 0.84 ml/min · kg.

Oral Single-Dose Kinetics

Mean plasma and tissue levels (AUC) of zopolrestat after single doses were higher for normal rats than for diabetic rats (Table II). Estimates of plasma half-life of zopolrestat were slightly longer in normal rats than in diabetic rats. Tissue half-lives were similar in normal and diabetic rats and, for each group of animals, were longer than plasma half-life (Table II). The collection intervals were insufficient for a reliable calculation of half-life in lens, but half-life in this tissue appeared to greatly exceed plasma half-life.

Normal rats excreted less than 0.4% of the dose from 0 to 48 hr after dosing into urine as unchanged drug, while diabetic rats excreted 2.5% of the dose into urine over the same period. Renal clearance of unchanged drug was 0.0015 ml/min · kg for normal rats and 0.016 ml/min · kg for dia-

Table I. Zopolrestat Pharmacokinetics in Normal Male Rats Following a Single 2-mg/kg iv Dose[a]

	Plasma zopolrestat concentration (μg/ml)
Time (hr)	
0.0	(4.32)[b]
0.5	3.97 ± 0.93
1.0	3.74 ± 0.79
2.0	3.19 ± 0.59
4.0	2.34 ± 0.39
7.0	1.77 ± 0.28
16	0.76 ± 0.07
24	0.41 ± 0.12
AUC (0–24), (μg · hr/ml)	35.3
AUC (0–∞), (μg · hr/ml)	39.9
Half-life (hr)[c]	7.9
Volume of distribution (L/kg)	0.57
Total-body clearance (ml/min · kg)	0.84

[a] Plasma concentrations are reported as mean ± standard deviation for four animals at each timepoint.

[b] The zero-point plasma concentration was extrapolated from the least squares regression curve of the logarithms of mean plasma concentrations between 0.5 and 4 hr after dosing.

[c] Half-life was determined over the 4- to 24-hr interval.

Table II. Pharmacokinetics of Zopolrestat After a Single Oral Dose of 50 mg/kg in Normal and Diabetic Sprague Dawley Rats

	Normal rats				Diabetic rats			
	Plasma	Nerve	Kidney	Lens	Plasma	Nerve	Kidney	Lens
T_{max} (hr)	4.0	12	4.0	16	2.0	8.0	4.0	12
C_{max} (μg/g)	127	12.9	79.0	0.53	144	10.0	65.7	0.37
AUC (0–24)[a]	1690	234	1240	7.95	1280	125	874	5.95
AUC (0–∞)[a]	2040	452	1800	—[c]	1370	212	1200	—
$t_{1/2}$ (hr)[b]	7.99	20.2	12.9	—	6.56	20.9	17.1	—

[a] AUC is reported in units of μg · hr/g.
[b] Half-life was calculated over the 12- to 48-hr interval.
[c] Insufficient data for reliable calculation of these parameters.

betic rats. No glucuronide conjugates could be detected in urine from either group of animals.

Oral Multiple-Dose Kinetics

Plasma AUC(0–24) for zopolrestat in diabetic rats after five daily doses (874 μg · hr/ml) at 50 mg/kg/day was similar to AUC(0–24) for diabetic rats after a single dose (871 μg/hr · ml) at 50 mg/kg (Table III), demonstrating that plasma levels of zopolrestat do not accumulate in diabetic rats with multiple dosing. Nerve, kidney, and lens concentrations, however, did increase after multiple dosing (Table III) reflecting the longer half-life of zopolrestat observed in tissues. Half-life of zopolrestat in liver was shorter than for other tissues, and based on the 24-hr liver concentrations after single and multiple dosing (Table III), accumulation did not occur for this tissue.

DISCUSSION

Although zopolrestat is highly bound to plasma proteins (~99% at 20 μg/ml), it has a moderate volume of distribution (0.57 L/kg), indicating a tissue affinity as well as plasma protein affinity. Tissue to plasma AUC(0–∞) ratios after a single oral dose at 50 mg/kg indicate that highly perfused organs, such as kidney with a ratio of 0.88, achieved drug levels approaching those of the plasma compartment, while poorly perfused tissues, such as lens with a ratio of 0.02, did not achieve high exposure levels. An important target tissue for this aldose reductase inhibitor, the nerve, with a ratio of 0.22, achieved an intermediate exposure. With the possible exception of the lens, the tissue concentrations were severalfold greater than the estimated unbound concentration in plasma. Tissue-to-plasma ratios for zopolrestat in diabetic rats were slightly lower than those for normal rats.

Zopolrestat was tightly bound to plasma proteins for both normal and diabetic rats, although diabetes was associated with decreased protein binding. Changes in plasma protein glycosylation (27) or plasma lipid composition (28,29) have been proposed as possible contributors to diabetes-induced changes in protein binding of acidic compounds in man. It may also be possible that increased concentrations of the ketone bodies, acetoacetate or β-hydroxybutyrate (not measured in this study), would compete with zopolrestat for protein binding sites. It is likely that one or more of these factors contributed to the changes in protein binding of the acidic compound, zopolrestat, observed in this study.

Orally administered zopolrestat was rapidly absorbed by normal and diabetic rats. If plasma AUCs in normal rats

Table III. Pharmacokinetics of Zopolrestat for Diabetic Rats After One or Five Daily Oral Doses at 50 mg/kg/day

	Single dose	Multiple dose
Plasma		
T_{max} (hr)	3.0	3.0
C_{max} (μg/ml)	95.7	65.0
AUC (0–24)[a]	871	874
AUC (0–∞)[a]	961	—
$t_{1/2}$ (hr)[c]	8.9	6.9
Nerve		
T_{max} (hr)	3.0	1.0
C_{max} (μg/g)	6.49	8.37
AUC (0–24)[b]	106	167
AUC (0–∞)[b]	315	—
$t_{1/2}$ (hr)[c]	42	44
Kidney		
T_{max} (hr)	3.0	1.0
C_{max} (μg/g)	60.2	55.5
AUC (0–24)[b]	726	881
AUC (0–∞)[b]	1110	—
$t_{1/2}$ (hr)[c]	17.0	13.2
Liver		
T_{max} (hr)	—[e]	—[e]
C_{max} (μg/g)	—[e]	—[e]
C-24 (μg/g)	18.3	16.6
AUC (0–24)[b]	—[e]	836
AUC (0–∞)[b]	—[e]	—
$t_{1/2}$ (hr)[c]	—[e]	8.3
Lens		
T_{max} (hr)	48	1.0
C_{max} (μg/g)	0.26	0.84
AUC (0–24)[b]	4.2	11.0
AUC (0–∞)[b]	—[d]	—
$t_{1/2}$ (hr)[c]	—[d]	—[d]

[a] AUC is reported in units of μg · hr/ml.
[b] AUC is reported in units of μg · hr/g.
[c] Half-life was calculated from 12 hr to the last quantifiable time point.
[d] Insufficient data for reliable calculation of these parameters.
[e] Livers were not collected at all time points from all animals and these parameters could not be determined.

following intravenous (2 mg/kg) and oral dosing (50 mg/kg) are compared, calculated absolute bioavailability exceeds 200%. There is a large difference in the dose levels used in this calculation, but this anomaly suggests that nonlinear kinetics may exist for zopolrestat.

AUC(0–∞) for total (bound and unbound) zopolrestat in plasma was higher in normal rats than in diabetic rats, but the free fraction was almost three fold greater in diabetic rats and consequently unbound plasma AUC was higher in the diabetic rats. Despite the apparently higher concentrations of unbound zopolrestat in plasma of diabetic rats, tissue concentrations of drug were higher in normal rats. Since the estimates of tissue half-lives of zopolrestat were similar for normal and diabetic rats, the differences in tissue concentrations may be due primarily to differences in tissue penetration of drug and not differences in tissue elimination. Diabetes has been shown to affect membrane structure (30,31) and it is possible that the mechanism for zopolrestat uptake is affected by such diabetes-associated membrane changes.

Plasma concentration profiles (data not presented) and AUC(0–24) after multiple oral dosing were essentially identical with those after a single dose (Table III). The lack of plasma accumulation of zopolrestat with once daily dosing is consistent with the 7- to 9-hr plasma half-life. Tissue profiles for nerve and lens were dramatically different from plasma profiles (Fig. 2). In classic pharmacokinetic models (compartmental or physiological), tissue and plasma profiles become parallel at some point. This did not occur with zopolrestat in nerve and lens (Fig. 2) by 72 hr after dosing (considering that plasma concentrations at 72 hr were below 0.2 μg/ml), even though plasma levels had declined over a period exceeding seven half-lives.

Thus, as with many drugs, plasma accumulation did not predict tissue accumulation. It is likely that steady state was not attained in nerve and lens after 5 days of administration. The AUC(0–24) in nerve on day 5 was considerably less than the single dose AUC(0–∞). Also the half-life in nerve and the immeasurable long half-life in lens are consistent with a longer required time to reach steady state. The accumulation in kidney and liver were minimal, consistent with the shorter half-lives in these tissues. AUC in liver was not determined after a single dose, although a 24-hr concentration was measured. The 24-hr concentrations after a single dose and after five doses were similar, indicating an absence of accumulation in liver. Another factor complicating the interpretation of these data is the unstable physiological state of the streptozotocin diabetic rat; the condition of the rat continues to deteriorate with time, which may also affect the distribution and disposition of zopolrestat.

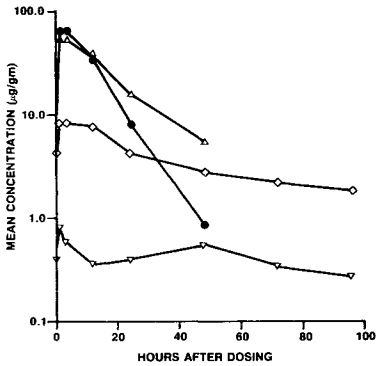

Fig. 2. Mean concentrations of zopolrestat in plasma (μg/ml) and tissues (μg/gm) from diabetic rats dosed orally with 50 mg/kg/day for 5 days. Each point represents the mean (four animals) concentration in plasma (●), nerve (◇), kidney (△), and lens (▽) following the last dose.

Urinary excretion of zopolrestat appeared to be a minor pathway of elimination for both normal and diabetic male rats. Renal elimination after a single 50-mg/kg dose of zopolrestat was 0.4 and 2.5%, respectively, of the total dose. The higher renal clearance in diabetic rats was most likely a reflection of the diabetes-induced polyuria (urine flow rates of 0.21 ml/min in normal rats vs 5.0 ml/min in diabetic rats). No urinary glucuronides of zopolrestat, which have been observed in human urine (32), could be detected in urine of either normal or diabetic male rats. It is possible that any such acylglucuronides excreted into the urine were hydrolyzed to zopolrestat by the alkaline conditions of rat urine. This phenomenon has been observed in female rats with ponalrestat, a structurally similar aldose reductase inhibitor (33). The major route of elimination of zopolrestat in rats remains to be elucidated.

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