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

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The effect of chronic cannulation of the rat jugular vein on the pharmacokinetics of amoxycillin and antipyrine administered by the i.v. and oral routes has been evaluated. Animals that received the i.v. dose of amoxycillin on the eighth day after jugular vein cannulation 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; amoxycillin; 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 bioavailability, 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 pharmacokinetic/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 introduced 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 administration. 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 anaesthesia.

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

Drugs, Dosing Schedules, Sampling Protocols, and Analytical Techniques

Amoxycillin

Amoxycillin 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

Drug and dose	Group	Day[a] (route)	Day[b] (route)	n[c]
Amoxycillin, 8.8 mg	1	4 (i.v.)	8 (oral)	5
	2	4 (oral)	8 (i.v.)	5
Antipyrine, 3.2 mg	1	1 (i.v.)	4 (oral)	6
	2	1 (oral)	4 (i.v.)	6
	3	1 (i.v.)[d]	4 (i.v.)	11
	4	4 (i.v.)	— —	11

[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.

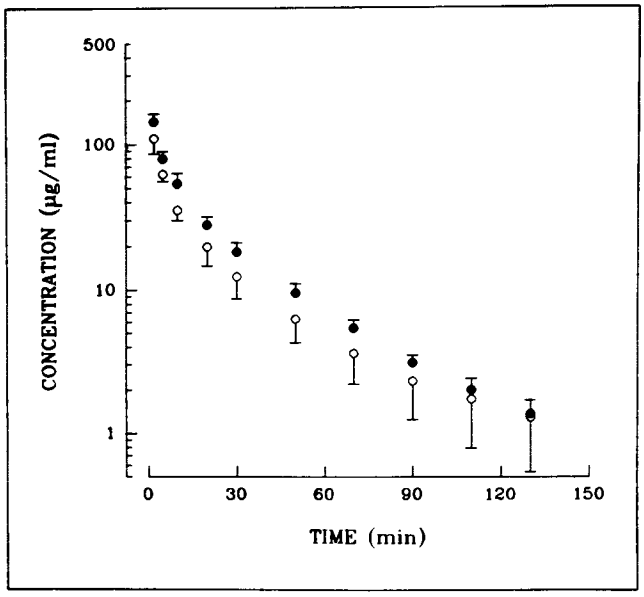

Fig. 1. Mean plasma levels and standard deviations of amoxycillin after intravenous injection of a 8.8-mg dose to animals of group 1 (○) and group 2 (●).

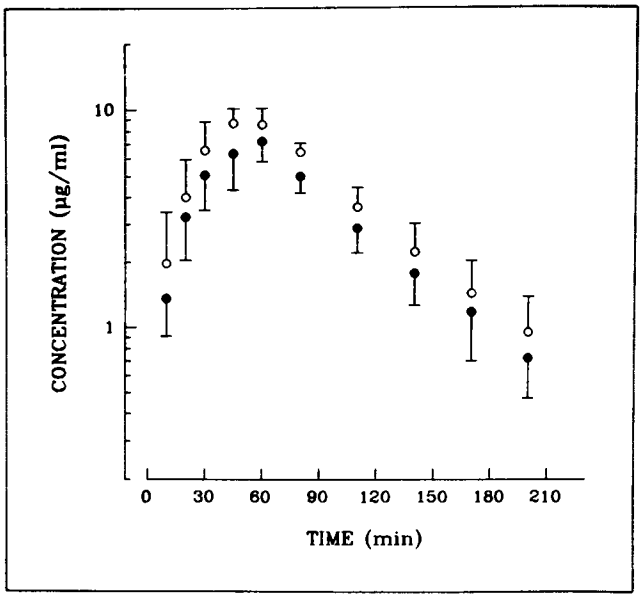

Fig. 2. Mean plasma levels and standard deviations of amoxycillin after oral administration of a 8.8-mg dose to animals of group 1 (○) and group 2 (●).

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 ($t_{1/2}$), the mean residence time (MRT), the steady-state volume of distribution (Vd_{ss}), 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

Parameter	Mean ± SD	
	Group 1 (4)[a]	Group 2 (8)[a]
Cl (ml/min)	5.5 ± 1.1	4.0 ± 0.3*
AUC (μg · min/ml)	1665 ± 393	2235 ± 188*
Vd_{ss} (ml)	155 ± 17	105 ± 8**
$t_{1/2}$ (min)	40 ± 6	30 ± 4*
MRT (min)	29 ± 7	27 ± 3

[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.

Table III. Area Under the Plasma Drug Concentration Versus Time Curves After Oral Administration of Amoxycillin and Antipyrine, and Bioavailability Estimates

Parameter	Amoxycillin		Antipyrine	
	Group 1 (8)[a]	Group 2 (4)[a]	Group 1 (4)[a]	Group 2 (1)[a]
AUC (μg · min/ml)	872 ± 129[b]	684 ± 147	2038 ± 285	1294 ± 386*
F (%)	54 ± 12	31 ± 6*	173 ± 43	74 ± 15*

[a] Days from jugular vein cannulation to drug administration.
[b] Mean ± SD.
* $P < 0.01$; different from rats in group 1, t test.

tio between the AUC values obtained after the oral and the intravenous dosing to the same animal.

Statistical comparisons between pairs of means were made via t test. For comparisons of more than two means, a one-way analysis of variance (ANOVA) was employed, and when statistically significant differences were found, Tukey's multiple-range test was applied in order to detect the statistically different means. A probability level of less than 0.05 was considered to be statistically significant.

RESULTS

Amoxycillin

Mean plasma concentrations of amoxycillin versus time after i.v. and oral administration of the drug are shown in Figs. 1 and 2. The mean plasma drug levels obtained on day 8 were appreciably larger than those obtained on day 4. Results of the pharmacokinetic analysis are summarized in Tables II and Table III. A significant decrease in Cl, Vd_{ss} and $t_{1/2}$ and a significant increase in AUC were observed on day 8 compared to day 4. After oral administration, the mean AUC on day 8 was larger than on day 4, although the difference was not statistically significant ($P = 0.063$). Apparent oral amoxycillin bioavailability was greater in group 1 animals than in group 2.

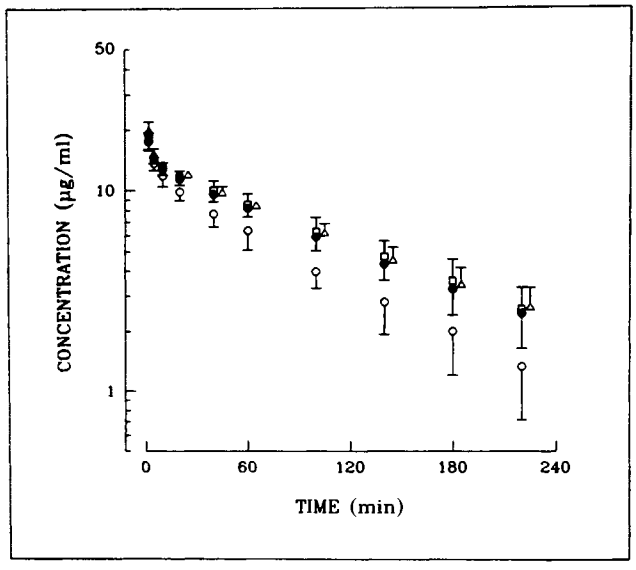

Fig. 3. Mean plasma levels and standard deviations of antipyrine after intravenous injection of a 3.2-mg dose to animals of groups 1 (○), 2 (●), 3 (□), and 4 (△). Data for group 4 have been slightly shifted to facilitate visual inspection.

Antipyrine

Mean plasma antipyrine concentrations after i.v. dosing are given in Fig. 3. Plasma drug concentrations for group 1 were lower than for the others, which suggests a larger antipyrine clearance for that group. Pharmacokinetic parameters calculated after intravenous antipyrine administrations are summarized in Table IV. Statistically significant differences between the four group parameters were found only for group 1. No statistically significant differences were found among pharmacokinetic parameters of groups 2, 3, and 4.

Plasma concentration time courses of antipyrine after oral administration of the drug to groups 1 and 2 are shown in Fig. 4. Plasma drug levels were higher for group 1 and a larger AUC value was obtained for this group than for group 2 (Table III). The bioavailability estimated for group 1 was larger than 100% and different from the bioavailability estimated for group 2.

The hematocrit of groups 3 and 4, before and after blood sampling, is shown in Fig. 5, where the hematocrit of normal rats is also displayed as a reference value.

DISCUSSION

The cannula implantation procedure used in the present study is based on the method of Bakar and Niazi (3) with some differences, concerning mainly the length of the cannula inserted into the blood vessel (3.4 instead of 3 cm). An inserted length of 3.4 cm was found to be necessary in order for the end of the cannula to reach the auricle and to achieve a long patency period of the implanted cannulas (several weeks).

Initial experiments with antipyrine (groups 1 and 2) revealed a smaller clearance of the drug in animals intravenously dosed on the fourth day after jugular cannulation than in those intravenously dosed on the first day (Table IV). There seemed to be three possible causes of the smaller antipyrine clearance on the fourth day: the prior drug treatment on the first day, the loss of blood by sampling during the first drug treatment, and physiological changes caused by a prolonged presence of the cannula in the bloodstream. The data in Table IV indicate that antipyrine pharmacokinetics on the fourth day were independent of the previous drug treatment and of the loss of blood by sampling 3 days before, since animal groups 2, 3, and 4 showed similar values

Table IV. Pharmacokinetic Parameters of Antipyrine in Rats After i.v. Administration

Parameter	Mean ± SD			
	Group 1 (1)[a]	Group 2 (4)[a]	Group 3 (4)[a]	Group 4 (4)[a]
Cl (ml/min)	2.7 ± 0.6	1.9 ± 0.3*	1.8 ± 0.4*	1.8 ± 0.3*
AUC (μg · min/ml)	1219 ± 262	1735 ± 325*	1843 ± 352*	1858 ± 288*
Vd_{ss} (ml)	256 ± 23	237 ± 10	231 ± 17*	232 ± 13*
$t_{1/2}$ (min)	71 ± 16	91 ± 18	93 ± 16	97 ± 18*
MRT (min)	98 ± 21	129 ± 26	132 ± 23*	135 ± 25*

[a] Days from jugular vein cannulation to drug administration.
* Significantly different from rats of group 1.

of pharmacokinetic parameters. The decrease in antipyrine clearance on the fourth day seems, therefore, to be due to the prolonged presence of the cannula in the bloodstream.

The decrease in the hematocrit caused by the blood sampling (approximately 2.5 ml of blood) on the first day after jugular cannulation was still apparent on the fourth day (group 3) (Fig. 5). However, the decrease did not affect the antipyrine pharmacokinetic parameters determined on the fourth day, since parameters obtained in group 3 were similar to those in group 4, which was not subjected to a previous blood sampling and showed a normal hematocrit at the beginning of drug administration.

Terao and Shen (4) observed a decrease in the apparent volume of distribution and an increase in the systemic availability of *l*-propranolol in the rat when the drug was administered on the seventh day after jugular vein cannulation instead of the day after cannulation. These changes were associated with a 50 to 60% decrease in the serum unbound fraction of *l*-propranolol between the 2 study days, caused by the presence of the catheter in the jugular vein.

Later, Chindavijak *et al.* (5) studied the pharmacokinetics of propranolol and antipyrine in rats, 2 and 48 hr after insertion of silicone catheters in both jugular veins. These authors concluded that the presence of indwelling catheters can alter the plasma concentration of drugs and that this can be partially due to a change in hepatic metabolism.

The decrease in clearance of antipyrine observed in the present study could be caused by a reduction in the hepatic intrinsic clearance of the drug, as pointed out by Chindavijak *et al.* (5). However, a decrease in clearance and steady-state volume of distribution of amoxycillin, a drug slightly bound to serum proteins and eliminated mainly through the renal route (10–12), was also detected. This result suggests that changes induced by the presence of a catheter in the bloodstream can be broader than previously proposed.

The apparent bioavailability of amoxycillin was larger for group 1 than for group 2 and the same occurred with regard to the apparent bioavailability of antipyrine (Table III). We attribute this to the decrease in clearance of both drugs on the second administration day rather than to alterations in the amounts of drug absorbed. If bioavailability is assessed using the mean AUC values corresponding to oral and i.v. administration on the same day after jugular vein cannulation, bioavailability estimates are similar regardless

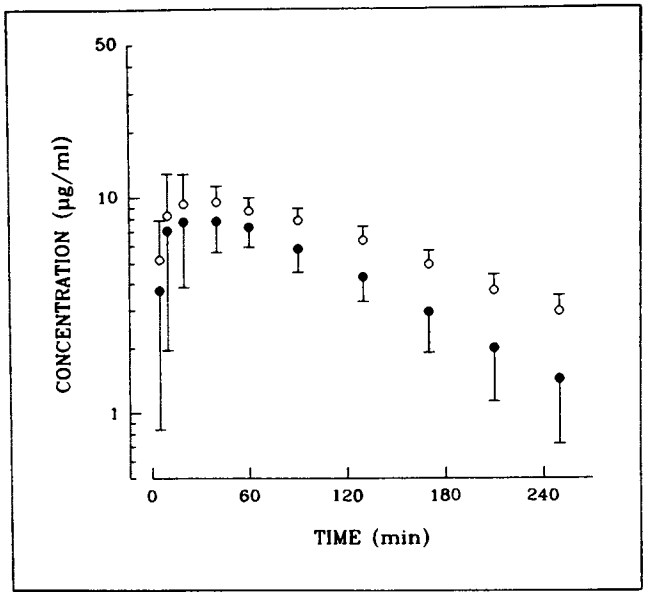

Fig. 4. Mean plasma levels and standard deviations of antipyrine after oral administration of a 3.2-mg dose to animals of group 1 (○) and group 2 (●).

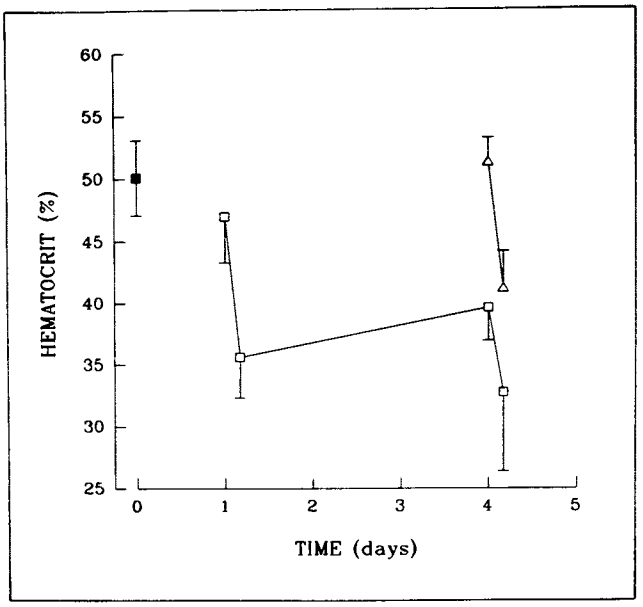

Fig. 5. Hematocrit values in rats in groups 3 (□) and 4 (△) of antipyrine assay before and after blood sampling. The hematocrit of normal rats is also shown (■; n = 6). Means ± SD.

of the administration day: 41 and 39% for amoxycillin (from mean AUC values obtained on days 4 and 8, respectively) and 106 and 112% for antipyrine (from mean AUC values obtained on the first and the fourth days, respectively). This result suggests that the bioavailability of drugs with a low hepatic extraction ratio (like those used in the present study) could be correctly estimated in chronically cannulated rats if both oral and i.v. drug administrations are performed in different animals at the same time after cannulation. However, for other drugs that undergo extensive metabolic first-pass effect, such as propranolol, this may not be the case, since the decrease in clearance with time could lead to a larger increase in AUC after oral administration than after i.v. administration (4) and, consequently, to an increased bioavailability with time.

Results presented here and those previously published show that pharmacokinetic crossover assays should not be done using rats with chronic implanted cannulas due to time-dependent changes in pharmacokinetics. An intriguing question concerns the adequate time period between cannulation and drug administration. Although the experiments of Chindavijak *et al.* (5) showed that there was no difference between the control rats and the rats after 2 hr of catheter implantation as far as metabolizing activity of the hepatocytes is concerned, this period may not be adequate for drugs eliminated by renal excretion. Walker *et al.* (13), for example, have reported that experimentation in rats 2–3 hr after ether anaesthesia and surgery is associated with a severe depression of renal hemodynamics (depression of renal blood flow and glomerular filtration rate), and they suggest that the effects of the ether and/or surgery have not completely subsided by this time.

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