Use of a Chronic Ureter Cannula in the Pig for the Determination of Renal Clearance

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ABSTRACT. The surgical implantation of chronic ureter cannula to determine the renal clearance was evaluated using 24 pigs. The silicon tubing was surgically implanted into both ureters of each pigs. Two types of thick tubing (the inside diameter 1.0 or 2.5 mm and the outside diameter 4.0 mm) were used for these cannulas. The tubing was exposed out of the pig's body on the flank, on the hypogastric zone near the umbilicus, or near the groin. The following steps were effective to minimize the opportunity of a bacterial infection in the kidney and to maintain the functional integrity of the chronic ureter cannula for as longer period as possible: 1) to use the tubing of larger opening as the ureter cannula, 2) to expose the tubing from the hypogastric zone near the groin, and 3) to apply the disinfectant frequently to the incision sites, cannula outlets and pig's metabolic cage. The oral ingestion of the GGES solution increased the urinary volume, which might in turn have resulted in the effective rinsing of the kidney and the chronic ureter cannula. The cannula served satisfactorily for more than 3 weeks in 13 of the pigs, up to a maximum of 7 weeks. The PSP clearances values were low during the first week of the postoperative period, which may be attributed to surgical stress. The chronic ureter cannula, associated with the postoperative period of more than 1 week, can be recommended for the evaluation of the renal clearance of drugs in the pig.—**KEY WORDS:** chronic ureter cannula, inulin clearance, pig, PSP test, renal clearance.

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The renal clearance of specific drugs is an important parameter of their pharmacokinetic evaluation. This value is calculated from the rate of urinary excretion of the specific drug divided by the drug's plasma concentration. The most accurate method for obtaining the renal clearance is the concurrent sampling of the urine, just after it is excreted from the kidney, and the collection of a blood specimen. The determinations of renal clearance were generally performed, using either anesthetized (*in situ*) or conscious animals (*in vivo*). For the *in situ* determinations, the urine was usually collected from a cannula that had been inserted into the bladder or ureter [1, 7, 9]. For most of the *in vivo* determinations, the urine was sampled as being naturally voided. For some of the *in vivo* determina-

tions, the urine was obtained from a catheter in the urinary bladder [4, 8]. In this study, a chronic ureter cannula was employed to permit the exact determination of the renal clearance of a specific drug in the conscious pig. The methods of maintaining the function or patency of this ureter cannula over the prolonged period of time and the assessment of its patency are discussed in this paper.

MATERIALS AND METHODS

Animals: Twenty-four female commercial breed pigs, weighing 28–45 kg and 2–5 months old, were utilized in this experiment. After the surgical implantation of the chronic ureter cannula, they were fed a commercial mashed feed (2% of body

weight per day) in which no antibacterial drug was added. The pigs were allowed to drink water *ad libitum*. Some pigs were allowed to drink the glucose glycine electrolyte solution (GGES) three times a day, with a total volume of three liters per day. The pigs were housed in metabolic cages.

Surgical operation: Two different sizes of thick silicon tubing were used for the ureter cannula in order to minimize the risk of the twisting or folding of the tubing in and out of the pig's body. One type of tubing had of a smaller opening (1.0 mm the inside and 4.0 mm the outside diameter) and the other tubing had a larger internal opening (2.5 mm the inside and 4.0 mm outside diameter). The animals were anesthetized with halothane. Ketamine was injected (10 mg/kg, i.m.) as a preanesthetics. Through a midline incision, the ureters were exposed. The tubing was inserted into both ureters, 5 cm rostral to the ureter ostium of the bladder and then advanced cranially to the proximal end of each ureter. The silicon tubing was fastened to the psoas minor with silk sutures. The distal ends of the two cannulas were subcutaneously advanced and fastened to the muscles transversus abdominis. The tubing was exposed out of the pig's body on the flank (F-zone), on the hypogastric zone near the umbilicus (U-zone) or near the groin (G-zone) and was fastened to the skin. The surgical scheme is presented in Fig. 1. Urine from the ureter cannula was allowed to flow passively out of the pig's body.

Postoperative management: Ampicillin and kanamycin sulfate were postoperatively provided by i.m. injection for two days. In order to minimize further the opportunity for the development of bacterial infection in these chronic ureter cannulas, the following protocol was followed. Povidon-iodine solution was applied to the surgical incision sites and the cannula outlets three times a day. Benzetonium chloride was utilized for the

Fig. 1. Scheme of the surgical implantation of the chronic ureter cannula.

a: cuff, b: skin, c: subcutaneous tissue, d: muscles transversus abdominis, e: peritoneal cavity, f: muscles psoas minor, g: kidney, h: fastened by silk.

disinfection of the metabolic cage housing the animals every day. The GGES solution was administered to hasten urination, which in turn, provided additional protection from bacterial infection to the kidney. The formulation of the GGES solution was 67.5% glucose, 14.3% NaCl, 10.3% glycine, 0.81% citric acid, 0.21% potassium citrate and 6.8% potassium phosphate monobasic. For the preparation of the GGES solution, 67 gm of the powder was dissolved in two liters of water [2]. A sequential surgical operation was performed whenever the ureter cannula became dislocated.

PSP clearance and BUN: The patency or maintenance of unrestricted urine flow in the chronic ureter cannula was monitored with the assistance of chemical tests, specifically the phenolsulfophthalein (PSP) clearance and the BUN value. A urine dipstick test was also performed, but was proved to be of little value because of its variation of the results and less sensitivity. The PSP clearance test was represented by the PSP-50 value, which is the elapsed time for 50% excretion of the injected PSP dye from the chronic ureter cannula. This value was calculated from the PSP concentration and the urine volume. The PSP dye was injected

at a dosage of 10 mg/kg (i.v.). The urine was fractionally sampled every 10 minutes up to a maximum of 180 minutes. The urine concentration of PSP was then assayed using a spectrophotometric method [5]. The urinary sample was centrifuged. From the supernatant obtained, 0.5 ml was added to 3 ml of acetone, mixed well using a vibrator and allowed to stand for 20 minutes. The absorbance of acetone layer at 550 nm was compared to a standard curve to obtain the PSP concentration. The BUN value was determined every week using the urease indophenol method [4]. A commercial kit (BUN-S, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) was used.

Inulin clearance test: The inulin clearance was calculated from the excreted volume of urinary inulin per minute divided by the inulin plasma concentration. In order to examine the influence of anesthesia on the renal function, the inulin clearance was determined for both the anesthetized group of pigs and the conscious group of pigs. For the determination of the anesthetized group, pentobarbital-Na (25 mg/kg, i.v.) was given, followed by the intravenous injection of inulin at a concentration of 100 mg/kg and then the constant infusion of inulin (1 mg/kg/min) in Ringer's solution with 5% mannitol. After the plasma inulin concentration had reached a steady-state condition, the urine was fractionally sampled for 10 minutes. The blood sample was collected at the midpoint of the urine sampling period. In order to obtain the inulin clearance for the conscious pig group, the inulin was intravenously injected at a concentration of 100 mg/kg, followed by the additional administration of 50 mg/kg of inulin every 2 hr. The urine was sampled for 2 minutes. The blood sample was obtained immediately after the urine collection. The inulin concentration in the blood or urine was determined by the spectrophotometric method [6]. After deproteinization with a

trichloroacetic acid solution, 0.1 ml of 3-indoleacetic acid and 4 ml of concentrated HCl were added to the diluted sample. This mixture was incubated for 70 minutes at 37°C and analyzed afterwards using a spectrophotometer.

RESULTS

1. Assessment of the patency of the ureter cannula

(1) *PSP and BUN test:* The PSP-50 was determined 86 times in 23 different pigs. The PSP-50 values ranged from 9.3 to 145 minutes. Fifty four of the PSP-50 values obtained were less than 30 minutes (54/86, or 63%). All of the pigs with a PSP-50 value of less than 30 minutes were vigorous, showed a healthy appetite, and had a glossy or brilliant skin and body hair. Their urinary flow was reasonably profuse and the urine was colorless. The PSP-50 value proved to be an effective indicator in the evaluation of the patency of the ureter cannula. The BUN values for this group of pigs were less than 20 mg/dl.

The BUN test was performed 103 times for the 24 different animals. The BUN values ranged from 4.3 to 81 mg/dl. Eighty five of the BUN values were less than 20 mg/dl (85/103, or 83%). Seven pigs out of 85 suffered from a serious illness, and became moribund. Even though the renal function was impaired, the BUN value could not increase so rapidly. The BUN values proved to be ineffective in the assessment of the functional integrity of the ureter cannula.

(2) *Correlation between the PSP-50 and BUN value:* The correlation between the PSP-50 and BUN determination is represented in Fig. 2. Two groups of pigs are shown in this figure. Group I (open circle) had a postoperative period of more than one week. Group II (filled square) had a postoperative period of less than one week, including sequential operations due to can-

nula dislocations. Group I was subdivided further into 3 subgroups. Group I(a) with a PSP-50 value of <30 minutes and a BUN value of <20 mg/dl. Group I(b) with a PSP-50 value of >30 minutes and a BUN value of <20 mg/dl. Group I(c) with a BUN value of >20 mg/dl. All of the experimental pigs from Group I(a) were vigorous and their ureter cannulas were assessed to be unobstructed. The pigs from Group I(b)

showed subclinical symptoms, including unvigorousness, reduced urinary flow and a dark or sallow skin and body hair. However, they had an appetite. These pigs became members of Group I(c) later. The animals of Group I(c) were not vigorous and had only a little appetite. Their urinary flow was intermittent and the urine excreted was turbid. They were sacrificed using pentobarbital.

The value of PSP-50 and BUN of the animals of Group I(a) and Group II are listed for comparison in Table 1. The PSP-50 values of Group II was significantly longer.

2. Inulin clearance test

The inulin clearance test was performed using both anesthetized and conscious groups of pigs. The pigs of Group I(a) were subjected to this determination. The inulin clearance values were lower in the anesthetized pigs, compared with those in the conscious pigs as shown in Table 2. The inulin clearance values of the conscious pigs were comparable with the values which have been published in the literature of previous experiments [10].

3. Histological observations

Table 3 lists the results from our clinical trials with the 24 experimental pigs. The patency of the chronic ureter cannula was assessed using the PSP-50 values. The experimental pigs were assigned the numbers corresponding to the order of the surgical implantation of their chronic ureter cannu-

Fig. 2. Correlation between the PSP value (PSP-50) and the BUN value. The (○) represents a postoperative period of more than 1 week (Group I). The (■) represents a postoperative period of less than 1 week (Group II). Group I was further subdivided into 3 groups.

Group I(a): PSP-50 value of <30 minutes and BUN of <20 mg/dl.

Group I(b): PSP-50 value of >30 minutes and BUN of <20 mg/dl.

Group I(c): BUN value of >20 mg/dl.

Table 1. Effect of the postoperative period on the PSP-50 and BUN values; comparison between Group I (a) and Group II

Postoperative period	n	PSP-50 value (minute) mean (SD) ^{a)}	BUN value (mg/dl) mean (SD)
Group I (a)	51	17.8 (12.2–25.4)	11.6 (9.1–15.2)
Group II	16	36.6 (19.3–59.0) ^{b)}	10.0 (7.6–13.3)

a) Geometric mean (standard deviation).

b) $p < 0.05$, compared with the value of Group I (a).

n: Numbers of determinations.

Table 2. Inulin clearance and urinary flow values obtained from 2 groups of pigs, either under anesthesia or conscious

Experimental Conditions	n	Urinary Flow (ml/minute) mean (SD) ^{a)}	Inulin Clearance (ml/minute/kg) mean (SD)
Anesthesia	18	0.95 (0.46–1.4)	1.5 (1.1–2.1)
Conscious	29	5.5 (3.4–7.5) ^{b)}	3.1 (2.3–4.5) ^{b)}

a) Geometric mean (standard deviation).

b) $p < 0.05$, compared with the values of the anesthesia group.

n: Number of determinations.

Table 3. Summary of our trials

Pig No.	Functional Period ^{a)} (weeks)	Subclinical Period ^{b)} (weeks)	The Inside Diameter of Cannula	Cannula Outlet of Cannula	GGES
1	1	3	1.0 mm	flank	not given
2	0	2	1.0	flank	not given
3	0	2	1.0	umbilicus	not given
4	1	2	1.0	umbilicus	not given
5	2	3	2.5	umbilicus	not given
6	2	1	2.5	umbilicus	given
7	5	3	2.5	groin	given
8	3	2	2.5	groin	given
9	3	2	2.5	groin	given
10	5	1	2.5	groin	given
11	2	1	2.5	groin	given
12	1	2	2.5	groin	given
13	3	2	2.5	groin	given
14	4	2	2.5	groin	given
15	0	3	2.5	groin	given
16	0	3	2.5	groin	given
17	4	0 ^{c)}	2.5	groin	given
18	3	2	2.5	groin	given
19	0	1 ^{d)}	2.5	groin	given
20	4	0 ^{c)}	2.5	groin	given
21	5	0 ^{c)}	2.5	groin	given
22	5	0 ^{c)}	2.5	groin	given
23	5	2	2.5	groin	given
24	7	3	2.5	groin	given

The pig was assigned numbers in the order of their surgical operation.

a) Functional period: the period of Group I (a); PSP-50 value <30 minutes, BUN value <20 mg/dl.

b) Subclinical period: the period of Group I (b); PSP-50 value >30 minutes, BUN value <20 mg/dl.

c) Sacrificed for autopsy.

d) Died from pneumonia.

las (number 1 to 24), as listed in Table 3. In the pigs numbers 3 through 6 the chronic ureter cannulas were exposed out of the umbilicus zone of the pig's body. These cannulas were often kicked off by the animal and had to be reimplanted. A renal

autopsy and histological examination were performed on all of the experimental pigs. Pathological changes, including pyelectasia, pyelonephritis, infarction and hemorrhage etc., were uncovered. In pigs numbers 1 and 2, the smaller silicon tubing (the inside

diameter of 1.0 mm) was implanted as the ureter cannula. Severe pyelectasia was observed later in these 2 pigs. Few pathological changes were observed in the kidney of pigs numbered 17, 20, 21 and 22. These pigs were members of Group I (a) at autopsy.

DISCUSSION

The surgical implantation of a chronic ureter cannula was performed on 24 commercial pigs. The following steps could minimize the opportunity of a bacterial infection in the kidney and maintain the functional integrity of the chronic ureter cannula as long as possible: 1) to use the tubing of larger opening as the ureter cannula which could relieve the burden of the kidney and urinary excretion. Too large opening relative to the outside diameter of the tubing, however, may run the risk of twisting or folding of the tubing in or out of the pig's body. The tubing in the present experiment was the largest to be inserted into the pig's ureter, 2) to expose the tubing from the hypogastric zone near groin, 3) to apply the disinfectant frequently to the incision sites, cannula outlets and pig's metabolic cage. More frequent disinfection may minimize the opportunity of bacterial infection. The oral ingestion of the GGES solution increased the urinary volume, which might in turn have resulted in the effective rinsing of the kidney and the chronic ureter cannula. The chronic ureter cannula served satisfactorily for more than 3 weeks in 13 pigs.

The PSP-50 values, as an indicator of the renal blood flow, assisted in the evaluation of the patency or unobstructed flow of urine through the chronic ureter cannula. The PSP-50 value was significantly longer in Group II pigs that had a postoperative period of less than 1 week, which might have been due to surgical stress. The inulin clearance values, representing the glomerular blood flow, were lower in the anesthe-

tized pigs, compared with the conscious pigs. Therefore, it appears that the renal clearance of a specific drug could be most accurately determined by using the conscious pigs that have surgically implanted the chronic ureter cannula following a post-operatively recuperative period of more than 1 week.

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要 約

ブタの腎クリアランス値測定のための慢性尿管カテーテル装着法：小久江栄一・下田 実・田中伸枝・吐山 豊秋(東京農工大学農学部家畜薬理学教室)——24頭のブタの両側尿管に、シリコンチューブを外科的に装着した。より正確な腎クリアランス値を得るためである。内径と外径がそれぞれ1.0×2.5mm、2.5×4.0mmの2種類のチューブをカニューレとして使った。太いチューブを使うこと、鼠径部から体外に出すこと、可能な限り細菌による感染を防ぐことが、慢性カニューレを長持ちさせるのに有効であった。この処置をした18頭の内13頭は3週間以上、正常の腎クリアランス値を示した。最長有効期間は7週間であった。フェノールレッド(PSP)クリアランス値は、カニューレの状態(腎の健康状態)の診断に有効であった。カニューレを装着した後1週間以内では、PSPクリアランス値は低かった。慢性カニューレを装着したブタの、麻酔下と無麻酔下でのイヌリンクリアランスを測定した。麻酔下ではクリアランス値は低かった。ブタで正確な腎クリアランス値を得たい場合は、慢性尿管カニューレを装着し、無麻酔下で測定する方法が推奨できる。但し、手術後1週間以内は測定に使わない方がいい。