Influence of Permanent Cannulation of the Jugular Vein on Pharmacokinetics of Amoxicillin and Antipyrine in the Rat

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The effect of chronic cannulation of the rat jugular vein on the pharmacokinetics of amoxicillin and antipyrine administered by the i.v. and oral routes has been evaluated. Animals that received the i.v. dose of amoxicillin on the eighth day after jugular vein cannu- lation showed decreased clearance (4.0 ± 0.3 ml/min) and steady-state volume of distribution (105 ± 8 ml) compared to animals that received the i.v. dose on the fourth day (5.5 ± 1.1 ml/min and 155 ± 17 ml, respectively). Rats first dosed by the i.v. route showed an oral bioavailability of 54 ± 12%, whereas for those first dosed by the oral route the calculated bioavailability was 31 ± 6%. Antipyrine was administered to rats by the i.v. and oral routes on the first and fourth days after jugular vein cannulation. Animals intravenously dosed on the fourth day showed a decreased clearance (1.9 ± 0.3 ml/min) compared to rats intravenously dosed on the 1st day (2.7 ± 0.6 ml/min). Antipyrine bioavailability was larger in animals first dosed by the i.v. route than in animals first dosed by the oral route (173 ± 43 and 74 ± 15%, respectively). These results argue against the use of crossover studies in rats with permanently implanted cannulas since kinetic changes induced by cannulation can be larger than previously proposed.

KEY WORDS: permanent cannulation; pharmacokinetics; amoxicillin; antipyrine.

INTRODUCTION

For serial blood sampling from unanesthetized, unrestrained rats, several procedures for chronic cannulation have been developed (1–3), with emphasis on a long patency period of the implanted cannulas. By using animals carrying cannulas that remain functional for several days, it is possible to perform crossover studies, for example, on bioavailabil- ity, in which each animal receives more than one treatment within a given time interval. However, changes in pharmacokinetics of propranolol and antipyrine in rats carrying implanted cannulas have been reported (4,5).

We have used jugular vein-cannulated rats in our pharma- cokinetic/bioavailability studies with different drugs and treatment schedules. The purpose of this report is to show that large changes in pharmacokinetic parameters can be induced in the presence of a venous implanted cannula.

MATERIALS AND METHODS

Experimentation Animals, Dosing, and Sampling Procedures

Male Wistar rats weighing 290–350 g were used for all experiments. Prior to assays, the rats were subjected to jugular vein cannulation with 12-cm-long fragments of a medical-grade silicon tubing (Silastic, Dow Corning Co.; internal diameter, 0.5 mm; outer diameter, 0.94 mm).

Under ether anaesthesia, 3.4 cm of the cannula was intro- duced into the jugular vein toward the heart. The free end of the cannula was subcutaneously conducted to the dorsal base of the neck, where it emerged, and the exteriorized end was closed with a polyethylene plug. The inside of implanted cannulas remained permanently filled with heparinized (20 IU/ml) normal saline.

Animals were fasted overnight prior to drug administra- tion. All drugs were administered in aqueous solution.

In order to facilitate blood sampling and intravenous dosing, a 15-cm-long silicon tubing (bridge-tubing) was connected to the free end of the cannula a few minutes before starting the experiments. Oral doses were administered by gastric intubation under light ether anesthesia.

Blood samples (0.2–0.3 ml) were drawn at fixed times with heparinized syringes. After each sampling, blood vol- ume was replaced with the same volume of saline. Plasma was immediately separated from erythrocytes by centrifuga- tion (1000g for 5 min) and stored at −20oC until analysis.

Drugs, Dosing Schedules, Sampling Protocols, and Analytical Techniques

Amoxicillin

Amoxicillin trihydrate, with a labeled potency of 861 μg/mg, was supplied by Gamir-Rottapharm Laboratories, Valencia, Spain. The drug was administered to the animals at a dose level of 8.8 mg of active antibiotic by the intravenous (0.5 ml of drug solution) and oral (2 ml of drug solution) routes.

Ten rats were randomly distributed in two groups of five. Animals of group 1 received the i.v. dose on the fourth day after the jugular vein cannulation and the oral dose 4 days later. The rats in group 2 received both doses at the same time intervals but in the opposite order (Table I).

Blood samples were drawn at 2, 5, 10, 20, 30, 50, 70, 90, 110, and 130 min after i.v. dosing and at 10, 20, 30, 45, 60, 80, 110, 140, 170, and 200 min after oral dosing.

Plasma samples were tested for amoxycillin content by a classic microbiological diffusion procedure (6) with Micrococcus luteus as the test organism, within 48 hr of sample collection (coefficient of variation, 5%; detection limit, 0.05 μg/ml).

Antipyrine

Antipyrine (supplied by Kabi-Fides Laboratories, Barcelona, Spain) was administered to the rats at a dose level of

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Table I. Dosing Schedules

<table>
<thead>
<tr>
<th>Drug and dose</th>
<th>Group</th>
<th>Daya (route)</th>
<th>Dayb (route)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin,</td>
<td>1</td>
<td>4 (i.v.)</td>
<td>8 (oral)</td>
<td>5</td>
</tr>
<tr>
<td>8.8 mg</td>
<td>2</td>
<td>4 (oral)</td>
<td>8 (i.v.)</td>
<td>5</td>
</tr>
<tr>
<td>Antipyrine,</td>
<td>1</td>
<td>1 (i.v.)</td>
<td>4 (oral)</td>
<td>6</td>
</tr>
<tr>
<td>3.2 mg</td>
<td>2</td>
<td>1 (oral)</td>
<td>4 (i.v.)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1 (i.v.)d</td>
<td>4 (i.v.)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4 (i.v.)</td>
<td>—</td>
<td>11</td>
</tr>
</tbody>
</table>

*a* Days from jugular vein cannulation to first drug administration.

*b* Days from jugular vein cannulation to second drug administration.

*c* Number of rats.

*d* Administration of normal saline free of antipyrine.

3.2 mg. A 0.5-ml volume of drug solution was injected through the bridge-tubing for the i.v. administration, and 1 ml of drug solution was used for the oral administration.

Four groups of rats were dosed with this drug. Initial experiments were performed on two groups of six animals. Each animal was dosed by the i.v. and oral routes with a time interval between doses of 3 days; the first dose was administered on the day after the jugular cannulation (Table I).

Two additional groups of animals (groups 3 and 4; Table I) were assayed in order to elucidate the cause of the changes in pharmacokinetics of antipyrine observed in groups 1 and 2. The rats in group 3 were intravenously injected with 0.5 ml of normal saline on the day after the jugular vein cannulation and an intravenous antipyrine dose was given 3 days later. After normal saline administration, blood samples were drawn at the same times as after i.v. drug administration, but they were thrown away. The animals in group 4 received a single i.v. antipyrine dose on the fourth day after the cannulation, without any previous treatment.

Fig. 1. Mean plasma levels and standard deviations of amoxycillin after intravenous injection of a 8.8-mg dose to animals of group 1 (○) and group 2 (●).

Fig. 2. Mean plasma levels and standard deviations of amoxycillin after oral administration of a 8.8-mg dose to animals of group 1 (○) and group 2 (●).

Blood samples were drawn at 2, 5, 10, 20, 40, 60, 100, 140, 180, and 220 min after i.v. dosing and at 5, 10, 20, 40, 60, 90, 130, 170, 210, and 250 min after oral dosing.

Antipyrine concentrations in plasma were determined by means of a slightly modified version of an HPLC method from the literature (7). The coefficient of variation and the detection limit of the analytical technique were 6% and 0.1 μg/ml, respectively.

Pharmacokinetic Methods and Statistics

The total area under the plasma drug concentration versus time curve (AUC) was calculated by means of a combination of the regular trapezoidal and the logarithmic trapezoidal rules (8).

The terminal disposition half-life (t1/2), the mean residence time (MRT), the steady-state volume of distribution (Vds), and the plasma clearance (Cl) were calculated by the usual procedures (9).

Systemic bioavailability (F) was estimated from the ra-

Table II. Pharmacokinetic Parameters of Amoxycillin in Rats After i.v. Administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (4)a</th>
<th>Group 2 (8)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl (ml/min)</td>
<td>5.5 ± 1.1</td>
<td>4.0 ± 0.3*</td>
</tr>
<tr>
<td>AUC (μg · min/ml)</td>
<td>1665 ± 393</td>
<td>2255 ± 188*</td>
</tr>
<tr>
<td>Vds (ml)</td>
<td>155 ± 17</td>
<td>105 ± 8**</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>40 ± 6</td>
<td>30 ± 4*</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>29 ± 7</td>
<td>27 ± 3</td>
</tr>
</tbody>
</table>

*a* Days from jugular vein cannulation to drug administration.

* P < 0.05; different from rats in group 1; t test.

** P < 0.001; different from rats in group 1; t test.