

Lessons from nanoscale drug delivery

There are health, safety and environmental concerns about using nanotechnology in drug delivery. **Joseph Chamberlain** reports from a meeting that considered the lessons which may be learnt from nanoscale drug delivery research to avoid potential adverse effects of the new materials

Opening the meeting, Anthony Seaton, of the University of Aberdeen, asked whether nanoparticles themselves imply a hazard to health. In 1995, Professor Seaton and colleagues had proposed a hypothesis which associated exposure to low concentrations of particles with cardiac death without evoking a primary affect on the lung. This hypothesis was based on earlier observations that urban air pollution always included very large numbers of nanometer-sized particles and that such particles were able to penetrate alveolar epithelia and cause inflammatory reactions. It was proposed that such reactions were responsible for altering blood coagulability and hence causing increased risks of cardiac infarction.

Professor Seaton described his own studies showing the ubiquity of nanoparticles wherever combustion takes place and subsequent changes in red blood cell concentration of exposed subjects, suggesting an effect of particles on endothelial function. Such possible changes have now been demonstrated in clinical experiments that have shown falls in tissue plasminogen activator and rises in tissue factor in response to inhalation of diesel exhaust; both these changes would predict increased coagulability as a consequence of endothelial activation.

Long-term exposure to air pollution is associated with increased risks of developing heart disease. This fits with the hypothesis in that changes in known cardiac risk factors, such as fibrinogen levels in blood, as a consequence of lung inflammation would be expected to have this effect. There is good evidence that particulate pollution is associated with rises in fibrinogen.

It has now also been demonstrated experimentally in rats that nanoparticles can penetrate to the brain via the nasal mucosa and olfactory nerve. This has important implications with respect to chronic neurodegenerative disease. Although this work leads to the conclusion that particulate air pollution has important implications for public health, it remains to be established that these effects are actually due to the particular properties of nanoparticles. Research to investigate these properties is an important priority in view of the rapid development of nanomedicine and of the increasing public health issues of chronic degenerative diseases, concluded Professor Seaton.

From air pollution to nanotechnology

The rise of the nanotechnology industry means that new types of nanoparticles are being developed and used, said Ken

Donaldson, of the University of Aberdeen. Little is known of the toxicology of these new materials but information is accumulating on the toxic potency of small particles and their mechanisms.

The medical and scientific literature suggests that particles can penetrate the brain directly through translocation of inhaled ultrafine particles. Other targets for particles may be the skin, which is the subject of much research, particularly in relation to cosmetics, and the gut, where only a little research is in progress.

The total harm delivered to tissue by particles may be considered to be related to the surface area and the inherent toxicity of that surface, plus factors for shape (diameter and length) and biopersistence.

However there remains a medical paradox: exposure to nanoparticles is linked to chronic pulmonary and systemic disease and to coronary artery disease, yet nanoparticles in nanomedicine preparations may be used to treat the same chronic diseases. Thus the pharmaceutical problem may be approached by understanding the toxicology of particles. Do medical nanoparticles fit into the oxidative stress paradigm or are they really "not particles as we know them", asked Professor Donaldson.

Where in the tissues do nanoparticles end up?

Polymer nanoparticles have been studied as potential drug delivery systems for at least 25 years, said Martin Garnett, of the University of Nottingham. During that time much work has been carried out on how nanoparticles can be delivered to various tissues following intravenous administration. Early experiments demonstrated that nanoparticles are normally taken up by macrophages responsible for protecting the body from invading bacteria and viruses, and that this uptake could be prevented by appropriate nanoparticle surface coatings. Earlier work using non biodegradable particles and polymer micelle-like nanoparticles demonstrated how the degree of coating can affect rate of uptake by the mononuclear phagocyte system and which liver compartment takes up nanoparticles.

Subsequent work from different laboratories showed that more subtle variations in coatings and their interaction with biological components can lead to a wider range of biodistributions. In some instances specific tissue uptake can be seen even without biological targeting, probably due to adsorption of specific biological components recognised by tissue receptors.

More recently the Nottingham group has shown organ and tissue biodistribution of polyethyleneglycol-coated particles and penetration and uptake of these particles by different cell types in culture models of normal brain cell preparations.

These results may at first sight seem to apply only to the nanotoxicology of specialist pharmaceutical preparations, said Dr Garnett. However, there is a link between stabilisation of particles for pharmaceutical and other nanoparticle applications through the key mechanisms that can be used to stabilise and prevent aggregation of nanoparticles, a major consideration for drug delivery scientists. Study of these systems may, therefore, give an insight into the final locations of a range of nanoparticles, which reach the bloodstream either adventitiously or by design.

Orally dosed nanoparticles

Sandy Florence, dean of the School of Pharmacy, University of London, drew on 40 years' experience of studying small particles in drug delivery. Of particular interest was the question of the biodistribution of orally administered particles, including nanoparticles,

and their potential for toxicity. Such studies may allow some level of predictive toxicology of the systems being studied and increase our knowledge of factors affecting uptake to aid in these predictions. The lack of research on the toxicity of very small particles in the past may be because our lack of understanding of oral uptake of insoluble particles has reduced our vigilance, said Professor Florence. Closer examination of the absorption potential of small particles is revealing some surprises. For example, there is clear evidence of orally administered titanium dioxide being absorbed and located in the liver. Professor Florence wondered about other pharmaceutical excipients, such as magnesium stearate, which may also be absorbed as small particles. The nature of the particles (charge, lipophilicity) has a bearing on whether a particle is well-absorbed, and environmental factors in the gut are the same as those classically considered for drug studies — physical and chemical stability of the particle, transit times, residence time at the site of absorption, interaction with food, transport through the mucus, adhesion to epithelial surfaces and stimuli for cellular uptake.

Transforming nanomaterials to pharmaceuticals: carbon nanotubes

Over the past few years, considerable advances have been made in the field of nanotechnology. The technique of introducing reactive groups into otherwise inert carbon nanotubes (termed "functionalisation") has paved the way for their potential application as a delivery system for diverse molecules, including peptides, proteins, plasmid DNA and synthetic oligodeoxynucleotides, said Kostas Kostarelos, of the School of Pharmacy, University of London. Carbon nanotubes are chemical structures based on fullerenes. A honeycomb sheet structure is folded to form the tubes, which may be single-walled, typically 0.4–2nm in diameter, 20–1,000nm in length or multiple-walled, typically 1.4–100nm in

diameter and lengths measured in micrometers. For comparison, toxic asbestos fibres are 200–1,000nm in diameter and 5–15µm in length.

The goal of the drug delivery scientist is to produce nanotubes that are good carriers of drugs and are biocompatible with the test subject or patient. The organic functionalisation of carbon nanotubes can improve their solubility and biocompatibility profiles so that their manipulation and integration into biological systems has become possible. Functionalisation can include a fluorescent label or a radiolabel so the distribution of the nanotube can be monitored. For example, water-soluble, single-walled carbon nan-

otubes have been functionalised with the chelating molecule diethylenetriaminepentaacetic and labelled with indium-111 for imaging purposes.

Intravenous administration of these functionalised products followed by gamma scintigraphy indicates that they are not retained in any of the reticuloendothelial system organs (liver or spleen) and are rapidly cleared from systemic blood circulation through the kidney. This observed rapid blood clearance and half-life of approximately three hours has major implications for the clinical uses of carbon nanotubes. Excretion studies indicate both types are excreted as intact nanotubes.

Developing nanopharmaceuticals for the clinic

Nanopharmaceuticals can be developed either as drug delivery systems or biologically active drug products. Such products can result in improved formulations for oral, pulmonary, nasal and topical administration, said Ruth Duncan (Cardiff University) in discussing some of the issues in bringing such nanotechnology products into clinical trials and eventually therapeutic application. She welcomed the opportunity for toxicologists and drug delivery scientists to work together.

The two groups shared the common aim of defining the potential hazards of nano-sized materials. Whereas toxicologists aim to understand why materials are toxic, nanomedicine design is aimed at finding a safe product and we must understand what needs to be done in both fields, share methodology, enable an adequate supply of reference compounds and define what these reference materials should be.

The community of nanotechnology is complex, involving chemistry and engineering for production, the biological sciences for application, and law and ethics for regulation and safety issues. Since 1990 there has been a steady stream of approved products in the nanomedicine field. Europe has an excellent reputation in the study of the toxicology of fine particles and has been at the leading edge of preclinical and clinical development of new nanopharmaceuticals. Yet first market approval of nanopharmaceuticals is typically through the Food and Drug Administration in the US. The UK and Europe must acknowledge their own excellence in nanomedicine and nanof ormulation, fund R&D, and ensure the benefits of improved health care.

Other issues mentioned by Professor Duncan included headline journalism, often contradictory and unhelpful, the importance of considering the toxicological implications of all components, and some specific examples of products developed by the Centre for Polymer Therapeutics in Cardiff in association with a wide variety of partners.

Nanopharmaceuticals must be safe with respect to the proposed application, route of administration, dose, toxicokinetics and long-term fate. They must mediate a therapeutic benefit, and be amenable to scaled-up GMP manufacture, concluded Professor Duncan.

Benefits and risks

Intravenous administration of colloidal drug carriers such as polymeric nanoparticles is followed by a rapid opsonisation, the process of coating micro-organisms with plasma proteins to increase their adherence to phagocytic cells in preparation for phagocytosis, mainly in the liver, said Elias Fattal (University of Paris Sud). This biodistribution profile has opened a way to the treatment of several diseases such as intracellular infections or hepatic metastasis. Despite the benefits provided by passive targeting, nanoparticles could potentially lead to the impairment of the mononuclear phagocyte system, resulting in severe damage of the host defence function.

Repeated administration of nanoparticles, however, does not result in toxicity or blockade of the mononuclear phagocyte system. Although a single injection of nanoparticles induced a depletion of opsonines resulting in a transient decrease in the phagocytic ability of Kupffer cells, the clearance of colloidal carbon particles from the circulation was normal after repeated injections. Stimulation of phagocytic activity of hepatic macrophages during polymeric nanoparticles uptake due to the rapid recovery of opsonic levels could result in maintaining a normal clearance function during repeated administration of

nanoparticles. Professor Fattal also reported alterations in the hepatocyte behaviour after repeated administration of polymeric nanoparticles.

The depletion of the antioxidant defences glutathione and superoxide dismutase in hepatocytes provided evidence of oxidative stress after intravenous injection of polymeric nanoparticles. This effect was accompanied by the stimulation of the synthesis of acute phase proteins in hepatocytes which resulted in the increase of serum levels of alpha-1-acid glycoprotein.

The inflammatory response and the oxidative stress were observed after treatment with all types of nanoparticles. These effects, related to the particulate form, could be a consequence of the release of inflammatory mediators and reactive oxygen intermediates by Kupffer cells after nanoparticle uptake. However, liver damage was only observed after treatment with polyalkylcyanoacrylate (PACA) nanoparticles. This was probably related to the polymer nature. Indeed, degradation products released from Kupffer cells after PACA nanoparticle degradation could be responsible for the diminution of hepatocyte metabolic activities.

Nevertheless, the alterations observed in the hepatocyte behaviour induced by PACA nanoparticles did not result in cell death. Simple histological examination or the measurements of specific blood parameters or liver damage (aminotransferases, bilirubin) are not sensitive enough to detect toxicity due to PACA nanoparticles. Professor Fattal suggested that hepatotoxicity related to such nanoparticles is slight and that all effects were reversible. Hepatocytes were able to restore their functions when the treatment was stopped.

An encouraging sign of acceptance of nanotechnology in drug delivery can be seen in the granting of orphan drug status for doxorubicin nanoparticles in hepatocellular carcinoma, said Professor Fattal.

The meeting on nanoscale technology was organised by Academy of Pharmaceutical Sciences of Great Britain and took place in London on 27 February