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TCDD adsorbed on silica as a model for TCDD contaminated soils: Evidence for suppression of humoral immunity in mice

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ABSTRACT

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the prototypical aryl hydrocarbon receptor (AhR) ligand, exhibits immune suppression *in vivo* and *in vitro*. Suppression of primary humoral immune responses in particular has been well characterized as one of the most sensitive functional immune endpoints in animals treated with TCDD. Previous studies have used purified TCDD to elucidate the mechanisms by which TCDD and dioxin-like compounds (DLC) impair IgM production by B cells, but did not represent the route by which animals and humans are likely to be exposed environmentally. In the studies reported here, mice were treated with TCDD adsorbed onto a well-defined synthetic silica phase of known purity and physical properties, followed by sensitization with sheep erythrocytes to initiate a humoral immune response. We found that surfactant-templated mesoporous forms of amorphous silica provided an ideal combination of purity, dispersibility and textural properties for immobilizing TCDD. TCDD-adsorbed silica distributed to the spleen and liver after oral administration as assessed by induction of *cyp1a1* gene expression. Most notably, TCDD delivered in the adsorbed state on amorphous silica and as a solute in corn oil (CO) produced similar suppression of the anti-sheep red blood cell immunoglobulin M antibody forming cell (sRBC IgM AFC) response at equivalent doses of TCDD. These results suggest that TCDD immobilized on silicate particles found in soils distributes to the spleen and suppresses humoral immunity.

1. Introduction

Soils and sediments are complex, heterogeneous mixtures consisting of inorganic mineral matter, and smaller amounts of organic matter, usually <10%. Clays and other silicate phases, both crystalline and amorphous, are among the most common naturally occurring inorganic mineral phases in soils and sediments. Organic matter is a structurally complex mixture of known biochemicals (e.g., carbohydrates, proteins, and lipids), humic substances (e.g., humic and fulvic acids), and high surface-area carbonaceous materials (e.g., chars). In soils and sediments, organic matter has often been viewed as playing a disproportionately large role, relative to its abundance, in the sequestration of sparingly soluble neutral organic contaminants. However recent investigations have shown that these fine-grained minerals function as important sorptive phases for the immobilization of several prominent classes of organic contaminants including dioxins under environmentally relevant conditions (Liu et al., 2009; Nolan et al., 1989). Dioxins and many other similar AhR ligands (e.g., PCBs and PAHs) have exceptionally low water solubility and hence exist predominantly as sorbed species in soils and sediments due to their interactions with inorganic and organic geosorbents (Ferrario et al., 2000; Green et al., 2004; Hoekstra et al., 1999).

There is good evidence that silicate minerals function as both an environmental sinks and as subsequent sources for polychlorinated-dibenzo-*p*-dioxins (PCDDs) and -dibenzofurans (PCDFs), and may even play a role in their *in situ* formation (Gu et al., 2008). Perhaps the best-known and well-documented association of PCDDs/PCDFs with naturally occurring silicate minerals occurs in a material commonly referred to as "ball clay". This is a commercial term used in reference to earthen materials comprised of mixtures of clays and other silicate minerals. They occur as prehistoric geo-

Abbreviations: AFC, antibody forming cell; IgM, immunoglobulin M; SPLC, splenocytes; sRBC, sheep red blood cells; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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logic deposits in nature, and are mined in several locations and used to make ceramics, tiles, bricks, etc. Ball clays have also been used widely as livestock feed additives. They impart certain favorable properties to feeds (e.g., anti-caking), and claims have been made that they may even promote animal health, for instance by adsorbing fungal toxins. However, it has now been realized that ball clays can be reservoirs for high levels of PCDDs, with concentrations as high as 15,000 pg WHO-TEQ/g (Gadomski et al., 2004). These clays have caused livestock contamination in several instances when they were used as feed additives (Hayward and Bolger, 2005). It was reported that 5% of national poultry production, and at least 35% of farm-raised catfish in the USA, was contaminated by PCDDs originating from clay added to animal feed (Ferrario et al., 2000; Hayward and Bolger, 2005; Hayward et al., 1999).

Although association of PCDDs with mineral phases does not render them biologically unavailable, the extent to which immobilized AhR ligands are available for metabolic uptake by microorganisms and mammals, and how this is dependent on the exact nature of the adsorbent phase (e.g., clays, silica, etc.), is not clear. Naturally occurring clays and related fine-grained inorganic minerals are complex materials of variable composition and purity and, as such, they are not ideally suited for quantitative studies of the biodistribution of adsorbed toxicants (Ehlers and Luthy, 2003; Semple et al., 2004). In order to better understand the toxicology of chemical environmental contaminants that function as AhR ligands, the objective of the present study was to investigate whether, and to what extent, TCDD immobilized on a well-defined synthetic silicate phase of known purity and physical properties, impairs immune competence as assessed by the anti-sRBC IgM AFC response. Administration of TCDD adsorbed on silica delivered in an aqueous solution by oral gavage mimics ingestion of TCDD-contaminated soils. Mesoporous amorphous silica with a surface area and pore size specifically engineered for sequestering TCDD was selected as the sorptive medium for the study.

2. Materials and methods

2.1. Reagents

Sodium silicate containing 27 wt.% SiO_2 and 14 wt.% NaOH, 1.3.5-trimethylbenzene, acetic acid and reagent grade dimethylsulfoxide (DMSO) were purchased from Aldrich Chemical Co. (Milwaukee, WI). TCDD in DMSO solution (100 μg/ml) was purchased from Accustandard (New Haven, CT). CO vehicle for delivery of nonadsorbed TCDD was purchased from Sigma (St. Louis, MO). Pluronic P123 surfactant was obtained from BASF (Wyandotte, MI). All reagents were used as received without further purification.

2.2. Synthesis of mesocellular foam silica, MSU-F

Amorphous silica in mesocellular foam form (Schmidt-Winkel et al., 1999) was assembled from aqueous sodium silicate as the SiO_2 source, Pluronic P123 as the structure-directing surfactant porogen, 1,3,5-trimethylbenzene (TMB) as a micelle expanding co-surfactant, and acetic acid as the requisite acid according to previously described methods (Kim et al., 2000). The overall molar composition of the reaction mixture was 1.0 SiO_2:0.78 NaOH:0.017 P123:0.83 CH_3COOH:0.69 TMB:230 H_2O. The general method of synthesis has been described previously (Kim et al., 2000). Briefly, a micellar solution of P123 porogen was mixed with an amount of aqueous acetic acid solution equivalent to the NaOH content of the silicate source and stirred for 2 h. TMB was added to the porogen solution and the resulting mixture was stirred for an additional hour. Sodium silicate then was added to the solution of P123 porogen solution and TMB pore expander under vigorous stirring at ambient temperature and the mixture was allowed to age at 25 °C for 24 h. The mixture was then heated at 100 °C for 24 h under static conditions. The porogen-intercalated mesostructured product was recovered by filtration and dried in air at ambient temperature. The final porogen-free mesostructure, denoted MSU-F, was obtained by ethanol extraction of the as-made product under reflux for 2 h. The ethanol-extracted product was allowed to dry in air at room temperature for several days prior to use.

2.3. Physical characterization of silica

N_2 adsorption–desorption isotherms for MSU-F mesocellular foam silica were measured at −196 °C on a Micromeritics ASAP 2010 sorptometer. The sample was degassed at 150 °C under vacuum (<10^{-6} Torr) overnight prior to analysis. The intra-particle pore volume of the silica was taken to be equal to the volume of nitrogen adsorbed at a partial pressure of 0.99. Pore size and window size distributions of the mesocellular foam structure were determined by fitting to the Barret–Joyner–Hallender (BJH) equation to the adsorption and desorption legs of the isotherm, respectively. Transmission electron microscopy (TEM) images were obtained on a JEOL 2200FS field emission microscope with a ZrO/W Schottky electron gun and an accelerating voltage of 200 kV. Sonification was used to disperse the powdered samples in ethanol, and the resulting suspension was dripped onto 300 mesh copper grids for imaging analysis.

2.4. Immobilization of TCDD on MSU-F silica

TCDD was immobilized on MSU-F silica by the incipient wetness method. In this procedure, an aliquot of TCDD solution equal to the intraparticle pore volume of the silica sample is dropped onto the surface of dry silica powder in a glass vial with the use of a hypodermic syringe. The vial is sealed with an aluminum-lined screw cap and the mixture is vigorously agitated on a vortex mixer until the liquid is uniformly dispersed in the powder and the powder is uniformly dry and returned to a free flowing state. The resulting mixture is then equilibrated overnight at 60 °C to ensure uniform distribution of the TCDD solution within the intra-particle pores of the silica. After each impregnation, the DMSO solvent is removed in a vacuum oven at 60 °C overnight in order to obtain a uniform distribution of TCDD molecules on the walls of the pores. The procedure is repeated for TCDD loadings requiring sequential impregnations to achieve the desired loading. The TCDD-impregnated silica is then used to prepare aqueous suspensions for use in the mouse dosing experiments.

2.5. Animals

Pathogen-free female B6C3F1 mice, 6 weeks of age, were purchased from Charles River Breeding Laboratories (Portage, MI). On arrival, mice were randomized, transferred to plastic cages containing sawdust bedding (5 animals/cage), and quarantined for 1 week. Mice were given food (Purina Certified Laboratory Chow) and water *ad libitum* and were not used for experimentation until their body weight was 17–20 g. Animal holding rooms were kept at 21–24 °C and 40–60% relative humidity with a 12-h light/dark cycle. All procedures involving mice were approved by the Michigan State University Institutional Animal Care and Use Committee.

2.6. In vivo antibody forming cell response (AFC)

Mice (5 per treatment group) were administered corn oil vehicle (CO VH), TCDD, silica alone, DMSO-adsorbed silica or TCDD-adsorbed silica (doses provided in figure legends) by oral gavage once per day for 5 consecutive days. The silica or TCDD-adsorbed silica was delivered in 200 μl water. On day 3, mice were sensitized with 5×10^8 sRBC per mouse by i.p. injection, which allowed for TCDD exposure surrounding antigen sensitization. Four days after sRBC sensitization, mice were sacrificed and total body and spleen weights were recorded. Enumeration of the antibody forming cells was based on the Jerne plaque assay (Jerne and Nordin, 1963). Briefly, 100 μl aliquots of the recovered splenocytes were combined with 0.5% melted agar (Difco/BD, Franklin Lakes, NJ), guinea pig complement (Gibco/Invitrogen, Carlsbad, CA) and sheep erythrocytes. The mixture was vortex mixed, poured onto a petri dish, overlaid with a 24 mm × 50 mm glass cover slip, and allowed to solidify. The petri dishes were incubated for at least 3 h at 37 °C, after which AFCs were enumerated at 6.5× magnification using a Bellco plaque viewer (Bellco Glass Co., Vineland, NJ). Cell number was determined using a Z1 Coulter particle counter (Beckman Coulter, Brea, CA).

2.7. Real time polymerase chain reaction (PCR)

Mice (5 per treatment group) were administered CO, TCDD (5 μg/kg/day), DMSO-adsorbed silica or TCDD-adsorbed silica (5 μg/kg/day) by oral gavage once per day for 5 consecutive days. Twenty-four hours after the last dose, spleens and livers were placed in TRI reagent (Sigma) and stored at −70 °C. On the day of RNA extraction, spleens and livers were homogenized. Following phase separation with bromochlorophenol, RNA was precipitated from the aqueous phase with isopropanol. The remainder of the extraction, purification and DNase treatment was done using the Promega SV Total RNA Isolation System (Promega, Madison, WI). Total RNA was reverse transcribed using random primers with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). cDNA was amplified with Taqman a primer/probe set for mouse *cyp1a1* purchased from Applied Biosystems and analyzed using a 7900 HT Fast Real-Time PCR System (Foster City, CA). Fold-change values were calculated using the ΔΔCt method (Livak and Schmittgen, 2001).

2.8. Statistical analysis

The mean ± S.E. was determined for each treatment group. Differences between means were determined with a parametric analysis of variance. When significant differences were detected, treatment groups were compared to the appropriate control using Dunnett's two-tailed *t* test. For real time PCR, statistical analysis was