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Effect of Grapefruit Juice on the Urinary 6 β -hydroxycortisol/cortisol Ratio

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The effect of grapefruit juice on the urinary 6 β -hydroxycortisol/cortisol ratio was investigated in healthy volunteers. The urinary concentration of cortisol was virtually unaffected by the administration of grapefruit juice whereas the concentration of 6 β -hydroxycortisol in the urine decreased when grapefruit juice was given every 3 hours. The administration of grapefruit juice was thus considered to result in a decrease in the urinary 6 β -hydroxycortisol / cortisol ratio. In addition, both diurnal and individual differences were also observed in the extent of the inhibitory effect of grapefruit juice.

Key words — grapefruit juice, CYP 3 A, cortisol, 6 β -hydroxycortisol, urine

Introduction

It has been shown that modulation of drug-metabolizing enzyme activity caused by either enzyme induction or enzyme inhibition have therapeutic and toxicological implication.

Recently it has been reported that plasma concentration of drugs which are metabolized by CYP 3 A enzymes were increased by grapefruit juice¹⁻⁴⁾. In addition, flavonoid components contained in grapefruit juice have been shown to inhibit the activity of CYP 3 A enzymes *in vitro*. Based on the findings, the information of the interaction with grapefruit juice has been described in the package insert of calcium antagonists such as nifedipine and nisoldipine since March 1995, in our country.

On the other hand, there are several reports indicated that urinary 6 β -hydroxycortisol/cortisol ratio can be used as a marker of metabolic activity of CYP 3 A enzymes *in vivo*⁵⁻⁷⁾. Therefore, in the present study, we investigated the effect of grapefruit juice on urinary 6 β -hydroxycortisol/cortisol ratio with healthy volunteers.

Materials and Methods

Two male and two female healthy subjects who were between the ages of 27 to 38 years and who weighed from 45 to 68 kg were selected for this study. All subjects provided written informed consent for participation in the study. To obtain the control level of concentration of cortisol, 6 β -hydroxycor-

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tisol and 6 β -hydroxycortisol/cortisol ratio, the urine was collected at the time of rising, 11 a.m., 3 p.m., 7 p.m., and 11 p.m., during three days, and the concentrations of cortisol and 6 β -hydroxycortisol in urine were measured. Then, mean $\pm$ S.D. of 6 β -hydroxycortisol/cortisol ratio and concentration of cortisol and 6 β -hydroxycortisol at each sampling points during three days was calculated as control level. The schedule of grapefruit juice administration and sampling was shown in Fig. 1. Grapefruit juice (150 ml) was given at every 3 hours from 10 hours before first sampling, and sampling was performed at same time as control study. Grapefruit juice was prepared from grapefruits just before use.

Fig. 1. Administration Schedule of Grapefruit Juice (GJ) and Sampling Points

Cortisol and 6 β -hydroxycortisol were purchased from Wako Chemical Co. (Osaka, Japan), and Steraloids Co. (Wilton, NH, USA), respectively. Other reagents were of highest grade commercially available.

The determination of 6 β -hydroxycortisol in urine was conducted as follows. After hydrochloride (1 N) was added to 5 ml of urine to adjust at pH 4.0, 6 β -hydroxycortisol was extracted with 15 ml of ethylacetate. The ethylacetate phase was treated with 1 ml of 0.25 N sodium hydroxide in saturated sodium sulfate. These procedure were repeated twice. The ethylacetate extract was evaporated to dryness under reduced pressure. The residue was dissolved in 200 ml of 10% ethanol which had been adjusted to pH 3.0 with TEAF (tetraethylammonium formate). An aliquot of the ethanol solution was applied to a high-performance liquid chromatograph (HITACHI L-6000) equipped with a UV absorbance detector (HITACHI L-4200). The reverse-phase chromatography was performed on an Inertsil ODS-2 column (5 μ m, 4.6 $\times$ 250 mm, GL Sciences Co.) preceded by a pre-column (4.6 $\times$ 10 mm) at 55°C. 6 β -hydroxycortisol was separated at a flow rate of 1 ml/min and by isocratic elution with 8% ethanol which had been adjusted at pH 3.0 with TEAF. The effluent was monitored at 240 nm. In this method, recovery percentage of 6 β -hydroxycortisol was 89%, and between-day coefficient of variation was found to be 5.1% (n=6).

For determination of cortisol in urine, 6 ml of chloroform was added to 3 ml of urine after the addition of 50 μ l of dexamethasone (2 μ g/ml) as the internal standard. The chloroform phase was treated with 6 ml of 0.3 N sodium hydroxide. These procedure were repeated twice. The chloroform extract was evaporated to dryness under reduced pressure. The residue was dissolved in 100 μ l of 50% methanol. As in the case of 6 β -hydroxycortisol determination, the reverse-phase chromatography was

performed on an Inertsil ODS-2 column (5 μ m, 4.6 $\times$ 250 mm, GL Sciences Co.) without a pre-column at 25°C. Cortisol was eluted at a flow rate of 1 ml/min and by isocratic elution with 30% acetonitril. The effluent was monitored at 247 nm. In this method, recovery percentage of cortisol was 80%, and between-day coefficient of variation was found to be 6.3% (n=6).

Results

Fig. 2 shows the effect of grapefruit juice intake on the concentration of cortisol in urine. The concentration of urinary cortisol which was measured without grapefruit juice administration, tended to decrease from morning to night. On the other hand, urinary cortisol which was measured 1 to 3 hrs after grapefruit juice administration, was also decreased from daytime to night, although there appeared individual difference in the urinary concentration. As shown in **Fig. 3**, the concentration of 6 β -hydroxycortisol which is one of the metabolites of cortisol, was observed to be decreased more pronouncedly in the two subjects (Age 36 and Age 27) by the administration of grapefruit juice regardless of the time of which 6 β -hydroxycortisol was measured. In addition, the urinary 6 β -hydroxycortisol/cortisol ratio without grapefruit juice was found to be time dependent, and tended to increase from daytime to night except that the highest value was observed at 15:00 p.m. in one case (**Fig. 4**). Furthermore, the administration of grapefruit juice resulted in the decrease in the urinary 6 β -hydroxycortisol/cortisol ratio in the two subjects (Age 36 and Age 27). **Fig. 5** shows the effect of grapefruit juice on the urinary 6 β -hydroxycortisol/cortisol ratio. The percent of decrease in the ratio by the administration of grapefruit juice was found to be greater in early morning and night than daytime, although the reverse relation observed in one case.

Fig. 2. Effect of Grapefruit Juice on Concentration of Urinary Cortisol in Four Volunteers

Open circles represent the data (mean $\pm$ S. D., n=3) without grapefruit juice, and closed circles represent the data (n=1) with grapefruit juice.

Fig. 3. Effect of Grapefruit Juice on Concentration of Urinary 6β-hydroxycortisol in Four Volunteers

Open circles represent the data (mean ± S. D., n=3) without grapefruit juice, and closed circles represent the data (n=1) with grapefruit juice.

Fig. 4. Effect of Grapefruit Juice on Urinary 6β-hydroxycortisol/cortisol Ratio in Four Volunteers

Open circles represent the data (mean ± S. D., n=3) without grapefruit juice, and closed circles represent the data (n=1) with grapefruit juice.

Fig. 5. Diurnal Change in the Effect of Grapefruit Juice on Urinary 6 β -hydroxycortisol/cortisol Ratio

Discussion

The grapefruit juice has been shown to cause the remarkable changes in the pharmacokinetic parameters of calcium antagonists including nifedipine, ferodipine and nisoldipine^{1,2)}. In the present study, we investigated effect of grapefruit juice on urinary 6 β -hydroxycortisol/cortisol ratio to obtain the information concerning the effect of drugs under consideration on the metabolism of other drugs. The results shown herein indicated that the intake of grapefruit juice resulted in the decrease in the 6 β -hydroxycortisol/cortisol ratio in urine, and the decrease in the urinary ratio by the administration of grapefruit juice was due to the change in the concentration of 6 β -hydroxycortisol rather than that of cortisol. Although there appeared individual difference in the extent of the inhibitory effect of grapefruit juice on 6 β -hydroxycortisol excretion in urine, the decrease in the concentration of urinary 6 β -hydroxycortisol was observed by the administration of grapefruit juice, and the inhibitory effect of grapefruit juice on the 6 β -hydroxycortisol/cortisol ratio seemed to be more pronounced when the ratio showed high values. In addition, the CYP 3A enzymes have been well known to catalyze the oxidative metabolism of cortisol to 6 β -hydroxycortisol. More recently, Lee et al. have shown that grapefruit juice inhibited 11 β -hydroxysteroid dehydrogenase, but its effect on urinary free cortisol did not exceed the normal range⁸⁾. Therefore, it is suggested that the effect of grapefruit juice on urinary 6 β -hydroxycortisol/cortisol ratio is due to the inhibition by grapefruit juice of the activity of CYP 3A enzyme (s).

It has been demonstrated that CYP 3A enzymes are one of the major forms of P450 in human livers, and that at least two forms of CYP 3A enzymes, CYP 3A4 and CYP 3A5, are expressed in adult human livers⁹⁾. Furthermore, the expression of CYP 3A5 has been shown to be varied between human cases. However, in our preliminary investigation, it was indicated that naringenin one of the flavonoids contained in grapefruit juice, inhibited both 6 β -hydroxylations of testosterone catalyzed by CYP 3A4 and CYP 3A5 expressed in COS-7 cells in a similar degree. It was, therefore, unlikely that the individual difference in the inhibitory effect of grapefruit juice on 6 β -hydroxycortisol forma-

tion was attributable to the difference in the expression level of CYP 3 A 5.

Since CYP 3 A enzymes are responsible for the metabolism of a wide variety and structurally unrelated drugs clinically used, particular attention should be given when the drugs which are metabolized by CYP 3 A enzymes are coadministered with grapefruit juice. In this study, we suggested that there was not only diurnal but individual differences in the extent of the inhibitory effect of grapefruit juice on CYP 3 A enzymes, although more precise investigations are required.

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