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Adverse health effects following exposure to engineered nanomaterials

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ABSTRACT. ***Adverse health effects following exposure to engineered nanomaterials. Advances in nanotechnology have enabled fabrication of nanomaterials with defined structures that areincreasingly being used for commercial purposes. Unlike chemical toxins, nanomaterials have unique interactions with macromolecules and cells based on their molecular size and interfacial physiochemistry. Adverse human health impacts due to occupational and environmental exposures to engineered nanomaterials (ENMs) are therefore of concern.***

Although the impact of ENMs on biological systems is still not clearly understood, strong evidence suggests that nanomaterials are present in human fluids and tissues. Effective and feasible hazard assessment of ENMs is urgently needed to guide regulation and policy-making and support the development of benign next generation ENMs. Beyond the physical parameters of the NMs themselves, the surrounding biological system has been shown to influence NM behavior, the nano-cellular interface, and subsequent biological responses.

It has recently been established that ENMs, upon entry into a physiological environment, exhibit a tendency of physical adsorption with proteins, peptides, lipids and amino acids to render a "biocorona" that may influence the bioavailability and distribution of ENMs within the host system, at the cellular, tissue and whole organism level. Consequently, research on the health and safety implications of ENMs must include assessments of how the biocorona may impact toxicity and lead to a new 'biological' identity of the ENM.

Although ongoing research suggests that almost every organ and organ system may be affected by ENMs, in this review we will focus on the main pathogenetic mechanisms and on key organ and organ systems such as the lung, the skin and the gastro-intestinal tract; we will also highlight several challenges associated with a comprehensive evaluation of their toxicity, including the vast and diverse array of ENM products, dependence on physicochemical characteristics and exposure matrices, and difficulties in quantifying dosimetry and dose-response. Possible attempts to overcome these challenges are also discussed.

Key words: *engineered nanomaterials, health effects, occupational setting.*

RIASSUNTO. Effetti sulla salute della esposizione a nanoparticelle ingegnerizzate. Il progresso delle nanotecnologie consente oggi la sintesi di nanoparticelle con struttura pre-definita, utilizzate per usi commerciali. A differenza dei comuni agenti chimici, hanno una particolare interazione con macromolecole e cellule, che è influenzata dalla dimensione e dall'interfaccia fisico-chimico. Per tale motivo si possono ipotizzare possibili conseguenze sulla salute dopo esposizione in ambito occupazionale e ambientale.

Nanoscience presents new possibilities for advancing technology, medicine and a number of other disciplines; on the other hand, in parallel with the development of nanotechnology, it is increasingly accepted that we are exposed to engineered and naturally occurring engineered nanomaterials (ENMs) on a daily basis (1, 2). Some properties of ENMs are of concern for human health: 1) their small size with high specific surface area which may make them more toxic than larger particles; 2) their likely bio-persistence, and 3) the potential to cross biological barriers (3-5). In addition, the interaction of ENMs with membranes and fluids inside living organisms may have profound effects on their biological activity. In fact, inside living organisms ENMs become coated with macromolecules (e.g., proteins, lipids) in what is essentially a corona around the particle. (6).This means that ENMs with a particular synthetic identity (e.g., ZnO) may have multiple biological identities (e.g., ZnO coated with serum proteins, ZnO coated with lipids).

Therefore, the greatest strength of nanoscience, the physical and chemical characteristics of materials at this scale, has the potential to be its greatest liability.

Although there have been proposed guidelines (7, 8), there is no universally accepted set of toxicity tests that can determine both the short term and potentially long term impacts of ENMs.

The fundamental shapes (sphere, tube/rod/belt, or sheet) and basic elemental composition (graphene, metal, metal oxide) of ENMs are nowdays well-defined, however subtle changes in their physicochemical properties can have profound influence on the biologic responses following exposures. For example, it has been reported that the effect on the developing embryo of two different form of nanocarbons (carbon black and carbon nanotubes) ranges from no adverse effect for carbon black to severe malformations for carbon nanotubes (9). It is clear that these subtle changes define ENM toxicity, but the ability to fully assess the toxicity of every ENM is impossible. Because toxicity assessments ultimately identify benign ENM and/or physicochemical properties, approaches to first rapidly identify and then characterize in depth specific hazards must be developed in terms that are most physiologically relevant to human health.

Sebbene i meccanismi della loro interazione biologica non siano stati del tutto elucidati, la loro presenza in fluidi biologici e in tessuti può considerarsi acquisita, per cui una valutazione attendibile del pericolo posto dalle nanoparticelle ingegnerizzate è ormai urgente per guidare gli enti preposti alla regolamentazione dell'esposizione ad agenti potenzialmente dannosi per gli esseri umani.
Oltre alla struttura fisico-chimica della nanoparticella, sono di particolare rilevanze, ai fini della tossicità, le modifiche che essa subisce quando viene a trovarsi a contatto con un sistema biologico. È infatti una caratteristica peculiare delle nanoparticelle quella di formare una sorta di corona superficiale con proteine, lipidi peptidi e aminoacidi che si trovano nei fluidi corporei, acquisendo così quella che viene definita "identità biologica" in contrapposizione all'"identità sintetica" rappresentata dalle caratteristiche chimico-fisiche della nanoparticella all'atto della sua sintesi industriale.
Sebbene siano riportati dati sperimentali di tossicità riguardanti in pratica tutti gli organi ed apparati, ci siamo focalizzati sul polmone, cute e tratto gastro-enterico per la loro rilevanza in caso di esposizione occupazionale ed ambientale. In questa review discuteremo le criticità legate ad una attendibile valutazione degli effetti sulla salute e le ricerche attualmente in corso per superare questi problemi.

Parole chiave: nanomateriali ingegnerizzati, effetti sulla salute, contesti occupazionali.

Lung adverse health effects

Inhalation is likely a major environmental route of exposure. To date, there exists no epidemiological or clinical evidence that inhalation of ENMs leads to adverse health effects in humans, however epidemiological data available for ambient ultrafine particle (AUFP), sharing with ENMs the size range (0-100 nm), show an association with adverse pulmonary effects, with increased morbidity and mortality in susceptible populations (10).

In animal experiments, pulmonary toxicological responses following ENM exposure largely result from the generation of reactive oxygen species (ROS), leading to inflammatory reactions (11, 12). It has also been reported that persistent particle-induced inflammatory responses in the lung – and presumably other tissues – lead to tissue injury, cell proliferation, and scarring and/or tumorigenic responses (13).

As far as the effects of individuals ENMs are concerned, it has been shown that acute exposure to different types of silica nanoparticles (SNP) alone or in conjunction with ovalbumin induced strong airway inflammation and airway hyper-responsiveness: (14). Furthermore, three types of metal oxide nanoparticles, TiO2, ZnO and SiO2, were shown to increase serum concentrations of total IgE, ovalbumin-specific IgE and ovalbumin-specific IgG1 after pharyngeal aspiration together with inhaled ovalbumin (15) suggesting that they have the potential to aggravate allergic reactions. A rapid onset of serious nickel allergy in a nano-nickel exposed individual has been recently reported (16).

Exposure to carbon nanotubes (CNT), especially in the occupational context, occurs mainly by inhalation. In the study by Rydman et al. (17), a short-term 4 days inhalation of the rod-like CNT induced novel innate immunity-mediated allergic-like airway inflammation. Marked eosinophilia was accompanied by mucus hypersecretion, AHR and the expression of Th2-type cytokines. Exposure to MWCNTs may cause airway fibrosis (18).

Skin adverse health effects

In occupational exposure scenarios, the skin is also a potential portal of entry for ENMs, particularly from surface contact, as has been shown, e.g., with beryllium compounds (19) A variety of engineered nanomaterials (ENMs) have the potential for dermal exposure. For example, the application of cosmetics and sunscreens containing TiO2 nanoparticles represents a major dermal exposure scenario for humans, but some dermal studies with TiO2 nanoparticles in rodents indicate a lack of penetration beyond the epidermis with no consequent health risks (20) Similar findings were reported for Quantum dots (QDs) (21)). Nevertheless, the interaction of nanoparticles with epidermal cells (keratinocytes and Langerhan's cells) could trigger immune responses in the skin that could in turn lead to systemic immune responses without penetration into the dermis or beyond (22). On the other hand, conditions that disrupt the skin barrier allow penetration into the viable skin layers and subsequent accumulation in local lymph nodes and liver (23).

Gastro-intestinal adverse effects

With potential for improving food quality and safety, the use of nanotechnology-based food additives, nutraceuticals, pesticides, fertilizers and packaging materials is growing rapidly, with hundreds of nano-enabled products (NEPs) already on the market (24). ENMs used in food products include inorganic and carbon-based materials. TiO2 is used to enhance color, texture and flavor (25); SiO2 is used as an anti-caking agent, and to clear beers and wines (26); and ZnO is added to dietary supplements and breakfast foods as a source of zinc (26). Nanocellulose (NC) is used in packaging to reduce bacterial growth and increase shelf life, and in foods as a non-caloric stabilizer for emulsions and foams, and to improve texture, flavor and appearance (27)). Conventional micron-sized forms of these materials have been approved for use in food by various regulatory authorities, and are generally regarded as safe, without characterization or definition of particle size. However, many of the approved food grade materials include nanosize particles. For example, food grade TiO2 (E171 - European designation) contains nanoscale particles (28)), and nano-sized TiO2 was found in dietary supplements (29)) and food products (30). Likewise, nano-sized SiO2 particles have been found in food grade SiO2 (E551) and at levels of up to 14.4 μg/g in commercial foods and dietary supplements (28).

The absence of labeling requirements for nano-size materials (29) makes identification of products containing

ENMs and estimation of ingested ENM exposure levels difficult. In addition to direct ingestion from food sources, another important source of gastrointestinal exposure is represented by the ENMs cleared through the muco ciliary escalator from the lung into the gastrointestinal tract (about 20% of lung exposure).

Only a few studies have investigated in vivo toxicity of iENMs. Studies on silver nanoparticles provided conflicting or inconclusive results (30, 31). In the few in vivo studies of metal oxide iENMs, TiO2 was found to have a variety of toxic effects within the GIT (32, 33) and to enter the systemic circulation to accumulate and cause inflammation and oxidative damage in the liver, kidney and spleen (34-36); ingested ZnO ENMs were reported to undergo size-dependent intestinal absorption with accumulation in multiple organs and damage to liver and pancreas (37, 38) and ingested SiO2 ENMs caused low-level hepatotoxicity in rats following a 10-week exposure (39). There is growing evidence that interaction of ENMs with gut microbiota mediates large part of intestinal and extra-intestinal effects of ENMs (40 e 41).

Challenging and emerging approaches in nanotoxicity evaluation

Our understanding of the toxicity mechanisms and potential impacts of most ENMs on humans is still in infancy (42) Adverse effects are influenced by nanoparticle composition, surface modification, dose, exposure route and animal species. Toxicity of some ENMs, such as metals have been more extensively evaluated, whereas toxicological information for other emerging ENMs such as 2D- and 3-D ENMs is much less extensive. Studies have been performed both in vitro and in vivo, on subjects ranging from bacteria and yeast to daphnia, rodents and humans. Most studies focused on single or a few effect endpoints or biomarkers, while systematic evaluations related to various exposure routes are rare. In this light, the development of high throughput screening (HTS) approaches to assess dose and time dependent cellular injury responses that are predictive of in vivo adverse outcomes is a key infrastructural requirement that will allow development of predictive algorithms and lead to the creation of safe next-generation ENMs.

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