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In Vivo X-ray Imaging of Transport of Renal Clearable Gold Nanoparticles in the Kidneys

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Abstract

With more and more engineered nanoparticles (NPs) being designed renal clearable for clinical translation, fundamental understandings of their transport in the different compartments of kidneys become increasingly important. Here, we report noninvasive X-ray imaging of renal clearable gold NPs (AuNPs) in normal and nephropathic kidneys. By quantifying the transport kinetics of the AuNPs in cortex, medulla and pelvis of the normal and injured kidneys, we found that ureteral obstruction not just blocked the NP elimination through the ureter but also slowed down their transport from the medulla to pelvis and enhanced the cellular uptake. Moreover, the transport kinetics of the NPs and renal anatomic details can be precisely correlated with local pathological lesion. These findings not only advance our understandings of nano-bio interactions in the kidneys but offer a new pathway to noninvasively image kidney dysfunction and local injuries at the anatomical level.

Graphical abstract

High-contrast noninvasive X-ray imaging of transport process of renal clearable gold nanoparticle allows quantitative understanding of nano-bio interactions in the normal and injured kidneys at the anatomical level.

Keywords

kidney; nano-bio interactions; NPs; renal clearance; X-ray imaging

Fundamental understandings of *in vivo* nano-bio interactions are key to future clinical translation of nanomedicines. In the past decades, significant progress has been made in quantitative understandings of nano-bio interactions in the liver^[1] and tumor

microenvironment.^[2] However, there are still limited studies on how engineered NPs interact with the kidneys^[3] even though more and more nanomedicines are designed renal clearable to expedite their clinical translation. For example, Choi et al. reported 3 nm cysteine-coated CdSe/ZnS quantum dots, 75% of which can be excreted in urine within 4 h after intravenous injection^[4]. Furthermore, they also demonstrated their applications in cancer imaging in the preclinical settings.^[5] Bradbury et al. developed 6-7 nm dye-doped silica NPs (C dots) that have renal clearance efficiency of 73% at 48 h post intravenous injection in mice.^[6] The whole body clearance of C dots was only 13~21 hours in humans.^[7] We reported the first renal clearable luminescent AuNPs^[8] and demonstrated their preclinical applications in cancer detection^[9] and noninvasive fluorescence kidney functional imaging.^[10] With the emergence of more and more renal clearable NPs,^[11] it becomes urgent to fundamentally understand how these renal clearable NPs are transported in the different compartments of the kidneys with normal or injured functions at the anatomical level; so that their future promise can be fulfilled while their potential hazards, in particular, to the patients with kidney dysfunctions, can be minimized.

Herein, we reported our pivotal studies on *in vivo* X-ray imaging of interaction between renal clearable AuNPs and the kidneys with normal function and unilateral ureteral obstruction (UUO). Our results show that the accumulation and clearance of renal clearable AuNPs in normal and obstructed kidneys can be quantitatively and noninvasively evaluated with planar X-ray imaging at contrast 6 times higher than iodine complexes. In the kidneys with normal function, transport kinetics of AuNPs in cortex, medulla and pelvis of kidney follow a simple one-component exponential decay with comparable half-lives. However, in the dysfunctional kidney with ureteral obstruction, the transport of the AuNPs from the cortex and medulla into the pelvis was significantly slowed down while the transport of the NPs into the cortex was not delayed. Moreover, high-penetration depth and high-spatial resolution of X-ray imaging allowed us to precisely identify the location of impairments, which caused significant alteration in the clearance kinetics of the AuNPs.

The synthesis and characterization of 2.5 nm renal clearable near-infrared-emitting glutathione-coated AuNPs (GS-AuNPs) were described in our previous work.^[9c] To test whether a simple planar X-ray imaging can readily detect the accumulation and clearance of GS-AuNPs in kidneys, we intravenously injected the CD-1 mice with GS-AuNPs at a dose of 1g/kg body weight (corresponding to 0.69 g Au/kg). This dose was 3.9 times less than that used by commercially available AuNPs for X-ray imaging of mice (2.7 g Au/kg).^[12] The histological study confirmed that the injected GS-AuNPs were well tolerated by CD-1 mice and no structural changes were found in major organs at Day 1 and Day 14 post injection (p.i.; Figure S1). With planar X-ray imaging, the elimination of GS-AuNPs through the urinary system indeed can be noninvasively monitored at high contrast: the GS-AuNPs were clearly observed in the renal cortex, medulla and pelvis of kidney at around 2 min p.i., followed by the clearance through the pelvis into the ureter and then into the bladder during 2-5 min p.i.; the bladder became detectable at ~2 min p.i. and was gradually filled with AuNPs until 30 min p.i. (Figure 1A&1B and Supplementary Movie S1). The limits of detection of GS-AuNPs were 2.0×10^{16} NPs per gram of kidney tissue *in vivo* and 9.6×10^{14} NPs in PBS (Figure S2&3 and Table S1). The dose of 1 g/kg was considered as the minimal dose for imaging the NP transport in the kidneys (Figure S4). On the other hand, it should be

noted that background tissues indeed have interference on the accurate qualification of the percentage injection dose (%ID) of GS-AuNPs in the kidneys and bladder *in vivo* (Figure S3 and Table S2&3) at low contrast.

Such high-contrast X-ray images of the renal clearance process cannot be readily achieved with diatrizoate meglumine (DM), a clinically used iodine agent, under the same imaging conditions. While the accumulation of DM in the bladder was observed, its accumulation in and clearance from the kidneys and ureters was barely detected after intravenous injection of DM at a dose containing the same amount of iodine atoms as gold atoms in the injected GS-AuNPs (DM 1×; Figure 1C&S5). Quantitative analysis shows that the percentage of kidney contrast enhancement by GS-AuNPs was nearly six times higher than that by DM at 4 min p.i. (27.9 % vs. 4.4%) (Figure 1D&S6). Since X-ray attenuation by gold is about four times of that by iodine (Figure S7), we intravenously injected the mice with four-time concentrated DM (DM 4×), which has equal X-ray attenuation as GS-AuNPs. While the kidney contrast indeed increased (Figure 1C&S8), the maximal percentage kidney contrast enhancement by DM was only 10.3%, still nearly three times less than that by GS-AuNPs (27.9%; Figure 1D). These results clearly indicate that high-contrast X-ray imaging of the kidneys enabled by GS-AuNPs *in vivo* is not only because of high X-ray attenuation of gold atoms but also due to longer retention of GS-AuNPs in the kidneys than DM, which allows us to further quantify the clearance kinetics of the NPs in the different kidney substructures. The clearance kinetics of GS-AuNPs from renal cortex and medulla to pelvis (nephrographic phase) and from pelvis to ureter (pyelographic phase)^[13] were analyzed by comparing the decay half-lives of the time-dependent X-ray density curves (TXDCs) derived from both kidneys, which were 8.6 ± 5.2 min and 8.9 ± 3.6 min for corticomedullary area and pelvic area, respectively (N=8, P>0.05; Figure 1E, Figure S9 and Table S4). No significant differences in the decay half-lives suggested that GS-AuNPs have no strong interactions with specific components of urinary system in normal mice.^[13–14]

To quantitatively understand how the renal disease affects the transport of GS-AuNPs through kidneys, we established a well-known kidney injury model, unilateral ureteral obstruction (UUO) model, where left ureter was ligated and tubular injury was induced in the left kidney while the right kidney remained intact (Figure 2A).^[2b] As control, the left ureter was exposed but not ligated in sham-operated group. One day after operation, UUO and contralateral kidneys showed distinct accumulation and transport kinetics of GS-AuNPs (Figure 2B and Supplementary Movie S2). The two elimination phases, nephrographic phase and pyelographic phase, were no longer distinguishable in the left UUO kidney while these two phases were still clearly visualized in the unobstructed right kidney (Figure 2B&S10A). For the UUO kidney, the accumulation of GS-AuNPs in the renal cortex and medulla reached the maximum at 6.5 ± 4.2 min (Figure 2C,2D&S10), statistically comparable to the time points in contralateral right kidneys (5.2 ± 1.5 min) and sham kidneys (8.7 ± 3.4 min) (P>0.05; Figure S11; Table S5). This indicated that the transport of GS-AuNPs from bloodstream to glomeruli in renal cortex was not significantly delayed in the obstructed kidney even though less GS-AuNPs entered into the diseased kidney due to reduced blood perfusion. The maximal total X-ray density (=average X-ray density times area of kidney) of the UUO left kidney (859.0 ± 253.3) was significantly less than those of contralateral right kidney (2893.3 ± 1074.3) and left kidney (1795.0 ± 420.2) in sham mice (P<0.05; Figure 2E

and Table S6). On the other hand, the elimination of GS-AuNPs from renal cortex and medulla into the pelvis was significantly slowed down in the obstructed kidney. For normal kidneys in sham mice and UUO model (unobstructed right kidney), X-ray density of cortex and medulla exhibited a sharp decay after 5-9 min with half-lives of 11.0 ± 6.9 min and 10.8 ± 7.0 min, respectively, representing efficient clearance of GS-AuNPs from cortex and medulla into the pelvis (Table S5) and nearly 65-70% of GS-AuNPs cleared out of the kidneys at 60 min p.i. (Figure 2F and Table S5). In contrast, the UUO kidneys generally showed no obvious clearance of particles from cortex and medulla despite variation among the mice. One UUO kidney exhibited no clearance at all during 0-60 min p.i., and only average $22.1 \pm 13.7\%$ of GS-AuNPs was cleared out of the kidneys in the other seven UUO kidneys, just one third of the value of normal kidneys (Figure 2F and Table S6).

The delayed clearance of GS-AuNPs from the injured kidneys altered the interaction between NPs and kidney cells. As shown in the X-ray image of the UUO kidney at 60 min p.i., GS-AuNPs were selectively retained on the interface between the pelvis and medulla while contralateral kidney showed very low X-ray density at the same time point (Figure 3A). To further understand this selective accumulation of GS-AuNPs in UUO kidney, we conducted histological studies on the both normal and obstructed kidneys with H&E staining and silver staining (Figure 3B). In unobstructed contralateral kidneys, even though the kidneys have been exposed to large amount of GS-AuNPs, the kidney tissue remained normal and GS-AuNPs were mainly accumulated in the cortex at 1 h p.i. (Figure S12A). For UUO mice on Day 1 post operation, while glomeruli and proximal tubules in cortex remained normal and contained particles (Figure S12B), extensive tubular dilatation and atrophy were found on the boundary of pelvis and medulla, where transport of GS-AuNPs was interrupted and NPs were retained in the tubular lumen (1 h p.i.; Figure S13). These results indicated that the slow transport of GS-AuNPs within the damaged medulla caused the unique distribution of GS-AuNPs in the UUO kidney. On Day 8 post operation when tubular dilatation and atrophy were more pronounced (Figure S14A), some AuNPs appeared inside the normal tubular epithelial cells at the junction of cortex and medulla, right below in medullary area where extensive tubular impairment was spotted (Figure S14B). These results indicate that the tubular cells started to internalize GS-AuNPs that could not be eliminated. Therefore, pathological change of kidneys not just altered the distribution pattern of GS-AuNPs but also changes their interaction with kidneys at the cellular level.

In summary, noninvasive dynamic X-ray imaging of renal clearance of GS-AuNPs allowed us to obtain quantitative understanding of NP transport and nano-bio interactions *in vivo* in the normal and injured kidneys. High contrast in the X-ray imaging of GS-AuNPs is not just because gold atom has a large X-ray attenuation coefficient but is also because GS-AuNPs have longer retention in the kidneys compared to iodine agents. Assisted with X-ray imaging, we found that in normal mice renal clearable AuNPs had uninterrupted transport in different components of urinary system, which all follows a simple one-component exponential decay with comparable kinetics. However, in the kidney with unilateral ureteral obstruction, the transport of renal clearable AuNPs from the cortex and medulla into the pelvis was significantly slowed down because of the dilatation and atrophy in medulla. Moreover, high-penetration depth and high-spatial resolution of X-ray imaging allowed us to precisely locate impairments noninvasively and correlate the injury with the changes in peak

time, maximal total X-ray density, clearance percentage at 60 min and relative renal function (%RRF) (Figure S15). Together with our ongoing studies on the correlation of kidney clearance kinetics of GS-AuNPs with glomerular filtration rate,^[15] these new findings not only advance our fundamental understandings of NP transport in normal and injured kidneys and further broaden renal clearable AuNPs in the kidney functional evaluation at anatomical level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A) A scheme showing the urinary system that consists of the kidneys, ureters and bladder. B) Representative noninvasive X-ray images of CD-1 mice before and after intravenous injection of 1g/kg GS-AuNPs before injection and at 2, 5 and 30 min p.i. (yellow arrows point to substructures of the kidney and components of urinary system ("C & M", cortex and medulla; "P", pelvis; "B", bladder). C) Images of the urinary system of mice after intravenous injection of diatrizoate meglumine (DM) solution containing same amount of iodine atoms as gold atoms in GS-AuNPs (DM, 1×) and four-time concentrated DM (DM, 4×) at maximum contrast enhancements. D) Percentage of contrast enhancement (%) of left kidney after intravenous injection of GS-AuNPs. Right kidneys showed the same trend (Figure S6). E) Representative time-dependent X-ray density curves (TXDCs) derived from cortex, medulla and pelvis in kidneys obtained from noninvasive X-ray images of mice after injection of GS-AuNPs during 0-60 min.

Figure 2.

A) A scheme showing the unilateral ureteral obstruction (UVO) mice model by complete ligating of left ureter. RK, right kidney; LK, left kidney. B) Representative X-ray images of the urinary system in UVO mice before and after intravenous injection of GS-AuNPs. C) Representative TXDCs derived from cortex and medulla in obstructed left kidney (UVO LK), and cortex, medulla and pelvis in contralateral right kidney in UVO mice. D) Statistical analysis of peak time, E) maximal total X-ray density, and F) clearance percentage at 60 min of cortex and medulla (CM) in kidneys in UVO mice and sham-operated mice. "ns", no significant difference; *P < 0.05, **P < 0.01.

Figure 3.

A) *In vivo* X-ray image of kidneys of 1 day UUO mouse taken at 60 min p.i. of GS-AuNPs zoomed in at kidney area. Arrow indicated the area where NPs were selectively retained, and dash line indicated contralateral kidney. B) H&E and silver-stained section from UUO kidney and contralateral kidney shown in panel A which were harvested right after 60-min dynamic X-ray imaging. Dash circles indicated medullary area; arrows pointed the dilated tubules where large amount of GS-AuNPs were accumulated.