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Malabaricone C-containing mace extract inhibits safrole bioactivation and DNA adduct formation both in vitro and in vivo

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ABSTRACT

Safrole, present in mace and its essential oils, causes liver tumors in rodents at high dose levels due to formation of a DNA reactive 1′-sulfooxysafrole. The present study identifies malabaricone C as a mace constituent able to inhibit safrole DNA adduct formation at the level of sulfotransferase mediated bioactivation. This inhibition was incorporated into physiologically based biokinetic rat and human models. Dosing safrole at 50 mg/kg body weight and malabaricone C-containing mace extract at a ratio reflecting the relative presence in mace, and assuming 100% or 1% uptake of malabaricone C-containing mace extract, the model predicted inhibition of 1′-sulfooxysafrole formation for rats and humans by 90% and 100% or 61% and 91%, respectively. To validate the model, mace extract and safrole were co-administered orally to Sprague-Dawley rats. LC-ECI-MS/MS based quantification of DNA adduct levels revealed a significant ($p < 0.01$) 55% reduction of safrole DNA adduct formation by malabaricone C-containing mace extract in the liver of rats exposed to safrole. The data obtained were used to perform a refined risk assessment of safrole. Overall, the results suggest a lower tumor incidence when safrole would be tested within a relevant food matrix containing sulfotransferase inhibitors compared to dosing pure safrole.

1. Introduction

Myristica fragrans Houtt. belongs to the family Myristicaceae and is the source for two important spices produced from different parts of the plant: nutmeg which originates from the seeds and mace which is made from the dried aril cover of the seeds. Mace as well as nutmeg are known to contain so-called alkenylbenzenes including safrole (Archer, 1988) which has been recognized to be DNA reactive and carcinogenic in rodent bioassays when given at high dose levels (Daimon et al., 1997, 1998). The use of safrole in food has been prohibited in the US by the FDA since 1960 and in Europe since 2008 (European Commission, 2008). As a result, main exposure currently results from dietary consumption of safrole containing herbs and spices and food products containing these herbs and spices or their essential oils (Siano et al., 2003; Choong and Lin, 2001).

Bioactivation of safrole is initiated by cytochromes P450 resulting in formation of the proximate carcinogenic metabolite 1′-hydroxysafrole followed by sulfonation mediated by sulfotransferases (SULTs) and resulting in formation of the ultimate carcinogenic metabolite 1′-sulfooxysafrole. 1′-Sulfooxysafrole forms covalent adducts with cellular macromolecules including DNA (Borchert et al., 1973a; Wislocki et al., 1977). The role of 1′-sulfooxysafrole in the formation of DNA adducts in the liver of rat and mice dosed with safrole is supported by the observation that this

Abbreviations: BMD, benchmark dose; $BMDL_{10}$, the lower confidence limit of the benchmark dose causing 10% extra tumor incidence; EDI, estimated daily intake; GSH, glutathione reduced; 7HC, 7-hydroxycoumarin; 7HCS, 7-hydroxycoumarin sulfate; HPLC, high performance liquid chromatography; 1′HSG, 1′-hydroxysafrole glucuronide; 1′HOS, 1′-oxosafrole; 1′HSS, 1′-sulfooxysafrole; MOE, margin of exposure; MTT, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PBBK, physiologically based biokinetic; PCP, pentachlorophenol; SCF, Scientific Committee on Food; S-3′-N^2-dG, N^2-(*trans*-isosafrol-3′-yl)-2′-deoxyguanosine; S-1′-N^2-dG, N^2-(safrol-1′-yl)-2′-deoxyguanosine; S-3′-N^6-dA, N^6-(*trans*-isosafrol-3′-yl)-2′-deoxyadenosine; E-3′-N^2-dG, N^2-(*trans*-isoestragol-3′-yl)-2′-deoxy-guanosine; SULT, sulfotransferase; SPDE, spleen phosphodiesterase; UDPGA, uridine 5′-diphosphoglucuronic acid; UPLC, ultra performance liquid chromatography; VPDE, venom phosphodiesterase.

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DNA adduct formation can be inhibited by co-administration of safrole with pentachlorophenol (PCP), a known SULT inhibitor (Boberg et al., 1983; Daimon et al., 1997; Randerath et al., 1984). Co-administration of 0.05% PCP simultaneously with 0.25% safrole via the diet to the adult female mice for 12 months resulted in the 6% of hepatoma incidence compared to 70% for those mice without PCP (Boberg et al., 1983). Fig. 1 schematically presents the pathway for bioactivation of safrole and the nature of the DNA adducts formed. The DNA adducts found in hepatic tissue of rats exposed to safrole were identified as N^2-(*trans*-isosafrol-3′-yl)-2′-deoxyguanosine (S-3′-N^2-dG), N^2-(safrol-1′-yl)-2′-deoxyguanosine (S-1′-N^2-dG) and N^6-(*trans*-isosafrol-3′-yl)-2′-deoxyadenosine (S-3′-N^6-dA) (Randerath et al., 1984). The S-3′-N^2-dG and S-1′-N^2-dG were the major adducts found in patients with oral squamous cell carcinoma resulting from the habit of betel quid chewing containing safrole (Chen et al., 1999; Ko et al., 1995; Hwang et al., 1992).

One may argue that risk assessment resulting from consumption of herbs and spices that contain safrole should take into account the possible modulating effects of other compounds present in these herbs or spices on the cytochrome P450 and/or sulfotransferase (SULT)-catalyzed bioactivation of safrole. It was recently demonstrated that a methanolic basil extract and its isolated constituent nevadensin inhibited the sulfation and DNA adduct formation of the proximate carcinogenic 1′-hydroxyestragole metabolite in studies using rat and human S9 protein, the hepatoma cell line HepG2, and/or rat hepatocytes (Alhusainy et al., 2010; Jeurissen et al., 2008). The inhibition of estragole DNA adduct formation by the basil ingredient nevadensin was also shown in the liver of rats orally exposed to estragole (Alhusainy et al., 2013). Therefore, the objective of the present study was to investigate whether ingredients present in safrole containing spices can inhibit the SULT-mediated bioactivation of safrole and the subsequent DNA adduct formation both in vitro and in vivo. Mace was chosen as the model spice of interest because it contains significant levels of safrole 0.43–1.99% (Archer, 1988). Upon identifying a major mace ingredient able to inhibit SULT mediated bioactivation and DNA adduct formation of 1′-hydroxysafrole in in vitro model systems, the possible effect of combined in vivo exposure was investigated by incorporating the kinetics for SULT inhibition into our recently developed physiologically based biokinetic (PBBK) models for formation of 1′-sulfooxysafrole in rat and human liver. Furthermore, the predicted inhibition of 1′-sulfooxysafrole formation by the ingredients present in mace extract was validated in a rat model. The results obtained were also used to perform a refined risk assessment of safrole assuming safrole would have been tested in the rodent bioassay in the presence of its matrix ingredients instead of as a pure compound.

2. Materials and methods

2.1. Materials

2.1.1. Caution

The following chemicals are hazardous and should be handled carefully: 1′-hydroxysafrole and 1′-acetoxysafrole.

Powder dried mace was obtained from a local supermarket. Pooled human and male Sprague-Dawley (SD) rat liver S9 were purchased from BD Gentest (Worburn, US). The HepG2 cell line was purchased from American type culture collection (Manassas, Virginia). Culture medium DMEM/F12 (L-glutamine, 15 mM HEPES) and phosphate buffer saline (PBS, pH 7.4) were purchased from GIBCO (Paisley, UK). The chemical compounds were obtained with highest purity available. 7-Hydroxycoumarin (7HC), 7-hydroxycoumarin sulfate potassium salt (7HCS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Tris(hydroxymethyl)aminomethane, uridine 5′-diphosphoglucuronic acid (UDPGA), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), 2′-deoxyguanosine (2′dG), PCP (purity 97%) and glutathione reduced (GSH) were obtained from Sigma–Aldrich (Steinheim, Germany). Methanol and ethanol (pro analysis), formic acid, and trifluoroacetic acid (Uvasol) were purchased from VWR (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) and dichloromethane were purchased from Acros Organic (New Jersey, US). Acetic anhydride was supplied by J.T. Baker (Deventer, The Netherlands). Chromatography grade acetonitrile and methanol were purchased from Biosolve BV (Valkenswaard, The Netherlands). 1′-Hydroxysafrole was synthesized and purified as described previously (Jeurissen et al., 2004; Martati et al., 2011). 1,2-Dihydroxy-4-allylbenzene was synthezised as described previously (Bolton et al., 1994). 1′-Acetoxysafrole was synthesized from 1′-hydroxysafrole as described previously for synthesis of 1′-acetoxyestragole from the related alkenylbenzene estragole (Borchert et al., 1973b; Punt et al., 2007).

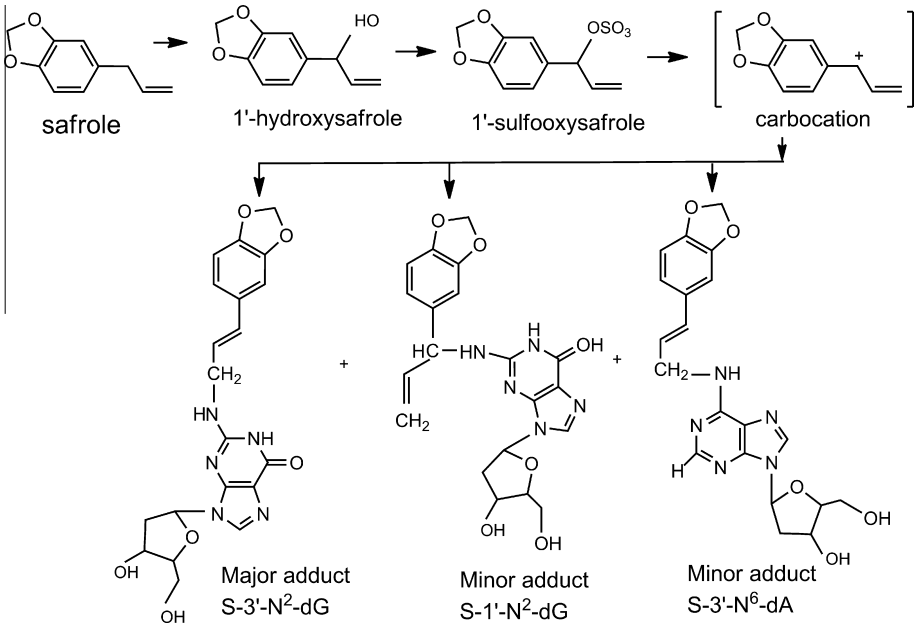

Fig. 1. Bioactivation pathways of safrole and the structure of DNA adducts formed (Chung et al., 2008; Daimon et al., 1998; Randerath et al., 1984; Phillips et al., 1981). S-3′-N^2-dG = N^2-(*trans*-isosafrol-3′-yl)-2′-deoxyguanosine, S-1′-N^2-dG = N^2-(safrol-1′-yl)-2′-deoxyguanosine and S-3′-N^6-dA = N^6-(*trans*-isosafrol-3-yl)-2′-deoxyadenosine.