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Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats

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ABSTRACT

Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity were studied in rats. A total of 28 Wistar albino male rats were used. Four study groups were randomly formed with seven animals in each. The groups were treated for 21 days with distilled water (control group), with water containing 100 ppm fluoride (fluoride group), with resveratrol (12.5 mg/kg i.p., resveratrol group), or with 100 ppm fluoride + 12.5 mg/kg resveratrol i.p. (fluoride + resveratrol group). At the end of the trial, blood samples were collected by cardiac puncture and tissue samples were taken simultaneously. The total antioxidant and oxidant status in plasma and tissues as well as plasma 8-hydroxydeoxyguanosine levels were measured. Histopathological analyses of rat liver and brain tissues were performed in all groups to identify any changes. In the fluoride group, the total oxidant levels increased in plasma, liver and brain and total antioxidant levels decreased, as did the plasma 8-hydroxy-deoxyguanosine levels. These changes were prevented by co-administration of resveratrol. In addition, fluoride-associated severe histopathological changes in brain and liver tissues were not observed in the fluoride + resveratrol group. Consequently, these data suggested that resveratrol had beneficial effects in alleviating fluoride-induced oxidative stress.

1. Introduction

Fluoride, the most electronegative element, forms ionized fluorides with several other elements (Chlubek, 2003; Rzeuski et al., 1998), and is an important natural and industrial environmental pollutant (Whitford, 1983). Living organisms are exposed to fluoride via food, drinking water, fluoride additives, toothpastes, and professional administration of fluoride gel (Edmunds and Smedley, 1996). Fluorosis is a serious health condition caused by intense and prolonged exposure to inorganic fluoride, which is particularly prevalent in regions where fluoride is released into the air by the burning of fluoride-loaded coal, industrial production of phosphate fertilizers, and volcanic activity (USNRC, 1993), as well as in regions where fluoride-containing groundwater is used for drinking water (Wang et al., 2004). Increased production of reactive oxygen radicals, increased lipid peroxidation, and impaired antioxidant defense mechanisms are involved in the pathogenesis of fluoride toxicity (Vani and Reddy, 2000). Free radicals cause toxicity by attacking membrane phospholipids, leading to membrane injury through lipid peroxidation, depolarization of the mitochondrial membrane, and apoptosis (Barbier et al., 2010).

Fluoride crosses the cell membrane (Carlson et al., 1960) and affects soft tissues, including the blood (Karadeniz and Altintas, 2008), brain (Shashi, 2003), and liver (Karaoz et al., 2003; Mittal and Flora, 2006). The liver has an active metabolism that renders it particularly sensitive to fluoride toxicity (Bouaziz et al., 2006; Guo et al., 2007). Many reports demonstrated that fluoride can induce lipid peroxidation and might cause change in the activity of some antioxidant enzymes in liver (Nabavi et al., 2012, 2013; Panneerselvam et al., 2013). Therefore, fluoride-induced oxidative stress and hepatotoxicity are associated with an imbalance in the oxidant/antioxidant systems of liver (Nabavi et al., 2012). Several studies performed on various animal species, including rats, sheep, and cattle, demonstrated that fluoride impaired liver function and

Abbreviations: 8-OHDG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; NaF, sodium fluoride; TAS, total antioxidant status; TOS, total oxidant status.

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metabolism, and caused histopathological changes (Grucka-Mamezar et al., 1997; Kapoor et al., 1993; Kessabi et al., 1986). Also, fluoride can accumulate in the brain (Geeraerts et al., 1986), which leads to abnormal behavioral patterns, impairment of neuronal and cerebrovascular integrity (Mullenix et al., 1995), and metabolic lesions (Vani and Reddy, 2000). Fluoride can also produce detrimental effects in brain tissue by inhibiting enzymes associated with energy production and transport, membrane transport, and synaptic transport (Vani and Reddy, 2000). Previous studies have also shown that fluoride increases lipid peroxidation and alters the levels of antioxidant enzymes in the brain (Flora et al., 2009; Shanthakumari et al., 2004) and liver (Guo et al., 2007; Hassan and Yousef, 2009; Mittal and Flora, 2006).

Resveratrol is a compound obtained from the roots of the *Polygonum cuspidatum* plant used in traditional Eastern medicine for the treatment of fungal diseases, skin inflammation, and cardiovascular and liver diseases (Arichi et al., 1982). Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a phenolic phytoalexin (Wang et al., 2002) that occurs naturally in various foods, including grapes, plums, cranberries, and peanuts. Its antioxidant effects have been demonstrated in the brain (Ates et al., 2007; Mokni et al., 2007; Sonmez et al., 2007), the liver (Dalaklioglu et al., 2013; Tunali-Akbay et al., 2010), and the kidneys (Silan et al., 2007). Resveratrol is a free radical scavenger that increases the activity of several antioxidant enzymes (Gusman et al., 2001; Leonard et al., 2003). Although previous reports have demonstrated that the harmful effects of fluoride reduced because of concomitant intake of antioxidants, including black tea extract (Trivedi et al., 2012), a combination of vitamin E, methionine, and L-carnosine (Agha et al., 2012), *Panax ginseng* (Karadeniz and Altintas, 2008), pineal proteins, and melatonin (Bharti and Srivastava, 2009), quercetin (Nabavi et al., 2012), gallic acid isolated from *Peltiphyllum peltatum* (Nabavi et al., 2013), ferulic acid (Panneerselvam et al., 2013). To the best of our knowledge there is no published studies on the protective effects of resveratrol against fluoride toxicity. To address this lack of information, the objective of this study was to investigate the protective effect of resveratrol against oxidative stress caused by fluoride treatment in rats.

2. Materials and methods

2.1. Chemicals

Sodium fluoride (NaF) was purchased from Merck (Darmstadt, Germany). *trans*-Resveratrol (>98% purity) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). All other chemicals were obtained from Merck & Co., Inc. (White House Station, NJ, USA) or Sigma–Aldrich Corporation (St. Louis, MO, USA).

2.2. Animals and treatment

Experiments were carried out using male Wistar albino rats weighing 180–200 g that were fed a standard chow diet with water available *ad libitum*. Seven animals were housed in each plastic cage under a 12-h light/dark cycle (lights on at 08:00 am) at a constant temperature of 25 ± 2 °C with 42 ± 5% relative humidity. The study protocol was in accordance with the guidelines for animal research and approved by the Ethical Committee of the Kirikkale University (10/155). Twenty-eight rats were randomly divided into 4 groups of 7 animals and treated as described below for 21 consecutive days. The control group received distilled water, the fluoride group received drinking water with 100 ppm fluoride, the resveratrol group received daily intraperitoneal (i.p.) administration of 12.5 mg/kg resveratrol, and the fluoride + resveratrol group received drinking water with 100 ppm fluoride and 12.5 mg/kg resveratrol i.p. daily.

2.3. Plasma collection

After 21 days, all animals were sacrificed under light ether anesthesia and blood samples were collected into heparinized tubes by cardiac puncture. Plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C and used for the determination of total antioxidant status (TAS), total oxidant status (TOS), and 8-hydroxydeoxyguanosine (8-OHDG) levels.

2.4. Tissue preparation

The livers and brains were removed, washed, and perfused with normal saline to remove residual blood. The liver and brain tissues were homogenized (model TH 220, OMNI, Warrenton, VA, USA) 1:10 (w/v) in ice-cold 140 mM potassium chloride at pH 7.4. The homogenates were centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatants were removed and stored at −80 °C until oxidative stress parameter analyses were performed.

2.5. Total antioxidant status assay

Total antioxidant status (TAS) was measured using a commercially available kit from Rel Assay Diagnostics (Gaziantep, Turkey) (Erel, 2004). The method was based on the reduction of colored 2,2′-azino-*bis*(3-ethylbenzotiazoline-6-sulfonic acid) (ABTS) radical to a colorless reduced form by antioxidants present in the sample. Absorbance was measured spectrophotometrically at a wavelength of 660 nm. The method was calibrated using the vitamin E analog Trolox, and data were expressed as mmol Trolox equivalent (eq.) per liter (mmol Trolox eq./L).

2.6. Total oxidant status assay

Total antioxidant status (TOS) was measured using a commercially available kit from Rel Assay Diagnostics (Erel, 2005). The method was based on the principle that the oxidants in the sample oxidized ferrous ions, previously bounded to a chelator, to ferric ions. In the acidic medium of the assay, these ferric ions formed a colored complex with a chromogen. The color intensity was measured spectrophotometrically at a wavelength of 530 nm. This assay was calibrated with hydrogen peroxide (H_2O_2), and the results were expressed as μmol H_2O_2 eq./L.

2.7. Determination of plasma 8-hydroxydeoxyguanosine

The levels of 8-hydroxydeoxyguanosine (8-OHDG) were determined using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (Eastbiopharm, Hangzhou, China). The plasma samples were quantified by interpolation using the 8-OHDG standard curve assayed on each plate. The sensitivity limit of the ELISA system was 0.25 ng/mL of 8-OHDG.

2.8. Histopathological analysis

Rat liver and brain tissues were fixed in 10% neutral formalin for 48–72 h. The tissues were then trimmed and processed for routine pathological examination. Then, they were embedded in paraffin wax and 4- to 5-μm-thick sections were cut. Hematoxylin and eosin staining was used for all tissue sections. Additionally, a 0.1% cresyl violet staining procedure was used to investigate neurodegenerative changes. Tissue slides were examined under a light microscope (Olympus BX51, Tokyo, Japan).

2.9. Statistical analysis

Statistical analyses of data were performed using GraphPad Prism 3.0 software (GraphPad Software, San Diego, CA, USA). The data were expressed as mean ± standard error. One-way analysis of variance (ANOVA) was used to analyze the differences between groups. Post hoc comparisons were performed using Tukey's multiple comparison test. P values less than 0.05 were considered as significant for all statistical calculations.

3. Results

3.1. Oxidative status in the plasma, liver, and brain

There were statistically significant differences in TAS, TOS, and 8-OHDG levels between the fluoride group and the control group. Exposure to fluoride significantly elevated plasma TOS and 8-OHDG levels. Additionally, plasma TAS significantly decreased in the fluoride group, compared to those in the other groups. However, in the resveratrol and fluoride + resveratrol groups, these values were not significantly different from those observed in the control group (Table 1).

In the fluoride group, TOS significantly increased in liver and brain tissues. In contrast, the TAS of these tissues in the fluoride group was significantly lower than that in the control group. However, TAS and TOS in the resveratrol group and in the fluoride + resveratrol group were not significantly different from those observed in the control group (Table 2). These findings indicated