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Effect of a Dietary Supplement Containing Raspberry Ketone on CYP3A Activity in Healthy Women

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Abstract

Objective: Raspberry ketone (RK) is available as a supplement with effect on weight gain suppression. Recent studies have found that various herbal products can affect the activities of drug metabolizing enzymes and drug efflux proteins, and provoke clinically relevant drug-drug interactions. Capsaicin, a molecule having a similar chemical structure to RK, is another well-known cytochrome P450 (CYP) inhibitor. On the other hand, it is totally unclear whether RK has any effect on human CYP activities. In this study, we evaluated the effect of orally administered RK on CYP3A activity by measuring 6beta-hydroxycortisol/cortisol ratio in urine samples.

Methods: This clinical study was conducted with approval by the Institutional Review Board at Gunma University Hospital. A total of 7 healthy women aged between 20 and 35 years were included and all of them provided written informed consent. Urine samples were collected from all subjects on the morning of day 5 (± 1 day) of menstrual cycle. In the subsequent RK phase, subjects took 3 tablets (16.7 mg/tab) of RK 3 times daily for 7 days, followed by urine sampling on the morning of day 8. In the control phase, the second morning urine sampling was performed 8 days after the first sampling. Urine 6beta-hydroxycortisol and cortisol concentrations were measured by HPLC UV method and the 6beta-hydroxycortisol to cortisol ratio was compared between the two phases.

Results: The mean basal and assessment ratios in the RK phase were 7.49 ± 4.76 and 9.20 ± 8.05, respectively, while the corresponding ratios in the control phase were 5.36 ± 3.17 and 5.19 ± 4.61, showing no significant difference in either phase.

Conclusion: RK does not affect CYP3A activity.

Keywords: Raspberry ketone; Supplement; CYP3A; Interaction; Oral contraceptives; 6beta-hydroxycortisol/cortisol ratios

Introduction

Raspberry ketone (4-(4-hydroxyphenyl) butane-2-on) is an aroma component of red raspberry. It has a chemical structure similar to that of capsaicin and has been shown to reduce weight gain in obese rats [1]. Wang et al reported that 40 rats given raspberry ketone with fattening diet were protected against fatty liver [2]. In another study conducted in human, the participants who takes raspberry ketone concomitant with caffeine, garlic, capsaicin, ginger and synephrine lost 7.8% of their fat mass, compared with the placebo group which lost 2.8% [3]. In Japan, it has been commercially available as a supplement for weight reduction since 2002.

A lot of studies have found that various herbal products, dietary supplements, herbal medicines and other plant-derived ingredients can affect the activities of drug metabolizing enzymes, such as cytochrome P450 (CYP), and drug efflux proteins, such as MDR1, and provoke clinically relevant drug-drug interactions. A major representative of these products is St. John's wort, which has been shown to induce the expressions of CYP2C9 [4], CYP2C19 [5] and CYP3A4 [6], thereby interfering with the efficacy of warfarin, cyclosporin and oral contraceptives. Capsaicin is another well-known CYP inhibitor [7], so that it is an important issue whether raspberry ketones with similar structures also have similar effects. In our previous study, raspberry ketone had shown little impact on CYP3A activity in rats [8]. On the other hand, it is totally unclear whether raspberry ketone has any effect on human CYP activities.

Renal clearance of hydroxycortisol has been reported to be an indicator of CYP3A activity [9], however, the measurement of blood concentration is required. To reduce stress for the subjects, it has been reported that the hydroxycortisol/cortisol ratio in urine samples is available as an indicator of CYP induction [10].

In this study, we investigated the effect of oral administration of raspberry ketone on 6beta-hydroxycortisol/cortisol ratio as an index of

CYP3A activity in young healthy women who are potential main user of raspberry ketone.

Materials and Methods

Chemicals and materials

Raspberry ketone was kindly supplied by Kanebo (Tokyo, Japan) as Vitarosso Tablet containing 16.7 mg of raspberry ketone in one tablet. The tablet also contains Gymnema sylvestris extract, adlay seed extracts, inositol and ascorbic acid as minor constituents. All other chemicals and reagents were obtained from commercial sources and used without further purification.

Clinical experiments

All clinical experimental procedures were approved by the Institutional Review Board in Gunma University Hospital. Seven healthy women aged 20-35 years were included in this randomized crossover open study. All participants gave written consent to participate in the study after they were informed of the study purpose and procedures. The exclusion criteria were use of any prescription drugs including contraceptives, pregnancy, and history of intake of raspberry ketone. We ascertained normal renal and hepatic function in all participants by routine clinical laboratory tests before the following procedures were performed.

The participants were requested to abstain from any supplements, herbal tea, St. John's wort, grapefruit juice, alcohol, coffee, and smoking during the study. Subjects were randomized into two study groups. Urine samples were collected from all subjects on the morning of day 5 (± 1 day) of menstrual cycle and the basal hydroxycortisol/cortisol ratio was measured. In the subsequent raspberry ketone phase, subjects took 3 tablets (16.7 mg/tab) of raspberry ketone 3 times daily for 7 days, followed by urine sampling on the morning of day 8 to calculate the assessment ratio. In the control phase, the second morning urine sampling was performed 8 days after the first sampling. In 3 of the 7 subjects, the first urine sampling was performed in the control phase and the second sampling in the raspberry ketone phase was performed in the subsequent menstrual cycle.

Measurement of 6beta-hydroxycortisol and cortisol in urine

To 1 mL of urine sample, 2 μg of dexamethasone as internal standard, 1 mL of 500 mM borate buffer (pH 8.0) and 5 mL of ethylacetate were added. The mixture was vigorously shaken for 10 min, then centrifuged at 2000 g for 10 min. The upper organic layer was taken and evaporated to dryness under reduced pressure, then the residue was dissolved with 6.5% acetonitrile, and injected into HPLC. The HPLC apparatus used was Waters 2960 separation module equipped with Waters 996 photodiode array detector (Waters, Milford, Massachusetts). The separation was performed on a YMC-Pack Pro C18 (250 mm × 4.6 mm i.d.) (YMC, Kyoto), monitored by UV absorbance at 245 nm and operated at 1.0 mL/min. The column temperature was maintained at 40°C. The mobile phase is the mixture of solution A (acetonitrile) and solution B (50 mM KH_2PO_4 / 10 mM acetic acid). The ratio of solution B was linearly increased from 6.5% at time 0 to 55% at 25 min, kept at 55% from 25 min to 35 min, then kept at 6.5% from 30 min to 40 min. The lower limit of quantification was 10 ng/mL for each compound.

Statistical analysis

The significance between basal and assessment ratio of urine 6beta-hydroxycortisol/cortisol ratios were evaluated by Wilcoxon t-test. $P<0.05$ was considered statistically significant. The results were expressed as the mean ± standard deviation (SD).

Results

Urine 6beta-hydroxycortisol/cortisol ratios in the raspberry ketone phase and control phase in seven subjects are shown in Table 1. In the raspberry ketone phase, the average basal ratio and average assessment ratio were 7.49 ± 4.76 and 9.20 ± 8.05, respectively. There was no significant difference in both ratios. Similarly, in the control phase, the average basal ratio and average assessment ratio were 5.36 ± 3.17 and 5.19 ± 4.61, respectively. There was also no significant difference.

		Subjects							Mean	S.D.	P-value
		No.1	No.2	No.3	No.4	No.5	No.6	No.7			
Raspberry ketone phase	Basal ratio	9.00	1.92	6.16	17.15	6.88	6.02	5.30	7.49	4.76	0.60
	assessment ratio	7.52	11.80	4.25	26.41	4.14	5.16	5.13	9.20	8.05	
Control phase	Basal ratio	8.60	2.68	10.71	3.52	4.47	5.27	2.24	5.36	3.17	0.60
	assessment ratio	1.89	6.82	14.82	3.80	1.39	3.15	4.51	5.19	4.61	

Table 1: Urine 6beta-hydroxycortisol/cortisol ratio in Rasberry ketone phase and control phase. S.D. means standard deviation.

Discussion

In this study, administration of raspberry ketone did not lead to a significant increase in the 6beta-hydroxycortisol/cortisol ratio, suggesting that raspberry ketone does not induce CYP3A expression.

Galteau et al. [9] have shown that renal clearance of hydroxycortisol can be a good indicator of CYP3A induction while the hydroxycortisol/cortisol ratio is susceptible to daily fluctuation and renal clearance and thus it cannot be a good indicator. Similar results have also been reported by Furuta et al. [11]. Chen et al. [12] reported the lack of correlation between hydroxycortisol/cortisol ratio and midazolam clearance, which is probably because they did not pay much attention to change in renal cortisol clearance associated with varying urine output or daily fluctuation [13]. Taking these factors into consideration, the hydroxycortisol/cortisol ratio in morning spot urine samples may be used as an indicator of CYP induction [10]. Cortisol

secretion is subject to daily fluctuation, which can be minimized by using the hydroxycortisol/cortisol ratio [14-18]. However, Ohno et al. [19] have demonstrated that diurnal variations of 6beta-hydroxycortisol and of cortisol were not really parallel, and therefore, the ratio varied from 4.3 to 12.6 as a daily fluctuation. It is therefore very important to collect samples at the same time of the day.

Katz et al. [20] and Burstein et al. [21] did not find any significant variation in the hydroxycortisol/cortisol ratio in 13 healthy females during the four different weeks of their menstrual cycle. Similarly, Lin et al. [22] did not find any significant difference in the ratio hydroxycortisol/cortisol in neither Caucasians nor in Asian women during the different periods of their menstrual cycle. Although hormonal status is unlikely to affect the hydroxycortisol/cortisol ratio, we eliminated this potential effect by collecting samples from subjects taking into account their menstrual cycle. Alcohol consumption may have some effects and thus was prohibited in this study [23]. Smoking [24-27] and caffeine-containing beverage [28] are unlikely to affect the parameter, but were prohibited just in case.

Without citing examples of St. John's wort, drug-food interactions are known to be of clinical significance. Co-administration of drugs with CYP3A4-inducing activity has been shown to reduce the AUC and hence efficacy of low-dose oral contraceptives [29,30]. Since raspberry ketone is a supplement taken for the purpose of body fat reduction and may be used preferably by young women it is particularly important to consider its effect on the metabolizing activity of CYP3A.

The results of the present study suggest that raspberry ketone does not affect CYP3A activity.

References

1. Morimoto C, Satoh Y, Hara M, Inoue S, Tsujita T, et al. (2005) Anti-obese action of raspberry ketone. Life Sci 77: 194-204.
2. Wang L, Meng X, Zhang F (2012) Raspberry ketone protect rats fed high-fat diets against nonalcoholic steatohepatitis. J Med Food 15: 495-503.
3. Lopez HL, Ziegenfuss TN, Hofheins JE, Habowski SM, Arent SM, et al. (2013) Eight weeks of supplementation with a multi-ingredient weight loss product enhances body composition, reduces hip and waist girth, and increases energy levels in overweight men and women. J Int Soc Sports Nutr 10: 22.
4. Jiang X, Williams KM, Liauw WS, Ammit AJ, Roufogalis BD, et al. (2005) Effect of St John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. Br J Clin Pharmacol 57: 592-599.
5. Wang LS, Zhou G, Zhu B, Wu J, Wang JG, et al. (2004) St John's wort induces both cytochrome P450 3A4-catalyzed sulfoxidation and 2C19-dependent hydroxylation ofomeprazole. Clin Pharmacol Ther 75: 191-197.
6. Sugimoto K, Ohmori M, Tsuruoka S, Nishiki K, Kawaguchi A, et al. (2001) Different effects of St John's wort on the pharmacokinetics of simvastatin and pravastatin. Clin Pharmacol Ther 70: 518-524.
7. Zhang Z, Hamilton SM, Stewart C, Strother A, Teel RW (1993) Inhibition of liver microsomal cytochrome P450 activity and metabolism of the tobacco-specific nitrosamine NNK by capsaicin and ellagic acid. Anticancer Res 13: 2341-2346.
8. Sekizuka M, Qi JW, Aomori T, Okada Y, Nakamura K, et al. (2014) The Effect of a Dietary Supplement Containing Raspberry Ketone on CYP3A Activity. Pharm Anal Acta 5: 6.
9. Galteau MM, Shamsa F (2003) Urinary 6beta-hydroxycortisol: a validated test for evaluating drug induction or drug inhibition mediated through CYP3A in humans and in animals. Eur J Clin Pharmacol 59: 713-733.
10. Furuta T, Suzuki A, Mori C, Shibasaki H, Yokokawa A, et al. (2003) Evidence for the validity of cortisol 6 beta-hydroxylation clearance as a new index for in vivo cytochrome P450 3A phenotyping in humans. Drug Metab Dispos 31: 1283-1287.
11. Chen YC, Gotzkowsky SK, Nafziger AN, Kulawy RW, Rocci ML Jr, et al. (2006) Poor correlation between 6beta-hydroxycortisol:cortisol molar ratios and midazolam clearance as measure of hepatic CYP3A activity. Br J Clin Pharmacol 62: 187-195.
12. Fenske M (2007) Poor correlation between 6beta-hydroxycortisol:cortisol molar ratios and midazolam clearance as measure of hepatic CYP3A activity: a comment. Br J Clin Pharmacol 63: 632.
13. Micuda S, Sispera L, Hodac M, Parízek P, Fuksa L, et al. (2007) Diurnal variation of 6beta-hydroxycortisol in cardiac patients. Physiol Res 56: 307-313.
14. Saenger P (1983) 6 beta-hydroxycortisol in random urine samples as an indicator of enzyme induction. Clin Pharmacol Ther 34: 818-821.
15. Joellenbeck L, Qian Z, Zarba A, Groopman JD (1992) Urinary 6 beta-hydroxycortisol/cortisol ratios measured by high-performance liquid chromatography for use as a biomarker for the human cytochrome P-450 3A4. Cancer Epidemiol Biomarkers Prev 1: 567-572.
16. Zhiri A, Mayer HA, Michaux V, Wellman-Bednawska M, Siest G (1986) 6 beta-hydroxycortisol in serum and urine as determined by enzyme immunoassay on microtitre plates. Clin Chem 32: 2094-2097.
17. Lee C (1995) Urinary 6 beta-hydroxycortisol in humans: analysis, biological variations, and reference ranges. Clin Biochem 28: 49-54.
18. Hunter DJ, Keane P, Walker C, YoungLai EV (1984) Variations in urinary levels of free 6 beta-hydroxycortisol, cortisol, and estrogens in late pregnancy. Gynecol Obstet Invest 18: 83-87.
19. Ohno M, Yamaguchi I, Ito T, Saiki K, Yamamoto I, et al. (2000) Circadian variation of the urinary 6beta-hydroxycortisol to cortisol ratio that would reflect hepatic CYP3A activity. Eur J Clin Pharmacol 55: 861-865.
20. Katz FH, Lipman MM, Frantz AG, Jailer JW (1962) The physiologic significance of 6beta-hydroxycortisol in human corticoid metabolism. J Clin Endocrinol Metab 22: 71-77.
21. Burstein AH, Reiss WG, Kantor E, Anderson GD (1998) Cytochrome P450 3A4 activity in premenopausal and postmenopausal women, based on 6-beta-hydroxycortisol: cortisol ratios. Pharmacotherapy 18: 1271-1276.
22. Lin Y, Anderson GD, Kantor E, Ojemann LM, Wilensky AJ (1999) Differences in the urinary excretion of 6-beta-hydroxycortisol/cortisol between Asian and Caucasian women. J Clin Pharmacol 39: 578-582.
23. Luceri F, Fattori S, Luceri C, Zorn M, Mannaioni P, et al. (2001) Gas chromatography-mass spectrometry measurement of 6beta-OH-cortisol/cortisol ratio in human urine: a specific marker of enzymatic induction. Clin Chem Lab Med 39: 1234-1239.
24. Vestal RE, Cusack BJ, Mercer GD, Dawson GW, Park BK (1987) Aging and drug interactions. I. Effect of cimetidine and smoking on the oxidation of theophylline and cortisol in healthy men. J Pharmacol Exp Ther 241: 488-500.
25. Beyeler C, Frey BM, Bird HA (1997) Urinary 6 beta-hydroxycortisol excretion in rheumatoid arthritis. Br J Rheumatol 36: 54-58.
26. Crowley JJ, Cusack BJ, Jue SG, Koup JR, Park BK, et al. (1988) Aging and drug interactions. II. Effect of phenytoin and smoking on the oxidation of theophylline and cortisol in healthy men. J Pharmacol Exp Ther 245: 513-523.
27. Moretti M, Villarini M, Scassellati-Sforzolini G, Monarca S, Libraro M, et al. (1996) Biological monitoring of genotoxic hazard in workers of the rubber industry. Environ Health Perspect 104: 543-545.

28. Caraco Y, Zylber-Katz E, Granit L, Levy M (1990) Does restriction of caffeine intake affect mixed function oxidase activity and caffeine metabolism? Biopharm Drug Dispos 11: 639-643.

29. Barditch-Crovo P, Trapnell CB, Ette E, Zacur HA, Coresh J, et al. (1999) The effects of rifampin and rifabutin on the pharmacokinetics and pharmacodynamics of a combination oral contraceptive. Clin Pharmacol Ther 65: 428-438.

30. Hall SD, Wang Z, Huang SM, Hamman MA, Vasavada N, et al. (2003) The interaction between St John's wort and an oral contraceptive. Clin Pharmacol Ther 74: 525-535.