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High-dose short-term administration of naringin did not alter talinolol pharmacokinetics in humans

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ABSTRACT

Naringin is considered the major causative ingredient of the inhibition of intestinal drug uptake by grapefruit juice. Moreover, it is contained in highly dosed nutraceuticals available on the market. A controlled, open, randomized, crossover study was performed in 10 healthy volunteers to investigate the effect of high-dose naringin on the bioavailability of talinolol, a substrate of intestinal organic anion-transporting polypeptide (OATP)-mediated uptake. Following 6-day supplementation with 3 capsules of 350 mg naringin daily, 100 mg talinolol were administered orally with 3 capsules of the same dietary supplement (1050 mg naringin) on the seventh day. This test treatment was compared to 100 mg talinolol only (control). The results showed that short-term high-dose naringin supplementation did not significantly affect talinolol pharmacokinetics. Geometric mean ratios of test versus control ranged between 0.90 and 0.98 for talinolol c_{max}, AUC_{0-48h}, $AUC_{0-\infty}$, $t_{1/2}$ and $A_{e(0-48h)}$. The high dose may provoke inhibition of the efflux transporter P-glycoprotein (P-gp) which counteracts the uptake inhibition. As disintegration and dissolution processes are required for the solid dosage form, dissolved naringin may arrive at the site of interaction after talinolol is already absorbed. In conclusion, the effect of nutraceuticals on drug pharmacokinetics can deviate from that observed when administered as food component due to the different dose and dosage form.

1. Introduction

During the last decades, fruit juice–drug interactions have drawn much attention in research and clinical practice (Hanley et al., 2011). In addition to the daily diet, components present in food and beverages such as flavonoids are increasingly consumed in dietary supplements due to their presumed health promoting effects, for example, anti-oxidative and anti-proliferative activities (Egert and Rimbach, 2011). These nutraceuticals represent a relevant source of interaction with drug transport and metabolism, among others, for two main reasons: First, specific components are ingested in doses which are several magnitudes higher than the normal dietary intake. Second, unlike prescribed drugs, their consumption is usually not reported to the physician upon visit. Consequently, uncontrolled pharmacokinetic nutraceutical–drug interactions enhance the risk of clinically relevant modulation of drugs' efficacy and adverse effects.

The flavanone glycoside naringin is naturally derived from citrus fruits and present in various food products, herbal formulations and dietary supplements (Sansone et al., 2009). As the predominant flavonoid in grapefruit juice, it was considered the major causative ingredient of transporter-mediated grapefruit juice–drug interaction (Bailey, 2010). In vitro and rat studies demonstrated that naringin inhibits both drug uptake and efflux transport by Oatp1a5 and P-gp, respectively (Shirasaka et al., 2009). In humans, Bailey et al. (2007) observed a significant decrease of fexofenadine bioavailability following concomitant administration with grapefruit juice which was explained by direct inhibition of enteric OATP1A2 by naringin. The concentration of naringin in commercial grapefruit juices ranges between approximately 50 and 1200 mg/l (Ho et al., 2000). Moreover, dietary supplements are available on the market along with dosing recommendations of 200–1000 mg naringin per day in its pure form or as grapefruit extract, for example, *100% Natural Naringin* (Swanson Health Products, Fargo, USA) or *Hesperidin Plus with Acerola and Naringin* (Warnke, Cologne, Germany). This raises concern about possible excessive intake of naringin through both diet and food supplements. On the other hand, naringin has been subject of interest

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from a pharmacological point of view. A study in humans confirmed that naringin supplementation positively influenced plasma cholesterol levels and the antioxidant capacity in hypercholesterolemic subjects (Jung et al., 2003). Also, formulation approaches such as pulmonary delivery and lipase-catalyzed acylation indicate its potential for clinical application (Sansone et al., 2009; Zhang et al., 2013).

To our best knowledge, the effect of highly dosed short-term naringin supplementation on drug transport in humans has not been investigated so far. This is an important gap with regard to the efforts being made to introduce naringin into clinical treatment complemented with the presence of this bioflavonoid in high concentrations in various food products and dietary supplements.

Talinolol was selected as the probe drug for our study in humans. The beta-1-adrenoceptor antagonist is a well-known substrate of intestinal P-gp-mediated efflux with a very low fraction metabolized of less than 1%, moderate protein binding of 50–70% and an oral bioavailability of 55–70% (Oswald et al., 2011). Assays in transfected HEK293 cells and *Xenopus leavis* oocytes revealed that talinolol is also substrate of the uptake transporters OATP1A2, OATP2B1 and Oatp1a5 (Naddaf et al., 2010; Shirasaka et al., 2010). The number of drugs sharing this characteristic is increasing, being discovered to be both substrate of epithelial uptake and efflux transport, for example, fexofenadine and aliskiren, while selective probe drugs for OATP1A2- and OATP2B1-mediated transport in humans are lacking (Dresser et al., 2002; Rebello et al., 2012). Shirasaka and co-workers (2009) reported concentration-dependent effects of naringin on the intestinal transport of talinolol in rats, with oral absorption being decreased at lower and increased at higher naringin concentrations which was explained by inhibition of Oatp-mediated uptake and P-gp-mediated secretion, respectively. Hence, we also aim to find out whether similar observations could be made in humans in this interaction study with highly dosed naringin compared to the lower naringin doses applied in grapefruit juice interaction studies.

2. Materials and methods

2.1. Materials

Naringin hard-gelatine capsules were purchased from Swanson Health Products (Fargo, USA). For testing content uniformity, naringin analytical reference material was purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany). Talinolol film-coated tablets (Cordanum® 100 mg) from AWD.pharma GmbH & Co., KG (Dresden, Germany) were provided by the University Medical Center, Johannes Gutenberg University (Mainz, Germany). Talinolol from AWD.pharma and carvedilol from Sigma Aldrich Chemie GmbH were used for the quantitative assay. For the HPLC analysis, all aqueous solutions were purchased from Sigma Aldrich Chemie GmbH and organic solvents from Fisher Scientific GmbH (Schwerte, Germany).

2.2. Test for content uniformity of naringin capsules

The purchased naringin capsules were tested for uniformity of content according to Ph.Eur. 7.0 section 2.9.6 (Uniformity of content of single-dose preparation). 10 capsules were assayed for naringin using naringin analytical reference material. In brief, the content of each capsule was removed, dispersed in 96% ethanol and diluted to 25 mg/l solutions. Three samples à 1 ml of each solution were assayed using UV/VIS spectroscopy at 285 nm (Lambda 35, PerkinElmer Inc., Rodgau, Germany). Linearity was confirmed for concentrations between 5 and 40 mg/l with linear correlation coefficients higher than 0.99. Coefficients of variation ranged between 0.33% and 1.12% of means and accuracy between −2.23% and 3.78% of the nominal values.

2.3. Participants

Ten healthy volunteers (2 females, 8 males, mean age 31.7 ± 9.8, mean body weight 73.7 ± 9.6 kg) passed the pre-study screening and completed the pharmacokinetic study after providing written informed consent. For the pre-study screening, an overall physical examination, medical history, electrocardiography, routine hematologic, biochemical and serologic tests were performed. Exclusion criteria included, among others, history of drug or alcohol abuse and cardiovascular disorders, *Q* interval of more than 220 ms, heart rate of less than 55 min^{-1} at rest and systolic blood pressure of less than 120 mm Hg in the lying position. Participants were not allowed to take any drugs and dietary supplements one month before and during the study period. An alcohol- and caffeine-free, low-flavonoid diet was to be kept one week before the study until its conclusion. A list of flavonoid-rich food and beverages to be avoided, for example citrus fruits and their juices, was handed out to each subject to guide the restricted diet.

2.4. Study design

The clinical study protocol was approved by the Federal Institute for Drugs and Medical Devices (Bonn, Germany) and the local Ethics Committee (State Chamber of Physician, Mainz, Germany). In the control period (talinolol only), subjects received a single tablet of 100 mg talinolol (Cordanum®) orally after overnight fasting for more than 9 h. In the test period (talinolol + naringin one-week treatment), each participant ingested 350 mg naringin three times daily with each meal for 6 days. On day 7, 100 mg talinolol and 3 capsules of 350 mg naringin were concomitantly administered at the clinical site. All subjects consumed a standardized flavonoid-free meal 4 and 10 h after drug intake in both control and test period. Peripheral venous blood was sampled just before and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48 h after drug administration. Total urine was collected at 2-h intervals between 0 and 16 h, then at 24, 36 and 48 h after medication. At every blood sampling time, blood pressure and heart rate were recorded for safety reasons. Adverse events were additionally monitored by means of periodic questioning. Post-study tests similar to the pre-study screening were performed to assess individual tolerability of the treatments.

2.5. Genotyping

After inclusion, subjects were genotyped for specific single nucleotide polymorphisms (SNPs) of the genes encoding transporters relevant for the uptake and secretion of talinolol in the gastrointestinal tract, namely the ABCB1 (P-gp), ABCC2 (MRP2), SLCO1A2 (OATP1A2) and SLCO2B1 (OATP2B1) gene. After obtaining additional written informed consent from each participant, the genotyping was carried out by polymerase chain reaction amplification and direct Sanger sequencing on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, USA) at the Institute of Human Genetics, Johannes Gutenberg University (Mainz, Germany). Genomic deoxyribonucleic acid prepared from venous blood samples was analyzed for the following SNPs due to their frequent occurrence among Caucasian subjects and their reported impact on drug pharmacokinetics (Siegmund et al., 2002; Haenisch et al., 2008; Nakanishi and Tamai, 2012): *ABCB1* c.2677G > T/A, *ABCB1* c.3435C > T, *ABCB1* c.1236C > T, *ABCC2* c.1249G > A, *SLCO1A2* c.516A > C, *SLCO1A2* c.38T > C, *SLCO2B1* c.1457C > T and *SLCO2B1* c.935G > A.