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The antigenotoxic effects of grapefruit juice on the damage induced by benzo(a)pyrene and evaluation of its interaction with hepatic and intestinal Cytochrome P450 (Cyp)1a1

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ABSTRACT

We determined the capacity of grapefruit juice (GJ) to inhibit the rate of micronucleated polychromatic erythrocytes (MNPE) in mice treated with benzo(a)pyrene (BaP), an environmental contaminant that is biotransformed by Cyp1a1 and is a strong genotoxic agent. For this study, we administered 4.1, 20.8, and 41.6 μl/g body weight (b.w.) of GJ to BaP-treated mice (340 mg/kg). We found a significant decrease in the frequency of MNPE at 48 and 72 h compared to BaP-only treated animals. In turn, no prevention of the cytotoxic damage induced by BaP was found. We next explored whether GJ's antigenotoxic mechanism of action was related to an inhibitory effect on the activity of the Cyp1a1 enzyme. A reduction in microsomal hepatic and intestinal ethoxyresorufin-O-deethylase (EROD) activity of 20% and 44%, respectively, was found in mice treated with BaP and GJ compared to BaP-only treated animals. Furthermore, when EROD inhibition was tested *in vitro*, we found a concentration-dependent EROD inhibition by GJ, which reached 85% of the maximum level. Together, these results suggest that the protective effect of GJ against the genotoxicity of BaP may be related to the inhibition of Cyp1a1 enzyme activity.

1. Introduction

Grapefruit (*Citrus paradisi* Macfad.) come from a perennial tree of about 6 m in height from the Rutaceae family, which grows mainly in subtropical climates. This tree produces a globular fruit of about 15 cm in diameter, which is consumed as juice, in combination with other fruits and vegetables or in preserves (Morton, 1987). Grapefruit contains carbohydrates, lipids, proteins, vitamins, minerals, and other compounds, such as flavonoids, coumarins, and hydroxicynamic acids. Its consumption has been suggested as beneficial for cardiac diseases, obesity, diabetes, and cancer chemoprevention (Adeneye, 2008; Diaz-Juarez et al., 2009). Investigations using various experimental models have shown the capability of various grapefruit juice (GJ) constituents to inhibit DNA damage induced by xenobiotics. This capability is also found in compounds such as vitamin C, vitamin E, naringin, zinc, and selenium (Alvarez-Gonzalez et al., 2001; Bronzeti et al., 2001; Record et al., 1996). In recent years, the antigenotoxic potential of GJ has also been investigated. A report showed GJ's protective effect against aflatoxin B1-induced liver DNA damage in rats (Miyata et al., 2004), and another study demonstrated suppression of DNA damage induced by 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) (Miyata et al., 2002) in the colon. Furthermore, a significant reduction in the levels of micronuclei induced by daunorubicin in mice was reported (Alvarez-Gonzalez et al., 2004).

Enzymes that belong to the Cytochrome P450 (CYP) superfamily are known for their diverse functions, including their participation in the biosynthesis and regulation of different molecules and in pathways involved in the metabolic disposition of chemicals (Alfin, 2003). With respect to this last function, modulating the action of compounds may lead to at least two scenarios: drug–drug interactions that may cause adverse reactions and the promotion or inhibition of the metabolic activation of carcinogens (Lin, 2006; Nassar et al., 2007). GJ is known to inhibit intestinal CYP3A4 when ingested together with a number of drugs, to modify their pharmacokinetics and to provoke an increase in the oral bioavailability of these drugs compound (Bailey et al., 1991; Ducharme et al., 1995; Kupferschmidt et al., 1995; Benton et al., 1996). However, this inhibition may also have beneficial consequences, as shown by the antimutagenic ability of GJ against aflatoxin B1 *in vivo* (Miyata et al., 2004). This effect could be attributed to the inhibitory effect of GJ on CYP3A activity.

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Overall, the above mentioned data indicate a complex action of GJ over different xenobiotics, which is probably related to the effects of specific constituents in the juice. Moreover, it is also known that GJ alters the metabolism of coumarin and caffeine, chemicals that are biotransformed by CYP2A6 and CYP1A2, respectively (Runkel et al., 1997; Maish et al., 1996). This suggests that drugs affected by GJ may not be limited to CYP3A substrates. In this context, it is pertinent to mention that Cyp1a1 is an enzyme involved in the oxidation of polycyclic aromatic hydrocarbons, including benzo(a)pyrene (BaP). BaP is an inducer of Cyp1a1 and is a well known environmental contaminant with high genotoxic potential (Miller and Ramos, 2001).

To gain a better understanding of the antigenotoxic capacity of GJ, GJ was administered to mice treated with BaP. To explore the mechanism of action involved, we evaluated whether GJ inhibited Cyp1a1 activity in the mouse, the homologous enzyme of CYP1A1 in the rat, and if this was related to a decrease in micronuclei function.

2. Materials and methods

2.1. Chemicals

Acridine orange (AO), BaP,7-ethoxyresorufin, NADPH, phosphate buffered saline (PBS), tween 20, tris, and corn oil were obtained from Sigma Chemicals (St. Louis, MO, USA). GJ was obtained from fresh *Citrus paradisi* Macfad. (Ruby red) and cultivated in a pesticide-free field in Albeciras, Veracruz, 400 km southwest of México City. A single oral administration of freshly prepared GJ was given to mice before each assay.

2.2. Animals

Male mice, weighing 25 g, were obtained from the National Institute of Hygiene (NIH, México City) and used in this study. The animals were maintained in polypropylene cages at 23 °C in a 12 h dark–light cycle, and they had access to food (Rodent Lab Chow 5001) and water *ad libitum*. The Committee of Ethics and Biosecurity in the National School of Biological Sciences approved this experimental protocol.

2.3. Determination of micronuclei

Thirty-six animals were obtained and organized into six groups receiving the following oral treatments: group 1 was given 340 mg/kg body weight (b.w.) of BaP; group 2 was given 0.01 ml/g of corn oil; and group 3 was administered GJ (41.6 μl/g b.w.). Groups 4, 5 and 6 were given GJ 4.1, 20.8, and 41.6 μl/g b.w. respectively, and 1 h later received 340 mg/kg b.w. of BaP. The doses of GJ selected in this study showed no genotoxicity or cytotoxicity in earlier studies using the same strain of mice and similar experimental conditions (Alvarez-Gonzalez et al., 2004). The dose of BaP was based on previous reports (Vanparys et al., 1992; Awogi and Sato, 1989) and data obtained in a preliminary experiment.

Micronuclei determination was made before the oral administration of the compounds and at 24, 48, and 72 h post-administration. For this purpose, two drops of blood from the tail of each mouse were smeared onto cleaned slides, fixed in methanol for 3 min, and stained for 1 min in AO (made in PBS, pH 6.8). The slides were then rinsed twice in PBS, air dried, and observed under a fluorescent microscope (Axioscop, Carl Zeiss). To analyze the antigenotoxic potential of GJ, we quantified the frequency of MNPE in 1000 polychromatic erythrocytes (PE) per mouse. To obtain information about GJ's anti-cytotoxic potential, we determined the proportion of PE with respect to the number of normochromatic erythrocytes (NE) in 1000 erythrocytes per mouse (PE/NE index). Comparisons between groups treated with BaP, GJ or both agents were completed with RM ANOVA and Student-Newman–Keuls tests, and an α value of 0.05 was considered significant.

2.4. Effect of GJ on the activity of Cyp1a1 in vivo

2.4.1. Experimental groups and microsomal preparation

Four groups of six mice were organized as follows: group 1 received corn oil (0.01 ml/g b.w., i.p.); group 2 was treated with 100 mg/kg b.w. of BaP i.p. (Cyp1a inducer); a third group received a p.o. administration of 41.6 μl/g b.w. of GJ; and a fourth group was administered BaP (100 mg/kg b.w., i.p.) and then, 6 h later, a p.o. dose of GJ (41.6 μl/g b.w.). Twenty-four hours after the last treatment, all mice were cervically dislocated, the liver and small intestine dissected, and microsomes obtained according to the procedure described by Maron and Ames (1983). Briefly, organs were placed in a 150 mM solution of KCl at 4 °C (3 ml/g of wet tissue), minced into small pieces and homogenized. The homogenates were then centrifuged at 10,000*g* for 10 min at 4 °C, and the supernatant (S9 fraction) was centrifuged at 105,000*g* for 1 h at 4 °C. The supernatant was then resuspended in PBS at pH 7.4 and centrifuged again under the same conditions, except the resuspension was made in PBS plus EDTA (1 mM), dithiothreitol (0.1 mM) and glycerol (20%). Protein concentrations were determined with the Bradford (1976) assay, and the microsomal fraction was kept at −70 °C until use.

2.4.2. Activity of Cyp1a1

The Cyp1a1-associated ethoxyresorufin-O-dealkylation (EROD) activity was measured according to the method described by Burke et al. (1994) with some modifications. The production of resorufin was spectrofluorometrically assessed with the microsomes previously obtained. For measuring EROD activity we used 2 ml of the reaction mixture (at 4 °C) constituted by 7-ethoxyresorufin (25 μM), 1 mg/ml of microsomal protein, and buffer (50 mM tris and 25 mM $MgCl_2$, pH 7.4). The mixture was pre-incubated at 37 °C for 3 min and the reaction began with the addition of 0.2 ml of 50 mM NADPH. The spectrofluorometric readings were recorded every 15 s for 3 min using excitation and emission filters of 530 and 585 nm, respectively. A resorufin solution was used for the calibration curve (5–250 pmol). All measurements were made in triplicate.

2.5. Effect of GJ on the activity of Cyp1a1 in vitro

2.5.1. Microsomal preparation

Two groups of four mice each were used. The negative control group was injected by i.p. with corn oil (0.01 ml/g), and the other group was treated by the same route with an inducer of cyp1a (50 mg/kg of BaP). Both groups were injected once a day for 3 days. Twenty-four hours after the last injection, the animals were sacrificed by cervical dislocation, their livers dissected, and the microsomal fraction was obtained as previously described for the *in vivo* assay.

2.5.2. Activity of Cyp1a1

The activity of Cyp1a1 was determined by quantifying EROD activity according to the procedure already described for the *in vivo* assay; however, this time freshly obtained GJ (0.5%, 2.5%, 3.25%, 4.25%, and 5% v/v) was added to the reaction mixture. Cyp1a1-associated EROD activities were compared by ANOVA and Tukey–Kramer tests ($\alpha = 0.05$).

3. Results

3.1. Effect of GJ on the frequency of micronuclei

The ability of GJ to inhibit the genotoxicity induced by BaP is shown in Fig. 1. A significant increase in the frequency of micronuclei was observed in response to treatment with BaP. This was determined to be 6.5 and 2.8 times higher than the frequency of MNPE observed in the control group at 48 and 72 h, respectively. On the other hand, we found that administration of GJ was not genotoxic to mice and showed a frequency of micronuclei in the range observed for the control group. Also, a statistically significant decrease in the amount of BaP-induced MNPE was observed with the three doses of GJ given. Protection against the genotoxicity of BaP was observed with a GJ dose of 4.1 mg/kg b.w., reducing by 29% and 57% the amounts of micronuclei after 48 h and 72 h of BaP treatment respectively.

Fig. 2 shows the bone marrow mitotic activity estimated with the PE/NE index. The GJ doses tested in the present study did not modify this index compared to control animals. However, a cytotoxic effect of BaP was evident at 48 h and 72 h after its administration to mice, showing an index decrease of 20% and 27%, respectively. The cytotoxic damage produced by BaP was not ameliorated by any of the three GJ doses tested.

3.2. Effect of GJ on the activity of Cyp1a1 in vivo

Table 1 presents the results of the GJ inhibitory effect on microsomal EROD activity. From the hepatic and intestinal microsomes collected in mice treated with BaP only, we found EROD activity increases of eight- and twofold, respectively, compared to the level in control microsomes. In contrast, administration of GJ to BaP-treated mice inhibited EROD activity by 20% and 44% in liver and intestinal microsomes, respectively.