PHARMACOKINETICS OF ORALLY ADMINISTERED DESFERRITHIOCIN ANALOGS IN CEBUS APELLA PRIMATES

RAYMOND J. BERGERON, WILLIAM R. WEIMAR, AND JAN WIEGAND

Department of Medicinal Chemistry, University of Florida, Gainesville, Florida

(Received June 17, 1999; accepted August 18, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:
The pharmacokinetic behavior of three iron chelators based on the desferrithiocin (DFT) pharmacophore, ([S]-4,5-dihydro-2-[2-(hydroxyphenyl)-4-thiazolecarboxylic acid (desmethyldesferrithiocin, DMDFT), 2; (S)-4,5-dihydro-2-[2,4-(dihydroxyphenyl)-4-thiazolecarboxylic acid [4-(S)-hydroxydesazaDMDFT], 3; and (R)-2-[2-(hydroxyphenyl)-4-oxazolinecarboxylic acid, the oxazoline analog of desazaDMDFT, 4, is described. Although 2 and 3 are comparably effective in inducing iron excretion upon oral administration, they exhibit markedly different plasma pharmacokinetics. Ligand 2 achieves a substantially higher plasma concentration than does 3, yet the renal clearance of these compounds is similar. The oxazoline analog 4 shows poor iron clearance when administered orally, although it remains in the plasma for extended periods. Chelator 4 demonstrates a marked capacity to bind to human serum albumin compared with the thiazoline derivatives. The possible implications for designing ligands for the treatment of transfusional iron overload are discussed.

Iron metabolism is characterized by a highly efficient recycling process (Finch et al., 1970; Hallberg, 1981; Finch and Huebers, 1982, 1986) with no specific mechanism for elimination. The introduction of “excess iron” (O’Connell et al., 1985; Thomas et al., 1985; Seligman et al., 1987) into this closed metabolic loop leads to chronic overload and ultimately to peroxidative tissue damage. Iron excretion can be promoted by therapy with ferric ion-specific chelators such as the hydroxamate desferrioxamine B (DFO)1, which is a bacterial siderophore and the drug of choice for the treatment of transfusional iron overload. Because DFO is poorly absorbed from the gastrointestinal tract and rapidly eliminated from the circulation, prolonged parenteral infusion is needed (Pippard, 1982, 1989; Lee et al., 1993). Patient compliance with such a demanding, expensive, and unpleasant regimen is a problem. Thus, considerable interest has developed in the search for an iron chelator that does not require parenteral administration.

Our laboratory has considerable experience in the study of iron chelators in the iron-loaded Cebus apella primate model. This model is highly predictive for the performance of ligands in patients with transfusional iron overload (Peter et al., 1994). We recently reported an extensive structure-activity relationship for orally active iron chelators based on the desferrithiocin (DFT) pharmacophore ([S]-4,5-dihydro-2-[3-hydroxy-2-pyridinyl]-4-methyl-4-thiazolecarboxylic acid, 1, Fig. 1) (Bergeron et al., 1999a,b). This and the other studies (Bergeron et al., 1991, 1993, 1994, 1996b) have made it possible to construct DFT analogs that are still orally effective, yet have substantially reduced toxicity compared with the parent ligand. Nonetheless, an even broader therapeutic window would be desirable, and it also may be advantageous to design chelators that selectively target one of the body’s two main pools of chelatable iron, the hepatocellular (parenchymal) and reticuloendothelial (RE) stores. Thus, an understanding of the pharmacokinetic behavior of iron chelators may allow the development of improved strategies of chelation therapy, including combination regimens targeting specific physiological compartments.

This paper describes the distinctive pharmacokinetic behavior of three prospective oral iron chelators based on the DFT pharmacophore (Fig. 1): desmethyldesferrithiocin ([S]-4,5-dihydro-2-[2-(hydroxyphenyl)-4-thiazolecarboxylic acid, DMDFT, 2; 4-(S)-hydroxydesazaDMDFT ([S]-4,5-dihydro-2-[2,4-(dihydroxyphenyl)-4-thiazolecarboxylic acid, 3; and the oxazoline analog of desazaDMDFT ([R]-2-[2-(hydroxyphenyl)-4-oxazolinecarboxylic acid, 4).

Experimental Procedures

Materials. Ligands 2, 3, and 4 were synthesized as described previously (Bergeron et al., 1991, 1999a,b). C. apella monkeys were purchased from World Wide Primates (Miami, FL). All reagents and standard iron solutions were obtained from Aldrich Chemical Co. (Milwaukee, WI). All hematological and serum chemical tests were carried out by Allied Clinical Laboratories (Gainesville, FL). Cremophor RH-40 was obtained from BASF (Parsippany, NJ).

Collection of Pharmacokinetic Samples in Monkeys. After an overnight fast, sedation with either ketamine and scopolamine or Telazol and atropine (3 and 4), and insertion of a lubricated 5 French, 16-inch urethral catheter, the compounds were solubilized in 40% (v/v) Cremophor RH-40/water and administered p.o. by gavage at a dose of 300 µmol/kg via an 8 French feeding tube. Sedation was maintained by either additional i.m. ketamine (2) or isoflurane gas given by intubation (3 and 4).
At times $t = 0, 0.5, 1, 2, 3, 4, 6,$ and 8-h postdrug, blood samples were taken from a leg vein. Urine was collected via catheter followed by rinsing the bladder with saline before drug administration and at 0.5-h intervals for 4 h thereafter. In some experiments with 3, 10-, 12-, and 24-h blood and urine samples were collected. The plasma and urine were stored frozen until HPLC analysis.

**Plasma and Urine Analytical Methods.** Plasma and urine pharmacokinetic samples were prepared for HPLC analysis by mixing with an equal volume of methanol, chilling at 0°C for 30 min, centrifugation at 3000 g, and filtration of the methanolic supernatant through a 0.2-μm filter before injection.

Analytical separation was performed on a C$_{18}$ reversed-phase HPLC system with UV-detection at 310 nm as described previously (Bergeron et al., 1993). The mobile phases and chromatographic conditions for each compound were as follows: ligand 2, solvent A, 5% aqueous CH$_3$CN; solvent B, 80% aqueous CH$_3$CN; isocratic 100% A (5 min) followed by a linear ramp to 100% B (10 min); analog 3, solvent A, 5% CH$_3$CN in 25 mM potassium phosphate buffer (pH 3.4); solvent B, 60% CH$_3$CN in 25 mM potassium phosphate buffer (pH 3.4); isocratic 100% A (10 min) followed by a linear ramp to 100% B (15 min); chelator 4, solvent A, 5 mM tetrabutylammonium phosphate in water (pH 7.0); solvent B, 80% aqueous CH$_3$CN; isocratic 100% A (7 min) followed by a linear ramp to 100% B (3 min).

**Pharmacokinetic Analyses.** The model-independent pharmacokinetic parameters, including the area under the time-concentration curve (AUC) from time zero to the time of the last measured plasma concentration (8 or 24 h), the total area under the first-moment curve (AUMC), mean residence time (MRT), and renal clearance (CLR) were estimated from plasma and urine concentration-time data as reported in Bergeron et al. (1995, 1996a, 1998).

**Human Serum Albumin Binding Assay.** The binding was carried out by incubation (30 min at 37°C) in 100 mM TRIS-chloride buffer, pH 7.4, such that the final concentrations of human serum albumin (Sigma Chemical Co., St. Louis, MO) and chelator were 1 and 300 μM, respectively. Ultrafiltration was performed with Centricron 3 filters (Amicon, Beverly, MA) to separate bound chelator from free ligand.

**Results and Discussion**

Compounds 2 (Bergeron et al., 1993) and 3 (Bergeron et al., 1999a) administered p.o. were at least as active in promoting iron excretion as an equivalent dose of parenteral DFO, whereas the oxazoline analog 4 was unexpectedly ineffective in inducing iron excretion (Table 1). Pharmacokinetic analysis provides some insight into the origin of the differences in iron clearance among the analogs.

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron clearance efficiency (%)$^a$</td>
<td>8.0 ± 2.5 [42 stool, 58 urine]</td>
<td>5.3 ± 1.7 [90 stool, 10 urine]</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>C$_{max}$ (μmol/l)$^b$</td>
<td>231 ± 90 (122–345)</td>
<td>9.5 ± 7.1 (3.1–22.9)</td>
<td>103 ± 22 (76–128)</td>
</tr>
<tr>
<td>AUC (μmol-h/l)$^b$</td>
<td>384 ± 88 (250–487)</td>
<td>33 ± 27 (12–94)</td>
<td>598 ± 62 (506–647)</td>
</tr>
<tr>
<td>AUMC (μmol-h$^2$/l)$^b$</td>
<td>723 ± 318 (404–1255)</td>
<td>154 ± 140 (61–463)</td>
<td>2788 ± 421 (2177–3141)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.88 ± 0.61 (1.37–2.90)</td>
<td>4.63 ± 1.23 (2.12–6.13)</td>
<td>4.65 ± 0.33 (4.30–5.07)$^c$</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td>50.7% ± 4.2%</td>
<td>5.7 ± 2.8%$^d$</td>
<td>1.0% ± 0.7%</td>
</tr>
<tr>
<td>(0–4 h) (% total dose)$^e$</td>
<td>492 ± 55</td>
<td>312 ± 161</td>
<td>12 ± 7</td>
</tr>
<tr>
<td>CLR$_{m}$ (ml/h-kg)$^f$</td>
<td>316 ± 61</td>
<td>250 ± 59</td>
<td>150 ± 30</td>
</tr>
</tbody>
</table>

$^a$ Iron clearance efficiency is the amount of iron excreted divided by the theoretical amount of 1 g-atom iron/2 mol of chelator (based on a 2:1 complex) × 100%. The efficiency values for 2 and 3 were taken from Bergeron et al. (1993) and Bergeron et al. (1999a), respectively. An equivalent dose of DFO given s.c. is 5.5 ± 0.9% efficient with 55% in stool and 45% in urine (Bergeron et al., 1992).

$^b$ The values for AUC and AUMC are for 0- to 8-h data for ease of comparison. The MRT value was calculated as AUMC/AUC. Because there was a very substantial level of 4 remaining in the plasma at 8 h, this value greatly underestimates that which would be obtained if reliable 0–∞ estimates were available for this drug. This is due to the heavy weighting of the AUMC value by significant plasma concentrations present at later times.

$^c$ Urine samples were obtained from the bladder via catheter followed by saline flush at 30-min intervals during the 4 h after drug administration.

$^d$ Because low plasma levels and urinary output was observed from 0 to 4 h for compound 3 in the first four experiments, four additional experiments were conducted in which urine and plasma samples were collected over a 24-h interval. The data reported herein for 3 represent total 24-h output.

$^e$ Urinary output was observed from 0 to 4 h for compound 3 in the first four experiments, four additional experiments were conducted in which urine and plasma samples were collected over a 24-h interval. The data reported herein for 3 represent total 24-h output.

**Figure 2.** Plasma pharmacokinetics of DMDFT (2, [□]), 4-(S)-hydroxydesazaDMDFT (3, [■]), and the oxazoline analog of desazaDMDFT (4, [●]).

Compounds were administered by oral gavage at 300 μmol/kg to male C. apella monkeys. For clarity, data are plotted as means of five ([□]), eight ([■]), or four ([●]) experiments.
Although 2 and 3 were comparably effective in inducing iron excretion, they exhibited markedly different plasma pharmacokinetics (Fig. 2). The $C_{\text{max}}$ and AUC$_{0-8 \text{ h}}$ of 3 were $\sim 4$ and <10%, respectively, of the corresponding values for 2 (Table 1). The amount of 3 eliminated in the urine in 24 h was only 10% of the amount of 2 eliminated in the first 4 h; this mirrored the low plasma AUC for 3.

The $CL_u$ of the two compounds was similar, 492 ± 55 versus 312 ± 161 ml/h/kg for 2 and 3, respectively. The low plasma levels observed for 3 are most plausibly the result of first-pass clearance by the liver. This explanation is consistent with the efficacy of 3 in inducing iron excretion and the predominantly fecal mode of that excretion (Table 1). In contrast, compound 2 achieved high plasma levels and was found to be extensively eliminated in the urine (50.7 ± 4.2% in 4 h), comparing favorably with the 58% urinary mode of excretion observed for 2 in iron clearance studies (Table 1). The different modes of excretion are not readily attributable to different distribution patterns of loaded iron because liver parenchymal and hepatocyte compartments appear to be loaded to a similar, massive extent in the primate model (R.J.B., G.M. Brittenham, H. Fujioka, W.R.W., and J.W., unpublished data).

Given the inability of oxazoline 4 to promote iron excretion (Table 1), the plasma pharmacokinetics of 4 were remarkable (Fig. 2, dashed line). This compound was well absorbed orally with a $C_{\text{max}}$ value of 103 μM and a very prolonged presence in the plasma; a terminal elimination phase was difficult to discern from these 0 to 8 h experiments. Also remarkable was the very poor $CL_u$ of 4 compared with 2 and 3 (Table 1). We suspected that plasma protein binding might be the basis for the prolonged residence of 4 in the plasma. Thus, the ability of human serum albumin to bind the ligands was evaluated (Table 2). Compound 4 was >99.5% bound to the albumin; this was a 10-fold greater extent than 2 or 3. Therefore, plasma protein binding may provide an explanation of the prolonged residence of 4, its poor $CL_u$, and its lack of clinical efficacy, i.e., such binding may simply render the drug unavailable to chelatable iron pools in the body. Alternatively, it may be that 4 is promoting iron excretion, but the iron chelate is eliminated at such a slow rate as to not be detected in our iron balance experiments. Certainly, the ultimate fate of 4 in the body remains to be determined.

Although the three ligands analyzed in these experiments are all DFT analogs, their iron-clearing properties and pharmacokinetic parameters are markedly different. It is clear that the oral bioavailability of a ligand does not guarantee iron-clearing efficacy. Because the pharmacokinetic behavior of the DFT pharmacophore can be manipulated, it follows that the site of iron chelation also can be altered. To find whether a chelator targets either of the two main pools of chelatable iron, the selective radiolabeling of hepatocellular and RE iron stores can be accomplished with a hypertransfused rat model (Zevin et al., 1992). In studies using this model, iron mobilized from hepatocellular pools was excreted directly into the bile. About one-third to one-half the iron derived from RE stores was eliminated in the urine; the remainder of this iron was recycled into the liver and excreted in the bile (Zevin et al., 1992). If this is the case, then our findings suggest that 2 particularly targets hepatocellular pools, whereas 2 promotes excretion of RE iron. Thus, it may be possible to design nontoxic, orally effective iron chelators that select either of the two major pools of chelatable iron.

**Acknowledgments.** We thank Michael Slusher, Katie Ratliff-Thompson, and Curt Zimmerman for their technical assistance and Eileen Eiler-Hughes for her editorial comments.

**References**


