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Editorial

Solubilization of Metal Particles and Lung Toxicity

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Inhaled particles are readily phagocytosed by alveolar macrophages (AMs) present in the lung. Other routes of particle exposure targeting the lung and frequently used with animal models include intratracheal instillation and oropharyngeal aspiration. The mechanism (s) by which AMs drive pulmonary toxicity downstream of particle uptake is not fully understood, as well as the contributing role of other phagocytic cell types present in the airways and lung including epithelial cells.

Metal-containing particles are often highly insoluble at physiological pH and are particularly relevant to environmental exposures and lung toxicity. Some of our recent work [1,2] has focused on indium-containing particles (ICPs) including indium phosphide (InP) and indium tin-oxide (ITO). InP and ITO have been suspected as causative agents of 'indium lung' disease following inhalation exposure in occupational settings within the microelectronics industry. ITO can be sintered or unsintered but the sintered form is most relevant to industrial use as a 'thin film' conductive coating on, for example, liquid crystal displays. InP is used in the manufacture of semiconductors, injector lasers and solar cells as examples. There are also well-established animal models of InP and ITO-induced pulmonary toxicity (injury, inflammation and fibrosis) and carcinogenicity. Whether or not the pulmonary toxicity of ICPs is dependent upon the total amount of indium metal present in the compound, the partner ion(s) and/or the particle's solubilization/dissolution potential (e.g. in AMs) is not clear.

Recent *in vitro* studies in our lab showed that InP and ITO particles were solubilized within mouse macrophages (RAW 264.7 cells) resulting in cytotoxicity (measured by MTT and LDH assays) and the release of ionic indium extracellularly (measured by atomic absorption spectroscopy) after 24 hours of culture. These events were dependent upon particle uptake and phagolysosomal acidification (inhibited by cytochalasin D and bafilomycin A1, respectively). Particle-induced cytotoxicity was similarly observed using primary (mouse-derived) alveolar macrophages. ITO is a combination of indium oxide and tin oxide at a ratio of ~90:10 (wt:wt), respectively. Indium oxide, but not tin oxide, particles were shown to be cytotoxic to macrophages suggesting that the cytotoxicity of ITO was not due to the presence of tin as a partner ion. Furthermore, the *in vitro* parameters of particle-induced cytotoxicity and release of ionic indium by macrophages were shown to correlate well with lung and pleural toxicity in mice and in general may be good predictors of particle-induced pulmonary toxicity *in vivo*. Despite containing similar amounts of indium, InP was much more toxic than sintered ITO (sITO) both *in vitro* (with greater indium release by macrophages) as well as *in vivo* suggesting that particle solubilization, and not the total amount of indium present in the compound, was a better determinant of toxicity.

InP and ITO were also shown in our studies to be phagocytosed by mouse lung (alveolar type II-derived) epithelial (LA-4) cells but, unlike with macrophages, were not cytotoxic after 24 hours of culture. No release of ionic indium was observed by the epithelial cells likely due to the absence of particle-induced cytotoxicity within this time frame. Lison et al. also showed that sITO was cytotoxic to rat macrophages (NR8383 cells) *in vitro* but not lung (alveolar type II-derived) epithelial (RLE) cells after 24 hours of culture; however, in contrast to the macrophages, the RLE cells did not readily phagocytose the particles [3]. Epithelial cells are probably less efficient in solubilizing particles following uptake compared to 'professional' phagocytes like macrophages but none the less are capable as we observed some particle-induced cytotoxicity of epithelial cells after 48 hours of culture. Using different particles, Ortega et al. showed that cobalt oxide was solubilized over time within human bronchial epithelial (BEAS-2B) cells following uptake via the phagolysosomal acidification pathway which resulted in the generation of ionic cobalt and cytotoxicity [4]. Ionic indium was shown in our studies (using soluble InCl3) to be cytotoxic to lung epithelial cells and even more so to macrophages after 24 hours of culture, compared to in particulate form as InP (containing equivalent amounts of indium), lending support to the notion that ionic indium is in fact the cytotoxic constituent of ICPs.

Based on our data with indium, we have proposed the following model. ICPs deposited in the lung via inhalation (or another route of exposure) are phagocytosed by AMs and alveolar type II epithelial cells. The particles are solubilized over time by both cell types following uptake via

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phagolysosomal acidification which generates cytotoxic ionic indium species, but solubilization occurs most efficiently and rapidly within AMs. Cellular apoptosis/necrosis results in the release of ionic indium extracellularly. Reactive ionic indium, which is much more toxic than in particulate form, together with other factors released from the dying cells then drive pulmonary toxicity downstream. The ionic indium may act directly on surrounding macrophages and/or other cell types (e.g. epithelial or stromal cells within the airways/lung) to induce injury and cell death and/or indirectly via another mechanism (e.g. induction of toxic-free radicals/reactive oxygen species (ROS) causing oxidative stress). Using human A549 (alveolar) epithelial cells, Tabei et al. showed *in vitro* that ITO nanoparticles (NPs) exhibited low cytotoxicity following cellular uptake but induced increased levels of intracellular ROS and DNA damage [5]. The rat studies by Lison et al. as well as Gottschling et al. [6] also suggested a role for ROS and oxidative stress in particulate indium-induced toxicity.

Badding et al. recently showed that sITO was cytotoxic to mouse macrophages (RAW 264.7 cells) and BEAS-2B cells *in vitro* after 24-48 hours of culture with apoptosis-mediated cell death (caspase activation) only occurring in macrophages [7]. Furthermore, they showed that sITO induced increased NFkB activity and cytokine production (IL-1β, IL-6, TNFα, and IL-8) in both cell types suggesting activation of the nod-like receptor protein 3 (NLRP3) inflammasome [8]. Thus, these data suggest that uptake of sITO particles by macrophages and epithelial cells results in a pro-inflammatory cascade (along with cell death) which may in part drive pulmonary toxicity (perhaps together with ionic indium released extracellularly). Indeed, it is likely that multiple mechanisms are involved in InP and ITO-induced pulmonary toxicity including the release of ionic indium, ROS and pro-inflammatory mediators from the apoptotic/necrotic cells. Similar mechanisms of toxicity may also apply to other metal-containing particles including cobalt oxide as previously mentioned. Additionally, zinc oxide (ZnO) nanowires [9] and NPs [10] were both shown to be cytotoxic to macrophages *in vitro* whereby cell death involved a phagolysosomal acidification-dependent increase in intracellular ionic zinc. In the case of the NPs, it was proposed that dissolution of ZnO under acidic conditions resulted in a local spike in ionic zinc, which damaged and permeabilized the lysosomes causing the contents (along with the ionic zinc) to leak out into the cytoplasm leading to cell death and the release of cytotoxic zinc ions extracellularly. Hamilton et al. also showed that uptake of silver nanospheres by macrophages (THP-1 cells) and lung epithelial cells (including LA-4 cells) resulted in cytotoxicity and NLRP3 inflammasome activation (IL-1β release) which was dependent upon particle dissolution (release of ionic silver) within acidic phagolysosomes [11]. As with ionic indium, ionic cobalt, zinc and silver may act directly and/or indirectly (together with pro-inflammatory mediators and/or ROS) on other cell types to promote lung toxicity.

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