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Olfactory mucosal necrosis in rats following acute intraperitoneal administration of 1,2-diethylbenzene, 1,2-diacetylbenzene and 2,5-hexanedione

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ABSTRACT

1,2-Diethylbenzene (1,2-DEB) is used in the manufacture of some plastics. Exposure to 1,2-DEB has been shown to induce peripheral neuropathy in rats. This neurotoxicity is thought to be caused by a metabolite, 1,2-diacetylbenzene (1,2-DAB), a γ-diketone-like compound. 1,2-DEB was previously shown to be extensively and rapidly taken up by the nasal mucosa in male rats. In the present study, the nasal mucosa in rats exposed to 1,2-DEB and 1,2-DAB were examined histologically. Results were compared to sections from rats exposed to two other DEB isomers – 1,3-diethylbenzene (1,3-DEB) and 1,4-diethylbenzene (1,4-DEB) – and to two other neurotoxic compounds – n-hexane and its γ-diketone metabolite, 2,5-hexanedione (2,5-HD). A single intraperitoneal dose of 1,2-DEB (200 mg/kg) induced time-dependent necrosis in the olfactory epithelium and Bowman's glands, with lesions appearing from the earliest observation time (4 h) in the dorsomedial olfactory mucosa. Lesions spread through the lateral and ventral parts of the ethmoturbinates over the following days. The dorsal and medial zones of the nasal cavity started to regenerate from 72 h after treatment, with the new epithelium showing metaplasia. One month after treatment, most of the olfactory epithelium had returned to normal. 1,2-DAB (40 mg/kg) caused the same lesions as those observed after treatment with 1,2-DEB. Treatment with 2,5-HD (1 g/kg) also caused lesions of the olfactory epithelium, mainly at level IV. However, these were comparatively less severe than those observed after exposure to 1,2-DEB. In contrast, intraperitoneal injection of 1,3-DEB (800 mg/kg), 1,4-DEB (800 mg/kg) and n-hexane (2 g/kg) did not affect the nasal mucosa. Pretreatment of rats with 5-phenyl-1-pentyne, an inhibitor of CYP2F2 and CYP2E1 completely inhibited the olfactory toxicity caused by 1,2-DEB. These results suggest that metabolic activation of 1,2-DEB may be responsible for the toxicity observed.

1. Introduction

Diethylbenzene (DEB; CAS No. 25340-17-4) is a yellow liquid with an aromatic odor. It is manufactured as a mixture of three isomers: 1,2-diethylbenzene (1,2-DEB), 1,3-diethylbenzene (1,3-DEB), and 1,4-diethylbenzene (1,4-DEB). It is almost exclusively converted to divinylbenzene, an intermediate used as a cross-linking agent in the manufacture of synthetic rubbers, polyester resins, ion exchange resins or casting resins. A very small percentage (<5%) is used as an industrial heat-transfer fluid, and low levels are found in motor fuels. Occupational exposure to DEB could occur in manufacturing or formulating operations. DEB is produced, distributed, stored, and used in closed systems. Exposure to this compound could thus occur at ethylbenzene or divinylbenzene manufacturing sites, facilities that formulate DEB into heat-transfer fluids, or at facilities using heat-transfer fluids containing DEB. Worker exposure is also possible during product transfer between industrial sites. Occupational exposure to diethylbenzene may occur through inhalation or dermal contact with this compound at workplaces where diethylbenzene is produced or used.

The 1,2-DEB (CAS No. 135-01-3) isomer has been shown to be neurotoxic in rats, while 1,3- and 1,4-isomers showed no toxicity (Gagnaire et al., 1990). Rats repeatedly exposed to 1,2-DEB exhibited blue discoloration of the skin and urine, paralysis of the hind limbs, severely impaired sensorimotor nerve conduction velocities, and alterations in auditory evoked potentials (Gagnaire et al., 1990, 1992a, 1992b). 1,2-DEB is mainly metabolized by direct oxidation of the ethyl side chain to form two enantiomers of 1-(2-ethylphenol) ethanol and their glucuroconjugates. It also forms a minor aromatic diketone metabolite, 1,2-diacetylbenzene

Abbreviations: 1,2-DEB, 1,2-diethylbenzene; 1,3-DEB, 1,3-diethylbenzene; 1,4-DEB, 1,4-diethylbenzene; 1,2-DAB, 1,2-diacetylbenzene; 2,5-HD, 2,5-hexanedione; HES, hematoxyline–eosine–safran; PAS, periodic acid–Schiff.

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(1,2-DAB) (Gagnaire et al., 1991; Payan et al., 1999, 2001; Thrall et al., 2007). Like 1,2-DEB, 1,2-DAB has been reported to induce electrophysiological deficits, blue discoloration of the skin, brain and spinal cord, limb weakness (Gagnaire et al., 1991, 1992b; Kim et al., 2001; Tshala-Katumbay et al., 2005, 2008), and nerve fiber alterations including proximal neurofilament-filled giant axonal swellings, demyelination, and myelin bubbles (Kim et al., 2001; Tshala-Katumbay et al., 2005, 2008). These were most prominent in the spinal cord and spinal roots. Because of these effects, 1,2-DAB is thought to be the active metabolite of 1,2-DEB, forming adducts with motor and cytoskeletal proteins required for axonal transport (Sabri et al., 2007).

A bio-distribution study of 1,2-DEB by whole-body autoradiography showed that after intravenous administration of [^{14}C] 1,2-DEB, the highest concentrations of non-volatile [^{14}C] metabolites were found in the nasal cavity, ethmoid turbinates and in the kidneys (Payan et al., 2008). The site-specific distribution of the radioactivity in the nasal cavity may be due to the high rate of metabolic activation and the accumulation of reactive metabolites in the olfactory mucosa, which could be related to the high levels of P450 cytochromes present in the nasal mucosa in rodents (Brittebo, 1993, 1997; Thornton-Manning and Dahl, 1997; Ling et al., 2004). Thus, in situ metabolism of 1,2-DEB to 1,2-DAB, a highly protein-reactive compound (Tshala-Katumbay et al., 2008, 2009), could lead to olfactory toxicity, as shown for other compounds actively metabolized in the olfactory mucosa (Reed, 1993; Brittebo, 1993, 1997; Genter et al., 1998; Green et al., 2001; Lee et al., 2005; Zhuo et al., 2009; Xie et al., 2010).

The main purpose of the present study was to examine the potential toxicity of 1,2-DEB on rat nasal mucosa after intraperitoneal treatment with this compound and to follow the kinetics of the damage induced by a single treatment. The potential toxicity of two other isomers, 1,3- and 1,4-diethylbenzene (1,3-DEB and 1,4-DEB, respectively), and of 1,2-DAB on rat nasal tissue was also investigated. The neurotoxic mechanisms proposed for 1,2-DEB and 1,2-DAB are thought to be similar to those of n-hexane and its γ-diketone metabolite, 2,5-hexanedione (2,5-HD) (Kim et al., 2001, 2002; Spencer et al., 2002; Sabri et al., 2007; Tshala-Katumbay et al., 2006, 2009). Therefore, we compared the effects of 1,2-DEB and 1,2-DAB on the nasal mucosa to those caused by intraperitoneal exposure to n-hexane and 2,5-HD. We also hypothesized that CYP2F4 (rat ortholog of mouse CYP2F2) and/or CYP2E1 might be involved in in vivo bioactivation of 1,2-DEB. To investigate the latter point, we performed histological studies in rats treated with both 1,2-DEB and 5-phenyl-1-pentyne, an inhibitor of CYP2F2 and CYP2E1.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Charles River, Domaine des Oncins, Saint-Germain-sur-l'Arbresle, France) were isolated for 6 days before the study period and were approximately 9 weeks old (body weight: 300–330 g) at the beginning of the study. Animals were housed in polypropylene cages (one per cage) on woodchip bedding. The room temperature (22 °C), humidity (55 ± 5%) and light cycle (7 a.m.–7 p.m.) were controlled automatically. Filtered tap water (pore size 0.3 μm) and food (UAR-Alimentation, Villemoisson, Epinay-sur Orge, France; sterilized with γ-rays) were available ad libitum.

All of the experiments in this study were performed in line with the European guidelines relating to the protection of animals used for experimental and other scientific purposes (86/609/EEC).

2.2. Chemicals

The following chemicals were used:1,2-DEB (99% purity; Aldrich, Steinheim, Germany); 1,3-DEB (99% purity; Fluka, Buchs, Switzerland), 1,4-DEB (99% purity; Fluka, Buchs, Switzerland), 1,2-DAB (99% purity; Aldrich, Steinheim, Germany), n-hexane (98% purity; Merck, Darmstadt, Germany) and 2,5-HD (99% purity, Fluka, Buchs, Switzerland), 5-phenyl-1-pentyne (Alfa-Aesar, Karlsruhe, Germany). All purity grades are as indicated by suppliers.

2.3. Animal treatment

A total of seventy three rats were assigned to the different groups. Twelve groups, of four male rats each, received single intraperitoneal injections of 1,2-, 1,3-, 1,4-DEB and 1,2-DAB. Each DEB isomer was diluted in olive oil before injection. 1,2-DAB was dissolved in saline and 5% acetone and administered in a volume of 4 mL/kg. DEB isomers were administered in a volume of 1 mL/kg body weight for 1,2-DEB and 4 mL/kg body weight for 1,3- and 1,4-DEB. The doses were chosen on the basis of the results obtained in preliminary range-finding studies demonstrating olfactory toxicity. The doses used produced no mortality and resulted in a reduction in body weight of less than 10%. Overall doses were as follows: 200 mg/kg body weight for 1,2-DEB, 800 mg/kg body weight for 1,3- and 1,4-DEB, and 40 mg/kg for 1,2-DAB. 1,2-DEB-treated animals were euthanized 4 h, 8 h, 14 h, 24 h, 48 h, 72 h, 1 week, 2 weeks or 1 month after treatment. Animals treated with the other two isomers and 1,2-DAB were euthanized 72 h after treatment. Six control rats received vehicle alone: olive oil (three rats) or saline/acetone (three rats) and euthanized 72 h after injection.

Two other groups, of three rats each, received single intraperitoneal injections of n-hexane (2 g/kg) and 2,5-hexanedione (2,5-HD, 1 g/kg). n-Hexane was dissolved in olive oil and 2,5-HD was dissolved in saline. The molar ratios used for 1,2-DEB and n-hexane or 1,2-DAB and 2,5-HD were chosen based on the known systemic toxicity and peripheral neurotoxicity of these compounds (Gagnaire et al., 1991, 1992b; Spencer et al., 1980). n-Hexane and 2,5-HD were administered in a volume of 4 mL/kg body weight. These animals were euthanized 48 h after injection.

To determine the role of metabolism in 1,2-DEB-induced olfactory toxicity, a group of five rats were given a single intraperitoneal dose of 200 mg/kg 1,2-DEB. These rats also received three doses of 200 mg/kg 5-phenyl-1-pentyne in olive oil (10 mL/kg) 16 h and 30 minutes before receiving 1,2-DEB, and 8 h after receiving 1,2-DEB. In this experiment, positive controls (5 rats) were treated with 1,2-DEB and olive oil (not containing enzymatic inhibitor), while negative controls (3 rats) were given 200 mg/kg 5-phenyl-1-pentyne in olive oil (10 mL/kg) alone. The same protocol was followed for all animals. Animals were euthanized 48 h after 1,2-DEB injection.

2.4. Histopathology procedure

Sodium heparin was administered intraperitoneally (500 units/100 g) thirty minutes before inducing deep anesthesia with sodium pentobarbital (10 mg/mL; 1 mL). Rats were perfused via the left ventricle with 0.1 M phosphate-buffered saline (PBS) containing sodium heparin (10 units/mL, pH 7.4), followed by Bouin's solution. Bouin's solution (10 mL) was also flushed through the trachea to fill the nasal cavity. Rats were decapitated, and the eyes, lower jaw, skin, and musculature were removed. Trimmed heads were postfixed in Bouin's solution with shaking for 24 h. After fixation, heads were decalcified for 6 days in a solution containing 17% disodium ethylenediaminetetraacetate, pH 7.4 (Osteosoft, Merck, Darmstadt, Germany) and 4% formaldehyde.