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Orally co-administrated oleo-gum resin of *Commiphora myrrha* decreases the bioavailability of cyclosporine A in rats

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Cyclosporine A is a narrow therapeutic indexed immunosuppressant used after organ transplantation. Several herbs have been reported to alter its pharmacokinetics.Myrrh, dried oleogum resin obtained from *Commiphora myrrha* (Burseraceae) has been used for many common ailments. The present study was carried out to investigate the effect of myrrh on the pharmacokinetics of cyclosporine A. The rats of the control group received 60 mg/kg, p.o. cyclosporine A, and blood samples were collected at predetermined time intervals. Rats of the test group were treated with an aqueous suspension of myrrh (380 mg/kg p.o.) for eight days and on 8th day a single dose of cyclosporine A was administered to the treated group after 1 h of myrrh administration. Blood samples were drawn at predetermined time points and the drug was analyzed in whole blood by using H-Class UPLC-TQD. Pharmacokinetic profiles of control and test group were compared.Statistically significant differences were observed between the pharmacokinetic parameters of control and treated groups. In the myrrh treated group, the $AUC_{0\text{-}t}$ and C_{max} of cyclosporine A was decreased by about 45% and 48%, respectively. The time to reach maximum concentration (T_{max}) remained almost unchanged in both groups. Results indicated that the bioavailability of cyclosporine A was reduced by about 45% when co-administered with myrrh. This observation suggests that concurrent consumption of myrrh and cyclosporine A should be avoided. To confirm the clinical relevance of these findings, P-gp and CYP3A based molecular investigations can be performed along with a well-planned clinical study.

1. Introduction

In last decades the safety, efficacy and drug interaction potential of herbs have been widely evaluated. The outcomes of these investigations revealed many unforeseen issues/fact of herb's safety and their ability to interact with prescription drugs. There are numerous studies highlighting the potential of herbs to modulate the activity of P-glycoprotein and cytochrome P450. Apart from enzyme based interactions, there are cases where the herbs/constituents interact with drugs through other physiological mechanisms.

Cyclosporine A (CsA), a narrow therapeutic indexed immunosuppressant indicated for the prophylaxis of organ rejection in kidney, liver and heart allogeneic transplants; and for the treatment of patients with severe active rheumatoid arthritis and psoriasis (Neoral®, Novartis) (Anonymous). Supratherapeutic blood concentration of cyclosporine may cause nephrotoxicity and hepatotoxicity, while subtherapeutic blood levels cause graft rejection. Log-term treatment with CsA may also increase cardiovascular and cardio-muscularproblems (Tang et al. 2011; Quin et al. 2014). Numerous herb drug interactions based on enzyme modulations have been reported over the years, many of them are of clinical relevance (Chen et al. 2012; Na et al. 2011). Cyclosporine is a substrate of P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4). The modulation of any of these two or both may alter the pharmacokinetics of cyclosporine (Saeki et al. 1993; Kronbach et al. 1988). In a review, Colombo et al., (2014) reported that most differences in cyclosporine A bioavailability are due to food ingestion, changes in gastric motility, diarrhea, diabetes, and CYP3A5 genetic polymorphism; while the modulation of P-gp and CYP3A4 activity also affect cyclosporine metabolism and absorption.

"There have been reports of a serious drug interaction between cyclosporine and the herbal dietary supplement, St. John's Wort. This interaction has been reported to produce a marked reduction in the blood concentrations of cyclosporine, resulting in subtherapeutic levels, rejection of transplanted organs, and graft loss (Neoral®, Novartis)" (Anonymous; Barone et al. 2001; Mai et al. 2000; Barone et al. 2000). Fukunaga and Orito reported that repeated administrations of St John's wort decreases the Cmax and AUC of cyclosporine A in dogs (Fukunaga and Orito 2012).

"Grapefruit and grapefruit juice affect metabolism of CsA and increase its blood concentrations, thus should be avoided (Neoral®)" (Anonymous; Yee et al. 1995). Grapefruitjuice significantly increases the cyclosporine and total metabolite concentrations; and the AUC of cyclosporine and metabolite (Ioannides-Demos et al., 1997). Co-administration of cyclosporine A with grapefruitjuice affects the formation and/or elimination of M1 and M9 metabolites (Hermann et al. 2002).

Therefore, there are chances of other herbs affecting the pharmacokinetics especially AUC and C_{max} of cyclosporine will be of clinical importance. Numerous herbs have been identified which interact with P-gp and CYP3A4 (Zhou et al. 2004; Fujita 2004). The bioavailability of cyclosporine has been affected by many traditional drugs and their extracts.

Hsu et al. (2013) reported that co-administration of mulberry significantly decreases the bioavailability of cyclosporine A through activation of P-glycoprotein and cytochrome P450 3A. Wuzhi tablets (ethanol extract of *Schisandra sphenanthera*) significantly increased the $AUC_{0\text{-}36\ h}$ and C_{max} of orally administered cyclosporine A (1.89 mg/kg) by 293.1% and 84.1%, respectively. This dramatic increase was observed at low doses of cyclosporine A, but at high dose of cyclosporine A (37.8 mg/kg) the increase in $AUC_{0\text{-}36\ h}$ and C_{max} was 40.1% and 13.1%, respectively (Xue et al. 2013). Aqueous extract of liquorice and its major ingredient glycyrrhizin decreases the C_{max} and AUC of cyclosporine. The studies reveal that glycyrrhetic acid (the major metabolite of glycyrrhizin) significantly activated the functions of P-gp and CYP3A4 (Hou et al. 2012).

Grenier et al. (2006) investigated that co-administration of pomelo juice increased the bioavailability ($AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max}) of cyclosporine, possibly by inhibiting CYP3A and\or P-gp activity. Ginger juice significantly decreased the oral bioavailability of cyclosporine in rats, and this interaction was expected to occur at the absorption phase, since ginger juice did not alter the pharmacokinetics of intravenous cyclosporine (Chiang et al. 2006). Plant flavonoids such as quercetin and rutin significantly decreased the C_{max} and AUC of cyclosporine A. The activation of P-gp and CYP3A by quercetin and rutin may be the responsible mechanism of action (Yu et al. 2011). Ginkgo and onion comprises quercetin and their co-administration with cyclosporine A markedly decreased the oral bioavailability of cyclosporine A (Yang et al. 2006).

Myrrh is a dried oleo-gum resin of *Commiphora myrrha*, family Burseraceae. Myrrh contains volatile oil, resins and gums which on hydrolysis yields arabinose, galactose, xylose, and 4-*O*-methylglucuronic acid (Kakrani 1981; Hanus et al. 2005; Ahmed et al.).

Myrrh could be consumed by patients treated with cyclosporine A. Based on the aforementioned modulation of P-gp and CYP3A4 by herbs, the present study was carried out to investigate the effects of co-administration of myrrh on oral pharmacokinetics of cyclosporine A.

2. Investigations, results and discussion

The effect of co-administered myrrh on the pharmacokinetics of cyclosporine A was investigated in rats. Pharmacokinetic profiles of cyclosporine A (Neoral®) control and myrrh treated test groups are illustrated in the Fig. 1 These pharmacokinetic profiles are expressed by plotting cyclosporine A blood concentration *versus* time. Comparative pharmacokinetic parameters of cyclosporine A control and test groups are presented in the Table 1. The concomitant administration of myrrh aqueous suspension significantly reduced the mean maximum blood concentration and area under curve of cyclosporine A by 45% and about 48%, respectively. Elimination rate constant was increased by about 57%. Increased elimination rate constant and decreased C_{max} and $AUC_{0\text{-}t}$ of cyclosporine in myrrh treated group suggest that the metabolism and intestinal efflux of cyclosporine A may bemodulated by myrrh. Myrrh may have potentiated the CYP3A enzymes in the intestine/liver and also may have modulated the P-glycoprotein efflux in the intestine. Time to reach the maximum blood concentration of cyclosporine A control group was

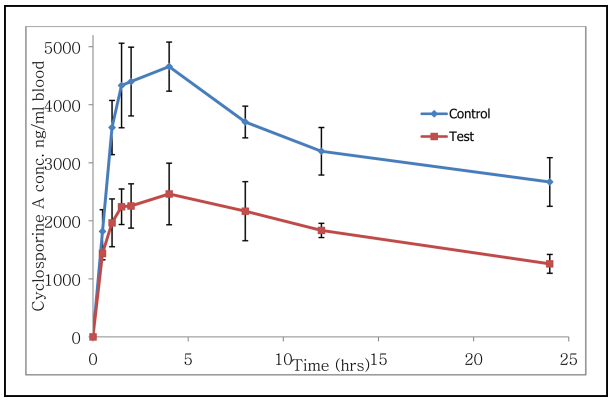

Fig. 1: Oral pharmacokinetic profiles of cyclosporine A control and myrrh treated test groups in rats.

calculated as 3.1 ± 1.24 h. In myrrh treated group, T_{max} was slightly increased to 4.4 ± 2.19 h which was not significant.

Numerous interactions studies of cyclosporine A with herbs and their products are already mentioned in the introduction part. Co-administration of some herbal products has modulated the pharmacokinetics of cyclosporine A. The leaflet of Neoral® also reported that co-administration of food with Neoral® decreases the cyclosporine AUC and C_{max}. To the best of our knowledge, this is the first investigation designed to evaluate the effect of myrrh aqueous suspension on the pharmacokinetics of orally administered cyclosporine A in rats. It revealed that co-administration of myrrh with cyclosporine A in rats reduces the oral bioavailability of cyclosporine to a significant level and should therefore be avoided.

P-Glycoprotein and CYP3A play a significant role in the bioavailability of cyclosporine A, since both are present at the first absorption site (enterocytes) of drugs. Further, there are reports that both work complementary to each other and have common substrates. The information about CYP3A and P-gp modulating substances present in myrrh is not yet complete. It has been reported that *Commiphora mukul*, another *Commiphora* species comprises quercetin and its derivatives (Kakrani 1981; Hanus et al. 2005). Quercetin has been reported to interact with CYP3A and P-gp. The chemical composition of *Commiphora myrrha* also comprises quercetin (Ahmed et al.).Quercetin has been reported todecrease the bioavailability of CYP3A and P-gp substrates.The present study did not confirm the exact mechanism behind myrrh and cyclosporine A interaction, but it raises the precautionary warning to avoid the concomitant oral administration of the these two.

Present observations suggested that co-administration of myrrh with cyclosporine A will reduce it bioavailability by significant margin. AUC0-t and Cmax of cyclosporine A were reduced by about 45% and 48%. In this interaction a possibility of CYP3A and P-gp involvement is expected. Based on the warnings written at the label of Neoral® and present findings, a precaution should be taken in mind to avoid co-administration of myrrh and other herbs with cyclosporine until a lack of substantial interaction is proven in human.

3. Experimental

3.1. Chemicals and reagents

Neoral® oral solution containing 100 mg/mL cyclosporine A (Novartis Pharma, Switzerland, MFD: 12-2010; EXP: 11-2013; LOT: H5108A) was obtained from the pharmacy of King Khalid University Hospital, Riyadh, Saudi Arabia. Cyclosporine A and cyclosporine D (internal standard) were purchased from USP™ reference standard (Rockville, MD) and Santa Cruz Biotechnology Inc., respectively. Zinc sulphate monohydrate and ammonium acetate were purchased from Merck (Germany) and Fluka Chemika

Table 1: Pharmacokinetic parameters of cyclosporine A control and myrrh treated groups

Parameters	Cyclosporine A control (mean ± SD)	Cyclosporine A test (mean ± SD)
C_{max} (ng/mL)	4925.58 ± 271.67	2561.53 ± 411.39*
T_{max} (h)	3.1 ± 1.24	4.4 ± 2.19
$AUC_{0\text{-}24}$ (ng.h/mL)	80331.68 ± 5028.94	43586.78 ± 4624.85*
Kel (h^{-1})	0.011 ± 0.008	0.017 ± 0.003

*Significant difference from cyclosporine A control group with t-test, $^{*}p \leq 0.01$

(Switzerland), respectively. The zinc sulphate and buffer solutions were prepared in ultra-purified water. Ultra-pure water was prepared by using Milli-QR Gradient A10R (Millipore, Moscheim Cedex, France). The HPLC grade methanol was purchased from Fisher Chemical (Loughborough, UK). Formic acid was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India).

3.2. Animals and study protocol

Ten healthy male rats weighing between 300 and 350 g were obtained from Animal Care and Use Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were randomly divided into two groups (5 each). The study protocol was approved and ethical permission was granted by the Director of "Experimental Animal Care and Use Centre, College of Pharmacy, King Saud University, Riyadh" in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publication No 80-23; 1996). All rats were maintained under standard laboratory conditions of a 12-hour light/dark cycle at 25 ± 2 °C. The animals were given pellet diet with water *ad libitum* and fasted overnight prior to the experiments.
Each rat of the control group received Neoral® (cyclosporine 60 mg/kg) orally and blood samples (~0.5 mL) were collected into heparinized vacutainer tubes at 0.0, 0.5, 1.0, 1.5, 2.0, 4.0, 8.0, 12, and 24 h intervals. The blood samples were stored at −80 °C until analyzed. Thereafter, rats were treated with a saline suspension of *Commiphora myrrha* (myrrh 380 mg/kg, p.o.) for the next eight consecutive days. The animals were fasted overnight after the 7th day of treatment. On the morning of day 8, the last dose of *Commiphora myrrha* (myrrh) was administered to the fasted animals. One hour lates Neoral® (cyclosporine 60 mg/kg, p.o.) was administered and the same sampling scheme was repeated as described previously. The blood samples were stored at −80 °C until analyzed.

3.3. Instrumentation

Cyclosporine A and cyclosporine D were analyzed with a Waters Acquity H-Class UPLC®-tandem quadrupole mass spectrometer (TQD) (Waters, Milford, USA). The H-Class UPLC® system comprises an Acquity quaternary solvent manager and an Acquity sample manager coupled with a column heater. TQD was equipped with electrospray ionization (ESI) probe. The system was controlled by MassLynx 4.1 Software. Data acquisition, processing and reporting was carried out automatically by using application manager 'QuanLynx' included with MassLynx 4.1 Software ((Version 4.1, SCN 714)). The sample tuning was assisted with the help of IntelliStart®. Other instruments include rotary pump (Sogevac, France) for assisting vacuum and a nitrogen generator (Peak Scientific, Scotland) to supply dessolvation gas.

3.4. Chromatographic conditions

The analyte elution was achieved on an Acquity UPLC®BEH Phenyl C18 1.7 μm, 2.1 x 50 mm column (Made in Ireland). A guard column [Acquity UPLC®BEH Phenyl 1.7 μm VanGuard™ Pre-column 2.1 x 5 mm (Made in Ireland)] was used to support the analytical column. The column was maintained at 55 ± 5 °C. The mobile phase comprises methanol (A) and 3 mM ammonium acetate buffer (B) comprising 0.1% formic acid. Mobile phase was pumped at 350 μl/min under gradient control. The gradient program of inlet method was: 80% A and 20% B for first 0-0.49 min, changed to 100% A for 0.5-0.8 min, then again changed to 80% A and 20% B for 0.81-3.0 min (Alam et al. 2015). The total analysis time was 3 min including re-equilibration of the column. Five μl of extracted sample were injected for the analysis. The temperature of auto-sampler was kept at 20 ± 2 °C.

3.5. Mass spectrometer conditions

Cyclosporine A and cyclosporine D were determined in the positive electrospray ionization (ESI^{+}) mode. The sodium adducts of cyclosporine A and cyclosporine D were estimated under single ion recording mode. Optimized conditions of tune page were set as: capillary voltage (kV) 3.6, cone voltage 92, extractor (V) 3, RF lens (V) 0.1, source temperature 150 °C, desolvation temperature 350 °C, dissolvation gas 600 L/H, cone gas 0.0, low mass resolution (LMR_1) 15, high mass resolution (HMR_1) 14, ion energy (IE_1) 1.0. The system was operated in single ion recording mode for estimating cyclosporine A-sodium adducts (m/z 1225.1) and cyclosporine D-sodium adducts (m/z 1239.1) (Alam et al. 2015).

3.6. Calibration curve and sample preparation

Protein precipitation method was used to prepare a calibration curve of cyclosporine A in rat whole blood. Standard stock solutions of cyclosporine A and cyclosporine D (IS) were prepared in methanol to give a final concentration of 1 mg/ml. These stock solutions were stored in a refrigerator below 8 °C and used for 20 days from the date of preparation. The stock solution of cyclosporine A was used for preparing a series of calibration standards. Series of diluted stock solutions or working solutions of cyclosporine A was prepared in methanol and 15 μl aliquot of each working solution was added to 300 μl blank rat blood. Ten μl of diluted internal standard (CyD) were added to each sample. Samples were vortexed for 20 s and then 150 μl of zinc sulphate (0.2 M) was added and vortexed again for another 20 s. Zinc sulphate was added to rupture the red blood cells (hemolysis). Finally the protein was precipitated by adding 525 μl of methanol; and then samples were vortexed again for about 30 s. The samples were left over for 5 min and then centrifuged at 10,000 rpm for 8 min. The supernatant was aspirated and analyzed according to the optimized conditions. Six replicates of calibration curves were prepared in the range of 250 to 5000 ng/ml blood. Blood samples stored at around −80 °C were thawed at room temperature and vortexed for 30 s to ensure homogeneity. A, similar procedure was followed for extracting the cyclosporine A from blood samples of control and test groups.

3.7. Pharmacokinetic analysis

The pharmacokinetic profiles of cyclosporine A control and myrrh treated groups were plotted between blood concentration of cyclosporine A and corresponding sampling time. The pharmacokinetic parameters of control and treated groups were obtained by using PK software on Microsoft Excel. Pharmacokinetic parameters such as maximum blood concentration (Cmax) and time to reach maximum concentration (Tmax), area under the curve from 0 to t hrs (AUC0-t) and elimination rate constant (Kel) were calculated.

3.8. Statistical analysis

The data is presented as mean with standard deviation of mean (SEM) for the individual group. Differences in pharmacokinetic parameters of cyclosporine A before and after treatment with myrrh were assessed by a Student's t-test. Statistical significance was assumed when $P < 0.05$.

Conflict of interest:The authors declare that there is no conflict of interests.

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