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Short communication

The improved everted gut sac: a simple method to study intestinal P-glycoprotein

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Abstract

P-glycoprotein (P-gp) is an energy-dependent transporter protein located in the apical membrane of intestinal mucosal cells. It is believed that it may limit the bioavailability of many orally administered drugs, by transporting them back into the intestinal lumen following their absorption by the enterocytes. To demonstrate the activity of the intestinal P-gp in vitro, we have used the 'improved' rat everted gut sac system, where good tissue viability and metabolic activity is maintained by incubating the everted sacs in tissue culture medium. Digoxin was used as the test drug. After 90 min incubation, its transport across the intestinal mucosa was enhanced 2.5-fold by the P-gp inhibitor verapamil, and 5-fold by the inhibitor quinidine. In the presence of the three drugs, the sacs showed uniform kinetics and good viability, as assessed by the active transport of glucose and lack of release of cellular enzymes. The improved everted gut sac system has potential as a simple and efficient tool to evaluate the role of P-gp in the intestinal absorption of drugs, and to screen for putative P-gp inhibitors that may improve the bioavailability of drugs susceptible to transport by P-gp.

Keywords: P-glycoprotein; Everted gut sac; Digoxin; Verapamil; Quinidine

P-glycoprotein (P-gp) is an energy-dependant efflux pump associated with the multidrug resistance in tumor cells, but also expressed in a variety of normal human tissues including liver, brain, kidney and the gastrointestinal tract (Thiebault et al., 1987). At the intestinal level, P-gp is located on the apical membrane of the

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mature intestinal cells and acts as a pump that transports drugs back into the lumen as they are absorbed across the intestinal mucosa (Hebert, 1997; Watkins, 1997). There is currently considerable interest in intestinal P-gp and the central role it plays in limiting the oral bioavailability of a wide class of drugs (Benet et al., 1996). Studies on different in vitro models, have shown that the cardiac glycoside digoxin is a substrate of P-gp in the intestine (Cavet et al., 1996; Su and Huang, 1996), and these observations have been confirmed in vivo using mice lacking the MDR 1-type P-glycoproteins (Mayer et al., 1997). Verapamil and quinidine are drugs that are known to inhibit P-gp (Ford and Hait, 1990). By comparing the transport kinetics of [^{3}H]digoxin in the absence or in presence of verapamil or quinidine, the objective of the study was to demonstrate that the improved everted gut sac method is a simple and efficient in vitro model to study the intestinal role of P-gp and to test its potential substrates and/or inhibitors.

The everted gut sac technique consisted of gently everting a freshly excised small intestine (rat), filling it with oxygenated tissue culture medium (TC199) at 37°C, and dividing it into sacs approximately 2.5 cm in length, using braided suture silk. Each sac was then placed in an Erlenmeyer flask (50 ml) containing 9.0 ml of pregassed (95% O_2; 5% CO_2) TC199 at 37°C with or without the P-gp inhibitor (verapamil 100 μM or quinidine 1.3 mM, final concentrations), to which had been added 1 ml of medium containing 100 μM digoxin 'spiked' with [^{3}H]digoxin. The inhibitor concentrations were chosen to allow comparison with other studies (see later). The use of tissue culture medium ensures excellent tissue viability and metabolic activity and is a considerable improvement on the basic technique (Barthe et al., 1998). 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, 5 ml of Ready Safe™ scintillation fluid was added, and the samples counted in a Beckmann LS 1801 scintillation counter. From the counts and the sac contents volume the nmoles of digoxin present in each sac were calculated. For the tissue samples, 0.8 ml of the digest was neutralised with 0.2 ml of 5 N HCl before being mixed with 5 ml of scintillant and counted. The protein content of the sac digest was determined spectrophotometrically using the method described by Peterson (1986), with bovine serum albumin as the standard. The transport of digoxin into the tissue and from the mucosal to the serosal side of the mucosa was calculated as nmol transported/mg of total sac protein. To verify the integrity of the sacs, glucose was measured both in the incubation medium and in the sac contents using a modification of the method described by Lloyd and Whelan (1969). As glucose is actively transported by small intestine, healthy metabolically active sacs that are not leaking, will accumulate glucose in the serosal medium. A similar increase in the serosal glucose concentration was observed with all the compounds tested, an increase of between 5- and 7-fold, after 90 min incubation. The potential toxicity of digoxin, verapamil and quinidine at the concentrations used was also assessed by measuring the release into the incubation medium of two enzymes: (1) the standard marker of cytotoxicity, lactate dehydrogenase (LDH) a cytosol enzyme and, (2) alkaline phosphatase (AP), a membrane enzyme localised on the brush border. None of the drugs caused any release above control of LDH and AP into the incubation medium, demonstrating no toxicity at the cellular level. Fig. 1 shows that verapamil (100 μM) and quinidine (1.3 mM) significantly increased the accumulation of digoxin (10 μM) in the mucosal tissue. Fig. 2 shows that the two drugs significantly increased the transport of digoxin (10 μM) across the mucosa into the sac contents. At 90 min, the enhancement of transport across the mucosa was approximately 5-fold in the presence of quinidine and 2.5-fold in the presence of verapamil ($p < 0.01$). These results confirmed that, in

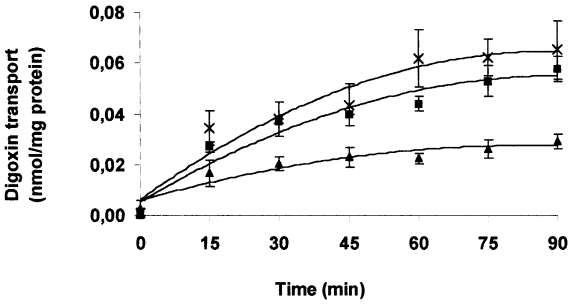

Fig. 1. Transport of digoxin into everted gut sac tissues in the presence of verapamil or quinidine in the incubation medium. (▲) Digoxin (10 μM) alone; (×) digoxin (10 μM) + quinidine (1.3 mM); (■) digoxin (10 μM) + verapamil (100 μM). The data represent the means ± S.E. ($n = 3$ sacs at each time point). R^2 values: 0.91, 0.94, 0.94, respectively. At 90 min, $p < 0.05$ (t-test).

the improved everted gut sac model, digoxin is a substrate of the intestinal P-gp, and shows uniform kinetics of uptake. With the Caco-2 monolayer model, using the same concentrations of verapamil as in the current study, Cavet et al. (1996) observed an enhancement of 7-fold for the transport across the cell layer of digoxin in the presence of verapamil. Caco-2 cells were originally derived from a human colon carcinoma, and this cancer-derived cell line has properties different from normal small intestinal cells. In particular, the rates of drug transport are very much lower, especially for poorly absorbed drugs. The difference in results obtained with the two models could be explained by the overexpression of P-gp on the Caco-2 cells, resulting in a more marked enhancement of the absorption of digoxin in presence of verapamil. Also the concentration of digoxin used was very low (3.6 nM). Using a different everted gut sac technique, Su and Huang (1996) observed a 2-fold increase in digoxin transport in the presence of 1.3 mM quinidine, compared with our 5-fold increase. However, these authors used a single 25-cm everted sac containing a very small volume of serosal fluid (3 ml), and an extremely low concentration of digoxin (0.74 nM). In addition, in their method, the sacs were incubated in a simple salt-containing media/buffer. Viability of the intestinal tissue may be very poor under such conditions. In early histological studies, Levine et al. (1970) had showed that after 30 min incubation in salt-buffered medium, 50–75% of the normal epithelium had disappeared, and at 1 h there was a total disruption of the epithelial border. Plumb et al. (1987) also observed that when everted gut sacs were incubated for 20 min in simple media, there was severe interstitial oedema and disruption of the villus epithelium. The use of tissue culture medium in our method greatly enhances the viability of the tissue, as shown by the active transport of glucose, as well as histology at the electron microscope level, and gives rates of transport much greater than Caco-2 cells (Barthe et al., 1998). Clearly if the sacs were not biochemically active, or, if they were not physically intact, a glucose concentration gradient would not be maintained. In conclusion, our everted gut sac technique, using a complex tissue culture media, is a simple and effective method to study the action of intestinal P-gp on intestinal drug absorption in vitro. The method has considerable potential for the rapid screening and evaluation of potential inhibitors of intestinal P-gp. Such inhibitors may have a significant role to play in the enhancement of the intestinal absorption of many drugs which have low or variable bioavailability due to the action of the mucosal P-gp.

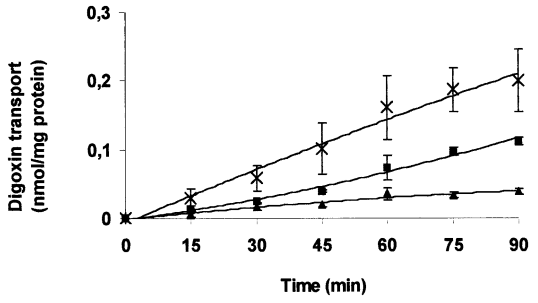

Fig. 2. Transport of digoxin across everted gut sac into the serosal space in the presence of verapamil or quinidine in the incubation medium. (▲) Digoxin (10 μM) alone; (×) digoxin (10 μM) + quinidine (1.3 mM); (■) digoxin (10 μM) + verapamil (100 μM). The data represent the means ± S.E. ($n = 3$ sacs at each time point). R^2 values: 0.96, 0.98, 0.98, respectively. At 90 min, $p < 0.01$ (t-test).

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