The Estimation of Drug Residue in Pig Kidney by Repeated Biopsy

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ABSTRACT. Usability of a repeated renal biopsy was examined for the estimation of drug residue in the pig kidneys. The renal biopsy was carried out under laparoscopic observation. Eleven castrated pigs were given intramuscularly 1 mg (potency) of gentamicin (GM) per kg body weight. The renal parenchyma was sampled by the biopsy 3, 10, 20, 30, 40 and 50 days after administration. Two pigs were sacrificed to obtain the kidneys immediately after each biopsy. No clinical manifestations were observed except transient hematuria in all biopsied pigs. The mean concentration of GM in the biopsied samples decreased similarly to that in the kidneys of sacrificed pigs. There was no significant difference in the analytical values between the biopsied samples and those obtained by sacrifice immediately after the biopsies. These results suggest that the estimation of drug residue in the pig kidneys by the repeated biopsy is useful to determine the withdrawal time of drugs remaining predominantly in the kidneys.—**KEY WORDS:** biopsy, drug residue, gentamicin, laparoscopy, pig kidney.

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A residue study of a drug remaining for a long period in a certain organ needs to use a large number of experimental animals, since the extensive residue data is required for the estimation of the withdrawal time [14]. We have already reported that the drug residue study by the method of the repeated biopsy is useful for the estimation of the withdrawal time in calves [13]. In the present study, the procedure of the repeated renal biopsy, the influence of the biopsy on urology and kidney histology, and the usability of the biopsy method for the estimation of the drug withdrawal time are examined after the administration of gentamicin (GM) known as a persistent residue in the kidneys [1, 7, 8].

MATERIALS AND METHODS

Animals and experimental protocol: Eleven castrated pigs, weighing 40–65 kg at the start of the experiment, were used. Gentamicin sulfate (Gentocin; Shionogi Pharmaceutical Co., Ltd., Osaka) was administered intra-

muscularly to the pig with a dose of 1 mg (potency)/kg body weight. The renal parenchyma was taken repeatedly by biopsy 3, 10, 20, 30, 40 and 50 days after administration. Two pigs were sacrificed by exsanguination to obtain the kidney samples immediately after every biopsy, except one pig after the final biopsy.

Biopsy method: The renal biopsy in pigs was performed according to the previous method [9] with some modifications. Pigs were anesthetized with co-medication of 2 mg/kg azaperon (Stresnil; Janssen Co., Ltd., Belgium) and 10 mg/kg metomidate (Hypnodil; Janssen Co., Ltd., Belgium [3]) or 2 mg/kg azaperon and 12.5 mg/kg pentobarbital sodium (Abbott Laboratories Co., Ltd., U.S.A.). In the latter case of the co-medication, local anesthesia was induced with 10 ml of 1 percent lidocaine injected into the superficial fascia, muscle and perirenal tissues. After the animal was fixed with either left or right side down on an operating table, the trocar was inserted into the peritoneal cavity

through an incision in the flank. The laparoscope connected to the cool light source was set through the trocar. It was advanced forward with an insufflation of air through the trocar until the kidney and the other abdominal organs were observed. Under the laparoscopic observation, the biopsy needle with a stylet was inserted into the peritoneal cavity from an another incision in the flank. The cutting prong replaced with the stylet was thrust into the renal cortex to clip a piece of the renal parenchyma. An antiseptic (povidon-iodine solution) was applied to the closed incision sites. The trocar, laparoscope, cool light source and biopsy needle were the same as those in calves [9].

Clinical and pathological examination: Clinical observation of pigs before and after the biopsy was performed on the following items; clinical manifestation (activity, body temperature, body weight, appetite, defecation, urination), urinalysis (occult blood, protein, glucose, ketone, urobilinogen, pH, urinary color) and a half life of plasma PSP [6]. Autopsy was carried out in all pigs. Histopathological examination of the kidneys was performed in 5 pigs sacrificed 30, 40 and 50 days after administration.

Drug assay: The concentration of GM was determined by RIA described in the previous paper [13].

The recovery of GM was 99 ± 1 and $83 \pm 1\%$ (mean $\pm$ S.D., $n=3$) from the 20 mg renal tissue added 0.003 and 0.03 μg (potency), respectively. The reliable detection limit in this assay condition was 0.05 μg (potency)/g.

Statistics: Parallelism of the decay curves of the renal GM concentrations obtained from the biopsied samples and sacrificed ones was examined by Parallel Line Assay [11]. Kinetic parameters were calculated by Damping Gauss-Newton method [12]. Difference of GM concentrations between the biopsied and sacrificed samples was evaluated by Two-Way Layout ANOVAR [11]. A P value less

than 0.05 was considered to be statistically significant.

RESULTS

The laparoscopic views from the right flank are sequentially shown in Fig. 1. A severe hemorrhage from the kidney was occasionally found immediately after the removal of the biopsy needle, but it terminated within 1 min. Most of the biopsy specimens contained only cortical tissue weighing 17.5 ± 5.9 mg (mean $\pm$ S.D.). Two failures in 34 biopsies were experienced through the study. One failure was caused by the adhesion of colon to the peritoneum, resulting in the perforation of the trocal to the adherent bowel. Another one was caused by an unusual excitement after the azaperon-pentobarbital administration. An adequate loss of the skin perception for the biopsy was hardly obtained by the azaperon-pentobarbital anesthesia without the application of lidocaine. On the other hand, an appropriate anesthesia was induced by the azaperon-metomidate medication.

No clinical manifestations were observed except macro- and microscopic hematuria in all biopsied pigs. The macroscopic hematuria was observed in 4 cases out of 34 and lasted for 1~2 days. The microscopic hematuria was observed in all cases during 1~6 days. Adhesion of colon and peritoneum was observed in one case. Hemorrhage, necrosis, proliferation of the fiber, cicatrization and regeneration of the renal tubule around the area of the centesis were observed in all cases.

The renal concentration of GM is shown in Fig. 2. The decreases of the mean concentrations appeared to be monoexponential in both of the biopsied and sacrificed samples and ran parallel each other. Pharmacokinetic parameters calculated from the mean concentration were almost identical in both cases (Table 1). There was no significant difference in the analytical values of GM between the

Fig. 1. Laparoscopic view from the right flank in a pig. (A : 1; Right kidney, 2; Liver, 3; Small intestine. (B): The needle (arrow) was inserted into the kidney. (C): Some pericapsular hemorrhage (arrow) was found at the biopsied site after the removal of the needle.

Fig. 2. The residual concentration of gentamicin in pig kidneys after the drug administration. The filled circles (●) show the concentrations of the samples from sacrificed animals. The circles (○) and vertical bars represent the mean concentrations $\pm$ S.D. of the biopsied samples. a) $n=9$, b) $n=5$, c) $n=3$.

Fig. 3. Comparison of renal gentamicin concentrations in the biopsied samples and the samples from animals sacrificed immediately after biopsy. The oblique line implies the complete consistence of both data.

Table 1. Pharmacokinetic parameters in the kidney

	$C_0[\mu\text{g}(\text{potency})/\text{g}]^{\text{a)}}$	$\alpha(\text{day}^{-1})^{\text{a)}}$	AUC ^{b)}
Biopsy	12.6	0.115	109
Sacrifice	20.1	0.187	108

a) Drug concentration in kidney = $C_0 e^{-\alpha t}$.

b) Area under the curve $[\mu\text{g}(\text{potency}) \cdot \text{day}/\text{g}]$.

Each parameter was calculated from the mean concentration at each time.

biopsied samples and those obtained by sacrifice immediately after the biopsies (Fig. 3).

DISCUSSION

The repeated renal biopsy under the laparoscopic observation has been performed in calves and reported to be safe and solid for the sampling of the renal tissue [9]. There were some differences in the conditions for the biopsy in pigs, compared with that in calves. In the first place, the general anesthesia was required when biopsied in pigs although only the local anesthesia was necessary in calves. Second, the air insufflation into the peritoneal cavity was necessary for

the laparoscopic observation of the abdominal cavity. Third, in the case of pigs, the performance of the biopsy was possible on both left and right kidneys.

It has been reported that adverse effects were not observed except transient hematuria after renal biopsy on food producing animals [2, 4, 5, 9, 10]. In this study, no remarkable complications were also observed except hematuria and an adhesion of colon and peritoneum after the biopsy. The adhesion might be caused by the blood clot since it was not encountered when the peritoneal cavity was rinsed enough with saline after biopsy. The hematuria in pigs was more severe and more frequent than that in calves, but it was only

transient.

These results suggest that the repeated removal of the renal parenchyma for drug assay are possible by the biopsy under the laparoscopic observation in pigs.

The mean concentration of GM in the biopsied renal samples decreased similarly to that in the samples obtained by sacrifice at various intervals (Fig. 2). The analytical values of GM from the biopsied samples reflected closely the residue level in sacrificed pig kidneys (Fig. 3). From both studies in calves [13] and pigs, it was concluded that the residue study by the repeated renal biopsy was useful to estimate the withdrawal time of drugs remaining in the kidneys.

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要 約

ブタにおける反復腎臓生検による薬剤残留試験法： 高橋雄二・城戸靖雅・直井昌之¹⁾・小久江栄一¹⁾（畜産生物科学安全研究所，¹⁾東京農工大学農学部）——ゲンタマイシン（GM）1 mg（力価）/kg を11頭の去勢豚に筋肉内注射後3，10，20，30，40及び50日目に生検により腎臓組織を採材するとともに各時点で2頭（50日目のみ1頭）を生検直後に屠殺解剖した。生検は腰けん部から腹腔鏡を挿入し，その観察下で行った。血尿以外に臨床症状の異常はみられなかった。生検試料から得たGMの経時的变化は屠体試料によるものとよく一致した。生検組織と生検直後に放血殺して得た屠体組織中のGM濃度の間に有意差はみられなかった。以上の成績から，ブタにおける反復腎臓生検による薬剤残留試験法は実用性があると考えられた。