High-Pressure Liquid Chromatographic Assay and Pharmacokinetics of HR 810 After Intramuscular Injection in Rabbits

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A high-pressure liquid chromatographic assay was developed for the detection of HR 810 in rabbit plasma. There was no interference in the high-pressure liquid chromatographic assay from other antibiotics. The method was accurate, reproducible, and capable of detecting <1 µg of HR 810 per ml in plasma. The assay correlated with the microbiological assay (correlation coefficient, 0.93) and was used to quantitate the concentration of HR 810 in rabbit plasma and determine its half-life subsequent to a 20-mg/kg intramuscular dose. The peak concentration of HR 810 was 51.2 ± 8.0 µg/ml at 1 h postdose. The half-life of the absorption phase (mean ± standard deviation) was 0.35 ± 0.10 h, and the half-life of the elimination phase was 0.75 ± 0.06 h. This is 54% less than the half-life of elimination of 1.38 h previously reported for the intravenous dose.

MATERIALS AND METHODS

HR 810 is a new aminothiazole semisynthetic cephalosporin with antimicrobial activity against Staphylococcus spp., enterococci, and Pseudomonas spp. (4). Initial pharmacokinetic studies in which animals were given intravenous doses have been completed with the microbiological assay for the quantitation of HR 810 in body fluids and tissue (4). In this study we developed a high-pressure liquid chromatographic (HPLC) assay for HR 810, compared the method with the microbiological assay, and used the rabbit model to determine the pharmacokinetics of a 20-mg/kg intramuscular dose.

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Five New Zealand White female rabbits were injected in the gluteus muscle with 20 mg of HR 810 (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.) per kg of body weight in 1 ml of 0.1 M sodium phosphate buffer (pH 6.1). Plasma specimens were collected from the auricular artery of the ear every 30 min through 4 h postinjection. All specimens were maintained at −70°C until assayed by both microbiological and HPLC assays.

The concentrations of HR 810 in plasma were determined by the microbiological assay by the method of Klesel and Seeger (4). The only modification in this procedure consisted of the use of Streptococcus pyogenes ATCC 19615 instead of S. pyogenes ATCC 19615. The HPLC assay of HR 810 consisted of the acetonitrile dichloromethane-phase extraction procedure, which has been used successfully for other beta-lactam antibiotics (1, 6). The procedure consists of adding 2.5 volumes of acetonitrile to 0.5 ml of plasma. The specimens were then vortexed and centrifuged for 5 min at 3,000 × g. The supernatant was removed to a clean screw-capped test tube (13 by 100 mm), and 5.0 ml of dichloromethane was added. The specimen tubes were capped, inverted several times, and centrifuged as previously described (1). The upper aqueous layer was injected into the HPLC instrument (Waters Associates, Inc., Milford, Mass.), which consisted of a model 510 pump attached to a C18 µ-Bond-a-Pak column, a model 710-B automated sample processor, a 481 variable wavelength detector, and a 1200 recorder (Linear Instruments Corp., Irvine, Calif.). The mobile phase consisted of 15% acetonitrile and 85% 0.1 M sodium phosphate buffer (pH 6.1). The flow rate was 2.5 ml/min. Fifty microliters was injected onto the column by the automated sample processor. The detector was set at 0.5 absorbance units (full scale). The detector wavelength was 270 µm. The standards consisted of normal rabbit plasma spiked with 0 to 50 µg of HR 810 per ml. The standards were prepared by adding HR 810 to the plasma with a microliter syringe (Hamilton Co., Reno, Nev.). All specimens were analyzed in duplicate, and within- and between-batch reproducibility studies were performed a minimum of five times. Other antimicrobial agents, including other beta-lactam antibiotics, vancomycin, chloramphenicol, and aminoglycosides, were assayed for interference with this procedure. A total of 50 µg of each antibiotic per ml was added to plasma, extracted, and assayed by the HPLC procedure described above. The stability of HR 810 was determined by reanalysis after the extracted specimens were stored overnight at 4°C under identical chromatographic conditions.

All HR 810 concentrations were calculated by peak height versus concentration and linear least-squares regression analysis. For comparative purposes, the HPLC and microbiological assays were done on 24 random specimens. These specimens were compared by use of the Wilcoxon paired-sample test. The correlation of the two test procedures was determined by linear least-squares regression analysis. Pharmacokinetic parameters were determined with NONLIN, an iterative least-squares regression computer program (5). A one-compartment model was used to analyze the data since no significant improvement in fit occurred when a two-compartment system was tested with the F-test as advocated by Boxenbaum et al. (2). Half-lives of the absorption and elimination phases were determined by dividing the natural logarithm of two by the respective rate constant.

Systemic clearance and volume of distribution cannot be calculated unless bioavailability is known. Consequently, we elected to express both terms relative to the fraction absorbed (F), realizing that the terms represent systemic clearance (CL) and volume of distribution (V) only if F = 1.0. Intramuscular clearance (CL/F) was calculated as (dose/area under the concentration-time curve), and vol-
The high-pressure liquid chromatogram of HR 810 was shown in Fig. 1. The retention time of HR 810 was 4.9 min, and there was no interference from body metabolites or other beta-lactam antibiotics, chloramphenicol vancomycin, or aminoglycoside antibiotics. HR 810 was stable after extraction at 4°C for at least 16 h. The concentration range tested when the microbiological assay was compared with the HPLC assay was 5.0 to 52.8 μg/ml. The mean value (± standard deviation) was 29.2 ± 13.8 μg/ml for the microbiological assay and 30.04 ± 14.6 μg/ml for the HPLC assay. The correlation coefficient for comparing the microbiological assay with the HPLC assay was 0.93. HPLC within-batch reproducibility studies for HR 810 from 5.0 to 47.6 μg/ml recovered at least 96.4% of the added antibiotic. Between-batch reproducibility studies recovered a minimum of 95.2% of HR 810. The HPLC assay could readily detect 1.0 μg of HR 810 per ml in plasma.

The mean weight of the rabbits was 4.4 ± 0.4 kg, and the summary of the pharmacokinetic parameters of HR 810 in rabbit plasma are shown in Table 1. Figure 2 depicts the time course of absorption and elimination of the 20-mg/kg intramuscular dose of HR 810.

DISCUSSION

The HPLC assay method was reproducible and correlated well with the microbiological assay of HR 810. We detected no metabolites in the rabbit plasma, but this was only a single-dose study, for only a short time and metabolites may be present only in long-term therapy. HR 810 was stable in the extracted material at 4°C for 16 h, and peak heights did not change at room temperature through several hours as the HPLC assay was completed.

### Table 1. Pharmacokinetics of a 20-mg/kg intramuscular dose of HR 810 in five rabbits

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Lag time for absorption (h)</th>
<th>$t_{1/2a}$ (h)</th>
<th>$t_{1/2b}$ (h)</th>
<th>AUC ($\mu$g · h/ml)</th>
<th>CLp (ml/h)</th>
<th>$V$ (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.33</td>
<td>0.20</td>
<td>0.71</td>
<td>108.1</td>
<td>832.6</td>
<td>812.7</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>0.91</td>
<td>101.4</td>
<td>867.9</td>
<td>660.1</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>0.17</td>
<td>0.64</td>
<td>74.1</td>
<td>998.7</td>
<td>1,081.4</td>
</tr>
<tr>
<td>4</td>
<td>0.08</td>
<td>0.61</td>
<td>0.86</td>
<td>126.1</td>
<td>745.4</td>
<td>600.7</td>
</tr>
<tr>
<td>5</td>
<td>0.17</td>
<td>0.43</td>
<td>0.65</td>
<td>109.3</td>
<td>841.7</td>
<td>897.4</td>
</tr>
<tr>
<td>Mean ± SD*</td>
<td>0.25 ± 0.07</td>
<td>0.35 ± 0.10</td>
<td>0.75 ± 0.06</td>
<td>103.8 ± 8.5</td>
<td>857.3 ± 91.5</td>
<td>810.5 ± 192</td>
</tr>
</tbody>
</table>

* Abbreviations $t_{1/2a}$, half-life of the absorption phase; $t_{1/2b}$, half-life of the elimination phase; AUC, area under the plasma concentration-time curve; CLp, intramuscular plasma clearance (systemic clearance/fraction absorbed); $V$, intramuscular volume of distribution (volume of distribution/fraction absorbed); ND, not determined.

* Values are means ± 1 standard deviation.
The maximum concentration of HR 810 in plasma after the 20-mg/kg intramuscular dose was administered was $51.2 \pm 8.0 \mu g/ml$ at 1 h postdose. At 4 h, the mean concentration in plasma was $5.5 \pm 2.9 \mu g/ml$. From the data it would appear that an intramuscular dose every 4 h would provide adequate antimicrobial coverage in most instances. The concentration-time curve of HR 810 in rabbit plasma best fits a one-compartment model. The mean half-life of the elimination phase was $45 \pm 3.6$ min, as compared with a half-life of 89 min in a previous study (4). In that study, the AUC was $86.4 \mu g \cdot h/ml$, whereas our AUC was $103.8 \pm 8.5 \mu g \cdot h/ml$. This difference in AUC is probably not significant. There are several reasons for the discrepancy in half-life (3). These reasons include different methods of curve fitting and weighting factors used in fitting plasma concentration versus time curves. In the previous study, the NONLIN program was not used (4). Overall, though, the differences between the two studies are not great. The methods described herein should prove useful in future clinical studies of this new antibiotic.

LITERATURE CITED