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Concentration dependency of modulatory effect of amlodipine on P-glycoprotein efflux activity of doxorubicin – a comparison with tamoxifen

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Abstract

Modulators of P-glycoprotein (P-gp) can enhance or limit the permeability of a number of therapeutic agents that are considered substrates of this efflux pump protein. The modulatory effect of amlodipine (4-dihydropyridine calcium antagonist) on P-gp efflux activity has not been fully elucidated. We have studied the concentration dependency of its modulatory effect and compared it qualitatively with tamoxifen (a non-esteroid anti-estrogen). The investigation was conducted on transmembrane efflux of doxorubicin at a fixed concentration of 5 μM across a Caco-2 monolayer in the presence of various concentrations of amlodipine or tamoxifen. The maximum flux of doxorubicin from basolateral to apical (*ba*) occurred at 4.5 μM amlodipine and at 0.02 μM tamoxifen. At higher concentrations, the apical to basolateral (*ab*) flux and the net flux of doxorubicin (*ba* – *ab*) declined steadily in a concentration-dependent manner. We analysed the observed net flux data by fitting different mathematical models to the data. A composite sigmoidal E_{max}/I_{max} (stimulatory/inhibitory) model was found to be the most appropriate to define the system. The observed and calculated parameters supported the modulatory role of both compounds and clearly indicated that the stimulation and inhibition of transmembrane efflux occurred simultaneously in the presence of amlodipine or tamoxifen. It was concluded that amlodipine, similar to tamoxifen, modulated the transporter-dependent transmembrane flux of the P-gp substrate in a concentration-dependent manner.

Introduction

P-glycoprotein (P-gp) has been recognized as a major barrier to the permeability of a number of compounds, known as substrates of this ATP-dependent efflux pump. Anthracycline antibiotics such as doxorubicin, epirubicin and daunorubicin are well known examples of P-gp substrates (Mankhetkorn et al 1999; Sonveaux et al 1999; Marbeuf-Gueye et al 2000).

Modulators of P-gp are compounds that are able to influence the drug efflux activity of this transmembrane, ATP-dependent efflux pump. Modulation can occur in terms of stimulation or inhibition of the efflux activity through different mechanisms. Modulation can be achieved by direct interaction of the modulator with ligand binding sites on P-gp (Borchers et al 1995), or the interaction with ATPase domains of P-gp (Conseil et al 2001). The lipid composition and properties of the plasma membrane surrounding P-gp have been shown to exert profound impact on the ability of the protein pump to interact with and transport its potential substrates (Sinicrope et al 1992; Romsicki & Sharom 1999), which in turn can change the P-gp-ATPase activity. Compounds that strongly interact with the plasma membranes and alter the physical properties of the bilayer, such as fluidity, have a lipid-mediated effect on transport properties of P-gp substrates across the bilayer membrane (Hendrich & Michalak 2003). Modulation can also be the result of regulation of multidrug resistance gene expression (Tokura et al 2002).

In-vitro transport studies have identified tamoxifen, a non-esteroid anti-estrogen, as an inhibitor of P-gp efflux activity (Kayyali et al 1994; Lavie et al 1997). Studies on reconstituted liposome-integrated P-gp have demonstrated that the effect of tamoxifen on P-gp-ATPase activity is biphasic and concentration dependent (Rao et al 1994). The

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stimulatory phase occurs at low concentrations of tamoxifen and its inhibitory phase takes place at high concentrations. Tamoxifen is known to modify the properties of the plasma membrane by reducing the membrane fluidity (Custodio et al 1993a; Hendrich & Michalak 2003), stimulating phosphatidylethanolamine hydrolysis (Kiss 1994), and inhibiting ethanolamine phosphorylation (Kiss & Crilly 1995). Thus, the modulation of P-gp by tamoxifen can be attributed to various and complementary mechanisms. The published data concerning the modulatory effect of tamoxifen are mostly related to its inhibitory effect and do not address the modes of its modulatory effects on P-gp efflux activity (Kayyali et al 1994; Wang et al 2000).

Amlodipine, a 1, 4-dihydropyridine calcium antagonist, is another example of a compound that incorporates itself into the lipid bilayer (Tulenko et al 2001). The only published study with regard to modulatory effect of amlodipine on P-gp efflux activity has suggested an inhibitory role for this calcium-channel blocker (Katoh et al 2000). Similar to tamoxifen, in-vitro studies have identified amlodipine as an inhibitor of P-gp efflux activity. Similar to tamoxifen, amlodipine also localizes within the plasma membrane and changes the organization and thermodynamic properties of the lipid bilayer (Tulenko et al 2001).

In this study, we have investigated the combined inhibitory and stimulatory effects of amlodipine in comparison with tamoxifen on P-gp efflux activity of doxorubicin, with the intention of determining the concentration dependency of their modulatory effects, and predicting the effect by a modulator-substrate net efflux relationship.

Materials and Methods

Materials

Doxorubicin was provided by Pharmacia Upjohn (Albuquerque, NM). Amlodipine was provided by Pfizer (Sandwich, UK). Tamoxifen (citrate salt) was obtained from Sigma Chemical Co. (St Louis, MO). All other chemicals used were obtained commercially and were of reagent grade.

Cell culture and transcellular transport

Caco-2 (human adenocarcinoma) cells were obtained from ATCC (HTB37, Rockville, MD). The cells were maintained in complete medium consisting of high glucose-Dulbecco's modification of Eagle's medium (DMEM) supplemented with 1% (v/v) non-essential amino acids, 2 mM glutamine, 100 IU mL^{-1} penicillin, 100 $\mu g\, mL^{-1}$ streptomycin (Mediatech Inc., Herndon, VA), and 10% heat-inactivated foetal bovine serum (FBS) (Biofluids, Rockville, MD), in a 5% CO_2/95% air atmosphere. The cells were treated with 0.25% (w/v) trypsin–0.03% (w/v) EDTA followed by incubation with trypsin solution (2.0 mL/50 cm^2 flask) at 37°C for 5–10 min. The dispersion of the cell layer was verified by observation under an inverted microscope. At the end of the incubation period, 8.0 mL of the complete medium was added to each flask, and cells were aspirated. Cell suspension (3 mL) and 6.0 mL fresh complete medium were added to a new flask and incubated at 37°C for 5–10 min.

The transcellular transport studies were conducted on confluent Caco-2 monolayers grown on polycarbonate membranes of cell culture inserts, Transwell (Costar, Cambridge, MA). The complete medium lacking antibiotics and FBS, designated as incomplete medium, was used for the transport studies. Caco-2 cell suspensions were seeded at a density of 1×10^5 cells cm^{-2} onto 12 mm-diameter polycarbonate membranes (0.4-μm pore size, 1 cm^2 growth area) of Transwell assembly. The upper and lower compartments of each Transwell contained 0.5 and 1.5 mL fresh medium, respectively, and was refreshed every four days. The monolayers were used for permeability studies 27 days after seeding. Triplicate monolayers were used per determination. Stock solutions of doxorubicin (5 mM), tamoxifen (5 mM), and amlodipine (10 mM) were diluted with incomplete medium to obtain the desired concentrations. The media in both compartments of the Transwells were replaced with fresh blank incomplete medium 3 h before the start of the experiments. The efflux of doxorubicin at a concentration of 5 μM was assessed across the monolayers in the presence of various concentrations of tamoxifen (0, 0.02, 0.2, 0.8 and 1.6 μM) or amlodipine (0, 1.0, 3.0, 4.5, 6.0, 9.0 and 20 μM). The donor compartments contained 650 μL doxorubicin solution and a concentration of amlodipine or tamoxifen. The receiver compartment had only 650 μL doxorubicin-free medium containing the corresponding concentration of amlodipine or tamoxifen. The Transwells were incubated at 37°C and the media in the receiver compartment was collected after 96 h. The viability of cells was monitored daily during the incubation. No cell death or sign of toxicity was observed.

Doxorubicin concentration in each sample was determined using HPLC (Andersen et al 1993). The chromatographic system comprised a Waters 6000A HPLC pump coupled to a Gilson fluorometer set at λ_{ex} 480 nm and λ_{em} 540 nm, and chromatograms were integrated on a Waters Data Module. Separation was performed on a Nova Pak C-18 cartridge column (3.9 × 50 mm, particle size 4 μm) in conjunction with a Radial Compression Module unit and a C-18 Nova Pak guard column (Waters, Milford, MA). The mobile phase contained a 0.28 M sodium formate buffer (pH 3.51 at room temperature), acetone, and isopropanol (72.5:25:2.5, respectively). The final pH of the solution was 4.0 at room temperature.

Statistical analysis

The transcellular transport data are reported as mean ± s.d. (n = 3). A two-way analysis of variance was used to evaluate the effect of the modulator concentrations on the unidirectional fluxes of doxorubicin across the Caco-2 monolayers (*ab* and *ba*), followed by post-hoc comparisons of the means of individual groups using Tukey's Honestly Significant Difference test (ezANOVA version 0.97, Columbia, SC). Effect of the modulator concentrations on the net flux of the substrate, and the ratio of fluxes (*ba*/*ab*) were evaluated using a one-way analysis of variance, followed by the post-hoc comparisons as described above. Statistical significance was accepted at $P < 0.05$ for all tests.

The parameters of the model are expressed as mean ± s.e. (n = 3). The differences in values of the parameters between tamoxifen and amlodipine treatments were analysed using an unpaired *t*-test (Microsoft Exel 97 SR-2, Microsoft Corporation, Redmond, WA).

Data modelling

The relationship between the concentration range of each modulator and efflux of doxorubicin across Caco-2 monolayers were analysed based on a composite sigmoidal E_{max}/I_{max} effect model (Lundstrom et al 1992). To apply the model, we assumed two mechanisms for doxorubicin flux across the monolayer, the bidirectional passive diffusion (a first-order reaction) and the unidirectional efflux via P-gp from basolateral-to-apical direction (a saturable process). The flux of doxorubicin from apical-to-basolateral (J_{ab}), and from basolateral-to-apical (J_{ba}) directions could be described by equations 1 and 2, respectively:

$$J_{ab} = (K_{diff}\,[\text{doxorubicin}] - J_{max}\,[\text{doxorubicin}])/(K_m + [\text{doxorubicin}]) \quad (1)$$

$$J_{ba} = (K_{diff}\,[\text{doxorubicin}] + J_{max}\,[\text{doxorubicin}])/(K_m + [\text{doxorubicin}]) \quad (2)$$

Where J_{max} is the maximum saturable flux through P-gp, K_m is the corresponding Michaelis–Menten constant, and [doxorubicin] is the initial concentration in the donor compartment.

We identified the net flux of doxorubicin across the monolayer (J_{net}) as the effect or in-vitro pharmacological response (equations 3 and 4).

$$J_{net} = J_{ba} - J_{ab} \quad (3)$$

$$J_{net} = 2J_{max}\,[\text{doxorubicin}]/(K_m + [\text{doxorubicin}]) \quad (4)$$

A composite E_{max}/I_{max} sigmoidal model involving one stimulatory and two distinct inhibitory effects achieved the best fit to the data. The model was found the most suitable to describe the relationship between the net flux and concentration of the amlodipine or tamoxifen (equation 5):

$$E = E_0 + \frac{E_{max}C^n}{EC_{50}^n + C^n} - \frac{I_{max_1}C^{n_1}}{IC_{50_1}^{n_1} + C^{n_1}} - \frac{I_{max_2}C^{n_2}}{IC_{50_2}^{n_2} + C^{n_2}} \quad (5)$$

Where C is the concentration of modulator (tamoxifen or amlodipine), E is the observed effect (J_{net}) at concentration C, E_0 is the net flux (J_{net}) in the absence of the modulator, E_{max} is the maximum modulator-induced stimulatory effect, I_{max} is the maximum modulator-induced inhibitory effect, and n, n_1 and n_2 are the exponents of the sigmoidal curve, which account for the curvature around the corresponding concentration at 50% of maximum response. The model was fitted to the observed data by using WinNonlin (Pharsight Corp., Mountain View, CA). This model took into account the observed net flux of doxorubicin across Caco-2 cell monolayer as a combination of one stimulatory and two inhibitory effects at the low and high concentrations of tamoxifen or amlodipine, respectively.

Results

The apical-to-basolateral (*ab*) or basolateral-to-apical (*ba*) fluxes of doxorubicin across cultured Caco-2 monolayers in the presence of various concentrations of tamoxifen are shown in Figure 1. The *ba* flux of doxorubicin was statistically greater than the *ab* flux at each given concentration of tamoxifen, with the exception of 1.6 μM. Comparison of the *ab* flux of doxorubicin in the absence and presence of 0.02 μM tamoxifen showed a significant increase in the unidirectional flux of doxorubicin in presence of tamoxifen. Similar comparisons revealed a significant reduction in the *ab* flux in 0.8 μM tamoxifen, while no significant difference was observed for 0.2 or 1.6 μM tamoxifen. The *ba* flux of doxorubicin was significantly increased in 0.02 μM tamoxifen, significantly decreased in 1.6 μM of the modulator, and showed no significant difference in 0.2 or 0.8 μM tamoxifen with respect to control (0 μM tamoxifen).

Tamoxifen at 0.02 and 0.8 μM increased the ratio of the unidirectional fluxes, designated as *ba*/*ab*, compared with the control (Figure 2). Tamoxifen at 1.6 μM reduced the ratio significantly, but had no significant effect on the ratio at 0.8 μM.

Effect of tamoxifen concentration on the net flux of doxorubicin, occurring in the *ba* direction, is shown in Figure 3 (solid circles). Tamoxifen significantly increased the net flux of doxorubicin at 0.02 μM, reduced it at 1.6 μM, and showed no significant effect on the net flux

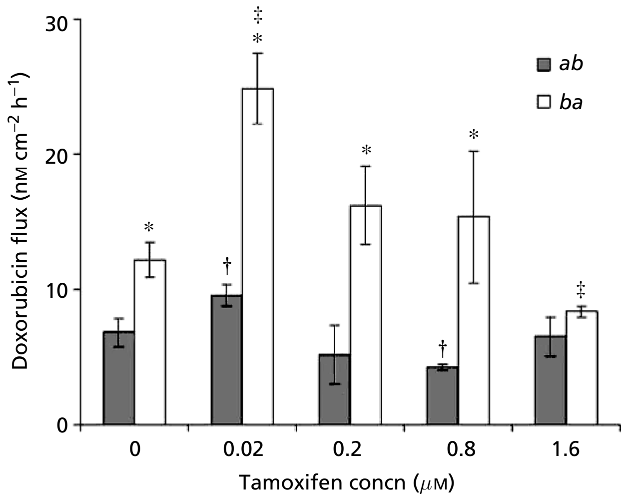

Figure 1 Comparison of the effect of various concentrations of tamoxifen on the apical-to-basolateral (*ab*) and basolateral-to-apical transport (*ba*) flux of doxorubicin across Caco-2 monolayers. $*P < 0.05$ compared with the corresponding *ab* flux, $^{\dagger}P < 0.05$ compared with the *ab* flux of the control, $^{\ddagger}P < 0.05$ compared with the *ba* flux of the control.

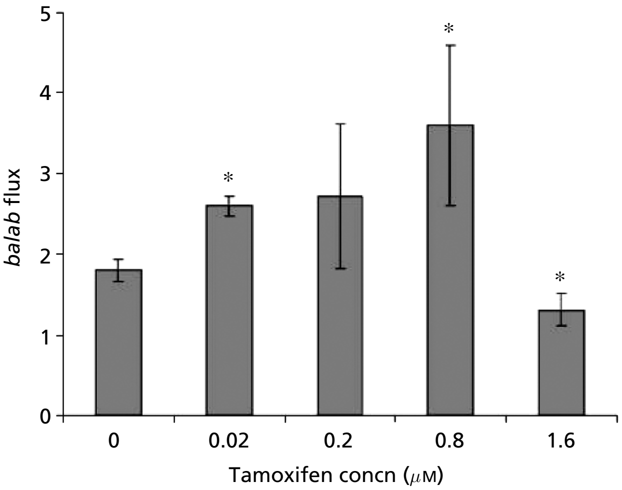

Figure 2 Comparison of the effect of various concentrations of tamoxifen on the ratio of fluxes (*ba*/*ab*) of doxorubicin across Caco-2 monolayers. *$P < 0.05$ compared with control.

Table 1 Model parameters of the effect of amlodipine, and tamoxifen on doxorubicin net efflux according to equation 5 (composite E_{max}/I_{max} model)

Parameters	Tamoxifen	Amlodipine
E_{max} (nM cm^{-2} h^{-1})	27.77 ± 3.94	55.11 ± 5.07*
EC50 (μM)	0.0036 ± 0.0004	4.64 ± 0.24*
I_{max1} (nM cm^{-2} h^{-1})	24.37 ± 2.05	35.46 ± 2.98*
$IC50_1$ (μM)	0.009 ± 0.001	5.07 ± 0.50*
I_{max2} (nM cm^{-2} h^{-1})	9.35 ± 1.73	27.39 ± 5.06*
$IC50_2$ (μM)	1.43 ± 0.19	13.48 ± 1.78*
E_0 (nM cm^{-2} h^{-1})	5.40 ± 0.73	6.02 ± 0.57

The parameters were estimated according to equation 5. The values represent mean ± s.e. E_{max} is the maximum modulator-induced stimulatory effect, I_{max} is the maximum modulator-induced inhibitory effect, $E_0 = J_{net}$ in the absence of the modulator. The model predicts an increase in the net flux with respect to that of the control (0 μM amlodipine) with an EC50 estimated as 4.64 μM, followed by two phases of reduction in the net flux of doxorubicin with IC50 values of 5.07 and 13.48 μM. *$P < 0.05$ compared with tamoxifen parameter.

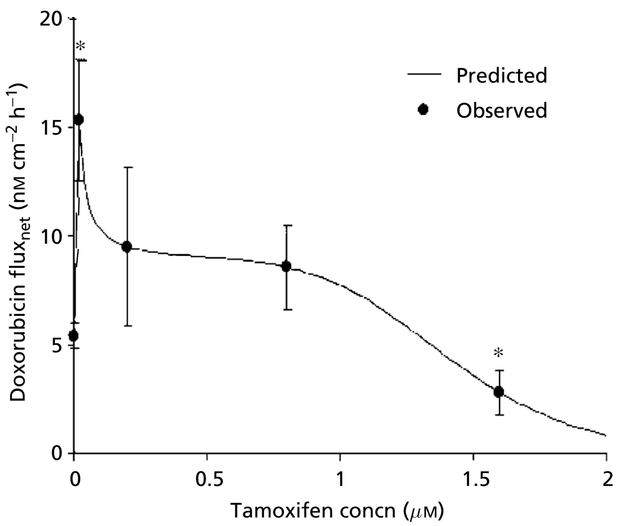

Figure 3 Concentration-dependent modulation of the net flux of doxorubicin by tamoxifen across Caco-2 monolayers, the predicted curve represents the fitted line obtained according to equation 5 (composite E_{max}/I_{max} model). *$P < 0.05$ compared with control.

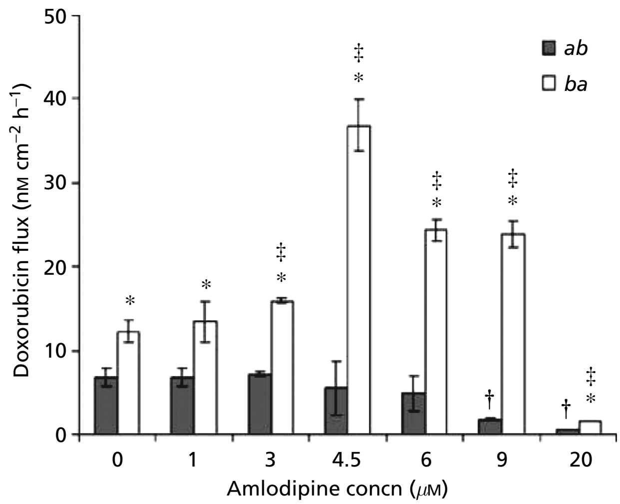

Figure 4 Comparison of the effect of various concentrations of amlodipine on the apical-to-basolateral (*ab*) and basolateral-to-apical transport (*ba*) of doxorubicin across Caco-2 monolayers. *$P < 0.05$ compared with the corresponding *ab* flux, †$P < 0.05$ compared with the *ab* flux of the control, ‡$P < 0.05$ compared with the *ba* flux of the control.

at 0.2 and 0.8 μM. Figure 3 also depicts the predicted net flux vs tamoxifen concentration data (solid line), estimated by fitting the composite E_{max}/I_{max} model described in the Materials and Methods section (eqn 5) to the observed data. The parameters obtained from the analysis of the data are summarized in Table 1. The model predicted an increase in the net flux with respect to control (0 μM tamoxifen) with an EC50 (the concentration which produces 50% of the maximum possible response) estimated as 0.0036 μM, followed by two phases of reduction in the net flux of doxorubicin with IC50 values (the concentration required for 50% inhibition) of 0.009 and 1.43 μM.

Figure 4 illustrates the effect of amlodipine concentration on the unidirectional fluxes of doxorubicin in either the *ab* or *ba* direction across cultured Caco-2 monolayers. As it was reported for tamoxifen, *ba* fluxes were significantly greater than the *ab* at all given amlodipine concentrations. Figure 5 depicts the ratio of fluxes estimated

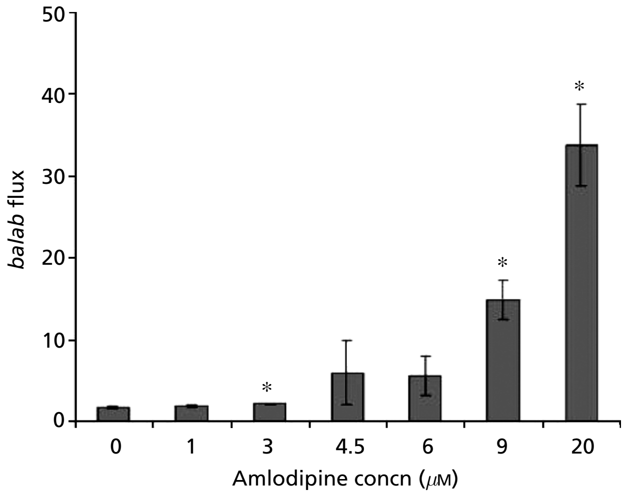

Figure 5 Comparison of the effect of various concentrations of amlodipine on the ratio of fluxes (*ba/ab*) of doxorubicin across Caco-2 monolayers. $*P < 0.05$ compared with control.

from *ba/ab* at various amlodipine concentrations. Statistical analysis of the data revealed significant increase of this parameter in 3, 9 and 20 μM amlodipine concentrations compared with control.

Effect of amlodipine on the net flux of doxorubicin, also occurring in the *ba* direction, is shown in Figure 6 (solid circles). The net flux was significantly increased at amlodipine concentrations ranging from 3 to 20 μM, and remained unaffected at 1 μM amlodipine with respect to its absence. The solid line in Figure 6 represents the predicted net flux of doxorubicin vs amlodipine concentration data,

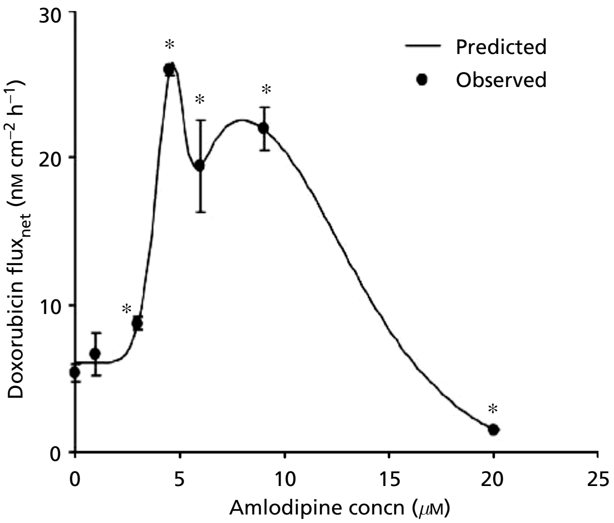

Figure 6 Concentration-dependent modulation of the net flux of doxorubicin by amlodipine across Caco-2 monolayers, the predicted curve represents the fitted line obtained according to equation 5 (composite E_{max}/I_{max} model). $*P < 0.05$ compared with control.

estimated by using the composite E_{max}/I_{max} model (eqn 5). The estimated parameters from analysis of the data are summarized in Table 1. The model predicted an increase in the net flux with respect to control (0 μM amlodipine) with an EC50 estimated as 4.64 μM, followed by two phases of reduction in the net flux of doxorubicin with IC50 values of 5.07 and 13.48 μM.

Statistical comparison of the model parameters reported for tamoxifen and amlodipine (Table 1) revealed a significant difference between all the parameters, with the exception of the modulator independent parameter E_0.

Discussion

Since the discovery of P-gp, many studies have been conducted to identify and classify the substrates as well as modulators of this transmembrane xenobiotic-efflux pump (Juliano & Ling 1976). Compounds with modulatory effects on ATPase activity of P-gp have been classified into three groups: those with stimulatory effect on P-gp ATPase activity at low concentrations and inhibitory effect at high concentrations (class I), those with only stimulatory effect (class II), and those with only inhibitory effect (class III). Tamoxifen has been identified as a class I modulator, as well as a substrate that is poorly transported by P-gp (Rao et al 1994).

The data obtained from doxorubicin flux across Caco-2 monolayers were analysed at three levels, namely the unidirectional fluxes of doxorubicin (*ab* and *ba*), ratio of the unidirectional fluxes (*ba/ab*), and the net flux (*ba* – *ab*). This was to elucidate the concentration-dependent mode of modulatory effect of tamoxifen and amlodipine.

In general, the *ba* fluxes were found to be greater than the corresponding fluxes from the *ab* direction (Figures 1 and 4). This observation confirmed that a transport process other than passive diffusion was involved in *ba* fluxes. We attributed the difference to the efflux activity of P-gp in Caco-2 cells. This was because the cell monolayers had been reported as an in-vitro model for drug transport studies due to their polarized efflux property (Augustijns et al 1993; Deferme et al 2002; Pfrunder et al 2003), and this efflux activity was mainly attributed to the presence of P-gp in the apical membrane (Stephens et al 2001).

Under the conditions of this study, no distinct pattern emerged as to the effect of tamoxifen on the *ab* flux of doxorubicin. The effect was either none (0.2 and 1.6 μM) or small (0.02 and 0.8 μM). For amlodipine, the same effect showed no significance over the range 1–6 μM, but there was a considerable reduction of the *ab* flux at 9 and 20 μM. Such an effect could be attributed to its membrane modulating properties through its localization within the plasma membrane (Mason et al 1999), especially in terms of membrane permeability.

The *ba/ab* value provided information regarding the extent of *ba* flux with respect to the corresponding *ab* flux of the substrate. Although useful in identifying the impact of the modulators of the transport mechanism, it lacked the ability of identifying the mode of modulation. A comparative analysis of the fluxes ratio with the magnitude of the *ab* fluxes in conjunction with that of the control revealed the impact of the modulators on the membrane-dependent transport. Analysis of the *ba*/*ab* data for tamoxifen suggested a stimulatory effect at 0.02 μM, a net inhibitory effect at 1.6 μM, and no apparent effect at 0.2 and 0.8 μM of the modulator, with no apparent impact on the membrane-dependent transport. However, the same method of analysis suggested a stimulatory effect for amlodipine with no net inhibitory effect on the transporter-dependent transport, and a significant impact on reduction of the membrane-dependent transport at 9 and 20 μM.

Comparison of the net flux of doxorubicin across Caco-2 monolayers in the presence of various tamoxifen concentrations over the range 0.02–1.6 μM with those obtained in the absence of tamoxifen (Figure 3) suggested a peak stimulatory effect on the efflux activity of P-gp at 0.02 μM tamoxifen. Such stimulatory mode was followed by reduction in the net flux of doxorubicin as the tamoxifen concentration increased, with a net inhibitory effect at 1.6 μM. The results demonstrated clearly a dual modulatory mode for tamoxifen with respect to P-gp efflux activity. This conclusion was consistent with published data concerning the effect of tamoxifen on ATPase activity of P-gp (Rao et al 1994).

It was interesting to observe that the mathematical relationship best fitted to the data (Figure 3) contained one stimulatory component with $EC50 = 3.6 \pm 0.4$ nM and two inhibitory components with $IC50_1 = 9.0 \pm 1.0$ nM, and $IC50_2 = 1.4 \pm 0.19$ μM (Table 1). This suggested two distinct inhibitory modes for tamoxifen with respect to its effect on the efflux of doxorubicin. The comparison of EC50 and $IC50_1$ revealed that the stimulation was more likely to occur simultaneously with the inhibition at low concentrations of tamoxifen. Depending upon the concentration of the drug, the overall outcome of simultaneous stimulation and inhibition could manifest itself as stimulation, no effect or inhibition. The significant difference between IC50 of the two inhibitory components might correspond to the two distinct inhibitory processes. Considering the calculated EC50 and IC50 values, tamoxifen was found to be a potent modulator of P-gp. Tamoxifen is known to be a substrate of the CYP3A family, and some members of this family such as CYP3A4 exist in Caco-2 cells (Benet & Cummins 2001). Thus, the potency of tamoxifen might be in part due to the co-existence of its active metabolite 4-hydroxy tamoxifen, which is formed during the experiment. This metabolite of tamoxifen is known to incorporate itself in the membrane (Custodio et al 1993b), and is considered a class I P-gp modulator (Rao et al 1994).

The effect of various concentrations of amlodipine on the net flux of doxorubicin across Caco-2 monolayers (Figure 6) indicated that amlodipine had no effect on the transport of doxorubicin at concentrations below 3 μM. However, at approximately 4.5 μM, a peak representing the stimulatory effect on the efflux activity of P-gp was observed, which declined at higher concentrations representing the reduction in the net flux. This observation was consistent with published data reporting the inhibitory effect of amlodipine at concentrations higher than the concentration range in this study (Katoh et al 2000).

A composite E_{max}/I_{max} model with one stimulatory ($EC50 = 4.64 \pm 0.24$ μM) and two inhibitory components ($IC50_1 = 5.07 \pm 0.50$ μM, $IC50_2 = 13.48 \pm 1.78$ μM) was best fitted to the data, within the selected amlodipine concentration range (Figure 6). Analysis of the net flux of doxorubicin and comparison of EC50 with $IC50_1$ and $IC50_1$ with $IC50_2$ suggested both stimulatory and inhibitory effects of amlodipine on the transmembrane transport of the P-gp substrate, thus identifying it as a class I modulator. However, the reducing effect of amlodipine on the *ab* flux at 9 and 20 μM with respect to control (Figure 4) suggested an additional mechanism for the molecule to impact the net flux through modification of the membrane-dependent transport. In this regard, the results of a recent publication have shown amlodipine to have the ability to inhibit doxorubicin-induced apoptosis in neonatal rat cardiomyocytes at 10 μM (Yamanaka et al 2003). Although the study focused on toxicity of doxorubicin in the presence of amlodipine and attributed the reduction of toxicity to antioxidant properties of amlodipine, the outcome coincides with our results with respect to the effect of amlodipine on doxorubicin transmembrane transport at 9 μM. Comparison of the model parameters estimated for tamoxifen and amlodipine (Table 1) identified amlodipine as a less potent modulator of P-gp.

Conclusions

The mathematical model presented in this study with the corresponding parameters (Table 1) would enable one to predict, isolate and simulate the stimulatory and inhibitory behaviour of amlodipine and tamoxifen. Thus, the concentration dependency of the modulatory effects of tamoxifen and amlodipine on P-gp efflux activity can be predicted and formulated for substrates of P-gp in cell culture systems. However, interpretation of the results obtained from the net flux data should be performed in conjunction with analysis of the unidirectional fluxes to determine the underlying mechanisms involved in modulation of the transport process. These findings can be related to clinical applications of co-administration of substrates and modulators of P-gp, considering the local drug concentration variations in the target tissues.

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