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Amorphous nanosilica particles induce ROS generation in Langerhans cells

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Generation of total intracellular reactive oxygen species (ROS) was measured in XS52 cells, a Langerhans cell-like line, treated with different sized amorphous silica particles. The results suggested that exposure to amorphous nanosilica particles (nSPs) with a particle size of 70 nm induced a higher level of ROS generation than did exposure to micron-sized amorphous silica particles. This finding means that it is essential to examine the biological effects of ROS generated after exposure to nSPs, which will provide useful information for hazard identification as well as the design of safer nanomaterials.

Recently, the development of nanomaterials (NMs) with particle sizes below 100 nm has received extensive interest. For example, titanium dioxide nanoparticles and amorphous nanosilica particles (nSPs) are colorless and reflect ultraviolet light more efficiency than micro-sized particles and have been already used in various applications such as cosmetics, medicines and foods (Fadeel et al. 2010). Thus, exposure to NMs is unavoidable for us in today's environment. In this respect, there is increasing concern regarding the potential health risks caused by exposure to NMs. In most cases, however, the evaluation of NMs has been insufficient for ensuring their safety. Hence, it is necessary to accumulate safety information, such as hazard data and exposure assessment to NMs, for risk evaluation to create safer forms of NMs.

Here, we have studied the relationship between the *in vivo/in vitro* distributions of NMs, together with their biological effects and physicality. In our previous study, we found that nSPs with particle size of 70 nm (nSP70) penetrated the stratum corneum of mice skin and were taken up by living cells such as keratinocytes and Langerhans cells after 28 days of exposure (Nabeshi et al. 2010). In another study, we showed that smaller sized amorphous silica particles induced greater cytotoxicity against XS52 cells, a Langerhans cell-like line (Nabeshi et al. 2009). Here, we have accumulated information to clarify amorphous silica particles-mediated cytotoxicity against XS52 cells in term of the levels of total intracellular reactive oxygen species (ROS) generated.

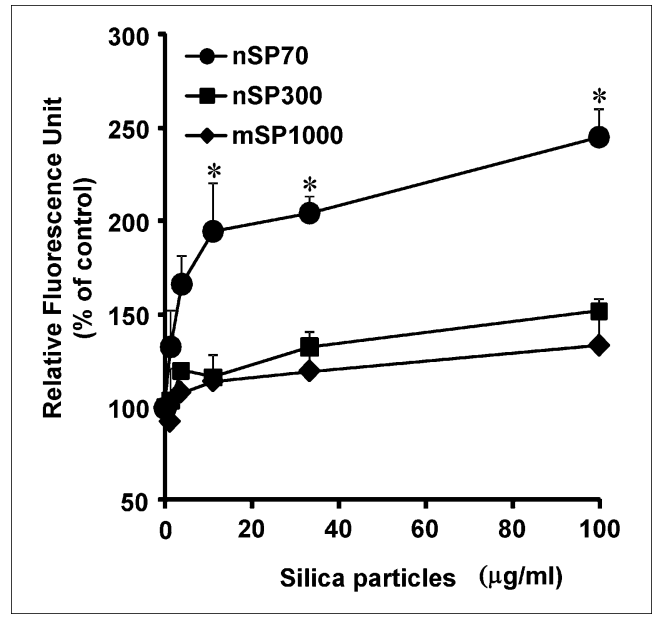

Fig.: Detection of ROS induced by silica particle treatment in XS52 cells. XS52 cells were incubated with various concentrations of nSP70 (circles), nSP300 (squares), and mSP1000 (diamonds) for 3 h. The total ROS induced by treatment with silica particles was expressed as relative fluorescence units in the DCFH assay. Data are presented as means ± SD (n = 4).*$P < 0.05$ versus the same dose of nSP300 or mSP1000

XS52 cells were treated with nSP70, and micro-sized amorphous silica particles with diameters of 300 or 1000 nm (nSP300 or mSP1000, respectively). As a result, all sizes of amorphous silica particles were found to induce intracellular ROS generation in a dose-dependent fashion. On the other hand, ROS generation by nSP70 treatment was significantly greater than that by nSP300 and mSP1000 treatment at the same particle concentration (Fig.). Thus, intracellular ROS generation in XS52 cells induced by amorphous silica particles was significantly increased by decreasing the particle size to less than 100 nm.

Some reports have indicated that ROS generation is related to a cellular stress response, such as DNA damage and apoptosis induction (D'Auteaux et al. 2007). The results of this study suggest that ROS generation may be involved in amorphous silica particles-mediated cytotoxicity in XS52 cells. In our previous study, we found that ROS generated by amorphous nanosilica particles induced DNA damage in human keratinocyte cells (Nabeshi et al. 2011). Therefore, these results suggest that DNA damage may be triggered by ROS induced by amorphous silica particles in XS52 cells. However, the detailed mechanism of NMs-mediated ROS generation remains unclear. Therefore, it is essential to analyze the mechanism underlying ROS generation induced by amorphous nanosilica particles to ensure the safety of NMs.

Recently, the mechanism of particulate matter-mediated ROS generation is gradually becoming clear. For example, it has been reported that inflammasomes are activated by actin-mediated endocytosis of crystalline silica or asbestos, which leads to NADPH oxidase activation and ROS generation (Dostert et al. 2008). Furthermore, many kinds of signaling pathways, such as Akt/P13K or MAPK pathways, are also related to ROS generation. On the basis of this information, we need to investigate whether these pathways are related to ROS generation induced by amorphous nanosilica particles. Furthermore, we should examine the relationship between physicality, exposure to amorphous silica particles and ROS generation. In the future, we

believe this information will be useful for the safety assessment and evaluation, as well as the design, of safer NMs.

1. Experimental

1.1. Silica particles

Suspensions of fluorescent (red-F)-labeled amorphous silica particles (Micromod Partikeltechnologie GmbH, Rostock, Germany) were used in this study; the particles had diameters of 70, 300, and 1000 nm (designated nSP70, nSP300, and mSP1000, respectively). The suspensions of silica particles were sonicated for 5 min and then vortexed for 1 min immediately prior to use.

1.2. Cell culture

Cells of the Langerhans cell-like line XS52 (a kind gift of Akira Takashima, University of Toledo, Health Science Campus, Toledo) were expanded in complete medium containing 2 ng/ml of murine GM-CSF and 10% culture supernatants from skin-derived stromal NS47 cells (a kind gift of Akira Takashima). The complete medium was RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 1% non-essential amino acids, 1% L-glutamine, 1 mM sodium pyruvate, 1% 2-mercaptoethanol, 10 mM HEPES buffer, and 1% antibiotic-antimycotic mix stock solution.

1.3. Detection of reactive oxygen species (ROS)

Generation of total intracellular ROS was measured by monitoring the increasing fluorescence of 2'7'-dichlorofluprecein (DCF) using 2'7'-dichlorodihydorofluorecein diacetate (DCFH-DA; Sigma, St, Louis, MO). 3×10^4 XS52 cells were seeded into each well of a 96-well plate. After 24 h incubation, the cells were treated with nSP70, nSP300, or mSP1000 for 3 h. The cells were then washed once with phenol red-free medium, and incubated in 100 μl of DCFH-DA at 37 °C 5% CO_2 for 30 min. The fluorescence of DCF was monitored at the excitation and emission wave-length of 485 nm and 530 nm, respectively.

1.4. Statistical analysis

All data are reported as the mean ± SD. The significance of variation among different groups was determined by one-way ANOVA. Differences between the experimental group and the control group were determined by Williams' test. A value of $P < 0.05$ was considered significant.

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