INFLUENCE OF SOME BIOFLAVONOIDS ON THE TRANSPORT OF NITRENDIPINE

Kandhagatla Rajnarayana, Allenki Venkatesham
and Devarakonda R. Krishna*

Department of Pharmacology and Clinical Pharmacy,
University College of Pharmaceutical Sciences,
Kakatiya University, Warangal, India

SUMMARY

Flavonoids form a large class of phenolic substances widely distributed in nature and exhibit several biological effects. P-glycoprotein is part of a large family of efflux transporters found in the gut, gonads and other organs. Male albino rats were used for this study. The whole small intestine was flushed with 50 ml of ice-cold saline after sacrificing the animal with an overdose of pentobarbital. The small intestine was isolated and divided into duodenum, jejunum and ileum. Each segment was everted, a 5-cm long sac was prepared, 1 ml of nitrendipine solution was 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 and the same concentration of different bioflavonoids, viz., diosmin, quercetin, chrysin, methyl hesperidin and gossypin, was introduced into the mucosal side. 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.

* Author for correspondence:
Devarakonda R. Krishna. M.Pharm, Ph.D.
Department of Pharmacology & Clinical Pharmacy
University College of Pharmaceutical Sciences
Kakatiya University
Warangal – 506 009, AP, India
e-mail: rkdevarakonda@gmail.com

Diosmin and quercetin decreased the transport rate of nitrendipine to nearly the same extent in all regions. Chrysin and gossypin decreased the transport rate of nitrendipine to a greater extent in the ileum than in the duodenum and jejunum. Methyl hesperidin caused inhibition of nitrendipine transport in the ileum and jejunum, but not in the duodenum. All bioflavonoids, i.e., quercetin, diosmin, methyl hesperidin, gossypin and chrysin, decreased the transport of nitrendipine, a P-gp substrate in the rat intestine. The highest expression of P-gp was found in the ileum followed by the jejunum and duodenum.

KEY WORDS

bioflavonoids, everted rat intestine, P-gp

INTRODUCTION

Flavonoids form a large class of phenolic substances widely distributed in nature, which exhibit several biological effects. Diosmin is a flavone, used for treating venous insufficiency, hemorrhoids and varicose veins. Quercetin has antioxidant, antithrombotic, anti-inflammatory and anticarcinogenic activities /1/. Chrysin is a flavone which reduces elevated blood pressure, cardiac hypertrophy and functional vascular changes, but shows no effect in normotensive Wistar Kyoto (WKY) rats. These protective effects are associated with reduced oxidative stress due to the antioxidant properties of the drug /2/. Gossypin is a flavonoid glycoside isolated from the flower of *Hibiscus pitifolius* (Malvaceae). Gossypin exhibits varied biological activities, viz., analgesic, antipyretic, and anti-inflammatory. Methyl hesperidin is a flavonone. It possesses anti-inflammatory and antioxidant activities /3-7/. Grapefruit contains different flavonoids in the form of glycosides. Among their aglycones, naringenin, quercetin, kaempferol, hesperidin and apigenin were reported to inhibit microsomal CYP3A-mediated oxidation of drugs such as nifedipine in rat and human liver /8/. It has been postulated that the drug transporter P-glycoprotein (P-gp) and CYP3A4 are functionally linked components of a xenobiotic detoxification cascade that limits the bioavailability of several drugs /9/. The flavonoids naringenin, kaempferol and quercetin inhibit CYP3A4-mediated dihydropyridine metabolism by human liver

microsomes *in vitro* /10/. P-glycoprotein is part of a larger family of efflux transporters found in the gut, gonads, kidney, biliary system, and luminal membranes of the endothelium of blood vessels in the brain and other organs. They appear to have developed a mechanism 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 transports structurally and functionally diverse compounds /11/. Intestinal P-gp, 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 /12,13/. Substrates transported by P-gp include a variety of compounds including certain anticancer agents, steroid hormones, calcium channel blockers, immunosuppressive agents and β-blockers /14,15/. P-gp is also expressed in other human and rodent tissues, including the adrenal gland, kidney, liver, colon, brain, testis and eye /16,17/. In these tissues, P-gp is reported to prevent the accumulation of xenobiotics by active efflux. However, there are only a limited number of studies on the *in vivo* function of P-gp in normal tissues, such as the intestine, and on the relationship between *in vivo* and *in vitro* P-gp function under various experimental conditions. Therefore, we examined the influence of some flavonoids, viz., chrysin, gossypin, methyl hesperidin, quercetin and diosmin, on everted rat intestine *in vitro* using the transport of nitrendipine as a model substrate for P-gp.

MATERIALS AND METHODS

Materials

Nitrendipine and nifidipine pure substances were a kind gift from Aristo Pharmaceuticals Ltd, Mumbai, India. Diosmin, methyl hesperidin and chyrsin pure substances were a kind gift from Elder Pharmaceuticals Ltd, Mumbai, India. Quercetin was purchased from SD Fine Chemicals, Mumbai, India. Gossypin pure substance was a kind gift from Andhra University, Vishakapatanam. Acetonitrile and methanol (HPLC grade) were purchased from E. Merck Ltd, Mumbai, India.

High performance liquid chromatograpy (HPLC) system

A Shimadzu high performance liquid chromatography unit equipped with 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 µ diameter, 100 Å pore diameter) was used for analysis of samples. The mobile phase consisted of methanol:water:glacial acetic acid (75:25:1 v/v/v) with a flow rate of 1 ml/min. The eluent was monitored at 235 nm and sensitivity of 0.001 a.u.f. was used for analysis.

Study design

Experiments were performed with Wistar rats weighing from 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. Approval for this study was given by the institutional animal ethics committee. The experimental procedure was performed by modified method /18/. The 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 overdose of pentobarbital. The small intestine was isolated and divided into duodenum, jejunum and ileum. Each segment was everted, a 5-cm long sac was prepared, 1 ml (2 mg/ml) of nitrendipine solution was 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 and the same concentration (25 mM) of different bioflavonoids, viz., diosmin, quercetin, chrysin, methyl hesperidin and gossypin in DMSO, was introduced into the mucosal side. DMSO was used in control experiments. The solution was pre-warmed at 37°C and pre-oxygenated with 5% CO_2 and 95% O_2 throughout the experiment. The transport of nitrendipine from the serosal to the mucosal surface 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. In the present study, different bioflavonoids, diosmin, quercetin, chrysin, methyl hesperidin and

gossypin, were added to the mucosal medium. Using this medium, the transport of nitrendipine in the absence (control) or presence (test) of the different bioflavonoids was determined.

Method of analysis

Nitrendipine transport was estimated by a modified reversed phase HPLC method /19/. To 490 µl of sample solution, 10 µl of nifedipine (1 mg/ml) was added as internal standard and vortexed for 2 minutes, then 20 µl of the supernatant was injected onto the HPLC column. The retention times of nitrendipine and nifidipine were 7.1 and 5.0 minutes, respectively.

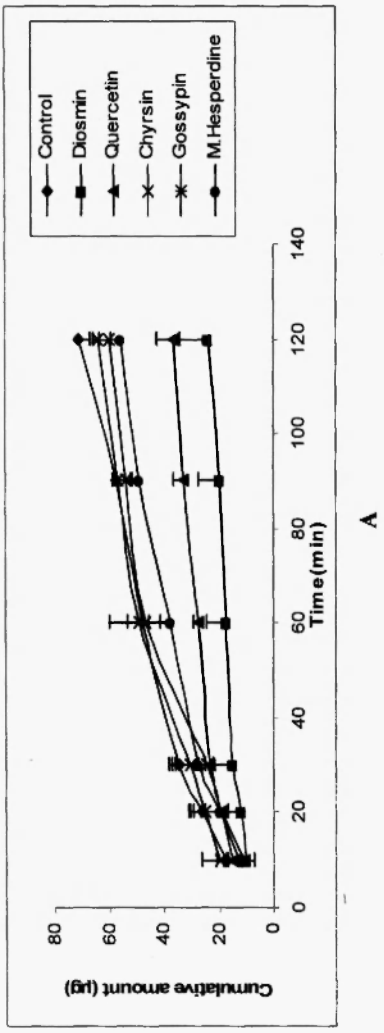
Different concentrations (0, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 32 and 40 µg/ml) of nitrendipine in D-PBS containing 50 mM of glucose solution were prepared for the calibration curve. Samples were treated as above and peak areas obtained at different concentrations of the drugs were plotted against the 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 sample. A linear calibration curve in the range 0.5-40 µg/ml nitrendipine in D-PBS containing 50 mM of glucose solution was established ($r^2 = 0.999$).

Statistical analysis

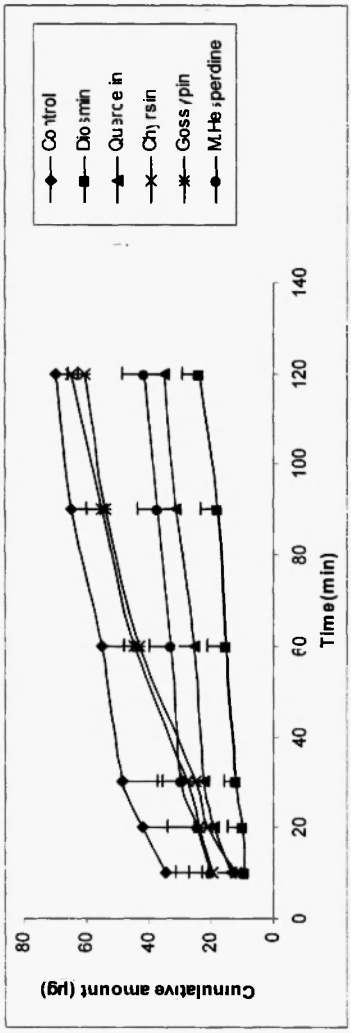
Transport of nitrendipine from the everted sac of rat intestine in the presence and absence (control) of different bioflavonoids was represented as means ± SD (n = 6) and was compared using Student's t-test for determining statistical significance ($p < 0.005$).

RESULTS

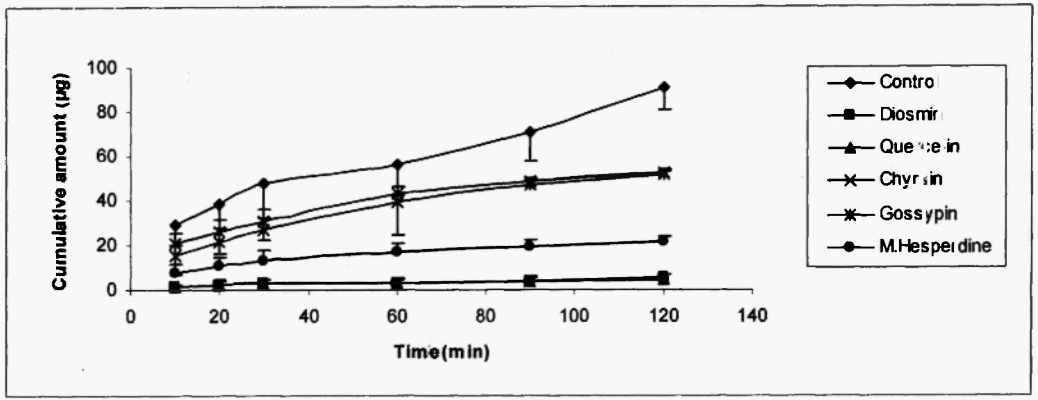
The transport of nitrendipine from the serosal to the mucosal side in everted parts of the duodenum, jejunum and ileum in the presence of bioflavonoid/DMSO (control) was determined (n = 6). In both segments, the transport was significantly inhibited by diosmin and quercetin. Thus, the expression of P-gp in rat intestine was functionally confirmed. Diosmin and quercetin decreased the transport rate of nitrendipine to nearly the same extent in all regions. Chrysin and



A



B



C

Data are expressed as means ± SD.

Fig. 1: Transport of nitredipine from the serosal to mucosal side in the everted gut preparation in the presence (test) and absence of different bioflavonoids. **A:** duodenum; **B:** jejunum; **C:** ileum.

gossypin decreased the transport rate of nitrendipine statistically significantly more in the ileum than in the duodenum and jejunum. Methyl hesperidin decreased the transport rate of nitrendipine statistically significantly more in the ileum and then the jejunum compared to the duodenum. This might be due to low expression of P-gp in the duodenum. Transport of nitredipine from the serosal to mucosal side in the everted duodenum, jejunum and ileum in the presence (test) and absence (control) of different bioflavonoids is shown in Figure 1.

DISCUSSION

P-gp-mediated nitrendipine transport showed regional variation and the transport rate in the ileum was about 5-6 fold higher than in other regions. The transport of nitrendipine when compared with control was 3.5-, 2.8- and 17-fold higher in the duodenum, jejunum and ileum, respectively. The transport rate of nitrendipine from the serosal to mucosal surface in the ileum was double that of the transport rate measured in the duodenum.

The transport of nitrendipine in rat intestine was inhibited in the presence of diosmin by 70%, 63% and 84% in the duodenum, jejunum and ileum, respectively. This inhibitor increased the transport of nitrendipine in the apical-to-basolateral direction by inhibiting P-gp function. The mechanism for the transport of nitrendipine in the presence of diosmin is not clear. However, in rat jejunum, the epithelial cell layer connected by tight junctions is relatively leaky with transepithelial electrical resistance (TEER) of approximately 30 Ohm.cm^2 /21/. Therefore, transport via the paracellular route may be involved in the intestinal transport of nitrendipine, which should be insensitive to diosmin. P-gp-mediated nitrendipine transport showed regional variation, and the transport rate in the ileum was about 9.1-10 fold higher than in other regions. The transport of nitrendipine when compared with control was 2.0, 1.9 and 19.2-fold higher in the duodenum, jejunum and ileum, respectively, to that of test.

The addition of natural rodent diet or quercetin increased etoposide absorption in everted sacs of jejunum or ileum, in comparison to those with artificial rodent diet. The enhancing effect of quercetin was compatible with the effect of natural rodent diet in the jejunum and was higher in the ileum. These *in vitro* data support the hypothesis that

certain dietary components, possibly flavonoid-related compounds, may influence P-gp's function in the intestine and thus increase the absorption of etoposide /22/. The transport rate of nitrendipine from the serosal to mucosal surface in the ileum was double that of the transport rate measured in the duodenum and jejunum. The transport of nitrendipine in rat intestine was inhibited in the presence of quercetin by 52%, 46% and 94% in duodenum, jejunum and ileum, respectively. The transport of nitrendipine when compared with control was 1.7-fold higher in the ileum to that of test. The transport rate of nitrendipine from the serosal to mucosal surface in the ileum was higher than in the duodenum and jejunum.

The transport of nitrendipine in rat intestine was inhibited in the presence of chrysin in duodenum and jejunum by 8% and 9%, respectively, but did not reach statistical significance, and 40% inhibition in the ileum was statistically significant. P-gp-mediated nitrendipine transport showed regional variation and the transport rate in the ileum was about 1.5-fold higher than in the other regions. The transport of nitrendipine when compared with control was 1.62-fold higher in the ileum than that of test. The transport rate of nitrendipine from the serosal to mucosal surface in the ileum was higher than in duodenum and jejunum. The transport of nitrendipine in rat intestine was inhibited by gossypin by 5% and 0.6% in duodenum and jejunum, respectively, statistically insignificant, whereas in the ileum inhibited was 37%, which was statistically significant. Chrysin and gossypin inhibited nitrendipine transport across the ileum but not in the duodenum and jejunum. This might be due to low expression of P-gp in the duodenum and jejunum. The transport of nitrendipine when compared with control was 4.1- and 1.7-fold higher in the ileum and jejunum, respectively, compared to that of test. The transport rate of nitrendipine from the serosal to mucosal side in the ileum was higher than in jejunum.

The transport of nitrendipine in rat intestine was inhibited in the presence of methyl hesperidin in the duodenum by 17%, statistically insignificant, whereas in the ileum and jejunum it was inhibited by 75% and 40%, respectively, statistically significant.

CONCLUSION

Inhibition of P-gp by bioflavonoids might increase the oral bioavailability of drugs which are metabolized by CYP3A isozymes. It might also decrease the development of drug resistance in cancer cells by inhibiting P-gp. All bioflavonoids tested, viz., quercetin, diosmin, methyl hesperidin, gossypin and chrysin, decreased the transport of nitrendipine, a P-gp substrate, in the everted rat intestine. The highest expression of P-gp was found in the ileum, followed by the jejunum and duodenum. The results of the present study suggest the highest expression level of P-gp is in the ileum. P-gp mediated transport of nitrendipine in the everted rat intestine in the presence of the above-mentioned bioflavonoids was as follows:

quercetin > diosmin > methyl hesperidin > gossypin > chrysin.

ACKNOWLEDGEMENT

The first author acknowledges the fellowship provided by UGC, New Delhi.

REFERENCES

1. Whalley DC, Rankin SM, Hoult JRS, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol 1990; 39: 1743-1750.
2. Villar IC, Jimenez R, Galisteo M, Garcia-Saura MF, Zarzuelo A, Duarte J. Effects of chronic chrysin treatment in spontaneously hypertensive rats. Planta Med 2002; 68: 847-850.
3. Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A, Tripodo MM. Biological effects of hesperidin, a citrus flavonoid. (Note I): anti-inflammatory and analgesic activity. Farmaco 1994; 40: 709-712.
4. Emim JA, Oliveira AB, Lapa AJ. Pharmacological evaluation of the anti-inflammatory activity of a citrus bioflavonoid, hesperidin, and the isoflavonoids, duartin and claussequinone, in rats and mice. J Pharm Pmarmacol 1994; 46: 118-122.
5. Lonchampt M, Guardiola B, Sicot N, Bertrand M, Perdrix L, Duhault J. Protective effects of a purified flavonoid fractions against reactive oxygen radicals. In vivo and in vitro study. Arzneimittelforschung 1989; 39: 882-885.
6. Damon P, Flandre O, Michel F, Pedrix L, Labrid C, Crastes PA. Effect of chronic treatment with a purified flavonoid fraction on inflammatory granuloma

in the rat. Study of prostaglanidin E2 and F2α and thromboxane B2 release and histological changes. Arzneimittelforschung 1987; 37: 1149-1153.
7. Ratty AK, Das NP. Effects of flavonoids on nonenzymatic lipid peroxidation: structure activity relationship. Biochem Med Metab Biol 1988; 39: 69-79.
8. Fukuda K, Ohta T, Yamazoe Y. Grapefruit component interacting with rat and human CYP3A: possible involvement of non-flavonoid components in drug interaction. Biol Pharm Bull 1997; 20: 560-564.
9. Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochromeP4503A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol Carcinogen 1995; 13: 129-136.
10. Guengerich FP, Brian WR, Iwasaki M, Sart MA, Baarnhielm C. Oxidation of dihydropyridine calcium channel blockers by human liver cytochrome P450 3A4. J Med Chem 1991; 34: 1838-1844.
11. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 1999; 39: 361-370.
12. Terao T, Hisanaga E, Sai Y, Tamai I, Tsuji A. Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier. J Pharm Pharmacol 1996; 48: 1083-1089.
13. Tsuji A, Tamai I. Carrier-mediated intestinal transport of drugs. Pharm Res 1996; 13: 963-977.
14. Hunter J, Hirst BH, Simmons NL. Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers. Pharm Res 1993; 10: 743-749.
15. Wils P, Phung-Ba V, Warnery A, Lechadeur D, Raeissi S, Hidalgo IJ, Scherman D. Polarized transport of docetaxel and vinblastine mediated by P-glycoprotein in human intestinal epithelial cell monolayers. Biochem Pharmacol 1994; 48: 1528-1530.
16. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 1987; 84: 7735-7738.
17. Holash JA, Stewart PA. The relationship of astrocyte-like cells to the vessels that contribute to the blood-ocular barriers. Brain Res 1993; 629: 218-224.
18. Yumoto R, Murakami T, Nakamoto Y, Risa H, Nagai J, Takano M. 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 Pharmacol Exp Ther 1999; 289: 149-155.
19. Narinkonic Y, Aqbaba P, Yladimiror S, Stankovic S. Simultaneous HPLC determination of nitrendipine and impurities of the process of synthesis. J Pharm Biomed Anal 2001; 24: 993-998.
20. Trezise AE, Romano PR, Gill DR, Hyde SC, Higgins CF. The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. EMBO J 1992; 11: 4291-4303.
21. Fromter E, Diomond J. Route of passive ion permeation in epithelia. Nature 1972; 235: 9-13.

22. Lo YL, Huang JD. Comparison of effects of natural or artificial rodent diet on etoposide absorption in rats. In Vivo 1999; 13: 51-55.