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Iohexol Clearance for Determination of Glomerular Filtration Rate in Cynomolgus Monkeys (*Macaca fascicularis*)

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The purpose of this study was to validate a method for determine the glomerular filtration rate (GFR) in healthy cynomolgus monkeys by using iohexol. Eighteen healthy cynomolgus macaque monkeys (age, 4 to 6 y [mean, 5 y]; weight, 2 to 6 kg [mean, 4 kg]) were randomly entered into 3 different doses groups (3 male and 3 female macaques per group) of 30, 60, 90 mg I/kg to receive an intravenous bolus injection of iohexol. Serum iohexol concentrations were determined by using liquid chromatography–tandem mass spectrometry, and clearance rate were determined by using WinNonlin software. The GFR value (mean ± SD) of each dose group was 2.50 ± 0.321, 2.65 ± 0.529, and 2.75 ± 0.385 mL/min/kg. These values did not differ significantly between dose levels or sexes. Iohexol clearance is a simple, precise method that is suitable for the determination of GFR in cynomolgus monkeys.

Abbreviation: GFR, glomerular filtration rate

Chronic kidney disease is a major public health problem throughout the world. For example, approximately 2300 Australians begin dialysis or undergo kidney transplantation each year due to this disease.[14] In 2007, more than 500,000 people were treated for end-stage renal disease in the United States,[20] where chronic kidney disease affects an estimated 27 million adults[21] and is associated with increased mortality, morbidity, and healthcare cost. This pernicious condition is often lacks significant symptoms or urinary abnormalities and is unrecognized in 80% to 90% of cases.[7,12,13] Awareness of chronic kidney disease among patients has modestly increased in recent years but remains low. In 2002, the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative published a guideline on chronic kidney disease that addressed its evaluation, classification, and stratification of risk to help primary care physicians identify patients with early chronic kidney disease and improve health outcomes.[16] Chronic kidney disease is defined as the presence of structural or functional abnormalities in the kidneys with or without an accompanying reduction in glomerular filtration rate (GFR).

GFR describes the flow rate of filtered fluid through the kidney; there are several ways to estimate GFR, including inulin clearance, radionuclide markers clearance, renal dynamic imaging, and serum creatinine levels, among others. Iohexol is a nonionic, monomeric, iodinated contrast agent, which it is not secreted or reabsorbed in the renal tubule and which is not synthesized or metabolized within the body; its protein binding is low (less than 2%). To date, it has been used as a marker in both renal and plasma clearance studies for the GFR assessment in veterinary medicine.[15,22]

Although NHP have been used extensively in pharmacokinetic and toxicologic evaluations of new chemical entities, few data are available regarding techniques for monitoring GFR in healthy animals. Here we estimated GFR in cynomolgus macaques by using an iohexol clearance method and investigated sex-associated differences and the dose proportionally of iohexol. Iohexol concentration can be determined by monitoring iodine by X-ray fluorescence,[5] inductively coupled plasma–atomic emission spectroscopy,[4] capillary electrophoresis,[18] and UV–HPLC;[19] the current study used liquid chromatography–tandem mass spectrometry.

Materials and Methods

GFR testing in cynomolgus macaques was conducted as approved by the IACUC of WuXi Apptec (Suzhou, Jiangsu Province, People's Republic of China), which is in compliance with the Animal Welfare Act and guidelines in the *Guide for the Care and Use of Laboratory Animals*[10].

The study population comprised 18 cynomolgus macaques (9 female and 9 male; age, 4 to 6 y [mean, 5 y]; body weight, 2 to 6 kg [mean, 4 kg], Hainan Jingang Biotechnology, Hainan, China) that were seronegative to simian T-lymphotrophic virus, SIV, simian retrovirus types 1 through 5, and B virus. The animal facility was on a 12:12-h light:dark cycle; macaques were fed twice daily with approximately 120 g Certified Monkey Diet (Vital Keao Feed, Beijing, China). Reverse-osmosis–purified water was available without restriction to all animals. Enrichment toys and treats were provided on a daily basis.

Iohexol injection (catalog no. 15062261) was purchased from Yangtze River Pharmaceutical Group (Taizhou, China), and 0.9% saline was purchased from Huai'an Shuanghe Pharmaceutical (Huai'an, China). To achieve the 30-, 60-, and 90-mg I/kg dose needed for the test groups, iohexol was diluted with 0.9% saline on the day of dosing, and the dose for each animal was given as 1 mL/kg, calculated according to body weight.

Iohexol powder (catalog no. BCBP6943V) and tolbutamide (catalog no. SZBA013XV) were obtained from Sigma-Aldrich (St Louis, MO) for use as standards in the analysis of iohexol concentration in serum by liquid chromatography–tandem

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Table 1. Serum chemistry results

		Sex	Albumin (g/L)	Glucose (mmol/L)	Urea (mmol/L)	Creatinine (umol/L)	Calcium (mmol/L)	Phosphorus (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chlorine (mmol/L)
	Normal	Male	40.8–53.7	1.84–5.23	3.89–9.04	38–88	2.29–2.77	1.29–2.71	144–157	3.8–6.6	101–112
	range	Female	39.5–52.3	1.77–4.99	3.66–9.09	40–78	2.27–2.73	1.13–2.41	144–155	3.7–6.4	102–112
Dose (mg I/kg)	Animal										
30	P1001	Male	49.4	3.08	4.92	66	2.73	1.88	150	4.7	106
	P1002		47.7	2.87	5.93	80	2.69	2.02	147	4.5	104
	P1003		41.9	3.16	4.66	75	2.58	1.94	149	5.2	107
	P1501	Female	47.7	3.14	6.94	59	2.62	1.74	148	4.2	105
	P1502		46.3	3.12	6.01	71	2.51	1.42	145	4.3	104
	P1503		45.2	2.63	6.28	66	2.60	1.76	151	4.5	108
60	P2001	Male	43.3	2.23	5.34	90	2.62	1.69	149	4.3	107
	P2002		50.0	2.87	6.30	78	2.66	1.86	150	4.3	103
	P2003		42.2	3.54	5.47	73	2.51	1.93	149	5.3	105
	P2501	Female	44.1	2.65	5.54	49	2.67	1.93	146	4.9	105
	P2502		43.9	2.34	5.92	41	2.55	1.72	147	4.4	105
	P2503		39.4	2.32	5.94	52	2.60	1.44	148	3.8	110
90	P3001	Male	46.3	2.85	5.81	70	2.62	1.77	147	4.8	104
	P3002		43.5	2.92	7.09	82	2.59	1.83	149	4.7	106
	P3003		43.9	2.73	5.59	77	2.42	1.85	147	4.7	105
	P3501	Female	42.1	2.33	4.83	46	2.48	1.86	145	4.0	105
	P3502		41.7	2.45	5.49	56	2.43	1.69	144	4.2	106
	P3503		44.9	2.60	4.87	52	2.47	1.47	146	4.3	106

The normal range given is the in-house standard reference.

mass spectrometry. A liquid chromatography–tandem mass spectrometry system (4000 Q TRAP, Applied Biosystems, Foster City, CA) equipped with a column (ACE AQ 2.1, 100 mm, 3 µm; Advanced Chromatography Products, Aberdeen, Scotland, United Kingdom) and positive ion-mode electrospray ionization was used for analysis of iohexol concentration in serum samples, which were collected from predose to 6 h postdose. Selected reaction monitoring transitions were: iohexol [M+H]+, m/z 821.9 and 804.2; tolbutamide (internal standard), m/z 271.2 and 155.1. Mobile phase A was 0.3% formic acid in water, and mobile phase B was 0.3% formic acid in acetonitrile. The column temperature was 45 °C, and the flow rate was 0.45 mL/min. The retention times for iohexol and tolbutamide were 1.42 min and 2.49 min, respectively.

An automated analyzer (model 7180, Hitachi, Japan) was used to assess serum biochemistries for general health information.

Study design. We evaluated GFR in conscious healthy adult cynomolgus macaques by administering iohexol solution at 3 dose levels: 30-, 60-, and 90- mg I/kg. Each dose group contained 3 male and 3 female macaques. The monkeys were trained to present an arm, for reproducible blood collection. A blood sample was collected prior to study initiation, and animals with any biochemical abnormality were excluded; in particular, serum creatinine, albumin, glucose, and electrolytes were monitored as markers of kidney function (Table 1).

On the day of study, a predose (time 0) blood sample was collected, and the appropriate dose of diluted iohexol solution was administered to restrained conscious animals via a cephalic vein. Then serial blood samples were collected at 5 and 30 min and 2, 4, and 6 h after dosing from peripheral vessels that were not used for iohexol administration. Animals were restrained by using a monkey chair for the 0-, 5-, and 30-min samples and then went back to their cages, where they were restrained by using a squeeze cage for collection of the remaining samples. The circulating iohexol concentration was assayed by liquid

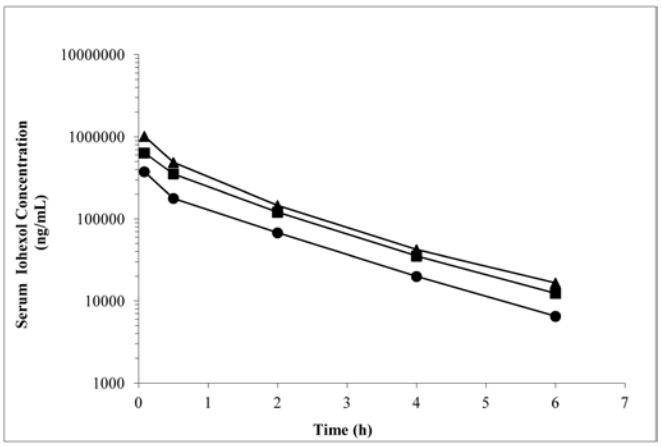

Figure 1. Mean activity serum concentration versus time profiles at 3 doses of iohexol. Iohexol was administered intravenously to 6 healthy cynomolgus monkeys each at 30 (circles), 60 (squares), or 90 (triangles) mg I/kg.

Table 2. Calculation of GFR by using noncompartmental and 1-compartment models

	Clearance (mL/min/kg; mean ± SD, n = 6)	
Dose (mg I/kg)	Noncompartmental	1-compartmental
30	1.72 ± 0.147	2.50 ± 0.321
60	2.17 ± 0.259	2.65 ± 0.529
90	2.07 ± 0.344	2.75 ± 0.385

At all dose levels, clearance values differed ($P < 0.01$) between models.

Table 3. GFR values from different dose levels and sexes

	Clearance (mL/min/kg) (Mean ± SD)			
	1-compartment model		Noncompartmental model	
Dose (mg I/kg)	Male (*n* = 3)	Female (*n* = 3)	Male (*n* = 3)	Female (*n* = 3)
30	2.51 ± 0.290	2.49 ± 0.417	1.74 ± 0.181	1.69 ± 0.140
60	2.32 ± 0.372	2.98 ± 0.486	2.09 ± 0.274	2.25 ± 0.275
90	2.48 ± 0.375	3.02 ± 0.127	1.84 ± 0.306	2.30 ± 0.210

Clearance did not differ between dose levels or sexes

chromatography–tandem mass spectrometry; GFR was determined by calculating the rate of iohexol clearance.

To process samples for bioanalysis, we followed the standard protocol at our institution. Briefly, blood was collected into commercially available serum separator tubes and allowed to clot at room temperature for about 30 min before centrifugation. The clotted samples were centrifuged at 3000 × *g* for 15 min at 2 to 8 °C; serum was transferred to prelabeled polypropylene tubes, frozen in the upright position immediately over dry ice, and stored at –60 °C or lower until analysis. Samples were processed under yellow light because of the light sensitivity of iodine.

For protein precipitation, 30 µL serum was added to 300 µL acetonitrile containing 200 ng/mL tolbutamide (internal standard); the mixture was vortex-mixed well, centrifuged at 3200 × *g* for 20 min at 4 °C. Then 80 µL of the supernatant was removed, transferred to another container, and evaporated to dryness under nitrogen. The residues were reconstituted with 160 µL 0.1% formic acid in water, vortex-mixed well, and centrifuged at 4 °C; 10 µL of the supernatant was injected for analysis by liquid chromatography–tandem mass spectrometry. Any samples in which the iohexol concentration exceeded the upper limit of quantitation were diluted by a factor of 10 by adding 27 µL diluent to 3 µL serum sample or by a factor of 100 by adding 27 µL diluent to 3 µL of the 10-fold diluted serum sample.

Data analyses. Retention times, chromatograms, and peak area integrations were obtained by using Analyst (version 1.4.2, AB SciEx, Framingham, MA). Serum concentration data of iohexol were analyzed by using Phoenix WinNonlin software (version 6.2.1, Pharsight, Mountain View, CA). Because there was almost no binding or metabolism of iohexol in blood and because nearly all of the iohexol dose was eliminated in the urine, the iohexol clearance rate was considered to be the GFR (given as mL/min/kg) in this study. The clearance data were expressed as mean ± SD. During the WinNonlin analysis, 2 pharmacokinetic models were used for calculation of clearance by using 6 blood-sample points: a noncompartmental model (linear log trapezoidal, best-fit model) and 1-compartment model (model 1).

The Student *t* test was used for comparing means of quantitative data. A *P* value of 0.05 was considered to be statistically significant, and *P* values below 0.01 were considered as highly statistically significant.

Results

All animals in this study tolerated iohexol well, and no adverse reactions were observed. The mean serum concentrations of iohexol are shown in Figure 1. As the iohexol dose increased from 30 to 90 mg I/kg, the $AUC_{0\text{-last}}$ increased dose-proportionally in both sexes. No sex-associated difference was observed at any of the 3 dose levels (data not shown).

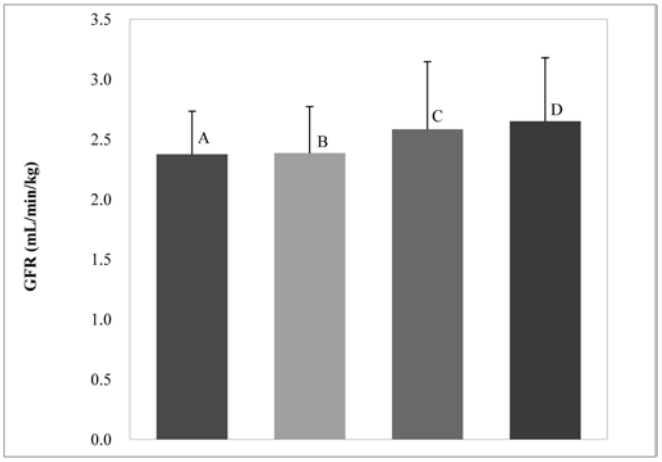

Figure 2. GFR values estimated by combining various time points (*n* = 6 per time point) in the 1-compartment model. (A) 0.5, 2, and 4 h. B) 0.5, 2, and 6 h. C) 2, 4, and 6 h. D) 5 min and 0.5, 2, 4, and 6 h.

The clearance values calculated from the noncompartmental and 1-compartment models differed significantly ($P < 0.01$; Table 2**)**. When compared within the same model, clearance values did not differ among the 3 dose levels or between sexes (Table 3).

We then used data from different combinations of time points in the 1-compartment model to calculate clearance for the 60-mg I/kg dose: 1) 0.5, 2, and 4 h; 2) 0.5, 2, and 6 h; 3) 2, 4, and 6 h; and 5 and 30 min and 2, 4, and 6 h. None of these clearance values differed from any of the others (Figure 2).

Discussion

In our cynomolgus macaques, iohexol clearance was a reliable method for evaluating GFR. Several studies have investigated the plasma clearance of iohexol in humans[6,8] and other species.[9] In the current study, we chose iohexol dose levels based on those in previous human studies[2,17] and extrapolated the human doses to our macaques according to their body surface area. Because systemic exposure (that is, $AUC_{0\text{-last}}$) increased dose-proportionally from 30 to 90 mg I/kg and in consideration of animal wellbeing, we recommend a dose of 30 mg I/kg (or even lower) for assessing GFR in cynomolgus macaques.

The GFR values we obtained ranged from 2.50 ± 0.321 to 2.75 ± 0.385 mL/min/kg by using a 1-compartment model and are consistent with the result (2.61 ± 0.24 mL/min/kg) from a previous study.[11] In addition, the calculated GFR did not differ among the 3 iohexol dosages used.

In addition, we compared the clearance values obtained by using a 1-compartment model with those from a noncompartmental model. The mean clearance values calculated by using the noncompartmental model were lower than those from the 1-compartment model. The differences between 2 models

might reflect differences in the calculation methods. The one-compartment model views the body as a single compartment and produces a straight-line plasma disappearance curve. In contrast, the noncompartmental model disregards the number of compartments and calculates AUC by adding the area of each trapezoid defined by the curve. Both of these methods have their benefits and limitations,[18,21] and the ideal calculation method is unknown at this time.

Several studies have investigated the minimal number time points needed to determine accurate GFR values.[21] Calculating iohexol plasma clearance by using 3 plasma samples was sufficiently accurate in healthy dogs.[3] We found that the iohexol clearance rates calculated by using 3 compared with 5 samples did not differ significantly, demonstrating that a 3-sample method is a reliable and useful procedure for determining GFR determine in cynomolgus macaques.

Our study showed that GFR evaluation by using iohexol clearance did not differ between sexes, unlike the situation for the creatinine clearance method.[1] However, we included only 3 male and 3 female macaques in each group. In addition, the iohexol-based GFR values we obtained did not differ from those obtained by using iodixanol.[11] Therefore, in the current study, we validated the use of iohexol for determining the GFR in healthy cynomolgus monkeys.

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