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Augmentation of antigen-stimulated allergic responses by a small amount of trichloroethylene ingestion from drinking water ☆

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ABSTRACT

In previous report, we have shown that trichloroethylene (TCE) increases histamine release and inflammatory cytokine production from antigen-stimulated mast cells. In this study, we examined the enhancing effect of a small amount of TCE ingestion from drinking water on antigen-stimulated allergic responses. After exposure of Wistar rats to TCE ingestion for 2 or 4 weeks, we performed a passive cutaneous anaphylaxis (PCA) reaction. TCE ingestion for 2 and 4 weeks enhanced PCA reaction in a dose-dependent manner. On histological examination, TCE ingestion for 2 weeks exacerbated inflammation characterized by infiltration of lymphocytes and accumulation of mast cells around the vessel in the skin. After TCE ingestion for 4 weeks, the mesenteric lymph nodes (MLNs) showed increase of the size and wet weight, and germinal centers changed distinctly. The interleukin-4 (IL-4) mRNA levels on spleen, MLNs and leukocytes were increased. Moreover, serum total IgE levels of TCE ingestion increased in a time-dependent manner. Our results suggest that TCE ingestion induces pro-inflammatory responses and causes Th1/Th2-type helper T-cell imbalance. And more, a small amount of TCE ingestion may lead to the initiation and acceleration of type I allergic reaction.

1. Introduction

Most investigators today agree that the prevalence rate of allergic diseases has clearly increased in industrial areas and countries (Beasley et al., 2003). Several epidemiological studies have reported associations between environmental pollutants, such as diesel exhaust particles and formaldehyde, and the prevalence of allergic diseases (Wantke et al., 1996; Takano et al., 1997; Terada et al., 1999; Strachan, 2000; Etzel, 2003). These pollutants have been reported to induce the production of inflammatory cytokines and Th2-type helper T cells lymphocyte-derived cytokines, enhance IgE production and increase histamine release from mast cells (Takenaka et al., 1995; Saneyoshi et al., 1997; Takizawa et al., 1999). Mast cells and basophils are well-known as a critical participant in various biological processes, including type I allergic reaction (Plaut et al., 1989; Stevens and Austen, 1989).These cells express IgE on their surface membrane receptors, with high affinity and specificity. The interaction of multivalent antigens with surface-bound IgE initiates a series of biochemical events, which culminate in the release of histamine, serotonin and the production of cytokines.

Volatile organic compounds are evaporated into the atmosphere at room temperature, and widely used in paint, adhesives, cosmetics, furnishings, dry cleaning and metal degreasing agent. Trichloroethylene (1,1,2-trichloroethene, TCE) has been the most popular substitute used in the dry cleaning industry. The most significant exposure to TCE occurs in the workspace. It has been estimated that more than 400,000 workers are exposed to TCE (NIOSH, 1994; IARC, 1995). And as a consequence of widespread use, TCE has been commonly detected as an environmental contaminant of groundwater, surface water and soil (Westrick et al., 1984; Josephson, 1986). Chronic exposure to TCE is accompanied by many severe toxicological and pathological problems. The toxic effects of TCE exposure are associated with disturbances in the central nervous system and changes in the parenchymal organs in the liver and kidneys (Wang et al., 2002). And TCE has been reported to induce hepatic lipid peroxidation and elevated oxidative DNA damage in mouse liver (Channel et al., 1998).

Previously, we reported that TCE increases histamine release and inflammatory mediator production from antigen-stimulated mast cells in vitro (Seo et al., 2008), but there are no reports of in vivo studies about the effect of TCE ingestion from drinking

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water on antigen-stimulated allergic responses. In the present study, after we treated Wistar rats with a small amount of TCE in drinking water for 2 or 4 weeks, we examined the enhancing effect of TCE on passive cutaneous anaphylaxis (PCA) reaction. And we investigated histological changes of the skin and immune tissues from the rats treated with TCE ingestion from drinking water. And we measured interleukin-4 (IL-4) and interferon (IFN)-γ mRNA levels of spleen, mesenteric lymph nodes (MLNs) and leukocytes isolated from whole blood of TCE ingested rats. Moreover, we measured total IgE levels in serum of TCE ingested rats.

2. Materials and methods

2.1. Reagents and animals

TCE (purity 98%) was purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan). A rat anti-dinitrophenol (DNP) monoclonal IgE antibody-producing cell line, REC established in Kissei Pharmaceutical Co., Ltd. (Hotaka, Japan), was maintained. The culture supernatant of REC was used as a source of IgE antibody. The IgE antibody titer of the preparation estimated by homologous PCA was 1:4000 or greater. DNP-conjugated bovine serum albumin (DNP–BSA) was prepared according to the method that was previously reported (Eisen et al., 1953). The number of DNP residues introduced was 13 per BSA molecule. Male Wistar rats weighing 250–300 g were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were housed three per cage and maintained on a 12-h light: dark cycle. Water and a pelleted diet were supplied *ad libitum* before TCE treatment. Experiments were undertaken following the guideline for the care and use of experimental animals produced by the Japanese Association for Laboratory Animal Science in 1987.

2.2. TCE ingestion from drinking water

A 30 g/L TCE stock solution was prepared in dimethyl sulphoxide (DMSO). This stock solution was diluted with distilled water, and 0.03 and 3 mg/L TCE solutions were prepared, and used as drinking water. The final concentration of DMSO in drinking water was less than 0.01% (v/v). In preliminary test, we confirmed that this level of DMSO in drinking water was not affect in all our experiments.

Eight-week-old Wistar rats were treated with TCE in drinking water at a concentration of 0.03 or 3 mg/L for 2 or 4 weeks. The control vehicle group of rats was given distilled water. The water was changed every other day to ensure dose maintenance.

2.3. PCA reaction

Before 48 h of each treatment endpoint (2 or 4 weeks) of TCE ingestion, 0.1 mL of rat anti-DNP IgE diluted to 1:1500 or 1:500 was injected subcutaneously into both abdominal sides. After treatment with TCE, 1 mL of antigen-Evans blue solution (1 mg of DNP–BSA and 5 mg of Evans blue were dissolved in 1 mL of saline) was injected into the tail vein. The rats were killed after 30 min, and skin with blue spots was removed. The activity of PCA was determined by measurement of the cutaneous concentration of Evans blue.

Evans blue extraction was performed according to the method described by Katayama et al. (Katayama et al., 1978). Briefly, the pigment spot was placed in a test tube and incubated overnight at 37 °C with 1 mL of 1 N KOH, and then 9 mL of a mixture of 0.6 N H_3PO_4-acetone (5:13) was added. The pigment was extracted by mixing. After centrifugation, the amount of dye was determined colorimetrically (the absorbance at 620 nm).

2.4. Histological evaluation and measurement of lymphocytes and mast cells

After treatment with TCE in drinking water for 2 weeks, the skin was fixed in 10% neutral-buffered formalin, and embedded in paraffin. After treatment with TCE in drinking water for 4 weeks, the immune tissues, such as spleen and MLNs, were removed from rats and wet weights of them were measured. These tissues were fixed in 10% neutral-buffered formalin, and embedded in paraffin. Serial sections (4 μm thick) were stained with hematoxylin–eosin and Alcian blue staining.

Histological analyses were performed using a microscope (Olympus). The numbers of lymphocytes infiltrating the dermis were counted per unit area (1 mm^2), and the numbers of mast cells accumulated perivascularly were counted per blood vessel.

2.5. Cytokine profile analysis

After TCE ingestion for 4 weeks, whole blood was obtained from each group of rat and then spleen and MLNs were extirpated from rats. Total RNA was isolated with PureLink™ Total RNA Blood Purification Kit (Invitrogen) or ISOGEN (Nippon Gene Co., Ltd.) according to the manufacturer's instructions. One microgram of total RNA from each sample was reverse transcribed using Oligo (dT) primers (Invitrogen) and Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions.

PCR amplification was performed with Ex Taq Polymerase (Takara Bio Inc.) using the IQ™-Cycler (BioRad). The primer sequences and annealing temperature of IL-4, IFN-γ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows: IL-4 (Accession No. X16058; forward primer, 5′-TCTCACGTCACTGACTG TA-3′; reverse primer, 5′-CTTTCAGTGTTGTGAGCGT-3′; annealing temperature, 53 °C; product size, 406 bp), IFN-γ (Accession No. AF010466; forward primer, 5′-AGAGCCTCCTCTTGGATATCTGG-3′; reverse primer, 5′-GCTTCCTTAGGCTAGATTCTGGTG-3′; annealing temperature, 60 °C; product size, 309 bp), GAPDH (Accession No. X02231; forward primer, 5′-GCATGGCCTTCCGTGTTCCTAC-3′; reverse primer, 5′-ACTCCTTGGAGGCCATGTAGGC-3′; annealing temperature, 62 °C; product size, 318 bp). The PCR was performed under the following conditions: 94 °C, 30 s; annealing temperature of each gene, 30 s; and 72 °C, 1 min, for 35–40 cycles. The PCR products were electrophoresed in a 1% agarose gel and visualized by ethidium bromide staining, and were semi-quantitatively analyzed with an image analyzer (Densitograph, ATTO). The relative expression intensity was calculated according to the following formula: IL-4 mRNA or IFN-γ mRNA/GAPDH mRNA in each sample.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Serum samples were obtained immediately from each TCE ingested group of rats at 0, 2 and 4 weeks. The concentrations of total IgE in serum were measured by using ELISA for Quantitative Determination of Rat IgE (CellTrend GmbH) according to the manufacturer's instructions. The assay was performed in duplicate. The concentration of total IgE was calculated from standard curve.

2.7. Statistical analysis

Data are shown as the means ± SEM. Statistical analyses were performed with StatView (SAS Institute, version 5.0). Fisher's protected least significant difference test or Scheffe's *F* test was applied, and a p-value <0.05 was considered significant.