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Evaluation of kidney repair capacity using ^{99m}Tc-DMSA in ischemia/reperfusion injury models

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ABSTRACT

Quantitative ^{99m}Tc-DMSA renal uptake was studied in different renal ischemia/reperfusion (I/R) mice models for the assessment of renal repair capacity. Mice models of nephrectomy, uni- and bi-lateral I/R together with sham-operated mice were established. At 1 h, 1 d, 4 d, 1, 2 and 3 wk after I/R, ^{99m}Tc-DMSA (27.7 ± 1.3 MBq) was injected via tail vein and after 3 h post-injection, the mice were scanned for 30 min with pinhole equipped gamma camera. Higher uptake of ^{99m}Tc-DMSA was measured in normal kidneys of uni-lateral I/R model and nephrectomized kidney I/R model at 3 wk post-surgery. Comparing the restoration capacities of the affected kidneys of nephrectomy, uni- and bi-lateral I/R models, higher repair capacity was observed in the nephrectomized model followed by bi-lateral then uni-lateral models. The normal kidney may retard the restoration of damaged kidney in uni-lateral I/R model. Moreover, 3 wk after Uni-I/R, the size of injured kidney was significantly smaller than non-ischemic contralateral and sham operated kidneys, while nephrectomy I/R kidneys were significantly enlarged compared to all others at 3 wk post-surgery. Very strong correlation between ^{99m}Tc-DMSA uptake and weight of dissected kidneys in I/R models was observed. Consistent with ^{99m}Tc-DMSA uptake results, all histological results indicate that kidney recovery after injury is correlated with the amount of intact tubules and kidney sizes. In summary, our study showed good potentials of ^{99m}Tc-DMSA scan as a promising non-invasive method for evaluation of kidney restoration after I/R injuries. Interestingly, mice with Bi-I/R injury showed faster repair capacity than those with uni-I/R.

1. Introduction

The kidney is vulnerable to diverse forms of injury that can lead to dysfunction and, in the most severe cases, acute renal failure (ARF). Renal ischemia/reperfusion (I/R) injury, which follows reduced renal blood flow, is a major cause of ARF. And if renal I/R injury is severe enough to lead to ARF, it can result in a very high mortality rate of 50% [1–3]. This form of renal injury has been extensively studied in animal models of renal artery cross-clamping and has been shown to be due to necrosis of the S_3 segment of the proximal tubule and, to a lesser degree, the thick ascending limb [4–6].

Renal scintigraphy has been used for a long time to measure the relative renal function. These methods can be performed with different radiopharmaceuticals such as technetium-99m dimercaptosuccinic acid (^{99m}Tc-DMSA), technetium-99m diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA), technetium-99m mercaptoacetyltriglycine (^{99m}Tc-MAG3), iodine-131 orthoiodohippurate (^{131}I-OIH) and more recently technetium-99m ethylenedicysteine (^{99m}Tc-EC) [7–10]. Although all these methods are accurate to measure this parameter, some differences can be observed among them [11]. These differences are caused by distinct biological properties of radiopharmaceuticals such as mechanisms of renal excretion, renal cells retention of radioactive material, level of plasma-protein bound and level of plasmatic clearance. However, ^{99m}Tc-DMSA as a static renal agent is considered as the most reliable method to measure relative renal function [12–14] and the most appropriate tracer for renal cortical imaging [15].

Recovery from ARF requires the replacement or regeneration of lost tubular epithelial cells. This process is accompanied by complex changes in gene expression of growth modulatory molecules, such as epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), and hepatocyte growth factor (HGF) [16,17]. It has been generally believed that some of the surviving renal tubular cells differentiate and re-enter the cell cycle to produce epithelial cells that rebuild the structure and function of the renal tubules. However, in some cases of either human ARF or experimental models of renal injury, a lack of correlation between histopathological evi-

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dence of injury and renal function has been found, and the relative contribution of functional versus structural changes to the evolving renal dysfunction has not been unequivocally substantiated [18,19]. Moreover, these histopathological methods are labor intensive, time consuming and most importantly are difficult for interpretation of the results. ^{99m}Tc-DMSA, on the other hand is a kidney imaging agent that provides more accurate information about the functional status of kidneys noninvasively. Its quantitative measurement is therefore, a good index for renal function. In this study, we used ^{99m}Tc-DMSA quantitative scan to assess the restoration capacities of renal tubular cells after experimental induction of renal I/R injury.

2. Materials and methods

Experiments were conducted with 8-week-old C57BL/6 male mice divided into four treatment groups ($n = 3$): uni-lateral I/R, bi-lateral I/R, uni-lateral nephrectomy and sham operated mice. The mice were permitted free access to water and standard mouse chow. In all cases, the studies were conducted in accordance with the guideline of the Animal Care and Use Committee of the Kyungpook National University School of Medicine.

To induce operation, the mice were anesthetized with pentobarbital sodium (60 mg/kg body weight; intraperitoneally) prior to surgery. Body temperature was maintained at 36–38 °C. Renal I/R injury was induced by a 30 min clamping of renal arteries as previously described [20]. Briefly, to induce uni-lateral or bi-lateral renal ischemia, kidneys were exposed via a flank incision and then the right renal pedicle for uni-lateral renal ischemia or both right and left renal pedicles for bi-lateral renal ischemia were clamped with a nontraumatic microaneurysm clamp (Roboz Surgical Instrument Co., USA) for 30 min. The incisions were then closed temporarily during ischemia. After 30 min, the clamps were removed and reperfusion of the kidneys was visually confirmed by visualizing dark color of ischemic kidneys. The left kidneys of mice in uni-lateral nephrectomy group were surgically removed (nephrectomized) and I/R was induced on the right kidney in the same way as indicated above. Animals in sham group underwent sham surgery and used as a control group.

Pertechnetate $^{99m}TcO_4^-$ was eluted from technetium-99m generator (Lantheus Medical Imaging, USA) which contain molybdenum-99 (^{99}Mo, half life 2.75 d). The extracted 740 MBq activity was added to ^{99m}Tc-DMSA kit vial (Mallickrodt Medical BV, Netherlands) and the volume of the vial was made 2 mL using saline water in accordance with the manufacturer's instructions. The reconstituted mixture was then well mixed by shaking for 1 min. After incubation for 10 min at room temperature, labeling efficiency was evaluated by thin layer chromatography on silica gel, with n-butanol/acetic acid/H_2O (3:2:3) as developing solvent, in which ^{99m}Tc-DMSA stays at the origin while the free $^{99m}TcO_4^-$ migrates slowly towards the solvent front.

One representative mouse from each animal group ($n = 3$) was followed using non-invasive radiopharmaceutical. At 1 h, 1 d, 4 d, 1, 2, and 3 wk after I/R injury, ^{99m}Tc-DMSA (27.7 ± 1.3 MBq) was injected via tail vein. After 3 h, the mice were scanned for 30 min with phinhole equipped gamma camera (Infinia, GE Healthcare, USA). Region of interest (ROI) was drawn in gamma camera images, and the ratio of ROI counts to total counts was measured. After last imaging study at 3 wk post-surgery, kidneys were harvested, weighted, taken picture to evaluate kidney gross morphology and then fixed in 4% paraformaldehyde for histological analysis. Kidneys of other two mice in each group were also harvested and weighted. Body weight of all mice was measured prior to the sacrifice in order to calculate the ratio of kidney weight over total body weight of mice.

Fixed kidneys were washed with PBS three times for 5 min each, paraffin-embedded, cut into 2 μm sections with a microtome (RM2165; Leica, Bensheim, Germany), and stained with Periodic Acid-Schiff (PAS). Tubular damages were assessed on PAS stained 2 μm thick sections by comparing tubular cell necrosis, tubular dilatation, cast deposition, cellular infiltration and brush border losses among the different treatment groups of kidneys. [20,21].

3. Results

The radiolabeling of ^{99m}Tc-DMSA was achieved without difficulty by following the manufacturer's instruction. The radiochemical purity of ^{99m}Tc-DMSA checked by radio-TLC was always higher than 99%.

Gamma scintigraphy imaging of ^{99m}Tc-DMSA uptakes by normal and damaged kidneys along with schematic drawing of I/R model of each animal group was represented in Fig. 1. And, ^{99m}Tc-DMSA kidney uptakes over 3 wk were analyzed by calculating the ratio of kidney to total body count in gamma scintigraphy (Fig. 2). At a glance, ^{99m}Tc-DMSA kidney uptake pattern in Fig. 2 can be categorized into three groups; gradual increase, no change, and gradual decrease. Even though there is no big difference in ^{99m}Tc-DMSA kidney uptake between I/R models up to 4 d, different tendency of ^{99m}Tc-DMSA uptake between the groups are clearly observed after 1 wk since I/R surgery. The normal left kidney in uni-lateral I/R group and I/R injured kidney in nephrectomy group showed gradual increase of ^{99m}Tc-DMSA uptake over 3 wk, especially after 4 d since I/R surgery. The percentage of kidney count over total body count in normal kidney in uni-lateral and I/R kidney of nephrectomized group was increased from 20% and 26% at 1 h post-injury to 37% and 39% at 3 wk post injury, respectively. The sham operated group showed consistent ^{99m}Tc-DMSA uptake in both left and right kidneys (17% and 19% at 1 h; 21% and 24% at 3 wk). In contrast, the I/R injured kidneys in uni-lateral and bi-lateral models showed gradual decrease of ^{99m}Tc-DMSA uptake over the 3-wk study period. Both uni-lateral and bi-lateral I/R injured kidneys showed similar gradual decrease pattern up to 1 wk. However, the ^{99m}Tc-DMSA uptake of right I/R injured kidney in uni-lateral model was dropped dramatically to ca. 7% at 2 and 3 wk from 16% at 1 wk, while left and right I/R injured kidneys in bi-lateral model showed 13% and 15% of ^{99m}Tc-DMSA uptake, respectively, at 3 wk. The patterns of change in ^{99m}Tc-DMSA uptake in the right and left kidneys of both bi-I/R and sham models closely paralleled, but the uptake was a bite higher at every time point in the right kidneys compared with the left.

All normal and I/R injured kidneys were dissected from four animal groups after 3 wk post-surgery, photographed, and weighted. Kidneys from the same group showed very good uniformity in size (Fig. 3A). Any significant difference in terms of kidney size and weight was not found between scintigraphy imaging group (first row in Fig. 3A) and others ($n = 2$) in each group. Kidneys in nephrectomized group were enlarged by more than 50% compared to sham group (227 ± 12 vs. 144 ± 19 mg), while the size of I/R injured kidneys in uni-lateral group (99 ± 15 mg) was shrunken compared to normal kidneys (156 ± 10 mg) in the same group and sham operated kidneys. Even though the kidneys in bi-lateral group seemed to be slightly smaller than sham controls, the actual kidney weight of bi-lateral I/R group was even slightly higher than that of sham group (150 ± 33 vs. 144 ± 19 mg).

In order to get rid of the body weight variation of individual mouse even in the same group, kidney weight was compared by using the ratio of kidney weight to total body weight of mouse (Fig. 3B). As seen in size comparison in Fig. 3A, the nephrectomized group showed the highest kidney weight ratios (1.01 ± 0.03) while the I/R injured right kidneys showed the lowest ratio of 0.44 ± 0.06.

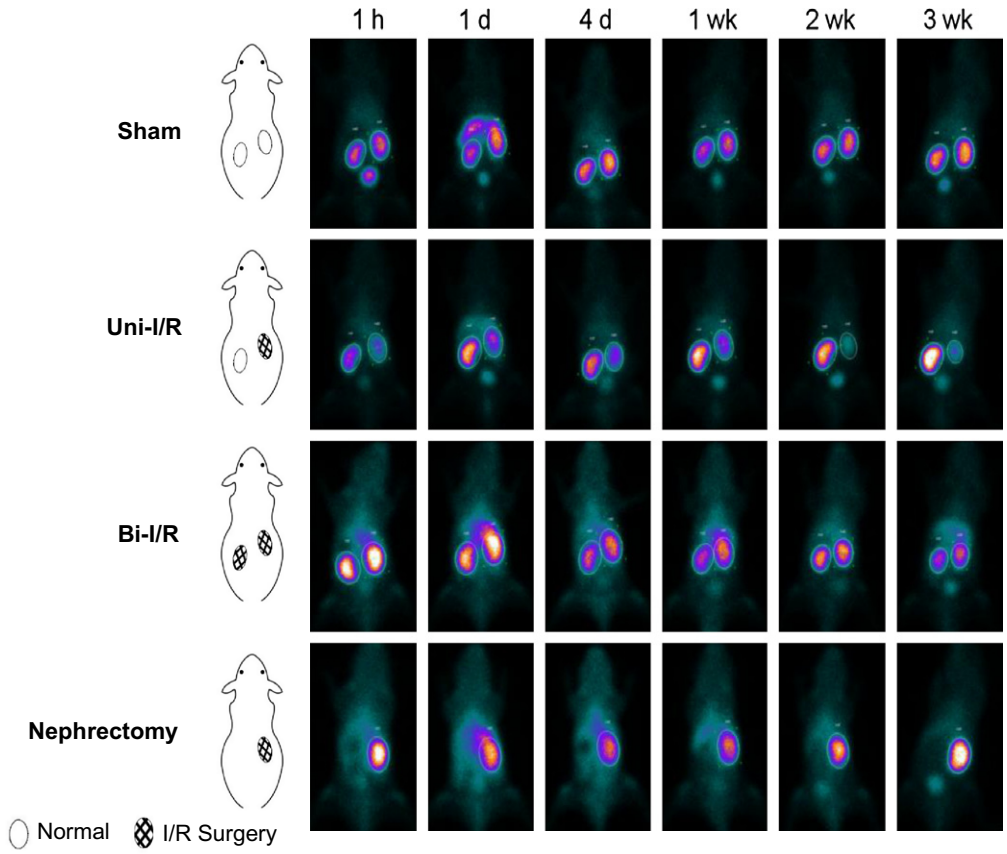

Fig. 1. Gamma camera images of mice model induced with sham, uni-lateral I/R, bi-lateral I/R and nephrectomy after ^{99m}Tc-DMSA injection along with schematic diagram of each I/R model.

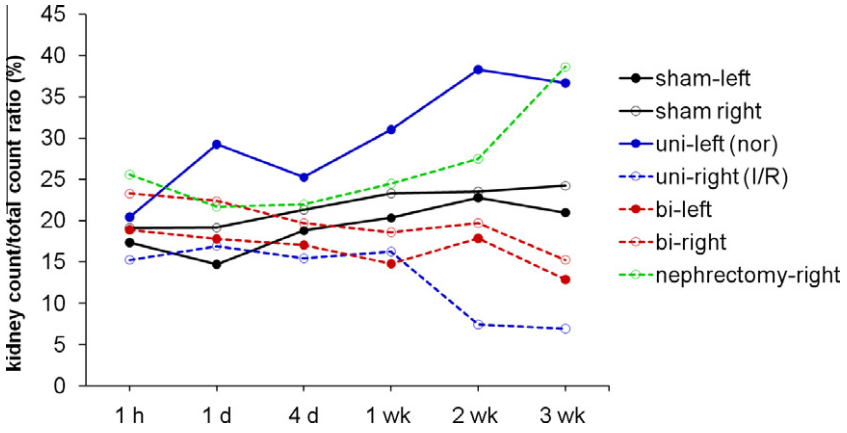

Fig. 2. Gamma camera imaging analysis of uptake of ^{99m}Tc-DMSA by kidneys treated differently over 3 wk. Data are presented as percentage kidney ROI counts/total counts.

The normal left kidney showed higher value of 0.70 ± 0.03 compared to counterpart left kidneys of sham group (0.58 ± 0.05) as well as I/R injured right kidney of the same uni-lateral group.

The relationship between ^{99m}Tc-DMSA uptake and kidney weight after 3 wk of I/R induction are graphically displayed in Fig. 3C. High correlation coefficient (R^2 = 0.947) was found between the ratio of ^{99m}Tc-DMSA kidney uptake over total count and the ratio of kidney weight over body weight.

PAS staining results of sham, uni-lateral, bi-lateral, and nephrectomy I/R injured kidneys after 3 wk recovery period since I/R injury are summarized in Fig. 4A–D. The ischemia affected kidney in uni-lateral model showed the highest kidney damage among the four animal groups as has been indicated by; numbers of congested, dilated and disrupted tubules with necrotic debris, great expansion of interstitium, increased interstitial cells and reduction of brush borders (Fig. 4B). As compared to the ischemic kidneys of uni-laterally ischemic mice, the ipsilateral kidney of bi-laterally ischemic kidneys also presented similar morphological changes (Fig. 4C). However, these morphological changes in the kidneys of bi-lateral ischemic mice were less than those in the ipsilateral kidneys of uni-lateral ischemic mice. Ischemic kidneys of uni-laterally nephrectomized mice presented much better morphological features than the ipsilateral kidneys of both bi-laterally and uni-laterally ischemic mice (Fig 4D).

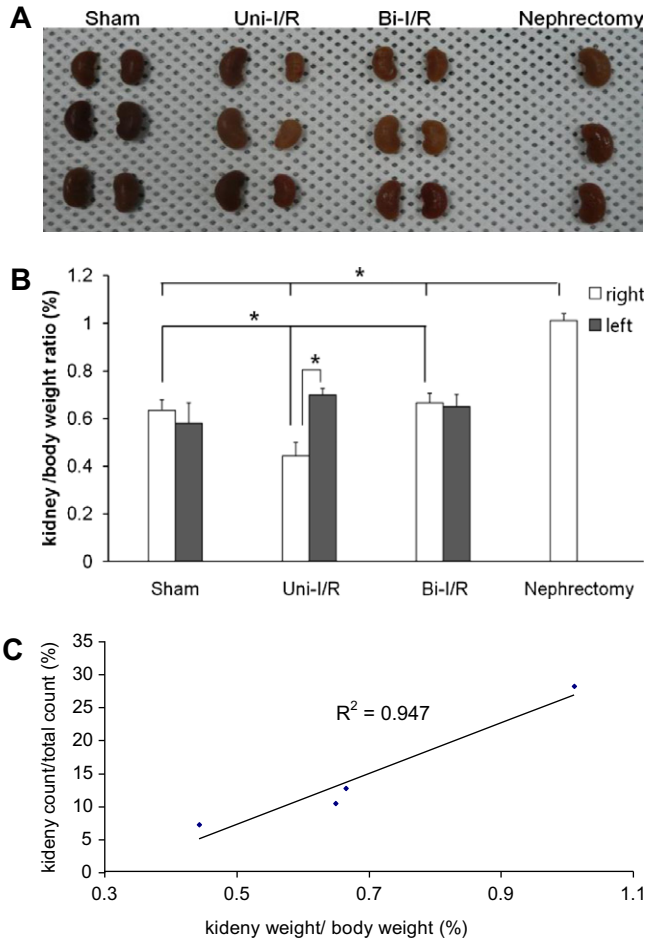

Fig. 3. (A) Comparison of size of kidneys harvested after last imaging study (3 wk post I/R). (B) Kidneys weight of mice after 3 wk of operation. The results are represented as ratios of kidneys to body weight of respective mice. Values were compared using student's *t* test (p values < 0.05 is indicated by asterisk). (C) Graph comparing ^{99m}Tc-DMSA uptake as measured by gamma counter and kidney weight after 3 wk of I/R induction (kidneys only with I/R were compared).

4. Discussion

Renal scintigraphy using ^{99m}Tc-DMSA is being advocated as the preferred method for assessment of renal functions following several types of kidney insults [9,22–24]. Majd et al. evaluated the advantages and disadvantages of imaging modalities for clinical use and confirmed the value of ^{99m}Tc-DMSA even at present, in the era of high technical capabilities of ultrasound, CT and MRI methodology [23]. Its uptake correlates with effective renal plasma flow, glomerular filtration rate, and creatinine clearance. Its quantitative measurement is therefore a good index for renal function. Previous studies have shown that ^{99m}Tc-DMSA uptake differentiates normal from diseased kidneys [9,25–28].

The aim of this study was therefore, to evaluate the correlation of ^{99m}Tc-DMSA renal uptake with renal tubular repair status in experimental renal ischemia/reperfusion mice models, and furthermore to see its reliability as a marker of kidney restoration capacity.

Quantitative ^{99m}Tc-DMSA renal uptake was studied in different renal I/R mice models. Mouse models of uni-lateral I/R, bi-lateral I/R and uni-nephrectomized I/R were established. As a control, sham operated mice were also used. Higher uptake of ^{99m}Tc-DMSA was measured in normal left kidney of Uni-I/R model followed by nephrectomized I/R kidney at all time point except at 1 h and 1 d post I/R, compared to the sham and Bi-I/R treated counterpart kidneys. Statistically significant increase in the size of kidneys of nephrectomized group was also measured compared with the other groups ($p < 0.05$) (Fig 3B). As a physiological compensatory mechanism for the removed kidneys (nephrectomy) and damaged kidneys (Uni-I/R), an increased functional status of the remaining contralateral kidneys is factual as was demonstrated by an increased ^{99m}Tc-DMSA uptake between 62% and 70% over sham operated counterpart kidneys at 3 wk post-I/R. This increase in uptake by the contralateral kidneys could be due to hyper-functional uptake, by existing nephrons and not functional volume enlargement. This result was in agreement with Yen's finding demonstrating hyper-functional uptake of ^{99m}Tc-DMSA by the contralateral kidney in response to uni-lateral kidney disease [29]. In this study, quantitative SPECT of ^{99m}Tc-DMSA uptake has showed a hyper-functional uptake of the radiotracer by the contralateral kidney which is proportional to the diminished uptake by the diseased kidney [29].

Comparing radiotracer uptake of the damaged kidneys of both uni-lateral and bi-lateral mice models, greater uptake was measured in the bi-lateral mice models almost at each time point of imaging, implying higher repair capacity in the right kidney of Bi-I/R model than its counterpart in Uni-I/R models. Three weeks after uni-lateral ischemia, the size of ipsilateral kidney was also significantly smaller than non-ischemic contralateral kidneys as well as sham and Bi-I/R counterpart kidneys ($p < 0.05$), indicating that the recovery of uni-laterally ischemic kidney is more delayed than bi-lateral ischemia model (Fig. 3). The presence of a normal contralateral kidney might negatively influence mechanisms contributing to restoration of affected renal tubular cells. This finding is in compatible with other studies on observations of the adverse effects of the presence of the contralateral normal kidney on the functional recovery of a uni-laterally ischemic kidney [30,31]. The uptake of both the affected kidneys in uni-lateral and bi-lateral I/R models were found to be continuously decreasing over 3-wk study period, indicating that longer time might be required for full recovery of the affected kidneys.

The patterns of reduced ^{99m}Tc-DMSA uptake in the right and left kidneys of the Bi-I/R models closely paralleled as compared to the sham operated counterpart kidneys. However, the uptake was a bite higher in the right kidneys compared with the left both in the sham and Bi-I/R models. This could be due to the fact that the size of right kidney of mice is normally bigger than its counterpart left kidney. The low uptake by the affected kidneys as compared to the sham operated kidneys could be the result of lost functional volume, not diminished uptake, by the surviving nephrons [32].

Consistent with ^{99m}Tc-DMSA uptake results, most severe histopathological changes characterized by dilated and disrupted tubules with necrotic debris, great expansion of interstitium, increased interstitial cells and reduction of brush borders were seen in kidney samples from Uni-I/R models stained by PAS (Fig. 4). These morphological changes were minimal in nephrectomized kidney samples while being intermediate for Bi-I/R models. This result was also in agreement with our comparative study of weight of each I/R injured kidney after 3 wk of study period. The plot approaches the line of identity, indicating a virtual one-to-one relationship between ^{99m}Tc-DMSA uptake and kidney weight gain (Fig. 3C). Accordingly, the ratio of nephrectomized kidney to body weight was significantly increased, compared with its counterpart from the other three groups, while that of injured kidney of Uni-I/R is dramatically decreased at 3 wk post-I/R ($p < 0.05$) (Fig. 3B). It is worth to note that even though bi-laterally affected kidneys showed much severe morphological change compared to sham operated kidneys, the size and weight of kidneys excised from sham operated group and bi-lateral group are comparable while ^{99m}Tc-DMSA kidney uptake of Bi-I/R group is significantly lower

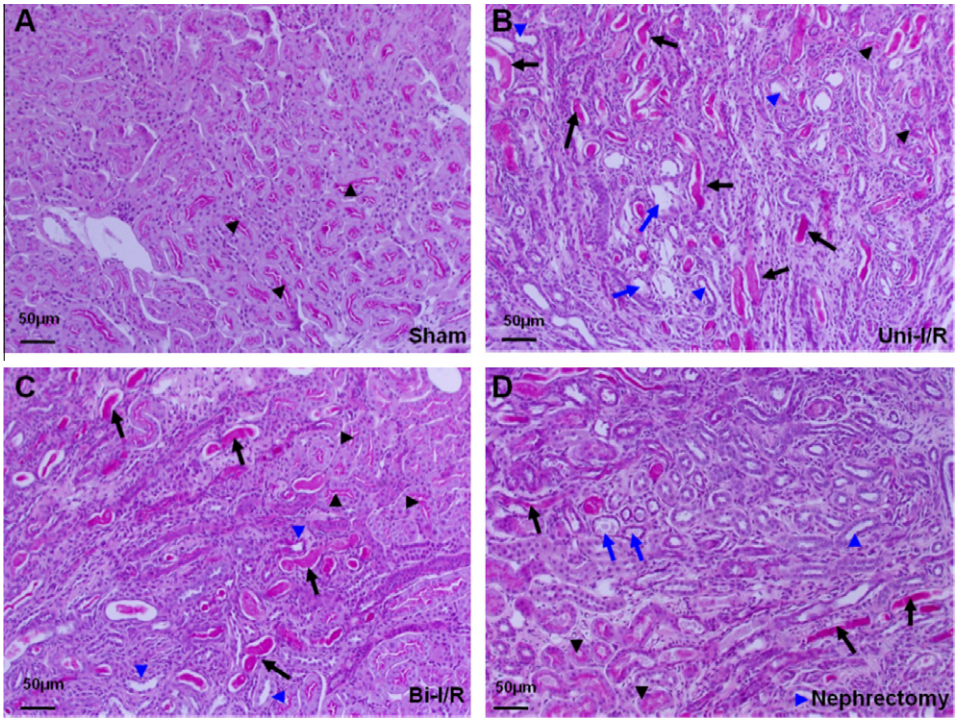

Fig. 4. Light microscopy showing representative sections of the kidney from sham (A), Uni-I/R (B), Bi-I/R (C) and Nephrectomy-I/R (D) operated mice after 3 wk of operation (PAS, ×40). Blue and black arrows indicate tubules with necrotic debris and cast formation respectively while arrow heads of same color indicate tubular dilatations and PAS positive brush borders respectively. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

than that of sham group. This fact clearly indicates that ^{99m}Tc-DMSA scan reflects more correctly the kidney damage/recovery status than gross examination of excised kidneys.

The results of the present study show that ^{99m}Tc-DMSA scan is a promising noninvasive method for evaluation of restoration status of damaged renal tubular cells. The ^{99m}Tc-DMSA uptake by renal tubular cells is directly correlated with degree of kidney damage as was confirmed by histological study. Interestingly our experiments also indicate that mice with Bi-I/R had higher repair capacity than those with Uni-I/R, which is contrary to what usually is anticipated.

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