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Original Article

Inhibition of P-glycoprotein expression and function by anti-diabetic drugs gliclazide, metformin, and pioglitazone *in vitro* and *in situ*

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Abstract

P-glycoprotein (P-gp) is a trans-membrane drug efflux pump. Several drugs are P-gp substrates. Some drugs may affect the activity of P-gp by inhibiting its function, resulting in significant drug-drug interactions (DDIs). It is critical to understand which drugs are inhibitors of P-gp so that adverse DDIs can be minimized or avoided. This study investigated the effects of gliclazide, metformin, and pioglitazone on the function and expression of P-gp. Rhodamine 123 (Rh 123) efflux assays in Caco-2 cells and western blot testing were used to study *in vitro* the effect of the drugs on P-gp function and expression. The *in situ* rat single-pass intestinal permeability model was developed to study the effect of the drugs on P-gp function. Digoxin and verapamil were used as a known substrate and inhibitor of P-gp, respectively. Digoxin levels in intestinal perfusion samples were analyzed by high-performance liquid chromatography. Intestinal effective permeability (P_{eff}) of digoxin in the presence of 0.1, 10, and 500 µM gliclazide, 100 and 7000 µM metformin, and 50 and 300 µM pioglitazone was significantly increased relative to the digoxin treated cells ($P < 0.01$). P-gp expression was decreased by gliclazide, metformin and pioglitazone. Intracellular accumulation of Rh 123 by the drugs increased, but the differences were not significant relative to the control cells ($P > 0.05$). It was found that gliclazide, metformin, and pioglitazone inhibited P-gp efflux activity *in situ* and down-regulated P-gp expression *in vitro*. Further investigations are necessary to confirm the obtained results and to define the mechanism underlying P-gp inhibition by the drugs.

Keywords: Gliclazide; Intestinal permeability; Metformin; P-glycoprotein; Pioglitazone

INTRODUCTION

The oral route is the most preferable and popular form of administration of drugs. After oral administration, the extent and rate of absorption of a drug depend on its concentration in the intestinal lumen and permeability across the intestinal epithelia. Sufficient intestinal absorption is necessary for successful therapy. The physicochemical properties of a drug, the physiological functions and properties of the gastrointestinal tract, and the epithelial barrier all influence intestinal drug absorption (1). The role of membrane transporters in drug permeability and bioavailability is very important.

P-glycoprotein (P-gp; MDR1; ABCB1) is a 170 kDa trans-membrane ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. Several drugs, for example digoxin, are P-gp substrates. P-gp is expressed in tissues such as intestinal epithelium, hepatocytes, renal proximal tubular cells, and the blood–brain and blood–testis barriers (2).

Some drugs, herbs, and food components may affect the activity of P-gp by inhibiting or inducing its function. P-gp inhibitors increase the permeability and subsequently serum concentration of the P-gp substrates and can cause unwanted and sometimes harmful effects. It is important to know which drugs are P-gp inhibitors or substrates to avoid or minimize unwanted drug-drug, herb-drug, and food-drug interactions (1,2). This study was designed and carried out to investigate the

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probable inhibitory effects of the anti-diabetic drugs gliclazide, metformin, and pioglitazone on P-gp.

Gliclazide has been used in the treatment of non-insulin dependent diabetes mellitus (DM) and is the drug of choice in effective long-term sulfonylurea therapy for treatment of type II DM. The slow absorption rate of the drug usually results from the poor dissolution rate of formulation and also poor permeability across the gastrointestinal membrane (3-5). The drug stimulates insulin release from pancreatic β-cells that lowers blood glucose (5,6).

Metformin is one of the most commonly-used drugs for the treatment of DM; especially type II DM. It reduces serum glucose level through non-pancreatic mechanisms without augmenting insulin secretion. As an insulin sensitizer, it increases the effects of insulin. Metformin also suppresses endogenous glucose production by the liver through reducing gluconeogenesis rate and glycogenolysis. It activates the adenosine monophosphate kinase (AMPK) enzyme, resulting in the inhibition of gluconeogenesis and glycogen synthesis in the liver. Metformin also stimulates insulin signaling and glucose transport in muscles and decreases the amount of blood sugar that the intestine or stomach absorbs (7-9).

Pioglitazone is a thiazolidinedione anti-diabetic drug that enhances insulin sensitivity in target tissues. The drug binds to the peroxisome proliferator activated receptor gamma (PPAR-γ), a nuclear receptor and, to a lesser extent, PPAR-α followed by modulation of the transcription of insulin-sensitive genes involved in the control of glucose and lipid metabolism. A slow rise in insulin sensitivity in skeletal muscle, hepatic, and fat cells results from this function. Pioglitazone does not increase insulin secretion, but increases the peripheral action of insulin (10-13). The drug reduces insulin resistance in the liver and peripheral tissues and also the quantity of glucose, insulin, and glycated hemoglobin in the bloodstream. It decreases withdrawal of glucose from the liver and increases the expense of insulin-dependent glucose (12).

The present study investigated the *in vitro* and *in situ* effects of different concentrations of gliclazide, metformin, and pioglitazone on P-gp function as a membrane transporter. Rhodamin 123 (Rh 123) and digoxin were used as known substrates and verapamil as a known inhibitor of P-gp. The *in vitro* study was performed by Rh 123 accumulation assay in Caco-2 cells after treatment with the drugs and verapamil. Expression of P-gp in Caco-2 cells was also assayed using the western blot method after treatment with the drugs and verapamil. The *in situ* study was carried out on rats for single-pass intestinal permeability to determine whether intestinal P-gp-mediated digoxin transport was affected by the drugs. Digoxin has been shown to be a typical substrate of P-gp and is widely-used as an *in vivo* and *in vitro* probe to measure P-gp activity (14,15). The effective permeability of digoxin was calculated and compared in the presence and absence of the drugs and verapamil. This study is the first to investigate the effects of gliclazide, metformin, and pioglitazone on the function and expression of P-gp *in situ* and *in vitro*.

MATERIALS AND METHODS

In vitro study

Cell culture

Caco-2 cells were purchased from Pasteur Institute of Iran (Tehran, Iran). The cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (Sigma Aldrich, USA). The cells were maintained in a CO_2 incubator (Memmert, Germany) at 37 °C with 90% relative humidity and 5% CO_2 atmosphere. The culture medium was replaced 2-3 times a week. After two weeks and reaching near confluence 80%-90%, the cells were detached from the culture flask by adding 0.25% trypsin EDTA solution. Cells were then seeded at the required density to wells or flasks for sub-culturing or testing (16).

MTT cytotoxicity study

Although the concentrations of drugs used in this study were in the therapeutic range, their cytotoxicity was tested by MTT (3-(4,5-dime-thylthiazol-2-thiazolyl)-

2,5-diphenyl-tetrazoliumbromide) assay. The Caco-2 cells were seeded into 96-well plates at a density of 15×10^3 cells per well. After 24 h, the medium was replaced with 200 µl/well of the drugs in the relevant concentrations diluted with complete culture medium or by complete culture medium as a control. After 24 h of incubation, MTT solution (2 mg/ml final concentration) was added and incubated for 4 h at 37°C in a CO_2 incubator. The MTT solution was removed and the resulting formazan crystals were solubilized IN 200 µl/well of DMSO and 25 µl/well Sorensen buffer. The optical densities measured using a microplate reader (Statfax 2100, Awareness; USA) at 570 nm with background subtraction at 630 nm. The following formula was used to calculate the percentage of cell viability. MTT assay was performed in triplicate for the control as well as each concentration. Cell viability mean ± standard deviation (SD) was calculated and analyzed statistically for each concentration.

$$Cell\ viability\ \% = \frac{OD\ value\ of\ the\ test}{OD\ value\ of\ the\ control} \quad (1)$$

Rhodamine 123 efflux assay

P-gp function in Caco-2 cells was assessed by measurement of intracellular accumulation of Rh 123, which is inversely proportional to P-gp activity. Caco-2 cells were seeded in 24-well plates and allowed to attach for 24 h in a CO_2 incubator. The old medium was removed and the cells washed with PBS. Gliclazide (500 µM), metformin (7000 µM), pioglitazone (500 µM), and verapamil (300 µM, as a P-gp inhibitor) in culture media were added to the wells. After 24 h, the old medium was removed and cells were washed three times with PBS. Rh 123 solution (DMEM containing 10 mM HEPES (pH = 7.4) and 5 µM Rh 123) was added and incubated for 3 h at 37 °C, then the cells were washed three times with ice-cold PBS (pH = 7.4). The cells were lysed in 1% Triton X-100 and centrifuged (3-18 k; Sigma) at 1500 g for 10 min. Supernatant was used to measure the Rho123 and protein contents. The intra-cellular accumulation of Rh 123 in each sample was determined quantitatively by fluorescence spectrophotometry at an exciting wavelength of 485 nm and emission wavelength of 532 nm (FP-750, Jasco, Japan). The protein content of the samples was assayed using a commercial kit (Pars Azmoon, Iran) and the Rho123 values were normalized using the protein values of supernatants (17,18).

Western blot analysis of P-gp expression

To assay the expression of P-gp in the Caco-2 cells after treatment with gliclazide, metformin, pioglitazone, digoxin, and verapamil, the Caco-2 cells were seeded into a 6-well plate in a density of 10^6 cells per well. After 24 h, the cells were treated with culture medium (control), containing a drug or verapamil (300 µM) as a P-gp inhibitor. After 48 h, the solutions were removed. The cells were washed with PBS and incubated for 5 min with 0.25% trypsin-EDTA at 37 °C. The detached cells were washed twice with PBS. Lysis buffer (Triton X-100 50 mM, NaCl 150 mM, EDTA 5 mM, 1 % protease inhibitor cocktail (Sigma Aldrich, USA)), and Tris-HCl, pH = 7.4 was added and the cell suspension centrifuged at 1500 g for 5 min.

The proteins were separated by electrophoresis through 12.5% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE, 80 V, 120 min). The gel was electro blotted to a poly-vinylidene di-fluoride (PVDF) membrane using the semi-dry western blotting system (Bio-Rad, USA). All membranes were blocked for nonspecific binding by incubation in 3% nonfat-dried milk for 1 h at room temperature. The membrane was then washed three times with Tris-buffered saline (TBS; with 0.1% Tween 20) and incubated overnight with mouse monoclonal anti-P-gp antibodies (1:1000 in TBS) (AB80594, Abcam, UK). On the next day, the membrane was washed with TBS and incubated with horseradish peroxidase-conjugated rabbit anti-mouse secondary antibodies (AP8036, Razi Biotech, Iran) for 2 h.

The membrane was washed and the proteins were detected using an ECL kit (Amersham, GE Healthcare, Italy). The proteins were visualized by exposing the membrane to medical x-ray film (Fuji, Japan) for 5 min in a dark room. β-actin was considered as the internal standard and was detected using rabbit polyclonal anti-β-actin (AB16039, Abcam) as

the primary antibody and HRP-conjugated goat anti-rabbit IgG (AP7181, Razi Biotech) as the secondary antibody. P-gp expression was presented as the ratio of intensity of the P-gp band to the β-actin band in the same blot run (P-gp/β-actin) (19).

In situ study

Intestinal permeability is described by the ability of a compound to move across the intestinal epithelial barrier and represents a direct measurement of the local absorption rate. It is expressed as centimeters per second (cm/s) (20).

Animals

Male Sprague-Dawley rats (200–250 g) were obtained from Pasteur Institute of Iran (Tehran, Iran). The animals were housed under standard conditions (22 ± 2 ℃, 12/12 h light/dark cycle, 55%–60% relative humidity) with free access to standard rodent chow and clean water. The rats were fasted for 12-16 h prior to experimentation (water was available *ad libitum*). All procedures were conducted in accordance with the human and animal ethical protocols. The animal study protocols were approved by the research ethics committee of Tabriz University of Medical Sciences (Ref No: TBZMED.REC.1394.378).

Single-pass intestinal perfusion

Single-pass intestinal perfusion (SPIP) was performed on a total of 33 animals divided into five groups. The groups consisted of: blank solution (n = 3), blank solution containing 20 µM digoxin (n = 3), blank solution containing digoxin and 300 µM verapamil as a typical p-gp inhibitor (n = 3), blank solution containing digoxin and 1, 10, and 500 µM gliclazide (n = 9 and 3 for each concentration), blank solution containing digoxin and 1, 100, and 7000 µM metformin (n = 9 and 3 for each concentration), and blank solution containing digoxin and 1, 50, and 300 µM pioglitazone (n = 9 and 3 for each concentration). The blank solution was PBS (pH = 7.4) containing 50 mg/l phenol red (without drug). Because water absorption and secretion and other parameters could change during testing and cause errors in the permeability results, 50 mg/l phenol red as a non-absorbable marker was added to the perfused solutions to correct the results.

The animals were anesthetized with an intra-peritoneal injection of thiopantal sodium (60 mg/kg). A 3-4 cm incision was made in the abdominal midline and approximately 9-11 cm segment of the jejunum was selected and cannulated at both ends with plastic tubes. The exposed segment was kept moist with body-tempered saline during the experiment. Before injecting the main drug solution, body-tempered normal saline was passed through the cannulated segment to wash and clear the lumen of the segment. The solution containing the substance of interest was then administered at a constant perfusion flow rate of 0.2 ml/min by a volumetric infusion pump (Argus Medical AG, Switzerland). After achieving a stable status, the perfusate was quantitatively collected at 10 min intervals (2 ml). Each perfusion experiment lasted for 90 min. At the end of the procedure, the animal was euthanatized under anesthetic conditions and the length of the segment was measured (cm). Samples were stored at -70 ℃ in an ultra-low-temperature freezer (Jal Tajhiz, Iran) for phenol red and digoxin analysis. Blank perfused solution was collected at the outlet and used to prepare the calibrate solutions as well as performing stability studies (20,21).

The concentration of phenol red in the perfused (outlet) samples was measured at 560 nm using an ultraviolet-visible (UV-VIS) spectrophotometer (Ultra-spec 2000, Pfizer USA) (22). The amount of digoxin in the perfused samples was detected by high-performance liquid chromatography (HPLC).

High-performance liquid chromatography analysis of digoxin in intestinal perfused samples

The HPLC system was composed of a Smartline manager 5000, Smartline UV detector 2600, and Smartline Pump 1000 (Knauer Advanced Scientific Instruments, Germany). The mobile phase for digoxin analysis was 35% (v/v) acetonitrile/water which was filtered through sintered a 1.0-1.6 µm pore glass filter P5 (Winteg, Germany) and degassed in a sonicator. The mobile phase was pumped in isocratic mode at a flow rate of 2

ml/min at ambient temperature. UV detection was used at 218 nm and samples of 20 µl were injected using a Hamilton injector syringe (Hamilton, Switzerland) onto the column (Knauer 15VE081ESJ, 150 × 4.6 mm with precolumn-eurospher 100-5 C8, Knauer Advanced Scientific Instruments, Germany) (21).

Data analysis

The corrected outlet concentration of the drug (C_{out}) was calculated as (23):

$$C_{out}(corr) = C_{out} \times \frac{CPR_{in}}{CPR_{out}} \quad (2)$$

where, C_{out} (corr) is the corrected outlet concentration of the drug, C_{out} is the outlet concentration of the drug, CPR_{in} is the concentration of phenol red entering the intestinal segment, CPR_{out} is the concentration of phenol red leaving the intestinal segment.

Effective permeability (P_{eff}) was calculated from the steady state concentrations of digoxin in the perfusate collected from the outlet. Steady state was confirmed by the ratio of the outlet to the inlet concentration (corrected for phenol red concentrations) versus time as shown in Fig. 1 (20, 21).

The effective steady-state intestinal permeability (P_{eff}) was calculated as:

$$P_{eff} = \frac{Q_{in} \times ln\,(C_{out}/C_{in})}{2 \times 60\pi rl} \quad (3)$$

where, P_{eff} is the effective permeability (cm/s), Q_{in} is the perfusion rate (0.2 ml/min), C_{in} is the concentration of the test drug entering the segment, C_{out} is the concentration of the test drug leaving the segment, r is the radius of the intestinal segment (≈ 0.18 cm), and l is the length of the intestinal segment (cm).

Statistical analysis

The data was expressed as mean ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The differences between groups were determined using the unpaired t-test with SPSS 13.0 and was considered significant at $P < 0.05$ and $P < 0.01$.

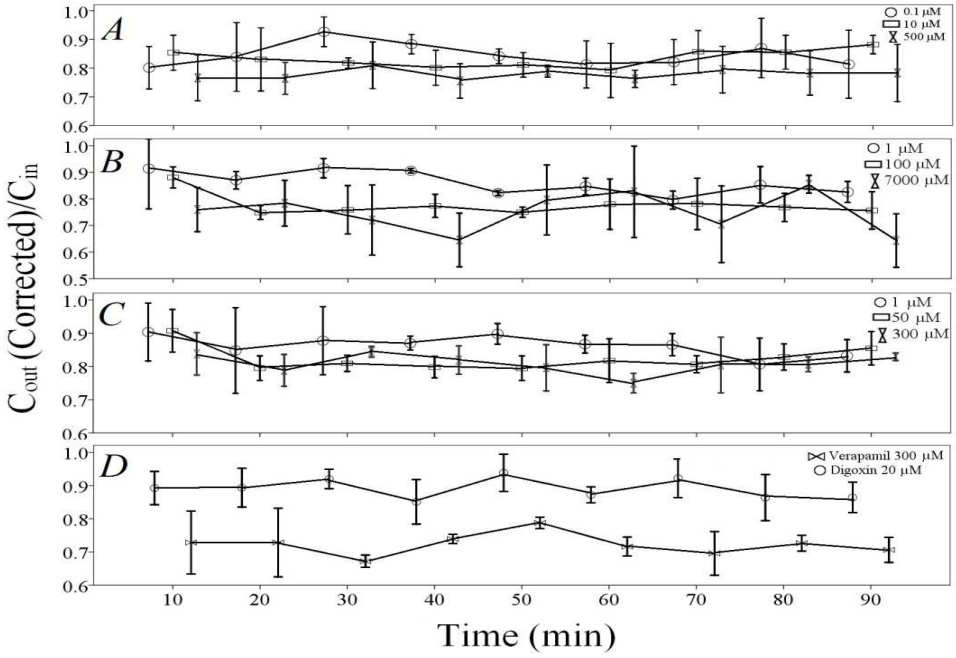

Fig. 1. Plots of outlet and inlet concentrations ratio (Cout/Cin) of digoxin (20 µM) *vs* time for A; gliclazide , B; metformin, C; pioglitazone, D; digoxin and verapamil in rat SPIP study (n = 3 for each concentration of each drug, error bars represent mean ± SD).

RESULTS

In vitro study

Cytotoxicity

Statistical analysis showed that there was no significant difference in cell viability among the treatment groups ($P < 0.05$) and the concentrations used were considered appropriate for use in the *in vitro* and *in situ* tests.

Rhodamine 123 efflux assay

Fig. 2 shows the results of treatment with 500 µM gliclazide, 7000 µM metformin, and 500 µM pioglitazone which increased the intracellular accumulation of Rh 123 in Caco-2 cells, but the differences were not statistically significant ($P < 0.05$). The mean intracellular concentration of Rh 123 in the control cells was 424.4 ± 37.3 and in the gliclazide-, metformin-, pioglitazone-, and verapamil-treated cells were 519.3 ± 109.8, 470.0 ± 109.7, 485.0 ± 39.0, and 749.4 ± 32.1 pg/mg protein, respectively.

Immuno-blotting

P-gp expression is the ratio of P-gp band intensity to β-actin band intensity (P-gp/β-actin) and was compared with the verapamil control bands in the same blot run (24). Gliclazide, metformin, pioglitazone, and verapamil treatment decreased P-gp protein levels relative to the untreated control as shown in Fig. 3.

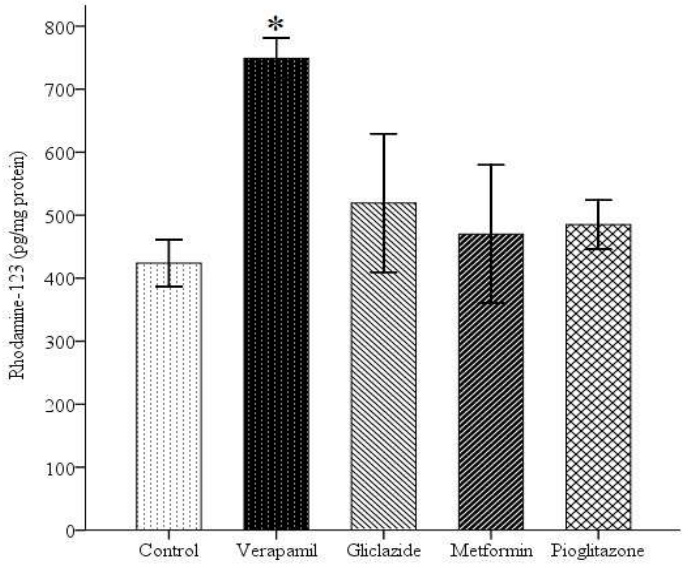

Fig. 2. Effects of gliclazide, metformin, pioglitazone, and verapamil on intracellular accumulation of Rh 123 in Caco-2 cells (n = 4). Bars show mean ± SD. * and ** represent significantly different from control (untreated) cells ($P < 0.05$ and $P < 0.01$, respectively).

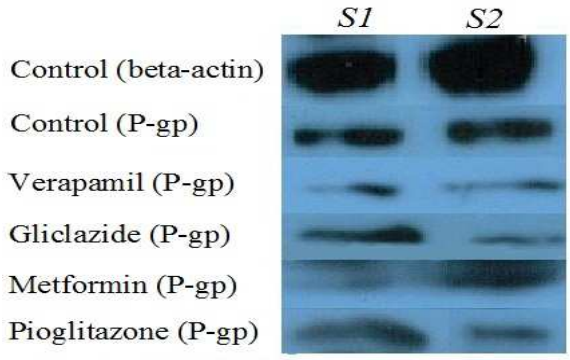

Fig. 3. Western blot of P-gp and β- actin in gliclazide (500 µM), metformin (7000 µM), pioglitazone (300 µM), and verapamil (300 µM) treated, and control (non-treated) Caco-2 cells (bands of 2 samples of each are shown).

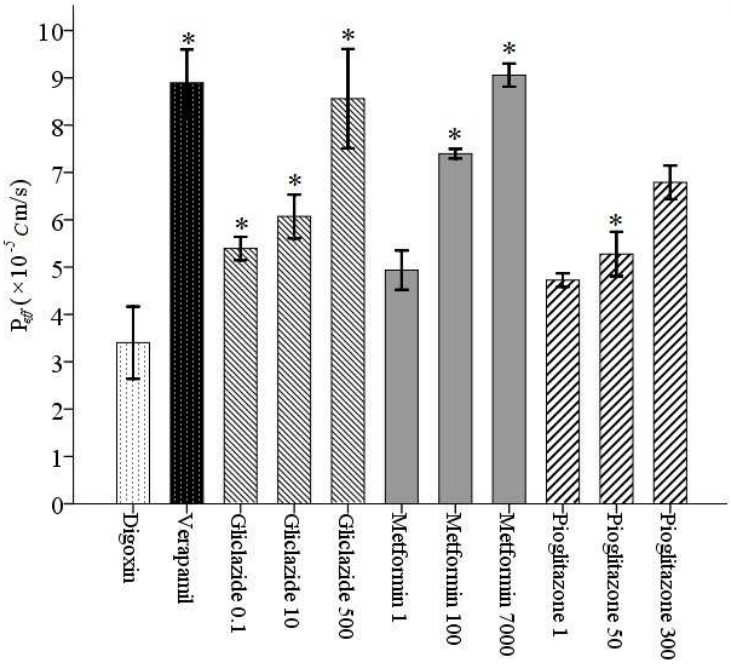

Fig. 4. Effects of different concentrations of gliclazide, metformin, pioglitazone, and verapamil (P-gp inhibition control) on the effective permeability (P_{eff}) of digoxin compared to the control group (n = 3). Bars show mean ± SD. * represent significantly different from control (digoxin) group ($P < 0.01$).

In situ study

Adsorption experiments

Preliminary adsorption experiments showed no considerable adsorption of compounds onto the tubing or syringe and that the stock and working standard solutions of digoxin and phenol red were stable during the experiments.

Single-pass intestinal permeability

The P_{eff} values of digoxin (20 µM) in the absence and presence of verapamil were 3.4 ± 0.8 and 8.9 ± 0.7 × 10^{-5} cm/s, respectively. These were 5.4 ± 0.2, 6.0 ± 0.5, and 8.6 ± 1.0 ×10^{-5} cm/s for 0.1, 10, and 500 µM gliclazide, respectively. The P_{eff} values for digoxin in the presence of 1, 100, and 7000 µM metformin were 4.9 ± 0.4, 7.4 ± 0.1, and 9.1 ± 0.3 ×10^{-5}cm/s, respectively. The P_{eff} values for digoxin in the presence of 1, 50, and 300 µM pioglitazone were 4.7 ± 0.2, 5.3 ± 0.5, and 6.8 ± 0.4 ×10^{-5}cm/s, respectively. Fig. 4 shows that gliclazide treatments significantly increased the P_{eff} of digoxin relative to the control group (digoxin alone) ($P < 0.01$). Treatment with 1 µM metformin and 1 µM pioglitazone did not significantly increase the P_{eff} of digoxin relative to the control group ($P > 0.05$), but P_{eff} at high concentrations, 100 and 7000 µM metformin, and 50 and 300 µM pioglitazone was significantly different ($P < 0.01$).

DISCUSSION

Although treatment of Caco-2 cells with 500 µM gliclazide increased the accumulation of Rh 123 by 22% compared with the control (Fig. 2), the difference was not statistically significant ($P > 0.05$). The results of the rat *in situ* SPIP showed that 0.1, 10, and 500 µM gliclazide significantly increased digoxin P_{eff} by about 59%, 76%, and 153%, respectively (Fig. 4). Digoxin is a well-known substrate of P-gp and enhancement of its permeability may be caused by inhibition of P-gp efflux. The results of the *in situ* study showed dose-dependent inhibition of P-gp with gliclazide in the range of 0.1-500 µM. Protein expression of P-gp for 500 µM gliclazide-treated Caco-2 cells was low relative to the control cells, confirming inhibition of P-gp by 500 µM gliclazide.

Maghrebi and colleagues monitored intestinal absorption in rats and recorded no

net intestinal absorption for gliclazide. They suggested a role for P-gp efflux pump transporter in limiting intestinal absorption of gliclazide (25). Previous studies have found that cytochrome P450 2C9 (CYP2C9) and CYP2C19 are involved in metabolism of gliclazide (10,26,27). Studies on the pharmacokinetics and pharmacodynamics of drugs affecting P-gp have found that there is considerable overlap in drug specificity for P-gp and some CYP enzymes (2). These findings are in accordance with the findings of the present study.

Metformin (7000 µM) treated Caco-2 cells exhibited 10.7% more accumulation of Rh 123 than the control cells (Fig. 2), but the difference was not statistically significant ($P > 0.05$). In contrast, in the rat SPIP test, 100 and 7000 µM of metformin significantly increased the P_{eff} of digoxin by 117.6% and 167.6% relative to the digoxin-alone treated control cells, respectively ($P < 0.01$). Although 1 µM metformin increased the P_{eff} of digoxin by about 44%, the effect was not statistically significant (Fig. 4). As shown in Fig. 5, the inhibition by metformin *in situ* on P-gp was dose-dependent. P-gp expression in Caco-2 cells treated with 100 µM metformin was less dense than that in non-treated cells (Fig. 3), illustrating the inhibitory effect of metformin on P-gp.

Previous *in vitro* and *in vivo* experimental reports revealed that metformin is a P-gp substrate in placental apical membranes (28), but not in rat intestinal epithelia (29). There is also evidence showing P-gp inhibitory effect of metformin, which confirms the present findings. The data obtained by Hemauer and colleagues indicate that metformin significantly inhibits the transport of [3H]-rosiglitazone with P-gp (28). Aldea and colleagues showed that treatment with metformin increased the concentration of Rh 123 in cancer cells as a result of the P-gp inhibitory effect of metformin (30). Kim and colleagues found that metformin down-regulated the expression of the P-gp through the AMPK-mediated inhibition of nuclear factor κB and cAMP response element transcriptional activity in MCF-7/adriamycin cells (31).

The results of the *in vitro* Rh 123 uptake tests indicate that treatment of Caco-2 cells with 500 µM pioglitazone increased the accumulation of Rh 123 by 14% compared with the control (Fig. 2), but the difference was not statistically significant ($P > 0.05$). It was also found *in situ* that 50 and 300 µM pioglitazone significantly amplified digoxin P_{eff} by about 51% and 100%, respectively ($P < 0.01$; Fig. 4). Although 1 µM pioglitazone increased digoxin P_{eff} by about 38%, this was not statistically significant; however, the results of the *in situ* study showed a dose-dependent effect of pioglitazone of 1-300 µM on P-gp. Protein expression of P-gp in pioglitazone-treated Caco-2 cells was low relative to the control cells, confirming the inhibition of P-gp by pioglitazone. In accordance with the current findings, Choi and colleagues reported that bioavailability of nifedipine was augmented in the presence of pioglitazone which could be attributed to the inhibition of P-gp-mediated efflux transporter of nifedipine with pioglitazone in the small intestine (13). On the other hand, Chang and colleagues found the role of P-gp as a barrier to pioglitazone and a substrate of P-gp reached the brain (32). Previous studies have demonstrated that CYP2C8 and CYP3A4 are enzymes involved in the metabolism of pioglitazone *in vivo*. Pioglitazone inhibits CYP2C8 and CYP3A4 *in vitro* (10). It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap in the limited absorption of drugs (13).

The present findings showed that the anti-diabetic drugs gliclazide, metformin, and pioglitazone inhibit P-gp activity *in situ* and also down-regulate P-gp expression *in vitro*. After oral administration and complete dissolution of the drugs, because local drug concentrations in the intestinal lumen may be high, intestinal P-gp can be effectively inhibited by P-gp inhibitor drugs. This can result in increased exposure of any co-administered drug that is a substrate of P-gp and may cause unwanted and sometimes harmful DDIs), thus, it is essential to predict and minimize potential DDIs in the intestine (16). P-gp expression and activity is dependent on factors such as animal species variability,

individual genetic makeup, age, and disease status. Further study is required to confirm the present findings and better understanding of the mechanism of P-gp inhibition by the drugs.

CONCLUSION

The results of the current study showed that anti-diabetic drugs gliclazide, metformin, and pioglitazone inhibited P-gp efflux activity *in situ* in a dose-dependent manner and also down-regulated P-gp expression *in vitro*. The inhibitory effects of the drugs on P-gp should be considered to predict potential DDIs when drugs are co-administered with drugs that are P-gp substrates. Further investigation is necessary to confirm these results and to define the underlying P-gp inhibitory mechanisms of the drugs.

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