Pharmacokinetics of Glucarpidase in Subjects With Normal and Impaired Renal Function

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Glucarpidase (formerly known as carboxypeptidase G2 or CPG2) is being evaluated for the adjunctive treatment of patients experiencing, or at risk of, methotrexate toxicity attributable to its delayed elimination. Delayed elimination of methotrexate can occur in patients with methotrexate-induced renal toxicity. In this study, glucarpidase pharmacokinetics were assessed in volunteer subjects with normal (n = 8) and severely impaired (n = 4) renal function. Each subject received a single intravenous dose of glucarpidase 50 U/kg (equivalent to 114.5 μg/kg) infused over 5 minutes. The mean maximum serum concentration (Cmax) for glucarpidase in renally impaired subjects was 2.9 μg/mL, the mean half-life (t1/2) was 10.0 hours, and the mean area under the serum concentration–time curve from time zero to infinity (AUC0–∞) was 24.5 μg×h/mL. Similar values were found in subjects with normal renal function (mean Cmax 3.1 μg/mL, mean t1/2 9.0 hours, and mean AUC0–∞ 23.4 μg×h/mL). The results indicated little effect of renal impairment on the serum pharmacokinetics of glucarpidase.

Keywords: Glucarpidase; methotrexate; renal; Voraxaze

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of MTX, ineffective rescue by LV, and a host of secondary toxicities.\textsuperscript{10}

Glucarpidase (formerly known as carboxypeptidase G2 or CPG2) is a bacterial enzyme that cleaves the MTX molecule into inactive metabolites, 4-deoxy-4-amino-N10-methylpteroylglutamate, which are metabolized by the liver. Use of glucarpidase thus provides an alternative route of MTX elimination, which is particularly important in patients who develop renal dysfunction attributable to MTX nephrotoxicity.\textsuperscript{11-14}

The effect of glucarpidase on the kinetics of MTX elimination in cancer patients has been reported elsewhere.\textsuperscript{15-18} It has not been feasible to investigate the kinetics of glucarpidase in patients, because those who receive it are in a potentially life-threatening situation, and taking serial blood samples at predefined time points is not practical. Therefore, the kinetics of glucarpidase has been investigated in healthy subjects, in the absence of MTX. The present study examined the pharmacokinetics of glucarpidase in subjects who had both normal and severely impaired renal function, the latter to more closely match the intended patient population.

METHODS

Subjects

Twelve subjects aged between 22 and 51 years participated in this study; 8 subjects (median age 42 years) had normal renal function (calculated creatinine clearance >80 mL/min) and 4 subjects (median age 41 years) had severe renal impairment (calculated creatinine clearance <30 mL/min). Subjects with normal renal function had a median weight of 83.4 kg (range 67.4-114.7 kg), and subjects with impaired renal function had a median weight of 87.5 kg (range 75.3-106.5 kg). Nine subjects (6 with normal renal function and 3 with impaired renal function) were male. Three subjects (2 with normal renal function and 1 with impaired renal function) were female. The self-reported races of the 12 subjects were 9 Black (6 with normal renal function and 3 with impaired renal function) and 3 Caucasian (2 with normal renal function and 1 with impaired renal function). Demographic details are shown in Table I. All subjects gave their written informed consent prior to commencing the study. The study protocol and consent form were approved by the Crescent City Institutional Review Board (New Orleans, Louisiana).

Design

This was an open-label, single-site study to characterize the pharmacokinetics and assess the safety and tolerability of glucarpidase. A study of this size was considered adequate to characterize the pharmacokinetics of glucarpidase and to determine whether the pharmacokinetics are affected by renal impairment.

Test Material

A single intravenous dose of glucarpidase (50 U/kg, Voraxaze\textsuperscript{TM} lot number 2090302, Cangene Corporation, Winnipeg, Canada) was administered to each study subject. Prior to administration, each vial of glucarpidase was reconstituted with 1.0 mL of sterile...
normal saline solution (sodium chloride 0.9% wt/vol). Following reconstitution, each milliliter contained approximately 1000 U of glucarpidase. Glucarpidase was reconstituted immediately prior to use and administered via bolus intravenous injection over 5 minutes.

Sample Collection for Pharmacokinetic Analyses

Serum glucarpidase profiles were evaluated for all subjects using 7-mL blood samples collected predose (immediately prior to the administration of glucarpidase), at the end of the 5-minute infusion of glucarpidase, and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 hours following the start of the glucarpidase infusion. Urinary excretion of glucarpidase was evaluated using samples obtained in the following intervals: 0 to 2, 2 to 4, 4 to 8, and 8 to 24 hours following the start of glucarpidase infusion.

Glucarpidase Concentration Measurements

Serum concentrations of glucarpidase were determined by Covance Laboratories Ltd (Harrogate, UK) by assaying both enzymatic activity using ultraviolet spectroscopy and total glucarpidase protein using a competitive enzyme-linked immunosorbent assay (ELISA). Urinary excretion of unchanged glucarpidase was evaluated using the ELISA method only.

The ELISA was a competitive enzyme immunoassay in which goat anti-rabbit antibodies are used to capture a specific glucarpidase complex in each sample. A biotinylated conjugate and glucarpidase in sample or standards compete for these specific binding sites. Because the concentration of glucarpidase increases, the amount of biotinylated conjugate captured by the antibody decreases. This amount is detected using a streptavidin alkaline phosphatase conjugate and a chromatogenic substrate reaction and is measured at 492 nm. The amount of glucarpidase in each sample is determined by comparing results from the sample wells with a standard curve. The lower limit of quantitation (LLOQ) of this assay was 0.26 μg/mL, and the interassay precision of quality control samples was 15.2% or better.

Pharmacokinetic Analyses

The following pharmacokinetic (PK) parameters were calculated, for both serum enzyme activity and serum ELISA data, according to the model independent approach\(^{19}\): maximum serum concentration (C\(_{\text{max}}\)), time to maximum concentration (T\(_{\text{max}}\)), area under the serum concentration–time curve from time zero to last measurable time point (AUC\(_{0-t}\)), area under the serum concentration–time curve from time zero to infinity (AUC\(_{0-\infty}\)), magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase (λ\(_z\)), half-life (t\(_{1/2}\)), total clearance (CL), and steady-state volume of distribution (V\(_{ss}\)). PK parameters were not calculated for urine samples because only 1 subject had measurable concentrations.

Analysis of variance (ANOVA) was performed on natural log (ln) transformed C\(_{\text{max}}\), AUC\(_{0-t}\), AUC\(_{0-\infty}\), and t\(_{1/2}\) to compare normal and renally impaired subjects. This analysis was performed separately for both the enzymatic and the ELISA methods. For all PK parameter calculations, serum concentrations that were below the lower limit of quantification were treated as zero values if they were present at the beginning or the end of the profile. In all other cases, LLOQ values were treated as missing values. All subjects were dosed and infused correctly; hence, a dose of 50 U/kg glucarpidase (equivalent to 114.5 μg/kg) and a 5-minute infusion time were used in all serum PK calculations. Actual sampling times were used in the calculation of serum PK parameters. All PK calculations were performed using WinNonlin\textsuperscript{®} (Pharsight Corporation, Mountain View, California, version 4.1) and/or SAS\textsuperscript{®} (version 9.2; SAS Institute, Cary, North Carolina).

Safety Assessment

The safety and tolerability of glucarpidase were monitored by collection of adverse event reports, physical examinations, vital signs, electrocardiograms, and clinical laboratory evaluations (hematology, clinical chemistry, and urinalysis).

RESULTS

All 12 enrolled subjects completed the study. The PK data for serum glucarpidase from both subject groups
using both assay methods are shown in Table II. The mean concentration ±SEM time course data from both groups using the ELISA method are shown in Figure 1. A comparison of the pharmacokinetic parameters showed no evidence of a difference between the 2 subject groups (P > .1, ANOVA; see Table III). The lack of association between creatinine clearance and total glucarpidase clearance is plotted in Figure 2. For consistency, the creatinine clearance was normalized by body weight prior to plotting.

Eleven of the 12 subjects had no quantifiable excretion of unchanged glucarpidase in urine. In 1 (renally impaired) subject, the concentrations of unchanged glucarpidase measured in the urine between 0 and 2 hours and between 2 and 4 hours were 13.3 and 15 ng/mL, respectively, which equates to 0.000568% when expressed as a fraction of the dose infused.

No adverse events were reported during this study. No changes in hematology, chemistry, urinalysis, or vital signs were observed postdose, and no significant

<p>| Table II  Serum Pharmacokinetics for Glucarpidase |
|-----------------|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Assay</th>
<th>PK Parameter</th>
<th>Impaired Renal Function (n = 4)</th>
<th>Normal Renal Function (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic method (enzyme activity)</td>
<td>C\textsubscript{max}, μg/mL</td>
<td>2.76 (0.552)</td>
<td>3.29 (0.812)</td>
</tr>
<tr>
<td></td>
<td>T\textsubscript{max}, h</td>
<td>0.550 (0.100, 4.00)</td>
<td>0.175 (0.100, 1.00)</td>
</tr>
<tr>
<td></td>
<td>AUC\textsubscript{0-t}, μg \cdot h/mL</td>
<td>17.3 (4.32)</td>
<td>19.7 (7.12)</td>
</tr>
<tr>
<td></td>
<td>AUC\textsubscript{0-∞}, μg \cdot h/mL</td>
<td>23.0 (5.78)</td>
<td>23.3 (7.24)</td>
</tr>
<tr>
<td></td>
<td>t\textsubscript{1/2}, h</td>
<td>8.17 (2.591)</td>
<td>5.64 (0.662)</td>
</tr>
<tr>
<td></td>
<td>CL, mL/min/kg</td>
<td>0.0873 (0.02376)</td>
<td>0.0891 (0.02736)</td>
</tr>
<tr>
<td></td>
<td>V\textsubscript{ss}, mL/kg</td>
<td>56.7 (14.02)</td>
<td>42.0 (11.98)</td>
</tr>
<tr>
<td>ELISA method (total glucarpidase)</td>
<td>C\textsubscript{max}, μg/mL</td>
<td>2.86 (0.828)</td>
<td>3.08 (0.843)</td>
</tr>
<tr>
<td></td>
<td>T\textsubscript{max}, h</td>
<td>0.550 (0.100, 1.00)</td>
<td>0.250 (0.100, 2.00)</td>
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<tr>
<td></td>
<td>AUC\textsubscript{0-t}, μg \cdot h/mL</td>
<td>21.5 (10.49)</td>
<td>20.2 (5.22)</td>
</tr>
<tr>
<td></td>
<td>AUC\textsubscript{0-∞}, μg \cdot h/mL</td>
<td>24.5 (9.43)</td>
<td>23.4 (6.85)</td>
</tr>
<tr>
<td></td>
<td>t\textsubscript{1/2}, h</td>
<td>9.97 (2.061)</td>
<td>9.00 (3.180)</td>
</tr>
<tr>
<td></td>
<td>CL, mL/min/kg</td>
<td>0.0860 (0.02855)</td>
<td>0.0892 (0.03018)</td>
</tr>
<tr>
<td></td>
<td>V\textsubscript{ss}, mL/kg</td>
<td>67.9 (29.64)</td>
<td>58.0 (18.08)</td>
</tr>
</tbody>
</table>

Values are mean (SD), except median (min, max) for T\textsubscript{max}, C\textsubscript{max}, maximum serum concentration; T\textsubscript{max}, time to maximum concentration; AUC\textsubscript{0-t}, area under the serum concentration–time curve from time zero to last measurable time point; AUC\textsubscript{0-∞}, area under the serum concentration–time curve from time zero to infinity; t\textsubscript{1/2}, half-life; CL, total clearance; V\textsubscript{ss}, steady-state volume of distribution; ELISA, enzyme-linked immunosorbent assay.
differences were observed between subjects with normal renal function and subjects with renal impairment. There were no abnormal, clinically significant electrocardiographic findings for any subject.

**DISCUSSION**

This is the first study explicitly designed to elucidate the pharmacokinetics of glucarpidase in human subjects. However, glucarpidase pharmacokinetics were also assessed in a small pilot study in patients with central nervous system lymphoma, in which glucarpidase concentrations were assessed using an enzyme activity assay. These patients were treated with HDMTX, and it is possible that the presence of MTX and its metabolites may have affected the assay measurements. Nevertheless, the results found by De Angelis et al. appear to be comparable to those of this study.

Because the ELISA method was specific to glucarpidase, PK parameter results using the ELISA method were considered as primary in summarizing the findings of this study. These results indicated a 7% lower mean $C_{\text{max}}$ for glucarpidase in subjects with impaired renal function relative to the normal renal function group; the total glucarpidase exposure, as measured by mean $\text{AUC}_{0-t}$, was marginally higher by about 5% in subjects with impaired renal function. However, no significant differences were noted in mean $C_{\text{max}}$ and $\text{AUC}_{0-t}$ between the 2 subject groups.

Median $T_{\text{max}}$ was short for both subject cohorts, indicating rapid equilibration of glucarpidase after completion of the short infusion. The ELISA results revealed no significant differences in mean $t_{1/2}$ between the 2 subject groups. Large variability was noted, especially for $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$, in the renally impaired subject group, with 1 subject having a higher exposure relative to the other 3 subjects. This finding may, however, be attributable to the limited number of subjects in this group ($n = 4$).

Our results indicate that glucarpidase at a dose of 50 U/kg is well tolerated in subjects with both normal and impaired renal function. Furthermore, the serum pharmacokinetics of glucarpidase are unaltered in subjects with renal impairment and therefore are not expected to be altered in the target population.

Financial disclosure: None declared.

**REFERENCES**

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