NOTE *Laboratory Animal Science*

Estimation of Glomerular Filtration Rate in Cynomolgus Monkeys (*Macaca fascicularis*)

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ABSTRACT. To estimate the glomerular filtration rate (GFR) in cynomolgus monkeys (*Macaca fascicularis*), a three-blood-sample method using iodixanol was assessed in comparison with the conventional multisample strategy using inulin. Iodixanol and inulin were coadministered intravenously 40 mg I/kg and 50 mg/kg, respectively, to male monkeys, followed by blood collection 60, 90 and 120 min later. A close correlation (r=0.96) was noted between the GFR values estimated by both methods. In clinically healthy monkeys, the basal values were determined to be 3.06 ± 0.50 m*l*/min/kg. This is the first report, suggesting that serum clearance of iodixanol is a ready-to-use tool for a screening the GFR in monkeys, although it is necessary to perform a more longitudinal study using animals with reduced renal function.
KEY WORDS: glomerular filtration rate, inulin, iodixanol, monkey, serum clearance

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Although the monkey has been used extensively in pharmacokinetic and toxicological evaluations with new chemical entities, there is only limited information dealing with the estimation of glomerular filtration rate (GFR). The GFR measurement is considered the best overall index for precise assessment of kidney function in humans [10] and other species [13]. However, application of classic urinary clearance using the standard tracer inulin to monkeys is complicated and time-consuming. For example, it relies on accurately timed repeated blood and urine collections and usually involves bladder catheterization to measure accurate urine volume. Moreover, inulin requires boiling immediately before use, because it has extremely low solubility. The non-ionic, monomeric, radiographic contrast medium iohexol has been utilized for the GFR assessment in veterinary fields [12, 13], instead of inulin, whereas it has been reported that concern exists regarding the nephrotoxic potential [12]. Here, we focused on the isotonic, non-ionic, dimeric iodine contrast medium iodixanol as a new tracer to estimate the monkey GFR. Iodixanol is physiologically inert, stable in serum and freely filtered at the glomerulus. Iodixanol is not secreted or reabsorbed in the renal tubule and is not synthesized or metabolized within the body [4, 8], when it is used as the GFR tracer. Thus, the amount of iodixanol filtered at the glomerulus is considered consistent with the amount excreted in urine. Furthermore, iodixanol is recognized to be less nephrotoxic than other non-ionic, non-isotonic contrast media, including iohexol, in randomized, double-blind, prospective, multicenter studies using high risk human patients with chronic renal diseases [1, 5, 11]. Generally, it is considered that classic urinary clearance is equal to serum (systemic) clearance, which is calculated as the dose of the tracer administered divided by the area under the serum tracer concentration versus time curve (AUC), when the tracer is filtered only from the renal glomerulus [13]. Taken together, serum clearance without urine collections can be utilized as the alternative standard method, instead of classic urinary clearance.

In the present investigation, we first examined an optimum dose of iodixanol with appropriate blood sampling times for the estimation of the monkey GFR based on the serum iodixanol clearance. Next, we determined the GFR in clinically healthy monkeys by using the established method.

Eight clinically healthy purpose-bred male cynomolgus monkeys (*Macaca fascicularis*), weighing 3.3–5.5 kg and aged 5–9 years old, were used. Monkeys were regarded as "healthy" from the results of clinical observations, hematology, serum biochemistry and urinalysis. Especially, serum urea nitrogen (BUN) and creatinine, and urinary creatinine, albumin, glucose and electrolytes were measured as kidney functional items. The monkeys were individually housed in stainless steel cages (60 cm wide × 68 cm deep × 75 cm high) incorporating squeeze-back system in an air-conditioned room [preset temperature, 24°C (acceptable range, 18–28°C); preset relative humidity, 60% (30–70%)]. The facility was kept in light/dark cycle of 12 hr (07:00–19:00) and ventilation rate of 10 to 20 air changes/hr. Basal diet (100 ± 2 g/day, PS-A, Oriental Yeast, Tokyo, Japan) was given to each monkey, and tap water was available *ad libitum*. All experimental manipulations were performed in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science [9].

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Iodixanol (Visipaque 320; 290 mOsm/kg H_2O, Daiichi-Sankyo, Tokyo, Japan) was administered intravenously at 20, 40 or 80 mg I/kg to the saphenous vein via a 24G indwelling catheter to assess the disappearance from blood. The dose levels of iodixanol were chosen on the basis of the previous feline reports [6]. Blood (0.5 m*l*) was collected from the femoral vein pre-dose and 5, 10, 15, 30, 45, 60, 90, 120 and/or 150 min later using a 25G-needle attached to a 2-m*l* disposable syringe. All procedures were performed under the conscious conditions. Serum iodixanol concentration was measured with reversed-phase high-performance liquid chromatography (HPLC) according to a previously reported procedure [4] with minor modifications [7]. Briefly, serum specimens (100 μl) were deproteinized by adding 20% trichloroacetic acid (TCA; Wako Chemicals, Osaka, Japan) at a ratio of 1:1 and placed at 4°C for 30 min to complete precipitation before removal of the proteins by centrifugation (14,000 × g, 4°C) for 10 min. The supernatant was centrifuged again under the same conditions, and then, it was used as the injectable sample. The HPLC system consisted of separation equipment (Alliance Waters 2690 Separations Module; Waters, Milford, MA, U.S.A.), a UV detector (Waters 996 Photodiode Array Detector; Waters) and analytical software (Millennium^{32}; Waters) equipped with a 250 × 4.6-mm C-18 reverse-phase column (RP-18 GP, 5 μm; Kanto Chemical, Tokyo, Japan). The stepwise mobile phase profile was composed of distilled water followed by 80% acetonitrile in distilled water, and the flow-rate was maintained at 1 m*l*/min. The detection wavelength was 244 nm, which is the approximate absorbance maximum for iodixanol. The detection limit of serum iodixanol concentration was 3.125 μg I/m*l*. Validation studies revealed no significant difference between serum and plasma iodixanol concentrations.

Inulin (Inulead; 100 mg/m*l*, Fuji Yakuhin, Saitama, Japan) as a semi-synthetic, diagnostic drug (chicory) for human use was administered intravenously at 50 mg/kg to monkeys. The dose of inulin was chosen based on the cat data [6]. Serum inulin concentrations were colorimetrically determined by an autoanalyzer method using a commercially available kit (Dia-color-inulin, Toyobo, Osaka, Japan). The assay was consigned to an independent testing service (SRL, Tokyo, Japan). The detection limit in serum inulin concentrations was 20 μg/m*l*. It was confirmed beforehand that there was no drug-interference to either iodixanol or inulin level in monkey serum.

A clearance value (Cl) was calculated from the following formula. The Cl term was considered the GFR for this study, and the GFR was represented as m*l*/min/kg.

Cl=Dose/AUC,

where Dose is the dose of iodixanol or inulin injected, and AUC was sought by linear trapezoidal rule with extrapolation using three serum samples.

The quantitative data are expressed as the mean and standard deviation (SD). Comparison of GFR values between the two methods was performed according to standard recommendations for comparing analytical techniques based on Deming's regression [3] and Bland-Altman bias presentation [2].

In monkeys (n=3) given 20, 40 or 80 mg I/kg iodixanol according to a 3 × 3 Latin square design, the mean serum concentration disappeared linearly by 150 min at all doses. No statistical difference in GFR values calculated was observed among these 3 doses. Considering the detectable sensitivity (mean serum iodixanol concentration at 150 min later in monkeys given 20 mg I/kg iodixanol: 10.5 μg I/m*l*), a dose of 40 mg I/kg iodixanol was chosen (Fig. 1A) as was the case with cats [6]. When 3 other monkeys were administered 40 mg I/kg iodixanol (Fig. 1B), no significant difference was noted between GFR values estimated from the 1-compartment model (2.61 ± 0.24 m*l*/min/kg) using 6 blood-sample points versus the 2-compartment model (2.41 ± 0.18 m*l*/min/kg) using 9 blood-sample points. The 1-compartmet model is likely to underestimate AUC, but the 2-compartment model requires multiple blood samples at the distribution phase. Since the present data resembled those from the 2-compartment model, the 1-compartmet model was selected for the further study.

The combination of blood-sample times was prepared as follows: a) 30, 45, 60, 90, 120 and 150 min later; b) 60, 90 and 120 min later; c) 30, 45, 60, 90 and 120 min later; d) 30, 45, 60, 90 and 150 min later; e) 30, 45, 60, 120 and 150 min later; f) 30, 45, 90, 120 and 150 min later; g) 30, 60, 90, 120 and 150 min later; h) 45, 60, 90, 120 and 150 min later. No significant difference was noted among the GFR values obtained from the aforementioned combinations (Fig. 1C). For subsequent investigations, a combination of 40 mg I/kg iodixanol with sampling times of 60, 90 and 120 min later was chosen, based on the previous cat data [6] showing excellent reproducibility with minimum sampling points.

When iodixanol and inulin were coadministered intravenously at 40 mg I/kg and 50 mg/kg, respectively, to 8 healthy monkeys, there was a close correlation (r=0.96, P<0.01) between their GFR values (Fig. 2A). On Bland and Altman analysis, all GFR values were within the 95% agreement plots (Fig. 2B), suggesting that the three-blood-sample method with iodixanol can be used in monkeys, instead of inulin.

In healthy monkeys, the reference values in GFR were determined to be 3.06 ± 0.50 m*l*/min/kg (n=8), although little report was available on the estimation of GFR in cynomolgus monkeys to date. To the best of our knowledge, this is the first reference value in cynomolgus monkeys.

The dose of 40 mg I/kg iodixanol corresponded to about one-thirteenth the human clinical dose (32 g I/60 kg body weight) as the contrast medium for angiography. The three-blood-sample method is considered to have many technical advantages, such as repeated application to the same animals, usage of non-radiolabeled agent and a small amount of serum specimen related to a high sensitivity for detection of iodixanol. However, further studies are warranted to collect cumulative background data including increased numbers of animals together with cases showing reduced renal function.

No adverse reactions related to iodixanol treatments were seen throughout the experiment period.

In conclusion, the result demonstrates that the three-blood-sample method with iodixanol is a versatile and practical procedure for screening the GFR in the monkey.

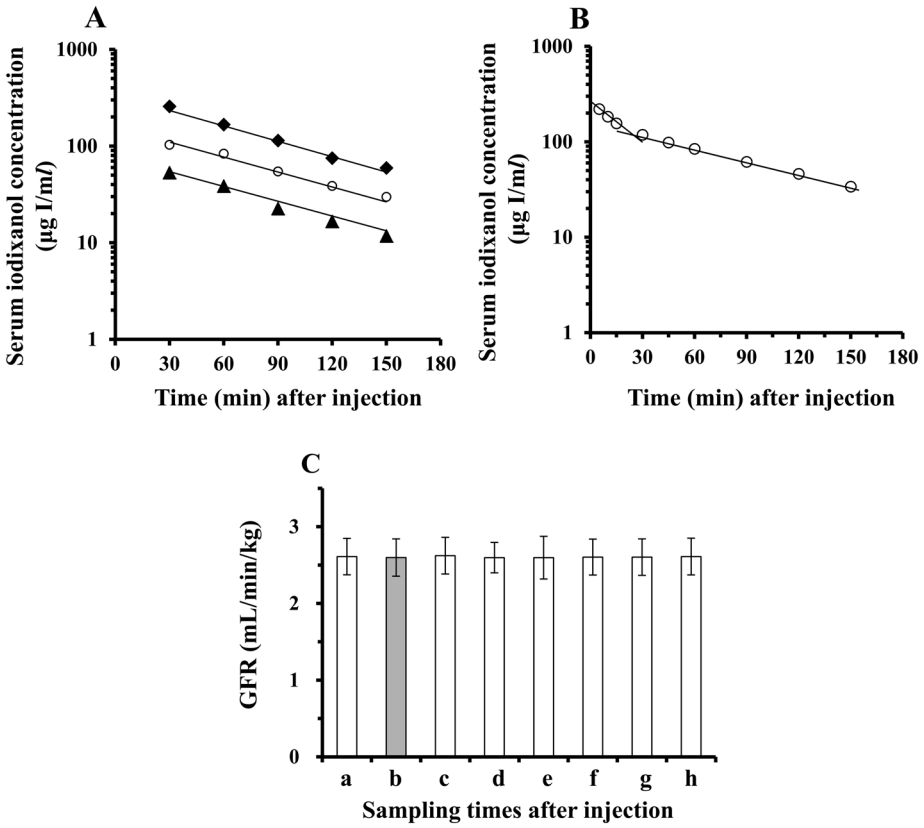

Fig. 1. Optimum dose and appropriate blood sampling time of iodixanol. (A) Mean serum disappearance curve at 3 doses of iodixanol. Iodixanol was administered intravenously to 3 healthy cynomolgus monkeys each at 20 (closed triangles), 40 (open circles) or 80 mg I/kg (closed diamonds). (B) Representative serum disappearance curve at 40 mg I/kg of iodixanol. (C) GFR values estimated by combinations of various sampling times (in min) after iodixanol injection in the 1-compartment model; a) 30, 45, 60, 90, 120 and 150 min later; b) 60, 90 and 120 min later (*gray bar*); c) 30, 45, 60, 90 and 120 min later; d) 30, 45, 60, 90 and 150 min later; e) 30, 45, 60, 120 and 150 min later; f) 30, 45, 90, 120 and 150 min later; g) 30, 60, 90, 120 and 150 min later; h) 45, 60, 90, 120 and 150 min later. n=3.

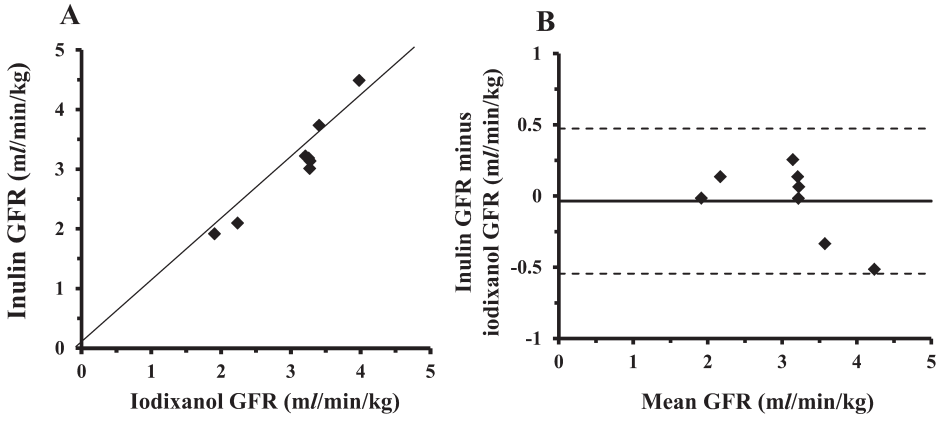

Fig. 2. Plots between GFR values estimated by the multisample method using iodixanol (iodixanol GFR) and inulin (inulin GFR) in healthy cynomolgus monkeys. (A) Scatter plot of GFR between the two methods. Deming's regression was y=1.18×–0.54. r=0.96. (B) Bland and Altman plot of the differences between the two methods. Mean bias (solid line): 0.04. Upper and lower values represent 95% agreement plots: mean bias ± 0.51 (dotted lines). n=8.

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