Short Communication

Pharmacokinetics of Aminolevulinic Acid after Oral and Intravenous Administration in Dogs

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ABSTRACT:

The purpose of these studies was to examine the pharmacokinetics, oral bioavailability, and systemic side effects of aminolevulinic acid (ALA) in beagle dogs after oral and i.v. administration. Oral and i.v. doses of ALA (128 mg of ALA hydrochloride, equivalent to 100 mg of ALA) were administered to four animals using a crossover design. Animals were allowed a 2-week washout period between doses. Plasma ALA concentrations were determined using precolumn fluorescent derivatization and reversed-phase HPLC. Plasma concentrations after i.v. administration declined rapidly with a terminal half-life of 19.5 ± 2.5 min (mean ± S.D.). Total body clearance and volume of distribution at steady state averaged 6.79 ± 1.77 ml/min/kg and 295 ± 128 ml/kg, respectively. Peak plasma concentrations of ALA after oral administration ranged from 1.27 to 9.42 μg/ml. Oral bioavailability in these animals averaged 41.2 ± 14.8% (range, 23.5–58.5%). These studies demonstrate that oral administration may provide a convenient and efficient route of delivery of ALA for photodynamic therapy in patients.

Photodynamic therapy (PDT) is a promising form of regional chemotherapy in which photosensitized cells can be selectively eradicated by exposure to light. A variety of porphyrins have been used for PDT (Benson, 1988; Harty et al., 1989). However, these agents require i.v. administration and cause prolonged photosensitization. These attributes have limited their clinical acceptance and intensified the search for orally bioavailable photosensitizing agents with improved safety profiles.

Aminolevulinic acid (ALA) is an endogenous metabolite present in virtually all mammalian cells as the first committed step in heme biosynthesis. The conversion of glycine and succinyl CoA to ALA is rate-limiting and is tightly regulated by feedback inhibition of ALA synthetase by heme. The next rate-limiting step in the heme biosynthetic pathway occurs at the conversion of protoporphyrin IX (PpIX) to heme. PpIX is a potent photosensitizer that accumulates in both normal and neoplastic tissues after exogenous administration of ALA (Loh et al., 1992; Bedwell et al., 1992; Loh et al., 1993a). Topical, oral, and i.v. administration of ALA followed by light treatment have been used in clinical trials to examine the utility of ALA PDT for the treatment of a variety of neoplasms, including superficial basal cell carcinoma, squamous cell carcinoma of the mouth, and colorectal adenocarcinoma (Marcus et al., 1996). Loh et al. (1993b) examined the tissue accumulation of PpIX-associated fluorescence after oral and i.v. administration of ALA and found that higher oral doses of ALA were required to achieve equal PpIX tissue concentrations. The pharmacokinetics and bioavailability of ALA have not been reported, but are needed to define appropriate dosing guidelines in patients. The goal of these studies was to examine the pharmacokinetics, bioavailability, and safety of ALA after single doses in dogs to guide oral and i.v. dosing studies in human volunteers at our institution.

Materials and Methods

Chemicals. ALA hydrochloride (Levulan) was provided by DUSA Pharmaceuticals, Inc. (Valhalla, NY). All other chemicals and solvents were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ) and were used as received from the manufacturer.

Animal Protocol. All animal procedures used in this research adhered to Principles of Laboratory Animal Care (National Institutes of Health publication no. 85–23, revised 1985) and were approved by the University of Tennessee Animal Care and Use Committee. Four male beagle dogs weighing 10.2 to 12.6 kg were purchased from Antech, Inc. (Dallas, TX). Animals were given two doses of ALA separated by a 2-week washout period. Two animals received an i.v. ALA dose on the first occasion, followed 2 weeks later by an oral ALA dose. The remaining two animals received an oral ALA dose on the first occasion, followed 2 weeks later by an i.v. ALA dose. The dosage form used for these studies, consisting of lyophilized ALA and sodium acetate/mannitol solution in separate vials, was provided by the University of Tennessee Parenterals Medication Laboratory. The vials contained 128 mg of sterile, lyophilized ALA-hydrochloride (equivalent to 100 mg of ALA). Diluent vials contained 10 ml of sodium acetate (9 mg/ml) with mannitol (12.5 mg/ml) solution. ALA was reconstituted with the diluent less than 30 min before administration to provide an aqueous dosing solution of pH 5.0. Oral (7.29 ± 1.15 mg/kg ALA) and i.v. doses (8.28 ± 1.05 mg/kg ALA) were administered by oral gavage and a jugular vein angiocatheter, respectively. This dose was approximately 6 times greater than the dose chosen (i.e., 1.34 mg/kg) for initial patient studies at our institution and resulted in similar plasma concentrations in dogs and humans (unpublished data). A smaller i.v. dose (1.77 ± 0.19 mg/kg) was used to examine the emetic side effects of ALA.

Angiocatheters with a heparin well were placed in the jugular and a...
saphenous vein before each dose. Blood samples (8 ml each) were withdrawn before each dose and at 2, 5, 10, 15, 30, 60, 120, 240, 480, and 720 min after i.v. doses and at 5, 10, 20, 30, 40, 50, 60, 70, 90, 120, 240, 480, and 720 min after oral doses. Blood pressure and heart rate were determined at various time points before and after ALA doses using a noninvasive device (Critikon Dinamap Vital Signs Monitor, model 8100; Critikon, Inc., Tampa, FL). Serum samples obtained immediately before and 12 h after ALA administration were submitted for routine clinical analysis including electrolytes, cholesterol, creatinine, bilirubin, alkaline phosphatase, serum glutamic-pyruvic transaminase, and creatine phosphokinase.

Analytical Method. An HPLC method was developed to quantitate ALA in dog plasma. This method was based in part on the precolumn derivatization method used by Okayama et al. (1990) to assay ALA in urine. The limit of detection of the published method was 10 μg/ml in urine, whereas the limit of detection for the assay reported here is 0.01 μg/ml in plasma. Briefly, an aliquot of plasma (0.01–1 ml) was diluted with human plasma to a final volume of 1.0 ml. The human plasma employed for the dilution also was utilized to prepare the standard curves and contained less than 0.02 μg/ml endogenous ALA. The dilutions were necessary to quantitate plasma concentrations that were greater than the upper concentration limit of the standard curve (1.0 μg/ml). Each plasma sample was treated with 2 ml of acetonitrile to precipitate the plasma proteins. After centrifugation, the supernatant was transferred to a clean culture tube, and solutions containing acetylacetone and formaldehyde were added. The tubes then were capped and placed in a boiling water bath for 1 h. After cooling, 1 ml of 1 N hydrochloric acid and 0.2 ml of internal standard were added. The internal standard, a fluorescent derivative of 3-amino-2-naphthoic acid, was synthesized using the same procedures utilized to derivatize ALA. The internal standard was not prepared in situ with ALA to avoid variability associated with competition for the reaction reagents. The acidified solution containing the internal standard and derivatized ALA was then extracted with 5 ml of ethyl acetate. The organic phase was extracted further with 1 ml of 1 N sodium hydroxide. The aqueous phase was transferred to another tube, acidified with 1 ml of 1 N hydrochloric acid, and re-extracted with fresh ethyl acetate. The ethyl acetate then was transferred to another tube and evaporated to dryness, and the residue was reconstituted with HPLC mobile phase.

Derivatized ALA and the internal standard were separated by isocratic reversed-phase HPLC. The stationary phase was a μBondapak C18 column (3.9 × 300 mm, 10-μm particle size; Waters Corporation, Milford, MA). The mobile phase contained 50% (v/v) methanol and 1% (v/v) glacial acetic acid in deionized water. The flow rate was 1.0 ml/min, and the analytes were monitored using a fluorescence detector (model 474; Waters Corporation) operating at an excitation wavelength of 363 nm and an emission wavelength of 470 nm. A standard curve was prepared in duplicate over a plasma concentration range of 0.01 to 1.0 μg/ml. Control samples (prepared at 0.03, 0.2, and 0.8 μg/ml) were stored frozen with the dog samples from the pharmacokinetic studies and were assayed in triplicate at the same time the dog samples were assayed. All analyses were performed at ambient temperature. The accuracy of the assay ranged from 110% of nominal at 0.01 μg/ml to 96% of nominal at 1.0 μg/ml. The precision (CV%) of the assay ranged from 20% at 0.01 μg/ml to 9% at 1.0 μg/ml.

Data Analysis. Endogenous ALA plasma concentrations are low and do not demonstrate circadian variation (Gorchein and Webber, 1987). For correct for endogenous ALA, the predose plasma concentration of ALA (0.025 ± 0.025 μg/ml, mean ± S.D.) was subtracted from plasma ALA concentrations determined during the pharmacokinetic studies (Marzo and Rescigno, 1993). Thus, pharmacokinetic parameters reported herein represent only exogenous ALA concentrations (i.e., total minus endogenous). The plasma concentration-time profiles were analyzed using established noncompartmental methods. The maximum plasma concentration observed after oral dosing and the time at which it was observed (Tmax) were determined by direct inspection of the individual plasma concentration-time profiles. The terminal slope of the ln(concentration) versus time plot was calculated by linear least-squares regression and the half-life was calculated as 0.693 divided by the absolute value of slope. The area under the plasma concentration-time curve from time zero to infinity (AUC∞) was calculated by the linear trapezoid rule. The total plasma clearance (CL) of i.v. ALA was calculated as the i.v. dose divided by the AUC∞ of i.v. ALA and the volume of distribution at steady state (Vdss) by the method of Benet and Galeazzi (1979). The relative bioavailability (F) of the oral doses was calculated by using the following equation:

\[
F = \frac{\text{AUC}_{\infty}^{\text{oral}}}{\text{AUC}_{\infty}^{\text{iv}}} \times \frac{\text{Dose}^{\text{oral}}}{\text{Dose}^{\text{iv}}}
\]

Statistical analyses were performed using a two-tailed t test at a 5% level of significance.

Results

Figure 1 shows the mean plasma ALA concentration–time profile in four dogs after administration of i.v. and oral doses of ALA hydrochloride. Plasma concentrations after i.v. ALA administration declined rapidly with a terminal half-life of 19.5 ± 2.50 min. CL and Vdss averaged 6.79 ± 1.77 ml/min/kg and 259 ± 128 ml/kg, respectively. The mean (±S.D.) peak plasma concentration of ALA observed after oral administration was 6.22 ± 3.48 μg/ml and occurred 28.1 ± 21.4 min after dosing. The terminal half-life after oral administration (40.7 ± 22.9 min) was not significantly different (P > .1) from that observed after i.v. administration, suggesting that elimination, and not absorption, is the rate-limiting process in ALA disposition after oral dosing. However, it is important to note that the lack of statistical significance between the half-lives observed after i.v. and oral dosing also may reflect the relatively small (n = 4) number of animals used in this study. Oral bioavailability in these animals averaged 41.2 ± 14.8%. Pharmacokinetic parameters characterizing the plasma concentrations of ALA after i.v. and oral doses are summarized in Table 1. We also monitored routine clinical chemistries.
ALA administration. Thus, conversion of ALA to PpIX by the gastrointestinal mucosa efficiently converts ALA to PpIX after i.v. other processes. Third and last, Loh et al. (1992) showed that the through the liver, leaving an additional 40% of the drug that is lost to most, about 20% of an oral dose of ALA is metabolized during transit in the dog (31 ml/min/kg; Davies and Morris, 1993), indicates that, at hepatic extraction ratio of ALA, as determined by comparison of the hepatic blood flow (6.79 ml/min/kg) to literature values for hepatic blood flow by transformed cells indicate that absorption is unlikely to present a significant barrier preventing the appearance of ALA in systemic circulation after oral administration. Because first-pass metabolism by intestinal microflora cannot be ruled out, further studies to examine the mechanisms limiting the oral absorption of ALA are needed.

One further point regarding the moderate oral bioavailability of ALA in these animals merits discussion. Emesis occurred in all dogs after oral ALA administration. Thus, emesis may have decreased the actual oral dose available for absorption and the apparent bioavailability. The emesis was not collected for analysis. However, comparison of the time at which maximal plasma ALA concentrations occurred (T\text{max}, Table 1) and the time at which the first episode of emesis occurred suggests that this was not the case. The time elapsed between when T\text{max} was achieved and emesis first occurred was 21, 140, 41, and 55 min in dogs 1, 2, 3, and 4, respectively. Thus, there appears to be no relationship between emesis and oral dose, because the dog with the lowest AUC value after oral dosing (Table 1) had the greatest time between T\text{max} and emesis and dogs with the highest AUC values after oral dosing had shorter times between T\text{max} and emesis.

In summary, these studies demonstrate that orally administered ALA is approximately 40% bioavailable after an oral dose in beagle dogs. Plasma pharmacokinetics are consistent with a low-extraction (extraction ratio about 0.2) and moderately distributed drug (V\text{dss} of about 0.2 liter/kg). The major barrier to systemic bioavailability of ALA after oral dosing appears to be its presystemic metabolism by the gastrointestinal mucosa. ALA produced no significant cardiovascular side effects after oral and i.v. administration, with dysgeusia and vomiting as the major side effects.

**Table 1**

**Pharmacokinetic parameters of ALA in dogs**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Half-life (min)</th>
<th>CL (ml/min/kg)</th>
<th>Vdss (ml/kg)</th>
<th>AUC (µg·min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.1</td>
<td>7.38</td>
<td>413</td>
<td>952</td>
</tr>
<tr>
<td>2</td>
<td>20.5</td>
<td>5.01</td>
<td>155</td>
<td>1769</td>
</tr>
<tr>
<td>3</td>
<td>22.2</td>
<td>8.99</td>
<td>316</td>
<td>1044</td>
</tr>
<tr>
<td>4</td>
<td>16.3</td>
<td>5.79</td>
<td>154</td>
<td>1353</td>
</tr>
<tr>
<td>Mean</td>
<td>19.5</td>
<td>6.79</td>
<td>259</td>
<td>1279</td>
</tr>
<tr>
<td>S.D.</td>
<td>2.50</td>
<td>1.77</td>
<td>128</td>
<td>369</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Maximum plasma concentration (µg/ml)</th>
<th>T\text{max} (min)</th>
<th>AUC (µg·min/ml)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.04</td>
<td>20.6</td>
<td>581</td>
<td>0.585</td>
</tr>
<tr>
<td>2</td>
<td>1.27</td>
<td>59.8</td>
<td>266</td>
<td>0.235</td>
</tr>
<tr>
<td>3</td>
<td>7.17</td>
<td>12.5</td>
<td>335</td>
<td>0.366</td>
</tr>
<tr>
<td>4</td>
<td>9.42</td>
<td>19.6</td>
<td>631</td>
<td>0.461</td>
</tr>
<tr>
<td>Mean</td>
<td>6.22</td>
<td>28.1</td>
<td>453</td>
<td>0.412</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.48</td>
<td>21.4</td>
<td>180</td>
<td>0.148</td>
</tr>
</tbody>
</table>

In the dog, a smaller ALA dose. In these studies, each animal received a 20-mg i.v. dose of ALA (i.e., one-fifth of the i.v. dose administered during the pharmacokinetic studies). Animals were monitored for 4 h after ALA administration. Three of four animals also vomited after administration of the smaller dose. Administration of the diluent solution (i.e., sodium acetate and mannitol solution) alone did not result in emesis, indicating that this effect was related to ALA administration.

**Discussion**

The moderate oral bioavailability of ALA (i.e., about 40%) could be the result of numerous factors, including poor absorption, first-pass metabolism by intestinal microflora, first-pass metabolism in the intestinal wall, and/or first-pass metabolism in the liver. Several facts suggest that first-pass metabolism in the intestinal wall is the major barrier preventing the appearance of ALA in the bloodstream after oral administration. First, numerous transport systems for amino acids are known to exist in the gastrointestinal tract. Although these transport systems, by nature, may be saturable, the fact that ALA is a small, water-soluble, five-carbon amino acid and that it is taken up rapidly by transformed cells indicate that absorption is unlikely to present a significant barrier (Peng et al., 1987; Bedwell et al., 1992). Second, the hepatic extraction ratio of ALA, as determined by comparison of ALA’s CL (6.79 ml/min/kg) to literature values for hepatic blood flow in the dog (31 ml/min/kg; Davies and Morris, 1993), indicates that, at most, about 20% of an oral dose of ALA is metabolized during transit through the liver, leaving an additional 40% of the drug that is lost to other processes. Third and last, Loh et al. (1992) showed that the gastrointestinal mucosa efficiently converts ALA to PpIX after i.v. ALA administration. Thus, conversion of ALA to PpIX by the gastrointestinal mucosa may be the major factor limiting the appearance of ALA in systemic circulation after oral administration. Because first-pass metabolism by intestinal microflora cannot be ruled out, further studies to examine the mechanisms limiting the oral absorption of ALA are needed.

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**Department of Pharmaceutical Sciences**

**College of Pharmacy**

**University of Tennessee**

**Guidelines, Inc.**

**Miramar, Florida**

**James T. Dalton**

**Marvin C. Meyer**

**Allyn L. Golub**
References


