Improved Tissue Distribution, Renal Clearance and Toxicity Profile of Functionalized Carbon Nanotubes

L. Lacerda[*], M. Prato[**], A. Bianco[***] and K. Kostarelos[*]

[*]Nanomedicine Lab, CDDR, The School of Pharmacy, University of London
29-39 Brunswick Square, WC1N 1AX London, UK, kostas.kostarelos@pharmacy.ac.uk
[**]University of Trieste, Trieste, Italy
[***]CNRS, Strasbourg, France

ABSTRACT

In this communication we discuss the *in vivo* parameters such tissue distribution, body clearance and toxicity following administration of carbon nanotubes (CNT). For this propose, therapeutically-relevant doses of serum protein-coated purified CNT (pCNT) and functionalized CNT (*f*-CNT) were administered intravenously in rodent species[1-3]. Striking differences were found between the liver and lung tissues of animals injected with nanotubes. pCNT exhibited significant accumulation in both tissues 24 h post-administration. *f*-CNT circulated in systemic blood circulation for 5 min, accumulated in the renal cortex and rapidly trespassed the glomerular filtering system and excreted in urine in the absence of any tissue accumulation or damage. The biocompatibility and biodistribution of CNT appears to be dramatically dependent on the degree of surface functionalization of these nanostructures, making *f*-CNT viable material for a variety of biomedical applications.

Keywords: carbon nanotubes, degree of functionalization, intravenous administration, *in vivo* toxicity, renal excretion

1 INTRODUCTION

Carbon nanotube (CNT) development for biomedical applications is in the nascent stages, however it is thought to lead to novel types of diagnostic, therapeutic and regenerative nanomedicines[4]. The construction of CNT-based delivery systems able to traffick intracellularly and deliver drug molecules including nucleic acids requires engineering nanotube features in order to achieve the efficiency in delivering the therapeutic molecules at the target sites and cellular compartments. However, it is imperative to determine critical *in vivo* parameters, namely their toxicological and pharmacological profiles, before any clinical application of CNT is contemplated[5]. Here we discuss the pharmacological and toxicological profiling of CNT, along with the limitations and opportunities these nanostructures bring in order to develop new delivery systems for nanomedicine.

2 CARBON NANOTUBES (CNT)

CNT have very interesting physicochemical properties such as: ordered structure with high aspect ratio, ultra-light weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behavior and high surface area. The combination of these characteristics make the CNT a unique material with the potential for diverse applications, including diagnostics and therapeutics[6].

One of the most important parameters in *in vitro* and *in vivo* studies with CNT is the type of nanotubes used, which is determined by the process by which they are made biocompatible. Interactions with cells and tissues have to be performed using biocompatible CNT, achieved by either covalent or non-covalent surface functionalization that results in water-dispersible CNT. A variety of different functionalization strategies for single and multi-walled CNT (SWNT and MWNT, respectively) have been reported by different groups[7]. In our laboratories, CNT were made compatible to physiological environments after functionalization by the 1,3-dipolar cycloaddition reaction (Figure 1)[8, 9].

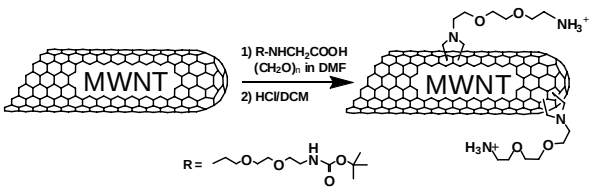

Figure 1. Scheme of 1,3-dipolar cycloaddition reaction performed on MWNT.

3 TISSUE DISTRIBUTION

In order to determine the tissue distribution of *f*-CNT, we have covalently attached onto the nanotube surface one of the most clinically established chelating molecule diethylentriaminepentaacetic dianhydride (DTPA), which was subsequently used to cage the γ-emitting radionuclide ^{111}In (Figure 2). Then, the [^{111}In]DTPA-CNT were dynamically tracked by single photon computed tomography (SPECT) imaging immediately after intravenous injection in rats[2]. In addition, the presence of *f*-CNT in several tissues was quantified by gamma counting at different time points after intravenous administration of the [^{111}In]DTPA-CNT in rats and mice[1, 2].

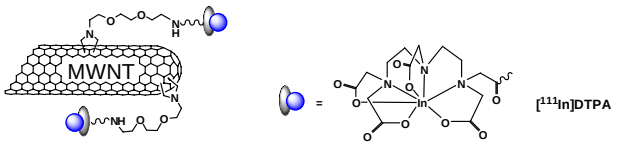

Figure 2. Molecular structure of [^{111}In]DTPA-CNT.

Following the administration by tail vein injection of [^{111}In]DTPA-CNT, these nanostructures were able to circulate for ~3 h[1]. Moreover [^{111}In]DTPA-MWNT accumulated in the kidneys and were excreted intact in the urine within 24 h post-administration[1, 2].

4 TOXICITY OF CNT

CNT are classified as 'nanoparticles' due to their nanoscale dimensions, therefore unexpected toxicological effects upon contact with biological systems may be induced. The nanometer-scale dimensions of CNT make quantities of milligrams possess a large number of cylindrical, fibre-like particles, with a concurrent very high total surface area. This total surface area will also depend on their degree of bundling and aggregation of nanotubes in solution. We examined whether the intravenous administration of different MWNT (Figure 3) induced any tissue injury or other histological or physiological abnormality on the organs that have previously been shown to interact with the nanotubes *in vivo* during the initial 24 h following injection in two different rodent species, rat and mouse[2, 3]. Non-functionalized pMWNT and water-dispersible *f*-MWNT were compared in order to verify the impact of functionalization on the *in vivo* profile of these nanostructures.

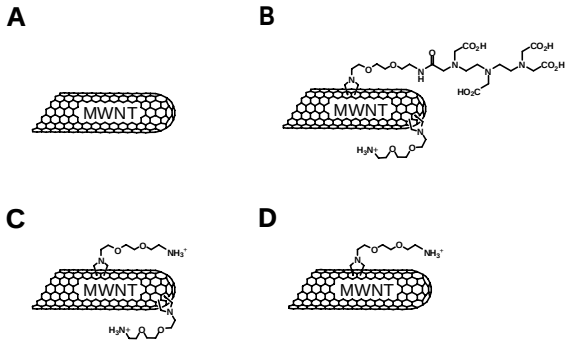

Figure 3. Molecular structures of pMWNT (A), MWNT-DTPA (B), MWNT-NH_3^+ with high loading of NH_3^+ (C), and MWNT-NH_3^+ with low loading of NH_3^+ (D).

Intravenous administration of non-functionalized pMWNT led to lung, liver and spleen accumulation. On the other hand, *f*-MWNT accumulation in organs was dependent on the degree of functionalization but was found to be independent of the characteristics of the functional group[2, 3].

5 PROS & CONS

The unique mechanical properties of CNT offer *in vivo* stability and the capacity to readily cross biological barriers opens the door to development of novel delivery systems. Moreover, the unique electrical and semiconducting properties and extremely large aspect ratio offers a template for the development of multimodal *in vivo* devices. In addition, the hollow, fibrous, light structure with different flow dynamics properties is advantageous in *in vivo* transport kinetics and lastly mass production – low cost makes CNT attractive to drug development.

However, pCNT (as prepared, non-functionalised) are inherently hydrophobic, therefore the main obstacle in the utilization of CNT in biology and medicinal chemistry is their lack of solubility in most solvents compatible with the biological milieu (aqueous based). To overcome this problem the modification of the surface of CNT (functionalisation) with different molecules is achieved by adsorption, electrostatic interaction or covalent bonding of different molecules and chemistries that render them more hydrophilic. Through such modifications, water solubility of CNT is improved and their biocompatibility profile completely transformed. Moreover, the bundling/ aggregation of individual tubes through van der Waals forces is also reduced by the functionalization of their surface. Nevertheless, severe limitations persist as the production of structurally and chemically reproducible batches of CNT with identical characteristics, high quality control and minimal impurities is still a challenge to the

pharmaceutical and clinical application of these nanomaterials.

6 CONCLUSIONS

The development of nanomedicine depends on determining the toxicological and pharmacological profiles of extremely promising and novel materials. The *in vivo* toxicological and pharmacological studies undertaken so far indicate that *f*-CNT can be developed, contrary to non-funtionalized, pCNT. Functionalization renders the surface of CNT water-soluble, compatible with biological fluids and leads to their rapid excretion through the renal route, minimising unwanted tissue accumulation. Therefore, the door of opportunity for the development of CNT as diagnostic and therapeutic nanomedicines has opened, and systematic study of their therapeutic efficacy and safety is expected in the next few years.

REFERENCES

[1] Singh R., Pantarotto D., Lacerda L., Pastorin G., Klumpp C., Prato M., Bianco A. and Kostarelos K., Tissue Biodistribution and Blood Clearance Rates of Intravenously Administered Carbon Nanotube Radiotracers. Proc. Natl. Acad. Sci. USA 2006, 103, (9), 3357-3362.

[2] Lacerda L., Soundararajan A., Pastorin G., Al-Jamal K. T., Herrero M. A., Bao A., Emfietzoglou D., Mather S., Phillips W. T., Prato M., Bianco A., Goins B. and Kostarelos K., Dynamic Imaging of Functionalised Multi-Walled Carbon Nanotube Systemic Circulation & Urinary Excretion. Adv. Mater. 2008, 20, (2), 225-230.

[3] Lacerda L., Ali-Boucetta H., Herrero M. A., Pastorin G., Bianco A., Prato M. and Kostarelos K., Tissue histology and physiology following intravenous administration of different types of functionalized multi-walled carbon nanotubes. Nanomed. 2008, 3, (2), In Press.

[4] Kostarelos K., Lacerda L., Partidos C. D., Prato M. and Bianco A., Carbon nanotube-mediated delivery of peptides and genes to cells: translating nanobiotechnology to therapeutics. J. Drug Deliv. Sci. Tec. 2005, 15, (1), 41-47.

[5] Lacerda L., Bianco A., Prato M. and Kostarelos K., Carbon nanotubes as nanomedicines: from toxicology to pharmacology. Adv. Drug. Deliv. Rev. 2006, 58, (14), 1460-1470.

[6] Bianco A., Kostarelos K., Partidos C. D. and Prato M., Biomedical applications of functionalised carbon nanotubes. Chem. Commun. 2005, (5), 571-577.

[7] Tasis D., Tagmatarchis N., Bianco A. and Prato M., Chemistry of carbon nanotubes. Chem. Rev. 2006, 106, (3), 1105-1136.

[8] Georgakilas V., Kordatos K., Prato M., Guldi D. M., Holzinger M. and Hirsch A., Organic functionalization of carbon nanotubes. J. Am. Chem. Soc. 2002, 124, (5), 760-761.

[9] Georgakilas V., Tagmatarchis N., Pantarotto D., Bianco A., Briand J. P. and Prato M., Amino acid functionalisation of water soluble carbon nanotubes. Chem. Commun. 2002, (24), 3050-3051.