ESF EpitopeMap Exchange Grant Final report

Project
Biodistribution of titania nanoparticles in mice – potential influence of protein corona resulting from different routes of exposure.

Dates
10th January – 9th April, 2011

Visitor
Bartlomiej Lukasz, Centre for Bionano Interactions, University College Dublin under the supervision of Prof. Kenneth Dawson

Host
Prof. Vyvyan Howard, School of Biomedical Sciences, Coleraine Campus, University of Ulster, Northern Ireland

Purpose of the visit
The aim of my visit in Prof. Howard's laboratory was to study the biodistribution of titanium dioxide (TiO_2) nanoparticles (NPs) in mice following intravenous injection or intratracheal instillation. Experience in Prof. Howard's team with mass spectrometry, specifically the ICP-MS technique which is frequently used in this type of study, appeared invaluable and would hopefully lead to further collaboration. Exploring the fate of nanoparticles delivered *in vivo* by different means is a significant step in drawing a comprehensive picture of risks and benefits behind NPs use, and for elucidating the role of the protein corona in determining nanoparticle fate and behaviour.

Description of the work carried out during the visit
10 weeks old wild type C57/black mice (n=3-6/group) were submitted to either intravenous (tail vain) injection (iv) or intratracheal instillations (it) with 40nm TiO_2 nanoparticles (8mg/ml suspension in saline). The injection volume was 100µl per animal, while instillations schedule comprised of four doses of 25 µl across four days, in order to deliver the same amount of nanoparticles in both cases. Both delivery methods included saline dosed controls, and nanoparticles were characterised in the saline solution prior to dosage.
The day following injection / last instillation animals were killed and dissected to obtain brain, lungs, heart, stomach, spleen, pancreas, liver, kidney, small and large intestines, testis, skeletal muscle, bone and blood samples, according to best practice and ensuring minimal pain to the animals (note: full ethical approval for these studies is held by both UU and UCD). All isolated tissue was washed with PBS (stomach and intestines were thoroughly cleaned from remaining content) and frozen at -20°C for later ICP-MS analysis.

Measurement of the titania content in the samples was performed with inductively coupled plasma mass spectroscopy (ICP-MS). Pre-weighed organs underwent digestion with ultrapure nitric acid in a microwave oven, with 10ppb indium spiked-in as an internal standard. Digested samples were resuspended in 2% nitric acid and subjected to measurement. Ti concentration was measured against a 0-500 ppb standard curve and

samples not fitting within its scope were diluted as required. Results were corrected against internal standard recovery.

Description of the main results obtained

Preliminary results show significant, although only a small percentage of the applied dose, amounts of titania (nanoparticles) detected in subjects' brain tissue resulting from both delivery methods. Following intravenous NPs application, the Ti levels in the brain tissue rose from 6.95 to 9.84 µg/g tissue ($p<0.05$ by Dunnett's post-hoc test) (Fig 1), which accounted for approx. 0.13% of the injected titania (800 µg). Repeated intra-tracheal instillations showed even an greater increase in brain Ti levels, 9.84 µg/g tissue ($p<0.001$ by Dunnett's post-hoc test) (Fig 1) constituting 0.24% of the original applied dose.

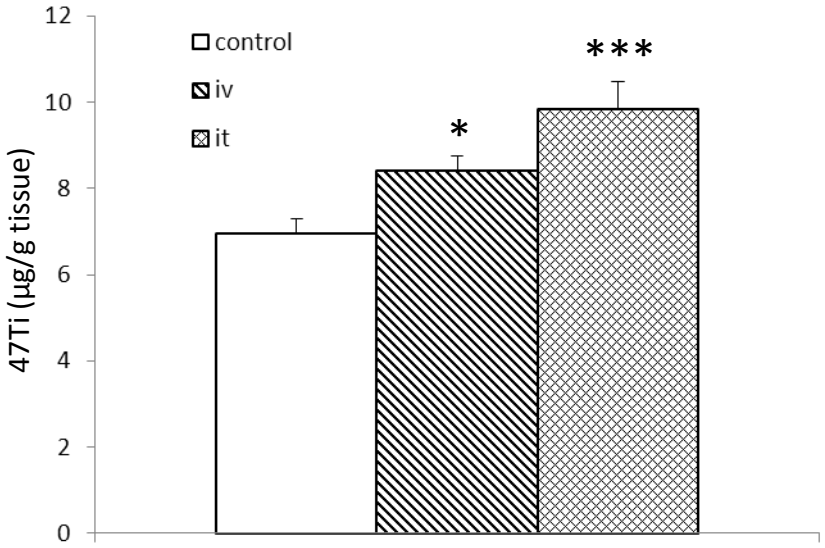

Fig.1 Titania (from anatase NPs) concentration (µg/g of tissue) measured in brains of intravenously injected (iv) or intratrachealy instilled mice. Measurements taken with ICP-MS on samples collected 24h after injection/last instillation and depicted as mean values ± SEM (n=4-6/group). The difference between groups was shown by one-way ANOVA ($F[2,15]=10.2$, $p=0.0015$) and Dunnett's post-hoc test (* for $p<0.05$, *** for $p<0.001$). Note that the significant levels of titania found in the control animals is a result of the prevalence of titania in food, water and the environment generally.

These results suggest that the 40 nm titanium dioxide NPs are able to cross the blood brain barrier (BBB) regardless of the *in vivo* introduction route. Within 24h of exposure to the NPs, the level of titania detected in brain tissue was significantly higher in comparison to sham-injected controls. The effects of intratracheal application are especially interesting, as NPs on their way to brain had to pass through not only the cells of the BBB but also the pulmonary alveolus wall. It suggests that nanoparticles might be actively transported from lungs lumen to bloodstream. NPs surface could also be altered during this process by interaction with the proteins and other biomolecules present in lungs, in the cells they pass through, and in the blood, which can affect their ability to cross the BBB.

Note that ICP-MS measures titania as a chemical, and as such does not give any information as to the form of the titania (i.e. nanoparticulate or otherwise). However, as we have specifically added titanium dioxide NPs, we can assume that any increase in the amount of titania detected is a consequence of transport of titanium dioxide NPs, as the dissolution

rate of these nanoparticles is low. However, further work using Electron Microscopy would be required to conclusively demonstrate the presence of nanoparticles of titanium dioxide.

Future collaboration with host institution

The presented results concern only brain samples, while titania quantification in the rest of collected material would significantly improve our understanding of the biodistibution of this type of NP. The next step to gain a better insight into mechanism of biodistribution will be characterisation of the protein corona on the NPs recovered from the collected tissue samples. We hope that both of these projects will include collaboration with Prof. Howard.

Projected publications/articles resulting or to result from your grant

Following full characterisation of the TiO_2 distribution in collected samples, data will be published.