Grapefruit Juice–Drug Interactions: Grapefruit Juice and Its Components Inhibit P-Glycoprotein (ABCB1) Mediated Transport of Talinolol in Caco-2 Cells

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ABSTRACT: To investigate the potential interaction between selected ingredients of grapefruit juice and, the transport of talinolol, a P-gp substrate, across Caco-2 cells monolayers was determined in the absence and presence of distinct concentrations of grapefruit juice, bergamottin, 6′,7′-dihydroxybergamottin, 6′,7′-epoxybergamottin, naringin, and naringenin. Talinolol permeability was selectively inhibited by grapefruit juice and its components. The furano coumarin, 6′,7′-epoxybergamottin, was the most potent inhibitor ($IC_{50} = 0.7$ μM), followed by 6′,7′-dihydroxybergamottin ($IC_{50} = 34$ μM) and bergamottin that did not show any inhibition at concentrations up to 10 μM. The flavonoid aglycone naringenin was around 10-fold more potent than its glycoside naringin with IC_{50} values of 236 and 2409 μM, respectively. The flavonoids and furanocoumarins tested in this study are in the same range of concentration they are present in the juice contributing, therefore, for the overall inhibitory effect of GFJ on P-gp activity. The *in vitro* data suggest that compounds present in grapefruit juice are able to inhibit the P-gp activity modifying the disposition of drugs that are P-gp substrates such as talinolol.
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Keywords: grapefruit juice; P-glycoprotein; ABCB1; talinolol; flavonoids; furanocoumarins

INTRODUCTION

Unlike other citrus juices concomitant intake of grapefruit juice (GFJ) has been demonstrated to modify greatly the disposition of a variety of medications taken orally, in most cases leading to elevation of their serum concentrations. The variety of drugs include dihydropyridine calcium channels blockers such as felodipine,[1] nifedipine,[1] nisoldipine,[2] and nitrendipine,[3] verapamil,[4] cyclosporine,[5,6] tacrolimus,[7] midazolam,[8] triazolam,[9] terfenadine,[10] diazepam,[11] saquinavir,[12] ethinylestradiol,[13] and caffeine.[14]

The enhancement of the oral bioavailability of some drugs such as felodipine, nicardipine, halofantrine after consumption of GFJ has been associated with higher incidence of dose-dependent side effects among the subjects.[15–17]

The main mechanism for grapefruit–drug interaction is the inhibition of the CYP3A4, the major enzyme catalyst of phase I drug biotransformation.[18] However, differently from the most potent orally administrated CYP3A4 inhibitors where the liver is the key site of action, GFJ when consumed in usual volumes, is an example of inhibitor that appears to act preferentially in the enteric CYP3A4, resulting in a significant reduction of the presystemic metabolism of drug.[19,20]

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Additionally, recent investigations have shown that the effect of GFJ are not restricted on the activity of the CYP3A4 but also involves the modulation of the intestinal influx and efflux membrane transporters the organic anion transporting polypeptides (OATP) and the permeability glycoprotein (P-gp/ABCB1), respectively.[21–24] These transporters have been demonstrated to alter the absorption of distinct classes of drugs.[24,25]

The modulation of P-gp activity by grapefruit juice or its components and the clinical relevance of such interaction is still unclear and is discussed controversially, since some authors have reported activation[26,27] and others inhibition of P-gp.[6,28,29] Thus, the extent to which GFJ modifies P-gp transporter activity remains unclear. Therefore, it was the aim of our study to systematically investigate *in vitro*, using a Caco-2 cell model, the potential drug interaction between selected ingredients of GFJ (Fig. 1) and the P-gp transporter system, which controls many barriers in the body. The β-blocker talinolol, a non-CYP substrate, yet a P-gp substrate, was used as a marker compound.

MATERIALS AND METHODS

Chemicals

The following materials were used: (rac)-verapamil hydrochloride, Hank's balanced salt solution (HBSS), 2-(*N*-morpholino)ethanesulfonic acid solution (MES); sodium pyruvate solution (1M), Lucifer yellow dipotassium salt, all of which were from Sigma Chemical Company (St. Louis, MO); Dulbecco's modified Eagle's medium (1×) high glucose without sodium pyruvate (DMEM), nonessential aminoacids, trypsin (0.05%)–EDTA (0.02%) solution, Fungizone® antimycotic solution containing 250 μg of amphotericin B and 205 μg of sodium deoxycholate per mL as solubilizer, penicillin G (10,000units/mL) and streptomycin sulfate (10,000 μg/mL) solution, (*N*-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) buffer solution (1M) (HEPES) were obtained from Gibco BRL Life Technology (Grand Island, NY); fetal bovine serum heat inactivated and Dulbecco's phosphate buffered saline (DPBS) (pH 7.4) were purchased from Mediatech Inc. (Herdon, VA, USA); talinolol (99.9% purity) was a gently provided by AWD-Pharma GmbH & Co. KG (Dresden, Germany), naringin (NAR) and naringenin (NAG), both >95% pure, were from Roth GmbH & Co. (Karlsruhe, Germany), bergamottin (BG) (98% purity) was bought from Indofine Chemical Company, Inc. (Somerville, NJ), 6′,7′-dihydroxybergamottin (DHB), and 6′,7′-epoxybergamottin (EPBG) were kindly supplied by Dr. John Manthey at the U.S. Department of Agriculture (USDA), Citrus and Subtropical Products Laboratory, Agricultural Research Service, Winter Haven, Florida. Upon isolation, the compounds identification and purity (>98%) were measured by analytical thin layer chromatography,

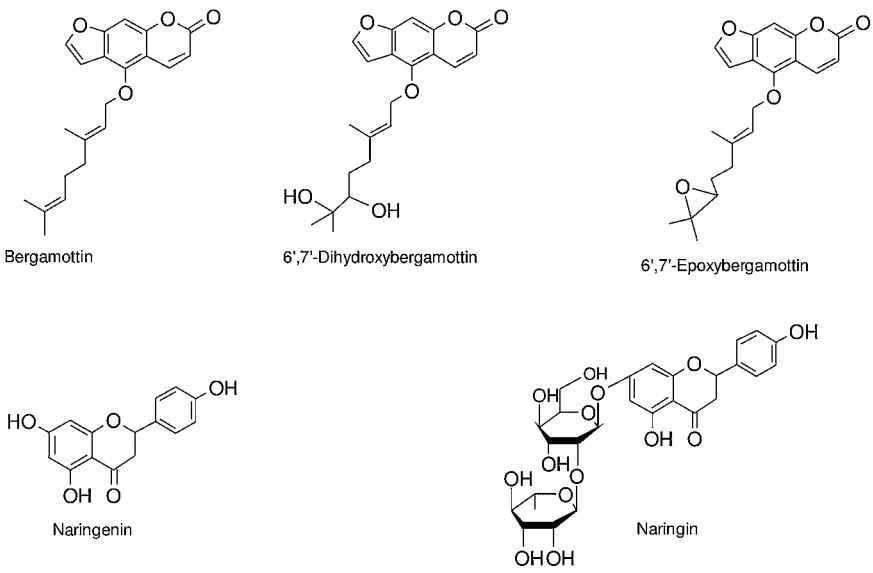

Figure 1. Structures of flavonoids and furanocoumarins present in grapefruit juice.