Food and Chemical Toxicology 74 (2014) 85–90

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effects of grapefruit juice on cortisol metabolism in healthy male Chinese subjects

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ARTICLE INFO

Article history:
Received 3 December 2013
Accepted 22 September 2014
Available online

Keywords:
Cortisol
Cortisone
Cytochrome P450 3A
Grapefruit juice
6β-Hydroxycortisol
11β-Hydroxysteroid dehydrogenase 2

ABSTRACT

Grapefruit juice (GFJ) inhibits intestinal CYP3A4 activity and it has been suggested that GFJ may also inhibit renal 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), which converts cortisol to cortisone. This study examined the effect of GFJ on the urinary excretion of cortisol, cortisone and 6β-hydroxycortisol (6β-OHC) and their ratios to asses these effects. Healthy male Chinese subjects took single doses of GFJ (200, 400, and 600 mL, respectively) at weekly intervals. Urine was collected over 24 h the day before and following GFJ intake. Subsequently, volunteers drank 400 mL GFJ for 7 days and urine was collected from 0 to 4 h daily. GFJ had dose-dependent effects on increasing cortisol excretion ($P < 0.05$) and the ratio of cortisol to cortisone ($P < 0.005$) and reducing 6β-OHC excretion ($P < 0.05$) and the ratio of 6β-OHC to cortisol ($P < 0.005$). There was no significant effect on cortisone excretion. Maximal effects were observed within 4 h after GFJ ingestion. Repeated doses had persistent but no cumulative effects. GFJ significantly reduced the ratio of 6β-OHC to cortisol. It increased the ratio of cortisol to cortisone and this appeared largely due to increased cortisol excretion related to impaired CYP3A4-mediated cortisol metabolism although a true inhibitory effect on 11β-HSD2 in the kidney cannot be excluded.

1. Introduction

Grapefruit or grapefruit juice (GFJ) is often recommended as a healthy dietary constituent, particularly in some weight reducing diets. Some recent studies support the benefits of the whole fruit or GFJ in potentiating the effect of weight reducing diets and also improving lipid metabolism (Dow et al., 2012; Silver et al., 2011). However, GFJ contains the furanocoumarin 6′7′ dihydroxybergamottin and the flavonoids naringenin and naringin which have been found to be inhibitors of the intestinal cytochrome P450 (CYP) 3A4 enzyme and can result in reduced presystemic metabolism and increased oral bioavailability of drugs that are highly influenced by this pathway, as originally discovered with felodipine (Edgar et al., 1992; Lown et al., 1997; Lundahl et al., 1997).

Cortisol is the major glucocorticoid hormone produced by the zona fasciculata of the adrenal cortex and excessive endogenous or exogenous cortisol can result in increases in body weight, blood pressure and glucose levels. Cortisol is metabolized by reduction of the A ring, reduction of the 20-ketone group and by 6β-hydroxylation, which occur mainly in the liver and possibly other organs (Galteau and Shamsa, 2003). Cortisol is metabolized to 6β-hydroxycortisol (6β-OHC) by CYP3A and cortisol, 6β-OHC and other metabolites are excreted in the urine. It has been demonstrated that GFJ decreases the urinary ratio of 6β-OHC to cortisol in healthy subjects (Li et al., 2010; Rouits et al., 2003; Seidegard et al., 1998), suggesting that endogenous cortisol metabolism occurs not only in the liver, but also in the gut mucosa or alternatively, GFJ inhibits not only intestinal but also hepatic CYP3A4.

It was reported that GFJ inhibited 11β-hydroxysteroid dehydrogenase (11beta-HSD) 2 in healthy volunteers assessed by measuring the ratio of cortisol to cortisone in urine and this was supported by showing inhibition of 11β-HSD2 by naringin in guinea pig kidney cortex microsomes *in vitro* (Lee et al., 1996). However, cortisone levels were not reported in that study and it would appear that the decrease in ratio of cortisol to cortisone was the result of cortisol levels increasing and the *in vitro* experiment showed that naringin at very high concentrations inhibited both 11β-HSD1 and 11β-HSD2, which have opposite effects on cortisol metabolism. 11β-HSD2 is predominantly expressed in the kidney and converts cortisol to inactive cortisone to protect the mineralocorticoid receptor from stimulation by cortisol. The activity of 11β-HSD2 is traditionally expressed as the ratio of cortisol to cortisone and the reverse reaction occurs in other tissues catalyzed by 11β-HSD 1 (Fig. 1), which has been considered as a drug target for obesity, diabetes and the metabolic syndrome (Pereira et al., 2012). Inhibition or deficiency

Abbreviations: GFJ, grapefruit juice; CYP, cytochrome P450; 6β-OHC, 6β-hydroxycortisol; 11β-HSD2, 11β-hydroxysteroid dehydrogenase 2; UPLC, ultra performance liquid chromatography; ANOVA, analysis of variance.

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http://dx.doi.org/10.1016/j.fct.2014.09.012
0278-6915/

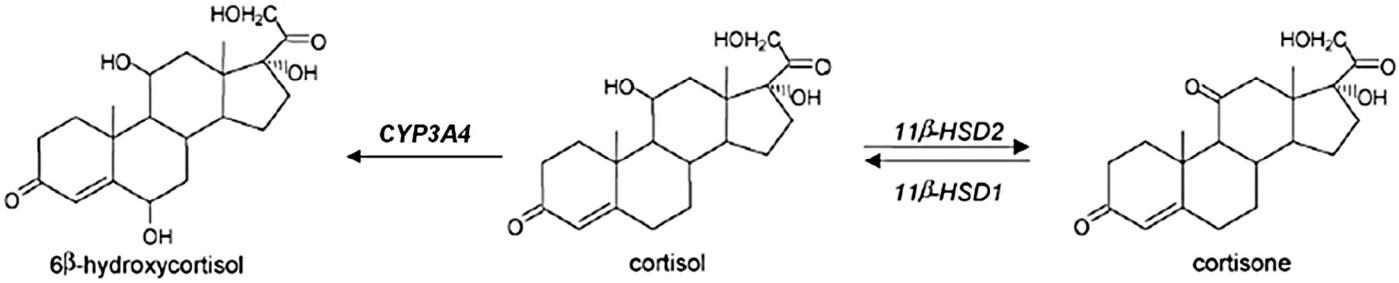

Fig. 1. Metabolism of cortisol.

of 11β-HSD2 causes high cortisol levels in the kidney and enhanced mineralocorticoid effects which may result in hypertension and hypokalaemia (Ferrari, 2010).

Interestingly, 11β-HSD2 is inhibited by licorice and herbal medicines derived from *Glycyrrhiza uralensis* and other *Glycyrrhiza* species (Stewart et al., 1987). There was also a case report of a 32-year-old Caucasian woman who drank 1 L GFJ daily who had low serum potassium, suppressed plasma renin activity and plasma aldosterone concentration, and an increased ratio of cortisol to cortisone in urine which appeared to return to normal after stopping the intake of GFJ (Palermo et al., 2003). Inhibition of 11β-HSD2 by GFJ was considered as the potential mechanism although the blood pressure of that patient was not increased by GFJ intake (Palermo et al., 2003). In these publications proposing that GFJ inhibits 11β-HSD2, urine 6β-OHC was not measured and no direct enzymatic studies were conducted, so it could not be confirmed whether the elevated urinary ratio of cortisol to cortisone was related to the inhibitory effect of GFJ on CYP3A4 or due to an effect on 11β-HSD2 in the kidney.

The present study examined the effects of single and multiple doses of GFJ on the urinary excretion of cortisol, cortisone and 6β-OHC and the ratios of 6β-OHC to cortisol and cortisol to cortisone in healthy male Chinese subjects to address this issue.

2. Materials and methods

2.1. Subjects and study design

This study comprised two parts: study 1 investigated the effects of increasing single daily consumption of GFJ (200 mL, 400 mL, and 600 mL) on the urinary excretion of cortisol, cortisone and 6β-OHC and urinary ratios of 6β-OHC to cortisol and cortisol to cortisone; study 2 examined the effects of repeated daily consumptions of GFJ (400 mL) for 7 days on these parameters. The 400 mL dose was chosen because it had a significant effect on the urinary ratio of 6β-OHC to cortisol for 24 h in the first part of the study. A total of 10 Chinese healthy male subjects (age range: 26–50 years, body weight: 62–82 kg) participated in study 1. Eight of these subjects participated in study 2. All subjects were nonsmokers and were in good health based on medical history, physical examination, electrocardiogram evaluation, and routine laboratory tests. The subjects were not taking any regular medications known to induce or inhibit CYP3A activity and did not take any medication including herbal medicines or grapefruit products for at least 2 weeks before and throughout the study. They were instructed to abstain from alcohol and caffeine-containing beverages for 48 hours prior to the study and during the study.

In study 1, subjects took increasing single doses of GFJ (Tropicana brand) at 09:00 h with a fixed dose sequence of 200 mL, 400 mL, and 600 mL on three different occasions with a 7-day washout period between the dosing days. A 24 h urine collection was performed on the days before the consumption of GFJ and on the dosing day at 4 h (09:00–13:00 h, 13:00–17:00 h, 17:00–21:00 h) and 12 h (21:00–09:00 h) intervals. In study 2, the volunteers drank 400 mL of GFJ once daily at 09:00 h for 7 consecutive days. Urine samples were collected at 09:00–13:00 h before and after the consumption of GFJ. The volumes of the urine samples were recorded. Water consumption of about 100–150 mL per hour was encouraged during the urine collection periods.

The study protocol was approved by the local Clinical Research Ethics Committee. Written informed consent was obtained from all the subjects before any study procedures were undertaken. The study was conducted in compliance with the Declaration of Helsinki.

2.2. Urinary cortisol, cortisone and 6β-hydroxycortisol measurements

The concentrations of cortisol, cortisone and 6β-OHC in urine samples were determined using a rapid ultra performance liquid chromatography (UPLC) method with ultraviolet detector as described previously (Xiao et al., 2012). Briefly, urine samples were cleaned up using Oasis HLB solid phase extraction columns (Waters, MA, USA). Cortisol, cortisone, 6β-OHC and the internal standard dexamethasone were separated on a Waters Acquity UPLC-Tunable UV system with an Acquity UPLC BEH C18 column (50 mm × 2.1 mm I.D., 1.7 μm) using a gradient elution of methanol and water (containing 0.01% formic acid). Peak areas of cortisol, cortisone and 6β-OHC and internal standard were measured. The volumes of the urine samples at each time interval were recorded for calculating the excretion rate of cortisol, cortisone and 6β-OHC (=urine concentration of cortisol, cortisone and 6β-OHC × volume of urine/time interval). The ratios of 6β-OHC to cortisol and cortisol to cortisone were calculated. The calibration curves for urinary cortisol, cortisone and 6β-OHC yielded good linearity with regression coefficients of >0.999 in the range of 5–200 ng/mL for cortisol and 10–1000 ng/mL for cortisone and 6β-OHC (Xiao et al., 2012). The limit of detection was 3 ng/mL for cortisol, and 5 ng/mL for cortisone and 6β-OHC (Xiao et al., 2012) and there was no sample with undetectable levels of urinary cortisol, cortisone or 6β-OHC. The general recoveries and accuracies of the three analytes determined at different concentrations were all above 90% and the method showed satisfactory overall intra-day (7.25%) and inter-day (8.75%) coefficients of variation (Xiao et al., 2012).

2.3. Statistical analysis

Urinary cortisol, cortisone and 6β-OHC excretion rates were calculated as μg/h. The effects of different doses or repeated doses of GFJ on the urinary cortisol, cortisone, 6β-OHC and the ratios of 6β-OHC to cortisol and cortisol to cortisone were assessed by repeated measures analysis of variance (ANOVA). Jonckheere–Terpstra trend test was performed to examine the dose effect of GFJ on these parameters. A P value of < 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA).

The sample size was calculated based on the results from previously published studies (Li et al., 2010; Seidegard et al., 1998). It has been shown that 600 mL of double strength GFJ reduced the urinary ratio of 6β-OHC to cortisol from 5.7 ± 2.4 to 4.2 ± 1.3 between 0 and 4 h after consumption of GFJ (Seidegard et al., 1998). The present study using normal strength GFJ in 8–10 subjects had >80% power to detect an effect of GFJ that reduced the ratio of 6β-OHC to cortisol by 1.0 assuming the SD is 1.3 with a type I error rate of 0.05.

3. Results

3.1. Effects of single doses of grapefruit juice on urinary cortisol and metabolites

The effect of single doses of GFJ on the urinary excretion of cortisol, cortisone, 6β-OHC and the ratios of 6β-OHC to cortisol and cortisol to cortisone are summarized in Table 1. The mean intraindividual variability of the basal urinary ratios of 6β-OHC to cortisol and cortisol to cortisone before GFJ intake at different time intervals were 22–31% and around 10%, respectively. Single exposure to GFJ at all the three doses significantly increased the urinary excretion of cortisol (14–30%, $P < 0.05$) and the ratio of cortisol to cortisone (12–44%, $P \leq 0.001$), and decreased the urinary excretion of 6β-OHC (up to 36.2%, $P < 0.05$ with the higher doses) and the ratio of 6β-OHC to cortisol (13–50%, $P \leq 0.001$) for the first 4 h