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Detection of early changes in renal function using ^{99m}Tc-MAG3 imaging in a murine model of ischemia-reperfusion injury

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Abstract

Accurate determination of renal function in mice is a major impediment to the use of murine models in acute kidney injury. The purpose of this study was to determine whether early changes in renal function could be detected using dynamic gamma camera imaging in a mouse model of ischemia-reperfusion (I/R) injury. C57BL/6 mice ($n = 5/\text{group}$) underwent a right nephrectomy, followed by either 30 min of I/R injury or sham surgery of the remaining kidney. Dynamic renal studies (21 min, 10 s/frame) were conducted before surgery (baseline) and at 5, 24, and 48 h by injection of ^{99m}Tc-mercaptoacetyl triglycine (MAG3; ~1.0 mCi/mouse) via the tail vein. The percentage of injected dose (%ID) in the kidney was calculated for each 10-s interval after MAG3 injection, using standard region of interest analyses. A defect in renal function in I/R-treated mice was detected as early as 5 h after surgery compared with sham-treated mice, identified by the increased %ID (at peak) in the I/R-treated kidneys at 100 s ($P < 0.01$) that remained significantly higher than sham-treated mice for the duration of the scan until 600 s ($P < 0.05$). At 48 h, the renal scan demonstrated functional renal recovery of the I/R mice and was comparable to sham-treated mice. Our study shows that using dynamic imaging, renal dysfunction can be detected and quantified reliably as early as 5 h after I/R insult, allowing for evaluation of early treatment interventions.

Keywords

acute kidney injury; nuclear imaging; mice models of acute renal failure

ACUTE KIDNEY INJURY (AKI) is a common clinical problem that is associated with a high incidence of morbidity and mortality (2–4, 16–18). Treatment options remain limited to supportive measures and renal replacement therapy with dialysis, necessitating the need for development of more viable therapies for AKI. Mouse models have been indispensable to our understanding of the pathophysiology AKI, since they provide the opportunity to perform mechanistic studies using genetically engineered mice. However, a major barrier to the use of mice in this field has been the inability to accurately determine renal function by

standard methods. The problems encountered with the use of serum creatinine concentration as a measure of renal function in a clinical setting are greatly exacerbated when applied to mice models. For example, obtaining blood from mice for a reliable creatinine measurement without causing hemolysis of the blood sample can be difficult. In addition, chromagens in mouse serum interfere with the picric acid-based creatinine assays, leading to a 5- to 8.5-fold overestimation of serum creatinine (10). Because of this problem, other methods for measuring serum creatinine in mice have been developed, such as an enzymatic method (7, 13), which is more accurate than the picric acid assay method, and the use of HPLC, which has been optimized with high accuracy specifically for mouse serum (7, 19). While an increase in blood urea nitrogen (BUN) is a marker of early AKI, other prerenal factors can also alter BUN levels. Changes in serum creatinine do not typically occur until at least 24 h after AKI, and hence such measures alone may be inadequate markers of intrinsic renal function.

The purpose of the present study was to evaluate the use of dynamic renal scintigraphy with ^{99m}Tc -mercaptoacetyltriglycine (MAG3) to detect early changes in renal function in a mouse model of renal ischemia-reperfusion (I/R) injury. ^{99m}Tc -MAG3 is already approved for human use and is used clinically for assessment of renal function. The ^{99m}Tc -MAG3 was prepared fresh using generator-eluted ^{99m}Tc (half-life = 6 h). ^{99m}Tc -MAG3 has a high extraction efficiency from functional kidneys, following active excretion by the tubular system. The renal imaging studies in the current report allowed accurate determination of %ID/kidney since the entire mouse was imaged, were minimally invasive, efficient (3 animals simultaneously), and cost effective. In addition, the second phase of the MAG3 curve provides an assessment of tubular secretion in a dynamic fashion that does not necessitate clearance studies or the infusion of a substance such as PAH. Therefore, the imaging enabled renal function to be assessed in the same animals at frequent intervals during the course of AKI.

MATERIALS AND METHODS

Chemicals

^{99m}Tc -MAG3 was obtained from the local Cardinal Health Pharmacy Service Center (Birmingham, AL). The ID was standardized to be ~1.0 mCi/25 g body wt.

Animals

Male C57BL/6 mice, aged 8–10 wk and weighing ~25 g, were purchased from Charles River Laboratories (Wilmington, MA). Animals were fed a standard diet and allowed free access to water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Renal I/R injury model

Mice were anesthetized using 2.5% isoflurane by inhalation. Under aseptic precautions, a right nephrectomy was performed via a right loin incision. A similar incision was made in the left loin, and the left renal pedicle was exposed, secured, and clamped with a microserrefine vascular clamp (Fine Science Tools) for 30 min. Blanching of the entire kidney ensured loss of blood flow. During this period, the kidney was kept moist, using sterile gauze soaked in saline. At the end of ischemia, the clamp was removed to allow reperfusion, which was confirmed visually, and the kidney was returned to the abdominal cavity in its original position. The incision was closed with 4-0 prolene sutures, and the animals were allowed to recover. An additional group of animals ($n = 5$) underwent sham surgery in which a right nephrectomy was performed and the left renal pedicle was exposed, but was not clamped. In separate experiments, additional mice underwent I/R or sham

surgery, and kidneys and blood were harvested at 5, 24, and 48 h after surgery. Mice were killed with a lethal dose of pentobarbital sodium (0.4 ml, 50 mg/kg), and blood was collected for measurement of BUN and creatinine.

Renal imaging

Whole-body imaging was accomplished using dynamic imaging protocols with ^{99m}Tc -MAG3 on three mice at a time. Mice were injected with 0.5 ml sterile saline (ip) while under isoflurane anesthesia to insure adequate hydration. After 15 min, three mice were positioned around a central isoflurane anesthesia delivery point on a parallel-hole collimator that was part of an Anger gamma camera (420/550 Mobile Radioisotope Gamma camera, Technicare, Solon, OH). ^{99m}Tc -MAG3, 1.0 mCi/25 g body wt, was injected via the tail vein, and the dynamic acquisition (Numa Station, Numa, Amherst, NH) was simultaneously started with the first injection. Total scan time was 21 min, with 125 frames collected at 1 frame/10 s. Renal scans with ^{99m}Tc -MAG3 were obtained 2–3 days before surgery for baseline measurements and then at 5, 24, and 48 h following I/R or sham surgery.

Image analysis

Image files were exported from Numa Station software and analyzed with a modified version of NIH Image software (NucMed-Image, Mark D. Wittry, St. Louis University, St. Louis, MO) using standard manual region of interest (ROI) analyses. An ROI was drawn for both the entire body and the left kidney (or both kidneys for presurgery imaging) for each animal, as well as a background region outside the mouse. The total pixels and counts/pixel data for each region of interest were tabulated for each frame collected, and the data were transferred into Microsoft Excel. The total counts in the kidney (pixels multiplied by the background corrected counts/pixel for kidney ROI) was divided by the total counts in the whole body (pixels multiplied by background corrected counts/pixel for body ROI), multiplied by 100%, gave %ID in the kidney for that time point. Peak %ID, time to peak (seconds), and the peak:10 min ratio were also calculated from the renograms.

Histological assessment

At 5 and 24 h after induction of renal I/R, the mice were anesthetized with isoflurane and the abdominal cavity was opened. The left kidney was removed and cut transversely into three to four slices and placed in 10% neutral buffered formalin. Tissues were embedded in paraffin, and 5- μm sections were stained with hematoxylin and eosin. Morphological assessment of injury was determined in the cortex and outer medulla by examining the tubules for loss of brush border, necrosis, casts, and areas of red blood cell extravasation into the interstitium.

Laboratory assays

BUN and creatinine were determined by Alfa Wassermann's VetACE Biochemistry Machine (West Caldwell, NJ). The alkaline picric acid reaction was used for the creatinine assay.

Statistical analysis

All results were expressed as means $\pm$ SE. The unpaired *t*-test was used to compare the control vs. treated groups. ANOVA and the Student-Newman-Keuls test were used to compare the mean values for multiple group comparisons. All values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

To confirm induction of renal dysfunction in the murine model of unilateral nephrectomy followed by contralateral I/R-induced renal injury, we measured BUN and serum creatinine at 5, 24, and 48 h, respectively, following I/R or sham surgery. As shown in Fig. 1, A and B, a significant increase in BUN and a modest increase in creatinine were noted at 5 h, with improvement in renal function at 24 and 48 h, following I/R. Evidence of renal injury was observed by histology performed at 5 and 24 h following I/R, but not in animals undergoing sham surgery (Fig. 1C).

Dynamic renal imaging using ^{99m}Tc -MAG3 assessed renal function in this model of I/R injury. Mice were injected sequentially, and uptake of the tracer in the kidneys and appearance in the bladder were monitored by capturing images at the rate of 1 frame/10 s. Figure 2 is a representative experiment showing every sixth frame in sequential order. Depending on their positioning during the experiment, a mouse on the *right* was injected first (*frame 1*), followed by a mouse in the *middle* (*frame 12*), and finally a mouse on the *left* (*frame 18*). Using ROI image analysis, the amount of injected radio-tracer that was retained in the sham (Fig. 2A) or I/R kidney (Fig. 2B) was visualized and quantitated over time, compared with the total amount of dose injected. As expected, sham-treated mice that underwent a unilateral nephrectomy had a delayed excretion of the tracer compared with the baseline measurements (Fig. 3A). Within 5 h following I/R injury, the percent dose of injected ^{99m}Tc -MAG3 that was retained in the kidney over 600 s was higher ($P < 0.05$) than sham-treated animals (Fig. 3A). At 24 and 48 h after I/R injury, the percent dose of injected ^{99m}Tc -MAG3 in the kidney did not differ from that observed with the sham-treated kidney (Fig. 3, B and C). Compared with baseline values, the peak value of the %ID differed ($P < 0.05$) in the animals undergoing I/R at all time points (Table 1). Differences were also observed in the animals undergoing sham surgery compared with baseline at 24 and 48 h after surgery. The time to peak and the peak:10 min ratios for the animals in the I/R group also differed ($P < 0.05$) from baseline measurements (Table 1). These data also allowed for assessment of renal blood flow. By calculating the average %ID at the initial phase (at 10, 20, 30, and 40 s), no significant differences were observed between sham and I/R at 5, 24, and 48 h after surgery (Table 1), suggesting that tubular function and not changes in renal perfusion was responsible for the findings. Interestingly, despite an absence of biochemical and histological alterations, MAG3 imaging detected subtle, but significant impairment in the kidneys of sham-treated mice, perhaps related to intravascular fluid shifts following unilateral nephrectomy and effects of anesthesia during the surgical procedure. In the model of I/R injury used in this study, 30 min of ischemia time resulted in mild increases in BUN and serum creatinine at 5 h; however, ^{99m}Tc -MAG3 scanning was able to detect significant impairment of renal function. The rates of clearance of ^{99m}Tc -MAG3 were similar at 24 and 48 h, indicating full functional recovery and was consistent with the BUN and creatinine values at these time points. These results demonstrate the utility of this imaging modality with ^{99m}Tc -MAG3 as an acceptable dynamic technique for detecting early changes in renal function in murine models of AKI.

MAG3 is useful in the assessment of renal function in that it is mostly excreted due to tubular extraction, in contrast to other compounds such as diethylenetriamine pentaacetic acid (DTPA), which are mostly filtered. It is for this reason that MAG3 is the recommended compound for assessment of impaired renal function (5). In a clinical setting, the curve generated from a renogram using ^{99m}Tc -MAG3 has two phases in the first minute of measurement. The initial, rapid rise to a peak phase is due to early tubular extraction and can provide an evaluation of renal perfusion. The second phase of the curve can be ascending, flat, or descending and is a measure of further tubular extraction, and thus, tubular function (1, 8).

Previous studies have used this technique in long-term studies to monitor renal function in rat models of chronic allograft rejection (14) and murine models of glomerular disease (6). Dynamic imaging using ^{99m}Tc -MAG3 over a period of time could allow one to assess not only tubular uptake of the tracer compound, but also the excretion of the compound. Since MAG3 is almost selectively extracted by tubular cells, acute tubular damage would affect the ability of the tubular cells to secrete MAG3 into the tubule, perhaps even after the tracer was successfully taken up by the cell. Indeed, recent studies have reported decreased expression of organic anion transporters (located at the basolateral surface of the proximal tubule) following renal I/R injury (9, 15). These transporters are also responsible for tubular secretion of agents such as PAH and MAG3. It is therefore possible that MAG3 accumulation in the kidney is due, at least in part, to decreased expression of organic anion transporters. Additional potential mechanisms of MAG3 accumulation in AKI may include tubular necrosis and intraluminal trapping due to tubular debris and cast formation, although cast formation was not prominent at 5 h following renal I/R injury (Fig. 1C, *top*).

An increased amount of MAG3 retained in the kidney following the first minute, relative to control animals, indicated a decreased ability of tubules to properly secrete MAG3. This phenomenon is shown in the graphs of %ID of ^{99m}Tc -MAG3 during the course of a 10-min renogram. The curve has an initial quick, rising phase to a peak and then a descending phase, which indicates excretion of the tracer. By comparing the percent dose being retained within the kidney over the course of the scan, we demonstrated that this technique detected early changes in renal function in mice following injury. The ability of the nuclear imaging technique to detect early changes in renal function in mouse models of renal I/R is consistent with the time points (<6–12 h) when increases in urinary neutrophil gelatinase-associated lipocalin (NGAL) have been described in AKI (11). In contrast to measuring urine or plasma biomarkers, such as NGAL, IL-18, or kidney injury molecule-1 (KIM-1) (12), the MAG3 technique provides dynamic and functional assessment. Because early detection and intervention are critical for successful treatment in AKI, future studies to evaluate novel therapies would benefit from this method to detect renal impairment/improvement at early time points following the insult/intervention.

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Fig. 1.

Renal function and histology of sham and ischemia-reperfusion (I/R)-treated mice. Blood urea nitrogen (BUN; *A*) and creatinine concentrations (*B*) for I/R and sham-treated mice at 5 ($n = 5$), 24 ($n = 5$), and 48 h ($n = 9$) are shown. * $P < 0.05$ vs. sham. *C*: renal histology at 5 and 24 h following I/R with classic changes in acute tubular necrosis (arrows) and cast formation (asterisks). Note the minimal cast formation at 5 h following I/R.

Fig. 2.

Representative scans from 3 mice following injection of ^{99m}Tc -mercaptoacetyltriglycine (MAG3) at baseline (*A*) and following I/R (*B*). Note the rapid uptake of the tracer in the kidneys and appearance in the bladder. The frames are numbered in sequential order, and every 6th frame is shown. Depending on their placement during the experiment, the mouse on the *right* was injected first (*frame 6*), following by the mouse in the *middle* (*frame 12*), and finally the mouse on the *left* (*frame 18*). Note that in *B* all animals underwent unilateral nephrectomy and contralateral I/R before imaging.

Fig. 3.

Percent injected dose (%ID) in the left kidney at baseline and following I/R or sham at 5 (A), 24 (B), and 48 h (C) after surgery: A: at 5 h following unilateral nephrectomy and contralateral I/R in mice, ^{99m}Tc -MAG3 imaging shows severe decreases in renal function (pink line at top). The middle (yellow) line represents sham animals that have undergone unilateral nephrectomy and hence the change from normal animals (blue line at bottom; $n = 5$ mice/group). A shows a significant difference in the amount of dose retained in the left kidney in I/R vs. sham-treated mice. $*P < 0.05$ vs. sham and baseline. $\#P < 0.05$ vs. sham. B and C show significant differences between baseline vs. sham and I/R ($\#P < 0.05$), but no significant difference between I/R and sham-treated mice.

Summary of extracted parameters of renograms from baseline and from animals undergoing uninephrectomy with sham or ischemia-reperfusion surgery

Table 1

Parameter	Baseline (n = 9)	Sham (n = 5)		I/R (n = 5)	
		5 h	24 h	48 h	5 h
Peak %ID	26.4±0.8	29.0±0.5 [#]	30.4±1.5 [*]	30.6±2.2 [*]	35.8±1.5 [*]
Time to peak, s	67±3.7	86±6.8 [#]	105±13 [*]	76±5.1	138±18 [*]
Peak: 10 min ratio	2.5±0.2	1.6±0.01 [*]	1.5±0.2 [*]	1.9±0.1	1.4±0.1 [*]
% ID (10–40 s)		16.7±0.9	16.8±2.2	18.6±1.6	21.9±1.9
					18.6±2.9
					22.8±2.2

Values are means ± SE. I/R, ischemia-reperfusion; ID, injected dose.

^{*} $P < 0.05$ vs. baseline.

[#] $P < 0.05$ vs. 5 h I/R.