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The complexity of intestinal absorption and exsorption of digoxin in rats

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Abstract

The potential multiple carrier-mediated mechanisms involved in the transport of digoxin in rat intestine were investigated by the rapid filtration method in rat intestinal brush-border vesicles (BBMV) and *in vitro* Ussing chambers. The uptake of digoxin showed a typical overshoot phenomenon in the presence of an inward proton gradient and an outward bicarbonate gradient, or an outward glutathione gradient in BBMV. Good fitting to an equation consisting of both saturable and linear terms was obtained using non-linear regression analysis. GF120918, a specific P-gp inhibitor, significantly increased the absorptive permeability of digoxin in rat ileum (7.02×10^{-7} cm/s versus 2.11×10^{-6} cm/s with GF120918) but the addition of DIDS (0.5 mM), an anionic transporter inhibitor, or bromosulfophthalein (0.1 mM), an Oatp inhibitor, in the presence of GF120918 decreased the absorptive permeability compared with GF120918 alone (2.11×10^{-6} cm/s versus 1.46×10^{-6} cm/s, $p < 0.01$ and 2.11×10^{-6} cm/s versus 1.60×10^{-6} cm/s, $p < 0.05$, respectively). The above results suggest the involvement of carrier-mediated uptake mechanism, possibly Oatp, in digoxin absorption. Interestingly, GF120918 (1 μM) did not abolish the polarized transport of digoxin in rat jejunum and ileum, and DIDS (0.5 mM), not a P-gp inhibitor, and MK571 (50 μM), an MRP-selective inhibitor, can also significantly decrease the exsorptive permeability of digoxin. This result indicates the involvement of non-P-gp efflux transporter in digoxin secretion and this transporter is DIDS and MK571-sensitive. Contrary to conventional concept, our studies show that intestinal absorption of digoxin may involve both active uptake and efflux transporters. Our study may have clinical implications in drug–drug or drug–food interactions involving transporters.
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Keywords: Digoxin; Brush-border membrane vesicles (BBMV); Carrier-mediated uptake; Organic anion transporting polypeptide (Oatp); P-glycoprotein (P-gp)

1. Introduction

Digoxin is a widely used, poorly water-soluble cardiac glycoside with a narrow therapeutic index. There is a substantial variation in absolute bioavailability (Shaw et al., 1972; Evans et al., 1992). For example, the reported intra-individual and mean inter-study bioavailability ranged from 40% to 90% (Yacobi et al., 1981; Meister et al., 1984) and 50% to 85% (Huffman, 1976; Kramer and Reuning, 1978; Marcus, 1978), respectively. Oral formulation factors such as particle size, polymorphism, and aging of products have long been regarded to contribute to this variable bioavailability (Jounela et al., 1975; Chiou and Kyle, 1979). The expected reduction in intra- and inter-individual variability in bioavailability was not realized when rapidly dissolving soft gelatin capsules were substituted for conventional tablets in a study (Johnson et al., 1986) suggesting that dissolution rate alone may not be the reason for variable bioavailability. Since digoxin metabolism after intravenous administration is insignificant (Hinderling and Hartmann, 1991), no significant first-pass metabolism is then expected (Magnusson et al., 1982; Rodin and Johnson, 1988).

Because of its lipophilicity and apparently linear absorption over a wide concentration range, digoxin has been commonly assumed to be absorbed across the apical membrane of enterocytes by passive diffusion (Caldwell et al., 1969). Recently, digoxin has been shown to be a substrate of intestinal P-glycoprotein (P-gp), a *MDR1* gene product (Mayer et al., 1996). P-gp has been commonly regarded to be able to retard intestinal absorption of its substrate drugs by pumping the absorbed drugs back into the intestinal lumen (Lin and Yamazaki, 2003). Therefore, P-gp may also contribute to the reported variable and incomplete bioavailability of digoxin. However, the impor-

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tance of P-gp in limiting digoxin intestinal absorption in humans seems somewhat unclear (Chiou et al., 2001). For example, co-administration of quinidine, a P-gp inhibitor, was reported to significantly increase digoxin bioavailability from 68.5% to 79.1% in one study (Pedersen et al., 1983), while failed to elicit significant changes (79.3% versus 73.5% in control) in another well-control study (Hager et al., 1981). It was also noted that distribution of P-gp is not uniform along the intestine and the mRNA levels appear to increase progressively from the stomach to the colon (Fojo et al., 1987) and that the predominant site for absorption after oral administration is the upper part of the small intestine (Hall and Doherty, 1971; Andersson et al., 1975), which harbors lower levels of P-gp. Thus, the uneven distribution of intestinal P-gp may significantly impact on digoxin absorption.

Recently, the organic anion transporting polypeptide (Oatp/OATP) (Noe et al., 1997; Reichel et al., 1999), originally found to transport digoxin in liver and brain, was also identified in the small intestine (Walters et al., 2000; Kobayashi et al., 2003). It is possible the intestinal Oatp/OATP may also contribute to the variable bioavailability of digoxin. If this is true, any factor affecting the active uptake of digoxin may present a new mechanism leading to its incomplete and variable oral absorption in patients. The purpose of this study was to explore the mechanisms of intestinal absorption and exsorption of digoxin using the rat intestinal BBMV and the conventional Ussing chamber with excised rat intestinal tissues. Although digoxin is a lipophilic compound, the uptake of digoxin into normal human placental BBMV has been successfully employed to study the transport kinetics of digoxin mediated by P-gp in a well-controlled study (Ushigome et al., 2003).

2. Materials and methods

2.1. Materials

Male Sprague-Dawley rats (260–320 g) were purchased from Harlan (Indianapolis, IN). Digoxin, quinidine, verapamil, 4,4′-diisothiocyanatostilbene-2,2′-disulfonate (DIDS), Tris base (2-amino-2-(hydroxymethyl)-1,3-propanediol), and MES (2-morpholinoethanesulfonic acid) were purchased from Sigma Chemical Co. (St. Louis, MO). D-Mannitol, and HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid) were purchased from Fisher Scientific (Hanover Park, IL). Bromosulfophthalein (BSP) and D-glucose were purchased from Acros Organics (Somerville, NJ). GF120918 was generously supplied by GlaxoSmithKline (Research Triangle Park, NC). MK-571 sodium salt was purchased from Alexis Biochemicals (San Diego, CA). D-[2-^{3}H]-glucose (15 Ci/mmol), [^{3}H(G)]-digoxin (37 Ci/mmol) and [^{3}H]-talinolol (17 Ci/mmol) were purchased from Amersham Biosciences (Piscataway, NJ), Perkin-Elmer Life and Analytical Sciences (Boston, MA) and Joerg Kix (Volxheim, Germany), respectively. Modified vertical Ussing chambers were purchased from Harvard Apparatus (Holliston, MA). All other chemicals were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO). All procedures involving animals were approved by the Institutional Animal Care and Use Committee at University of Illinois at Chicago.

2.2. Transport studies with rat intestinal brush-border membrane vesicles (BBMV)

The rat intestinal BBMV was prepared using calcium-precipitation method (Kessler et al., 1978; Yuasa et al., 1993). The protein contents of BBMV were determined using Modified Lowery Protein assay kit (Pierce Biotechnology, Inc., Rockford, IL) with BSA standard solution (the mean protein concentration was 12 mg/ml). The purity of BBMV was determined by measurement of the alkaline phosphatase activity compared to that of homogenate (the enrichment factor was about 10-fold). D-Glucose uptake was done to validate the transport capacity of the BBMV. Vesicle preparations were stored at −80 °C before uptake experiments.

The uptake of various concentrations of digoxin into BBMV was measured at 37 °C by the rapid filtration method (Yuasa et al., 1993). All uptake solutions were kept in 37 °C water bath for 5 min prior to the study. The uptake was initiated by adding 80 μl of the uptake solution into 20 μl of the BBMV suspension (5 mg protein/ml) and studied up to 5 min. At 3, 5, 10, 15, 30, 60, and 300 s the reaction was terminated by the addition of 1 ml ice-cold stop solution (30 mM potassium gluconate, 200 mM mannitol, and 25 mM HEPES) with incubation media. The mixture was immediately placed on a prewetted membrane filter (Millipore filter, HVLP, 0.45 μm pore size and 25 mm in diameter) and washed three times with 3 ml ice-cold stop solution. The filter was removed and transferred to a scintillation vial followed by the addition of 10 ml of scintillation cocktail, ScintiSafe 30% (Fisher Scientific) and the radioactivity associated with a filter membrane was determined in a liquid scintillation counter (LS6500; Beckman Coulter, Fullerton, CA). Non-specific binding of digoxin to the filter and BBMV was studied by adding the uptake solution containing digoxin to the vesicle suspension after adding 1 ml ice-cold stop solution. The amount adsorbed to the filter and BBMV was used to correct the amount of uptake by vesicles.

For studying the effects of various potential inhibitors on uptake of 5 μM digoxin, the BBMV suspension (double strength of the final concentration) was diluted with the loading solution with or without inhibitors to make 5 mg protein/ml of final concentration. The initial uptakes at 5 s were determined after adding 80 μl uptake solutions containing digoxin alone as control or with inhibitors into 20 μl vesicle suspensions.

2.3. In vitro transport and inhibition studies in the Ussing chambers

Male Sprague-Dawley rats were allowed free access to food and water prior to sacrifice by decapitation under light ether anesthesia. After laparotomy the entire gastrointestinal tract was quickly removed and the lumen of the intestine was rinsed with ice-cold Krebs–Ringer bicarbonate buffer. Segments of intestinal tissue (n = 4–6 from 2–3 rats) from different intestinal regions were used for each set of studies. Each segment was

cut opened along the mesenteric border and resulting flat sheets were mounted into Ussing chambers that provided an exposed area of 1.78 cm^2. Both mucosal (M) and serosal (S) sides were filled with 5 ml of pre-warmed and oxygenated Krebs–Ringer buffer (duodenum and proximal jejunum: pH 7.5 in S side and pH 6.0 in M side; distal ileum: pH 7.5 in both M and S sides) and allowed to equilibrate at 37 °C for 20–25 min prior to the start of the experiment. Measurement of transepithelial electrical resistance (TEER) value was conducted by EVOM^{TM} Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL) prior to the beginning and at the end of the transport studies to access the integrity of the intestinal tissue.

Prior to the initiation of an experiment, 1 ml of bathing solution in the donor chamber was removed and replaced with 1 ml of test solution (27 nM [^{3}H(G)]-digoxin in Krebs–Ringer buffer). The final digoxin concentration in the donor side was 5.4 nM. The duration of the transport experiment was 120 min. Samples (1 ml) were taken from the receiver chamber at designated times (40, 80, and 120 min) and replaced with the same volume of fresh Krebs–Ringer buffer without/with inhibitor. In inhibition studies, the potential inhibitor was added to both compartments 5 min prior to starting the transport study. In the talinolol transport study, 11.8 nM [^{3}H]-talinolol was used instead of [^{3}H(G)]-digoxin and 0.5 mM DIDS was used as an inhibitor in the inhibition study.

2.4. Analytical method

The samples from transport studies in the Ussing chambers were mixed with 8 ml of liquid scintillation cocktail, Scinti-Safe 30%. Radioactivity of [^{3}H(G)]-digoxin and [^{3}H]-talinolol in each sample was determined by Beckman LS6500 liquid scintillation counter.

2.5. Data analysis

The uptake kinetic parameters of digoxin by BBMV were obtained by fitting the data to an equation consisting of both non-linear (saturable) and linear terms using non-linear regression analysis by SigmaPlot 2000 (SPSS Inc., Chicago, IL):

$$v = \frac{V_{max} C}{K_m + C} + k_d C \quad (1)$$

where v represents the initial uptake rate, C the digoxin concentration, V_{max} the maximal uptake rate, K_m the apparent Michaelis constant, and k_d is the first-order uptake rate constant by the passive diffusion.

The effective permeability (P_{eff}) or transport clearance per unit gross surface area (CL_{tp}) was calculated using the following equation:

$$P_{eff} = CL_{tp} = \frac{\Delta C}{\Delta t} \frac{V}{C_0} \frac{1}{A} \quad (2)$$

where $\Delta C/\Delta t$ is the change in drug concentration in the receiving compartment over a time period between 40 and 120 min, V the volume of the solution in the receiving compartment, A the exposed area of the tissue in the Ussing chamber, and C_0 is the initial drug concentration in the donor compartment. The sink condition in the receiving compartment was maintained during the study since only 1% or less of [^{3}H(G)]-digoxin was transported.

Efflux ratio was calculated as follows:

$$\text{efflux ratio} = \frac{\text{mean } P_{eff\,S \to M}}{\text{mean } P_{eff\,M \to S}} \quad (3)$$

2.6. Statistical analysis

The effective permeability (P_{eff}) obtained from intestinal transport studies was expressed as mean ± standard deviation (S.D.). To compare the effect of various inhibitors versus control and the regional difference on digoxin transport, the unpaired Students' t-test was conducted. All statistics were performed at the alpha level of 0.05.

3. Results

3.1. Uptake of digoxin by BBMV

The uptake-time profiles of digoxin (Fig. 1) were studied under different concentration gradients. In the presence of an inwardly directed proton gradient and an outwardly directed bicarbonate gradient (pH_{out}/pH_{in} = 5.5/7.5 and $[gluconate]_{out}/[bicarbonate]_{in}$), the uptake profile showed a typical overshoot phenomenon with a peak time of 5 s. The uptake profile in the presence of only an outward bicarbonate gradient did not show an overshoot phenomenon. In the presence of an outwardly directed glutathione gradient, the uptake profile also showed a typical overshoot phenomenon with a peak time of 3 s. The above results suggested involvement of carrier-mediated transporter(s) in digoxin absorption.

The effect of various compounds (0.1 mM) on the digoxin (5 μM) uptake was tested in the presence of proton/bicarbonate gradients or an outward glutathione gradient (Table 1). In the presence of proton/bicarbonate gradients, digoxin uptake was significantly inhibited by DIDS (50.6% of the control value), an anion transporter inhibitor (Takanaga et al., 1996), as well

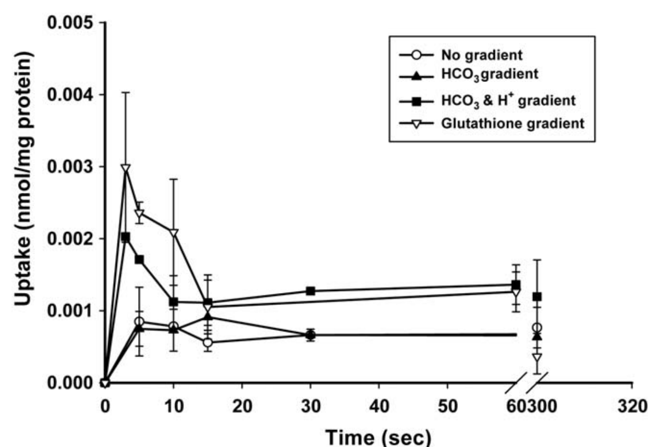

Fig. 1. Time profiles for the uptake of 5 μM digoxin in the different conditions. Each time point represents the mean ± S.D. (n = 3–4).

Table 1
The inhibitory effects of DIDS, verapamil, quinidine on the initial uptake of digoxin

Inhibitor	Concentration (mM)	Percent (%) of control values	
		Proton and bicarbonate gradients	Glutathione gradient
Control		100	100
DIDS	0.1	50.6 ± 32.1[a]	13.8 ± 10.5[b]
Verapamil	0.1	68.9 ± 20.1[a]	41.3 ± 11.9[a]
Quinidine	0.1	37 ± 36[a]	N/A[c]

Each value represents the mean ± S.D. ($n = 3$).
[a] $p < 0.05$, significant difference from the control.
[b] $p < 0.01$, significant difference from the control.
[c] Not applicable.

as by verapamil and quinidine (68.9% and 37% of the control value, respectively), reported inhibitors for Oatp (Cvetkovic et al., 1999). DIDS and verapamil also significantly reduced the digoxin uptake to 13.8% and 41.3% of the control value in the presence of an outward glutathione gradient, respectively. These results suggested that most of the uptake by rat intestinal BBMV was carrier-mediated.

The initial uptake of digoxin into BBMV in the presence of proton/bicarbonate gradients was concentration dependent and saturable (Fig. 2). Eq. (1) was used to estimate the kinetic parameters and an r^2 value of 0.91 was obtained. The kinetic parameters V_{max}, K_m, and K_d for digoxin were 49.4 pmol/mg protein/5 s, 135 μM, and 1.29×10^{-12} ml/mg protein/5 s, respectively.

3.2. Regional differences in bi-directional transport of digoxin in the Ussing chambers

The result of our bi-directional transport studies across different regions of rat intestinal track is depicted in Fig. 3. Judging from the permeability values as demonstrated in Fig. 3, S → M transport appeared to be site-dependent with the highest permeability in distal ileum (1.00×10^{-5} cm/s) and the lowest permeability in duodenum (1.92×10^{-6} cm/s). In contrast, M → S transport seemed to be site-independent with slightly higher permeability in duodenum and proximal jejunum (1.38×10^{-6} and 1.32×10^{-6} cm/s, respectively) compared with distal ileum (1.15×10^{-6} cm/s). Taken together, digoxin absorption was maintained throughout the different segments of the gut despite the progressive increase of the function/expression of efflux transporter(s) such as P-gp. These findings led us to hypothesize that factors other than efflux transporter(s) may modulate the variable digoxin bioavailability as well.

Results of the effect of potential inhibitors on M → S transport of 5.4 nM digoxin across rat intestinal tissue are summarized in Table 2. GF120918, a specific P-gp inhibitor (Hyafil et al., 1993), significantly increased the absorptive permeability of digoxin in rat ileum (1.15×10^{-6} cm/s ver-

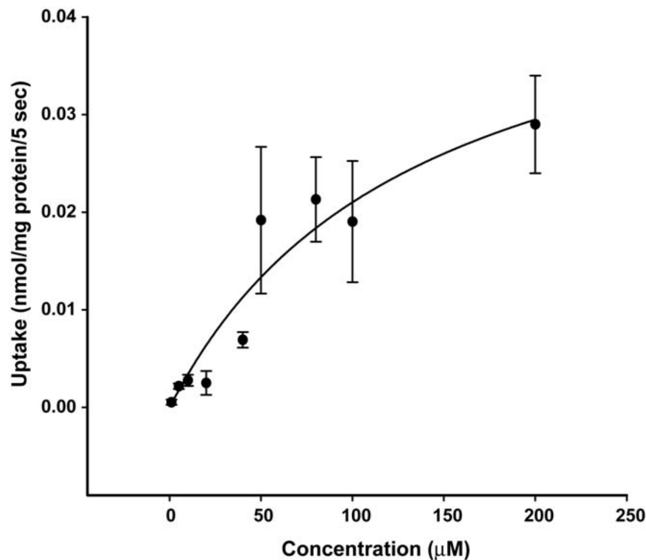

Fig. 2. Concentration dependent initial uptake of digoxin into BBMV in the presence of inwardly directed proton gradient and outwardly directed bicarbonate gradient. Each point represents the mean ± S.D. ($n = 3$–5).

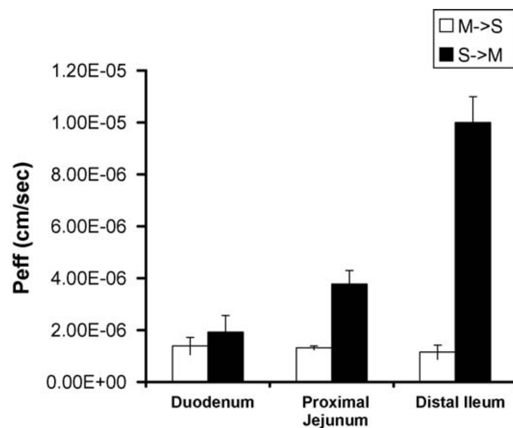

Fig. 3. The bi-directional permeability (cm/s) of 5.4 nM digoxin in different regions of small intestine (duodenum, proximal jejunum and distal ileum) measured by *in vitro* Ussing chambers. Each data represents the mean ± S.D. ($n = 4$–6).

Table 2
Summary of the effects of potential inhibitors on M → S transport of digoxin

Inhibitor	Concentration	Segment	Permeability (10^{-6} cm/s)
Control		Distal ileum	1.15 ± 0.27
Control		Proximal jejunum	1.32 ± 0.74
GF120918	1 μM	Distal ileum	2.11 ± 0.36[a]
GF120918	1 μM	Proximal jejunum	1.53 ± 0.69
DIDS	0.5 mM	Distal ileum	0.94 ± 0.18
BSP	0.1 mM	Distal ileum	1.11 ± 0.18
GF120918 + DIDS	1 μM + 0.5 mM	Distal ileum	1.46 ± 0.25[b]
GF120918 + BSP	1 μM + 0.1 mM	Distal ileum	1.60 ± 0.24[b]
Verapamil	0.1 mM	Distal ileum	3.07 ± 0.51[a]
Verapamil	0.1 mM	Proximal jejunum	2.82 ± 0.25[a]
Quinidine	0.2 mM	Distal ileum	4.74 ± 0.17[a]
Quinidine	0.2 mM	Proximal jejunum	1.29 ± 0.15
Apple juice	20%	Proximal jejunum	0.37 ± 0.12[a]
Apple juice	5%	Proximal jejunum	0.86 ± 0.19[a]

Each value represents the mean ± S.D. ($n = 3$–5).
[a] $p < 0.05$, significant difference from the control.
[b] $p < 0.05$, significant difference from 1 μM GF120918 in distal ileum.

Table 3
Summary of the effects of potential inhibitors on S → M transport of digoxin

Inhibitor	Concentration	Segment	Permeability (10^{-6} cm/s)
Control		Distal ileum	10.00 ± 0.1
Control		Proximal jejunum	3.77 ± 0.52
GF120918	1 μM	Distal ileum	6.29 ± 1.33[a]
GF120918	10 μM	Distal ileum	6.21 ± 0.34[a]
GF120918	1 μM	Proximal jejunum	3.11 ± 0.36[a]
DIDS	0.5 mM	Distal ileum	5.98 ± 0.35[a]
MK571	50 μM	Distal ileum	5.58 ± 0.56[a]
Verapamil	0.1 mM	Distal ileum	2.96 ± 1.32[b]
Verapamil	0.1 mM	Proximal jejunum	2.64 ± 0.34[b]
Quinidine	0.2 mM	Distal ileum	5.38 ± 0.73[a]
Quinidine	0.2 mM	Proximal jejunum	2.63 ± 0.44[a]

Each value represents the mean ± S.D. (n = 3–5).
[a] $p < 0.05$, significant difference from the control.
[b] $p < 0.01$, significant difference from the control.

sus 2.11×10^{-6} cm/s with GF120918). Although GF120918 can also inhibit the breast cancer resistance protein (BCRP) (de Bruin et al., 1999), digoxin is not the substrate of BCRP (Pavek et al., 2005). Interestingly, the addition of 0.5 mM DIDS, an anionic transporter inhibitor (Takanaga et al., 1996), or 0.1 mM BSP, an Oatp/OATP inhibitor (Cvetkovic et al., 1999) in the presence of 1 μM GF120918 further decreased the absorptive permeability of digoxin compared with GF120918 alone (2.11×10^{-6} cm/s versus 1.46×10^{-6} cm/s, $p < 0.01$ and 2.11×10^{-6} cm/s versus 1.60×10^{-6} cm/s, $p < 0.05$, respectively). Apple juice at 20% and 5% of normal strength also drastically reduced the absorptive permeability of digoxin in rat proximal jejunum (1.15×10^{-6} cm/s versus 0.37×10^{-6} cm/s and 0.86×10^{-6} cm/s, respectively). These results pointed to the potential involvement of carrier-mediated uptake mechanisms in digoxin absorption, which are sensitive to DIDS, BSP and apple juice.

The determined exsorptive permeabilities across rat intestinal tissue in the presence of several different inhibitors are presented in Table 3. GF120918, a specific P-gp inhibitor, decreased the exsorptive permeability of digoxin at a concentration of 1 μM. Raising the concentration of GF120918 (10 μM) did not further reduce the permeability in the S → M direction, suggesting that the complete inhibition of the P-gp-mediated transported process was achieved. In contrast, GF120918 failed to abolish the polarized transport of digoxin in rat ileum (efflux ratio: 3.0 versus 8.7 in the control, Tables 2 and 3) suggesting the involvement of non-P-gp efflux transporter(s) in digoxin secretion. Indeed, both 0.5 mM DIDS and 50 μM MK571, an MRP-selective inhibitor (Chen et al., 1999; Renes et al., 1999), significantly suppressed the exsorptive permeability of digoxin (10.0×10^{-6} cm/s versus 5.98×10^{-6} cm/s and 10.0×10^{-6} cm/s versus 5.58×10^{-6} cm/s, respectively). As a control, the exsorptive permeability of talinolol, a P-gp substrate (Spahn-Langguth et al., 1998), was not affected by 0.5 mM DIDS (Fig. 4) suggesting that DIDS had no effect on P-gp activity. The above results suggest the involvement of non-P-gp efflux transporter(s) in digoxin secretion in small intestine, which is/are sensitive to DIDS and MK571.

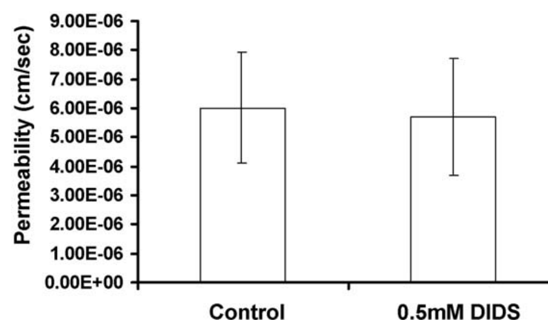

Fig. 4. The effect of 0.5 mM DIDS on exsorptive permeability of 11.8 nM talinolol in rat distal ileum. Each point represents the mean ± S.D. (n = 4–6).

4. Discussion

For a long time digoxin has been commonly assumed to be absorbed by passive diffusion in the intestine because of its apparently linear absorption and lipophilicity. The present surprising findings in rat BBMV study clearly suggest for the first time that most of the intestinal absorption of this bulky (MW 781) neutral compound in rat may involve a carrier-mediated active process. In our inhibition studies, 0.1 mM DIDS was able to decrease the uptake of digoxin to only 13.8% of the control value suggesting that the carrier-mediated process may contribute at least about 86% to the total uptake of digoxin by rat BBMV under our study conditions. Moreover, initial uptake profile as a function of drug concentration showed a saturable uptake over a 200-fold concentration range (1–200 μM) in the presence of proton and bicarbonate gradients. The above results strongly indicate the involvement of carrier-mediated mechanism in digoxin absorption in rats.

Oatps/OATPs have been well accepted as sodium-independent bile salt and organic anion transporters (Hagenbuch and Meier, 2003). The driving force for the Oatp/OATP has not been investigated in detail, but for Oatp1 and Oatp2, some experimental evidences indicate that intracellular bicarbonate or glutathione play an important role (Satlin et al., 1997; Li et al., 2000). Furthermore, Kobayashi et al. (2003) recently reported that the activity of OATP-B localized at the apical membrane of human intestinal epithelial cells may be optimal at the acidic surface pH of the small intestine. Our results show that uptake of digoxin (a neutral compound) into BBMV was significantly increased in the presence of proton and bicarbonate gradients ($pH_{out}/pH_{in} = 5.5/7.5$ and $[gluconate]_{out}/[bicarbonate]_{in}$) and an outwardly directed glutathione gradient (Fig. 2). Therefore, based on the above results, one can conclude that the uptake of digoxin may involve the Oatp in rat small intestine.

Based on the conventional concept, co-administration of a P-gp inhibitor such as quinidine or verapamil should increase the oral absorption of digoxin. However, two recent animal studies (Budihna and Strojan, 1993; Su and Huang, 1996) seemed to show the unexpected opposite results. In the guinea-pig study using the everted gut sac method (Budihna and Strojan, 1993), compared to controls the addition of 50 μM verapamil on the mucosal side significantly decreased concentrations of digoxin on the serosal side (12.8 nM versus 6.4 nM, 16.9 nM versus

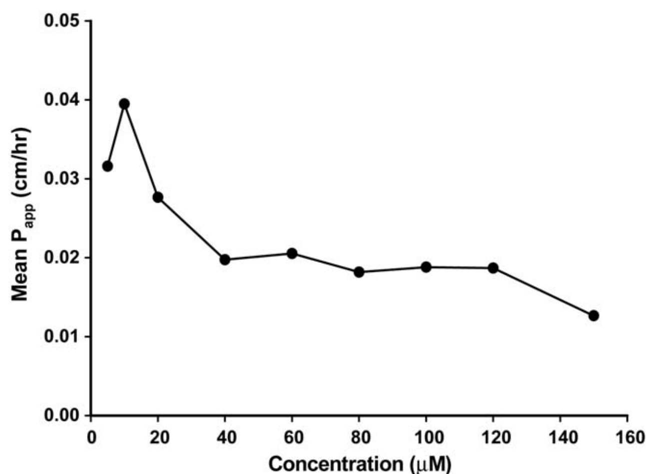

Fig. 5. The mean calculated absorptive permeability at various concentrations. Permeability was calculated from the flux data of Fig. 4B in Stephens et al. (2001).

8.3 nM, and 26.9 nM versus 12.0 nM at 30, 60, and 120 min, respectively). In another rat study using *in situ* circulated perfusion method (Su and Huang, 1996), co-administration of quinidine significantly decreased the absorption clearance of digoxin (6.4 ml/h versus 4.8 ml/h). Our results may help explain the findings from these two studies. It is possible that verapamil and quinidine, reported inhibitors for Oatp (Cvetkovic et al., 1999), might decrease the absorption of digoxin by inhibiting the active uptake mechanism in the intestine.

In order to further support our findings, the mean absorptive permeabilities were recalculated from the rat ileal flux data of Fig. 4B in Stephens et al. (2001). As shown in Fig. 5, the absorptive permeability of digoxin increased as the digoxin concentration was increased from 5 to 10 μM. At higher concentrations up to 150 μM, the permeability was decreased to 30% of that at 10 μM. These results indicate the participation of a saturable influx transport process in digoxin absorption (Tamai et al., 1997).

Digoxin has long been used as an example to demonstrate the P-gp-limiting effect on intestinal absorption due to its high efflux ratio as shown in Caco-2 cell studies (Crowe and Lemaire, 1998). Since the distal region of small intestine has a higher P-gp mRNA expression in rat (Takara et al., 2003), it is postulated that regional differences exist in the absorptive permeability of digoxin. Although we observed a regional difference in the directional secretory transport of digoxin in our study with the greatest polarized transport occurring in distal ileum, there were no significant differences in the absorptive permeability between duodenum, proximal jejunum and distal ileum (Fig. 3). This may indicate that the influence of P-gp on the rate or extent of the intestinal absorption of digoxin is rather limited. Using quinidine as a P-gp inhibitor, others (Hager et al., 1981; Pedersen et al., 1983) have previously reported conflicting results on digoxin bioavailability. In our study, 0.2 mM quinidine did not significantly increase the absorptive permeability of digoxin in proximal jejunum (Table 2), where digoxin was predominantly absorbed. Our observation rendered it unlikely that digoxin is efficiently transported by P-gp in the gut wall. Therefore, we proposed an alternative explanation that digoxin may be absorbed by carrier-mediated mechanisms overriding the efflux process. Thus, the net absorption is unaffected despite the presence of efflux transporters.

The absorptive permeability of digoxin in mdr1a (−/−) mouse was thought to represent the "true" passive permeability in intestine because of the complete abolition of P-gp (Stephens et al., 2002). In our study, co-administration of GF120918, a specific P-gp inhibitor, did increase the absorptive permeability of digoxin in rat ileum as expected. However, the addition of 0.5 mM DIDS or 0.1 mM BSP in the presence of 1 μM GF120918 further reduced absorptive permeability of digoxin, suggesting the involvement of other uptake transporters. We hypothesized that Oatp may play a role in digoxin absorption in rat because both DIDS and BPS have been found to inhibit Oatp/OATP (Takanaga et al., 1996; Cvetkovic et al., 1999). Consistent with this, apple juice, a potent Oatp/OATP inhibitor (Dresser et al., 2002), at 20% and 5% of normal strength significantly reduced the absorptive permeability to 28% and 65% of the control, respectively, in proximal jejunum. Thus, digoxin may be absorbed by a carrier-mediated uptake transporter, possibly Oatp, in rat intestine. Indeed, apple juice significantly reduced oral availability of fexofenadine, a dual substrate of P-gp and Oatp/OATP (Cvetkovic et al., 1999), in both humans and rats, possibly through inhibition of intestinal Oatp/OATP (Dresser et al., 2002; Kamath et al., 2004). If digoxin absorption is also mediated by Oatp in rat intestine, our study may suggest a new mechanism of food–drug interaction in humans.

Digoxin has been commonly assumed to be absorbed across the apical membranes of enterocytes by passive diffusion and secreted into the luman by P-gp in the intestine. Under such assumption, digoxin is expected to exhibit polarized transport *in vitro*, which should be blocked by specific P-gp inhibitors, such as GF120918. However, in our study using the Ussing chamber method, 1 μM GF120918 (a concentration where complete inhibition of P-gp function is achieved) failed to abolish the polarized transport of digoxin in rat ileum (efflux ratio: 8.7 versus 3.0 with GF120918, Tables 2 and 3). Thus, our results suggest the involvement of non-P-gp efflux transporter(s) in digoxin secretion in rat intestine. It is worth noting that the non-P-gp efflux transporter(s) involved in digoxin secretion may be sensitive to DIDS and MK571, since both 0.5 mM DIDS, an anionic transporter inhibitor, and 50 μM MK571, an MRP-selective (MRP2) inhibitor, significantly reduced the exsorptive permeability of digoxin. Indeed, Lowes et al. (2003) also reported previously that in Caco-2 cells digoxin secretion was mediated by an MK571-sensitive mechanism other than MRP2 and MK571 had a minimal effect on P-gp-mediated transport. However, the identity of this transporter remains to be elucidated.

DIDS did not influence the function of P-gp since it did not reduce the exsorptive permeability of talinolol, a P-gp substrate (Spahn-Langguth et al., 1998), in our study (Fig. 4). The digoxin secretion by the intestinal epithelium has been reported to involve both passive diffusion and Na^+/K^+ ATPase-mediated endocytosis at the basolateral membrane, followed by active

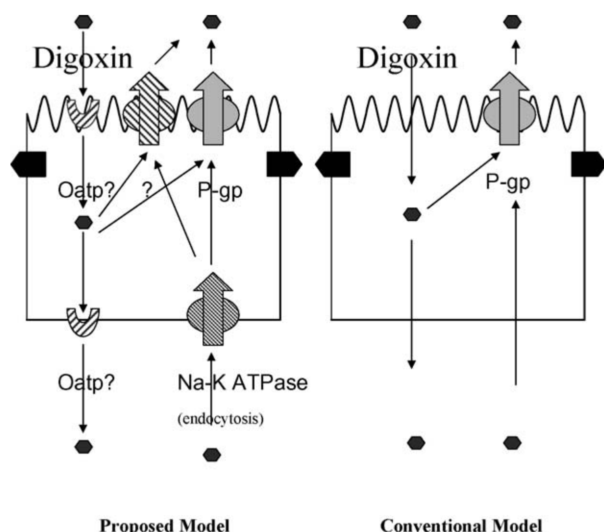

Fig. 6. Proposed and conventional digoxin transport pathways across the rat enterocytes.

efflux at the apical membrane (Cavet et al., 1996). Interestingly, one study has reported that DIDS inhibited Na^+/K^+ ATPase in the basolateral membrane of jejunum enterocytes (Faelli et al., 1984). Taken together, DIDS may reduce the net secretion of digoxin by inhibiting the Na^+/K^+ ATPase-mediated endocytosis at the basolateral membrane (Fig. 6).

5. Conclusion

The present studies using rat intestinal BBMV successfully demonstrated the potential dominant involvement of carrier-mediated uptake mechanism in digoxin absorption. Our results also indicated that this carrier-mediated uptake process may be proton and bicarbonate gradients-dependent or glutathione gradient-dependent, suggesting potential involvement of the Oatp in digoxin absorption in rat. Our results using the Ussing chambers method indicate that the absorptive permeability of digoxin is site- and P-gp-independent in rat intestine. In contrast, the intestinal absorptive permeability of digoxin is inhibited by DIDS, an anionic transporter inhibitor, and BSP, an Oatp inhibitor, in the presence of GF120918 suggesting the involvement of carrier-mediated uptake transporter, possibly Oatp, in digoxin absorption. This notion is further supported by the observation that both 20% and 5% apple juice, a potent Oatp/OATP inhibitor, also reduced the absorptive permeability of digoxin in rat intestine. Furthermore, 1 μM GF120818, a specific P-gp inhibitor, did not abolish the polarized transport of digoxin suggesting the involvement of non-P-gp efflux transporter(s), which might be DIDS and MK571-sensitive, in digoxin secretion. In summary, our studies highlight the potential involvement of multiple carrier-mediated uptake and efflux transporters in digoxin absorption and exsorption. The digoxin–drug and digoxin–food interactions involving both uptake and efflux transporters may be of clinical significance because of the narrow therapeutic range of digoxin. Further studies are needed to identify definitively those uptake and efflux transporters of digoxin.

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