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Modulation of lipid peroxidation and antioxidant defense systems in rat intestine by subchronic fluoride and ethanol administration

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Abstract

Excessive consumption of fluoride and ethanol has been identified as injurious to human health. Fluoride and ethanol co-exposures are commonly seen among the alcoholics residing in endemic fluoride areas worldwide. This study was undertaken to examine the modulation of lipid peroxidation and antioxidant defense systems in rat intestine by subchronic fluoride and ethanol administration. Female Sprague-Dawley rats were divided into four groups: group I (control), group II (fluoride was given orally at a dose of 25 mg/kg body weight), group III (30% ethanol was given orally at a dose of 1 mL/kg body weight), and group IV (a combination of fluoride and ethanol was administered orally at the dose described for groups II and III). Lipid peroxidation was elevated ($P < .05$) in intestine of rats by fluoride or ethanol treatments for 20 or 40 days. However, glutathione content was reduced by fluoride (32 and 44%) and ethanol (21 and 40%) treatments after 20 and 40 days, respectively. Fluoride-exposed animals showed reduction ($P < .05$) in the activities of superoxide dismutase (22 and 42%), catalase (30 and 37%), glutathione peroxidase (22 and 35%), glutathione reductase (32 and 34%), and glutathione-*S*-transferase (24 and 30%) after 20 and 40 days. A similar decrease ($P < .05$) in the activities of these enzymes was also noticed in animals exposed to ethanol for 20 or 40 days. The observed changes in lipid peroxidation, reduced glutathione levels, and enzyme systems were further augmented in intestine of rats exposed to fluoride and ethanol together. Intestinal histology showed large reactive lymphoid follicles along with mild excess of lymphocytes in lamina propria of villi, villous edema, focal ileitis, and necrosis of villi in animals exposed to fluoride and ethanol for 40 days. These findings suggest that fluoride and ethanol exposure induces considerable changes in lipid peroxidation, antioxidant defense, and morphology of rat intestine, which may affect its functions.

Keywords: Fluoride; Ethanol; Co-exposure; Lipid peroxidation; Antioxidant defense; Rat intestine

Introduction

Reactive oxygen species (ROS) are produced naturally as a part of intracellular metabolic processes and induce oxidative damage to cell membranes, lipids, proteins, and nucleic acids (Ames et al., 1993; McCord, 1993). However, cellular antioxidant defense systems control the damage caused by these harmful species (Bondy and Orozco, 1994). The rate of free radical generation and their elimination by antioxidant defense mechanisms are balanced under normal conditions (Rubin, 1993). However, when the generation of ROS in cells impairs antioxidant defenses or exceeds its ability to eliminate them, oxidative stress ensues (Jenkins and Goldfarb, 1993). Many reports have revealed enhanced ROS/free radical formation by fluoride exposure (Chlubek, 2003; Inkielewicz and Czarnowski, 2010; Rzeuski et al., 1998) and ethanol ingestion in the body (Mehta et al., 1998; Wetscher et al., 1995).

The absorption of fluoride occurs by passive diffusion process from intestine (Gharzouli and Senator, 1994; Whitford, 1996). Acute and chronic studies in experimental animals and humans have shown that sodium fluoride intake causes gastrointestinal damage (Shashi, 2002; Sondhi et al., 1995; Susheela et al., 1992). Gastric aberrations including loss of appetite, nausea, anorexia, abdominal pain, flatulence, constipation, and intermittent diarrhea are often reported in humans residing in endemic fluoride areas (Susheela et al., 1993). It has also been demonstrated that fluoride induces excessive production of oxygen free radicals, and may cause the depletion of some antioxidant enzyme systems (Chlubek, 2003). Toxic effects of fluoride on various biochemical parameters have been described (Chlubek, 2003; Singh et al., 1985), which are mediated by generation of free radicals (Rzeuski et al., 1998).

During alcohol ingestion, the gastrointestinal tract is exposed to ethanol concentrations several times higher than

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those attained in other tissues (Beck and Dinda, 1981). Ethanol ingestion alters intestinal morphology affecting its functions (Kaur et al., 1994; Persson, 1991). Alcohol is primarily metabolized in the liver (Maher, 1997) and generates ROS that causes oxidative stress and are capable of attacking cell membranes and biomolecules (Balkan, et al., 2001; Lindros, 1995). Enhanced free radical activity in the pathogenesis of ethanol-induced damage to various extrahepatic tissues such as gastric mucosa, the heart, and the testis has been demonstrated (Nordmann et al., 1990). In view of these observations, we investigated the effects of subchronic fluoride and ethanol exposure, alone and in combination on lipid peroxidation, antioxidant defense systems, and morphology of the intestine in rats.

Materials and methods

Chemicals and reagents

Sodium fluoride (NaF, molecular weight 41.99) and ethanol (CH_3CH_2OH, molecular weight 46.08) were procured from Sisco Research Laboratories (SRL) Pvt. Ltd. Mumbai, India and Changshu Yangyuan Chemicals, China, respectively. All other analytical grade chemicals and reagents were purchased from Merck (Germany), Sigma, or SRL Chemicals (India). Ultra pure water prepared by labPURE-Series Analytica6 & Ultraplusuf (BIO-AGE, Mohali, India) was used throughout the experimental period.

Animals

Female Sprague-Dawley rats ($N = 32$), obtained from Central Animal House of Panjab University, Chandigarh, India, were randomly divided into four groups. Animals were housed in polypropylene cages, equipped with a wire lid. Rats were maintained under a 12-h dark/light cycle with ad libitum access to rodent chow diet and tap water. The experimental protocol was approved by the Ethical Committee of the Institute on the use of laboratory animals. Experiments on animals were performed in accordance with guidelines for use of laboratory animals, approved by Indian Council of Medical Research, New Delhi.

Treatments

There were eight rats in each group: Group I (control), group II (Fluoride treated; fluoride was given orally at a dose of 25 mg/kg body weight), as described by Maurer et al. (1990). Group III (ethanol treated; 30% ethanol was given orally at a dose of 1 mL/kg body weight) and group IV (co-exposed to fluoride and ethanol; a combination of fluoride and ethanol was administered by the route and at the dose previously mentioned). Four animals from each group were fasted overnight and killed on days 20 and 40, respectively. All treatments were given daily at 9 a.m. and there was no time gap between fluoride and ethanol treatment in co-treated group, as they were given simultaneously. Body weight and daily food intake were recorded during experimental period.

Procedures

Overnight fasted animals were sacrificed under light ether anesthesia. Intestine was removed, rinsed with ice-cold isotonic saline (0.9% wt/vol NaCl). Ten percent (wt/vol) tissue homogenate was prepared in 50 mM sodium phosphate buffer (pH 7.4) using Potter-Elvehjam-type glass homogenizer. Two milliliters of 10% fresh homogenate was used for nonenzymatic assays and remaining solution was centrifuged at $1{,}000 \times g$ for 10 min at 4°C. The pellet was discarded and a portion of supernatant was again centrifuged at $12{,}000 \times g$ for 20 min to obtain postmitochondrial supernatant (PMS), as described by Shirkey et al. (1979).

Lipid peroxidation

Lipid peroxidation was quantified in tissue homogenates by the method of Wills (1966). The amount of malondialdehyde (MDA) formed was determined by the reaction with thiobarbituric acid and optical density was read at 532 nm. The results were expressed as nmoles of MDA/mg protein using molar extinction coefficient of MDA–thiobarbituric chromophore ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

Superoxide dismutase

Superoxide dismutase activity was assayed in PMS by the method of Kono (1978). Reaction mixture contained 1.90 mL of sodium carbonate buffer (50 mM in 0.1 mM *e*thylene*d*iamine*t*etra*a*cetic acid [EDTA], pH 10.8), 0.7 mL of nitro blue tetrazolium (96 μM), 0.15 mL of Triton X-100 (0.6% vol/vol), and 10 μL of PMS. The reaction was initiated by adding 0.15 mL of hydroxylamine hydrochloride (20 mM) and the development of blue-colored complex was followed with 30-s intervals at 560 nm for 3 min. The enzyme activity was expressed as units/mg protein, where one unit of enzyme activity is defined as the amount of enzyme inhibiting the rate of reaction by 50%.

Catalase

Catalase activity was assayed by the method of Aebi (1984). Reaction mixture contained 3 mL of H_2O_2 phosphate buffer (12.5 mM, pH 7.0) and 100 μL of PMS and change in color was monitored by recording absorbance (240 nm) at 30-s intervals for 3 min. The enzyme activity was expressed as mmoles of H_2O_2 decomposed/min/mg protein using molar extinction coefficient of H_2O_2 ($71\ M^{-1}\ cm^{-1}$).

Glutathione peroxidase

Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler (1984). The assay mixture consisted of 2.60 mL phosphate buffer (50 mM, pH 7.0, containing 15 mM EDTA), 0.1 mL reduced nicotinamide