Comparison of the Pharmacokinetics and Pharmacodynamics of the Aldose Reductase Inhibitors, AL03152 (RS), AL03802 (R), and AL03803 (S)

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The pharmacokinetics of AL03152 (RS) and its enantiomers, AL03802 (R) and AL03803 (S), were studied in the Sprague–Dawley rat following intravenous bolus administration. The enantiomers had differing pharmacokinetic profiles, while the racemic compound exhibited pharmacokinetic parameters approximating the mean values of the individual enantiomers. The total clearance (CL_T) values of the two enantiomers were similar, but the intrinsic clearance (Cl_{int}) was much greater for the S-enantiomer than for the R-enantiomer. The volume of distribution (V_{ss}) for AL03802 (R) was threefold greater than that for AL03803 (S). The stereoselectivity in V_{ss} could not be totally accounted for by the slight difference in serum protein binding of the isomers and resulted in a difference in the half-lives of the enantiomers. Only the R-isomer exhibited a persistent terminal elimination phase, consistent with more extensive tissue binding than the S-isomer. AL03152 enantiomers were equivalent in potency assessed from *in vitro* IC_{50} values toward rat lens aldose reductase and rat kidney L-hexonate dehydrogenase and lens EC_{50} values in diabetic rats.

KEY WORDS: aldose reductase inhibitor; enantiomers; pharmacokinetics; IC_{50}; EC_{50}; AL03152 (RS); AL03802 (R); AL03803 (S).

INTRODUCTION

AL03152 [2,7-difluoro-4-methoxyspiro(9H-fluorene-9,4′-imidazolidine)-2′,5′-dione] is a racemic methoxy analogue of the aldose reductase inhibitor imirestat (AL01576/HOE843). This compound was designed to increase clearance via facile metabolic O-demethylation and to reduce any potential toxicity caused by the long residence time of imirestat in the body, while maintaining comparable efficacy. Imirestat exhibited a low clearance, an intermediate volume of distribution, and a long biologic half-life in several species, including man (1,2). As hypothesized, AL03152 (RS) was cleared two- to fivefold more rapidly than imirestat in cynomolgus monkeys and rats via O-demethylation followed by glucuronidation (3,4). However, its potency in *in vitro* and *in vivo* was comparable to that observed with imirestat (3,5).

In this study, we have examined the differences in pharmacokinetics and pharmacodynamics of the individual enantiomers of AL03152 in Sprague–Dawley rats.

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MATERIALS AND METHODS

Materials

AL03152 and its enantiomers, AL03802 (R) and AL03803 (S) (structures in Fig. 1), were synthesized at Alcon Laboratories, Inc. All chemicals and solvents for GC-EC and biochemical assays were analytical grade. For intravenous (iv) and oral dosing, each drug was dissolved in a 0.1 *N* sodium carbonate solution, adjusted to pH ~11 with 1 *N* sodium hydroxide. This solution was stable for at least 3 weeks as determined by HPLC.

Intravenous Pharmacokinetic Study

Male Sprague–Dawley rats weighing 220–300 g were divided into three groups of 42 rats each. After fasting (water, *ad libitum*) overnight prior to and for 4 hr after dosing, rats received single 2 mg/kg iv bolus injections of the individual enantiomers or the racemate via the tail vein. At the following times postdose, three rats from each group were anesthetized and plasma samples were collected by cardiac puncture from each animal: 0 (predose), 1, 4, 8, 24, 31, 48, 72, 96, 120, 144, 168, 216, and 336 hr.

Serum Protein Binding

Protein binding of AL03152 and the individual enantiomers was determined by equilibrium dialysis as previously published (6). Briefly, pooled normal rat (Sprague–Dawley) serum samples were spiked with each isomer separately in a concentration range of 0.5–10 μg/mL and were incubated at 37°C for 1 hr with shaking. A duplicate set of 1-mL incubated serum aliquots at each concentration was dialyzed overnight against 2 vol of 0.133 *M* sodium–potassium phosphate buffer of pH 7.35 at 37°C with shaking. Postdialysis samples of both the buffer and the serum sides were analyzed to determine drug concentrations. The free fractions of the enantiomers and racemate were concentration independent over a 20-fold range (0.5–10.0 μg/mL).

Plasma, Serum, and Lens Drug Analyses

The gas chromatographic-electron capture method (nonstereoselective) described previously for plasma AL03152 determinations (3) was used for the determination of individual enantiomers and the racemate in all plasma, serum and lens samples. The lens sample preparation has been published (7). It is not believed that the enantiomers are interconvertable. The chiral center is a quaternary carbon and such a transformation would require the mechanistically unlikely cleavage and reformation on either the C(9)-nitrogen or C(9)-carbonyl bond (see Fig. 1). No examples of such a interconversion of enantiomers of closely related structures have been reported to our knowledge.

In Vitro Efficacy Study

The *in vitro* IC_{50} values of the enantiomers and racemate were measured with rat lens aldose reductase and rat kidney L-hexonate dehydrogenase preparations, using sensitive fluorescence assays as described previously (8).

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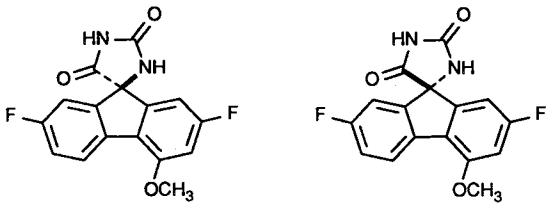

ALO3802 (R) **ALO3803 (S)**

Fig. 1. Structures of AL03802 (R) and AL03803 (S).

In Vivo Efficacy Study

Details of the quantitative evaluation of *in vivo* aldose reductase inhibitor activity in lens by measurement of tissue sorbitol content in the Sprague–Dawley diabetic rat model have been published (9). About 3 weeks after induction of diabetes, groups of six rats per each dose were administered individual enantiomers or the racemate orally at the following doses once per day for a total period of 8 days: 0.05, 0.15, and 0.50 mg/kg for AL03802 (R); 0.15, 0.50 and 1.50 mg/kg for AL03803 (S); and 0.15 and 0.50 mg/kg for the racemate. The racemate was used here as a control with only two doses since this compound has been tested previously with several doses in the same animal model (5). Because of the shorter half-life of S-isomer, the dose range for the S-isomer was greater than that of the R-isomer to provide comparable lens and plasma concentrations at the tissue collection time. Lens and plasma samples were collected approximately 24 hr after the last dose for drug and sorbitol (lens only) analyses. The oral bioavailability of the three compounds in rats was nearly quantitative (>95%) (3,4).

Pharmacokinetic Calculations

Plasma drug concentration–time data were analyzed using NONLIN 84 (Statistical Consultants, Lexington, KY); proper fit of the data to the selected model was confirmed statistically by the minimum Akaike's information criterion estimation (10). Total clearance (CL_T) was calculated as dose divided by AUC_0^∞. The area under the first moment curve ($AUMC_0^\infty$) was calculated from the area under the curve observed for the product of time and concentration versus time. Apparent volume of distribution at steady state (V_{ss}) was calculated by the following equation:

$$V_{ss} = \frac{\text{Dose}_{iv} \times (\text{AUMC})_0^\infty}{[(\text{AUC})_0^\infty]^2}$$

The intrinsic clearance (CL_{int}) was calculated by the following equation:

$$CL_{int} = \frac{CL_T}{f_u}$$

where f_u is the unbound fraction of the drug in plasma.

RESULTS

Pharmacokinetics

The mean plasma concentration–time profiles of AL03152 and its enantiomers in Sprague–Dawley rats are shown in Fig. 2 and the pharmacokinetic parameters calculated from the mean data are presented in Table I. AL03802 (R) was eliminated from plasma in triexponential fashion, exhibiting a persistent elimination phase, while AL03803 (S)

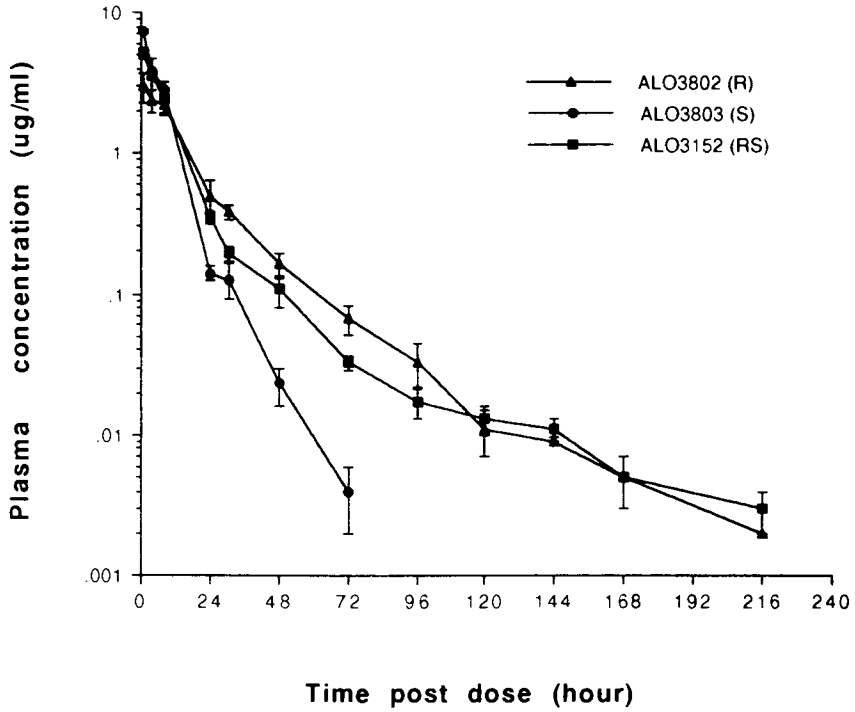

Fig. 2. Plasma concentration–time profiles of AL03152 and its enantiomers in rats following 2 mg/kg intravenous bolus injections. Each time point is the mean ± SD of three rats.

Table I. Pharmacokinetic Parameters[a] of AL03802 (R), AL03803 (S), and AL03152 (RS) Following a Single 2 mg/kg Intravenous Bolus Injection to Sprague–Dawley Rats

Parameter	AL03802 (R)	AL03803 (S)	AL03152 (RS)
CL_T (mL/hr/kg)	41.9	40.6	38.2
f_u, $\times 100$[b]	5.3 ± 0.8	3.7 ± 1.6	4.8 ± 0.1
CL_{int} (mL/hr/kg)	759	1103	914
V_{ss} (L/kg)	0.59	0.17	0.47
$t_{1/2\alpha}$ (hr)	6	3	4
$t_{1/2\beta}$ (hr)	15	9	11
$t_{1/2\gamma}$ (hr)	37	NA[c]	42

[a] Estimated from a single plasma concentration–time profile where each time point was the mean of three rats.
[b] Mean ± SD (n = 7); P = 0.058, Student's t test.
[c] Not applicable due to lacking a persistent γ phase.

was eliminated in a biexponential manner lacking the persistent phase. The racemate profile matched very closely the arithmetic means of the two enantiomer profiles during the first 96 hr, followed by a persistent terminal phase, reflecting the slow elimination of AL03802 (R).

Total clearance (CL_T) was not significantly different for the enantiomers; 42 mL/hr/kg for the R-enantiomer versus 41 mL/hr/kg for the S-enantiomer. The unbound fraction (f_u) was slightly higher for the R-isomer than for the antipode (mean ± SD; n = 7; 5.3 ± 0.8% vs 3.7 ± 1.6%; P = 0.058). The CL_{int} of AL03803 (1103 mL/hr/kg) was significantly greater than that of AL03802 (759 mL/hr/kg). The volume of distribution (V_{ss}; 0.59 L/kg) for AL03802 (R) was 3.5-fold greater than the 0.17 L/kg observed for AL03803 (S). The $t_{1/2\alpha}$ and $t_{1/2\beta}$ were shorter for the S-enantiomer (3 and 9 hr, respectively) than for the antipode (6 and 15 hr, respectively). The terminal half-life of the R-enantiomer was 37 hr. The racemate exhibited a CL_T (38 mL/hr/kg) similar to those of the enantiomers and a little larger V_{ss} (0.47 L/kg) than the mean values for the enantiomers. The CL_{int} of the racemate was close to the arithmetic mean of the enantiomers. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ of AL03152 were between those observed for the enantiomers and the terminal half-life was slightly longer than that of AL03802 (R).

Pharmacodynamics

The *in vitro* IC_{50} (drug concentration producing 50% inhibition of enzyme activity in the assay) values toward rat lens aldose reductase and rat kidney L-hexonate dehydrogenase are presented in Table II. The three compounds are very potent inhibitors of the two enzymes and showed virtually indistinguishable *in vitro* activities within the limits of the assays employed: 5.4 to 6.6 nmol/L for lens aldose reductase and 2.2 to 3.0 nmol/L for kidney L-hexonate dehydrogenase.

The inhibition of lens sorbitol accumulation vs plasma and lens drug concentration (~24 hr after the last dose) plots are shown in Figs. 3A and B, respectively; all data fit a single linear relationship, independent of the stereochemistry of the compounds, with r^2 = 0.967 for Fig. 3A and r^2 = 0.920 for Fig. 3B. From these curves, the drug concentration required for 50% inhibition of the lens sorbitol accumulation (EC_{50}) was estimated to be approximately 0.09 μg/mL in plasma and 0.96 μg/g tissue wet weight in the lens for all three compounds. The 10-fold greater EC_{50} value in lens than in plasma reflects the higher drug accumulation in the tissue than in plasma. Both lens and plasma AL03802 (R) concentrations were four- to sixfold greater than the corresponding AL03803 (S) concentrations when equal doses (0.15 and 0.5 mg/kg/day) of the enantiomers were administered. The concentrations of the racemate AL03152 were almost equal to the mean values of the enantiomers in both plasma and lens. The higher concentration of the R-isomer can be explained by its longer half-life.

Table II. *In Vitro* Inhibition Activities of AL03802 (R), AL03803 (S), and AL03152 (RS) Toward Rat Lens Aldose Reductase and Rat Kidney L-Hexonate Dehydrogenase

	IC_{50} (nmol/L)[a]	
Compound	Lens aldose reductase	Kidney L-hexonate dehydrogenase
AL03802 (R)	5.4 ± 0.2	3.0 ± 0.4
AL03803 (S)	6.2 ± 0.3	3.0 ± 0.4
AL03152 (RS)	6.6 ± 1.7	2.2 ± 0.9

[a] Mean ± SE (n = 3).

DISCUSSION

The results of this study demonstrate stereoselective pharmacokinetics of AL03152 enantiomers in the Sprague–Dawley rats. However, no stereoselectivity was observed in the activity of the compounds with regard to the inhibition of lens aldose reductase or kidney L-hexonate dehydrogenase *in vitro* or lens sorbitol accumulation *in vivo*.

The stereoselectivity in V_{ss} and terminal half-life was greater than the apparent difference in intrinsic clearance between the enantiomers. Since the 3.5-fold larger V_{ss} of AL03802 (R) cannot be accounted for by its 1.4-fold higher free fraction in plasma, it is apparent that the tissue binding of AL03803 (S) is substantially less than its optical antipode. Imirestat and AL03152 (RS) have exhibited extensive and slowly reversible binding in tissues (11,12). Because the total clearance of the two enantiomers is similar, the half-life of AL03802 (R) is prolonged, apparently due to its retention in tissue, as has been observed with imirestat (2,3). Studies in albino rabbits have demonstrated a fourfold longer lens retention for AL03802 (R) in comparison to AL03803 (S) (13), supporting the pharmacokinetic inference of greater tissue binding of AL03802 (R) in rats in this study.

The difference in intrinsic clearance between the enantiomers was only about 50%, substantially less than the difference in V_{ss} or in half-life. The conclusion that the intrinsic clearance of AL03803 (S) is in fact greater than that of its antipode is supported by *in vitro* evidence. AL03152 is eliminated mostly by O-demethylation followed by glucuronidation in rats (3,4). In rat liver microsomes, AL03803 (S) is demethylated at approximately 1.6 times the rate of AL03802 (R), when the substrate concentration is equal to the K_m (14).

In contrast to the substantial difference in pharmacokinetics is the very similar concentration–effort profile of the enantiomers and the racemate. Both *in vitro* IC_{50} values

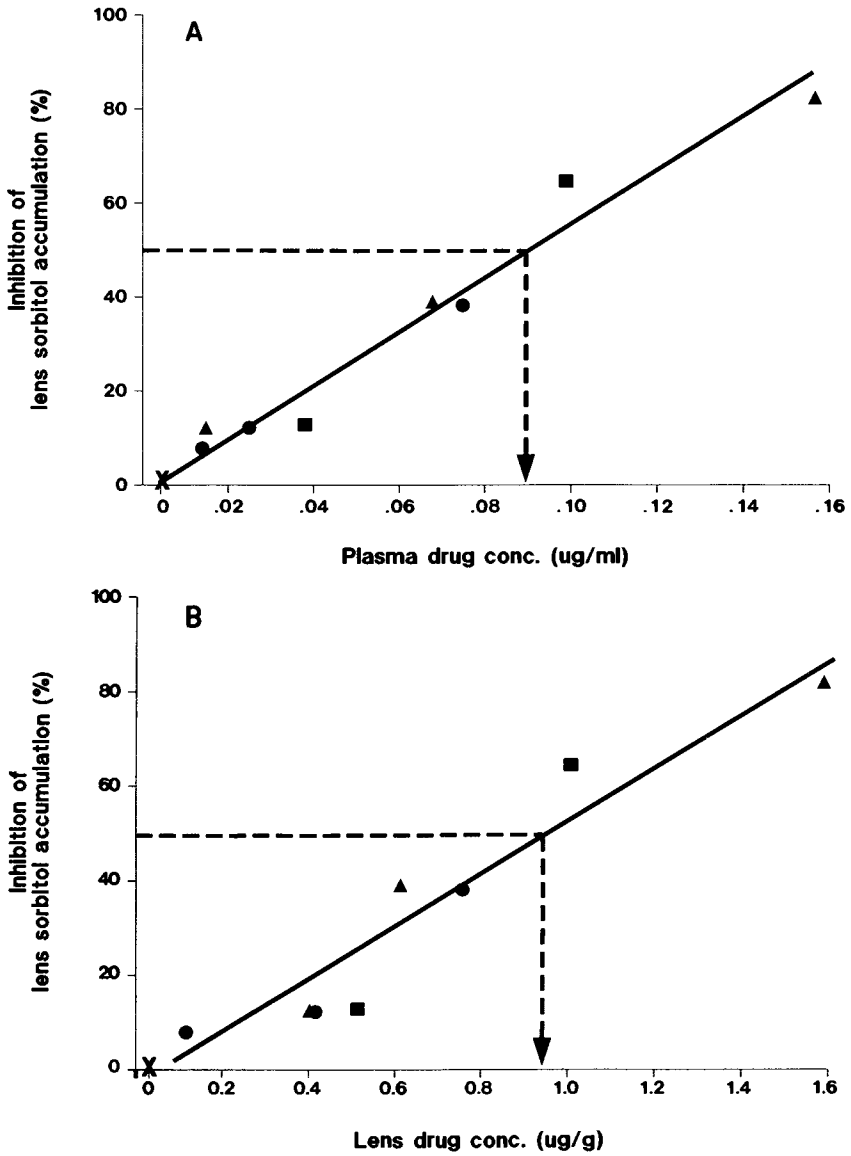

Fig. 3. Inhibition of the lens sorbitol accumulation of diabetic rats treated with AL03152 and its enantiomers vs plasma drug concentration (A) and lens drug concentration (B). Each point is the mean of six rats. The coefficient of variation was typically 20–30% for all parameters (plasma drug, lens drug, and lens sorbitol concentrations), except for 50% for the two lowest plasma drug concentrations. (**X**) Vehicle control; (▲) AL03802 (**R**); (■) AL03152 (**RS**); (●) AL03803 (**S**).

against lens aldose reductase and kidney L-hexonate dehydrogenase were similar for the enantiomers. In addition, the concentration–lens sorbitol relationship in the diabetic rat model appeared to be similar for the two enantiomers, although data were insufficient to completely characterize the concentration–effect relationship *in vivo* for any single compound.

In conclusion, AL03152 enantiomers exhibit stereoselective pharmacokinetic properties in rats but are equivalent in effect assessed from plasma or lens drug concentration or *in vitro*. The apparently diminished residence of AL03803 (S) in tissue without the loss of effect at a given concentration in plasma might confer a therapeutic advantage for this enantiomer.

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