The Serial Biopsy Technique for Estimation of Drug Residue in Calf Kidney Using Gentamicin as a Model Drug

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(Received 8 May 1984/Accepted 22 November 1984)

ABSTRACT. Residual levels of orally administered gentamicin (GM) in the calf kidney were serially determined using the renal biopsy technique. Twelve male calves were given GM per os 1 mg (potency)/kg of body weight twice a day for a week. Three calves were used for serial biopsy which was carried out at 3 hr and 3, 10 and 15 days after the final drug administration. Two calves were sacrificed to collect the kidney samples at each time mentioned above. The renal GM concentration was determined with gentamicin RIA kit, with which more than 95% of GM added was recovered from the renal tissue *in vitro* and the concentration less than 0.05 μ g (potency)/g was detectable in 20 mg of the tissue sample. There was no difference between the renal concentration of GM in the biopsied samples and the samples from the animals sacrificed immediately after the biopsy. The time courses of the decrease in renal drug concentrations were monoexponential in both the serially biopsied samples and the samples from the sacrificed animals. These results suggested that the biopsy technique might have a value for prediction of the withdrawal time of drugs in the food producing animals.—**KEY WORDS:** calf kidney, drug residue, gentamicin, serial biopsy.

Jpn. J. Vet. Sci. 47(2): 179–183, 1985

Although the pharmacokinetic prediction of drug level in the edible tissue is useful to estimate the withdrawal time of drug for the food producing animals, this method is not effective in some cases [6, 8, 9, 12]. The renal residue of aminoglycoside antibiotics, known as a persistent residue [5, 7], is such a case in which the decay curve of the kidney turns biexponentially to a slow depletion line at low concentrations [5]. To establish the proper withdrawal time for such a drug, a large number of target animals have to be sacrificed, since the direct measurements of the tissue residue at a certain interval after the drug withdrawal are necessary.

In the present study, the time course of the renal concentration of gentamicin, one of the aminoglycoside antibiotics, in calves was examined by the serial biopsy technique of the kidney, and the analytical data were compared with those from the sacrificed animals.

MATERIALS AND METHODS

Animals and experimental protocol: The experimental protocol is shown in Table 1. Twelve castrated male calves, weighing 60–120 kg at the start of the experiment, were used. Gentamicin (GM; Gentocin, Shionogi Pharmaceutical Co., Ltd., Osaka) was given per os to calf 1 mg(potency)/kg of body weight twice a day for a week. Three calves were serially biopsied. One calf was biopsied at 3 hr and 3 and 10 days, and 2 calves at 3 hr and 3, 10 and 15 days after the final drug administration. To collect the kidney samples, two calves were sacrificed at 3 hr and 3, 10, 15 and 30 days and one calf was sacrificed at 20 days after the final drug administration.

Biopsy technique: Detail of the biopsy technique used in this study was reported in another report [11]. The calves were starved

Table 1. Experimental protocol

Calf No.	Time after final drug administration (days)					
	0	3	10	15	20	30
1	B ^{a)}	B	B, S ^{b)}			
2	B	B	B	B, S		
3	B	B	B	B		
4	S					
5	S					
6		B, S				
7		S				
8			S			
9				S		
10					S	
11						B, S
12						B, S

a) Biopsy.

b) Sacrifice.

for 24 hr prior to the biopsy. They were loosely restrained in the stanchion. The right flank including the paralumber fossa was shaved and disinfected, and anesthetized with lidocaine. The trocar assembly was inserted into the peritoneal cavity through an incision in the paralumber fossa. The laparoscope (Type A5218, Olympus Co., Ltd.) was put into through the outer cannula of the trocar and advanced until the kidney and the other abdominal organs came into view. The biopsy needle with stylet (Franklin-Silverman type, Yayoi Co., Ltd., Tokyo) was inserted into peritoneal cavity at the site approximately 5 cm cephalad-dorsal from the center of paralumber fossa, and advanced to the kidney under the laparoscopic observation. After the needle was inserted 1~2 mm into the kidney through renal capsule, the cutting prong placed in the stylet was thrust to the renal cortex, and a piece of the renal parenchyma was clipped. After closing up the incision, a topical antiseptic (povidon-iodine solution) was applied to the incision sites.

Drug assay: The extraction procedure of GM from kidney is shown in Fig. 1. The GM concentration was determined by RIA using gentamicin RIA kit (Clinical Assay Lab. Ltd., U.S.A.). After the extraction, 0.1 ml

Fig. 1. Extraction procedure of gentamicin from the kidney.

of the extract and 1.0 ml of ^{125}I -GM tracer were put into an anti-GM serum coated tube and mixed using a vortex mixer. The tube was incubated for 60 min at 37°C and then the inner solution of the tube was discarded by decantation. The radioactivity bound to the tube was measured by a well type scintillation counter (Auto Well Gamma System, Aloka Co., Ltd., Tokyo) and GM concentration was calculated from the standard curve (ranging from 200 to 5 ng (potency)/ml) made by the same procedure using a standard solution. The standard solution was prepared by adding the standard GM (Lot No. USQ-GMC-O-M-6207 590 μg (potency)/mg, Shionogi Pharmaceutical Co., Ltd.) into the extract obtained from blank kidney. A duplicate assay was performed and the average is shown as the result. More than 95% of GM added to the 20 mg renal tissue *in vitro* was recovered by the present assay method (Table 2). The detection limit was 0.05 μg (potency)/g when the sample weight was 20 mg.

Statistical analysis: Statistical analysis was performed using unpaired Student's *t* test. A P value less than 0.05 was considered

Table 2. Recovery of gentamicin from calf kidney *in vitro*

Added gentamicin $\mu\text{g (potency)/g}$	Number of samples	Recovery (%) $M \pm SD$
0.20	3	96.0 ± 1.7
1.00	3	97.3 ± 3.5

to be statistically significant. Regression lines of the renal concentration of the residual drug versus time after drug administration were fitted by the perpendicular least-squares method by means of program SALS in Computer Centre, University of Tokyo [10].

RESULTS

In the present study, 11 out of 12 renal biopsies were successfully performed during the serial biopsy. One false case was caused by inserting the trocar into wrong direction of the abdominal wall and the trocar did not reach the abdominal cavity. In most cases the operations were completed about 15 min after restraining the calves in the stanchion. No remarkable clinical manifestations except transient hematuria were observed in all biopsied calves. The samplable amounts per biopsy were 19.5 ± 7.3 mg (mean $\pm$ S.D.) and 35.4 mg at the maximum. In order to secure the minimum amount of tissue required for the duplicate assay (40 mg) the biopsy needle was inserted into kidney 2.8 times per operation on the average. Most of the biopsy specimens contained only cortical tissue and some contained cortical tissue at each end and cortico-medullary tissue in the center.

There was no difference between the assay values of GM from the biopsied samples and those of samples from the sacrificed animals which were exsanguinated immediately after the biopsy (Table 3).

The time courses of the renal residue of GM from the serially biopsied samples and that from the sacrificed samples are shown in Table 4 and Fig. 2. The decreases of the

Table 3. Comparison of renal gentamicin concentrations in the biopsied samples and the samples from animals sacrificed immediately after biopsy

Calf No.	Time after final administration (days)	Gentamicin concentration $\mu\text{g (potency)/g}$	
		Biopsied	Sacrificed*
6	3	0.62	0.74
1	10	0.41	0.49
2	15	0.05	0.06
11	30	0.04	0.03
12	30	0.02	0.04

*: The sample was collected with biopsy needle.

concentrations in both data appeared to be monoexponential on the semilogarithmic paper. There were no statistical differences in the renal concentration of GM at time 0 (intercept) and the elimination rate constant from the kidney (k_{el}) between the biopsied and sacrificed samples. The assay values approximately converged to the allowable tolerance level of $0.05 \mu\text{g (potency)/g}$ at 15 days after the final drug administration in both data.

DISCUSSION

The biopsy technique under the laparoscopic observation used in the present study enabled us to complete the serial sampling of renal tissue. Moreover, it had the advantage of choosing a sampling portion of the kidney. The blind percutaneous methods [2~4, 11, 13], which are the usual renal biopsy techniques in the veterinary field, may require ripe experience for the proper tissue sampling [15].

The surgical detriments by the biopsy might affect the physical function, especially the function of renal elimination, and offer the erroneous information on the drug residue. With regards to the problem, many papers on the renal biopsy in food producing animals have reported that any adverse effect

Table 4. Time courses of renal gentamicin concentrations

Sampling method	Calf No.	Gentamicin concentration [$\mu\text{g (potency)/g}$]				
		Time after final administration (days)				
		0	3	10	15	20
Biopsy	1	—*	1.24	0.41	—	—
	2	0.78	0.26	0.13	0.05	—
	3	0.33	0.10	0.10	0.07	—
	intercept** (M $\pm$ SD): 0.622 $\pm$ 0.199 [$\mu\text{g (potency)/g}$]					
		kel*** (M $\pm$ SD): 0.108 $\pm$ 0.072 (day ⁻¹)				
Sacrifice	4	0.70				
	5	0.51				
	6		0.74			
	7		0.53			
	1			0.49		
	8			0.12		
	2				0.06	
	9				0.05	
	10					0.04
	intercept** (M $\pm$ SD): 0.686 $\pm$ 0.099 [$\mu\text{g (potency)/g}$]					
		kel*** (M $\pm$ SD): 0.112 $\pm$ 0.042 (day ⁻¹)				

* The sample was not able to obtain.

** The renal concentration of GM at time 0.

*** The elimination rate constant of GM from kidney.

was seldom encountered after the biopsy [4, 11, 13, 14]. Also, in the present study, no remarkable clinical manifestations except transient hematuria were observed. And drug residue might not be affected by the serial biopsy because the time course of the renal residue of GM from the serially biopsied samples was similar to that from the samples obtained by sacrifice at a various interval (Table 4, Fig. 2).

Residual concentration of GM in kidney have been determined by various methods following the extraction under several conditions [1, 5, 7, 16, 17, 18]. The binding of GM with some inner components of tubular epithelial cells was reported to be electrostat-

Fig. 2. The residual concentration of gentamicin in calf kidney after the drug administration. ◇: biopsied sample from calf No. 1, ○: biopsied sample from calf No. 2, □: biopsied sample from calf No. 3, ●: sample from sacrificed animal.

ic, not covalent [5], so that the GM residue in the kidney might be extractable by a proper solvent. In the present study, the extraction was carried out with glycine buffer containing an electrolyte to dissociate GM from cell components.

The assay method must have a high sensitivity because the amount of a sample obtained by biopsy is small in quantity. RIA method used in this study had a sufficient sensitivity.

To establish the proper withdrawal time for a drug, a large number of target animals have to be sacrificed. With this method, the result obtained from a few animals can use for a prediction of the withdrawal time.

ACKNOWLEDGEMENTS. We are indebted to Professor A. Ohga, of Hokkaido University and Professor T. Hayama, of Tokyo University of Agriculture and Technology for their kindness in correcting the manuscript.

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要 約

ゲンタマイシンをモデル薬剤としたウシ連続腎臓生検による残留試験法: 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12頭の去勢子牛に1日2回, 1週間経口投与し, 連続腎臓生検により, 経時的に GM 濃度を GM・RIA キットで測定した。3頭については最終投薬後3時間, 3日, 10日, 15日に, 合計3または4回, 組織を採取した。さらに20日に1頭, 30日に2頭を屠殺解剖して腎臓組織を採取した。用いた測定法による回収率は95%以上, 20 mgの組織で 0.05 ppm までの定量が可能であった。生検組織と生検直後に放血屠殺して得た組織中 GM の濃度に差はみられなかった。生検組織および屠体組織中の GM 濃度は全実験期間中ほぼ一致し, いずれも monoexponential な減少を示した。以上の成績から, 連続腎臓生検による残留試験法は有用であると考えられた。