

# Impact of citrus soft drinks relative to grapefruit juice on ciclosporin disposition

Ute I. Schwarz, Philip E. Johnston,<sup>1</sup> David G. Bailey,<sup>2</sup> Richard B. Kim, Gail Mayo & Aaron Milstone<sup>3</sup>

Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University Medical School and <sup>1</sup>Pharmaceutical Services, Vanderbilt University Medical Center, Nashville, TN, USA, <sup>2</sup>Lawson Health Research Institute, London Health Sciences Centre and Departments of Medicine and Physiology & Pharmacology, University of Western Ontario, London, Ontario, Canada, and <sup>3</sup>Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

## Correspondence

Ute I. Schwarz MD, Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University School of Medicine, 23rd Ave. at Pierce, RRB 542, Nashville, TN 37232, USA.  
Tel.: +1 615 343 3512  
Fax: +1 615 343 7605  
E-mail: ute.i.schwarz@vanderbilt.edu

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## Aims

A recent case report had suggested a citrus soft drink (Sun Drop<sup>®</sup>) may have caused clinically relevant elevations in ciclosporin levels through a grapefruit juice-like mechanism via inactivation of intestinal cytochrome P450 3A4 (CYP3A4). This study was conducted to investigate the effect of grapefruit juice and citrus sodas Sun Drop<sup>®</sup> and Fresca<sup>®</sup>, the latter soda containing 83-fold higher concentration of the proposed CYP3A4 inhibitor bergamottin than Sun Drop<sup>®</sup>, relative to water on oral ciclosporin pharmacokinetics.

## Methods

In a randomized four-way crossover study with a washout of at least 1 week, 12 healthy volunteers received a single oral dose of ciclosporin (Neoral<sup>®</sup>) with Sun Drop<sup>®</sup>, Fresca<sup>®</sup>, grapefruit juice and water (control). Each drink (591 ml) was consumed twice on the prior day and three times on the study day. Whole blood concentrations of ciclosporin were measured up to 24 h with a fluorescence polarization immunoassay.

## Results

Grapefruit juice increased area under the concentration–time curve by 186% ( $P < 0.0001$ ; 95% confidence interval of mean difference 3302–6240 ng ml h<sup>-1</sup>) and peak concentration by 150% ( $P < 0.0001$ ) of ciclosporin with a significant decrease in oral clearance of 43% ( $P < 0.0001$ ) when compared with water. Neither citrus soda altered significantly ciclosporin pharmacokinetic variables; changes in mean values ranged from  $\pm 3$  to 11% of the corresponding water value.

## Conclusion

Although our results do not support a clinically relevant grapefruit juice-like interaction between oral ciclosporin and citrus constituent containing sodas Sun Drop<sup>®</sup> or Fresca<sup>®</sup>, an effect in the setting of chronic ciclosporin therapy cannot be ruled out.

## Introduction

Ciclosporin, a calcineurin immunosuppressant, is widely used in the setting of organ transplantation. Because it has a narrow therapeutic concentration range, continuous monitoring of drug level and adjust-

ment of dose are required to prevent suboptimal concentrations that result in loss of desired immunosuppression or to avoid excessive levels associated with organ toxicities [1]. Ciclosporin oral bioavailability is highly variable and significantly dependent on multiple

factors such as drug formulation, metabolism by cytochrome P450 3A4 (CYP3A4) and active transport by the efflux pump P-glycoprotein in the intestine and liver [2–4].

There have been numerous reports of clinically relevant drug–ciclosporin interactions [5]. In addition, dietary constituents including grapefruit juice can augment blood ciclosporin concentrations via inhibition of intestinal CYP3A4, probably through irreversible enzyme inactivation mediated by furanocoumarins such as bergamottin and/or 6',7'-dihydroxybergamottin [6, 7].

Consumption of the carbonated soft drink Sun Drop® was recently reported to be temporally associated with clinically relevant doubling of serum ciclosporin trough levels in a lung transplant patient on Neoral® maintenance therapy on two occasions [8]. Moreover, intentional avoidance of this soft drink prevented the recurrence of this problem. Indeed, the makers confirmed that this citrus soda contained furanocoumarins. Our analysis of Sun Drop® and Fresca®, two of 15 citrus soda drinks screened for furanocoumarins, confirmed that both drinks potentially possessed concentrations of bergamottin capable of inhibiting CYP3A4 metabolism.

It was hypothesized that Sun Drop® and Fresca® would enhance systemic ciclosporin availability by a grapefruit juice-like CYP3A4-mediated interaction as a potential mechanism. Accordingly, we studied the effect of two different bergamottin-containing citrus sodas, Sun Drop® and Fresca®, relative to grapefruit juice and water in healthy volunteers on oral ciclosporin pharmacokinetics.

## Materials and methods

### Subjects

Twelve healthy nonsmoking Caucasian subjects (six men, six women; mean age  $28.1 \pm 7.3$  years, age range 19–40 years; mean weight  $70 \pm 9$  kg, weight range 56–80 kg, body mass index  $<30$ ) were enrolled. Health status was determined by medical history, physical examination and routine haematological and serum chemical testing. In females, pregnancy was excluded by  $\beta$ -human chorionic gonadotropin test in urine prior to each study day. No subject had had a significant illness within the preceding 4 weeks, had used prescription or over-the-counter medications within 7 days prior to the study, or had a history or evidence of cardiac, renal, hepatic or gastrointestinal disease or drug or alcohol abuse. The protocol was approved by the Vanderbilt University Institutional Review Board–Health Sciences and written informed consent was obtained.

### Study design

The study utilized an open, randomized, four-way crossover design. All beverages were purchased as a single lot with the exception of Fresca®. Grapefruit juice (Minute Maid®, The Coca-Cola company Houston, TX, USA) was given at normal strength after reconstitution from frozen concentrate. Bergamottin concentrations measured in Sun Drop® (Dr Pepper/Seven Up, Inc. Plano, TX, USA) and Fresca® (The Coca-Cola company, bottled by Coca-Cola Enterprises, Joplin, MO, USA) were  $0.078$  and  $6.5 \text{ mg l}^{-1}$ , respectively. Bergamottin concentration in grapefruit juice is typically  $5.6 \text{ mg l}^{-1}$ , range  $4.1$ – $10 \text{ mg l}^{-1}$  [9]. Dihydroxybergamottin was not detected in either soft drink.

One day prior to each study day, subjects consumed, based on a randomized schedule, 591 ml of the appropriate drink at 08.00 h and 20.00 h. The following study day, after an overnight fast, the same drink (591 ml) was ingested 2 h before, and 4 and 12 h after ciclosporin, which was administered as the microemulsion formulation Neoral® ( $2.5 \text{ mg kg}^{-1}$ ; Novartis, Basel, Switzerland) with 227 ml of water. Peripheral venous blood (4 ml) was sampled into EDTA (ethylenediaminetetraacetic acid) tubes before, and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after drug dosing and stored at  $4^\circ\text{C}$  pending analysis. Standard meals were served at 4 and 10 h. All subjects were asked not to consume citrus or fruit products for 1 week before and throughout testing. They abstained from alcohol and caffeine-containing beverages for 3 days before and during testing. The washout period was at least 1 week between study days.

### Furanocoumarin, blood ciclosporin and data analysis

Bergamottin and dihydroxybergamottin in Sun Drop® and Fresca® were measured by high-performance liquid chromatography according to a previously published method [10]. The furanocoumarins were assayed in triplicate and the coefficient of variation of the standard curve was 1.7% at  $5 \text{ mg l}^{-1}$  and 5.8% at  $9 \text{ mg l}^{-1}$  for bergamottin and dihydroxybergamottin, respectively.

Ciclosporin whole blood concentrations were measured with a fluorescence polarization immunoassay (Abbott Diagnostics Inc., Abbott Park, IL, USA) as described by the manufacturer.

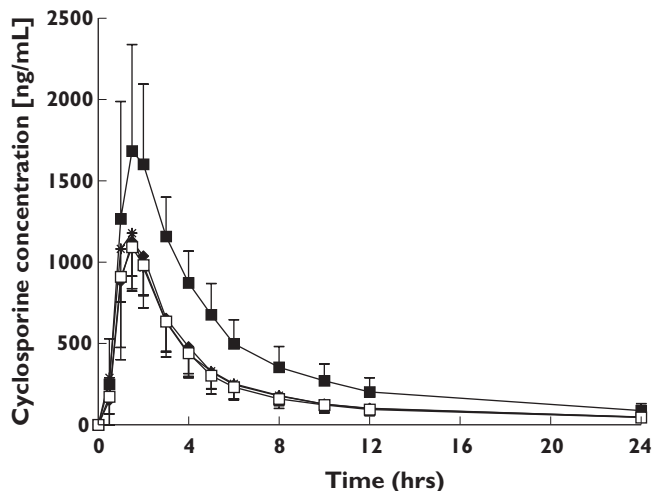
Pharmacokinetic parameters were analysed using a noncompartmental model. The terminal log-linear phase of the ciclosporin concentration–time profile was identified visually for each subject. The terminal elimination rate constant ( $k_e$ ) was determined by log-linear regression of the final data points (at least 4). The apparent elimination half-life of the log-linear phase ( $t_{1/2}$ ) was calculated as follows:  $0.693/k_e$ . The area under the drug

concentration–time curve from 0 to 24 h ( $AUC_{(0-24)}$ ) was calculated using the linear logarithmic/trapezoidal method. The AUC from 24 h extrapolated to infinity ( $AUC_{(24\text{ h}-\infty)}$ ) was determined by dividing the final ciclosporin concentration by  $k_e$ , and the AUC from 0 h to infinity ( $AUC_{(0\text{ h}-\infty)}$ ) by the sum of ( $AUC_{(0-24)}$ ) and ( $AUC_{(24\text{ h}-\infty)}$ ). The peak concentration in blood ( $C_{\max}$ ) and the time to reach  $C_{\max}$  ( $t_{\max}$ ) were obtained directly from the experimental data. The oral clearance ( $CL_o$ ) was determined by the following equation:  $\text{dose}/AUC_{(0-\infty)}$ .

Statistical comparisons between the three treatments and water were determined after confirmation of data's normal distribution using the parametric paired *t*-test and corrected for multiple comparisons (GraphPad Prism®, Version 4, 2003). A value of  $P < 0.016$  (0.05/3) was considered statistically significant; *P*-value and 95% confidence interval for the difference between treatments and water (control) are noted. Data are expressed as mean value  $\pm$  standard deviation (SD).

## Results

The results demonstrate that grapefruit juice markedly increased blood ciclosporin concentrations compared with water, whereas Sun Drop® and Fresca® did not alter ciclosporin blood concentrations (Figure 1). A summary of pharmacokinetic values for ciclosporin is shown in Table 1.



**Figure 1** Mean ( $\pm$  SD) blood ciclosporin concentration–time profiles ( $N = 12$ ) after orally administered ciclosporin ( $2.5\text{ mg kg}^{-1}$  body weight) with consumption of either 591 ml of water ( $\square$ ), grapefruit juice (GFJ) ( $\blacksquare$ ), Sun Drop® ( $*$ ), or Fresca® ( $\blacklozenge$ ). Treatments consisted of consumption of test fluids twice daily (08.00 h and 20.00 h) prior to each study day and then 2 h before and 4 and 12 h after the ciclosporin dose on each study day

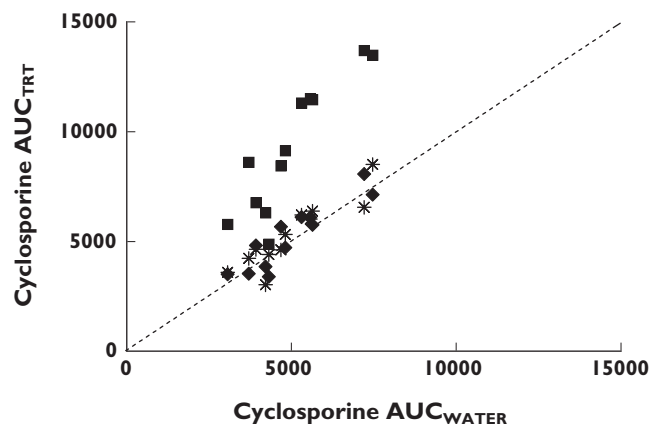
Grapefruit juice consumption produced a 43% reduction (range 60–0%;  $P < 0.0001$ ) in the oral clearance of ciclosporin with a corresponding increase in AUC over 24 h of 186% (range 110–230%;  $P < 0.0001$ ) and in  $C_{\max}$  of 150% (range 100–200%;  $P < 0.0001$ ) compared with water. A variable but consistent enhancement of ciclosporin's AUC was observed in every individual (Figure 2). Elimination half-life ( $P = 0.038$ ) and  $t_{\max}$  values ( $P = 0.096$ ) after grapefruit juice intake were not different in comparison with water, even though there was a trend towards a shorter elimination half-life with juice.

By contrast, consumption of the bergamottin-containing citrus sodas Sun Drop® and Fresca® was not associated with alterations in mean blood concentrations (Figure 1), pharmacokinetic parameters (Table 1) or individual AUCs of ciclosporin (Figure 2). The changes in the average pharmacokinetic variables of  $AUC_{(0-24)}$ ,  $C_{\max}$ ,  $t_{\max}$ ,  $t_{1/2}$  and  $CL_o$  after Sun Drop® and Fresca® ranged from  $\pm 3\%$  to 11% of the corresponding value during water control and did not reach statistical significance (Table 1). As was seen with water, after oral administration of ciclosporin as a microemulsion formulation, absorption was rapid, with peak levels being attained within 1–2 h (Figure 1).

No sex-related differences were noted in ciclosporin's blood concentration–time profile at baseline (water consumption).

## Discussion

In contrast to our previous findings in a lung transplant recipient, where repeated consumption of Sun Drop®



**Figure 2** Individual AUCs of S with water plotted against the corresponding value with grapefruit juice (GFJ) ( $\blacksquare$ ), Sun Drop® ( $*$ ), or Fresca® ( $\blacklozenge$ ). The diagonal dashed line represents the line of identity

**Table 1**

Pharmacokinetic variables of oral ciclosporin (2.5 mg kg<sup>-1</sup>) in 12 subjects after consumption of water, grapefruit juice (GFJ), Sun Drop<sup>®</sup> or Fresca<sup>®</sup>

Variable	Water	GFJ	Sun Drop	Fresca
AUC <sub>(0–24)</sub> (ng h ml <sup>-1</sup> )	4991 ± 1334	9281 ± 2999	5293 ± 1515	5220 ± 1514
Fold-change (range)	–	1.86 (1.1–2.3)	1.06 (0.7–1.2)	1.05 (0.8–1.2)
95% CI (P-value)	–	3082, 5498 (<0.0001)	–113, 718 (0.138)	–161, 619 (0.223)
AUC <sub>(0–∞)</sub> (ng h ml <sup>-1</sup> )	5635 ± 1376	10407 ± 3451	6007 ± 1702	5944 ± 1730
Fold-change (range)	–	1.85 (1.1–2.3)	1.07 (0.7–1.2)	1.05 (0.8–1.2)
95% CI (P-value)	–	3302, 6240 (<0.0001)	–134, 878 (0.134)	–160, 777 (0.175)
C <sub>max</sub> (ng ml <sup>-1</sup> )	1185 ± 298	1780 ± 542	1242 ± 288	1217 ± 255
Fold-change (range)	–	1.50 (1.0–2.0)	1.05 (0.7–1.5)	1.03 (0.6–1.4)
95% CI (P-value)	–	375, 818 (<0.0001)	–77, 192 (0.370)	–123, 188 (0.655)
t <sub>max</sub> (h)	1.54 ± 0.33	1.75 ± 0.45	1.38 ± 0.38	1.50 ± 0.43
Fold-change (range)	–	1.14 (0.8–1.5)	0.89 (0.5–1.3)	0.97 (0.5–2.0)
95% CI (P-value)	–	–0.04, 0.46 (0.096)	–0.45, 0.12 (0.220)	–0.46, 0.37 (0.830)
t <sub>1/2</sub> (h)	9.9 ± 1.9	8.2 ± 1.8	9.3 ± 2.2	9.4 ± 2.2
Fold-change (range)	–	0.82 (0.3–1.2)	0.94 (0.4–1.2)	0.95 (0.4–1.3)
95% CI (P-value)	–	–3.36, –0.12 (0.038)	–1.93, 0.74 (0.354)	–1.82, 0.78 (0.391)
CL <sub>o</sub> (ml min <sup>-1</sup> )	560.4 ± 133	319.0 ± 105	534.3 ± 136	542.9 ± 152
Fold-change (range)	–	0.57 (0.4–1.0)	0.95 (0.8–1.4)	0.97 (0.8–1.3)
95% CI (P-value)	–	–308, –175 (<0.0001)	–84, 31 (0.340)	–66, 30 (0.439)

Paired *t*-test, comparison between treatments and water, values given as mean ± SD; fold-change, ratio of mean values (range of individual ratios); 95% confidence interval (CI) for the mean difference (treatment minus water).

was associated with increased ciclosporin trough levels [8], we observed no effect of either Sun Drop<sup>®</sup> or Fresca<sup>®</sup> on ciclosporin blood levels in 12 healthy men and women compared with water, whereas grapefruit juice, as expected, augmented ciclosporin's AUC by more than 180%.

How can the discrepancy in findings between the case report and our formal research study in healthy subjects be explained? First, expressed levels of intestinal CYP3A4 and P-glycoprotein content may differ between the volunteer subjects and those of the reported lung transplant patient. Since the interaction between drugs and grapefruit juice occurs predominantly in the gut mucosa by inhibition of CYP3A4 and perhaps P-glycoprotein [6, 11], and the small intestine is a major site for ciclosporin's presystemic elimination [2, 3], differences in intestinal enzyme and efflux transporter expression could have accounted for this. Indeed, CYP3A4 protein has been reported to vary 30-fold in 20 donor small intestines [12]. Likewise, 10-fold variability in intestinal P-glycoprotein levels has been noted [3]. Moreover, in 19 kidney transplant patients 56% of the variability in steady-state oral ciclosporin clearance has been explained by the variability in liver CYP3A4

activity, and another 17% by the variability in intestinal P-glycoprotein levels [13], whereas 10-fold difference in intestinal CYP3A4 levels did not affect interpatient differences.

Second, since the patient was already on ciclosporin maintenance therapy, a chronic effect of ciclosporin on metabolic enzyme and the efflux transporter needs to be considered. Recently, with a non-invasive *in vivo* approach, both induction of intestinal CYP3A4 activity and reduction in hepatic and intestinal P-glycoprotein activity have been noted in transplant patients and healthy individuals on ciclosporin maintenance therapy in comparison with untreated healthy controls [14]. Accordingly, chronic ciclosporin therapy may result in differential response to ingestion of dietary constituents, such as citrus flavour-containing soft drinks.

Third, genetic factors may be responsible for the patient's susceptibility towards an interaction of ciclosporin with Sun Drop<sup>®</sup>. Genetic variants have been reported to alter the expression or function of the ciclosporin-metabolizing enzymes CYP3A4 and CYP3A5, and of P-glycoprotein [15], but conflicting results have been reported in clinical settings concerning the role of single nucleotide polymorphisms (SNPs)

in such genes as factors relating to variability in ciclosporin pharmacokinetics. The SNPs assessed include the *CYP3A4* promoter variant *CYP3A4\*1B* (A-392G) [16–20], the noncoding *CYP3A5* variant *CYP3A5\*3* [17, 21], and three common multidrug resistance (*MDR1*) gene variants in exon 12 (Gly412Gly), exon 21 (Ala893Ser/Thr) and exon 26 (Ile1145Ile) [17, 20–23]. Overall, single *CYP3A* or *MDR1* gene variants do not appear to be major determinants of oral ciclosporin disposition, but consideration of a more complex haplotype structure of these genes, including regulatory regions or linkage among variants of different genes (such as *CYP3A4* and 5), may provide further insight into the factors governing variability in ciclosporin disposition.

Finally, potentially active Sun Drop<sup>®</sup> components such as bergamottin could vary between the different lot numbers used by the patient and in the study, depending on the source of citrus fruit/juice used for extraction. This is a distinct possibility, since furanocoumarin content in grapefruit and other commercial citrus fruit juices or fresh grapefruit varies substantially among brands and different batches [9, 24, 25].

The furanocoumarins bergamottin and 6',7'-dihydroxybergamottin, identified in high levels in grapefruit juice, are likely candidate inhibitors of *CYP3A4* and possibly P-glycoprotein. Whereas bergamottin was found in Fresca<sup>®</sup> and Sun Drop<sup>®</sup>, 6',7'-dihydroxybergamottin has not been detected [26]. Bergamottin acts as substrate-dependent competitive (reversible) as well as substrate-independent mechanism-based (irreversible) inhibitor of *CYP3A4* [6, 7, 27]. With ciclosporin being a substrate of *CYP3A4* and P-glycoprotein, it is subject to grapefruit juice-type interactions *in vivo* which manifest as augmented blood levels, as shown in many clinical studies [28–32]. However, the contribution of other citrus fruit extracts or isolated juice constituents to ciclosporin disposition is still uncertain. In this study, neither bergamottin-containing citrus soda altered the pharmacokinetics of ciclosporin. Thus, it seems less likely that Sun Drop<sup>®</sup> alone could have doubled trough ciclosporin concentrations in the originally reported lung transplant recipient [8] by the hypothesized mechanism, since Fresca<sup>®</sup> containing 83-fold higher concentration of bergamottin (6.5 mg l<sup>-1</sup>, equivalent to grapefruit juice) was also without effect. The lack of effect by bergamottin in this study was similar to that recently reported with felodipine, another *CYP3A4* substrate [33]. The reported effect of a large amount of pure bergamottin (6 or 12 mg) on felodipine drug levels was not substantially greater than that of 2 mg bergamottin and less than that of grapefruit juice

(1.7 mg bergamottin). The authors suggested that additional active components are likely to contribute considerably to the drug-whole juice interaction. Alternatively, the observed lack of an interaction by the two studied soft drinks and ciclosporin might be explained by the absence of 6',7'-dihydroxybergamottin, another abundant furanocoumarin in grapefruit juice, that has been reported to be as potent as bergamottin as an inhibitor of intestinal *CYP3A4 in vitro*. However, it is present mainly in the supernatant fraction of grapefruit juice, which was less active than the particulate fraction, which suggests that 6',7'-dihydroxybergamottin is not the primary cause of this mechanism of drug interaction [34]. According to *in vitro* observations, other furanocoumarins such as dimer derivatives of 6',7'-dihydroxybergamottin are more potent inhibitors of *CYP3A4*, and may account for the clinical interaction of ciclosporin with whole grapefruit juice [25, 35–37].

Interestingly, for the lipophilic bergamottin a delayed onset of mechanism-based *CYP3A4* inactivation was reported in Caco-2 cells, suggesting that with foods containing bergamottin only, minimal interaction should occur with rapidly absorbed drugs [7]. In our study, in order to maximize the likelihood of detecting an effect, grapefruit juice as well as water, Sun Drop<sup>®</sup> and Fresca<sup>®</sup> were repeatedly consumed at higher than the usually ingested volume before and after administration of ciclosporin to allow an almost complete deactivation of intestinal *CYP3A4*. Since the inhibitory effect of grapefruit juice on intestinal *CYP3A4* is prolonged, with an estimated half-life for *CYP3A4* enzyme recovery of about 23 h after only 300 ml of grapefruit juice [38], it was not necessary to administer juice and ciclosporin simultaneously. Indeed, with this grapefruit juice intake regimen we observed increased blood ciclosporin exposure, supporting inactivation of intestinal *CYP3A4* as a relevant mechanism.

Since ciclosporin is also subject to P-glycoprotein transport, the effect of grapefruit juice on this efflux protein needs to be considered [26]. In agreement with our findings [11], the majority of *in vitro* studies have shown that grapefruit juice inhibits P-glycoprotein function, although with variable potency. Bergamottin alone did not alter P-glycoprotein activity at concentrations up to 50 µmol l<sup>-1</sup> (16.9 mg l<sup>-1</sup>) [11], whereas other investigators characterized bergamottin as a potent P-glycoprotein inhibitor [37, 39]. Although the interindividual variability of ciclosporin had been reported to depend to a certain extent on P-glycoprotein [13], changes in P-glycoprotein levels or *MDR1* mRNA in the intestinal biopsies following chronic consumption of grapefruit

juice (5–6 days, 227–300 ml thrice daily) were not observed in humans [6, 40].

It is also possible that the apparent association between Sun Drop<sup>®</sup> consumption and elevated trough ciclosporin concentrations [8] occurred only by chance. Data from any case report should not be viewed as definitive, but, as outlined in the current report, serve as the basis for a systematic prospective clinical study.

In conclusion, given their widespread consumption in North America, this study addressed a clinically important question relating to citrus soft drinks and their potential for interaction with ciclosporin. Although our results do not support a grapefruit juice-like CYP3A4-mediated interaction between Sun Drop<sup>®</sup> and oral ciclosporin, it cannot be stated with certainty that other citrus constituent containing soft drinks will not cause such an effect, nor can we rule out the possibility that certain individuals or patients on chronic ciclosporin therapy respond differently to citrus constituent-containing soft drinks. Furthermore, while the soft drinks assessed in this study are reflective of North America, presumably a variety of citrus soft drinks are also available in Europe and other continents, and the potential of subsets of such drinks to produce a grapefruit juice-like effect can not be ruled out. Therefore, additional studies are needed to determine more fully the interaction potential of citrus constituent-containing soft drinks on ciclosporin disposition.

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