

## Trough concentrations of cyclosporine in blood following administration with grapefruit juice

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Components of grapefruit juice have been shown to inhibit CYP3A4 activity, the enzyme involved in cyclosporine metabolism. Eleven medically stable patients (seven males, four females) receiving cyclosporine following kidney transplantation were instructed to take their usual dose of cyclosporine with water for 1 week (Phase 1), with grapefruit juice (8 ounces) for 1 week (Phase 2) and again with water for 1 week (Phase 3). Trough blood samples were obtained at the end of each phase for measurement of cyclosporine concentration using a specific monoclonal whole blood radioimmunoassay. Cyclosporine trough concentrations averaged  $116.9 \pm 51.6$  ng ml<sup>-1</sup> in the first phase,  $145.3 \pm 44.7$  ng ml<sup>-1</sup> with grapefruit juice ( $P < 0.05$  compared with the first and third phases) and  $111.2 \pm 56.1$  ng ml<sup>-1</sup> in the third phase. Cyclosporine concentrations increased in 8 of 11 patients when given with grapefruit juice (mean increase 32%; range -4 to 97%) and declined in 10 of 11 when subjects resumed taking cyclosporine with water (mean decrease 27%). These results suggest that grapefruit juice increases trough concentrations of cyclosporine in blood, possibly by inhibiting pre-hepatic gut wall metabolism, and could be useful in optimizing therapy with this drug.

**Keywords** cyclosporine grapefruit juice CYP3A4 cytochrome P-450

### Introduction

Cyclosporine is a cyclic undecapeptide of fungal origin which forms the cornerstone of therapy for maintenance immunosuppression following transplantation. However, blood concentrations of cyclosporine must be maintained within a relatively narrow range (approximately 100-300 ng ml<sup>-1</sup>) in order to achieve effective immunosuppression with a minimum of nephrotoxicity. This can be a difficult task due, in large part, to the wide inter-patient variability in oral bioavailability ranging from less than 5% to as much as 90% [1].

Recent studies have suggested that the poor and variable bioavailability of cyclosporine may relate to pre-systemic metabolism of cyclosporine in the gut wall [2-3]. The major cyclosporine-metabolizing enzyme appears to be CYP3A4 [4], a cytochrome P-450 enzyme present in high concentrations in the gut wall [5]. Erythromycin, a potent inhibitor of CYP3A4, has been found to increase substantially the oral bioavailability of cyclosporine possibly by inhibition of gut wall metabolism [6].

It has been suggested that a compound which inhibits

the pre-systemic metabolism of cyclosporine without systemic effects could be of clinical value [7]. This would improve bioavailability and decrease the cost of therapy through dose reduction in some patients. Most drugs known to inhibit CYP3A4 have significant adverse effects that make them less than ideal for co-administration with cyclosporine. However, recent reports have indicated that grapefruit juice is a potent inhibitor of the CYP3A4-mediated metabolism of a number of calcium channel blockers [8, 9]. The purpose of this investigation was to determine if administration of cyclosporine with grapefruit juice would alter trough concentrations of the drug in blood.

### Methods

The subjects of the study were patients receiving cyclosporine for immunosuppression following kidney transplantation. Transplants had been performed between 4

months and 5 years prior to the study. All were being followed as out-patients and were medically stable. They had been receiving oral cyclosporine for at least 4 weeks and were at steady state on their cyclosporine regimen prior to beginning the study. Each subject gave written informed consent to participate. The study was reviewed and approved by the St John Hospital and Medical Center Human Investigation Committee (Detroit, MI).

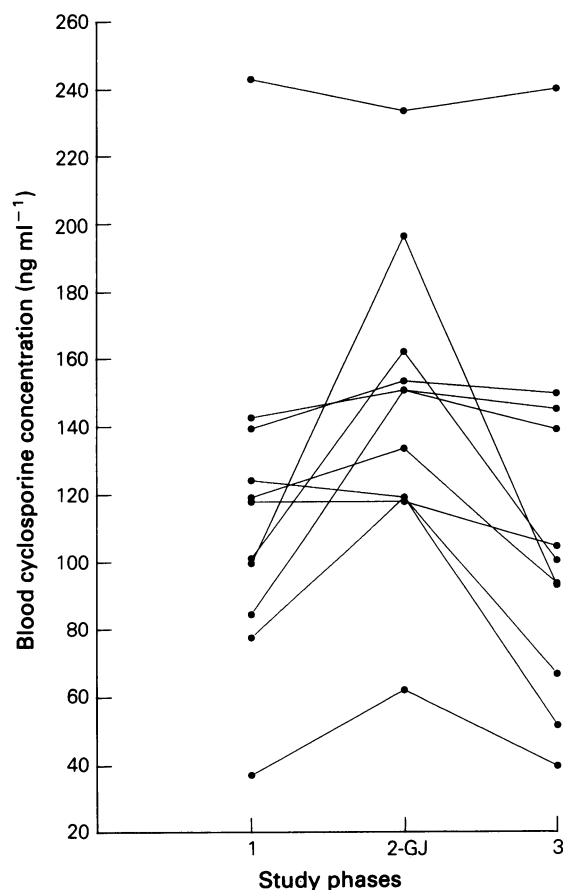
The study consisted of three consecutive phases (each lasting 1 week) with the first and third phases serving as control periods. Subjects were instructed to take their regular cyclosporine dose (patients were receiving the drug twice daily) with eight ounces of water for 1 week during the first phase of the study. During the second phase, each dose of cyclosporine was ingested with eight ounces of grapefruit juice (prepared from frozen concentrate) while in the third phase, cyclosporine was once again taken with water. At the end of each phase, a single blood sample (10 ml) was collected into EDTA-containing blood collection tubes (Vacutainer) prior to the next scheduled dose (trough concentration). Compliance was assessed using a record sheet on which subjects noted the time of ingestion of each dose of cyclosporine during the study.

Blood samples were frozen at  $-20^{\circ}\text{C}$  prior to analysis. Cyclosporine concentrations were determined in each sample using a specific monoclonal whole blood radioimmunoassay (CYCLO-TRAC SP, Incstar Corporation, Minnesota, MN). This method measures only cyclosporine and exhibits no significant cross-reactivity with metabolites. At concentrations between 100 and 400  $\text{ng ml}^{-1}$ , the within-day and between-day coefficient of variation for this assay averages less than 5% and less than 10%, respectively. Trough concentrations were compared using one-way repeated measures analysis of variance (ANOVA) with contrast testing for differences between individual phases.

## Results

Eleven subjects (seven males, four females) completed the study (two subjects started the study but failed to return for the final phase and were not included in the data analysis). The mean age of the subjects was 39.5 years (range 24–51 years) and they were receiving cyclosporine doses ranging from 150 to 350  $\text{mg day}^{-1}$  (mean 250  $\text{mg day}^{-1}$ ). Subjects were taking a variety of concomitant medications but only one was receiving a drug known to affect CYP3A4 activity (verapamil). The dosage of verapamil remained constant in this individual throughout the study.

Individual cyclosporine trough concentrations in blood for each of the three phases are presented in Figure 1. The concentration during the first (control) phase averaged  $116.9 \pm 51.6 \text{ ng ml}^{-1}$  (86.4–147.4  $\text{ng ml}^{-1}$ ) (mean  $\pm$  s.d. and 95% confidence interval). This was not significantly different from the mean concentrations of  $111.2 \pm 56.1 \text{ ng ml}^{-1}$  (78.1–144.3  $\text{ng ml}^{-1}$ ) observed in the final phase. Ingestion of cyclosporine with grapefruit juice resulted in an increase in trough concentrations to  $145.3 \pm 44.7 \text{ ng ml}^{-1}$  (118.9–171.7  $\text{ng}$



**Figure 1** Individual trough cyclosporine concentrations in blood for each of the three phases of the study (Phase 1–Water; Phase 2–Grapefruit juice; Phase 3–Water). Concentrations in Phase 2 were significantly higher than in either Phase 1 or 3.

$\text{ml}^{-1}$ ). This was significantly higher compared with both control phases. The average increase was 32% from Phase 1 to Phase 2 with a range of  $-4\%$  to  $97\%$  while the average change from Phase 2 to Phase 3 was  $-27\%$  (range of  $+3\%$  to  $-62\%$ ). Cyclosporine concentrations increased in 8 of 11 patients (one patient experienced no change) when subjects began taking the dose with grapefruit juice and decreased in 10 of 11 when subjects resumed taking the drug with water (Figure 1).

## Discussion

The results of this study indicate that trough concentrations of cyclosporine are elevated significantly when cyclosporine is ingested with grapefruit juice. The interaction appears to be similar in magnitude to the effect observed with some other inhibitors of CYP3A4. Brockmüller *et al.* [10] reported that diltiazem increased trough concentrations of cyclosporine by 45% in 19 of 22 patients receiving the combination.

Previous studies with the calcium antagonists felodipine [8] and nitrendipine [9] have demonstrated a dramatic decrease in hepatic first-pass metabolism with no change in drug half-life when these CYP3A4 substrates are administered with grapefruit juice. This is consistent with short-lived inhibition of hepatic enzyme

activity, and *in vitro* studies using human liver microsomes have established that several flavonoids found in grapefruit juice, in particular quercetin, kaempferol and naringenin [11, 12], are significant inhibitors of CYP3A4. However, since the hepatic extraction ratio of cyclosporine is low [1], it is unlikely that this could account for the observations in this study. Although this study was not designed to assess the mechanism of effect, a more likely explanation would appear to be inhibition of cyclosporine metabolism in the gut wall. Watkins and co-workers [5] have demonstrated that enzymes of the CYP3A group appear to be the most abundant forms of cytochrome P-450 present in the jejunal mucosa and that significant quantities of cyclosporine metabolites can be detected in portal venous blood following instillation of cyclosporine into the small intestine [2]. Hoppu *et al.* [3] have also reported a significant difference in metabolite profile between oral and intravenous administration of cyclosporine, an observation consistent with pre-hepatic metabolism in the gut wall. Another inhibitor of CYP3A4 activity, erythromycin, increased the oral bioavailability of cyclosporine from 36% to 60% [6], presumably by inhibition of gut wall metabolism.

The results of this investigation could have direct clinical application. There has been much interest in the co-administration of cyclosporine with inhibitors of CYP3A4 in order to increase concentrations in patients having difficulty attaining therapeutic levels and to decrease the cost of therapy with this expensive drug. Since grapefruit juice presumably lacks adverse systemic effects which could be encountered with CYP3A4 inhibitors like erythromycin, verapamil, diltiazem and ketoconazole, it may be an ideal substance for this purpose. However, the effect of inhibitors on concentrations of potentially active metabolites of cyclosporine needs to be explored. Further study is needed to determine if the timing of administration of grapefruit juice relative to cyclosporine affects the magnitude of the interaction. This would seem to be a reasonable possibility since cyclosporine is slowly absorbed over several hours. In addition, the wide range in cyclosporine bioavailability between patients would suggest that gut wall metabolism is more prominent in some patients and that these individuals might be more likely to exhibit significant inhibition of metabolism with grapefruit juice.

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