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Grapefruit juice effects on the bioavailability of cyclosporin-A in rats

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Abstract. – **Previous studies indicate that blood levels of cyclosporin-A are increased by concomittant administration of grapefruit juice in healthy subjects and patients. It was suggested that grapefruit juice could inhibit the metabolism of cyclosporin-A by CYP3A4, the predominant cytochrome P450 enzyme in the gut wall and liver. However, up to date, the mechanism of action of grapefruit juice has not been conclusively identified and no work has been conducted in animals to quantify its effect on cyclosporin-A metabolism. This study compared the disposition of cyclosporin-A (5 mg/kg) coadministered with grapefruit juice, orange juice or water (10 ml/kg) in male Sprague-Dawley rats. Time to peak concentration was about 5 h for each group. Area under the blood concentration-time curve and peak concentration of cyclosporin-A were increased by 31% and 20%, respectively, with grapefruit juice ($P < 0.05$). The effects of grapefruit juice were not duplicated by orange juice which did not differ significantly from water for any of the parameters tested. These results confirm that grapefruit juice may act as an inhibitor of drug metabolism altering the disposition of concomittantly administered cyclosporin-A in rats. Nonetheless, it was demonstrated that, under appropriate experimental conditions, rats may be suitable models for in vivo investigation of the interaction mechanism between grapefruit juice and cyclosporin-A.**

Key Words:

Cyclosporine, Food-Drug Interactions, Orange juice, Grapefruit juice.

Introduction

Cyclosporin-A, a cyclic undecapeptide of fungal origin, is a potent immunosuppressant which is currently the drug of choice in preventing graft rejection and graft versus host disease in organ transplantation[1]. Cyclosporin-A has gained a prominent role, mainly because of its lack of myelotoxicity and hormonal interferences. However, it is well known that several drugs and foods may alter cyclosporin-A pharmacokinetic. There is evidence that this may potentiate its adverse effects or reduce its immunosuppressive action[2,3,4]. Different mechanisms are involved in cyclosporin-A interactions. Changes in gastrointestinal motility may have an effect on the rate and extent of cyclosporin-A absorption after oral administration[5]. Moreover, induction or inhibition of cytochrome P450 in intestinal mucosa or liver influences cyclosporin-A biotransformation, respectively increasing or decreasing blood levels[6,7].

The interaction between grapefruit juice and some drugs was discovered by chance during a study of the potential interaction between alcohol and the calcium-channel blocker felodipine[8]. Since this finding, subsequent studies have found that grapefruit juice increases plasma concentrations of several other drugs as well as all calcium channel blockers, sedatives, protease inhibitors, and also cyclosporin-A. These drugs are metabolized by CYP3A4, the predominant cytocrome P450 enzyme in the gut wall and liver[9]. Cyclosporin-A also undergoes extensive first-pass metabolism by intestinal and hepatic CYP3A4 and grapefruit juice increases cyclosporin-A serum concentrations in both healthy subjects and patients[10]. Grapefruit juice contains high concentrations of bioflavonoids and furanocoumarins, which have been shown to inhibit CYP3A4 in vitro[11], and grapefruit juice has no effect on drug disposition after intravenous administra-

tion[12]. The data suggest that grapefruit juice could inhibit the metabolism of cyclosporin-A by acting on cytochrome P450 enzymes of the small intestine. Main efforts to identify the inhibitory substance present in grapefruit juice largely focused on naringin, but other components (6',7'-dihydroxybergamottin, quercitin, kaempferol) could be responsible for the grapefruit juice inhibition as observed in in vitro studies using rat liver microsomes[13,14]. These observations suggest that rats may be a good model species to futher investigate the in vivo interaction of grapefruit juice with cyclosporin-A. Preliminary studies from our laboratory showed that grapefruit juice may increase cyclosporin-A bioavailability in rats[15]. To date, the inhibitory substance or mechanism of action of grapefruit juice has not been conclusively identified and, to our knowledge, no studies have been conducted in animals to quantify the effect of grapefruit juice on cyclosporin-A metabolism.

The mechanism of action of grapefruit juice might be better understood by comparing grapefruit juice with orange juice. Based on these considerations, we sought to determine the interaction between grapefruit juice or orange juice and orally-administered cyclosporin-A metabolism in male Sprague-Dawley rats, to evaluate the feasibility of a rat model in the research of the interaction between cyclosporin-A and citrus fruit derivatives.

Material and Methods

Chemical and drugs

Cyclosporin-A microemulsion formulation (Sandimmune Neoral®) was supplied by Novartis. Grapefruit and orange juice were prepared by squeezing fresh fruits from commercially available single batch. Heparin and sodium pentobarbital (Sigma, Italy).

Animals and surgical cannulation procedure

Male Sprague-Dawley rats (250-270 g, Morini, San Polo d'Enza, Italy) were housed under constant environmental conditions (22 ± 1° C room temperature; 65 ± 5% relative humidity; 12-h light/dark) for at least 2 days and given food and water ad libitum. The day prior to dosing experiments, rats were subjected to surgical cannulation. During anesthesia induced by sodium pentobarbital (65 mg/kg i.p.), a small longitudinal incision was made in the skin of each rat over the right jugular vein, which was then made accessible by clearing the surrounding tissues. The vein was catheterized with laboratory tubing containing heparinized (100 IU/ml) normal saline and fixed in place with two nonabsorbable surgical sutures. Each cannula was terminated with a suitable length of polyethylene tubing and the free end exteriorized to the dorsal side of the neck. The exposed areas were then closed using nonabsorbable surgical suture. The implanted cannulas permitted frequent blood sampling from each rat. The rats were fasted 6 h before cyclosporin-A administration and for the duration of the experiment. All the experiments were conducted blind to treatment in conformity with the European Communities Council Directive 86/609/EEC in agreement with the Helsinki declaration.

Study design

1 ml of cyclosporin-A microemulsion (100 mg/ml) was diluted to 200 ml with water, grapefruit juice or orange juice to obtain a concentration of 5 mg/ml.

On experimental day, cannulated rats were assigned to three groups of 7 animals each and received a single oral dose of 10 ml/Kg cyclosporin-A by gastric gavage, using grapefruit juice, orange juice or water as vehicle, respectively.

Blood draws (150-200 µl) were made using 1 ml syringes connected to the jugular cannula at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 15 and 24 hours after cyclosporin-A administration. Blood samples were collected into anticoagulant tubes that contained ethylenediaminetetraacetic acid (EDTA) and were stored frozen at -20°C until whole blood cyclosporin-A concentrations were measured.

Cyclosporine analysis

Whole blood cyclosporin-A was assayed using TDx® cyclosporine monoclonal kit for analysis by fluorescence polarization immunoassay (FPIA) from Abbott Diagnostics Laboratories. Calibration curves were developed using a whole blood matrix spiked with

0, 100, 250, 500, 1000 and 1500 ng/ml cyclosporin-A according to the manufacturer's instructions. Assay quality was assured using concurrent analysis of three levels (150, 400 and 800 ng/ml) of quality control material. To obtain cyclosporin-A concentrations within the range of the standard curve, the blood sample was diluted before analysis with blood obtained from untreated rats. The intra-assay and inter-assay variation of this assay system were < 2.5% and 5%, respectively; a lower detection limit is 25 ng/ml for a whole blood sample.

Pharmacokinetic analysis

Area under the whole blood concentration-time curve (AUC) was calculated using the linear trapezoidal rule from time zero (t_0) to time t, where t is the last concentration estimated. Peak concentration (C_{max}) and time to peak concentration (t_{max}) were obtained directly from the data. Relative bioavailability (F_A/F_B) after cyclosporin-A administration at the same dosing level was determined according to the expression

$$F_A/F_B = AUC_A/AUC_B$$

where AUC_A and AUC_B are the areas under the cyclosporin-A plasma concentration-time curve for treatments A and B, respectively.

Statistical analysis

All the calculated pharmacokinetic parameters – except t_{max} – were assumed to follow log-normal distribution, and therefore log-transformed before statistical analysis. Pharmacokinetic data were analyzed using an analysis of variance and two-tailed Student's paired t-test. Statistical differences were considered to be significant when $P < 0.05$. Data are expressed by arithmetic means ± SD.

Results

The pharmacokinetic parameters for cyclosporin-A when given with water, grapefruit juice, and orange juice are summarized in Table I. The area under the whole blood concentration-time curve (AUC) was significantly increased by 31% with grapefruit juice compared to water. Peak concentrations (C_{max}) also showed a 20% increase with grapefruit juice ($P < 0.05$). No statistically significant differences were observed in these parameters between water and orange juice. Time to peak concentration (T_{max}) and elimination half-life ($t_{1/2}$) were about 5 h and 6.5 h respectively for all three groups. The mean whole blood cyclosporin-A concentration-time profiles obtained from each of the three treatment groups are shown in Figure 1. The profiles indicate that AUC and C_{max} are elevated with grapefruit juice, whereas no differences are apparent between water and orange juice groups. These findings confirm the interaction between cyclosporin-A and grapefruit juice in rats, and also show wide fluctuations in cyclosporin-A serum concentrations.

Discussion

Cyclosporin-A is an immunosuppressive agent that is useful in organ transplantation

Table I. Cyclosporin-A whole blood concentrations after oral coadministration with water, grapefruit juice or orange juice in rats. Data are mean values ± SD.

	Water	Grapefruit juice	Orange juice
AUC (ng/ml/h)	23067 ± 3301	30307 ± 4234*	25550 ± 2842
C_{max} (ng/ml)	1964 ± 205	2349 ± 215*	2032 ± 193
T_{max} (h)	4,86 ± 0,89	5,00 ± 1,00	4,71 ± 0,76
CLp (ml/min/Kg)	3,67 ± 0,51	2,79 ± 0,36	3,29 ± 0,38
Relative bioavailability	1,00	1,31	1,11

* Significantly different ($P < 0.05$).
AUC, area under the plasma drug concentration-time curve; C_{max}, maximum plasma drug concentration; T_{max}, time to reach the maximum drug concentration following drug administration; CLp, oral plasma clearance of drug.

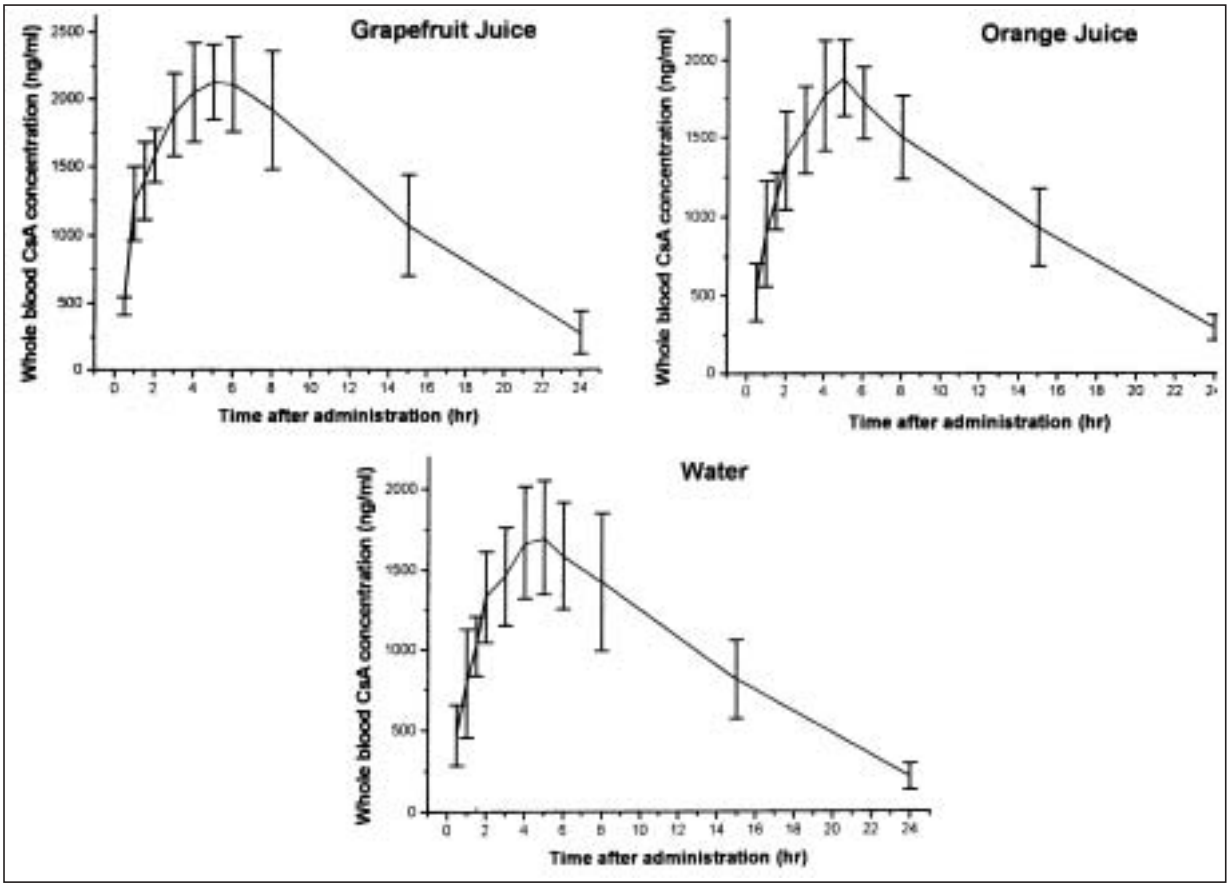

Figure 1. Mean (± SD) whole blood cyclosporin-A concentration-time profiles following single oral administration with grapefruit juice, orange juice or water (controls) in rats (n = 7 per group).

but, unfortunately, is expensive. Given the low cost of grapefruit juice, the coadministration of cyclosporin-A and grapefruit juice might allow for the use of significantly lower maintenance doses of this medication and would offer a cost-effective treatment to transplant recipients. However, one difficulty with the use of grapefruit juice is quality control in the content of active substances and its uniformity of effects upon cyclosporin-A disposition[12,16]. Therefore, before the mechanism of interaction of grapefruit juice with cyclosporin-A is determined, an intentional use of grapefruit juice to reduce the dosage and cost of cyclosporin-A could be dangerous. Sporadic use of grapefruit juice in patients taking cyclosporin-A should be discouraged since it may result in fluctuations in cyclosporin-A serum concentrations potentially responsible for rejection or nephrotoxicity. In fact, the present report shows an ample variability in the interaction between grapefruit juice and cyclosporin-A in rats. Similar data were also observed in clinical studies, in which it was associated with many factors, such as study population, timing of administration, dosage of grapefruit juice, and variability of grapefruit juice brands[17].

In the present study, relative bioavailability of cyclosporin-A in the grapefruit juice group was about one third more than in the control group. It is worth noting that the determination of relative bioavailabilities in this study assumes that cyclosporin-A systemic clearance was unchanged by each treatment. Support for this assumption comes from clinical studies in which grapefruit juice did not alter the kinetics of an intravenous cyclosporin-A dose but increased the AUC and bioavailability of cyclosporin-A given perorally, and by the fact that the elimination half-life of the drug was unchanged[12]. The increased bioavailability has been explained by a selective inhibition of intestinal cytochrome

P450 enzymes. In fact, grapefruit juice contains a variety of flavonoid molecules, such as naringin, quercitin and kaempferol, and some nonflavonoid molecules such as 6'-7'-dihydroxybergamottin, which are known to inhibit CYP3A4 activity[11,13,14]. However, the data about the inhibitory substances present in grapefruit juice are discordant and it is not yet completely worked out what compound in grapefruit juice markedly increases cyclosporin-A availability.

Naringin is the main bioflavonoid in grapefruit juice. Naringin is not a potent CYP inhibitor, but is partially metabolized by enteral bacteria to naringenin, which is a potent inhibitor of P450 enzymes, and was originally thought to be the component of grapefruit juice responsible for the interactions[18]. Although it was thought that another unidentified component in grapefruit may also have been responsible, since giving naringin alone does not seem to cause the same degree of inhibition as grapefruit juice. Moreover, a study on extracts of grapefruit juice interacting with rat and human P450 found that naringin accounted for only 10% of the inhibition of CYP activity seen in grapefruit juice[19].

In our study cyclosporin-A was administered with grapefruit juice and orange juice, both of which contain 6',7'-dihydroxybergamottin[20]. The AUC for cyclosporin-A was increased 31% with grapefruit juice, whereas orange juice did not significantly affect the AUC of cyclosporin-A. Our data suggest that 6',7'-dihydroxybergamottin is not responsible for the increased cyclosporin-A levels when given with grapefruit juice. It might be possible that grapefruit juice contains other inhibitory agents.

In vitro studies show that 6',7'-dihydroxybergamottin has no effect on P-glycoprotein[21] and that grapefruit juice significantly activates P-glycoprotein-mediated reduction in bioavailability, partially counteracting the CYP3A4 inhibitory effects of grapefruit juice[22]. P-glycoprotein is known to play a significant role in cyclosporin-A availability. P-glycoprotein is an efflux pump that, like CYP3A enzymes, is present at high concentration in intestinal enterocytes, the primary site of oral absorption, where it actively secretes absorbed drugs back into the gut lumen. It is possible to speculate that grapefruit juice may contain factors that inhibit P-glycoprotein activity. However, it should be emphasized that the experiments suggesting an effect of intestinal P-glycoprotein on cyclosporin-A metabolism, were performed in vitro[20,23].

Further studies are needed to identify inhibitors of P-glycoprotein in grapefruit juice. Nonetheless, our study suggest that, under appropriate experimental conditions, rats may be a suitable model for in vivo investigation of the relative contribution of reduced P-glycoprotein and CYP3A4 activity to the increased oral bioavilability of cyclosporin-A.

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