Letter

Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice

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ABSTRACT — Trichloroethylene (TCE) and tetrachloroethylene (perchloroethylene; PCE) are commonly identified as environmental contaminants of groundwater. Previously, we investigated the enhancing effects of TCE and PCE on antigen-induced histamine release and inflammatory mediator production in rat mast cells. In this study, to examine the potential effect of TCE and PCE on antigen-induced histamine release from mouse mast cells, mouse bone marrow-derived mast cells (BMMC) were sensitized with anti-dinitrophenol (DNP) monoclonal IgE antibody and then stimulated with DNP-BSA containing with TCE or PCE. Both TCE and PCE significantly enhanced antigen-induced histamine release from BMMC. Next we investigated the effects of TCE and PCE on the passive cutaneous anaphylaxis (PCA) reaction *in vivo* using ICR mice. TCE and PCE significantly enhanced the PCA reaction in a dose-dependent manner. In addition, we examined the enhancing effects of ingesting small amount of TCE and PCE in drinking water on antigen-stimulated allergic responses. After the ICR mice had ingested TCE or PCE in their drinking water for 2 or 4 weeks, we performed the PCA reaction. Both TCE and PCE ingestion enhanced the PCA reaction in a dose-dependent manner for 4 weeks. These results suggest that exposure to TCE and PCE leads to the augmentation of type I allergic responses in many species.

Key words: Trichloroethylene, Tetrachloroethylene, BMMC, Histamine release, PCA reaction

INTRODUCTION

In recent years, many epidemiological studies have shown that the incidences of allergic diseases such as asthma and pollinosis are increasing (Beasley *et al.*, 2003; Frei and Gassner, 2008). Allergic diseases are caused by both genetic and environmental factors (Burney *et al.*, 1990; Peat and Li, 1999). In particular, exposure to ambient environmental pollutants is known to be associated several allergic symptoms. For example, experimental evidence has shown that exposure to diesel exhaust particles (Inoue *et al.*, 2007; Liu *et al.*, 2008) and phthalate (Yanagisawa *et al.*, 2008) increase allergic responses, and these pollutants have adjuvant activity.

Trichloroethylene (TCE) and tetrachloroethylene (perchloroethylene; PCE) are categorized as volatile organic compounds and are used as dry-cleaning and metal-degreasing agents in industrial operations. As a consequence of their widespread use, they are commonly identified as environmental contaminants of groundwater, soil, and indoor or outdoor air (Cooke, 1991; Westrick *et al.*, 1984; Wu and Schaum, 2000). In humans, they are well known to disturb the central nervous system and induce changes in the parenchymal organs, especially the liver and kidneys (Lash *et al.*, 2007; Wang *et al.*, 2002). However, the immunotoxic effects of TCE and PCE are scarcely known. Previously, we reported the enhancing effects of TCE and PCE on antigen-induced

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allergic responses *in vitro* and *in vivo* using rats (Seo *et al.*, 2008a, 2008b, 2008c). On the other hand, there have been no reports on the species differences in the enhancing effects of TCE and PCE on antigen-induced allergic responses.

In the present study, we investigated the effects of TCE and PCE on antigen-induced histamine release from mouse bone marrow-derived mast cells (BMMC). In *in vivo* studies, we investigated the effects of TCE and PCE on the passive cutaneous anaphylaxis (PCA) reaction using ICR mice. Furthermore, after the ICR mice had ingested low concentrations of TCE or PCE in their drinking water for 2 or 4 weeks, we performed the PCA reaction.

MATERIALS AND METHODS

Reagents and animals

TCE (purity 98%) and PCE (purity 98%) were purchased from Nacalai Tesque Co. Ltd. (Kyoto, Japan). The stock solutions (TCE; 30 g/l, PCE; 10 g/l) were prepared in dimethyl sulfoxide (DMSO). A mouse anti-dinitrophenol (DNP) monoclonal IgE antibody-residue was prepared by culturing EC1 cells, as reported previously (Sakurai *et al.*, 1994). The EC1 culture supernatant was stored at -80°C and used as a source of IgE. The anti-DNP IgE antibody content of the preparation, as estimated by enzyme-linked immunosorbent assay, was 1.5 µg/ml. DNP-conjugated bovine serum albumin (DNP-BSA) was prepared according to the previously reported method (Eisen *et al.*, 1953). The number of DNP residues introduced was 13 per BSA molecule. Male ICR mice and male BALB/c mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). They were housed four per cage and maintained on a 12-hr light: dark cycle. Water and a pelleted diet were supplied *ad libitum*. All experiments were performed according to the guidelines for the care and use of experimental animals produced by the Japanese Association for Laboratory Animal Science in 1987.

Preparation and culture of BMMC

Bone marrow cells were obtained by flushing the femurs of 6 week-old BALB/c mice. The cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 20 ng/ml recombinant murine interleukin (IL)-3 (PeproTech, Rocky Hill, NJ, USA), 10% heat-inactivated fetal bovine serum (FBS, Thermo Trace. Ltd., Melbourne, Australia), and 1% penicillin/streptomycin (Gibco) at 37°C in a humidified atmosphere containing 5% CO₂. Non-adherent cells were passaged every 5-6 days for 4 weeks. After

4 weeks, the recovered populations were composed of > 90% mast cells, as judged by an assessment of their morphology under Alcian Blue staining.

Measurement of histamine release from BMMC

The stock solutions of TCE and PCE were diluted with Tyrode solution (137 mM NaCl, 2.7 mM KCl, 1.6 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 0.05% gelatin, 0.1% glucose, 0.41 mM NaH₂PO₄, pH 7.4) to a DMSO concentration of less than 0.1%. In preliminary tests, we confirmed that this level of DMSO in Tyrode solution did not affect any of our experiments. The BMMC (1 × 10⁵ cells/ml) were sensitized by incubating them overnight at 37°C with 0.15 µg/ml anti-DNP IgE antibody in RPMI-1640 medium containing 2% FBS. After washing the BMMC with Tyrode solution, they were pre-treated with various concentrations of TCE or PCE for 30 min at 37°C. After treatment with TCE or PCE, the cells were stimulated with 10 µg/ml of DNP-BSA in Tyrode solution at 37°C for 30 min to induce antigen-stimulated histamine release. The supernatants of cell suspensions were then collected, and the histamine content of the supernatants was determined according to our previous method (Sato *et al.*, 1998). Histamine release (% of total) was calculated as the ratio of the fluorescence intensity of the sample to that of total histamine, and the percentage enhancement was calculated as the ratio of histamine release (% of total) in the presence of TCE or PCE to that observed in their absence.

PCA reaction

The stock solutions of TCE and PCE were diluted with phosphate buffered saline (PBS) to a DMSO concentration of less than 0.1%. Various concentrations of TCE and PCE were intraperitoneally injected into the ICR mice (8 week-old). The mice were then intradermally injected with 40 µl of anti-DNP IgE antibody diluted to 1:50 with saline into dorsal skin sites that had been shaved 24 hr earlier. At 24 hr after the passive sensitization, the mice were challenged intravenously with 200 µl of antigen-Evans blue solution (1 mg of DNP-BSA and 3 mg of Evans blue were dissolved in 1 ml of saline). The reaction was examined at 30 min after the challenge, and skin with blue spots was removed. PCA activity was determined by measurement of the concentration of Evans blue extracted from the cutaneous layer of the removed skin. The extraction of Evans blue was performed according to a previously described method (Katayama *et al.*, 1978). Briefly, each pigment spot was placed in a test tube and incubated overnight at 37°C with 1 ml of 1 N KOH, then 9 ml of 0.6 N H₃PO₄-acetone (5:13) were added, and the pigment

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was extracted by mixing. After centrifugation, the amount of dye was determined colorimetrically (the absorbance at 620 nm).

TCE or PCE ingestion from drinking water

The stock solutions of TCE and PCE were diluted with distilled water, and 0.03 and 3 mg/l TCE solutions and 0.01 and 1 mg/l PCE 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 tests, we confirmed that this level of DMSO in drinking water did not affect any of our experiments.

Eight-week-old ICR mice were treated with TCE or PCE in drinking water for 2 or 4 weeks. The vehicle group of mice was given distilled water. The water was changed every other day to ensure dose maintenance. Twenty-four hours before each treatment endpoint (2 or 4 weeks) of TCE or PCE ingestion, the mice were intradermally injected with 40 μ l of anti-DNP IgE antibody diluted to 1:50 with saline into dorsal skin sites. PCA reaction was performed as described above.

Statistical analysis

Data are shown as the mean $\pm$ S.E. Statistical analy-

ses were performed with StatView (SAS Institute, version 5.0). Fisher's protected least significant difference test or Schéffe's F test was applied, and a *P*-value < 0.05 was considered significant.

RESULTS

Enhancing effect of TCE and PCE on histamine release from antigen-stimulated BMMC

To evaluate the enhancing effects of TCE and PCE on antigen-induced histamine release from BMMC, we examined histamine release from antigen-stimulated BMMC treated with TCE or PCE. Low concentrations of TCE and PCE significantly increased the amount of histamine released from the antigen-stimulated BMMC. Treatment with 0.1 mg/l TCE caused the biggest enhancement of histamine release from the antigen-stimulated BMMC (Fig. 1A, enhancement ratio compared with the control: 1.3). Moreover, treatment with PCE enhanced histamine release from the antigen-stimulated BMMC in a dose-dependent manner (Fig. 1B, the peak enhancement ratio compared with the control: 1.7).

Fig. 1. Effects of TCE (A) and PCE (B) on antigen-induced histamine release from BMMC. After being sensitized with anti-DNP IgE, the cells were treated with TCE (0.01–1 mg/l) or PCE (0.01–1 mg/l) and then stimulated with DNP-BSA for 30 min. The percentage histamine release was calculated as the ratio of the fluorescence intensity of the test sample to that of total histamine (*n* = 3). Data are expressed as the mean $\pm$ S.E. of 3 separate experiments (**P* < 0.05, ***P* < 0.01).

Enhancing effects of TCE and PCE on the PCA reaction

In our *in vivo* study, the effects of TCE and PCE on the PCA reaction were investigated. The results are shown in Fig. 2. Varying doses of TCE and PCE were intraperitoneally injected at 24 hr before the antigen challenge. PCE and TCE significantly increased the PCA reaction in a dose-dependent manner, and the doses that had the strongest effects were 0.1 mg/kg body weight (b.w.) TCE (Fig. 2A, enhancement ratio: 2.0) and 1 mg/kg b.w. PCE (Fig. 2B, enhancement ratio: 2.6).

TCE or PCE ingestion from drinking water for 2 or 4 weeks enhanced the PCA reaction

The volumes of drinking water ingested by the TCE and PCE groups each day were not different from that ingested by the vehicle group (data not shown). In the TCE ingestion groups, each group of mice ingested a mean volume of drinking water of 6.0 ml/day. This equates to a mean dose of 0.18 μ g TCE/day (0.03 mg/l TCE treatment group) or 18.7 μ g TCE/day (3 mg/l TCE treatment group). In the PCE ingestion groups, each group ingested a mean volume of drinking water of 6.9 ml/day. This equates to a mean dose of 0.07 μ g PCE/day (0.01 mg/l PCE treatment group) or 7.2 μ g PCE/day (1 mg/l PCE treatment group).

To evaluate the effects of TCE or PCE ingestion from drinking water on type I allergic reactions, we performed

the PCA reaction. The results are shown in Figs. 3 and 4. The ingestion of 0.03 or 3 mg/l TCE in drinking water for 2 weeks significantly enhanced the PCA reaction in a dose-dependent manner (Fig. 3A, enhancement ratio: 1.5 at 0.03 mg/l TCE, 2.2 at 3 mg/l TCE), and a longer period (for 4 weeks) of TCE ingestion led to further enhancement (Fig. 3B, enhancement ratio: 2.3 at 0.03 mg/l TCE, 3.2 at 3 mg/l TCE). The ingestion of PCE in drinking water for 2 weeks did not enhance the PCA reaction compared with the vehicle treatment (Fig. 4A), whereas PCE ingestion for 4 weeks enhanced the PCA reaction in a dose-dependent manner (Fig. 4B, enhancement ratio: 2.1 at 0.01 mg/l PCE, 2.4 at 1 mg/l PCE).

DISCUSSION

In this study, we found that TCE and PCE markedly increased antigen-induced histamine release from mouse BMMC. In an *in vivo* study, a single intraperitoneal injection of TCE or PCE enhanced the PCA reaction, which showed a good correlation with the amount of histamine released from the antigen-stimulated BMMC. Furthermore, the ingestion of TCE or PCE from drinking water also enhanced the PCA reaction.

The lower concentrations of TCE and PCE used in our experiments represent the standard values of the Japanese standard for drinking water, which are similar to those outlined in the WHO drinking water guidelines (World

Fig. 2. Effect of TCE (A) or PCE (B) treatment on the PCA reaction. TCE (0.01–1 mg/kg body weight) or PCE (0.01–1 mg/kg body weight) was intraperitoneally injected at 24 hr before antigen challenge. Two separate experiments were performed ($n = 4$). Data are expressed as the mean $\pm$ S.E. (* $P < 0.05$, ** $P < 0.01$).

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Fig. 3. Effects of ingesting TCE in drinking water for 2 or 4 weeks on the PCA reaction. Twenty-four hours before the end of treatment for 2 weeks (A) or 4 weeks (B), mouse anti-DNP IgE antibody diluted to 1:50 or PBS were intradermally injected. DNP-BSA-Evans blue solution was injected into the tail vein. Skin with blue spots was removed, and the supernatant of the Evans blue was measured. Data are expressed as the mean $\pm$ S.E. (* P < 0.05, ** P < 0.01).

Fig. 4. Effects of ingesting PCE in drinking water for 2 or 4 weeks on the PCA reaction. Twenty-four hours before the end of treatment for 2 weeks (A) or 4 weeks (B), mouse anti-DNP IgE antibody diluted to 1:50 or PBS were intradermally injected. DNP-BSA-Evans blue solution was injected into the tail vein. Skin with blue spots was removed, and the supernatant of the Evans blue was measured. Data are expressed as the mean $\pm$ S.E. (** P < 0.01).

Health Organization, 1996). The other concentrations of TCE and PCE used in these experiments have sometimes been detected in groundwater from contaminated areas (Leschber *et al.*, 1990; Wu and Schaum, 2000).

Our results show that TCE and PCE enhanced histamine release from antigen-stimulated BMNC. BMNC are mucosal mast cells that are distributed in the nasal, pulmonary, and gastrointestinal mucosa. In our previ-

ous studies, the enhancement of antigen-induced histamine release by TCE and PCE was evaluated using connective mast cells, rat peritoneal mast cells (RPMC), and rat basophilic leukemia (RBL-2H3) cells. These different types of mast cells and basophils share many common features, such as they all express the high-affinity IgE receptor (FcεRI), and activation of FcεRI leads to histamine release and cytokine gene expression. From our present results, the enhancement ratio of histamine release from BMMC was slightly lower than those found for RPMC and RBL-2H3 cells. However, the pathogenesis of pollinosis is caused by the activation of mucosal mast cells; therefore, the enhancement of antigen-induced histamine release from BMMC by TCE and PCE is an important finding.

In our *in vivo* studies, it was shown that a single intraperitoneal injection of TCE or PCE markedly enhanced the PCA reaction in ICR mice compared with Wistar rats (Seo *et al.*, 2008a). Furthermore, the ingestion of a low concentration of TCE or PCE from drinking water also markedly enhanced the PCA reaction in ICR mice compared with Wistar rats (Seo *et al.*, 2008b, 2008c). In terms of metabolism, both TCE and PCE serve as substrates for cytochrome P450 (P450) and glutathione S-transferases, and the metabolic pathways of TCE and PCE are very similar (Lash *et al.*, 2007). The kinetics of the formation of TCE and PCE metabolites are significantly different, and species differences in the rates of P450-dependent metabolism have been found, e.g., the rate of trichloroacetate (TCA) excretion is markedly slower in humans than in rats (Völkel *et al.*, 1998). In our preliminary examinations, the main metabolites of TCE and PCE from the P450 pathway, TCA and chloral, did not enhance histamine release from antigen-stimulated BMMC, RPMC, or RBL-2H3 (data not shown). Therefore, we speculate that TCE and PCE themselves enhance antigen-induced allergic responses. When humans are transiently exposed to TCE or PCE, very little TCE or PCE accumulates in their tissues, as they are rapidly excreted into the urine and expelled during exhalation. However, chronic exposure to TCE or PCE may induce serious enhancement of allergic responses in humans. Further study is needed to confirm using human mast cells.

In conclusion, we demonstrated the enhancing effects of TCE and PCE on histamine release from antigen-stimulated BMMC and the PCA reaction in mice. These results suggest that the ingestion of TCE or PCE from drinking water enhances allergic responses in many species, even at low concentrations such as the values set for the Japanese standard for drinking water, and may accelerate the augmentation of allergic diseases.

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