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Dose–response relationship for the pharmacokinetic interaction of grapefruit juice with dextromethorphan investigated by human urinary metabolite profiles

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ABSTRACT

Grapefruit juice (GFJ) has been shown to affect the pharmacokinetics of a large number of drugs, essentially by inhibition of efflux transporters and CYP3A4 monooxygenase in the small intestine. The GFJ dose usually used in human studies was one glass single-strength (1×). Information on a respective dose–response relationship is not available. We investigated the effect of GFJ of different concentration (0.25×, 0.5×, 1×, 2×) dosed in biweekly intervals in 19 volunteers. Components considered responsible for drug interactions, naringin, naringenin, bergamottin, and 6′,7′-dihydroxybergamottin were determined by LC–tandem mass spectrometry. Immediately after ingestion of GFJ, participants took an aqueous solution of dextromethorphan (DEX) as probe drug. Urine was collected in two sampling periods, 0–2 and 2–4 h, and excreted amounts of DEX and five metabolites associated with CYP3A4 and/or CYP2D6 enzyme activity were determined. Effects of GFJ were analyzed by the Wilcoxon matched-pairs signed-rank test against an average of four water control experiments. Two effects were highly significant: (i) a delay of total metabolite excretion in the first 2 h and (ii) an inhibition of the CYP3A4-dependent metabolic pathways. Effect magnitude and significance levels were dose-dependent and indicated 200 ml 1× GFJ as "lowest observed effect level" LOEL.

1. Introduction

Since 1989 (Bailey et al., 1989), more than 200 reports on interactions of grapefruit juice (GFJ) with over 50 drugs have been reported (Mertens-Talcott, 2006; Saito et al., 2005). Inhibition of intestinal transport and metabolism are considered to be the major mechanisms involved, which typically results in an increase in plasma AUC and c_{max}. The elimination half-life is less frequently affected, which indicates that liver metabolism is usually not involved (Bailey et al., 1991; Ducharme et al., 1995; Sigusch et al., 1994), except for excessive GFJ consumption, as shown with triazolam and midazolam (Lilja et al., 2000; Veronese et al., 2003). On the other hand, similar high-dose studies using lovastatin or simvastatin as probe drug did not indicate a hepatic effect (Kantola et al., 1998; Lilja et al., 1998).

GFJ acts on a number of target proteins in the small intestine. Most affected drugs are substrates of CYP3A4. In view of the fact that this monooxygenase is expressed in enterocytes (Kolars et al., 1994; Watkins et al., 1987) its contribution to the grapefruit effect is implied. Inactivation of CYP3A4 may include irreversible binding, as deduced from degradation and de novo synthesis of the protein (Hollenberg et al., 2008; Lown et al., 1997). Esterases (Li et al., 2007), efflux transporters (P-GP and MRP-2) (Honda et al., 2004; Takanaga et al., 1998), and influx transporters (OATP) (Dresser et al., 2002) are also inhibited. The interaction with the transporters is considered to be reversible, as suggested by the finding that P-GP and OATP protein levels were not affected (Glaeser et al., 2007; Lown et al., 1997).

GFJ components identified as inhibitors include furanocoumarins, such as bergamottin and 6′,7′-dihydroxybergamottin (Schmiedlin-Ren et al., 1997; Tassaneeyakul et al., 2000), and flavonoids, such as naringin and naringenin (De Castro et al., 2008; Ho et al., 2001; Ofer et al., 2005). The type of interaction with the target protein is compound-specific (Bailey et al., 2000). Storage and treatment of grapefruits affect the amount of these ingredients (Vanamala et al., 2005), which calls for the respective analysis of GFJ batches used in interaction studies.

Abbreviations: AUC, Area under the plasma concentration time curve; c_{max}, maximal plasma concentration; CV, coefficient of variation; CYP, cytochrome P450; DEX, dextromethorphan; DOR, dextrorphan; DORGlu, dextrorphan-O-glucuronide; GFJ, grapefruit juice; GI tract, gastrointestinal tract; HOM, 3-hydroxymorphinan; HOMGlu, 3-hydroxymorphinan-O-glucuronide; IS, internal standard; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; LOD, limit of detection; LOEL, lowest observed effect level; LOQ, limit of quantification; MOM, 3-methoxymorphinan; MR, metabolic ratio; MRM, multiple reaction monitoring; MRP-2, multidrug resistance associated protein 2; OATP, organic anion transporting polypeptides; OCT, organic cation transporter; P-GP, Pglycoprotein; Q1, first quartile; Q3, third quartile; Q-trap, linear ion trap mass spectrometry; tmax, time to achieve maximal plasma concentration; UGT, UDP-glucuronosyltransferase.

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The interaction is effective immediately after GFJ ingestion, remains at a maximum level for about 4 h (Lundahl et al., 1995), and disappears within 3 days (Greenblatt et al., 2003). Daily intake of GFJ does not increase the effect (Lundahl et al., 1998).

Most studies in humans had been performed with 200 ml of regular-strength or double-strength GFJ. It is therefore not known whether doses below 200 ml regular-strength GFJ still have an effect on orally administered drugs. Investigations of dose–response relationships are limited to in vitro studies. They focus on specific GFJ components (Tassaneeyakul et al., 2000) and specific CYP enzymes and transporters (Girennavar et al., 2007; Takanaga et al., 1998) and do not allow any extrapolation to the situation in humans.

In order to fill the toxicologically relevant gap of knowledge regarding GFJ dose–response we performed a human study. As probe drug, we chose dextromethorphan (DEX), an over-the-counter antitussive. The metabolic scheme for DEX is shown in Fig. 1. *N*-Demethylation is catalyzed by CYP3A4 and generates 3-methoxymorphinan (MOM), whereas CYP2D6 catalyzes *O*-demethylation of DEX to dextrorphan (DOR). The bi-demethylated product is 3-hydroxymorphinan (HOM). Major urinary excretion products are conjugates of DOR and HOM, i.e., dextrorphan-*O*-glucuronide (DORGlu) and 3-hydroxymorphinan-*O*-glucuronide (HOMGlu). By calculating fractions of the urinary excretion of appropriate metabolites, the effect of grapefruit juice on both types of monooxygenases could be investigated. The high sensitivity of the LC–MS/MS analysis enables measurement of all metabolites in urine after use of a pharmacologically ineffective low DEX dose (Lutz et al., 2004). Collection of urine in short time windows allows following a time course that mirrors plasma levels. Abstain from repeated blood sampling helps to recruit volunteers for a dose–response study that requires repeated experiments.

An investigation of DEX–GFJ-interactions on the basis of urinary excretion had been published before, using 250 ml regular GFJ and a therapeutic dose of DEX. With an HPLC-fluorescence analysis, they deduced a marked increase of the CYP3A4-specific metabolic ratio DEX/MOM. A change in the ratio DEX/DOR was not noted, indicating a lack of inhibition of CYP2D6 activity (Ducharme et al., 1996). Six years later, the same group published additional data that indicated a significantly increased bioavailability of DEX after 200 ml regular-strength GFJ (Di Marco et al., 2002).

In our study, we recruited a total of 19 participants and performed eight experiments at weekly intervals, switching between 200 ml water and 200 ml GFJ, at a concentration of 0.25×, 0.5×, 1×, and 2×. The dose of DEX was one-third of a normal therapeutic dose. Two urine samples were collected at 2 and 4 h after ingestion of GFJ and probe drug, and were analyzed for all compounds shown in Fig. 1.

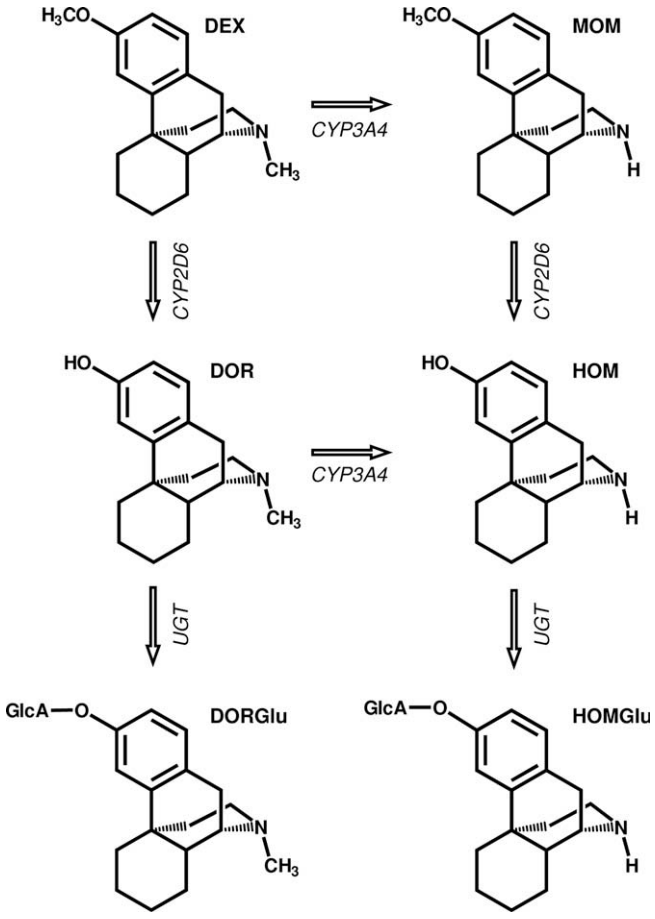

Fig. 1. Metabolism pathways of dextromethorphan catalyzed by cytochrome P450 monooxygenases, CYP3A4 and CYP2D6, as well as UDP-glucuronosyl-transferase (UGT). DEX: dextromethorphan, DOR: dextrorphan; HOM: 3-hydroxymorphinan; MOM: 3- methoxymorphinan; DORGlu: dextrorphan-*O*-glucuronide; and HOMGlu: hydroxymorphinan- *O*-glucuronide.

2. Materials and methods

2.1. Reagents and chemicals

Dextromethorphan hydrobromide for human use was from FAGRON GmbH (Barsbüttel, Germany). Dextromethorphan hydrobromide monohydrate and dextrorphan tartrate salt for analytics were from Sigma-Aldrich (Taufkirchen, Germany). 3-Methoxymorphinan hydrobromide and 3-hydroxymorphinan hydrobromide were a gift from Roche (Basel, Switzerland). The internal standard (IS) compounds [$^2H_3$]dextromethorphan and [$^2H_3$]dextrorphan were from Toronto Research Chemicals Inc. (North York, Canada). Bergamottin, naringin, and naringenin were from Sigma–Aldrich, whereas 6′,7′-dihydroxybergamottin was a gift from J.A. Manthey (Agricultural Research Service, USDA, FL, USA). β-Glucuronidase type HP-2 from *Helix pomatia* with an activity of 100 000 U/ml was from Sigma–Aldrich (Taufkirchen, Germany) as well as ethyl acetate 99.8% HPLC grade. Rotisolv™ HPLC gradient grade water and acetonitrile, methanol, Rotipuran™ formic acid (98%) and sodium acetate were from Roth (Karlsruhe, Germany). Hydrochloric acid (25%) was from Merck (Darmstadt, Germany).

2.2. Grapefruit juice

The grapefruit juice was ordered as white grapefruit juice concentrate (Nr. 75488, white, 58 Brix, cloudy, 5.8× concentrated) from Mainfrucht (Gochsheim, Germany). The concentrate was stored at −20 °C in aliquots. For usage, aliquots were diluted with tap drinking water to 200 ml of juice of 0.25×, 0.5×, 1×, and 2× concentration.

2.3. Human study design

2.3.1. Study population

Nineteen healthy Caucasians of age 22–63 (13 females and 6 males) volunteered for the study that was approved by the local ethics committee. Three were occasional smokers. There were no vegetarians and vegans or individuals with other special eating behavior. Their body mass indexes were between 19.8 and 25.7, calculated as weight (kg) divided by the squared height (m^2). All participants regularly drank between 1 and 2 l of non-alcoholic beverages per day.

Participants had been selected to be within certain limits regarding polymorphic CYP2D6 enzyme activity. Individual metabolic ratios DEX/DOR determined in urine after administration of 10 mg dextromethorphan hydrobromide monohydrate (Lutz et al., 2004) were kept within a factor of 20.

2.3.2. Study protocol

The experiment lasted for 8 weeks. Every Tuesday morning, after fasting overnight, the individuals voided the bladder, drank 200 ml liquid containing different GFJ concentrations, immediately followed by 10 mg dextromethorphan hydrobromide monohydrate (27 μmol DEX) dissolved in 50 ml water. The sequence of experiments started with a water control (0×) on the first Tuesday, followed by 1×, 0.5×, 0.25×, and 2×, separated weekly by three additional water controls. Consumption of alcoholic beverages in the night before and during the study was prohibited. Breakfast was allowed 1 h after DEX intake at the earliest. Amount and kind