Pharmacokinetics of Oral Zidovudine Entrapped in Biodegradable Nanospheres in Rabbits

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The pharmacokinetic profile of oral zidovudine entrapped in a 50:50 polylactide-coglycolide matrix (nanospheres) was compared to those of standard oral and parenteral zidovudine formulations in rabbits. The bioavailability of zidovudine nanospheres at 50 mg/kg of body weight was 76%, and this dose achieved prolonged exposure to zidovudine compared to standard formulations without an increase in the drug's peak concentration.

The pharmacokinetic profile and intracellular metabolism of zidovudine (ZDV) provide a strong rationale for the development of a sustained-release formulation. Orally administered ZDV is rapidly absorbed, and 65% of the dose is bioavailable (3, 4), but its short half-life (t1/2) (1 h) necessitates dosing every 4 to 6 h in the initial clinical trials. With current dosing regimens, which employ twice- or thrice-daily administration, plasma ZDV concentrations are below optimal antiretroviral concentrations (1 µM) for more than half of the dosing interval (4, 5). Although the active intracellular metabolite, ZDV-triphosphate (ZDVTP), has a longer t1/2 (4 h) than the parent drug, the phosphorylation of ZDV to ZDVTP is inefficient and saturable at doses exceeding 100 mg (6). ZDVTP accounts for only 10% of total intracellular ZDV nucleotides, and peak ZDVTP levels are similar after doses of 100 and 300 mg, suggesting that higher doses of ZDV administered less frequently may be suboptimal.

A novel formulation of ZDV entrapped in biodegradable, submicrometer-diameter polymers has been developed as a sustained-release delivery system. A similar nanosphere preparation of heparin, administered orally with mucoadhesive, improved the bioavailability of heparin and resulted in prolonged exposure to the drug (7). We compared the pharmacokinetic profile of ZDV administered orally in nanospheres to those of standard oral and parenteral ZDV formulations in rabbits.

ZDV nanospheres were provided by Verex Laboratories, Inc. (Englewood, Colo.), as a white powder containing 19.4% ZDV by weight entrapped in a 50:50 polylactide-coglycolide. The ZDV nanospheres were suspended in normal saline in a 1:4 ratio. A mucoadhesive gel (adjuvant) composed of methocel, carbopol-934-P, 40% sodium hydroxide, yellow lake no. 6, oil of orange, glycerin, corn syrup, and purified water was added to the nanosphere suspension to promote adhesion of the nanospheres to the intestinal epithelial surface. Oral ZDV was administered as syrup (Retrovir; Burroughs Wellcome Co., Research Triangle Park, N.C.). ZDV was administered intravenously (i.v.) as the parenteral solution at a concentration of 4 mg/ml over 10 min.

Fifteen female New Zealand White rabbits weighing 2.5 to 3.0 kg were used. Animals were individually housed, provided food and water ad libitum, and managed according to National Institutes of Health guidelines (11). A silastic central venous catheter was surgically placed in each rabbit under general anesthesia for repeated, atraumatic venous access as previously described (13).

Three rabbits were assigned to each of five study groups: i.v. ZDV, 10 mg/kg of body weight; oral ZDV, 10 mg/kg; oral ZDV nanospheres, 10 mg/kg with adjuvant; oral ZDV nanospheres, 50 mg/kg with adjuvant; and oral ZDV nanospheres, 50 mg/kg without adjuvant. The oral ZDV syrup and ZDV nanospheres were administered in 3-ml volumes for all groups. Two-milliliter blood samples were taken at baseline, at 15 and 30 min, and at 1, 2, 4, 6, 8, 24, 48, 72, and 96 h after the drug was administered. Plasma was separated by centrifugation for 10 min at 2,500 × g and was stored at −70°C.

ZDV concentration in plasma was measured by an enzyme immunoassay (Sigma Immunochemicals, Sigma Chemical Co., St. Louis, Mo.) which utilizes rabbit anti-ZDV antiserum and ZDV conjugated to horseradish peroxidase (HRP). Free ZDV-HRP and antibody-bound ZDV-HRP were separated by immunoprecipitation with goat anti-rabbit immunoglobulin G antisera. Plasma samples and standards made up with rabbit plasma underwent solid-phase extraction prior to undergoing the enzyme immunoassay in order to remove endogenous rabbit immunoglobulin G which could interfere with immunoprecipitation. C18 solid-phase extraction cartridges (Waters Corporation, Milford, Mass.) were primed with methanol and phosphate-buffered saline. After sample application, the cartridges were washed with phosphate-buffered saline and eluted with methanol. The methanol was evaporated to dryness under nitrogen at 38 ± 2°C with a TurboVap LV evaporator (Zymark Corporation, Hopkinton, Mass.). The samples were reconstituted with buffer containing bovine serum albumin, and the enzyme immunoassays were performed on the extracted standards and samples. The lower limit of detection for ZDV was 2.5 ng/ml (0.01 µM).

The area under the concentration-time curve (AUC) was derived by the trapezoidal method and extrapolated to infinity (AUC∞→∞) (8). The t1/2 was determined by linear regression of

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the log-transformed ZDV concentrations on the terminal portion of the decay curve. Clearance was calculated from the dose divided by the AUC for the i.v. dose, and the volume of distribution at steady state \( (V_s) \) was estimated by using the area under the moment curve for the i.v. dose (12). Bioavailability \( (F) \) was estimated by using the mean AUC\(_{\text{i.v.}}\) from three animals for each group receiving ZDV syrup orally (p.o.) or ZDV nanosphere preparation relative to the AUC\(_{\text{i.v.}}\) for the i.v. dose with the equation below:

\[
F = \frac{\text{AUC}_{\text{i.v.}}}{\text{AUC}_{\text{i.v.}} \cdot \text{dose}_{\text{i.v.}}} \cdot 100
\]

The plasma ZDV concentration-time profiles for the i.v. solution and conventional oral syrup are shown in Fig. 1A. The mean plasma ZDV concentration 5 min after the end of the 10-min i.v. infusion was 4.4 \( \mu \text{M} \) (range, 3.7 to 5.7 \( \mu \text{M} \)). ZDV was rapidly eliminated after the i.v. dose. The mean \( t_{1/2} \) was 1.2 h (0.9 to 1.8 h), and the clearance was 6.1 liters/kg/h (3.7 to 7.3 liters/kg/h). The mean \( V_s \) of ZDV in rabbits was 7.3 liters/kg (5.4 to 8.4 liters/kg). The conventional oral syrup was rapidly and completely absorbed by the rabbits (Fig. 1A; Table 1). The median time to peak after administration of the oral ZDV syrup was 1 h and the mean peak concentration was 3.6 \( \mu \text{M} \) (range, 2.0 to 4.8 \( \mu \text{M} \)). The clearance, \( V_{\text{ss}}, t_{1/2} \), and bioavailability of i.v. and conventional oral ZDV in this animal model, when scaled for species differences, are similar to the disposition of ZDV in humans (3). The nanosphere formulation administered with or without adjuvant was well tolerated by the animals. The plasma ZDV concentration-time profiles for the 10-mg/kg oral dose administered as conventional oral syrup and entrapped in nanospheres with adjuvant are shown in Fig. 1B. The bioavailability of the nanosphere preparation was excellent (Table 1), but the concentration-time profile at the 10-mg/kg level was not consistent with sustained-release delivery of ZDV. However, the 50-mg/kg dose (equivalent to 250 mg/m\(^2\) in humans) achieved prolonged exposure to ZDV without an increase in the peak concentration in plasma (Fig. 1C; Table 1). The plasma ZDV concentration exceeded 1 \( \mu \text{M} \) for more than 6 h and was \( >0.1 \mu \text{M} \) at 24 h after the nanosphere and adjuvant formulation was administered. Plasma ZDV concentrations were sustained longer and bioavailability appeared to be higher when the ZDV nanospheres were administered with adjuvant (Table 1). The nanosphere formulation confers sustained-release characteristics on ZDV at doses that are useful in humans. In addition, the adjuvant appears to enhance this effect, possibly by delaying the absorption from the gastrointestinal tract. This is consistent with the findings of other studies of microsphere formulations of ZDV (1, 2, 10).

Antiretroviral treatment regimens combine multiple agents, and the potential for simplifying the ZDV dosage schedule by less frequent administration of a sustained-release preparation could improve patients’ compliance and quality of life. In addition to the potential pharmacokinetic advantages of this formulation, preliminary results from a randomized clinical trial suggest clinical benefits, including equivalent efficacy with reduced toxicity and a lower rate of development of ZDV resistance with a sustained-release formulation of ZDV (Aztec) (9).

**REFERENCES**


**TABLE 1. AUC and bioavailability of ZDV administered i.v., orally as a syrup, and orally entrapped in nanospheres**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>ZDV AUC (( \mu \text{M-h} ))</th>
<th>( F ) (%)</th>
<th>( C_{\text{max}} ) (( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenteral solution</td>
<td>i.v.</td>
<td>10</td>
<td>6.8 (5.1–10)</td>
<td>97</td>
<td>3.6 (2.0–4.8)</td>
</tr>
<tr>
<td>Syrup</td>
<td>p.o.</td>
<td>10</td>
<td>6.6 (4.7–9.6)</td>
<td>84</td>
<td>2.6 (1.5–3.4)</td>
</tr>
<tr>
<td>Nanospheres with</td>
<td>p.o.</td>
<td>10</td>
<td>5.7 (3.5–9.1)</td>
<td>76</td>
<td>4.7 (2.8–6.6)</td>
</tr>
<tr>
<td>Nanospheres with</td>
<td>p.o.</td>
<td>50</td>
<td>25.6 (14–32)</td>
<td>55</td>
<td>4.8 (3.3–6.3)</td>
</tr>
<tr>
<td>Nanospheres without</td>
<td>p.o.</td>
<td>50</td>
<td>18.5 (11–26)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) Values are means, with ranges in parentheses.

\( ^b \) \( F \), fraction of the oral dose absorbed; \( C_{\text{max}} \), peak plasma ZDV concentration; p.o., orally.
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In Vitro Activities of Ketolides HRM 3647 and HRM 3004, Levofloxacin, and Other Quinolones and Macrolides against Neisseria spp. and Moraxella catarrhalis
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