Acta Pharmaceutica Sciencia
50: 213-218

Influence of Silymarin Pretreatment on Absorption of Nitrendipine

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Abstract

Silymarin was a flavonoid extracted from the medicinal plant *Silybum marianum*. Male albino rats were used for the study. Silymarin (50mg/kg, *p.o*) was given orally for 9 days and on day 10, animals were fasted overnight prior to sacrifice. The whole small intestine was flushed with 50 ml of ice-cold saline after sacrificing the animal with an over dose of pentobarbital. The small intestine was isolated and divided in to duodenum, jejunum and ileum. Each segment was everted and a 5 cm long sac was prepared and 1ml of nitrendipine solution introduced into the everted sac (serosal side) and both ends of the sac were ligated tightly. The sac containing nitrendipine solution was immersed in 30 ml of Dulbecco's phosphate buffer solution (D-PBS) containing 25 mM glucose. The transport of nitrendipine from serosal to mucosal surfaces across the intestine was determined by collecting samples from the mucosal medium periodically at different intervals 0, 10, 20, 30, 60, 90 and 120 minutes. The samples were analyzed by HPLC. Silymarin pretreatment significantly increased the transport rate of nitrendipine to greater extent in ileum than jejunum and with no effect in duodenum. It might be due to induction of P-glycoprotein (P-gp) in the intestine, with highest expression of P-gp being found in ileum followed by jejunum and duodenum.

Keywords: Silymarin, nitrendipine, everted rat intestine, P-glycoprotein.

Introduction

Flavonoids form a large class of phenolic substances widely distributed throughout the plant kingdom and can be detected in 3000 varieties (Kuhnau 1976). Silymarin (Fig. 1) was a flavonoid obtained from the medicinal plant Silybum marianum (Milk thistle) belonging to the family Asteracease. It was used in treatment of acute or chronic hepatitis and cirrhosis induced by drugs or toxins (Pepping 1999). Silymarin appeared to enhance the recovery from CCl_4-induced hepatotoxicity and also reduce lipid peroxidation in the liver (Germano et al. 2001). P-glycoprotein (P-gp) is part of a larger family of efflux transporters found in the gut, gonads, kidney, biliary system, luminal membranes of the endothelium of blood vessels in the brain and other organs. P-gp is also expressed in other normal human and rodent tissues, including the adrenal gland, kidney, liver, colon, brain and testis (Thiebaut et al. 1987). The transporters appear to protect the body from harmful substances. P-gp, encoded by the human MDR1 and rodent MDR1a/1b gene, is constitutively expressed in the brush border membrane of intestinal enterocytes and in the canalicular membrane of hepatocytes and transportersstructurally and functionally diverse compounds (Ambudkar et al. 1999).

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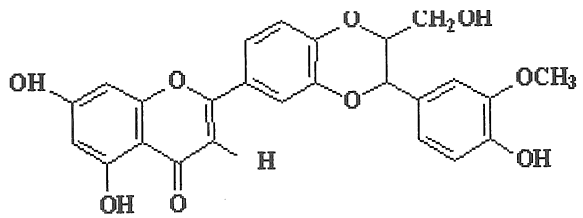
Intestinal P-glycoprotein, an ATP-dependent multidrug efflux pump, can be an active secretion system or an absorption barrier by transporting some drugs from intestinal cells into the lumen (Terao et al. 1996). Substrates transported by P-gp include a variety of compounds such as some anticancer agents, steroid hormones, calcium channel blockers, immuno-suppressing agents and β-blockers (Hunter et al. 1993). Nitrendipine has been used as a marker to study the function of P-gp in various multidrug resistant cells and various normal tissues including the intestine, but no clear reports are available on action of silymarin on P-gp transporter system.

Figure 1: Structure of silymarin



In this paper we report, the action of silymarin pretreatment on nitredipine (P-gp substrate) transport in rat intestine.

Materials and Methods

Materials

Nitrendipine and nifidipine pure substances were a kind gift from Aristo Pharmaceuticals Ltd, Mumbai, India. Silymarin was purchased from Brown & Burk Pharma Ltd, Mumbai, India. Acetonitrile and methanol (HPLC grade) purchased from E. Merck Ltd, Mumbai, India.

HPLC instrumentation

Shimadzu high performance liquid chromatography unit equipped with the LC-8A solvent delivery module, SPD-10AVP UV-Visible spectrophotometer detector, Class CR-10 Data Processor, Rheodyne (with 20 µl capacity loop). Injection Port and Wakosil II C-18 Column (stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5□ diameter, 100 °A pore diameter) were used for analysis of samples. Mobile phase consisting of methanol: water: glacial acetic acid (75:25:1 v/v/v) with a flow rate of 1ml/min. The eluent was monitored at 235nm and sensitivity of 0.001 a.u.f.s was used for the analysis.

Study design

Experiments were performed with Wistar rats weighing between 180 to 210 g. The animals were housed in colony cages under conditions of standard lighting (lights on from 07.00 to 19.00 h), temperature (22 ± 1°C) and humidity (60 ± 10%) for at least one week before the experiments. The experiment is planned after getting the approval from the institutional animal ethical committee. The experimental procedure was performed by modified method (Yumoto et al. 1999). Silymarin (50mg/kg, *p.o*) was given orally for 9 days and on day 10, animals were fasted overnight prior to sacrifice. The whole small intestine was flushed with 50 ml of ice-cold saline after sacrificing the animal with an over dose of pentobarbital. The small intestine was isolated and divided in to duodenum, jejunum and ileum. Each segment was everted and a 5 cm long sac was prepared and 1ml of nitrendipine solution (2 mg/ml) introduced into the everted sac (serosal side), and both ends of the sac were ligated tightly. The sac containing nitrendipine solution was immersed into 30 ml of Dulbecco's Phosphate Buffer Solution (D-PBS) containing 25 mM glucose. The solution was pre-warmed at 37°C and pre-oxygenated with 5% carbon dioxide (CO_2) and 95% oxygen (O_2) throughout the experiment. The transport of nitrendipine from serosal to mucosal surfaces across the intestine was measured by collecting samples from the mucosal medium periodically at different intervals 0, 10, 20 ,30, 60, 90 and 120 minutes. Control animals were without any pretreatment

with silymarin. DMSO used for control animal experiments. The transport of nitrendipine in the absence (control-DMSO)/presence of silymarin pretreatment (test) were determined.

Method of analysis

Nitrendipine levels were estimated by a modified reversed phase high performance liquid chromatography (HPLC) using Narinkonic et. al. (2001) method To 490 µl of sample solution 10 µl of nifedipine (1 mg/ml) was added as internal standard and vortexed for 2 minutes and 20 µl of the supernatant were injected onto the HPLC column. The retention times of nitrendipine and nifidipine were 7.1 and 5.0 minutes respectively.

Calibration curve of nitrendipine in D-PBS (Dulbecco's Phosphate Buffer Solution) containing 50mM of glucose solution: Different concentrations (0, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 32 and 40 µg/ml) of nitrendipine in test solution were prepared for calibration curve. The samples were treated as above and peak areas were obtained at different concentrations of the drugs. The areas of the peaks were plotted against the concentration of drug. The slope of the plot determined by the method of least square regression analysis was used to calculate the nitrendipine concentration in the unknown samples. A linear calibration curve in the range of 0.5 to 40 µg/ml was established (r^2=0.999) in D-PBS containing 50 mM of glucose solution.

Statistical analysis: Transport of nitrendipine from everted sac of rat intestine in the absence and presence of silymarin pretreatment represented in mean ± SD (n=6) and was compared using Student's paired t-test for determining the statistical significance.

Results

The transport of nitrendipine from serosal to mucosal side in everted parts of duodenum, jejunum and ileum in the absence and following pretreatment silymarin was determined. The nitrendipine transport was greater in the ileum and jejunum and statistically significant, whereas in duodenum it was found to be insignificant (Figures 2, 3 and 4). Exsorption rate constant, half life of nitredipine transport in everted intestinal region and 50% nitredipine transport time (T50) in intestinal region are given (Table 1 and 2). Silymarin pretreatment signifantly increased the transport rate of nitrendipine to greater extent in ileum than in jejunum with no effect in duodenum.

Figure 2. Transport of nitredipine from serosal to mucosal side in the everted duodenum in the presence (test) and absence of silymarin pretreatment. (n=6)

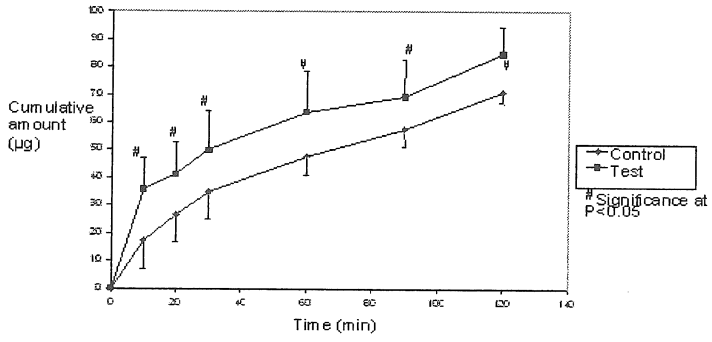


Figure 3. Transport of nitredipine from serosal to mucosal side in the everted jejunum in the presence (test) and absence of silymarin pretreatment. (n=6)

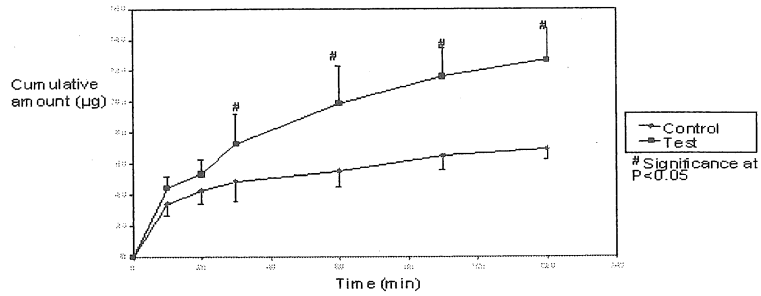


Figure 4. Transport of nitredipine from serosal to mucosal side in the everted ileum in presence (test) and absence of silymarin pretreatment. (n=6)

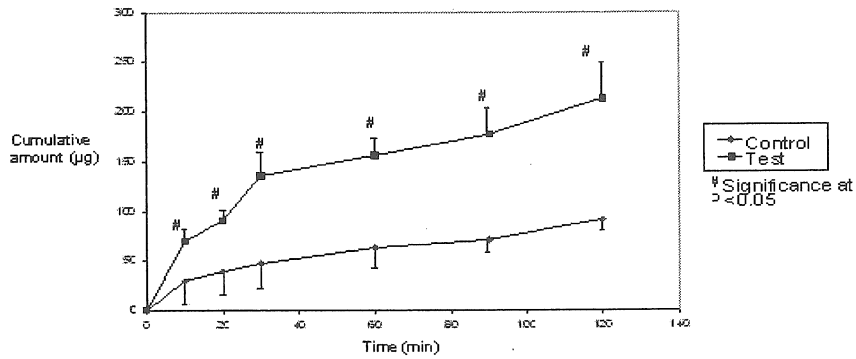


Table 1. T50 value of nitredipine transport in everted intestinal region. (n=6)

Intestinal region	T50 (min)
Ileum	6
Jejunum	13
Duodenum	30

Table 2. Mean ± SD value of exsorption rate constant and half life of nitredipine transport in everted intestinal region. (n = 6)

Intestinal region	Exsorption rate constant (min^{-1})	Exsorption half-life (min)
Duodenum	0.0099 ± 0.001	69.71 ± 13.75
Jejunum	0.0094 ± 0.007	93.21 ± 39.01
Ileum	0.0177 ± 0.058	51.87 ± 16.32

Discussion

Silymarin may influence the metabolic capacity of CYP3A4, a CYP 450 isoenzyme responsible for the hepatic and intestinal metabolism of many important classes of drugs (Venkataramana et al., 2000). Silymarin has little effect on the metabolism of erythromycin (CYP3A4), chlorzoxazone (CYP2E1), S (+)-mephenytoin (CYP2C19), caffeine (CYP1A2) and coumarin (CYP2A6). A moderate effect was observed for high affinity dextromethorphan metabolism (CYP2D6). Clear inhibition was found in case of dinitro nifedipine oxidation (CYP3A4) and S (-)-warfarin 7-hydroxylation (CYP2C9) (Beckman et al. 2000).

In earlier studies it was reported that silymarin pretreatment for 9 days increased the clearance of metronidazole and its major metabolite, hydroxy-metronidazole (HM) by 29.5% and 31.9%, respectively, with a concomitant decrease in half-life, C_{max} and AUC (0-48) in healthy volunteers. Urinary excretion of acid-metronidazole (AM), HM as well as metronidazole during 48h following administration was decreased. It could be due to induction of intestinal P-gp (Rajnarayana et al. 2004).

In another study conducted on pretreatment with silymarin for 7 days revealed that it had no effect on pharmacokinetics of ranitidine in healthy volunteers, therefore at the dose given in the study did not alter ranitidine C_{max} and AUC $_{(0-\infty)}$ that has clinical relevance. There was a significant difference in area under the first moment curve (AUMC) and mean residence time (MRT). It indicates that there was no significant influence on CYP3A4 mediated hepatic or intestinal metabolism of ranitidine (Nageshwar Rao et al. 2007).

The results of the present study indicate that silymarin might induced intestinal P-glycoprotein upon multiple dose administration. Along the length of rat small intestine, the P-gp-mediated nitrendipine transport showed a regional variation and the transport rate in the ileum was about 2.3- fold higher than in the other regions. The transport of nitrendipine when compared with test (silymarin pretreatment) was 2.3- and 2.1- fold higher in ileum and the jejunum respectively, than in control. The transport rate of nitrendipine from serosal to mucosal side in the ileum was higher than in jejunum. The transport of nitrendipine in rat intestine was greater in the presence of silymarin by 5.5 % in duodenum was not significant. Whereas, in the case of ileum and jejunum the transport was increased by 57.7% and 52.5% respectively, and was statistically significant. Silymarin pretreatment showed induction of nitrendipine (a P-gp - substrate) transport in the everted ileum and jejunum and no effect in duodenum. It might be due to little expression of P-gp in the duodenum.

Conclusion

Silymarin pretreatment increases the transportation of nitrendipine in the everted rat intestine. It might be due to the induction of P-gp in the intestine. Highest expression of P-gp was found in ileum followed by jejunum and duodenum.

Acknowledgement
The first author acknowledges the fellowship provided by University Grants Commission (UGC), New Delhi.

References

Ambudkar, S.V., Dey, S. Hrycyna, C.A., Ramachandra, M., Pastan I. and Gottesman, M.M. (1999). Biochemical, cellular and pharmacological aspects of the multidrug transporter, *Annu Rev. Pharmacol. Toxicol.* 39: 361-370.

Beckman, K.S., Rietbrock, S., Weyhenmeyer, R., Bocker, R.H. and Beckurts, K.T. (2000). Inhibitory effects of silybinin on cytochrome P450 enzymes in human liver microsomes, *Pharmacol. Toxicol.* 86: 250-261.

Germano, M.P, Angelo, D.V, Sanogo, R., Morabito, A. and Pasquale, D.R. (2001). Hepatoprotective activity of *Trichilia roka* on carbon tetrachloride-induced liver damage in rats, *J.Pharm.Pharmacol.* 53: 1569-1574.

Hunter, J., Hirst, B.H. and Simmons N.L. (1993).Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers, *Pharm. Res.* 10:743-749.

Kuhnau, J. (1976) The flavonoids: A class of semi-essential food components: their role in human nutrition, *World Res. Nutr. Diet. 4*:117-191.

Nageshwar Rao, B., Srinivas, M., Shravan Kumar, Y. and Madhusudhan Rao, Y. (2007). Effect of silymarin on the oral bioavailability of ranitidine in healthy volunteers, *Dru. Metab. Drug Interact.* 22:151-22:164.

Narinkonic, Y., Aqbaba, P., Yladimiror, S. and Stankovic,S. (2001).Simultaneous HPLC determination of nitrendipine and impurities of the process of synthesis,J. *Pharm. Biomed. Anal.* 24: 993-998.

Pepping, J. (1999). Milk thistle: *Silybum marianum. Am. J. Health Syst. Pharm.* 56: 1195-1197.

Rajnarayana, K., Reddy, M.S., Vidyasagar, J. and Krishna, D.R. (2004). Study on the influence of silymarin pretreatment on metabolism and disposition of metronidazole, *Arzneimittelforschung.* 54:109-113.

Terao, T., Hisanaga, E., Sai,Y., Tamai,I and Tsuji, A. (1996). Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier, *J. Pharm. Pharmacol.* 48: 1083-1089.

Thiebaut, F., Tsuruo,T., Hamada H., Gottesman, M.M., Pastan, I. and Willingham, M.C. (1987). Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues, *Proc.Nat. Acad. Sc. USA* . 84: 7735-7738.

Venkataramana,R., Ramachandran,V., Komoroksi, B.J., Zhang, S., Schiff, P.L. and Strom, S.C. (2000). Milk thistle, a herbal supplement, decreases the activity CYP3A4 and uridine diphosphoglucronyl transferase in human hepatocytes cultures, *Drug. Metab.Dispos.* 28: 1270-1273.

Yumoto,R.,Murakami,T., Nakamoto,Y., Risa,H., Nagai, J. and Takano, M. (1999).Transport of Rhodamine 123, a P-glycoprotein substrate, across rat intestine and Caco-2 cell monolayers in the presence of Cytochrome P450 3A-related compounds, *J.Pharmaco. Exp.* Ther. 289:149-155.

Received:24.12.2007
Accepted:28.08.2008