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Antioxidant and detoxifying enzymes in the liver of rats after subchronic inhalation of the mixture of cyclic hydrocarbons

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Abstract

The activity of antioxidant and detoxifying enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSHPx), glutathione-S-transferase (GST), the SOD isoenzyme patterns and the contents of thiobarbituric acid reactive substances (TBARS), were determined in the livers of male and female rats after subchronic inhalation of mixtures of benzene, cyclohexanone and cyclohexane. Except for decreased GSHPx (with substrate cumene hydroperoxide) and GST activities in female rats, no differences in the activities of antioxidant and detoxifying enzymes and TBARS content occurred. Between the activities of GSHPx and GST was observed an indirect relationship. The activities of GSHPx-cum and GST were influenced by sex.

Keywords: Cyclic hydrocarbons; Rats; Liver; Antioxidant enzymes; Isoenzyme pattern SOD

Introduction

Benzene, cyclohexanone and cyclohexane are the important substances widely used in an industry. Benzene is fairly soluble in water and is removed from the atmosphere in rain. The primary routes of exposure are inhalation of contaminants, especially in the areas with high traffic, and consumption of contaminated drinking water. Benzene is converted into a variety of metabolites, but the major one is phenol. Benzene can be enzymatically bioactivated to reactive intermediates that can lead to increased formation of reactive oxygen species (ROS) (Winn, 2003).

Some studies have demonstrated that a lot of chemical compounds such as pesticides, herbicides, metals, many organic compounds can generate extremely ROS (Winzer et al., 2002). ROS damage cellular macromolecules causing lipid peroxidation and nucleic acid and protein alterations. Their formation is considered as a pathobiochemical mechanism involved in the initiation or progression phase of various diseases such as atherosclerosis, ischemic heart diseases, diabetes, initiation of carcinogenesis or liver diseases (Halliwell and Gutteridge, 1999; Hoffman et al., 1989; Southorn and Powis, 1988; Yagi, 1994). Therefore, the concentrations of ROS have to be controlled by several defense mechanisms, which involve also a number of antioxidant and detoxifying enzymes. Antioxidant enzymes play a crucial role in maintaining cells homeostasis.

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Their induction reflects a specific response to pollutants (Cheung et al., 2001).

Superoxide dismutase (SOD) is a scavenger of superoxide radicals that are converted to H_2O_2 (McCord and Fridovich, 1969). Glutathione peroxidase (GSHPx) is involved in the reduction of hydrogen peroxide, lipid hydroperoxides and other organic hydroperoxides (Tappel et al., 1982). Glutathione-S-transferase (GST) represent a major group of detoxifying enzymes (Hayes and Pulford, 1995), which form a family of multifunctional proteins involved in the cellular detoxification of cytotoxic and genotoxic compounds and in the protection of tissues against oxidative damage (Mannervik and Danielson, 1988; Pickett and Lu, 1989). Besides certain roles in the endogenous metabolism, these enzymes are associated with the detoxification of xenobiotics such as drugs, carcinogens and environmental pollutants in man and animals, and with pesticide and herbicide resistance in insects and plants (Hayes et al., 1990).

The expression above mentioned enzymes is known to be influenced by many variables including species, organ, age, sex and several environmental factors (Prohaska and Sunde, 1993).

In the present study we evaluated the biochemical responses of the mixture of benzene, cyclohexanone and cyclohexane after subchronic inhalation exposure in rats on the activities of antioxidant and detoxifying enzymes as well as on the isoenzyme patterns of SOD in liver. Activities were observed in the liver, because it is one of the tissues showing a high rate of free radical generation, with high metabolic capability and a detoxifying capacity.

Material and methods

Chemicals

Perhydrol (30% H_2O_2), cumene hydroperoxide, 1-chloro-2, 4-dinitrobenzene (CDNB) were from Merck (Darmstadt, Germany). NADPH was purchased from the Boehringer Mannheim Biochemicals (Germany). Cytochrome *c*, xanthine, xanthine oxidase, nitroblue tetrazolium, bovine serum albumin, thiobarbituric acid, sodium azide, glutathione reductase, GSH were obtained from Sigma Chemical Company (St. Louis, MO).

Animals

Twenty-four clinically healthy male and female Wistar rats SPF, 6-week-old, were obtained from Velaz Praha, Czech Republic. There were 6 males weighing 350 ± 20 g and 6 females weighing 240 ± 20 g in the experimental group (S). The control group (C) consisted of 6 males with mean b. wt. 330 ± 20 g and 6 females with mean b. wt. 240 ± 10 g. The animals were housed individually in plastic cages and acclimatized for 1 week before dosing. Animals were observed before initiation of the study to ensure that they were healthy. Only animals found to be in a clinically acceptable condition were assigned to the study. Food and water were offered ad libitum. Animal quarters were maintained at 22 °C (± 2 °C); 30–70% relative humidity on a 12 h light/dark cycle (OECD, 1981).

Chemical substances in tested mixture

The tested mixture contained the chemical substances benzene, cyclohexanone and cyclohexane in the ratio (the mass) 4.2 (benzene): 8.08 (cyclohexanone): 59.8 (cyclohexane). The ratio was calculated according to a long-term measurement of the concentrations of these substances in the air of the chemical plants (the means of 5 years). This mixture represents ten times higher concentration than that found in the environment of chemical plants. Literary data about possible effects of this mixture of chemical substances on organisms are not available, but may be predicted from the knowledge of each of the constituents (Marhold, 1986).

Dose and exposure

The tested mixture was given by the inhalation apparatus at a dose of 0.72 g/m^3 for 2 h/day, 5 days/week for 105 days. Whole-body of animals was exposed.

Preparation of tissue extracts

Livers were washed two times with cooled physiological solution, cut into pieces, and homogenized in Ultra-Turrax T-25 homogenizer to make a 25% (w/v) homogenate in 5 mmol l^{-1} Tris-HCl buffer pH 7.8, containing 0.15 mol l^{-1} KCl, 1 mmol l^{-1} EDTA, and 2 mmol l^{-1} GSH. Homogenates were centrifuged 60 min at 105,000*g* using Beckman L8-60 ultracentrifuge. Supernatants were stored at −50 °C until used for later assays. All procedures were performed at 4 °C.

Enzyme assays

SOD (EC 1.15.1.1; SOD) was measured according to McCord and Fridovich (1969), at 550 nm (25 °C), through the inhibition of cytochrome *c* reduction using the xanthine–xanthine oxidase $O_2^{\cdot-}$ generating system. One unit of SOD was defined as the amount of enzyme that inhibits the rate of cytochrome *c* reduction by 50%, under the condition specified.

GSHPx (EC 1.11.1.9; GSHPx) was measured according to Flohé and Günzler (1984) in a coupled assay with