Pharmacokinetics of Pentaerythritol Tetrinitrate Following Intra-arterial and Oral Dosing in the Rat

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Abstract □ The pharmacokinetics of pentaerythritol tetrinitrate (2,2-bis(hydroxymethyl)-1,3-propanediol tetrinitrate, 1) were studied in rats following a single intra-arterial or oral dose (2 mg/kg) of the 14C-labeled drug. Blood levels of the tetrinitrate and its metabolites were determined using a thin-layer radiochromatographic procedure. The apparent systemic clearance of 1 was 0.61 ± 0.16 L/min/kg (mean ± SD, n = 6) which exceeded the value of normal cardiac output in rats. The steady-state volume of distribution was 4.2 ± 1.1 L/kg (n = 6), and the elimination half-life was estimated at 5.8 ± 0.6 min (n = 6). Blood levels of 1 were only detectable (higher than 4.0 ng/mL) in three of the six rats examined after the oral dose. The trinitrate derivative (2,2-bis(hydroxymethyl)-1,3-propanediol trinitrate, 2) the active metabolite of 1, was not detectable following oral dosing with the tetrinitrate. The oral bioavailability of 1 was in the range of 0–8%. In spite of the low water solubility of 1 (i.e., 1 µg/mL), a rather high fraction of the radioactive oral dose (25.7 ± 10.3% (n = 4) versus 62.4 ± 14.5% (n = 4) from the intra-arterial dose) was recovered in the urine. A significant portion of the intra-arterial dose (32.7 ± 11.0%, n = 4) was eliminated in feces, indicating enterohepatic recycling of radioactivity. Analysis of the metabolite pattern in urine indicated extensive metabolism of 1, 2, and the dinitrate derivative 3 (2,2-bis(hydroxymethyl)-1,3-propanediol dinitrate). Less than 0.2% of the dose was recovered as unchanged drug and 2 following either route of administration.

Pentaerythritol tetrinitrate (1) is an important member of the vasodilating organic nitrates. The results of several clinical trials have shown the effectiveness and prolonged duration of action following oral doses in the management of patients with angina pectoris1–3 and chronic congestive heart failure. The prolonged duration of action of 1 is believed to be partially attributable to its active metabolite, the trinitrate derivative 2, which has been shown experimentally to be as active as nitroglycerin, and clinically to have a longer duration of action.b The biotransformation of 1 has been examined with the use of 14C-labeled drug. In spite of their reported clinical efficacy, 1 and 2 have been shown to be absent from the blood following a single oral dose of 1 in humansa and in rats. Blood radioactivity has been shown to account for only a few percent of the 14C-labeled 1 absorbed in the rat.a In view of these observations, Davidson et al.4 proposed that the major portion of the absorbed 1 dose is rapidly distributed into tissue cells in which active nitrates are further denitrated with the release of metabolites into systemic circulation.

Although the metabolism and clinical uses of 1 have been extensively examined, quantitative information is lacking regarding its pharmacokinetics, particularly after systemic administration. This study was initiated to address this void, using the rat as the experimental animal.

Experimental Section

Materials—Compound 1 labeled with carbon-14 at the 1 and 2 positions with a specific activity of 1.71 µCi/mmol (Warner Lambert Co., Ann Arbor, MI) was used in this study. The radiochemical purity of 1 was checked by thin-layer chromatography (85% pure). The dioxane layer was separated from protein precipitates by centrifugation. The remaining blood sample was assayed immediately after mixing. The recovery of radioactivity was 87.6 ± 6.0% (n = 6, range from 84.5 to 92.3) for 1 and its metabolites in the urine and fecal samples were determined by a reported assay procedure9 with slight modifications.11 Recoveries of total radioactivity for this assay procedure were 93.3 ± 13.9% (mean ± SD, n = 66) and 76.5 ± 8.9% (n = 47) for blood samples after intra-arterial and oral doses of 1, respectively. Blood samples spiked with 14C]1 was dissolved in polyethylene glycol 400 (1.0 mL/kg) and injected over a period of 1 min. Rats were under light ether anesthesia immediately before dosing, and were conscious during the collection of blood samples. All animals were individually housed in plastic metabolism cages during the first 2-h intervals for the collection of blood samples. Animals were then housed in stainless steel metabolism cages during the first 2-h intervals for the collection of blood samples and allowed ad libitum food and water afterwards. Blood samples (~0.5 mL) were collected from the venous cannula at selected times after dosing by using 1.0-mL disposable plastic syringes. A similar volume of normal saline was used to flush the cannula and injected into the animal after each collection. A 50-µL aliquot of each blood sample was solubilized using Soluene-350 (Packard Instrument Co., Downers Grove, IL) for measurement of total radioactivity.1 The remaining blood sample (0.5 mL) was then mixed with 3.0 mL of 75% dioxane in water, and the dioxane layer was separated from protein precipitates by centrifugation. The residue was washed three times with dioxane. The combined dioxane "extract" (8.0 mL) was stored at −20°C pending assay. The blood concentrations of 1 and its metabolites were determined by a reported assay procedure19 with slight simplifications.

Thin-Layer Chromatography and Radiochemical Assay—Glass plates (Brinkmann Instruments, Inc., Westbury, NY) (5 × 20 cm) coated with silica gel (250 µm) were used. The solvent system for separation of 1 from its metabolites12 was toluene:ethyl acetate:ethanol:water (75:25:4:0.4)

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1-butano:water (10:5:2:2, upper phase). The plate was divided into 12-20 regions after development. The silica gel was scraped off the plate and mixed with 10 mL of Dioxscint (National Diagnostics, Somerville, NJ). Each sample was counted for 10 min with a Packard TriCarb model 3255 liquid scintillation counter. The drug and metabolite regions were separated by a region of background radioactivity on each plate. The counting efficiency of each sample was determined using the channels ratio method. An internal standard, [14C]toluene (Packard), was used to correct for quenching in samples with counting efficiencies of <60%.

Pharmacokinetic and Statistical Analyses—The apparent systemic clearance ($CL_B$) of 1 was calculated as the dose ($D$) divided by total area under the blood concentration–time curve (AUC). The disappearance of 1 in the blood followed a slight biphasic pattern as determined by the computer program CSTRIP. The first-order rate constant for decay of 1 in the terminal log-linear phase ($k_2$) was estimated using the last four blood concentration–time points of the disposition curve. The terminal elimination half-life ($t_{1/2}$) of 1 disposition was calculated as $0.693/k_2$. The steady-state volume of distribution ($V_{ss}$) was calculated using eq. $1^{17}$

$$V_{ss} = \frac{D \times AUMC}{AUC^2}$$

where AUMC is the area under the first moment curve. The AUC and $V_{ss}$ for 1 were computed by the method of LaGrangian polynomial approximations$^{16}$ using the computer program LA-GRAN.$^{15}$ The oral bioavailability of 1 was computed as the AUC after an oral dose divided by the AUC after an intra-arterial dose. An unpaired $t$ test was employed to evaluate the possible significant difference between parameter means.

Results

Pharmacokinetics of Pentaerythritol Tetranitrate—The detection limit of [14C]1 in blood was calculated according to Wang et al.$^{20}$ who defined this parameter as eight standard deviations above the system background. After correcting for both counting efficiency (~80%) and recovery of radioactivity for the assay of 1 in blood (~75%), the sensitivity of the assay could be estimated as 4 ng/mL.

Figure 1 shows the profiles of total radioactivity of 1 and its metabolites in rat whole blood following administration of a single intra-arterial dose of 1. Unchanged 1 could be followed for 25 min after parenteral dosing. In comparison, blood concentrations of 1 >4 ng/mL were detectable in only three of the six rats given the oral dose (Fig. 2). The pharmacokinetic parameters for 1 and the apparent terminal half-lives of 2 and 3 after both intra-arterial and oral administration of 1 are summarized in Table I. Pentaerythritol trinitrate, the active metabolite of 1, was not detectable in blood after oral administration. However, blood concentrations of 2 could be followed during the 2-h sampling period after parenteral administration of 1. Compared with the blood levels of 1 and 2, higher levels of pentaerythritol dinitrate were found in blood after either route of administration. The maximum concentrations of 3 were observed at 14 and 12 min following intra-arterial and oral administration of 1, respectively. The apparent 3 terminal half-life was shorter after oral dosing of 1 than that observed after parenteral dosing. Pentaerythritol mononitrate, 4, concentrations in blood started to rise and reached an apparent plateau during the 2-h sampling period. Blood levels of the polar metabolites (other than 2, 3, and 4) were lower than the levels of 3 and 4 after both routes of administration of 1. The metabolite patterns in urine and feces following intra-arterial and oral dosing of 1 were summarized in Table II. After oral dosing, urinary recovery of total radioactivity amounted to ~40% of that observed after intra-arterial
Figure 2—Blood concentrations of (■) radioactivity, (○) pentaerythritol tetranitrate, (△) pentaerythritol dinitrate, (▲) pentaerythritol mononitrate, and (X) polar metabolites after a single oral dose (2 mg/kg) of [14C]1 in rats. The left and right panels show results from an animal with and without detectable blood levels of 1, respectively.

Table I—Summary of Estimated Pharmacokinetic Parameters for Pentaerythritol Tetranitrate (1) and Its Metabolites in Venous Blood Following a Single 2 mg/kg Intra-arterial or Oral Dose of [14C]Pentaerythritol Tetranitrate to Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1*</td>
</tr>
<tr>
<td>CLa, L/min/kg d</td>
<td>0.62 ± 0.16</td>
</tr>
<tr>
<td>Vdav, L/kg g</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>t1/2, λ2, Intra-arterial, min g</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>t1/2, λ2, Oral, min g</td>
<td>—*</td>
</tr>
<tr>
<td>F, %</td>
<td>0.0–8.2±</td>
</tr>
</tbody>
</table>

*Pentaerythritol tetranitrate. tPentaerythritol trinitrate. △Pentaerythritol dinitrate. ▲Mean ± SD; n = 6. *Cannot be estimated from present study. tSignificantly different from oral value at p < 0.025. △n = 6.

dosing. A significant portion of the intra-arterial dose was eliminated in feces, indicating the presence of enterohepatic recycling of radioactivity.

Discussion

The recovery efficiency of radioactivity in the present study represents a substantial improvement over those previously reported in the literature. DiCarlo et al.12 used 8.0-mL aliquots of human blood in their study and reported a recovery of ±60%. In a later study with rat blood,21 the sample size used was 1.4–1.8 mL, but the recovery was not explicitly stated. In the present study, the sample size was 0.5 mL and the radioactivity recovery was 93.3 ± 13.9% and 76.5 ± 8.9% (mean ± SD) after intra-arterial and oral administration of 1, respectively. The poorer recovery of radioactivity from samples after oral dosing was probably due to loss of the more polar metabolites (e.g., glucuronides) during work-up, since radioactivity recovery of spiked 1 blood samples was satisfactory, i.e., at ~68%.

The large apparent clearance of 1, ~620 mL/min/kg, is larger than the reported cardiac output of about 400 mL/min/kg in the rat.22 Since blood (not plasma) samples were directly assayed, and intra-arterial (not intravenous) dosing was used, unusual erythrocyte/plasma partitioning of the drug and first-pass lung metabolism could not have contributed to this phenomenon. Pentaerythritol tetranitrate degradation in rat blood exhibited a half-life of ~15 min,21 compared with an in vivo disappearance half-life of 5.8 min. Thus, the in vivo degradation clearance could be estimated by multiplying the in vitro blood degradation rate constant (0.045 min−1) by the blood volume of 65 mL/kg. This in vitro blood clearance of 2.9 mL/min/kg was only 0.48% of the total in vivo 1 apparent clearance. Thus, blood degradation per se is not the main reason for the observed large clearance of 1.

We have shown recently, however, that when nitroglycerin was administered intravenously to rats,23 drug concentration in blood vessels decreased as a function of distance from the site of injection. Furthermore, when nitroglycerin was injected at two different locations in the inferior vena cava, the blood vessel segment immediately downstream of the injection site always bore the higher drug concentration. Armstrong et al.24 also showed that extraction of nitroglycerin occurs across the vascular bed in humans. The physicochemical and metabolic properties of nitroglycerin and 1 are similar, and it is likely that vascular extraction also exists for 1. Since blood vessels avidly metabolize organic nitrates,25 the extremely large apparent clearance of 1 might, in part, be caused by the process of "first-pass" blood vessel extraction. This mechanism would be consistent with an apparent clearance rate that is larger than the total flow rate in the system, i.e., the cardiac output.

In spite of the low water solubility of 1, >25% of the oral
radioactive dose was excreted in urine. However, only a small fraction (<8.2%) of intact drug reached the systemic circulation. It has been shown\textsuperscript{21} that 1 is stable in the contents of ligated stomach and small intestine, but is presumably metabolized by microflora in the ligated large intestine. Some of the oral dose of 1 may be metabolized in the large intestine to form water soluble 2 and 3 which are subsequently absorbed. Some of the metabolites present in the large intestine may have also originated from enterohepatic recirculation of radioactivity which, for 2, has been shown\textsuperscript{28} to be only 1.5% as active as 2 in the large intestine. The apparent terminal half-life of 3 after intra-arterial dosing of, was 2 mg/kg intra-arterial or oral dosing. Blood clearance of 1 was large and exceeded the normal cardiac output in rats. Compound 1 had a large volume of distribution and a short elimination half-life. Both 1 and 2 appeared to undergo extensive first-pass metabolism. The large total body clearance of 1 was likely attributable to extensive extra-hepatic metabolism.

**References and Notes**

15. The urinary and fecal excretion data obtained in the present study have been reported in a paper (ref. 13) which addressed the microbial degradation of organic nitrates and the methodological problems involved in measuring 1 and its metabolites in these excreta.
27. Colburn, W. A.; Hirom, P. C.; Parker, R. J.; Milburn, P. Drug Metab. Dispos. 1979, 7, 100–102.

**Acknowledgments**

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