

[Chem. Pharm. Bull.]
32(7)2851—2854(1984)

Chronic Blood Vessel Catheterization in Göttingen Miniature Pigs and Application to a Preliminary Bioavailability Study of Nalidixic Acid

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(Received November 1, 1983)

A surgical procedure for chronic jugular vein cannulation of Göttingen miniature pig for repeated blood samplings is described. Cannulated minipigs G were used for a preliminary study of the bioavailabilities of nalidixic acid tablets showing different dissolution rates and to investigate the elimination profiles of nalidixic acid in blood after intravenous administration of the drug. The results indicate the usefulness of these catheterized minipigs G for bioavailability studies requiring frequent blood samplings.

Keywords—pig; Göttingen minipig; bioavailability; jugular vein; cannulation; nalidixic acid; oral administration; intravenous administration

Although the similarity of many physiological functions of swine and man (cardiovascular system, gastrointestinal tract, lipid metabolism, skin features *etc.*)^{1,2)} has increased the use of pigs as a model animal in various studies, they are inconvenient as laboratory animals because of their size. However, Withey *et al.*,^{3,4)} successfully used a miniature swine for bioavailability studies. Recently, the Göttingen miniature pig (minipig G), a small minipig compared with usual ones, has become available in Japan. Thus, we decided to investigate the usefulness of minipig G as a model animal for bioavailability studies. The experiments require frequent blood samplings after drug administration, and this means that blood vessel cannulation is necessary because no major vein runs near the body surface of the pig. This report describes a chronic jugular vein catheterization technique applied to minipigs G, according to the method reported by Christison and Curtin⁵⁾ with some modifications, as well as the use of the cannulated pigs for a preliminary study on the bioavailability of nalidixic acid.

Experimental

Surgical Procedure—The following drugs were used in the experiments: Droleptan (2.5 mg/ml droperidol, Sankyo Co., Ltd.), Somnopentyl (64.8 mg/ml sodium pentobarbital, Pitman-Moore Inc.), Xylocaine 1% (10 mg/ml lidocaine hydrochloride, Fujisawa Pharmaceutical Co., Ltd.), 20% kanamycin sulfate (Banyu Pharmaceutical Co., Ltd.), and 15% sodium ampicillin (Toyama Chemical Co., Ltd.). Silicon rubber tubing (o.d., 3 mm; i.d., 1 mm; length, more than 50 cm) with a plastic anchor (diameter, 5 mm; length, 2 cm) which was fixed to the tube with silk sewing thread and paste at a point 10—15 cm from the end of the tube was used as the catheter (Fig. 1).

Minipigs G (Nihon Clea Co., Tokyo) ranging from 6 to 12 months of age and 15—30 kg in weight, were used in the study. Each pig was kept in an individual cage, and given 500—1000 g/d of the diet (M-16, Nihon Clea Co.), depending on the age of the animal. The pigs were fasted overnight before surgery. The skin at the surgical site was shaved with clippers and washed with tincture of iodine and alcohol for disinfection, 15—30 min after intramuscular administration of 0.1 mg/kg of droperidol to the animal for premedication. Then, 20 mg/kg of sodium pentobarbital

was injected into the jugular vein to anesthetize the animal. An incision (about 10 cm) was made in the neck from a point on the shoulder to a point slightly above the angle of the jaw, after several injections of 1 ml of 1% lidocaine hydrochloride into the muscle of the incision area. The jugular vein was isolated by blunt dissection with a homostat dorsal to the muscle around the incision. Two O-silk ligatures were placed around the vein, and the ligature furthest from the heart was tied in order to halt the blood flow through the vein. The vein was cleaned by stripping the connective tissues from a 2—3 cm length. A small cut was made in the wall of the vein between the ligatures with iris scissors. The catheter, filled with heparinized saline, was inserted through the nick into the vein toward the heart for 10—15 cm, depending on the size of pig. After aspiration of blood through the catheter had been confirmed, the ligature closest to the heart was tied around the catheter and connected with the anchor of the catheter and adjacent tissue to stabilize the catheter. A small incision was made at the dorsum of the pig's neck and a trocar was inserted from the dorsal nick to the jugular region. The distal end of the catheter was inserted into the trocar, and the catheter was pulled to the back of the neck after washing of the outside surface of the catheter with a few ml of 20% kanamycin sulfate. A metal adapter with a rubber cap was inserted onto the dorsal end of the catheter, and the catheter was stabilized in a loop on the back of the animal with adhesive tape (Fig. 1). The skin incision was closed with surgical silk, followed by intramuscular administration of 1.5 mg/kg of sodium ampicillin to the pig. All skin sutures were removed 7—14 days after surgery.

Bioavailability of Nalidixic Acid—Three different tablets A, C and E containing 250 mg nalidixic acid per tablet⁶ were employed for the bioavailability study. The *in vitro* dissolution rates (expressed as the time for 50% of the drug to be dissolved from the tablets) are given in Table I. The dissolution rates were determined by the oscillating basket method using a JP-X disintegration device (30 cpm)⁷ and by the JP-X paddle method (120 rpm) at 37°C, with 1000 and 900 ml of pH 7.2 sodium phosphate buffer (0.1 M) in the former and latter cases, respectively. According to a randomized block design, one tablet of the test products was orally administered with 200 ml of water to three pigs, NO. 145, 148 and 295 (7, 7 and 9 months of age and 19, 17 and 23 kg in weight, respectively) after they had been fasted overnight. No food was given until 6 h after dosing. Blood samples of approximately 5 ml were taken from the pigs through the catheter at 1, 2, 3, 4, 5, 6, 8 and 11 h after the drug administration and plasma samples were kept frozen until assayed. The experiments were repeated every week according to the indicated dosage schedule. Two of the pigs were intravenously given 250 mg of nalidixic acid dissolved in 10 ml of pH 10.0 sodium phosphate buffer (0.1 M) through the catheter, and blood samples were taken at 10, 20, 30, 45, 60, 90, 120 and 180 min after dosing. The pharmacokinetic parameters of nalidixic acid in plasma were calculated with a microcomputer using the MULTI program (weighting factor = 1).⁸ The plasma samples were kept frozen until the assay. The plasma concentrations of nalidixic acid were determined by liquid chromatography⁶ using a column (3 mm × 25 cm) of Hitachi Gel 3011N, a strong anion-exchange resin. The mobile phase consisted of 0.09% boric acid buffer (pH 9.2), 1.3% NaCl, 0.05% sodium benzoate and 20 v/v% acetonitrile. The drug was detected at 335 nm.

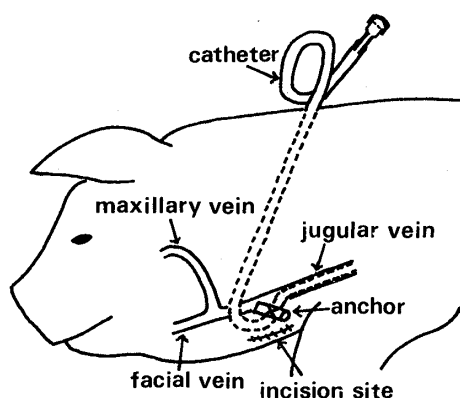


Fig. 1. Surgical Placement of the Cannula in Minipig G

TABLE I. Time (min) Required for 50% of the Drug to be Dissolved from Nalidixic Acid Tablets

Method	Tablet		
	A	C	E
Paddle	2.5	81.1	27.6
Oscillating basket	1.9	9.7	77.0

Results and Discussion

Minipigs G, like the other pigs, grow rapidly during infancy and reach sexual maturity at 5–6 months, which suggests that their physiological condition will not be stable till about 6 months. Thus, 6- to 12-month-old minipigs G were used in our bioavailability studies. The cannulated pigs took food the day after surgery, and were used for the experiments at least 10 days later. The catheter was flushed every day with heparinized sterile saline (10 units/ml) to prevent coagulation of blood within the catheter. The cannula usually remained functional for 1–4 months.

Cannulated minipigs G were used for a preliminary bioavailability study of nalidixic acid. Figure 2 shows the elimination profiles of nalidixic acid in plasma following intravenous administration of 250 mg of nalidixic acid each to two pigs. The pharmacokinetic parameters calculated on the basis of a two-compartment open model are listed in Table II. The half-lives of the initial phase (about 3 min) were very rapid and those of the second phase in the pigs

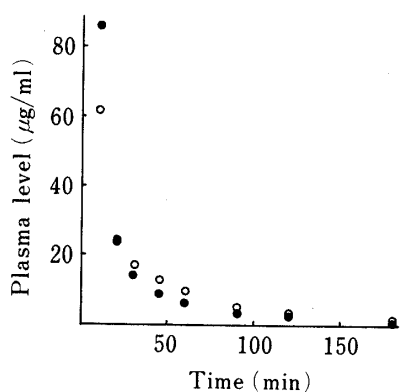


Fig. 2. Plasma Levels of Nalidixic Acid Following Intravenous Administration of 250 mg of Nalidixic Acid to Each of Two Pigs, No. 148 (●) and No. 145 (○)

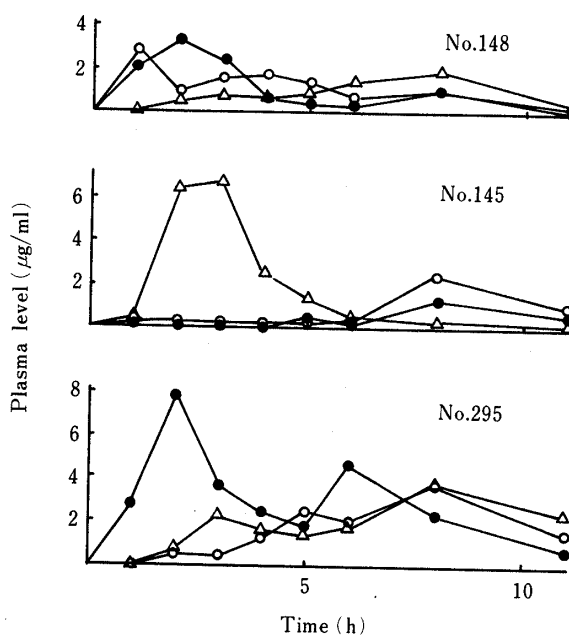


Fig. 3. Plasma Levels of Nalidixic Acid Following Oral Administration of Three Different Tablets A (●), C (○) and E (△) to Each of Three Minipigs G

TABLE II. Pharmacokinetic Data for Nalidixic Acid Following Intravenous Administration of 250 mg of Nalidixic Acid to Each of Two Minipigs G

Pig No.	Weight (kg)	A (µg/ml)	B (µg/ml)	α (min ⁻¹)	β (min ⁻¹)	$V_c^{(a)}$ (l)	$V_{ss}^{(a)}$ (l)	AIC ^(b)
148	17	527.9	26.3	0.217	0.0243	0.474	1.182	-8.01
145	19	401.5	29.9	0.239	0.0196	0.580	2.069	-2.01

a) V_c and V_{ss} are central compartment and steady-state distribution volumes, respectively.

b) Akaike's information criterion.⁹⁾
 $C_p = Ae^{-\alpha t} + Be^{-\beta t}$

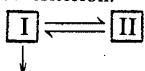


TABLE III. C_{\max} , T_{\max} and AUC_{0-11h} Following Oral Administration of 250 mg Nalidixic Acid Tablets (A, C, E) to Each of Three Minipig G

Pig	C_{\max} ($\mu\text{g/ml}$)			T_{\max} (h)			AUC_{0-11h} ($\mu\text{g}\cdot\text{h/ml}$)		
	A	C	E	A	C	E	A	C	E
No. 148	3.272	1.791	1.987	2	4	8	9.804	9.904	11.354
No. 145	1.188	2.418	6.810	8	8	3	4.275	9.371	19.927
No. 295	7.818	3.676	3.837	2	8	8	32.766	19.235	22.758
Means	4.093	2.549	4.211	4	6.7	6.3	15.615	12.837	18.013

(approximately 30 min) were faster than the half-life in humans (90—100 min).^{6,10} The calculated central distribution volumes in the pigs were very low. Figure 3 shows the plasma levels of nalidixic acid following oral administration of three different tablets to each of three pigs, and Table III summarizes the bioavailability data. As expected from the dissolution rates, the fast-dissolving product A gave higher peak plasma levels (C_{\max}) and smaller peak times (T_{\max}) than the slow-dissolving ones, C and E, in two of the pigs.

The successful drug administration and blood samplings in this study demonstrate the usefulness of minipigs G subjected to cannulation surgery for bioavailability studies requiring frequent blood samplings. The estimation of bioavailability of griseofulvin tablets using minipigs G will be described in a separate report.¹¹⁾

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