Detection and evaluation of impact of instilled carbon nanotubes. A 3-months follow-up investigation using helium-3, proton lung MR, optical and electronic microscopy

A. Al Faraj[1], A. Bessaad[1], K. Cieslar[1], S. Peyrol[2], G. Lacroix[3], E. Canet-Soulas[1], and Y. Crémillieux[1]

[1]Université Lyon1, CREATIS-LRMN, CNRS 5220, INSERM U630, Lyon, France, [2]CeCIL Centre Commun d'Imagerie Laennec, Lyon, France, [3]Institut National de l'Environnement et des Risques Industriels, Verneuil-en-Halatte, France

Introduction:

Due to their distinctive properties, novel engineered nanoparticles such as carbon nanotubes are more and more extensively used in manufactured materials. It appears then essential to develop tools for assessing their biodistribution and biological impact when accidently inhaled. Non-invasive hyperpolarized (HP) ^{3}He and proton lung MRI combined to ex-vivo optical and electron microscopy were used in a 3 months follow-up study to assess the biodistribution and biological impact of intrapulmonary instilled SWCNT (single-walled carbon nanotubes).

Material and methods:

Chemical composition of commercial raw-SWCNT samples were determined using Inductively Coupled Plasma–Mass spectrometry (ICP-MS). SWCNTs, suspended in 150 µl saline, were intra-tracheally instilled into male Sprague-Dawley rats (n=36). Three different doses of SWCNT were applied (0.1, 0.5 and 1mg). The animals were imaged at days D1, D7, D30 and D90. Lung ventilation was assessed with HP ^{3}He MRI at 2 Tesla. Ventilation images were acquired using a free breathing imaging protocol to avoid repeated animal's intubation [1]. Images were obtained at end-inspiration to visualize the largest fraction of ventilated airspaces. Proton MRI was performed at 4.7 Tesla. Gradient echo sequence (TE/TR=3.2/7-ms) was used to monitor inflammation events in lungs [2]. Lungs, liver, spleen and kidneys were removed at the different investigation time points. Histological analysis was carried out to assess the integrity of tissue and transmission electron microscopy (TEM) to characterize SWCNT effect at sub-cellular scale.

Results and discussion:

Iron content, measured by ICP-MS, was larger than 10 % (w/w) in SWCNT samples. A dose-dependent effect was observed at D1 in HP ^{3}He ventilation images with average SNR values varying from 34 (control instilled with saline) to 12 (1 mg SWCNT) (Fig.1a). The effect on SNR was not observed on later time points (D7 to D90). One month after SWCNT exposition, proton MRI revealed the presence of small detectable inflammatory nodules (assessed from hypersignal intensity pixels) in animal group instilled with the highest concentration of SWCNT (Fig1b). Histopathological images at endpoint (Fig1c) revealed the presence of multifocal granulomas encapsulating CNT bundles. TEM images (Fig1d) confirmed the dose and time dependent accentuation of inflammation with collagen fiber (CF) deposition and indicated that pneumocyte type II (ATII) and alveolar macrophages (AM) were the principal mechanism of defense to CNT acute toxicity.

We hypothesize that the detection of CNT with HP ^{3}He relies on the magnetic susceptibility effects induced by iron impurities. This assumption is supported by the absence of effects in animals instilled with iron-free purified SWCNTs (data not shown here). The transient effect on SNR could be due to CNT bundles encapsulation, homogenization of CNT distribution and incorporation of iron impurities in ferritin molecules or in hemosiderin deposits.

Conclusion:

Non-invasive HP ^{3}He and proton lung MRI combined to ex-vivo analysis (presently optical and electron microscopy) can provide an in-depth and complementary view on the biological impact of iron-containing CNTs. More generally, similar approach can be applied to any magnetic-labeled nanoparticles [3] for which toxicity investigation or diagnostic use are envisioned.

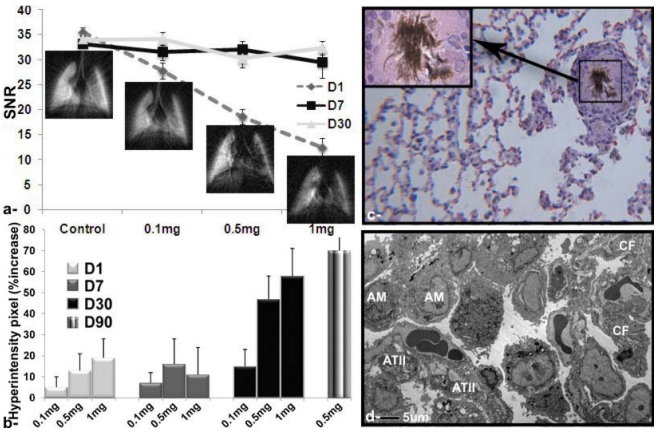

Fig.1: a- Signal-to-noise ratio (SNR) decrease with SWCNT concentration in HP-^{3}He MR lung images. b- histogram of hyperintensity pixel in proton lung images. c- histopathological lung images with CNT aggregates and associated multifocal granulomas. d- TEM micrograph showing an amplification of inflammatory reaction.

Reference: *1: Stupar et al. MRM 2007; 2: Beckmann et al. MRM 2001; 3: Al Faraj et al. MRM 2008*