

# Effects of Orange, Tangerine, and Grapefruit Juices on Pharmacokinetic Parameters of Cyclosporine A in Rats

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## Abstract

This study was designed to investigate the effects of tangerine, orange, and grapefruit juice on the pharmacokinetic pattern of cyclosporine A in rats. Forty male Sprague-Dawley rats were divided into four groups to receive water, orange, tangerine, or grapefruit juices respectively. Water or juices (10 ml/kg) were given orally twice a day for 3 days. Three hours after the 5<sup>th</sup> dose, cyclosporine A was given as a single oral dose (5 mg/kg). Blood samples (2 ml) were collected at 30, 120, and 240 min after administering cyclosporine A, and area under time-concentration curve (AUC), Maximal serum concentration ( $C_{max}$ ), and time to reach maximal concentration ( $T_{max}$ ) were calculated using one-compartment method. Orange juice, but not tangerine or grapefruit juices, significantly increased the AUC and  $C_{max}$  of cyclosporine A compared with water. The three juices significantly decreased the  $T_{max}$  of cyclosporine A. The findings suggest that Thompson Novel's orange juice increased the bioavailability of oral administration of cyclosporine A formulation in rats.

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**Keywords** • Pharmacokinetics • cyclosporine A • oranges • grapefruit

## Introduction

Cyclosporine A is a major immunosuppressive agent widely used in patients subjected to organ transplantation. Cyclosporine A undergoes extensive first-pass metabolism, in intestinal mucosa and liver. It is highly lipophilic and metabolized by CYP3A4 enzymes.<sup>1-3</sup> The pharmacokinetics of cyclosporine A is influenced by various factors including CYP3A4 enzyme,<sup>4</sup> and shows wide inter- and intra-subject variability. It was suggested that poor bioavailability of cyclosporine A might be a contributing factor to poor transplant outcomes seen in African patients.<sup>5</sup>

Grapefruit juice was reported to markedly improve the oral bioavailability of several dihydropyridine calcium channel blockers including felodipine, nifedipine, nitrorendipine, and nisoldipine.<sup>6</sup> Co-administration of grapefruit juice was shown to increase the serum levels of cyclosporine A and other orally administered CYP3A4 substrates.<sup>7</sup> The components in grapefruit juice responsible for the interaction with CYP3A4 and/or P-glycoprotein have not been fully determined, but furanocoumarins and furanocoumarin dimmers are strong candidates, as they were strong inhibitors of CYP3A4 in vitro. Moreover, grapefruit juice contains high concentrations of flavonoids, particularly naringin, which was shown to inhibit CYP3A activity in vitro.<sup>8,9</sup> Grapefruit, orange, and tangerine are species of citrus family. Despite various studies on the

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effect of grapefruit juice on the pharmacokinetics of cyclosporine A, studies on the effects of orange and tangerine juices are negligible. Since, tangerine, orange, and grapefruit are from the same family, it is likely that orange and tangerine juices might affect the cyclosporine A metabolism in the same way as grapefruit juice. Therefore, the objective of the present study was to investigate the effects of tangerine and orange juices on the pharmacokinetics pattern of cyclosporine A relative to that of grapefruit in rats.

## Material and Methods

Forty male Sprague-Dawley rats (Pasture institute, Iran) weighing 200-270 g were housed in light-(12 hr light/12 hr dark cycle) and temperature-(21-25°C) controlled condition with rat chow and water ad libitum. All experiments were conducted in a randomized manner between 8 and 12 in the morning.

Orange (*Citrus Sinensis* var. *Thompson Novel*), tangerine (*Citrus Reticular* var. *Unshio Satsuma*), and grapefruits (*Citrus Paradisi* var. *Marsh Seedless*) were purchased from certified local groceries. The fruits were characterized by an agriculture specialist at Agriculture Institute in Amol. The juices of the fruit were prepared using an electric juicer. Cyclosporine A syrup was obtained from *Sandimmun Neoral*, *Novartis Co*, *Switzerland*.

The rats were divided into four groups. Group 1 (control, n=8) received water, and groups 2, 3, and 4 received orange (n=12), tangerine (n=12) or grapefruit (n=8) juice, respectively. Water or Juices (10 ml/kg) was given orally twice a day for 3 days and 3 hours after the 5<sup>th</sup> dose a single oral dose (5 mg/kg) of cyclosporine A was given.

Blood samples (2 ml) were collected from each rat at 30, 120, and 240 min after the administration of cyclosporine A. The sera of the blood samples were separated and kept frozen at -20°C for further analysis. The serum cyclosporine A levels were determined using radioimmunoassay (kit Radim, Italy).

Pharmacokinetic parameters were calculated using a one-compartment method. The areas under the serum cyclosporine A level curves (AUC) were measured using trapezoidal method.<sup>10</sup> Maximal serum concentration ( $C_{max}$ ) and time to reach maximal concentration ( $T_{max}$ ) were obtained directly from the concentration-time profile.

The AUC,  $C_{max}$ , and  $T_{max}$ , presented as

mean±SD, were compared using one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. A P value of ≤0.05 was considered statistically significant.

## Results

The pharmacokinetic parameters are summarized in table 1. The administration of orange juices did result in a significant increase in area under the cyclosporine A curve relative to that of the control group. However, the administration of tangerine, or grapefruit juice did not change area under the cyclosporine A curve compared with that of the control group. Moreover, the  $C_{max}$  of groups receiving tangerine or grapefruit juices were not significantly different from each other or from the control group. The administration of tangerine, orange, or grapefruit juice was associated with significant decreases in  $T_{max}$  of cyclosporine A relative to that of the control group.

## Discussion

The main finding of the present study was that the administration of orange juice significantly increased AUC and  $C_{max}$ , and significantly decreased the  $T_{max}$  of cyclosporine A. The effects of orange juice observed in our study are not consistent with some previous studies.<sup>11,12</sup> This may be because of the use of different species of orange, while they used Seville variety of orange and in this study Thompson Novel variety was used.

The metabolism of cyclosporine A is probably inhibited by the effect of some citrus juice or CYP3A enzymes in the gut wall of the small intestine and to a lesser extent by the inhibition of these enzymes in the liver.<sup>3,13</sup> The increase of AUC,  $C_{max}$  and decrease of  $T_{max}$  by orange juice in the present study might be due to the above-mentioned effect. The orange juice in the area, where the present study was performed, may have flavonoids or other substances capable of changing cyclosporine A pharmacokinetics.

The study showed that grapefruit juice reduced the  $T_{max}$  of cyclosporine A significantly, but failed to change the AUC or  $C_{max}$  significantly. Some previous studies reported the effects of grapefruit juice on blood cyclosporine A levels. Ducharme and colleagues showed that grapefruit juice increased  $C_{max}$  and AUC of an old formulation of cyclosporine A by 62%.<sup>14</sup> Hermann and coworkers reported that the administration of

**Table 1:** Values (Mean ± SD) of AUC<sub>0-4</sub> (ng/ml),  $C_{max}$  (ng/l) and  $T_{max}$  (hr) of groups receiving water (n=8), orange (n=12), tangerine (n=12), or grapefruit juice (n=8).

	Water	Orange	Tangerine	Grapefruit
AUC <sub>0-4</sub>	2770±433	4555±398*	2911±586	3287±487
$C_{max}$	953.3±179.7	1182.8±59.6*	1105.7±19518	1148±67.52
$T_{max}$	3.85±0.14	2.30±0.23*	0.63±0.11*	1.98±0.14*

AUC<sub>0-4</sub> = area under the plasma concentration versus time curve in the interval 0 to 4 hours,  $C_{max}$  = maximum concentration,  $T_{max}$  = time to reach to  $C_{max}$ . \* Denotes significant difference (P≤0.05) from control group in one-way ANOVA.

cyclosporine A with grapefruit juice was associated with a significant increase in AUC, without changing  $C_{max}$  or  $T_{max}$  significantly.<sup>15</sup> Moreover, it was shown that grapefruit juice did increase  $C_{max}$  of cyclosporine A.<sup>16</sup> As well, In renal transplant patients, ingestion of grapefruit juice was shown to increase the oral bioavailability of cyclosporine A.<sup>16,17</sup> In addition, Mangone and colleagues reported that grapefruit juice increased AUC and  $C_{max}$  of cyclosporine A in rats.<sup>12</sup>

The different findings of the present study from that of previous ones might be due to the type of study, geographical origin, cultivar harvest, or storage time of grapefruit. The present study showed that tangerine juice reduced the  $T_{max}$  of cyclosporine A significantly, but failed to make significant changes in the  $C_{max}$  or AUC. In other study, in renal transplant recipients, we observed that intake of tangerine juice did not have any significant influence on the AUC or  $C_{max}$  of cyclosporine A.<sup>18</sup>

In summary, concurrent administration of *Thompson novel* orange juice and to some extent, tangerine juice increase the bioavailability of oral administration cyclosporine A formulation. However, further studies are needed in this area with the juices especially in a clinical feature of the drug pharmacokinetic interaction.

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