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Dermal absorption of amorphous nanosilica particles after topical exposure for three days

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The skin penetration and cellular localization of well-dispersed amorphous nanosilica particles (nSPs) with a diameter of 70 nm was analyzed in mice. Our results suggest that after topical exposure for three days the particles penetrate the skin barrier and are transported to the lymph nodes. These findings underscore the need to examine biological effects following dermal exposure to nSPs for the development of safer use of nSPs.

Nanomaterials (NMs) are defined as substances that have at least one dimension of less than 100 nm in size. NMs exhibit unique physicochemical properties that distinguish them from submicron-sized materials. These unusual properties have facilitated the development of innovative applications for NMs, which are already used in a wide variety of fields. For example, amorphous nanosilica particles (nSPs) and titanium dioxide nanoparticles ($nTiO_2$) are colorless and reflect ultraviolet light very effectively. Consequently, these substances are used in the cosmetics industry as a foundation and sunscreen (Lansdown and Taylor 1997; Napierska et al. 2010).

The reduced prticle size of NMs, however, poses new risks induced by changes in their biological reactivity and kinetics, which differ from those of bulk materials. For example, exposure of cells or animals to NMs, such as carbon nanotubes or $nTiO_2$, can induce cytotoxicity and inflammation that is different from that caused by exposure to submicron-sized materials (Nel et al. 2006; Xia et al. 2006). Furthermore, we have previously shown that nSPs display a different intracellular localization compared with submicron- or micro-sized silica particles, and induce a greater cytotoxic response. (Nabeshi et al. 2010; Yamashita et al. 2011; Nabeshi et al. in press) In general, risk is determined by the integrated value of a potential hazard with the amount of exposure. Hence, materials that are not absorbed and that have no effect on the application area pose no risk. Although there are a number of reports that focus on the biological response resulting from exposure to NMs, there is a paucity of information concerning the absorbency or localization of these materials. Thus, to accurately identify hazards associated with exposure to NMs, we must first analyze the *in vitro* and *in vivo* biodistribution of nSPs, especially with regard to penetration of biological barriers. Although we previously reported that topical application of nSP70 caused systemic exposure, absorbability of nSP70 after short term exposure has not yet been clarified.

In this study, we evaluated the intradermal absorption of well-dispersed nSPs with a particle size of 70 nm (nSP70) following topical exposure for three days. Specifically, nSP70 was applied to the inner side of the ears of female BALB/c mice for three days. The ears and regional lymph node were excised 24 h after the last administration and then analyzed by transmission electron microscopy. Our results show that nSP70 not only enter epidermal Langerhans cells, but also the cells in the dermis and regional lymph node.

Our findings suggest the particles can be dispersed throughout the body via the lymphatic transport system. Thus, exposure to nSPs in cosmetics poses an unknown risk that is not restricted to the application area. Hence, studies investigating the biological effects of dermal exposure to nSPs should include the whole body and not only the skin. Further studies are required to perform an appropriate risk analysis of nSPs e.g. quantitative determination of skin permeability and mechanisms for penetrating the skin barrier of nSP70. It is also important to investigate how nSPs behave after entering the body (e.g. accumulation potential, metabolism and excretion). Thus, we plan to collect ADME (absorption, distribution, metabolism and excretion) information as soon as possible. We believe that our study will provide useful information for developing safer NMs in the future.

Experimental

1. Silica particles

Suspensions of fluorescent (red-F)-labeled amorphous silica particles (Micromod Partikeltechnologie GmbH, Rostock, Germany) (25 mg/ml)

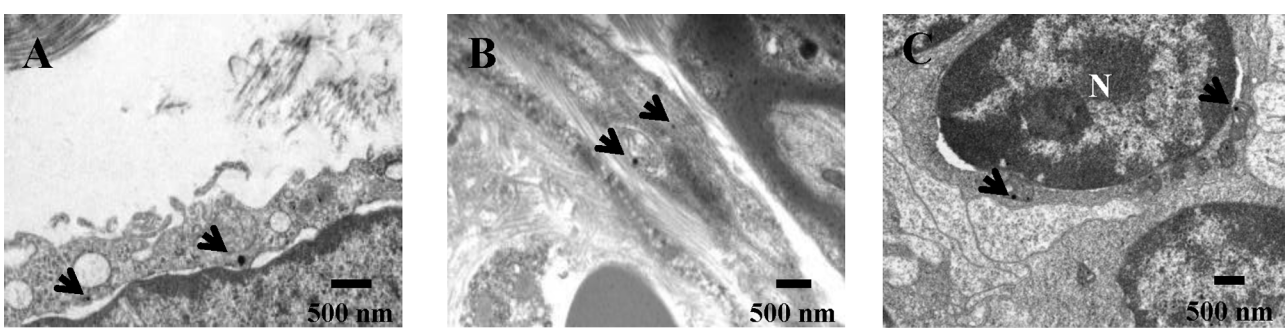

Fig.: Transdermal absorption test of nSP70 using the transmission electron microscopic (TEM) analysis. TEM analysis of skin and lymph node samples from mice after three-days of dermal exposure to nSP70. A-C, nSP70 (arrows) were present in the Langerhans cells (A), dermis (B) and cervical lymph node (C). N: nucleus. Scale bars: 500 nm (A–C)

were used in this study. Their particle diameter was 70 nm (nSP70). The suspensions of silica particles were sonicated for 5 min and then vortexed for 1 min immediately before use. Mean particle size was determined as described previously (Nabeshi et al. 2011), which confirmed that nSP70 remained as stable well-dispersed particles in water, rather than forming aggregates.

2. Dermal administration of silica particles and transmission electron microscopy analysis of skin and lymph node

nSP70 (250 mg/ear/day) were applied to the inner side of both ears of BALB/c mice for 3 days. Lymph nodes from each mouse were excised 24 h after the last administration of nSP70 and then fixed in 2.5% glutaraldehyde for 2 h. Small pieces of tissue sample were washed with phosphate buffer (three washes) and post-fixed in sodium cacodylate-buffered 1.5% osmium tetroxide for 60 min at 4 °C. The sample was block staining in 0.5% uranyl acetate and dehydrated by dipping through a series of solutions containing increasing concentrations of ethanol. Finally, the sample was embedded in Epon resin (TAAB). Ultrathin sections were stained with uranyl acetate and lead citrate. The stained samples were subsequently observed using a Hitachi electron microscope (H-7650; Hitachi, Tokyo, Japan).

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