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Short-term biodistribution and clearance of intravenously administered silica nanoparticles

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ABSTRACT

Recently, concerns have been raised about potential adverse effects of synthetic amorphous silica, commonly used as food additive (E551), since silica nanoparticles have been detected in food containing E551. We examined the biodistribution and excretion in female Sprague-Dawley rats of NM-200, a well characterized nanostructured silica representative for food applications. A single intravenous injection of NM-200 was applied at a dose of 20 mg/kg$_{bw}$, followed by autopsy after 6 and 24 h. The main organs where silicon accumulated were liver and spleen. The silicon concentration significantly decreased in spleen between 6 and 24 h. In liver the tendency was the same but the effect was not significant. This could be due to clearance of the spleen to the liver via the splenic vein, while liver clearance takes more time due to hepatic processing and biliary excretion. In treated animals the liver showed in addition a prominent increase of macrophages between both evaluation moments. Within the first 24 h, silicon was mainly excreted through urine. Further studies are necessary to evaluate the toxicokinetics of different types of silica nanomaterials at lower exposure doses in order to be able to predict kinetics and toxicity of silica nanoparticles depending on their physicochemical characteristics.

1. Introduction

Silicon dioxide, briefly referred to as silica, is widely used in the food industry. In Europe, synthetic amorphous silica is an approved food additive (E551), used as anticaking agent or as carrier for emulsifiers and colors [1]. Part of this silica may be in nanoparticle form [2,3] and some types of nanosilica have shown liver toxicity in animal studies after a long-term oral exposure to high doses [4,5]. It is currently still unknown whether this toxicity is due to the silica nanoparticles present, to (partially) dissolved silica or to a combination of both [2,5], as the oral absorption and biokinetics of food-grade nanosilica have not been well explored. Lee et al. [6] demonstrated a gastrointestinal absorption < 4% after a single oral dose administration of 500 mg/kg food-grade nanosilica, while other studies report absorption levels below 0.25% [7], and references therein]. It seems that gastrointestinal absorption of nanosilica decreases with increasing treatment dose, possibly due to intestinal gelation of silica [5], although changes in particle stability, aggregation and surface properties following interaction with luminal factors present in the gastrointestinal tract may influence the bioavailability of nanosilica as well [8]. Lee et al. [6] demonstrated that the oral absorption of silica nanoparticles highly depends on the presence of sugar or proteins, presumably due to their surface interaction on nanoparticles, while Sakai-Kato et al. [9] found differences in nanosilica agglomeration depending on fed/fasting conditions. All these factors complicate the comparison of different studies on biodistribution and toxicokinetics of food-grade nanosilica after oral ingestion.

Upon intravenous (IV) administration, gastrointestinal interactions and absorption are by-passed and besides toxicity, other aspects of toxicokinetic processes, such as tissue distribution and elimination, can be studied in detail. Single dose IV studies noted no or low reversible histological or tissue effects of silica nanoparticles in mice [10,11], while long-term repeated dose IV studies showed significant tissue injury or potential dysfunction of biliary excretion and glomular filtration

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in mice [12,13]. Three consecutive IV injections of the silica nanomaterial NM-203 in rats induced hepatotoxicity, thrombocytopenia and even animal death at the highest dose of 3 × 20 mg/kg [14], but no DNA damage was induced in liver, spleen or lung.

After IV treatment of mice, nanosilica mainly accumulates in liver and spleen, but some accumulation is also reported in lung or in kidney [10–12,15–17]. Zhuravskii et al. [17] reported the retention of amorphous nanosilica in rat liver up to 60 days after IV injection, causing liver tissue remodeling and development of fibrosis. The observed nanosilica concentrations in liver 24 h after IV injection are in general higher than or equal to the nanosilica concentrations in spleen for rats and mice ([18–20], but a study in which mice tissue concentrations were measured at different moments within the first 24 h suggests that silica is already rapidly cleared from both organs within this timeframe and that nanosilica concentrations might initially be higher in spleen [19]. Quantitative information about the tissue distribution within the first 24 h after IV treatment is, however, still scarce, especially for food-grade nanosilica.

Silica nanoparticles have been shown to be actively excreted by mice within a few days after IV administration, mainly by the urinary tract but also within feces, due to particle excretion through the biliary route [19]. Nevertheless, part of the silica nanoparticles can reside relatively long in the body: in some studies, particles were still detected in the liver and spleen up to 8 weeks after injection [16,19,21]. The biodistribution and the excretion rate of nanosilica after IV administration is not uniform among different animal studies and may be affected by the particle size [10,15,21–22], the particle shape [12,22–23], the presence (and type) of a coating [10–12,22] and the gender of the animal [18].

The majority of the nanosilica toxicokinetic studies are performed in the context of biomedical applications and have not used food-grade nanosilica, which may have different kinetics than e.g. mesoporous nanosilica [22]. The purpose of the present study was therefore to examine the short-term ($\leq$ 24 h) toxicokinetics after IV administration of a well characterized food-grade silica nanomaterial, NM-200, selected in the OECD (Organization for Economic Co-operation and Development) testing program [24], as quantitative information about the tissue distribution within the first 24 h is still scarce. NM-200 has already been studied in the NANOGENOTOX project where a single dose of NM-200 (precipitated silica) or NM-203 (fumed silica) was administered intravenously to male and female rats. Biodistribution and excretion of silicon (Si) was recorded after 24 h up to day 90 [18]. The toxicokinetics within the first 24 h after a single dose of NM-200 were, however, not yet studied. To be complementary to the NANOGENOTOX study, the same animal model (rat) and an identical treatment dose of 20 mg/kg_{bw} was applied.

2. Methods

2.1. Preparation of nanomaterial dispersions used for IV injections

NM-200, a representative test material of uncoated precipitated synthetic amorphous silica (SAS) was obtained from the Joint Research Centre Repository (JRC, Ispra, Italy). This material was produced by precipitation. NM-200 has been well characterized previously in the OECD testing program [25]. NM-200 was dispersed in phosphate buffered saline (PBS; Sigma-Aldrich) to mimic the pH, ionic strength and osmolarity of body fluids and dispersed using the generic NANOGENOTOX dispersion protocol [26] as described in [3]. The silica concentration in the dispersion used for IV injection was 8 mg SiO_2/mL. All suspensions were prepared freshly just prior to use. PBS was used as vehicle control.

2.2. Nanoparticle dispersion characterization

The morphological characteristics of the dispersed NM-200 in PBS were analyzed qualitatively and quantitatively by transmission electron microscopy (TEM) as described in [3].

The hydrodynamic diameter of the dispersed NM-200 particles was analyzed by particle tracking analysis (PTA) as described in [27].

2.3. In vivo experimental design

Nine-week-old female specific pathogen free Sprague-Dawley rats were purchased from Harlan (Horst, The Netherlands). The animals were allowed to acclimatize for one week and at the start of the experiment they were individually housed in metabolic cages at room temperature (21 ± 2 °C) with a relative humidity of 50 ± 20% and a 12 h light/dark reversed cycle. The body weight of the animals at the beginning of the study was 220 ± 13 g. Water and standard food (RMH-B, AbDiets, The Nederland) were given *ad libitum*, except for the 12-hour period prior to the IV injections where they were deprived from food. The study was designed according to the European and national guidelines for the care and use of laboratory animals as well as the OECD guidelines for chemicals [28] and the study protocol was approved by the joint animal welfare committee of the Veterinary and Agrochemical Research Centre (CODA-CERVA) and the Scientific Institute of Public Health (ISP-WIV) prior to the onset of the study.

At the start of the trial rats were randomly divided into four groups of five animals. Two groups received 519 ± 58 µl (equaling a dose of 20 mg/kg bw) of NM-200 suspended in PBS while the two control groups received 546 ± 42 µl of PBS. The administered volumes were adapted to the weight of the animals. The intravenous injection was given in the tail vein while animals were under anesthesia using isoflurane. One group of each was necropsied after 6 h and the remaining two groups after 24 h.

At each time interval the selected animals were euthanized by intramuscular injection of ketamine-medetomidine, followed by exsanguination via the jugular vein. Blood was collected in a tube coated with K_2EDTA preventing blood clotting (BD vacutainer EDTA, Becton Dickinson) for silicon measurements. Following tissues were sampled at autopsy: liver, kidney, pancreas, spleen, brain, heart, lungs, leg muscle, uterus, ovaries, bladder, small intestine, colon, stomach and the contents of stomach and intestines, as well as urine and feces produced during the experiment. The walls of the stomach and intestines were washed twice with PBS to remove the remaining content. A subsample of the organs was fixed in formaldehyde for histopathology while the rest was frozen at −20 °C for silicon analysis.

2.4. Histological and immunohistochemical analysis

For histopathology, attention was paid to sample for each type of organ similar parts in all rats. The samples were fixed in a 4% phosphate-buffered formaldehyde solution (Sigma-Aldrich, 40% solution diluted 10 times), processed routinely, paraffin-embedded, and sectioned at 5-µm thickness by a microtome. Sections were then stained with haematoxylin-eosin staining. The samples were light microscopically (Olympus BX50) assessed. For the immunohistochemical staining of macrophages, the monoclonal antibody CD68 (MCA341R; AbD Serotec, Biorad, France) was applied on paraffin sections of liver, spleen and lungs at a dilution of 1:100. For visualization Envision/HRP mouse (DAB+) kit (K4007; DAKO, Glostrup, Denmark) was used for immunolabeling. This kit also blocks endogenous peroxidase. To demask the epitope we used citrate buffer pH 6 (S2369; DAKO, Glostrup, Denmark) in a pressure cooker. The slides were evaluated qualitatively and quantitatively by the pathologist (e.g. number and concentration of microgranulomas).

2.5. Silicon analyses

Organ samples were thawed prior to their preparation for silicon analysis. Organs and tissue samples with a mass greater than 0.55 g,