NOTE *Pharmacology*

Serum Clearance of Iodixanol for Estimating Glomerular Filtration Rate in Calves

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ABSTRACT. To evaluate serum clearance of iodixanol, applicable to the estimation of glomerular filtration rate (GFR), clinically healthy and experimentally-induced nephropathy calves were prepared. Iodixanol was administered intravenously at 40 mg I/kg, and blood was withdrawn 60, 120, and 180 min later. Serum iodixanol concentration was determined by high-performance liquid chromatography. No statistical difference in GFR was noted between strains (Holstein vs. Japanese Black) or sexes, and the α_2-adrenergic agonist xylazine increased GFR. In calves subjected to right renal vessel ligation, followed by a left nephrectomy, a marked reduction in GFR was observed with renal ischemic changes. These results suggest that the GFR estimation by serum iodixanol clearance is a ready-to-use tool in calf research and practice owing to the ease of monitoring serial renal function.

KEY WORDS: calves, glomerular filtration rate (GFR), iodixanol, nephropathy, serum clearance.

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Despite the fact that glomerular filtration rate (GFR) is the best overall index of renal function, applying the standard inulin clearance to large animals owns potentially daunting problems such as urine collection and bladder washout. Therefore, only a few studies have reported GFR measurements in calves [15]. Plasma clearance of the nonionic monomeric X-ray contrast medium iohexol is used as a standard for estimating GFR in small animal practice [3, 5, 7], whereas a concern regarding further deterioration of impaired renal function has been reported in humans [2], presumably due to its relatively high osmolarity (osmolar ratio: approximately 2). Here, we focused on the isotonic nonionic dimeric X-ray contrast medium iodixanol as a new tracer for estimating calf GFR. Iodixanol is rapidly excreted in urine without metabolic degradation and no or very little protein binding with a very short half-life in animals [8] and humans [16], as with iohexol. In contrast, iodixanol has significantly fewer nephrotoxic effects in high-risk patients undergoing arteriography than other nonionic contrast media, including iohexol, in a randomized, double-blind, prospective, multicenter studies [2, 14]. Chemically, iodixanol has twice the amount of iodine in one molecule and low osmolarity compared to iohexol and is assumed to show the same pharmacodynamic action at half the exposure dose to the whole body as iohexol, enabling a reduction in the administration volume to large animals. Additionally, the pharmacologically active ingredient of iodixanol is iodine, which has been excluded from the Japanese "positive list", a regulatory law [10] on substances with potential to cause damage to human health.

In the present study, clinically healthy and experimentally-induced nephropathy calves were prepared to assess serum iodixanol clearance in estimation of calf GFR. According to our previous study [11], the GFR estimated by a three-blood-sample method using iodixanol was in good agreement with that obtained from a conventional inulin clearance in clinically healthy and nephropathy rats. Accordingly, this study was essentially the same as that used for rats but was scaled up for calves.

Iodixanol (Visipaque 320; 320 mg I/m*l,* 290 mOsm/kg H_2O) was purchased from Daiichi-Sankyo (Tokyo, Japan). All other chemicals and reagents were of the highest grade available from commercial sources, unless otherwise stated. Iodixanol was administered intravenously at a dose of 40 mg I/kg into the jugular vein, and blood (1 m*l*) was withdrawn at pre-dose and 60, 120, and 180 min later via the opposite jugular vein (three-sample method). The dose (40 mg I/kg) of iodixanol was chosen based on the detectable sensitivity and minimum exposure of the whole body with its injection volume, and these dosage regimes were used for all subsequent investigations.

Seven female and 10 male Holstein-Friesian calves (body weight: *ca.* 60 kg) and 1 female and 4 male Japanese Black calves (60–70 kg), aged 2–3 months old, were included. They were housed in free-stall barns around the Shizukuishi areas (Iwate, Japan), and at the research farm of the Field Science Center and the Veterinary Teaching Hospital, Iwate University (Morioka, Japan). All animals were declared healthy after clinical and hematological examinations with urinalysis. All experimental procedures were approved by the Animal Experimental Ethics Committee of Iwate University.

As a diuretic model, the α_2-adrenergic agonist xylazine (2% Selactar; Bayer Healthcare, Osaka, Japan) was administered intravenously at 0.2 mg/kg to 4 male Holstein calves, followed 5 min later by a bolus injection of 40 mg I/kg

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iodixanol. An additional 4 calves received 0.9% saline solution (1 m*l*/100 kg) in the same way to serve as corresponding controls.

For the nephropathy model, 2 male Holstein calves (cases 1 and 2) were subjected to epidural anesthesia [12, 13]. An 18G × 80 mm disposable epidural needle (Tuohy needle; Hakko Medical, Nagano, Japan) was inserted into the first interlumbar space from the dorsal midline. After confirming the absence of blood or cerebrospinal fluid in aspirates, a solution (5 m*l*) containing 0.025 mg/kg xylazine and 0.1 mg/kg lidocaine (2% Xylocaine; AstraZeneca, Tokyo, Japan) was administered at a rate of 0.5 m*l*/sec, preceded by an intravenous administration of 0.1 mg/kg xylazine, to keep sedative conditions with analgesia. The animals were positioned in a left recumbency, and a right-flank laparotomy was performed with local infiltration analgesia using 2% lidocaine (10 m*l*). Subsequently, both the renal artery and vein of the right kidney were fastened firmly using a vinyl lock-tie (5 × 300 mm, Ohm, Saitama, Japan) until the pulse disappeared in the renal artery. The day the vessel ligation was performed was regarded as day 1. On day 4 (case 2) or 14 (case 1), the animals were positioned in a right recumbency using the same epidural and regional anesthesia with lidocaine as described above, and the left kidney was removed to evoke severer renal failure. After completion of each operation, 0.05 mg/kg atipamezole (Antisedan; Nippon Zenyaku Kogyo, Fukushima, Japan) was administered intravenously to reverse sedation. During the experimental periods, antibiotics, nonsteroidal anti-inflammatory drug, and/or nonnarcotic analgesic agent were given to protect against infection and to control pain, if needed. In the present protocol, if either blood urea nitrogen (BUN) or serum creatinine concentrations reached 60 or 6 mg/d*l*, respectively, the animal was euthanized immediately by exsanguination under sodium pentobarbital (50 mg/kg, i.v.) anesthesia, preceded by xylazine (0.2 mg/kg, i.v.). GFR was estimated on days 0 (before the study commenced), 1 (pre-ligation), 2, 3, 6, 8, 11, 12, 13, 15, and 16 for case 1, and on days 0, 1(pre-ligation), 2, 3, 4 (pre-operation), 5, and 6 for case 2. In case 1, GFR was not estimated at predose on the operation day (day 14) because many basal GFR values were obtained.

At termination (on day 16 for case 1, and day 6 for case 2), the right kidney was excised, fixed in 10% formalin, embedded in paraffin wax, cut at 3 μm thickness, stained with hematoxylin and eosin (HE), and examined histopathologically.

Serum iodixanol concentration was measured with reversed-phase high-performance liquid chromatography according to a previously reported procedure [9] with some modifications [11].

The clearance calculations were based on a 1-compartment model [4]. Briefly, the area under the concentration curve (AUC) was calculated by the linear trapezoidal rule with extrapolation using three serum samples, and a clearance value (Cl) was calculated with the following formula. The Cl term was considered the GFR for this work.

$$Cl = Q_{tot}/AUC,$$

where Q_{tot} is the dose of iodixanol injected. BUN and serum creatinine concentrations were measured with a Hitachi 7350 autoanalyzer (Hitachi, Tokyo, Japan) on the same days that GFR was estimated.

In calves given 40 mg I/kg iodixanol, serum iodixanol concentrations disappeared linearly within 180 min (data not shown). The GFR in clinically healthy claves was determined to be 2.82 ± 0.36 m*l*/min/kg for Holstein (mean ± SD, n=17) versus 2.92 ± 0.42 m*l*/min/kg for Japanese Black (n=5), and 2.93 ± 0.41 m*l*/min/kg for females (n=7) versus 2.73 ± 0.30 m*l*/min/kg for males (n=10) in Holstein. Neither strain nor sex differences were seen under the conditions of this study. The AUC is normally determined by the 2-compartment and non-compartment models, but requires multiple blood samples. Although the 1-compartment is likely to underestimate AUC compared to the aforementioned 2 methods, the present results resembled calf historical data using inulin (2.61 m*l*/min/kg) [1] or isotope-labeled EDTA (2.31 m*l*/min/kg) [17] as a tracer, in which the experimental conditions were very different.

Treatment of calves with xylazine exhibited significantly elevated GFR (3.24 ± 0.19 m*l*/min/kg) relative to the corresponding control (2.71 ± 0.35 m*l*/min/kg), indicating that an α_2-adrenergic agonist dramatically increases urinary output and reduces urinary osmolality, probably due to decreased antidiuretic hormone concentration [6]. However, since xylazine has a wide range of pharmacological effects such as increasing central blood pressure initially, as well as decreasing it later, iodixanol was administered 5 min after xylazine injection in this study.

In calves subjected to right renal vessel ligation, followed by a left nephrectomy, only the vessel ligation did not affect GFR, BUN and serum creatinine levels, except for a subtle reduction in GFR on day 11 in case 1. In the previous study [11], the GFR in unilaterally nephrectomized rats reversed to the basal value within 24–48 hr after the operation without changes in BUN and serum creatinine concentrations. The added load with a left nephrectomy showed markedly decreased GFR along with increases in BUN and serum creatinine concentrations from 1 day post-operation (Fig. 1), suggesting stenosis in the right renal vessels. At pre-dose in sequential GFR estimations, serum iodixanol residue was found at levels of more than approximately 50 and 1.5 mg/d*l* for BUN and creatinine concentrations, respectively. In certain blood-sample times when serum residue existed, therefore, serum iodixanol concentrations at 60, 90 and 120 min were subtracted by the pre-dose value, and the compensated value was used to estimate GFR. Histopathologically, severe necrosis in the proximal tubular epithelia of the right kidney was observed with mononuclear cell infiltration in the interstitium and/or hyaline cast deposits (Fig. 2), indicating ischemic changes. The findings demonstrate that the three-sample method using iodixanol can apply to calf GFR estimations.

No adverse clinical signs related to the iodixanol admin-

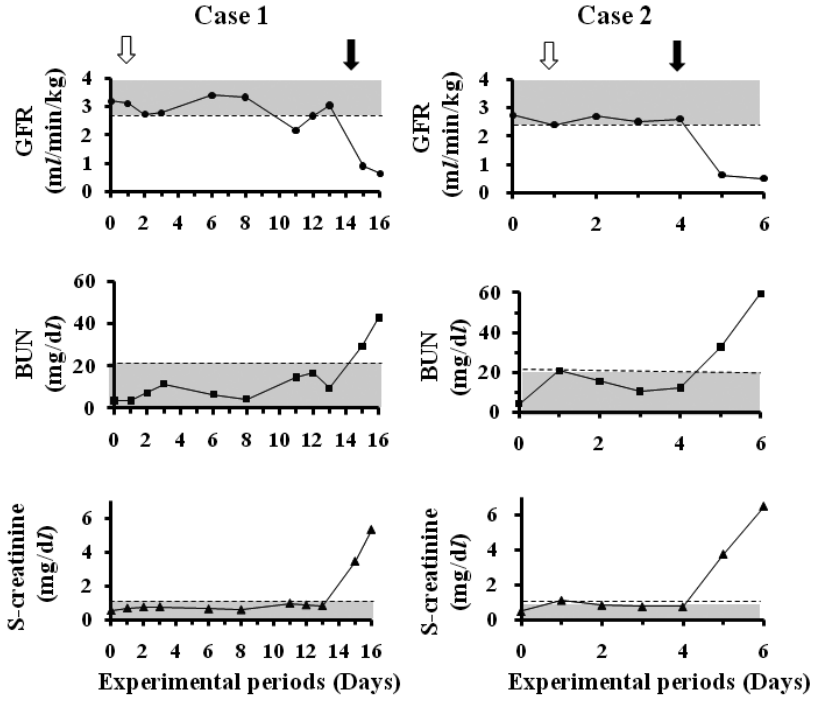

Fig. 1. Changes in glomerular filtration rate (GFR), blood urea nitrogen (BUN) and serum creatinine levels in calves subjected to right renal vessel ligation, followed by a left nephrectomy (cases 1 and 2). Stippled areas represent the reference ranges based on our background laboratory data. Open and closed arrows show time-points for right renal vessel ligation and a left nephrectomy, respectively. S: serum.

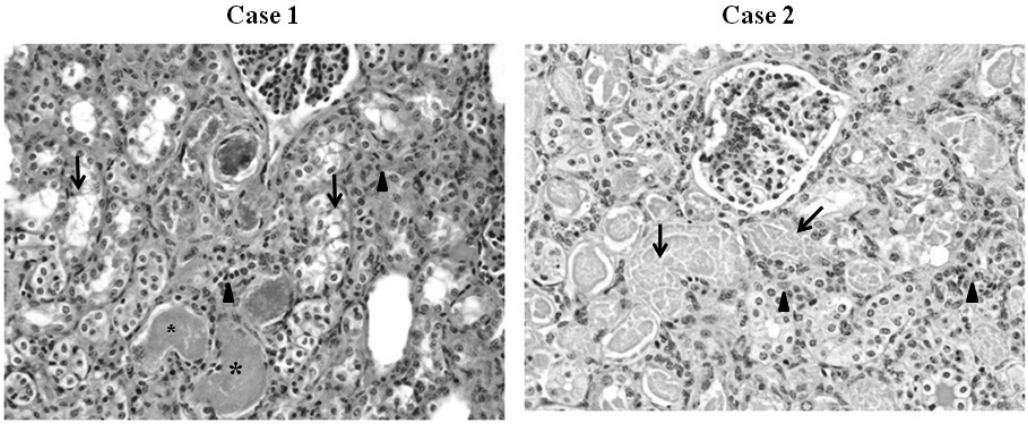

Fig. 2. Histopathological findings in the right kidney of calves subjected to right renal vessel ligation, followed by left nephrectomy (cases 1 and 2). Note severe necrosis (arrows) in the proximal epithelial cells with hyaline casts (asterisks), in addition to mononuclear cell infiltration in the interstitium (arrowheads). × 40, HE stain.

istrations were observed throughout the study. The three-sample method using iodixanol possessed practical advantages such as repeated application to the same animal, use of a non-radiolabeled agent and the small amount of serum specimen (80 μl). However, further studies are necessary to collect cumulative background data including age as well as using dairy cattle.

In conclusion, the results suggest that the GFR estimation using serum iodixanol clearance is a ready-to-use tool for calf pharmacological researches and practical settings because of the ease of monitoring serial renal function.

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