Measurement of glomerular filtration rate in anesthetized and conscious rhesus monkeys (Macaca mulatta)

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Objective—To validate a method to assess glomerular filtration rate (GFR) in conscious monkeys via transcutaneous radiation detection after IV injection of technetium Tc 99m pentatate (99mTc-DTPA).

Animals—4 healthy rhesus monkeys.

Procedures—On day 1, each monkey was anesthetized. Iothalamate sodium I 125 (125I-iothalamate) was administered via continuous rate infusion (0.0037 MBq/min); blood and urine samples were obtained for determination of 125I-iothalamate plasma clearance variables and estimation of GFR. One dose of 99mTc-DTPA (7.4 MBq/kg, IV) was also administered during the 125I-iothalamate plasma clearance test, and transcutaneous measurements of technetium 99m-emitted radiation were obtained by use of an ambulatory renal monitor (ARM) applied to a brachium of each monkey. Determination of GFR by use of the ARM was repeated on days 8 and 45 in the same monkeys without anesthesia.

Results—Sensitivity, accuracy, and precision of the 2 methods were similar. By use of the ARM, GFR determined by use of the renal rate constant (kGFR) was calculated; the value obtained on day 1 under anesthesia was similar to values determined via 125I-iothalamate plasma clearance testing on the same day, but was 16% to 23% less than that measured on days 8 and 45 in conscious monkeys.

Conclusions and Clinical Relevance—The ARM method for assessment of GFR was less invasive, faster, and more convenient than the standard clearance method, but yielded comparable results. The need to train animals and size restrictions of the device may limit the use of this technique in other nonhuman animals. (Am J Vet Res 2010;71:1492–1499)
determined by collection of multiple samples of blood and urine over several hours followed by deferred assays, which increase the duration and complexity of the test. Attempts have been made to simplify the procedures (eg, by testing a single blood sample); however, at best, an estimate of GFR by use of these techniques requires a minimum period of 4 to 8 hours. The inconvenience of clearance techniques further increases when they are used to determine GFR in animals that have a high acquisition cost; such animals are generally in limited supply and require special housing and handling. Studies in nonhuman primates have particularly raised concerns about animal well-being, especially with the use of more invasive techniques. Consequently, the alternative of a more convenient and less invasive means to measure renal function in these animals would be welcomed.

The purpose of the study reported here was to validate a method to assess GFR in conscious rhesus monkeys via transcutaneous radiation detection after IV injection of $^{99m}$Tc-DTPA. This method is based on a single-injection technique\(^{1,9,10}\) in which continuous and instantaneous measurement of radiation is performed transcutaneously by use of an ARM instead of intermittent and deferred assays of blood and urine. Because the supply of rhesus monkeys was limited, the results of the present study were also compared with similar measurements reported in studies\(^{11,12}\) of humans. Although the ideal marker for evaluation of GFR is controversial,\(^{13}\) we used $^{99m}$Tc-DTPA (a radiopharmaceutical agent with clearance characteristics similar to those of inulin) and $^{125}$I-iothalamate\(^{14,18}\) to test the new method. We compared GFR values obtained using this method with those obtained in the same monkeys via a standard clearance method with a continuous rate infusion of $^{125}$I-iothalamate.

**Materials and Methods**

**Animals**—Four healthy adult rhesus monkeys (Macaca mulatta) [2 males and 2 females; weight range, 2.6 to 3.3 kg]\(^{a}\) were used in the study. Monkeys were determined to be healthy on the basis of physical examination by a veterinarian and were housed individually. Their maintenance, housing, and monitoring were in accordance with national guidelines for the use and care of laboratory animals,\(^{19}\) with feed and tap water available ad libitum. The monkeys were trained to sit quietly for 1 to 2 hours on a restraining primate chair but were not accustomed to wearing the ARM used to estimate GFR. The study protocol was approved by the Animal Care and Use Committee of Charles River Laboratories.

**Study protocol**—On day 1, anesthesia was induced with ketamine hydrochloride\(^{e}\) (10 mg/kg, IM) and maintained with 2.5% isoflurane\(^{e}\) in oxygen via a face mask. Sterile physiologic saline (0.9% NaCl) solution was administered IV (30 mL/kg/h) during the experiment, and the monkeys were monitored until recovery. Simultaneous measurements for estimation of GFR were performed in anesthetized monkeys via 2 methods. A direct IV bolus of $^{99m}$Tc-DTPA\(^{f}\) was administered for transcutaneous radiation detection by use of the ARM secured at the brachium of each monkey, while $^{125}$I-Iothalamate\(^{e}\) was administered simultaneously via continuous IV infusion for evaluation of renal clearance by use of blood and urine samples as described elsewhere.\(^{20}\) On days 8 and 45, GFR estimation was repeated by use of the ARM in the same monkeys in the conscious state after IV injection of $^{99m}$Tc-DTPA without other treatments.

Quality control of the radioactive pharmaceutical agents used in this study was performed by the manufacturer of the products. The agents were assessed via measurement of the labeling efficiency and determination of the radionuclide, chemical, and radiochemical purity of the compounds.

**ARM preparation**—An ARM\(^{11,12}\) (intended for use on humans) was altered for use on the monkeys as previously described.\(^{21}\) The device was modified to fit the small size of a monkey’s arm; the number and type of detectors used were also changed. The modified ARM basically consisted of 2 radiation detectors with a data collection and analysis system (Figure 1).
Each detector comprised a CsI(Tl)-crystal-photodiode array connected to a low-noise, high-gain shaping amplifier and a low-level discriminator. This assembly was shielded by a 4-mm lead housing with the crystal surface exposed. Each detector was mounted on a printed circuit board that contained the amplifier and discriminator circuitry. Detector assemblies were surface mounted on a hemicylindrical metal armband with the exposed crystal surface oriented to the inside. A computer module (to record and store detector data and allow data streaming to a personal computer) and the power supply (four 9-V batteries) were also surface mounted on the arm band. To provide comfort and support to reduce variability in radiation counts caused by motion, the inside of the arm band was padded with high-density memory foam with 2.06-cm (0.8-inch) thickness. The combined weight of the unit was 0.34 kg. After placement on the monkey’s brachium, the instrument was secured with a hook-and-loop fastening strap (imitating the placement of a blood pressure cuff). Compression from the metal hemicylinder and tension from the hook-and-loop fastening strap provided an almost motion-free assembly between the monkey’s brachium and the ARM. A software program developed in our laboratory was used to start and stop data collection by the ARM and to adjust settings for plotting data during acquisition.

Quality-control testing of the experimental technique—Radiation counts of 4 test sources (3 obtained by dilution of the same agent used in monkeys and 1 cobalt 57 from a commercial source) were performed in vitro by use of a dose calibrator and subsequently analyzed by use of the ARM for quality control of the method. Source A contained 1.85 MBq of 99mTc-DTPA, which was accepted as having a radiation count rate similar to that measured transcutaneously at the brachium of monkeys immediately after IV administration of 7.4 MBq of 99mTc-DTPA/kg; source B contained 1.85 MBq of 125I-iothalamate; and source C (a sample of physiologic saline solution) contained no radiopharmaceutical agent. A radiation count (over a 10-second period) was obtained 5 consecutive times for each test sample. The signal-to-noise ratio was assessed as A:C, where A and C represented the radioactivity detected (counts/s) for sources A and C, respectively. Similarly, the interference of iodine 125 (125I) counts with technetium 99m (99mTc) counts was assessed as the ratio B:A, where B represented the radioactivity detected (counts/s) for source B. Radiation counting efficiency of the device was assessed as the ratio of A:D, where A represented the radioactivity detected (counts/s) for source A and D was the expected disintegration rate of the 99mTc-DTPA source (1.85 × 10^6 disintegrations/s) derived from measured activity. Sensitivity of the technique was defined by use of the limit of detection and limit of quantification from a source containing 0.3 MBq of cobalt 57 (terminal half-life, 271.79 days).

Quality-control testing of the ARM—Performance of the device was evaluated before its use on monkeys via measurement of λ (reference value, 1.925 × 10^13/min) for different dilutions of 99mTc-DTPA dissolved in 250 mL of physiologic saline solution. For these tests, the decay correction factor in the ARM (which automatically adjusts each measurement by λ) was inactivated.

Intra-assay accuracy was determined by comparison of the 15-minute slopes (from 0 to 15, from 16 to 30, from 31 to 45, and from 46 to 60 minutes) of the correlation between the natural logarithm of radiation counts versus time of a single source containing 1.85 MBq of 99mTc with the expected λ of 99mTc. Intra-assay accuracy was defined as the range of differences between the mean ± 2 SDs of the measured rate constants and the expected λ for 99mTc expressed as a percentage of the mean. Interassay accuracy was calculated on the basis of the 15-minute slope (from 0 to 15 minutes) of the correlation between the natural logarithm of radiation counts versus time of sources containing 1.4, 1.5, 1.8, and 1.9 MBq of 99mTc. Interassay accuracy was defined as the range of differences between the mean ± 2 SDs of the measured rate constants in multiple sources and the expected λ for 99mTc expressed as a percentage of the mean. Precision was determined by comparison of the slope of the natural logarithm of radiation counts versus time measured for 8 consecutive periods of 15 minutes each in a single source containing 1.85 MBq of 99mTc-DTPA (intra-assay) or the sources containing 1.4 to 1.9 MBq of 99mTc-DTPA (interassay), each recorded for a total of 120 minutes. Precision was expressed as the coefficient of variation calculated as (SD/mean) × 100 at the 15- and 60-minute time points.

Estimation of GFR via use of the ARM—The same ARM protocol was used on day 1 in anesthetized monkeys and on days 8 and 45 in conscious monkeys. Before each use, the ARM was connected to a personal computer via a parallel port to load the operating program. The device was then disconnected and placed and secured on the monkey’s left brachium in a manner similar to the placement of a blood pressure cuff. A single dose of 7.4 MBq of 99mTc-DTPA/kg was administered via direct IV injection, and transcutaneous measurements of radiation in the contralateral limb were performed over 10-second intervals without interruption for 30 minutes (a total of 180 measurements). Measurements were recorded as the natural logarithms of radiation count versus time. After the final measurement, the ARM was removed and reconnected to the personal computer and the data were transferred to a spreadsheet for analysis.

Renal clearance of the radioactive GFA (99mTc-DTPA) was assessed via analysis of the slope of activity versus time (ie, κ values) determined from transcutaneous radiation measurements after automatic correction for λ. The kgFR was calculated as the product of κ × 16.74 minutes (ie, the slope of correlation between a previously reported GFR value and κ).

Estimation of GFR via 125I-iothalamate clearance—A continuous rate infusion of 125I-iothalamate and timed blood and urine sample collections were used to determine GFR in the anesthetized monkeys. After dilution of the radiopharmaceutical agent (0.222 MBq/kg in 10 mL of saline solution), a priming injection of this diluted solution (3.0 mL IV) was initiated, followed by the INF, at a rate of 0.0037 MBq/min. The
urinary bladder was catheterized by use of a pediatric-sized urinary catheter.

Samples of blood (2 mL obtained via an indwelling catheter placed in a cephalic vein contralateral to the infusion site) and urine (obtained via syringe attached to the urinary catheter; the urinary bladder was emptied and flushed with 10 mL of saline solution) were collected at 15-minute intervals after a 30-minute equilibrium period. The 125I radioactivity of samples was determined by use of an γ radiation counter until UVfA and PGfA each reached steady state (ie, similar count rates were determined in 2 consecutive samples). The UVfA was determined as the product of UfA (counts/min/mL) \( \times V_{UP} \) (mL/min; determined from sample volume vs time of collection). Plasma was obtained via centrifugation of blood samples in untreated glass tubes (15 minutes at 1,200 \( \times g \)), and 0.5 mL of plasma was used to measure PfA (counts/min/mL).

Once steady state was determined, 2.0-mL blood samples were collected via IV catheter at 15-minute intervals for 45 minutes (for a total of 3 samples). Urine samples were also obtained via urinary catheter (with some difficulty) by completely emptying and flushing the urinary bladder with 10 mL of saline solution every 15 minutes; plasma and urine samples were stored at 4°C until use. A 10-μL sample of the infusate was also obtained. The 125I radioactivity of samples was measured 7 days after the experiment to allow for complete decay of 99mTc (which has a terminal half-life of 6 hours and was administered concurrently for the comparison experiment), and GFR was calculated according to the formula \( \kappa GFR = \lambda \) for individual sample values at each time point. Values for UVfA and PfA were determined as previously described; the GFR was calculated again as \( UfA/PfA \). Values for GFR determined via both methods were normalized by body surface area calculated on the basis of body weight and height.23

**Statistical analysis**—The GFR data are expressed as mean ± SD. Comparisons between samples were performed via paired Student t test analysis, and linear regression analysis of natural logarithms of radiation counts versus time was used to calculate the rate constants via a commercially available statistical analysis software program.24 A value of \( P < 0.05 \) was considered significant.

### Results

**Quality-control tests**—Sources containing 1.85 MBq of \(^{99m}\)Tc-DTPA (source A), 1.85 MBq of \(^{125}\)I-iothalamate (source B), or no radiopharmaceutical agent (source C) were analyzed by use of the ARM for quality control of the experimental method. Measured (mean ± SD) radioactivity was 1530 ± 0.9 counts/s, 7 ± 0.2 counts/s, and 2 ± 0.2 counts/s for sources A, B, and C, respectively. Results of analysis of the signal-to-noise ratio of a source with a radiation count rate similar to that measured transcutaneously at the brachium of the monkeys immediately after injection of 7.4 MBq of \(^{99m}\)Tc-DTPA/kg (A:C ratio, 765) and the ratio of interference of 125I counts over 99mTc counts (B:A ratio, 5 × 10^-3) indicated that almost all of the radiation detected by the ARM when both radiopharmaceutical agents were administered was emitted from \(^{99m}\)Tc-DTPA. The radiation counting efficiency of the ARM determined via comparison of actual radiation detection versus the expected disintegration rate of test source A was 0.083%. Limits of detection and quantification used to evaluate sensitivity were 0.2349 \( \times 10^{-3}\) min and 0.4087 \( \times 10^{-3}\) min, respectively.

The measured \( \lambda \) of a single \(^{99m}\)Tc source obtained by use of the ARM in vitro (mean ± SD intra-assay val-
The measured 99mTc radioactivity, 89% to 110%; precision, 5.4%) were comparable.

and precision was 5.3%; values for 60 minutes (accuracy, 92% to 114%, and precision was 5.3%). The mean κ GFR determined in monkeys by use of the κ rate infusion of 125I-iothalamate was 16%

κ ARM ranged from 6.5% to 22.7%. The mean κ value determined on day 1 in anesthetized monkeys was 16% to 23% less than that measured on days 8 and 45 in conscious monkeys. The κGFR was also less on day 1 than on days 8 and 45.

Estimation of GFR via the ARM—The natural logarithms of radiation count versus time were recorded by use of the ARM following administration of 99mTc-DTPA to monkeys (Figure 2). A rapid increase in count rate was detected during the first 5 minutes after injection; this was attributable to mixing of the radioactive GFA in the vascular space. After 5 minutes, the radiation count rates decreased versus time with first-order kinetics; this decrease represented the physical decay of the 99mTc as well as renal clearance of the radioactive GFA.

Individual κ values obtained via analysis of the slope of 99mTc-DTPA activity versus time determined from radiation counts obtained via ARM showed less variability than the values of GFR determined via analysis of 123I-iothalamate clearance after blood and urine collections (Tables 1 and 2). The coefficient of variation of κGFR determined in monkeys by use of the ARM ranged from 6.5% to 22.7%. The mean κ value determined on day 1 in anesthetized monkeys was 16% to 23% less than that measured on days 8 and 45 in conscious monkeys. The κGFR was also less on day 1 than on days 8 and 45.

Estimation of GFR via 123I-iothalamate clearance—Measurements obtained from blood and urine samples collected after IV administration of 123I-iothalamate were used to calculate GFR values (Table 2). The mean GFR values obtained by use of UVGFA and PGFA measurements were smaller than obtained by use of values for INFA, particularly in the 2 male monkeys. This variability was likely attributable to the variability in volumes of urine produced between collection periods. The substantial reduction in variability observed when calculations of GFR were performed with INFA values instead of UVGFA measurements was independent of the sex of the animals and collection periods.

Discussion

Quality-control testing of GFR estimations obtained via standard clearance techniques that rely on collection of blood and urine samples is difficult and often unreliable. This is largely due to the technical complexity of the tests. Other factors contribute to reduced accuracy when values are obtained and recorded by use of transcutaneous radiation detection devices. For instance, a method for estimation of GFR by measurement of 99mTc-DTPA radiation with transcutaneous radiation detection devices was reported to be the least accurate of several methods tested in 1 study. However, accuracy in that study was compromised by the large amount of noise obtained with CdTe detectors, compared with...
that of the CsI(Tl)-crystal-photodiode array detectors used in the study reported here. An additional factor that contributed to this difference was the substantial reduction in the frequency of measurements (1/120 seconds vs 1/10 seconds, respectively) between that study and the present study. The long counting interval required by the CdTe detectors is likely a reflection of the reduced efficiency and increased noise of that type of detector. The accuracy and response time of each technique depend on the type of detectors and settings used in the instrument during data collection. Consequently, it is critical that the performance of the instrument and settings for transcutaneous detection of radiation be tested before use in monitors of this type.

Although not entirely comparable to measurements obtained in animals, the determination of λ by use of the ARM in vitro provides a simple means to test the performance of the device in the low range of GFR values and independent of biological variability. In effect, this is a suitable method to establish a standard to which the κ values obtained via transcutaneous measurements in monkeys could be compared. The test can be performed easily via measurement of λ of a 99mTc source with data collected without decay correction. This type of quality-control test may help to reduce variability among transcutaneous measurements obtained in monkeys. Because the λ of 99mTc happens to be similar to the κ value obtained in patients with as much as a 70% reduction in GFR, the measurement of λ is an excellent method to perform quality testing of ARM even in a range that corresponds to stage III or moderate renal insufficiency in the classification of chronic kidney disease in humans.25

The results of analysis of the signal-to-noise ratio and of the interference of 125I counts with 99mTc counts indicated that almost all (99.5%) of the radiation detected by the ARM when both agents were present was emitted from 99mTc-DTPA. The difference in cumulative doses (7.4 MBq/kg for 99mTc-DTPA vs 0.222 MBq/kg for 125I-iothalamate), difference in photon energy between radiopharmaceutical agents, and use of a correct set of γ energy discriminator were factors that contributed to this difference was the substantial reduction in variability that follows a single injection of a radiopharmaceutical agent. In contrast to the extremely short time (≤ 5 minutes) in the present study likely resulted from incomplete urine collection, any residual urine volume would have accounted for a large fraction of the small urine volumes produced by the monkeys during the 15-minute sampling intervals. This possibility was supported by the substantial reduction in variability detected when estimates of GFR were calculated on the basis of values for INFΔ instead of UV.30,31 Independent of sex of the monkeys. This was also in agreement with results of other studies,7,26,27 which indicated that GFR values calculated on the basis of values for INFΔ were more precise and accurate than those calculated on the basis of the UV.24 The GFR values obtained during INFΔ in the study reported here were consistent with values obtained in rhesus monkeys by use of other techniques and various GFAs.20,21

The rather simple kinetics detected by use of the ARM in the present study are in contrast to the more complex patterns of blood radiation counts versus time. The decrease with time in blood sample radioactivity that follows a single injection of a radiopharmaceutical agent is represented by 3 stages.4 The first stage is detected immediately after injection and corresponds to the mixing of the agent in the vascular compartment. The second stage is represented by a rapid decrease in blood sample radioactivity, which corresponds to diffusion of the agent from the vascular compartment to the interstitial compartment. Finally, the third stage is represented by a slow decrease in blood sample radioactivity that follows first-order kinetics and corresponds to clearance of the agent by the kidneys. As a result of these more complex kinetics, attempts to simplify and shorten the duration of the test by reducing the number of blood samples obtained have been partially successful5,27 because several hours are required for the renal clearance of the agent to reach the third stage. This is in contrast to the extremely short time (≤ 5 minutes) required to reach first-order kinetics with the ARM detection technique. This unique characteristic of transcutaneous whole-tissue recording is a consequence of the simultaneous detection of radioactive signals from the interstitial and vascular compartments.7 Because transcutaneous whole-tissue radiation detection cannot resolve the vascular and interstitial compartments as separate entities, the system operates as a single...
compartment that comprises only the extracellular element.

Because of the variability of the data, it is unclear whether the difference detected between day 1 and days 8 and 45 represented changes in GFR induced by general anesthesia, as has been described in reports of anesthesia and surgery in rats and humans. Nevertheless, the ability to determine GFR in conscious monkeys would eliminate the concern for any effect of anesthesia as a confounding factor in nephrotoxicity tests. The coefficient of variation of κGFR determined in monkeys by use of the ARM ranged from 6.5% to 22.7% with a mean value of 14.7%, which is similar to the value obtained in human studies that involved the same technique and approximately half the coefficient of variation obtained with standard clearance techniques. Although the measurement of κ is less accurate and precise than the measurement of λ, results are at least similar to measurements in multiple blood and urine samples in this and a previous study. Moreover, the use of the λ determination as a standard for sample analysis should help to determine at what step in the measurement of κ a decrease in accuracy or precision may occur. With this approach, we were previously able to identify motion as one of the most important factors in κ variability. Consequently, we expect the accuracy and precision of κ values to reach a similar quality to that determined for the measurement of λ with better training and improved immobilization of the ARM.

Body size is indirectly measured via determination of variables such as weight and body surface area. The κ value obtained in the study reported here were highly similar to rate constants obtained in rhesus monkeys with twice the body weight of the monkeys used in the present study and similar to the rate constant determined in humans with normal renal function and body size approximately 20 times as great as that of monkeys in the present study. This indicates, as contended in another report by our group, that no adjustment of κ is necessary to correct for differences in body size as is typically done for clearance techniques. The similarity of κ values between humans and monkeys indicates that the rate constant for the clearance of 99mTc-DTPA measured transcutaneously with the ARM likely represents a measure of the relative efficiency of the kidneys to clear the GFR from body compartments independent of body size, as was reported for 125I-iodothalamate after normalization on the basis of body surface area. In other terms, the measurement of κ likely represents a more pertinent way to measure renal function than to measure the absolute value of GFR by use of standard clearance techniques. A meaningful comparison of renal function can be performed independent of age, sex, and size of the subjects by use of this alternative method.

Measurements of GFR by use of the ARM are indirect. In the authors’ experience in a human intensive care unit setting, the correlation detected between the rate of excretion of 99mTc-DTPA and GFR is observed only in hemodynamically stable patients under steady-state circumstances. Space or volume variation imposed by fluid resuscitation or extreme local blood flow changes at the site of detection could affect the values for GFR. The correlation detected via this method is valid only when the rate-limiting step in the excretion of 99mTc-DTPA is attributable to kidney function.

Because the ARM radiation detection method requires minimal restraint, has a brief measurement time of only 15 to 30 minutes, and is minimally invasive, we conclude that it represents a fast, sufficiently accurate, convenient, and less-invasive way to measure GFR in conscious nonhuman primates.

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