Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 1 (1): 54-63, 2006
ISSN 1816-496X

Bioavailability Enhancement of Cyclosporine in Rats Pretreated with Endotoxin

V. Prasad, V. Jain and P.R. Mishra
Pharmaceutics Division, Central Drug Research Institute, Lucknow, India

Abstract: The present study was aimed to measure absorption and disposition of cyclosporine in endotoxin pretreated rats. Cyclosporine (Sandimmune, 10 mg kg^{-1}) was orally administered with and without a concomitant dose of endotoxin (5 mg kg^{-1}) i.p. to rats. Cyclosporine concentrations in blood samples were determined by HPLC UV detector. The pharmacokinetic parameters were calculated by noncompartmental analysis using WINNONLIN. The pretreatment of endotoxin in rats significantly increased cyclosporine AUC_{0-t} (area under the concentration-time curve from time zero to the last point) by 33% in rats, indicating that the rats pretreating with endotoxin significantly increased cyclosporine oral bioavailability. *In vitro* absorption study was done to assess the effect of endotoxin on cyclosporine absorption. However, the significant increase in permeation drug into serosal side was noticed in rats, when pretreated with endotoxin, thus the inverted sac study also supported the finding of endotoxin inhibited the function of intestinal P-gp thus making cyclosporine more absorbed into lumen. The increased absorption of cyclosporine in both *in vitro* and *in vivo* may be due to inhibitory activity of endotoxin on intestinal P-gp and Cytochrome P4503A. This is significant in cases when cyclosporine was administered as immunosuppressant agent in patients suffering from septimea. A paired Student's t-test was conducted for statistical comparison in all experiments.

Key words: Endotoxin, Cyclosporine A, interaction, rats, P-glycoprotein, absorption

Introduction

Cyclosporin A (CsA) is a neutral, lipophilic, cyclic undecapeptide (Fig. 1) with a low aqueous solubility. In spite of a good stability in the gastrointestinal tract and a high partition coefficient, the absolute bioavailability of CsA after oral dosing is low with a high variability (10-60%) (Ptachcinski *et al*., 1986; Lindholm *et al*., 1988). This complicates the use of CsA, as the therapeutic index for this drug is low. The variability of CsA absorption is related to chemical and physical properties of vehicles (Takada *et al*., 1985; Reymond and Sucker, 1988). Cyclosporine is a widely used immunosuppressant with a narrow therapeutic window. It is also used for the therapy of autoimmune diseases like psoriasis and rheumatoid arthritis. Cyclosporine and its metabolites have nephrotoxic, hepatotoxic and neurotoxic side effects. Any factor affecting the absorption or disposition of cyclosporine is therefore of therapeutic importance.Cyclosporine is metabolized by CYP3A4 (Kronbach *et al*., 1988) and is also a substrate of P-glycoprotein (P-gp), the multi-drug efflux transporter (Edwards *et al*., 1999). The large variation in bioavailability ranging from 2 to 89% might primarily be due to the varying CYP3A4/Pgp level among individuals. CYP3A4 is mainly present in

Corresponding Author: V. Prasad, Pharmaceutics Division, Central Drug Research Institute, Lucknow, India

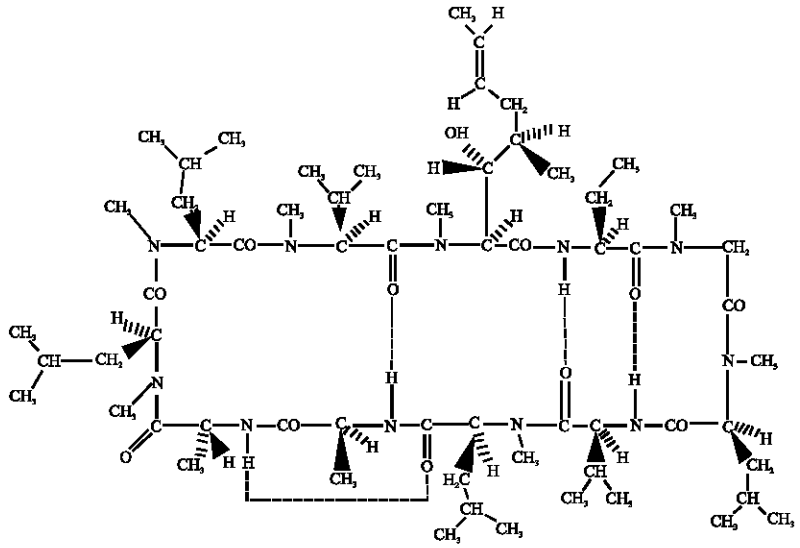

Fig. 1: Chemical structure of cyclosporine A

the intestine and liver. The significant role of CYP3A4 for drug- drug interactions has been well recognized. P-gp is a 170 kDa membrane protein expressed in various normal human tissues such as small intestine, kidney, liver and capillary endothelial cells of brain and testes (Sugawara *et al*., 1998; Thiebaut *et al*., 1987).

Inflammation is a complex immunological response that is a component of many disease states, making it an important consideration in clinical therapeutics. An acute inflammatory reaction is initiated by a wide variety of pathological stimuli, including infection, tissue damage, trauma, or cellular stress resulting in the release of pro-inflammatory cytokines and modulation in the expression of many hepatic proteins. Numerous clinical reports indicate that drug biotransformation is compromised during infection and inflammation due to a down-regulation of cytochrome P450 caused by the elicited inflammatory response (Morgan *et al*., 1997; Slaviero *et al*., 2003). Inflammation is also known to alter the expression and activity of several drug efflux transporters (Hartmann *et al*., 2002; Goralski *et al*., 2003). The concomitant roles (i.e., removing xenobiotics from cells) and close cellular localization of metabolic enzymes and efflux transporters indicate that these proteins may function as a coordinate protective mechanism to limit systemic access of xenobiotics, likely contributing to the high interindividual variability that is observed for numerous drugs.

A variety of pharmaceutical and chemical agents, immune mediators and disease states affect drug disposition by modulation of metabolism and/or transport. Cytochrome P450 3A is involved in the metabolic clearance of approximately 50% of drugs currently on the market and accounts for approximately 70% of total cytochrome P450 content in the small intestine (Wacher *et al*., 1998). Expression of P-gp, MRP2 and CYP3A are all reduced in animal livers during infection or inflammation (Tang *et al*., 2000; Payen *et al*., 2000). *In vivo* and *in vitro* studies indicate that interleukin-6 (IL-6) and other pro-inflammatory cytokines released during the inflammatory response are primarily involved in mediating this downregulation (Lee and Piquette, 2001; Warren *et al*, 2001).

Such reports of LPS induced inflammation are responsible for down regulation (Sukhai *et al*., 2000), although the molecular mechanism of this phenomenon has yet to be elucidated, it is possible that these proteins share common regulatory pathways. Indeed, the Pregnane X Receptor (PXR) has been shown to regulate a network of drug-metabolizing and drug transporter genes in the liver and intestine, including CYP3A and MDR1 and MRP2 (Synold *et al*., 2001). With respect to several acute phase protein markers, the intestine exhibits an acute phase response in a manner similar to that observed in the liver (Molmenti *et al*., 1993; Wang *et al*., 1998). However, the role of inflammation on the metabolic and transport proteins in the intestine has received little attention, despite the fact that the intestine is the first major barrier to xenobiotic absorption and that modulation of drug transport and/or metabolism likely impacts oral bioavailability. We conducted the present study to observe the effect of LPS pretreatment on cyclosporine bioavailability, as we hypothesize LPS induced acute inflammation on the expression and activity of PGP, MRP2 and CYP3A in intestinal tissue, which may further affect cyclosporine absorption and thus bioavailability.

Materials and Methods

Materials

CsA powder, the oily solution Sandimmun (CsA-S) and the new oral microemulsion Sandimmun Neoral ® (CsA-SN) were kindly provided by Sandoz Laboratories (Mumbai, India). Sodium chloride and Potassium dihydrogen phosphate were obtained from Sigma Chemicals, USA. Polyethylene Glycol was obtained from Aldrich Chemical Company, USA. Ortho phosphoric acid was obtained from Qualigens. All the solvents used in this study were obtained from Merck, India Ltd.

Animals

Male Sprague Drawley rats (250–275 g) were used for *in vitro* and *in vivo* studies were conducted in accordance with the guidelines of the Institutional Council of Animal Care. (As per protocol approved by institutional animal ethics committee) and experiments were carried out in Central Drug Research Institute, Lucknow. Rats were injected with 5 mg kg^{-1} i.p. endotoxin (LPS from *Escherichia coli* serotype O55:B5; Sigma, USA) dissolved in 0.5 mL of sterile saline, drinking water was provided but food was withheld after treatment to control for potential differences in food intake.

HPLC Method for Estimation of Cyclosporine

The apparatus used for this work was an LC-6A solvent delivery pump equipped with an SPD-10A UV-Vis detector and a C-R4A integrator (all from Shimadzu, Japan). The detector was set to 210 nm. The samples were applied by a Rheodyne 7725 loop injector with an effective volume of 100 µL. A Shimpack CLC-ODS (150 x 6 mm i.d.; 5 µm particle size) (from Shimadzu Co., Kyoto, Japan) was used for the chromatographic separation. The mobile phase comprised of acetonitrile and 5 mM disodium hydrogen phosphate (75:25, v/v), adjusted to pH 5.1 with concentrated phosphoric acid and 1M sodium hydroxide. Analyses were run at flow rate of 1.5 mL^{-1} min at 70°C for 12 min and then 10 min at flow rate of 2.5 mL^{-1} min. Before the next injection, the flow rate was decreased to 1.5 mL^{-1} min. The samples were quantified using CyA peak area (Hossein *et al*., 2003; Oellerich *et al*., 1995).

Procedure for Extraction of Cyclosporine from Blood

Five hundred microliters acetonitrile was added to a 250 μL of whole blood in a 1.5 mL polypropylene microcentrifuge tube. The tube was vortex-mixed for 30 s and centrifuged for 2 min at 11,000xg. The supernatant was transferred to another clean tube and a 50 μL volume was injected into the chromatograph.

In Vitro Studies

Everted Gut Sac Method

The animals were divided into two groups first group was used for endotoxin pretreated followed by cyclosporine administration this study was performed 24 h after LPS administration and second group was used for cyclosporine administration only. The everted gut sac technique consisted of gently everting a freshly excised small intestine (rat), filling it with oxygenated tissue culture medium at 37°C and dividing it into sacs approximately 2.5 cm in length, using braided suture silk. Each sac was then placed in a flask (50 mL) containing 9.0 mL of pregassed (95% O_2, 5% CO_2) then placed in Sandimmune. The sacs were incubated at 37°C in an oscillating water bath (60 cycles: min). At the appropriate time points, sacs were removed, washed four times in 0.9% NaCl solution and blotted dry. The sacs were cut open and the serosal fluid drained into small tubes. Each sac was weighed before and after serosal fluid collection to calculate accurately the volume of medium inside the sac. The sacs were then digested individually in 25 mL of 1 M NaOH at 37°C for 2 h. Samples of the external medium (0.5 mL) and serosal fluid (0.4-0.5 mL) were made up to 1 mL with water (Benet *et al*., 1996).

Extraction of Cyclosporine in Serosal Fluid

The samples collected were then extracted twice using hexane, then ether was added, vortexes for 2 min and ether layer was collected and then reconstituted with 1 mL of mobile phase. It was then analyzed by using HPLC.

Viability Assay of Everted Gut Tissue

In order to assess the duration of the gut sac tissue viability, glucose transport study was performed to demonstrate active transport against a concentration gradient from the mucosal to the serosal fluid and glucose was estimated by reported method (Lloyd and Whelan *et al*., 1969).

In Vivo Studies

Animals and Drug Administration

Eight male Sprague-Drawley rats (250-350 g) were randomly divided into two groups, respectively. Animals were given a dose of 10 mg kg^{-1} cyclosporine (Sandimmune, diluted with olive oil) with and without endotoxin pretreated (5 mg kg^{-1} i.p.) in crossover designs. Food was withheld for 12 h before and 4 h after dosing but free access to drinking water was allowed during the experiment. One week was allowed for wash out. Blood samples were withdrawn by cardiopuncture or from the jugular vein at 1, 3, 5, 8, 12, 24 and 36 h for rats. Cyclosporine A was given orally and LPS was given through intraperitoneal route. The entire study adhered to the Principles of Laboratory Animal Care (animals used were approved by institutional animal ethics committee). Blood cyclosporine concentration was measured by HPLC as described previously. The assay was calibrated for concentrations ranging from 25.0 to 1500.0 ng mL^{-1}. Samples were analyzed by comparing this standard curve and pharmacokinetic parameters were calculated using WINNONLIN software.

Data Analysis

Both the mean peak cyclosporine concentration (C_{max}) and the time to reach Cmax (T_{max}) were derived directly from the observed individual blood levels. Values for area under the concentration-time curve (AUC_{0-t}) of cyclosporine were calculated by noncompartmental analysis using WINNONLIN software. The paired Student's t-test and ANOVA with Scheffe's test were used for statistical comparison of the *in vivo* results and everted sac study, respectively, taking $p<0.05$ as significant.

Results

Figure 2 depicts the blood profiles of cyclosporine after administration of cyclosporine with or without endotoxin pretreated in rats. Because the elimination constants of cyclosporine in some rats could not be estimated, we therefore presented the observed exposure of cyclosporine (AUC_{0-t}) as given in Table 1 for rats after two treatments. Present results showed that endotoxin pretreated rats; cyclosporine mean residence time would have increased that lead to increase in bioavailability (AUC_{0-t}) by 33%. However, no significant changes on T_{max} of cyclosporine were found after endotoxin pretreatment in rats. The everted intestine sac study showed and further supported the data that pretreated endotoxin in rats would have inhibited the efflux transports like P-gp and even may be by inhibiting CYP4A in intestine and liver,which are responsible for the transport of cyclosporine from the serosal to mucosal side for both jejunum and ileum in a time-dependent manner as shown in Fig. 3 and 4 respectively, indicating that LPS pretreatment in rats significantly inhibited the function

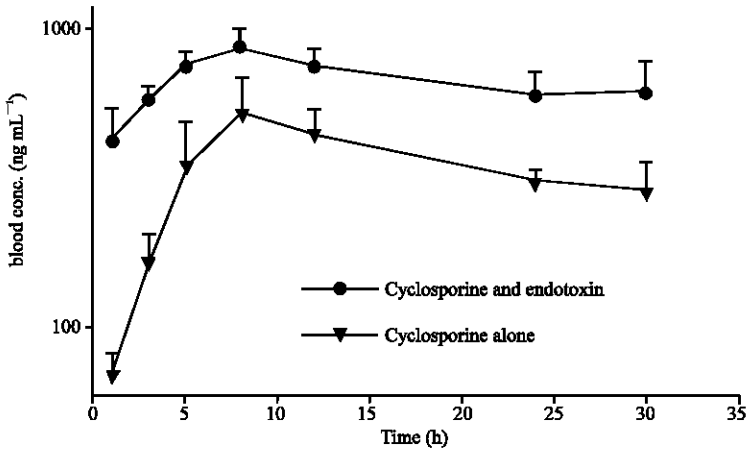

Fig. 2: Mean (FSE) blood concentration time profiles of cyclosporine alone and cyclosporine after treated with endotoxin in eight rats

Table 1: The pharmacokinetic parameters of cyclosporine after administration of cyclosporine (10 mg kg^{-1}) alone and with endotoxin pretreated (5 mg kg^{-1}) i.p. in rats (n = 8)

	Parameters	Cyclosporine alone	Cyclosporine pretreated with endotoxin	Difference
Rats	C_{max}[a]	770.1±152.9	950.1±121.9	+19%
	AUC_{0-t}[b]	11188±907	16614±2942	+33%*

C_{max}[a] (nmol mL^{-1}); peak serum concentration. AUC_{0-t}[b] (nmol min mL^{-1}); area under the serum concentration-time curve from time zero to the last point.* $p<0.05$

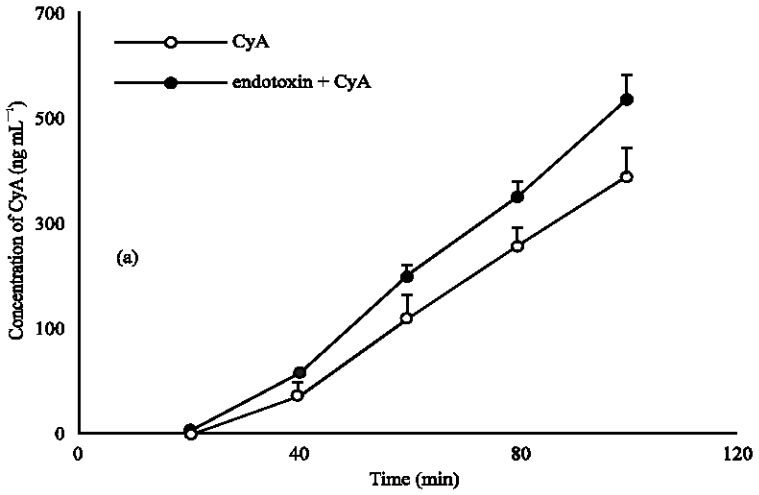

Fig. 3: Mean concentration of cyclosporine (ng mL^{-1}) transport across (a) jejunum (•) pretreated with endotoxin followed by cyclosporine (o) and cyclosporine alone; (n = 3, *p<0.05)

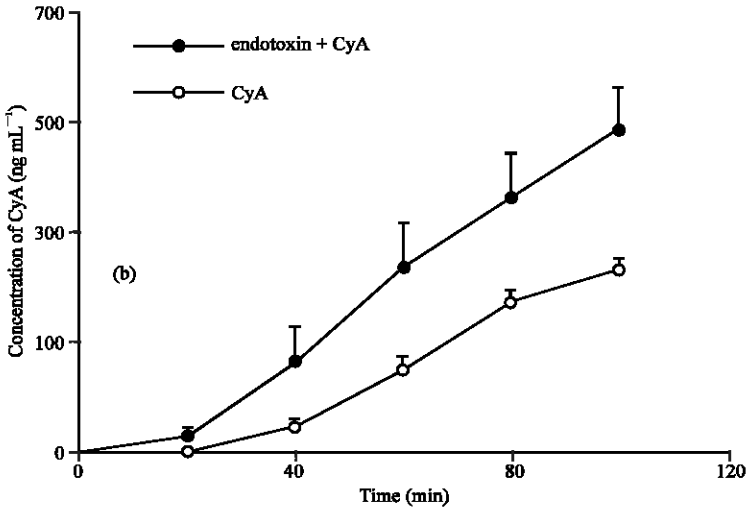

Fig. 4: Mean concentration of cyclosporine (ng mL^{-1}) transport across (b) ileum in the (•) pretreated with endotoxin followed by cyclosporine (o) and cyclosporine alone; (n = 3, *p<0.05)

of intestinal P-gp thus making cyclosporine more bioavailable. The intestinal membrane integrity was seen in control rats and endotoxin treated rats by glucose transport from mucosal side to serosal side and vice versa as there was no change in bidirectional transport as shown in Fig. 5, indicating that intestinal membrane viability was same in control and treated.

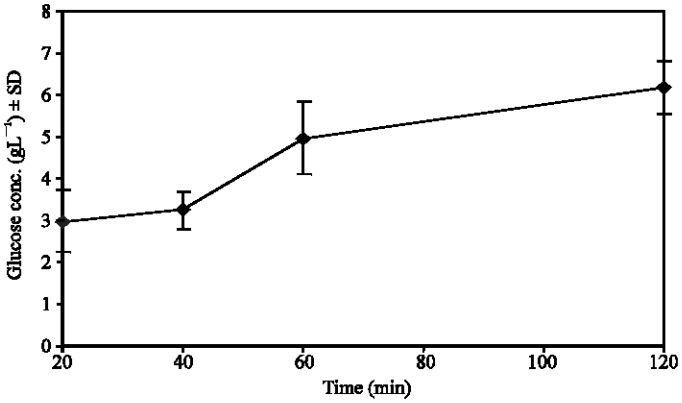

Fig. 5: Glucose transport from mucosal to serosal in (gL) in everted gut tissue

Discussion

Although rats administered LPS displayed a pronounced formation of exudate around the eyes and nostrils and many developed diarrhea, there was no mortality in either the treated or the control groups. Similar cell viability and membrane integrity were seen in intestinal segments obtained from LPS-treated and control rats. The directional transport of glucose remained unchanged and LPS treatment did not affect glucose permeability in intestinal segments mounted on everted sac method (Fig. 5). Nevertheless, similar cell viability and integrity were seen in treated and control samples.

Potential mechanisms of the reduced oral bioavailability of CsA are as follows: (1) poor solubility and intestinal permeability, (2) enhanced metabolism in the intestinal wall and the liver (Kolars *et al*., 1991) and (3) extrusion of CsA into the intestinal lumen through the activated P-glycoprotein in gut wall, CYP3A4 in liver microsomes. Based on the results of our everted sac study, pretreated rats with LPS enhance the transport of cyclosporine from intestinal barrier to blood circulation. The synergistic barrier function of CYP3A4 and Pgp in the small bowel played an important role for the biological defense mechanism to xenobiotics. Based on *in vitro* evidence, endotoxin is causing the suppression of CYP 3A4 and Pgp and thus is likely to enhance the oral bioavailability of cyclosporine, a known substrate of CYP3A4/Pgp. However, expectedly, our *in vivo* results indicated that endotoxin pretreated rats significantly increased cyclosporine oral bioavailability, suggesting that the effect of endotoxin on the fate of cyclosporine may be attributed to its suppression of CYP3A4 or Pgp. The *in vitro* evidence could therefore would be supporting to the *in vivo* effects of endotoxin treated. Prior to proceeding with the endotoxin-cyclosporine interaction study, if we are going to actual role of endotoxin in Pgp and CYP3A4.

Previous studies have demonstrated that inflammation reduces the hepatic expression and activity of the drug efflux transporters *mdr1* and *mrp2* (Tang *et al*., 2000; Payen *et al*., 2000). This suppression is mediated primarily by the pro-inflammatory cytokines, particularly IL-6 (Lee and Piquette, 2001). Whether inflammation-mediated changes in *mdr1* or *mrp2* expression occur in epithelial tissues such as the intestine is not known. However, there is increasing evidence that the intestinal mucosa responds to endotoxin in a manner similar to the well characterized hepatic acute phase response (Wang *et al*., 1998). Endotoxin is known to stimulate the production of IL-6 in liver

(Billiar *et al*., 1992). Expression of IL-6 mRNA in the intestinal segments of LPs-treated rats reported by (Hossein *et al*., 2003), downregulation also enhancement of cyclosporine in blood (Goralski *et al*., 2003).

Although levels of *mdr1a* mRNA were reduced in all intestinal regions of LPS-treated rats, suggests that a net increase in bioavailability of P-gp and MRP2 substrates is likely to occur *in vivo* during an inflammatory response. The expression and activity data of Pgp/*mdr1a* and MRP2 are in agreement, leading us to believe that the reductions in both Pgp and MRP2 activities are the result of a regulatory mechanism of acute inflammation. Furthermore, these studies indicate that statistically significant alterations in intestinal drug absorption can be predicted as a result of altered drug transporter levels. Conversely, it could be argued that mucosal damage and enhanced cell sloughing could result from LPS. However, since tissue viability and glucose permeability were not significantly different between LPS-treated and control animals, it is unlikely that alterations in membrane integrity are responsible for the observed changes in expression and activity of Pgp and MRP2. Present data demonstrated that that bioavailability enhancement of cyclosporine could be attributed to pretreated rats with endotoxin in rats would have suppressed the efflux transporters and absorption barriers or some other barriers. Although delineation of the individual roles of Pgp and CYP3A in inflammatory-mediated changes in drug disposition is important, it is particularly critical to ascertain the impact of their coregulation during an inflammatory response. Intestinal efflux transporters and metabolic enzymes also contribute to drug clearance, including secretion of drugs into bile and direct absorption of drugs into the intestinal lumen. Hence inflammatory stimuli are likely to impose changes in the bioavailability and clearance of numerous drugs that are substrates of PGP, MRP2, or CYP3A, thus increasing the possibility of adverse drug reactions or therapeutic failure. Our findings indicate that the intestine is affected during an inflammatory response. Alterations in the drug transport or metabolism occurring within the intestine, as well as the liver, should therefore be considered when predicting drug disposition during inflammation. Increased and variable drug absorption is likely to occur in inflammation and proper precautions must be taken when determining dose and regimen of drug therapy. Understanding this phenomenon may aid in better predicting therapeutic efficacy or toxicity and raises the importance of acknowledging the presence of drug-disease interactions.

Conclusions

It was interesting to observe the enhancement of bioavailability of cyclosporine in inflammation induced by LPS. By this experiment we can conclude that proper precautions should be taken predicting drug disposition during inflammation conditions, understanding this phenomenon may lead to predict the therapeutic efficacy and toxicity.

Acknowledgements

The authors thank the Sandoz Laboratories for providing the gift sample, V. Jain for skilled technical support during HPLC analysis and Indian Council of Medical Research for providing fellowship during the course of study.

References

Billiar, T., R. Curran, D. Williams and P. Kispert, 1992. Liver nonparenchymal cells are stimulated to provide IL-6 for induction of the hepatic acute-phase response in endotoxemia but not in remote localized inflammation. Arch. Surg., 127: 31-37.

Benet, L.Z., C.Y. Wu, M.F. Hebert and V.J. Wacher, 1996. Intestinal drug metabolism and antitransport processes: A potential paridigm shift in oral drug delivery. J. Control Release, 39: 139-143.

Edwards, D.J., E.M. Fitzsimmons, E.G. Schuetz, K. Yasuda, M.P. Ducharme, L.H. Warbasse, P.M. Woster, J.D. Schuetz and P. Watkins, 1999. 6V, 7V-Dihydroxybergamottin in grapefruit juice and Seville orange juice: Effects on cyclosporine disposition, enterocyte CYP 3A4 and P-glycoprotein. Clin. Pharmacol. Ther., 65: 237-244.

Goralski, K., G. Hartmann, M. Piquette-Miller and K.W. Renton, 2003. Downregulation of Mdr1a Expression in the Brain and Liver During CNS Inflammation Alters the *in vivo* Disposition of de Montellano, P.R. (Ed.). Plenum Press, New York, pp: 473-535.

Hartmann, G., A. Cheung and M. Piquette-Miller, 2002. Inflammatory cytokines, but not bile acids, regulate expression of murine hepatic anion transporters in endotoxemia. J. Pharmacol. Exp. Ther., 303: 273-281.

Hossein, A., H. Abol and D. Ahma, 2003. Simple determination of cyclosporine in human whole blood by high-performance liquid chromatography. J. Chromatogr. B., 795: 209-214.

Kronbach, T., V. Fischer and U.A. Meyer, 1988. Cyclosporine metabolism in human liver: Identification of a cytochrome P-450 III gene family as the major cyclosporine metabolizing enzyme explains interactions of cyclosporine A with other drugs. Clin. Pharmacol. Ther., 43: 630-635.

Kolars, J.C., W.M. Awni, R.M. Merion and P.B. Watkins, 1991. First-pass metabolism of cyclosporine by the gut. Lancet, 338: 1488-1490.

Lloyd, J.B. and W.J. Whelan, 1969. An improved method for enzymic determination of glucose in the presence of maltose. Anal. Biochem., 30: 467-470.

Lindholm, A., S. Henricsson, M. Lind and R. Dablqvist, 1988. Intraindividual variability in the relative systemic availability of cyclosporine after oral dosing. Eur. J. Clin. Pharmacol., 34: 461-464.

Lee, G. and M. Piquette-Miller, 2001. Influence of IL-6 on MDR and MRP-mediated MDR in human hepatoma cells. Can. J. Physiol. Pharmacol., 79: 876-884.

Morgan, E.T., 1997. Regulation of cytochromes P450 during inflammation and infection. Drug Metab. Rev., 29: 1129-1188.

Molmenti, E.P., T. Ziambaras and D.H. Perlmutter, 1993. Evidence for an acute phase response in human intestinal epithelial cells. J. Biol. Chem., 268: 14116-14124.

Oellerich, M., V.W. Armstrong, B. Kahan, L. Shaw, D.W. Holt, R. Yatscoff, A. Lindholm, P. Halloran, K. Gallicano and K. Wonigeit, 1995. Ther. Drug. Monit., 17: 642.

Ptachcinski, R.J., R. Venkataramanan and G.J. Burckart, 1986.Clinical Pharmacokinetics of cyclosporine A. Clin. Pharmacokin, 11: 107-132.

Payen, L., L. Sparfel, A. Courtois, L. Vernhet, A. Guillouzo and O. Fardel, 2002. The drug efflux pump MRP2: regulation of expression in physiopathological situations and by endogenous and exogenous compounds. Cell. Biol. Toxicol., 1 18: 221-233.

Reymond, J.P. and H. Sucker, 1988. *In vitro* model for cyclosporine intestinal absorption in lipid vehicles. Pharm. Res., 5: 673-676.

Sugawara, I., I. Kataoka and Y. Morishita, 1998. Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody. MRK. Cancer Res., 48: 1926-1929.

Sukhai, M., A. Yong, J. Kalitsky and M. Piquette-Miller, 2000. Inflammation and IL-6 mediate reductions in the hepatic expression and transcription of the mdr1a and mdr1b Genes. Mol. Cell. Biol. Res. Commun., 4: 248-256.

Synold, T., I. Dussault and B. Forman, 2001. The orphan nuclear receptor SXR coordinately regulated drug metabolism and efflux. Nat. Med., 7: 584-590.

Slaviero, K., S. Clarke and L. Rivory 2003. Inflammatory response: an unrecognised source of variability in the pharmacokinetics and pharmacodynamics of cancer chemotherapy. Lancet Oncol., 4: 224-232.

Takada, K., N. Shibata, H. Yoshimura, Y. Masuda, H. Yoshikawa and S. Muranishi, 1985. Promotion of the selective lymphatic delivery of cyclosporine A by lipid-surfactant mixed micelles. J. Pharmacobio. Dyn., 8: 320-323.

Thiebaut, F., T. Tsuruo, H. Hamada, M.M. Gottesman, I. Pastan and M.C. Willingham, 1987. Cellular localization of the multidrugresistance gene product P-glycoprotein in normal human tissues. Proceedings of the National Academy of Sciences of the United States of America, 84: 7735-7738.

Tang, W., C. Yi, J. Kalitsky and M. Piquette-Miller, 2000. Endotoxin down regulates hepatic expression of PGP and MRP2 in 2-acetylaminofluorene-treated rats. Mol. Cell. Biol. Res. Commun., 4: 90-97.

Wacher, V., J. Silverman, Y. Zhang and L. Benet, 1998. Role of PGP and CYP 3A in limiting oral absorption of peptides and peptidomimetics. J. Pharm. Sci., 87: 1322-1329.

Wang, Q., J. Wang, S. Boyce, J. Fischer and P. Hasselgren, 1998. Endotoxemia and IL-1 stimulate mucosal IL-6 production in different parts of the gastrointestinal tract. J. Surg. Res., 76: 27-31.

Warren, G., P. Van Ess, A. Watson, M. Mattson and R. Blouin, 2001. Cytochrome P450 and antioxidant activity in interleukin-6 knockout mice after induction of the acute-phase response. J. Interferon. Cytokine. Res., 21: 821-826.