Renal excretion

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Carbon-Nanotube Shape and Individualization Critical for Renal Excretion**

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In the past few years there has been an accumulating amount of evidence to suggest that the structural features of nanoparticles are responsible for dramatically different pharmacokinetic and body-excretion profiles. For example, a recent study[1] convincingly illustrated that the mean hydrodynamic diameter of quantum dots (spherically shaped nanocrystals) is a determinant factor in achieving effective urinary excretion through the renal filtration barrier. In that study, however, the effect of nanoparticle shape on renal filtration was not examined at all. Our group has investigated the pharmacokinetic and excretion profiles of nonspherical, fibrilous carbon nanotubes (CNTs) and, in the present study, we have attempted to elucidate the mechanism by which such cylindrical nanoparticles can be excreted through the renal route.

We have previously reported that surface-functionalized, water-dispersible, single-walled carbon nanotubes (SWNTs; average diameter 1 nm; average length 300–1000 nm) were capable of rapid and effective renal clearance and urinary excretion with a blood-circulation half-life of a few hours.[2] These observations were in agreement with other studies performed independently by Wang et al.[3] using hydroxylated SWNTs (SWNT-ols) with an alternative functionalization chemistry and McDevitt et al.[4] with SWNTs functionalized similarly to ours using cycloaddition chemistry. Both of these studies reported that the majority of the injected SWNTs rapidly cleared the body with the majority of the radioactivity found in urine. More recently, we have reported that systemic administration of larger multi-walled carbon nanotubes (MWNTs: average diameter 20–30 nm; average length 500–2000 nm) led to clearance from the bodies of rodents via the kidneys and bladder, as studied by dynamic whole-body single-photon-emission-computed tomography (micro-SPECT/CT) imaging.[5] In both cases, SWNTs and MWNTs have also been observed intact in the excreted urine of the animals by transmission electron microscopy (TEM).[2] Interestingly, our reports of carbon-nanotube urinary excretion have also been reported by different groups, all using water-dispersible, functionalized CNTs.[6] However, the elimination mechanism of cylindrical-shaped structures, such as CNTs, through the glomerular filtration membrane remains to be directly determined. In this work, we focused on the elucidation of the possible mechanism by which CNTs could translocate through the glomerular filtration system into the urine by histological TEM in the absence of any probe molecules.

In order to investigate the crucially important mechanism of CNT elimination from the blood compartment and, in particular, their translocation through the kidneys, we carried out TEM imaging of ultrathin renal cortex sections (70 nm) of BALB/c mice 5 min and 30 min after intravenous (tail vein) administration of MWNTs. Figure 1a shows the characteristic structure of the renal glomerulus formed by a mesh of capillaries (insert of Figure 1a) and depicts all the components that constitute the glomerular filtration barrier: the fenestrated endothelium (FE), basal membrane (BM), and podocytes (P). Individualized, well-dispersed MWNTs were observed in the renal capillary lumen and during translocation through the glomerular filtration barrier with their longitudinal axis vertically oriented to the endothelial fenestrations, as can be seen in Figures 1c and d (arrows indicate MWNTs). As previously reported, the MWNT excretion lasts for a few hours (blood circulation half-life $\approx$3 h) and, as shown in Figure 1, we observed MWNTs translocating both at 5 min and at 30 min post injection.

We have also repeatedly observed that administration of CNT dispersions that were not adequately individualized, or that have aggregated in vivo, could be found in the glomerular capillaries and were not able to translocate through the kidney filtration system, as seen in Figure 1b (see dashed line around the MWNT bundle and Supporting Information, Figure S1). This indicates that dimension, shape, and structural characteristics of the individual nanoparticles are extremely important, provided that adequate individualization is achieved in vivo. If the injected nanomaterial is in aggregates or bundles, the latter will not be able to cross the glomerular filter and will accumulate in the liver, spleen, or lungs, as has been observed by others.[7,8] Differences in the individualization of CNTs before administration or varying degrees of CNT aggregation in vivo (after administration) are thought to be responsible for the dramatically different pharmacokinetic and excretion profiles

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Supporting Information is available on the WWW under http://www.small-journal.com or from the author.

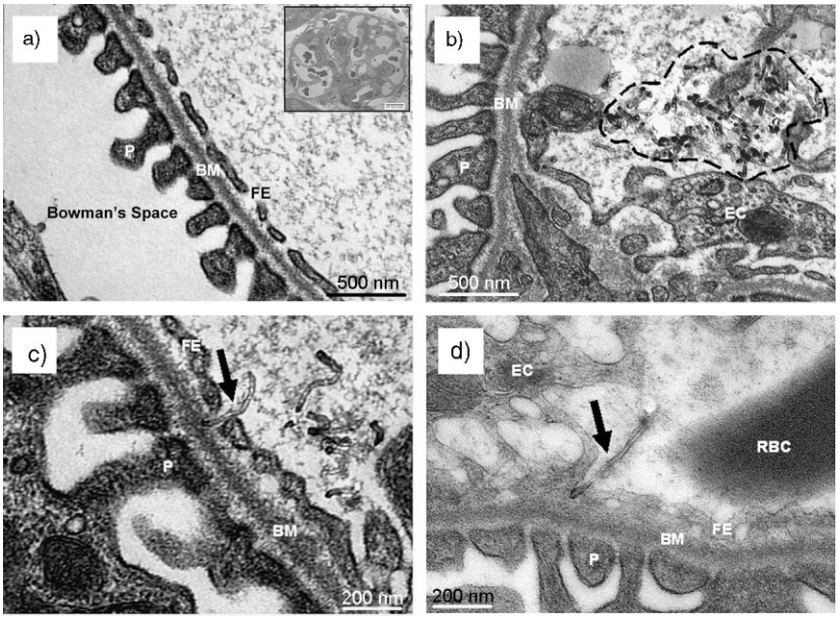

Figure 1. Renal clearance of carbon nanotubes. a) Glomerular filtration barrier of BALB/c mice: P, podocyte; BM, basal membrane; FE, fenestrated endothelium. Inset: section of renal glomerulus (scale bar is 10 μm); b) bundled MWNT agglomerate (circled by dashed black line) in the lumen of glomerular capillary; c) individualized MWNTs (black arrow) 5 min after intravenous (tail vein) injection; d) individualized MWNT (black arrow) 30 min after intravenous (tail vein) injection crossing the renal filtration membrane. EC, endothelial cell; RBC, red blood cell.

that have been reported in the literature recently using different types of CNT.

The mechanism by which CNT renal clearance occurs indicates the sharply different elimination characteristics that individualized, well-dispersed nanotube structures can exhibit in vivo compared to globular proteins and spherically shaped nanoparticles. Moreover, we speculate that rigid, long proteins will also behave differently because they lack the hollow, light cylindrical structure of the CNT. Since the MWNT length imaged in Figure 1 is significantly larger than the dimensions of the glomerular capillary wall (minimum diameter of fenestra is 30 nm; thickness of the glomerular basement membrane in rodents and humans is 200–400 nm, and width of the epithelial podocyte filtration slits is 40 nm),[9] the longitudinal nanotube dimension does not appear to be a critical parameter in renal clearance.

The mechanism by which nanotubes pass through the glomerular filtration barrier involves the acquisition of a spatial conformation in which the longitudinal CNT dimension is perpendicular to the endothelial fenestrations, since only the traverse dimension of CNT (cross section is between 20–30 nm) is small enough to allow permeation. This suggests that MWNTs are capable of reorientation while in blood circulation. They are readily able to pass into Bowman's space and subsequently accumulate in the bladder and be excreted in urine. Our observations further reinforce the view that glomerular capillary permeability is dependent on a multitude of factors including size, surface charge, and shape of materials,[10,11] and that nanofiber-shaped materials, such as the MWNTs used here, are able to permeate through this barrier almost readily.

The atypical pharmacological profiles of cylindrical-shaped, filamentous block-copolymer micelles in blood circulation have also been reported recently,[12] indicating that the shape of injected nanoparticles is critically important. In concert with such observations, we believe that a fine balance between CNT shape, backbone structure, surface character, and degree of individualization will determine the pharmacological and excretion profile of these fibrilous nanomaterials in vivo. Moreover, recent reports have experimentally described water flow rates through CNTs that, due to the almost frictionless water–carbon-backbone interface, are orders of magnitude faster than what would be predicted from conventional hydrodynamics theory.[13,14] Such unique CNT properties are also thought to contribute towards the considerable glomerular hyperpermeability observed in our studies, particularly in view of the hydraulic permeability and resistance properties of the glomerular capillary wall.[15]

The direct imaging of CNT at the blood–urine interface, and their translocation through the glomerular filter, illustrates the mechanisms by which novel nanomaterials interact with the biological milieu in vivo and experimentally indicates the critical role played by nanomaterial shape and the need for nanoparticle individualization in vivo. Clinical and pharmaceutical development of nonbiodegradable nanomaterials, such as quantum dots and CNTs, will only be made possible if body clearance and excretion of the vast majority of administered doses is achieved, so as to minimize organ accumulation and the duration of nanomaterial contact with healthy tissue.

Experimental Section

MWNTs: Nonfunctionalized, purified MWNTs (p-MWNTs) were purchased from Nanostructured & Amorphous Materials Inc. (Houston, USA). Regular pMWNTs used in this study were 94% pure, stock No. 1240XH. Outer average diameter was between 20 and 30 nm, and length between 0.5 and 2 μm. Pre-incubation in mouse serum (Sera Laboratories International Lda., UK) led to bundled aqueous dispersions of p-MWNTs. Functionalized MWNTs (f-MWNTs) were prepared following the 1,3–dipolar cycloaddition reaction, leading to significant increases in unbundling and individualization of the MWNTs in phosphate buffered saline (PBS), as previously described.[2] All MWNTs used in this study have been fully characterized and reported previously. For detailed information regarding the characterization of the nanotubes before and after functionalization, please see Reference [5].

Briefly, the level of elemental impurities was found to be 10.6% (Fe and Ni) in the p-MWNTs and 8.1% in f-MWNTs, as determined by atomic absorption analysis and thermogravimetric analysis. A mixture of closed- and open-ended nanotubes was found in all samples by TEM and cryo-TEM.

Animal handling procedures: Six-week-old, female BALB\c mice (Harlan, UK) were bedded on wood shavings and maintained on a standard rodent chow diet with mains drinking water ad libitum. A temperature of 19–22 °C was maintained, with a relative humidity of 45–65% and a 12 h light/dark cycle (lights on at 7:00 am). The animals were acclimatized for seven days before experiments. All procedures were approved by the Home Office (1989) Code of Practice for the Housing and Care of Animals used in Scientific Procedures (UK). Before the administration of MWNTs, the animals were anesthetized by inhalation with isoflurane (3% in 100% oxygen). The animals were intravenously injected in the tail vein with 400 μg mouse^{-1} of highly individualized f-MWNTs or 400 μg mouse^{-1} of bundled, nonfunctionalized pMWNTs. At 5 and 30 min post injection, the animals were euthanized by cervical dislocation under deep isoflurane anesthesia.

TEM imaging of kidney sections: Kidneys were collected from mice and samples of approximately 1 mm^3 in volume immediately fixed in phosphate buffered 2.5–3% glutaraldehyde (pH 7.3). Later, the kidney samples were post fixed in 1% aqueous osmium tetroxide for 90 min. After dehydration of the tissue in a series of ethanol immersions, the samples were embedded in resin, which was allowed to polymerize at 60 °C for at least two days. Ultrathin sections (70 nm) of kidney samples were cut with an ultramicrotome (diamond knife) and placed on copper TEM grids. The grids were stained with uranyl acetate and lead citrate and imaged with a Philips CM 10 TEM or a Hitachi H7600 TEM. Images were captured using a high-sensitivity digital camera.

Keywords:
carbon nanotubes · excretion · kidneys · nanoparticles · pharmacology · TEM

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