Clinical Pharmacokinetics of 5-Aminolevulinic Acid in Healthy Volunteers and Patients at High Risk for Recurrent Bladder Cancer

JAMES T. DALTON, CHARLES R. YATES, DONGHUA YIN, ARTHUR STRAUGHN, STUART L. MARCUS, ALLYN L. GOLUB, and MARVIN C. MEYER

Department of Pharmaceutical Sciences, College of Pharmacy, The Ohio State University, Columbus, Ohio (J.T.D., D.Y.); Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, University of Tennessee, Memphis, Tennessee (C.R.Y., A.S., M.C.M.); DUSA Pharmaceuticals, Inc., Valhalla, New York (S.L.M.); and Guidelines Integrated Services, Inc., Miramar, Florida (A.L.G.)

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ABSTRACT

5-Aminolevulinic acid (ALA) is a precursor of protoporphyrin IX (PpIX) that is being evaluated for use in photodiagnosis and phototherapy of malignant and nonmalignant disorders. Previous clinical studies using topical, oral, and intravesical administration have been conducted in attempts to determine the optimal route of administration for ALA. The purpose of these studies was to examine the systemic pharmacokinetics and elimination of ALA, the bioavailability of ALA after oral and intravesical doses, and the factors that affect ALA concentrations in the bladder during intravesical treatment. The disposition of ALA was evaluated in six healthy volunteers receiving single intravesical and oral doses (100 mg) and eight patients at high risk for recurrent bladder cancer receiving an intravesical dose (1.328 g) of ALA. The mean (±S.D.) plasma area under the plasma concentration-time curve from time 0 to infinity of PpIX (0.20 ± 0.11 µg · h/ml) after intravenous administration of ALA was not significantly different from that observed after oral administration of ALA (0.15 ± 0.11 µg·h/ml; P = 0.49). ALA terminal half-life was approximately 45 min after intravenous or oral administration. The oral bioavailability of ALA was approximately 60%. After intravesical administration, urine production was largely responsible for decreases in ALA concentration in the bladder, with less than 1% being absorbed into the systemic circulation. In summary, oral and intravenous administration of ALA at these doses results in modest plasma levels of PpIX. Regional administration (i.e., intravesical) of ALA resulted in a significant pharmacokinetic advantage, with urinary bladder being exposed to concentrations approximately 20,000-fold higher than systemic circulation.

ALA is present in virtually all human cells. It represents the first committed intermediate in heme biosynthesis and is a metabolic precursor of the endogenously formed photosensitizer protoporphyrin IX (PpIX). The synthesis of ALA is normally tightly controlled by feedback inhibition of ALA synthetase by intracellular heme levels. Exogenously administered ALA bypasses this control point and induces intracellular accumulation of PpIX (Kennedy and Pottier, 1992). Selectivity of PpIX accumulation in malignant and premalignant tissue has been demonstrated in a number of animal models and in human clinical studies, and may be useful for enhancing visual detection of tumors, including bladder malignancies (Marcus et al., 1996). ALA may also have a role in the treatment of esophageal cancer and squamous cell carcinoma (Ackroyd et al., 2000; Hinnen et al., 2000). Clinical studies using topical, oral, and intravesical administration have been conducted for a variety of disorders. In many cases, the pharmacokinetics of ALA and/or PpIX was examined. Popken et al. (2000) recently reported the systemic pharmacokinetics of ALA after intravesical administration (2- and 4-h bladder dwell times) in 20 patients with confirmed bladder carcinoma (Popken et al., 2000). Maximal plasma ALA concentrations were reached in 0.55 and 0.62 h after 2- and 4-h dwell times, respectively. The terminal elimination half-life of ALA in plasma was approximately 45 min, regardless of the dwell time of the intravesical dose in the bladder. Systemic absorption of ALA after the intravesical dose was estimated as less than 1%. However, the pharmacokinetics of ALA inside the bladder during intravesical treatment was not examined. Webber et al. (1997) examined the pharmacokinetics of ALA and PpIX after oral administration of ALA in a small number of patients (n = 4) (Webber et al., 1997). To our knowledge, there have been no ALA

ABBREVIATIONS: ALA, 5-aminolevulinic acid; PpIX, protoporphyrin IX; HPLC, high-performance liquid chromatography; AUC, plasma area under the plasma concentration-time curve; tlast, last sampling time; CL, clearance; MRT, mean residence time; F, bioavailability; Vd, volume of distribution.
pharmacokinetic studies after intravenous administration of ALA in humans. Thus, absolute ALA oral bioavailability has not been determined in humans.

The purpose of the current studies was to examine the pharmacokinetics of ALA in humans after intravenous, oral, and intravesical administration. The studies reported herein provide new information regarding the systemic pharmacokinetics and elimination of ALA, the absolute bioavailability of ALA after oral and intravesical doses, and the factors that affect ALA concentrations in the bladder during intravesical treatment.

Materials and Methods

Clinical Procedures for Intravenous and Oral Dosing. This study was completed using a two-way crossover design in six healthy volunteers. The study was reviewed and approved by the University of Tennessee Institutional Review Board. Before entry into each study, potential subjects received a complete blood and urine analysis, an electrocardiogram, and a physical exam. Subjects were excluded for any abnormal findings. Each subject received an intravenous and oral dose of ALA, with a 1-wk washout period between doses. The ALA dosage forms used for intravenous and oral dosing were identical and were provided by DUSA Pharmaceuticals, Inc. (Valhalla, NY). The dosage form consisted of a vial containing 128 mg of sterile, lyophilized ALA hydrochloride (equivalent to 100 mg of ALA) and a vial containing 10 ml of sodium acetate (9 mg/ml) and mannitol (12.5 mg/ml) diluent solution. ALA was reconstituted with the diluent less than 30 min before administration to provide an aqueous dosing solution of pH 5.0.

The subjects collected urine for two consecutive 12-h periods, beginning 24 h before each dose. The subjects were admitted to the hospital on the evening before each dose, and began the second 12-h urine collection. Subjects were not permitted to eat during this time but were allowed to drink water. On the morning of each study, an angiocatheter was placed in a forearm vein for blood sampling and subjects were transferred to a totally dark room, with the exception of a single red light. Subjects then received an intravenous or an oral dose of ALA. The intravenous dose was administered over 1 min via a contralateral angiocatheter, whereas the oral dose was administered with 120 ml of water. Blood samples (15 ml each) were collected immediately before dosing and 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h after the intravenous dose. The same blood-sampling scheme was used after administration of the oral dose, with the exception that no sample was collected 0.1 h after the dose. Blood samples were collected in vacuum tubes containing heparin, stored on ice in the dark, and transferred to a dark room for sample processing. Blood samples were then centrifuged at 500 g for 10 min, and the plasma layer removed. The subjects also collected urine for two consecutive 12-h periods after administration of each dose. At the end of each of the four 12-h urine collection periods (i.e., two predose and two postdose), the total volume and pH of the urine were determined. An aliquot (5 ml) of each urine sample and plasma samples were prepared and stored frozen with the patient samples from the pharmacokinetic studies. Stability studies showed that there was minimal degradation of ALA in plasma when frozen for up to 247 days. The control samples were assayed in triplicate at the same time the patient samples were assayed. Standard curves were linear over the tested concentration range, with interday coefficients of variation for the standards ranging from 4.5% at 0.01 µg/ml to 0.1% at 10.0 µg/ml. Interday coefficients of variation for the plasma controls samples were 12.6, 9.3, and 14.8% at concentrations of 0.02, 0.2, and 4.0 µg/ml, respectively.

Control and standard curve samples for urine analysis were prepared in water over a concentration range of 0.1 to 15.0 µg/ml. Stability studies showed that there was minimal degradation of ALA in diluted human urine under these storage conditions for up to 187 days. Urine control samples were prepared and stored frozen with the patient samples from the pharmacokinetic studies. Standard curves were linear over the tested concentration range, with interday coefficients of variation for the standards ranging from 10.4% at 0.1 µg/ml to 4.7% at 15.0 µg/ml. Interday coefficients of variation for the plasma controls samples were 4.7, 4.3, and 3.7% at concentrations of 0.04, 0.26, and 10.3 µg/ml, respectively.

Materials and Methods

Clinical Procedures for Intravesical Dosing. This was a single-dose, open-label study. Eight patients at high risk for recurrent bladder cancer were recruited from the Urology Clinic at the Veterans Administration Hospital in Memphis, TN. Inclusion criteria for this study included patients who are at high risk of recurrence as judged by symptomatology, cystoscopy, urine cytology, biopsy, and/or history of recurrent bladder tumors. Patients with newly diagnosed bladder cancer were not included in the study. All patients were male and completed informed consent before study entry. The study was reviewed and approved by the University of Tennessee Institutional Review Board.

On the morning of treatment, a urethral catheter was placed into the bladder of each patient to drain urine, and an angiocatheter with heparin well placed in the arm for venous blood sampling. A filter-sterilized solution (total volume 55 ml) of ALA hydrochloride was instilled into the bladder via the urethral catheter, and the catheter clamped shut for 1 h. The total dose of ALA hydrochloride was 1.698 g, equivalent to 1.328 g of ALA (final concentration 24,100 µg/ml). The pH of the intravesical dosing solution was 5.0. Serial blood and urine samples were collected before, during, and after the intravesical instillation period. Seven blood samples (15 ml each) were withdrawn from the venous angiocatheter, including one immediately before the ALA dose and six additional samples at 0.25, 0.5, 0.75, 1, 2, and 3 h after the intravesical ALA dose. Blood samples were collected in vacuum tubes containing heparin and stored on ice. Blood samples were then centrifuged at 500g for 10 min, and the plasma layer removed.

Urine samples were obtained from the urethral catheter during the instillation period using methods previously described (Wientjes et al., 1991; Dalton et al., 1999a). Three urine samples were collected, including one before the dose and two additional samples at 0.33 and 0.67 h. At the end of the 1-h instillation period, the bladder was drained via the urethral catheter. The volume and pH of the urine was recorded and an aliquot (5 ml) collected for HPLC analysis. Urine and plasma samples were stored at −20°C until HPLC analysis. Plasma and urine samples collected after intravesical dosing were assayed only for ALA.

Analysis of ALA in Plasma and Urine. ALA concentrations in the plasma, urine, and bladder instillate of subjects receiving ALA were determined using methods previously developed and reported in our laboratories (Dalton et al., 1999a,b). Standard curves were prepared over a plasma concentration range of 0.01 to 10.0 µg/ml. For plasma samples containing ALA concentrations greater than 10 µg/ml, an aliquot of plasma (0.01–1 ml) was diluted with human plasma to a final volume of 1.0 ml. The human plasma used for the dilution was also used to prepare the standard curves, and contained less than 0.02 µg/ml endogenous ALA. In addition, plasma control samples were prepared and stored frozen with the patient samples from the pharmacokinetic studies. Stability studies showed that there was minimal degradation of ALA in plasma when frozen for up to 247 days. The control samples were assayed in triplicate at the same time the patient samples were assayed. Standard curves were linear over the tested concentration range, with interday coefficients of variation for the standards ranging from 4.5% at 0.01 µg/ml to 0.1% at 10.0 µg/ml. Interday coefficients of variation for the plasma controls samples were 12.6, 9.3, and 14.8% at concentrations of 0.02, 0.2, and 4.0 µg/ml, respectively.

Control and standard curve samples for urine analysis were prepared in water over a concentration range of 0.1 to 15.0 µg/ml. Stability studies showed that there was minimal degradation of ALA in diluted human urine under these storage conditions for up to 187 days. Urine control samples were prepared and stored frozen with the patient samples from the pharmacokinetic studies. Standard curves were linear over the tested concentration range, with interday coefficients of variation for the standards ranging from 10.4% at 0.1 µg/ml to 4.7% at 15.0 µg/ml. Interday coefficients of variation for the plasma controls samples were 4.7, 4.3, and 3.7% at concentrations of 0.04, 0.26, and 10.3 µg/ml, respectively.

Analysis of PpIX in Plasma. An HPLC method was developed to quantitate PpIX concentrations in human plasma. Several literature reports indicate that PpIX is unstable in plasma (van Gog and Schothorst, 1973; Brazier, 1990). We therefore investigated the stability of PpIX in various media and storage conditions (e.g., light, yellow light, red light, temperature, and solvents). These studies indicated that blood and plasma samples 1) must be shielded from light at all times, 2) could be stored in the dark for up to 4 h at room temperature before extraction with ethyl acetate, and 3) could be stored after extraction in ethyl acetate for up to 53 days at −20°C.
with minimal loss of PpIX. For analysis of PpIX in plasma, blood, and plasma samples were processed in a room lit only with low-level red light, which had previously been determined to preclude PpIX degradation. Briefly, an aliquot (1 ml) of plasma was placed in a glass tube with 0.1 ml of an internal standard solution (a 2-μg/ml solution of mesoporphyrin IX in ethyl acetate), 0.1 ml of glacial acetic acid, and 5 ml of ethyl acetate. The mixture was briefly vortexed, sealed with Teflon-lined caps, and shaken on a horizontal shaker for 25 min at room temperature. The tubes were then centrifuged at approximately 500g for 20 min. The organic phase was transferred to a silanized conical tube and the ethyl acetate was evaporated under nitrogen. The aqueous phase was retained and extracted a second time with an additional 5 ml of ethyl acetate, as described above. This second portion was combined with the residue from the first extraction and evaporated to dryness under nitrogen in the same tube. The residue was reconstituted with 0.1 ml of dimethyl sulfoxide and injected onto the HPLC. PpIX and the internal standard were separated using reversed phase chromatography, with retention times of 9.2 and 14.3 min, respectively. The mobile phase contained 30% methanol and 46% tetrahydrofuran in 0.05 M phosphate buffer, pH 4.4, and was pumped at a flow rate of 1 ml/min. The stationary phase was a C18 column (μBondapak, 300 × 3.9 mm; Waters, Milford, MA). Analytes were detected by using a standard ultraviolet HPLC detector (model 481; Waters) set at 400 nm. Standard curves were prepared and were linear over a plasma concentration range of 0.01 to 10 μg/ml. In addition, plasma control samples were prepared at concentrations of 0.0, 0.021, 0.425, and 8.5 μg/ml. The control samples were assayed in triplicate at the same time the patient samples were assayed. Interday coefficients of variation for the standard curves and controls ranged from 7.7 to 2.6% at 0.01 and 1 μg/ml, respectively, during pretest validation and from 11.5 to 27.4%, respectively, during analysis of the subject samples. These data more than likely demonstrate the inherent difficulty in measurement of PpIX using procedures that must be performed in the dark.

**Data Analysis.** The plasma concentration-time profiles were analyzed using noncompartmental methods and WinNonlin software, version 3.0 (Pharsight, Cary, NC). Endogenous plasma and urine concentrations of ALA were low and were ignored during pharmacokinetic analysis. The maximal plasma concentration of ALA (Cmax) and the time at which it was achieved (Tmax) were determined by visual inspection of the plasma concentration-time profile for each patient. The terminal slope of each ln (concentration) versus time plot was calculated by linear least-squares regression of the plasma concentration-time data obtained after 2 h. The half-life was calculated as 0.693 divided by the absolute value of slope. The area under the plasma concentration-time curve (AUC0–tlast) from time 0 to the last sampling time (tlast) was calculated by the linear trapezoidal rule. The AUC from tlast to time infinity (AUCtlast–∞) was calculated as the concentration at tlast divided by the absolute value of slope. The total AUC from time 0 to infinity (AUC0–∞) was calculated as the sum of AUC0–tlast and AUCtlast–∞. Total plasma clearance (CL) of ALA was calculated as the (intravenous dose) divided by the (AUC0–∞) observed after the intravenous dose. Renal clearance (CLR) was calculated as total amount of ALA recovered in the urine divided by plasma AUC0–∞. The volume of distribution (Vd) was calculated as (dose) divided by the product of the absolute value of slope and AUC0–∞. The percentage of the extravascular doses that was absorbed into the plasma (i.e., bioavailability, F) was calculated as the product of the AUC0–∞ observed after the oral or intravesical dose and CL, divided by the dose administered by the oral or intravesical route, respectively. The relative exposure of the bladder urothelium to the drug was estimated as the ratio of the AUC0–tlast in urine to the AUC0–tlast in plasma after intravesical administration of ALA. For oral doses, the values of CL and Vd were calculated as described above, except that the values were also multiplied by the F observed in individual subjects. Statistical differences in the pharmacokinetic parameters were determined, as appropriate, using a t test or analysis of variance with the α value set a priori at P < 0.05.

**Results**

**Pharmacokinetics of ALA after an Intravenous Dose.** Plasma concentrations of ALA declined rapidly after intravenous administration, decreasing from an average value of 16.9 ± 6.2 μg/ml at 0.1 h to undetectable levels within 7 h (Fig. 1). The geometric mean terminal half-life of ALA was about 50 min (Table 1). The rapid disappearance of ALA from the plasma was likely due to its small Vd, as opposed to rapid metabolism or renal elimination of unchanged drug. The average Vd of ALA after intravenous dosing was 9.3 liters, or 0.12 ± 0.04 l/kg in these subjects. The mean (±S.D.) CL of ALA after intravenous dosing was 7.8 ± 2.5 l/h. Urinary excretion data showed that renal elimination of unchanged ALA accounted for approximately 27% of its CL.

**Pharmacokinetics of ALA after an Oral Dose.** Peak plasma concentrations of ALA (4.6 ± 0.9 μg/ml) were achieved at 0.83 ± 0.20 h after administration of the oral dose. Plasma ALA concentrations declined with a terminal half-life of approximately 45 min, similar to that observed after intravenous administration. The MRT of ALA after oral administration (i.e., about 90 min) was only slightly longer than that observed after intravenous administration (i.e., about 60 min). These and other pertinent pharmacokinetic parameters are summarized in Table 1. Based on plasma data, approximately 60% of ALA was absorbed after oral administration. The bioavailability of the oral dose was also estimated by comparing the urinary excretion of ALA after intravenous and oral doses. Urinary data showed that 50.3 ± 6.5% was absorbed after an oral dose, reasonably approximating the bioavailability estimate obtained using plasma drug concentrations. As observed after the intravenous dose, about 25% of the drug reaching the systemic circulation (i.e., amount in urine divided product of dose and bioavailable fraction) was excreted as unchanged drug in the urine. Analysis of variance was performed to examine the differences in the pharmacokinetic parameters due to treatment (i.e., route of administration), sequence, and/or period effects. No significant differences with regard to sequence or period effects were observed in any of the pharmacokinetic variables. As
TABLE 1  
Mean (± S.D.) pharmacokinetic parameters of ALA after intravenous and oral administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous Dose</th>
<th>Oral Dose</th>
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<tbody>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µg · h/ml)</td>
<td>13.7 ± 3.4</td>
<td>7.4 ± 1.2</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</td>
<td>15.44 ± 6.60</td>
<td>4.65 ± 0.94</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>Not applicable</td>
<td>0.875 (0.5 to 1.0)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>7.8 ± 2.5</td>
<td>13.8 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL&lt;sub&gt;n&lt;/sub&gt; (l/h)</td>
<td>2.1 ± 1.9</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>Vd (liters)</td>
<td>9.3 ± 2.8</td>
<td>14.5 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt; (leters)</td>
<td>50.1 ± 28</td>
<td>44.4 ± 10.1</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>59.7 ± 15.1</td>
<td>92.0 ± 9.7</td>
</tr>
<tr>
<td>F (%)</td>
<td>100</td>
<td>58.4 ± 15.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Median (range).  
<sup>b</sup> These values represent mean values of CL/F and Vd/F calculated using the concentration-time profiles observed after oral administration of ALA.

expected, differences (P < 0.05) in AUC, CL, estimated steady-state volume of distribution (V<sub>ss</sub>), and MRT were observed when comparing the pharmacokinetic parameters after intravenous and oral administration, whereas no statistically significant differences in half-life or CL<sub>n</sub> were found.

**Plasma Concentrations of PpIX after Intravenous and Oral Doses of ALA.** Plasma concentrations of PpIX observed after intravenous and oral administration of ALA were low and erratic (Fig. 2), ranging from below the limit of quantitation (i.e., 0.01 µg/ml) to 0.25 µg/ml. In fact, at this dose of ALA (100 mg), PpIX was not detected in the majority of samples (e.g., 39 of the 84 samples collected between 0.1 and 10 h after administration of the intravenous dose of ALA). The mean (± S.D.) plasma AUC<sub>0-∞</sub> of PpIX (0.20 ± 0.11 µg · h/ml) after intravenous administration of ALA was not significantly different from the AUC<sub>0-∞</sub> of PpIX (0.15 ± 0.11 µg · h/ml) observed after oral administration of ALA (P = 0.49; paired t test).

![Fig. 2. Plasma concentrations of PpIX after intravenous and oral doses of ALA. Six healthy human subjects received oral and intravenous doses of ALA using a crossover experimental design. An aqueous solution containing 128 mg of ALA hydrochloride (i.e., 100 mg of ALA) was administered during each phase of the study, with a 1-week washout period between doses. Plasma concentrations of PpIX at various times after administration were determined using a validated HPLC assay. Error bars represent the standard deviation of the mean plasma concentration observed at each time point.](Image 61x132 to 279x284)

**Pharmacokinetics of ALA after an Intravesical Dose.** Eight patients at high risk for recurrent bladder cancer received an intravesical dose (1.328 g dissolved in 55 ml of buffer; target concentration 24,100 µg/ml) of ALA. HPLC analysis showed that the concentration of the intravesical dosing solution was 20,700 ± 2,310 µg/ml (mean ± S.D.). The urine concentration of ALA declined from this value over the course of the intravesical instillation period due to urine production, systemic absorption, and degradation (Fig. 3). The mean (± S.D.) concentration of ALA present in the urine that was withdrawn from the bladder at the end of the instillation period was 5530 ± 1940 µg/ml, or about 25% of the initial concentration of the intravesical dosing solution. The mean volume of urine withdrawn from the bladder at the end of the 60-min instillation period was 169 ml (Table 2). Thus, urine production alone accounted for a decrease in the concentration of the intravesical dosing solution to 32% of its initial value (i.e., approximately 3-fold dilution of the ALA dosing solution by urine produced during the instillation period). The pH of the urine withdrawn at the end of the instillation period was determined and averaged 5.6, indicat-

![Fig. 3. Urine and plasma concentrations of ALA after intravesical dosing. Eight patients at high risk for recurrent bladder cancer received an intravesical dose of ALA. A filter-sterilized solution (total volume 55 ml) of ALA hydrochloride was instilled into the bladder via the urethral catheter, and the catheter clamped shut for 1 h. The total dose of ALA hydrochloride was 1.698 g, equivalent to 1.328 g of ALA (theoretical final concentration 24.1 µg/ml). Serial blood and urine samples were collected before, during, and after the intravesical instillation period. ALA concentrations were determined using a validated HPLC method. Error bars represent the standard deviation of the mean (n = 8) ALA concentration observed at each time point.](Image 326x148 to 542x484)
ing that the buffered dosing solution maintained the pH within an appropriate range (i.e., less than pH 7) to avoid degradation of ALA. The majority (78.4 ± 34.0%; mean ± S.D.) of intravesically administered ALA was recovered in the urine collected at the end of the instillation period, further supporting the idea that degradation played a minor role in the observed decrease in urine concentrations of ALA. Together, these data indicate that urine production was largely responsible for the observed decrease in ALA concentrations in the urine, and that ALA degradation contributed little, if any, to the observed decrease in urine ALA concentration.

Predose plasma concentrations of ALA were 0.028 ± 0.018 μg/ml (mean ± S.D.). A small fraction of the dose was absorbed into the bloodstream after intravesical administration of ALA. Peak plasma ALA concentrations were 0.54 ± 0.61 μg/ml (mean ± S.D.) and occurred 38 ± 17 min after intravesical instillation (Table 2). The mean plasma ALA concentration-time profile from these patients is also presented in Fig. 3. The geometric mean terminal plasma half-life of ALA after intravesical administration was 58 ± 16 min (Table 2). This value closely approximated the half-life of ALA observed after intravenous and oral administration. Only 0.52 ± 0.61% (i.e., less than 1%) of the intravesical dose of ALA was absorbed into the plasma, indicating that the urinary bladder protects the systemic circulation from exposure to ALA during intravesical administration.

Clinical Observations. The administration of intravesical, oral, and intravesical doses of ALA did not result in any adverse reactions, with the exception of pain at the injection site reported by two subjects after intravesicular injection.

Discussion

The rapid appearance of ALA in plasma after oral administration, similar terminal half-lives, and modest increase in MRT observed after oral and intravesical administration suggest that ALA is rapidly absorbed after oral administration. Assuming a hepatic blood flow of 90 l/h (Davies and Morris, 1993) and that the nonrenal clearance of ALA occurs mainly due to hepatic metabolism (i.e., nonrenal CL = hepatic CL = 5.3 l/h), this study indicates that hepatic first-pass metabolism is not the major factor limiting the oral bioavailability of ALA (i.e., one would predict an oral bioavailability of 94% based on a hepatic extraction ratio of 0.06 for ALA). van den Boogert et al. (1998) showed that significant concentrations of PpIX accumulate in the duodenal aspirate and jejunum of rats after oral administration of ALA. Thus, this suggests that gastrointestinal conversion of ALA to PpIX may be the major factor limiting its oral absorption. However, it is important to note that van den Boogert et al. (1998) and the majority of clinical investigations performed to date (Webber et al., 1997; Ackroyd et al., 2000; Hinnen et al., 2000) used much larger doses of ALA (e.g., 40–200 mg/kg). Thus, it is possible that solubility and/or capacity-limited absorption play a greater role in limiting the oral absorption of ALA at higher doses. We chose much smaller doses of ALA during our studies to preclude drug-related toxicity in our healthy volunteers and to preclude dose-dependent pharmacokinetic effects when comparing our intravenous, oral, and intravesical plasma concentration-time data.

The observation that PpIX levels after intravesical administration of ALA were not significantly higher than those observed after oral administration, despite the fact that only 60% of the oral dose of ALA was absorbed, suggests that the short MRT of ALA in the systemic circulation after intravenous and oral administration of 100 mg of ALA does not allow for significant conversion to PpIX in the systemic circulation. This hypothesis is corroborated by the fact that the values for AUC\textsubscript{0–∞} of ALA were about 50-fold higher than those for PpIX after intravenous and oral administration of ALA. However, it is important to note that tissue concentrations of PpIX (especially in the gastrointestinal mucosal) after oral and intravesical administration are likely higher than those observed in plasma (van den Boogert et al., 1998; Loh et al., 1993). Furthermore, the pharmacokinetics of ALA at higher doses (e.g., 40–60 mg/kg used clinically) may not be linear with dose, meaning that the MRT of ALA in plasma may be longer and allow for greater conversion to PpIX. Additional studies to characterize the pharmacokinetics of ALA will be needed to address these questions. Nonetheless, assuming that the pharmacokinetics of ALA and PpIX do not vary with dose, our data indicate that oral administration of ALA provides an equivalent degree of systemic PpIX accumulation as intravesical administration and supports the rationale for use of orally administered ALA during treatment of esophageal and gastrointestinal cancers (Ackroyd et al., 2000; Hinnen et al., 2000).

Studies using intravesical administration of ALA demonstrated that high concentrations of the drug can be effectively maintained in the urinary bladder with minimal exposure to the systemic circulation. Popken et al. (2000) recently reported the pharmacokinetics of ALA after intravesical administration. Our data corroborate their findings regarding limited absorption of ALA into the systemic circulation, and provide new information on the factors that influence bladder concentrations of ALA during intravesical therapy. The ma-

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Urine</th>
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<tbody>
<tr>
<td>AUC\textsubscript{0–∞} (μg · h/ml)</td>
<td>0.89 ± 1.03</td>
<td>10,280 ± 2,240</td>
</tr>
<tr>
<td>C\textsubscript{max} (μg/ml)</td>
<td>0.54 ± 0.61</td>
<td>20,700 ± 2,310</td>
</tr>
<tr>
<td>T\textsubscript{max} (min)</td>
<td>37.5 (15 to 60)*</td>
<td>Volume at 1 h (ml)</td>
</tr>
<tr>
<td>t\textsubscript{1/2} (min)</td>
<td>58 ± 16</td>
<td>pH at 1 h</td>
</tr>
<tr>
<td>F (%)</td>
<td>0.52 ± 0.61</td>
<td>% Recovered at 1 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC\textsubscript{0–60} urine/AUC\textsubscript{0–180}, plasma</td>
</tr>
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* Median (range).
jor factor determining the exposure of the urinary bladder to ALA after an intravesical dose was urine production. In these studies, the dose was diluted approximately 3-fold by urine produced during the 1-h instillation period. Nonenzymatic degradation of ALA and systemic absorption contributed little to the observed decrease in urine ALA concentrations during instillation. This claim is supported by the three facts: 1) the pH of the urine removed at the end of the instillation period was in proximity to the pH of the original buffered ALA dosing solution, 2) the low intravesical bioavailability and plasma concentrations of ALA, and 3) the high (approximately 80%) recovery of unchanged ALA in the urine collected at the end of the 1-h intravesical instillation period.

Despite urinary dilution of the intravesical ALA dosing solution, the bladder was exposed to significantly higher concentrations of ALA than the systemic circulation, a fact most readily demonstrated by comparison of the AUC_{t→∞} of ALA in the urine and plasma after intravesical administration (Table 2). The ratio of the AUC_{t→∞} in urine to AUC_{t→∞} plasma ranged from 2.88 to 41.484, with an average of 20,000-fold greater exposure of the bladder compared with the systemic circulation (plasma). Although maximal plasma concentrations of ALA achieved after intravesical administration were higher than endogenous predose plasma concentrations of ALA, less than 1% of the intravesical dose was absorbed from the bladder. As such, it is unlikely that one would observe measurable plasma concentrations of PpIX after an intravesical dose of ALA. These data indicate that systemic photosensitization should be of little concern after intravesical administration of ALA.

In summary, these data show that regional administration (i.e., intravesical) of ALA resulted in a significant pharmacokinetic advantage, with the urinary bladder being exposed to concentrations approximately 20,000-fold higher than the systemic circulation. Future preclinical and clinical studies with intravesical ALA that use techniques (Dalton et al., 1991; Au et al., 2001) to improve bladder exposure to this agent and measure PpIX accumulation in the bladder are needed and should provide insight into the role of ALA in phototherapy and photodiagnosis of urinary bladder cancer.

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Address correspondence to: Dr. James T. Dalton, The Ohio State University, 242 L. M. Parks Hall, 500 West 12th Ave., Columbus, OH 43210. E-mail: dalton.1@osu.edu.