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Research paper

Effect of oral contraceptives on the transport of chlorpromazine across the CACO-2 intestinal epithelial cell line

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Abstract

In previous chlorpromazine pharmacokinetic studies a dramatic elevation in blood plasma levels of this drug was observed when taken in combination with oral contraceptives. Different mechanisms have been postulated to explain this observation. The aim of the study was to investigate whether oral contraceptives such as ethinyloestradiol and progesterone enhance the absorption of chlorpromazine by means of inhibiting P-glycoprotein (P-gp) and if this effect is mainly due to ethinyloestradiol or progesterone or their combination. The Caco-2 cell line was used as an in vitro model to study the effects of these compounds on the transport of chlorpromazine. Both apical to basolateral (AP-BL) and basolateral to apical (BL-AP) transport studies were done on chlorpromazine in combination with different compounds. Ethinyloestradiol enhanced the AP-BL cumulative transport of chlorpromazine by 11.5% compared to the control group, which was also statistically significantly higher than the effect caused by progesterone (0.8%). A combination of these two steroidal hormones enhanced the cumulative transport of chlorpromazine by only 2.0% compared to the control group. This indicates the possible existence of separate drug-binding sites for these two hormones and chlorpromazine on P-gp. The drug-binding site (or receptor) for progesterone probably interacts allosterically with the binding site for ethinyloestradiol and thereby decreasing its transport enhancing effects on chlorpromazine.

Keywords: P-glycoprotein; Chlorpromazine; Ethinyloestradiol; Progesterone; Allosteric interaction; Caco-2 cells

1. Introduction

In a recent chlorpromazine pharmacokinetic study a dramatic elevation of this drug's blood plasma concentration was observed in one of the female patients, resulting in severe tremors and dyskinesia on day 12 of the study. Further investigation revealed that she had taken a combined oral contraceptive containing norgestrel and ethinyloestradiol 8 days after starting the chlorpromazine therapy [1]. A similar increase in chlorpromazine levels caused by these female steroidal hormones was also observed in a group of postmenopausal schizophrenic patients receiving hormone replacement therapy. In this group of patients the average daily dose of chlorpromazine was lower compared to the dosage received by a group of postmenopausal schizophrenic patients not receiving hormone replacement therapy. The lower dosage of chlorpromazine in the hormone replacement therapy group had a similar therapeutic effect compared to the group receiving the higher dose, but with significantly fewer adverse effects [2]. Since chlorpromazine is a substrate for P-glycoprotein (P-gp), it was hypothesised that P-gp may be involved in the mechanism by which steroidal hormones increase the absorption of chlorpromazine.

Active extrusion of absorbed drugs from the intestinal epithelium has been recognised as one of the major factors determining oral drug bioavailibility [3]. P-gp is a membrane bound, adenosine triphospate (ATP) dependant efflux transporter (molecular weight = 170 kDa), expressed at high levels in the villus tip of enterocytes of the small intestine [4].

One major characteristic of P-gp that distinguishes it from the other transporters of the adenosine triphosphate binding cassette super family is its ability to efflux a wide

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variety of commonly used drugs that are structurally and functionally unrelated [5,6]. This phenomenon termed multidrug resistance is associated with a reduction in the intercellular concentrations of drugs and an increase in their cellular efflux, both energy dependent processes [7].

P-gp substrates can be divided into the following two groups: (1) substrates that interact with P-gp and thereby activate P-gp ATPase; and (2) a group that exhibits a high affinity for P-gp, but fails to activate P-gp ATPase [8]. Both chlorpromazine and its metabolite, chlorpromazine sulfoxide, are substrates for P-gp [9,10]. Since both the female steroidal hormones (progesterone and ethinyloestradiol) as well as chlorpromazine have the potential to interact with P-gp, the possible effect of this efflux-transporter on the transport of chlorpromazine across epithelial cells warrants investigation.

Currently there are two models explaining the action by which P-gp actively efflux compounds into the lumen of the small intestines, which include the flippase and the hydrophobic vacuum cleaner model [11]. According to the hydrophobic vacuum cleaner model, P-gp acts like a vacuum cleaner by carrying its substrate into a cavity within the P-gp itself and then expelling the substrate into the extra-cellular medium [11].

The basis of the flippase model is that substrates do not interact with the transporter directly from the aqueous phase, but rather interacts with the lipid bilayer thereby gaining access to the core of the membrane associated P-gp. The drug is then flipped from the inner leaflet to the outer leaflet of the lipid bilayer. Alternatively, P-gp flips the drug from the inner leaflet of the bilayer directly into the extracellular medium. The net result of flipping drugs will be the same as that for the vacuum cleaner model, reducing the intracellular concentration of the drug and increasing the concentration of drug in the external medium [12].

Coinciding with these two proposed models is the characterisation of drug binding sites on P-gp and their interaction with each other. A possible third binding site was recently discovered which is capable of allosteric interactions with the other two sites. It is possible that the allosteric site interacts with the ATP binding sites, enhancing the rate of ATP hydrolysis or tightening the coupling between hydrolysis and drug transport [13]. Another study found the existence of three substrate-binding sites on P-gp and a fourth site that acted as a regulatory site due to its specificity for the binding of modulators, but not substrates. It was found that the four binding sites are able to allosterically communicate in a negative heterotropic manner with each other. This model suggests that each of the binding sites has a high and a low affinity state for its ligands/substrates with an equilibrium existing between the two states. Allosteric interactions between these sites may alter the equilibrium between these states, increasing the low affinity state and decreasing the high affinity state [14].

In this study the presence of a polarised efflux of chlorpromazine from Caco-2 cell monolayers was determined and the effect of ethinyloestradiol and progesterone individually as well as in combination on the transport of chlorpromazine across the Caco-2 cell monolayers were investigated.

2. Materials and methods

The human adenocarcinoma cell line, i.e. Caco-2 cells, (passage 36, American Type Culture Collection, VA) were grown in 25 cm^3 cell culture flasks (Corning Costar Corporation, USA) at 37°C in an atmosphere of 5% CO_2. The growth medium consisted of Dulbeco's Modified Eagle's Medium (Bio Whittaker, Walkersville, MD) supplemented with 10% foetal bovine serum (Delta Bioproducts Kempton Park, South Africa), 1% non-essential amino-acids (NEAA) (Bio Whittaker, Walkersville, MD) and a 1% pen/strep fungizone mixture (10 000 U penicillin/ml, 10 000 μg streptomycin/ml and 25 μg fungizone/ml, Bio Whittaker, Walkersville, MD). The growth medium was changed every 2nd day and the cells were trypsinated and split in a 1:3 ratio on a weekly basis.

Caco-2 cells (passage 36) were seeded on tissue culture treated polycarbonate filters in Costar Transwell six-well plates (Corning Costar Corporation, USA) consisting of an apical and basolateral chamber with a surface area of 4.7 cm^2. The volume of growth medium used on these filters is 2.5 ml in each chamber and the cell concentration seeded out on these filters was 1.77×10^4 cells/ml.

2.1. Experimental design

Experiments were conducted in a neutral environment (pH 7.4) such as found in some regions of the small intestine. All transport studies across the Caco−2 cell monolayers were done in triplicate. The medium for the transport experiments consisted of Hanks Balanced Salt Solution (HBSS) buffered at pH 7.4. The cell monolayers were washed once with the transport medium and then incubated for 1 h, prior to the start of the experiments. Following this initial incubation period, the transepithelial electrical resistance (TEER) was measured using a Millicell ERS meter (Millipore, USA) to determine if the cells reached confluent monolayers. All experiments were done on cell monolayers that had reached TEER values above 170 Ω/cm^2.

The transport of chlorpromazine across the Caco-2 cell monolayers was evaluated in five experimental groups, including group 1: chlorpromazine alone (Sigma Chemical Company Ltd., St. Louis, MO); group 2: chlorpromazine in the presence of verapamil (Sigma Chemical Company Ltd., St. Louis, MO); group 3: chlorpromazine in the presence of ethinyloestradiol (Sigma Chemical Company Ltd., St. Louis, MO); group 4: chlorpromazine in the presence of progesterone (Sigma Chemical Company Ltd., St. Louis,

MO) and group 5: chlorpromazine in the presence of both ethinyloestradiol and progesterone.

2.2. Apical to basolateral (AP-BL) transport studies

To study the apical to basolateral (AP-BL) transport of the test substances, the growth medium was removed from the apical chamber and replaced with an equal volume of transport medium (HBSS), which contained the substance for which transport was to be evaluated. Samples of 200 μl were drawn from the basolateral chamber at the following time intervals: 20, 40, 60, 80, 100, 120, 150, 180 and 240 min and each sample was replaced with an equal volume of fresh pre-heated transport medium at every time interval.

2.3. Basolateral to apical (BL-AP) transport studies

To study basolateral to apical (BL-AP) transport of the test substances, the transport medium was removed from the basolateral chamber and replaced with an equal volume of transport medium containing the substance for which transport was to be evaluated. Samples of 200 μl were removed from the apical chamber at the following time intervals: 20, 40, 60, 80, 100, 120, 150, 180, 240 min and each sample was replaced with an equal amount of fresh pre-heated transport medium every time.

2.4. Group 1: chlorpromazine alone

This experimental group served as a normal control for the AP-BL and BL-AP transport studies. Chlorpromazine hydrochloride was dissolved in HBSS in a concentration of 100 μM [15] and applied to the apical chamber of the transport filters for the AP-BL studies and applied to the basolateral chamber for the BL-AP studies.

An indication of a polarised efflux of chlorpromazine was determined by comparing the apparent permeability coefficient (P_{app}) of the AP-BL transport with the P_{app} of the BL-AP transport of chlorpromazine.

2.5. Group 2: chlorpromazine in the presence of verapamil

In this study group the AP-BL as well as the BL-AP transport of 100 μM chlorpromazine hydrochloride was evaluated in the presence of 500 μM verapamil [16], a known P-gp inhibitor. If the polarised efflux of chlorpromazine is not present in this experimental group, it would indicate the involvement of P-gp in the efflux of chlorpromazine.

2.6. Group 3: chlorpromazine in the presence of progesterone

In the third experimental group, the AP-BL and BL-AP transport of chlorpromazine hydrochloride was investigated in the presence of 10 μM progesterone [17]. This experimental group serves to give an indication of the contribution by progesterone on the enhancement of the transport of chlorpromazine hydrochloride across the Caco-2 cell monolayers.

2.7. Group 4: chlorpromazine in the presence of ethinyloestradiol

In this study group the AP-BL as well as the BL-AP transport of chlorpromazine hydrochloride is measured in the presence of 1 μM ethinyloestradiol (calculated in ratio to the concentration of progesterone as it appears in oral contraceptive products, i.e. a ratio of 10:1). This group was included to evaluate the effect of ethinyloestradiol on the transport of chlorpromazine across the Caco-2 cell monolayer possibly due to P-gp inhibition.

2.8. Group 5: chlorpromazine in the presence of both progesterone and ethinyloestradiol

In group five, the AP-BL and BL-AP transport of 100 μM chlorpromazine hydrochloride was evaluated in the presence of both 1 μM ethinyloestradiol and 10 μM progesterone. This group would give an indication of the synergistic effect (if any) of ethinyloestradiol and progesterone on the transport enhancement of chlorpromazine across epithelial cells, possibly due to their P-gp interaction.

2.9. Analysis of samples

Samples withdrawn during the transport experiments were stored in test tubes under nitrogen at −4°C for a period not longer than 10 days. The samples were then analysed by using an Agilent Hewlett Packard 1100 Series Chemetrix, high performance liquid chromatography (HPLC) system, consisting of a binary pump, auto-sampler, diode array detector and a thermostatic column compartment. Data obtained from this HPLC system were analysed and integrated with Hewlett Packard software. A Phenomenex Luna C_{18} (150 × 4.6 mm) column was used with an injection volume of 20 μl and the absorbance was measured at a wavelength of 254 nm. The mobile phase consisted of aqueous sodium-pentasulfonic acid (concentration = 0.001 g/ml and set at pH = 3.5) and acetonitrile in a 40:60 ratio with a flow rate of 1 ml/min (method adapted from the USP, 2002 [18]). The samples were analysed without an internal standard at room temperature. The repeatability of the system, when six measurements were made on the same day and under the same conditions, did not exceed a standard deviation of 0.002. The limit of quantification for chlorpromazine with this method was 150 ng/ml. The linearity (r^2) of the standard curve obtain for chlorpromazine in a concentration range of 150–30 000 ng/ml was 0.9995.

2.10. Data analysis

The transport of chlorpromazine was assessed across Caco-2 cell monolayers in the AP-BL and BL-AP direction in each of the five experimental groups. The cumulative transport values obtained for chlorpromazine in the five experimental groups were statistically analysed by means of a one-way repeated analysis of variance analysis and post-hoc Duncan tests. Values were considered statistically significantly different if $P < 0.05$.

2.11. Apparent permeability coefficient (P_{app})

The apparent permeability coefficient (P_{app}) for chlorpromazine across the Caco-2 cell monolayers in all the experimental groups were calculated from the cumulative transport profiles using the following equation:Where:

$P_{app} =$ $dQ/dt\{1/(A \cdot 60 \cdot C0)\}$,
$P_{app} =$ apparent permeability coefficient (cm s^{-1}),
$dQ/dt =$ permeability rate (amount permeated per minute),
$A =$ diffusion area of the monolayer (cm^2),
$C_0 =$ initial concentration of the drug.

3. Results and discussion

The total cumulative transport values (% of initial dose) of chlorpromazine in the AP-BL and BL-AP directions across the Caco-2 cell monolayers in all five experimental groups after a 4-h period are presented in Table 1.

Table 1
Cumulative transport values (% of initial dose) of chlorpromazine in the different experimental groups across Caco-2 cell monolayers in the AP-BL and BL-AP directions

Experimental group	Chlorpromazine (% of initial dose)	
	AP-BL[a,b]	BL-AP[a,b]
Group 1: chlorpromazine alone	18.20 ± 0.70[a]	32.57 ± 1.67
Group 2: chlorpromazine and verapamil	28.59 ± 4.57[b]	26.11 ± 6.84
Group 3: chlorpromazine and progesterone	19.00 ± 2.93[a]	30.75 ± 8.17
Group 4: chlorpromazine and ethinyloestradiol	29.68 ± 1.63[b]	27.97 ± 4.89
Group 5: chlorpromazine, progesterone and ethinyloestradiol	20.71 ± 2.93[a]	28.96 ± 0.95

[a] Values with the same letter do not differ statistically significantly (i.e. values with different letters are statistically significantly different, $P < 0.05$).
[b] Each value represents the average of three measurements ± SD.

3.1. Group 1: transport of chlorpromazine alone

The transport experiment of chlorpromazine alone (control group) across the Caco-2 cell monolayers achieved a total cumulative transport value of 18.20% of the initial concentration in the AP-BL direction and 32.57% of the initial concentration in the BL-AP direction after the 4-h period (Table 1).

The cumulative transport of chlorpromazine in the BL-AP direction was therefore 1.78 times higher compared to the cumulative transport in the AP-BL direction across the Caco-2 cell monolayers. This indicates a polarised efflux (i.e. an efflux in the BL-AP direction) of chlorpromazine across cell monolayers possibly mediated by an active transporter such as P-gp.

Furthermore, the results from the control group indicate therefore that the in vitro system (Caco-2 cell monolayer) used in this study is a suitable model for the investigation of the effects of other compounds (e.g. verapamil and oral contraceptives) on the transport of chlorpromazine as possibly mediated by interaction with P-gp.

3.2. Group 2: transport of chlorpromazine in the presence of verapamil

When verapamil (at a concentration of 500 μM) was applied to the apical side of the Caco-2 cell monolayers in combination with chlorpromazine, it increased the total cumulative transport of chlorpromazine in the AP-BL direction significantly to 28.59% as compared to the value of 18.20% in the control group (Table 1). This represents a 1.57-fold increase in the transport of chlorpromazine in the AP-BL direction in the presence of verapamil as compared to the control group (chlorpromazine alone).

In contrast to the increase of chlorpromazine transport in the AP-BL direction, the transport of chlorpromazine decreased in the BL-AP direction from 32.57% (as obtained in the control group) to a value of 26.11% of the initial concentration (Table 1).

These transport results of chlorpromazine in combination with a known P-gp inhibitor, verapamil, indicate that chlorpromazine is a substrate for P-gp. This is in accordance with the results obtained by Syed et al. [10], which showed an interaction between chlorpromazine and P-gp.

This experimental group serves as a positive control in this study to indicate that a proven P-gp inhibitor, verapamil, does affect the transport of chlorpromazine across the Caco-2 cell monolayers.

The significant increase in the transport of chlorpromazine in the presence of verapamil can possibly be explained by inhibition of P-gp by the verapamil molecules and thereby inhibition of the efflux of chlorpromazine back into the BL-AP direction. Results from recent studies indicate that the binding of verapamil at a concentration above 20 μM inhibits the ATPase activity of P-gp and is also capable of lowering the ATPase activity to a level below basal value

[19]. This lowering of the ATPase activity possibly inhibits the P-gp mediated efflux of chlorpromazine without directly affecting the binding of chlorpromazine to P-gp. This nucleotide independent binding of a substrate to P-gp was also observed with vinblastine. The binding of verapamil or vinblastine alone causes conformational changes in P-gp, but the changes induced by the binding of a combination of these drugs is different from that induced by nucleotide binding [20].

3.3. Group 3: transport of chlorpromazine in the presence of progesterone across Caco-2 cell monolayers

Progesterone alone had only a negligible effect on the transport of chlorpromazine in the AP-BL direction across the Caco-2 epithelial cell monolayers compared to the transport obtained in the control group (i.e. an increase from 18.20% to a value of 19.0% as indicated in Table 1).

Polarised efflux of chlorpromazine is still indicated in this experimental group by the fact that the total cumulative transport of chlorpromazine is substantially higher in the BL-AP direction (30.75% of the initial value) compared to the AP-BL direction (19.0% of the initial value). It seems that progesterone, in a concentration of 10 μM, does not have an inhibitory effect on the P-gp mediated efflux of chlorpromazine in the Caco-2 cell monolayers.

A possible explanation may be that at a concentration of 10 μM, the extent of the effect of progesterone on ATPase activity is too low to affect the transport of chlorpromazine across the Caco-2 cells. Although the progesterone did not cause an increase in the transport of chlorpromazine, the possibility exists that it may still interact with the P-gp transporter and by means of allosteric effects influence the interaction of other substances such as ethinyloestradiol with P-gp. This may have an indirect effect on the transport of chlorpromazine across the Caco-2 cell monolayers when progesterone is applied in combination with other compounds such as ethinyloestradiol.

3.4. Group 4: transport of chlorpromazine in the presence of ethinyloestradiol

The total cumulative transport values of chlorpromazine in the AP-BL and BL-AP directions after a 4-h period in the presence of ethinyloestradiol were 29.68 and 27.97% (of the initial concentration), respectively (Table 1). This represents a 1.63-fold increase in the cumulative transport of chlorpromazine in the presence of ethinyloestradiol compared to the transport when chlorpromazine was administered alone in the control group. This is a statistically significant increase in the cumulative transport of chlorpromazine across the epithelial cell monolayers in the AP-BL direction compared to its transport in the control group ($P < 0.05$, Duncan test).

Furthermore, the difference between the cumulative transport of chlorpromazine in the AP-BL and the BL-AP direction was only 1.7% in this experimental group compared to a difference of 14.4% in the control group. This clearly indicates that the P-gp mediated efflux of chlorpromazine as shown in the control group was inhibited by adding ethinyloestradiol.

3.5. Group 5: transport of chlorpromazine in the presence of ethinyloestradiol and progesterone

The total cumulative transport of chlorpromazine after a 4-h period in the AP-BL direction was slightly higher in the presence of a combination of ethinyloestradiol and progesterone compared to its transport in control group. This effect represents only a 1.14-fold increase in the transport of the chlorpromazine in the AP-BL direction. It is also clear from these results that the effect of ethinyloestradiol in combination with progesterone on the transport of chlorpromazine across Caco-2 cell monolayers is markedly lower than the effect obtained when ethinyloestradiol was added alone. A combination of ethinyloestradiol and progesterone does not inhibit the efflux of chlorpromazine to an extent where a significant increase in the transport of chlorpromazine in the AP-BL direction could be observed.

This can possibly be explained by the fact that ethinyloestradiol and progesterone affect each other's action on the efflux function of P-gp. Ethinyloestradiol and progesterone probably interact with different binding sites on P-gp and thereby allosterically modulate each other's affinity for P-gp, which can indirectly lead to a decrease in the effect of ethinyloestradiol on the transport of chlorpromazine.

In addition, the cumulative transport of chlorpromazine in this experimental group was 28.96% (of the initial concentration) in the BL-AP direction compared to the cumulative transport of 20.71% (of the initial concentration) in the AP-BL direction (Table 1). This indicates that a polarised efflux of chlorpromazine, most probably by means of P-gp, was still present in this experimental group.

3.6. Comparison of the transport of chlorpromazine in the different experimental groups

The cumulative transport of chlorpromazine in the AP-BL direction across Caco-2 cell monolayers plotted as a function of time is shown in Fig. 1, while the cumulative transport of chlorpromazine in the BL-AP direction plotted as a function of time is depicted in Fig. 2.

From Fig. 1 it is clear that the extent and rate of the transport of chlorpromazine was higher in the AP-BL direction when ethinyloestradiol (group 4) and verapamil (group 2) were added to the chlorpromazine. In contrast to the increase in the transport of chlorpromazine in the AP-BL direction, its cumulative transport in the BL-AP direction was decreased when ethinyloestradiol and verapamil were added. Therefore, the transport of chlorpromazine in the AP-BL direction across the Caco-2 cell monolayers was

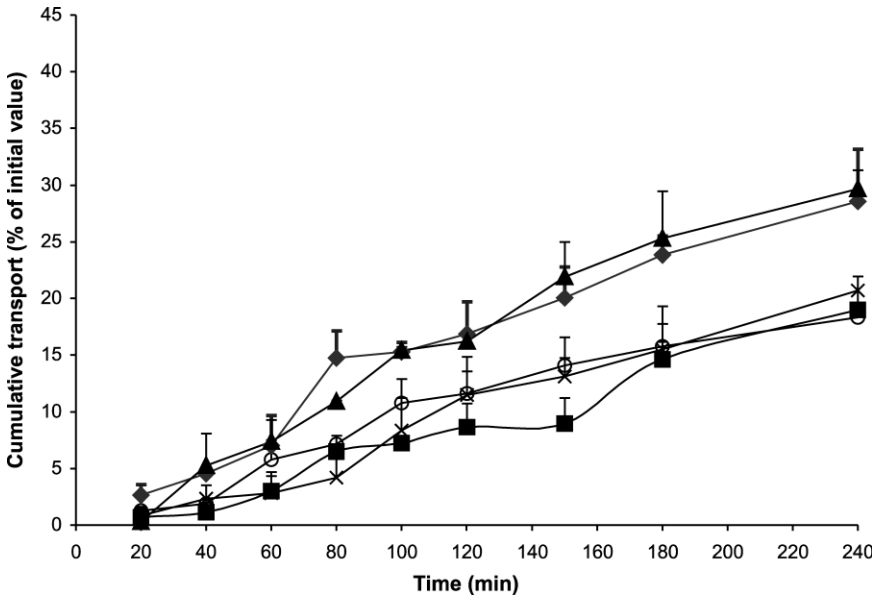

Fig. 1. Cumulative transport of chlorpromazine across Caco-2 cell monolayers in the AP-BL direction as a function of time in the different experimental groups. Key: Chlorpromazine alone (○), chlorpromazine and verapamil (◆), chlorpromazine and progesterone (■), chlorpromazine and ethinyloestradiol (▲), chlorpromazine and a combination of ethinyloestradiol and progesterone (×).

increased by an inhibition of the transport in the BL-AP direction or the efflux effect of P-gp.

The ethinyloestradiol-induced increase of chlorpromazine transport in the AP-BL direction across Caco-2 cell monolayers was achieved at a concentration of 1 μM compared to a similar order transport increasing effect caused by verapamil at a concentration of 500 μM. This indicates that ethinyloestradiol has a more potent effect than verapamil on the transport of chlorpromazine, possibly due to a higher affinity for P-gp.

A combination of ethinyloestradiol and progesterone did not have a synergistic effect on the transport of chlorpromazine across the cell monolayers. This indicates that progesterone probably counteracts the transport increasing effect on chlorpromazine as exhibited by ethinyloestradiol. This interaction between progesterone and ethinyloestradiol can possibly be explained by an allosteric modulation of the drug-binding site of ethinyloestradiol on P-gp, shifting its equilibrium to the low affinity state and thereby decreasing its binding affinity for ethinyloestradiol.

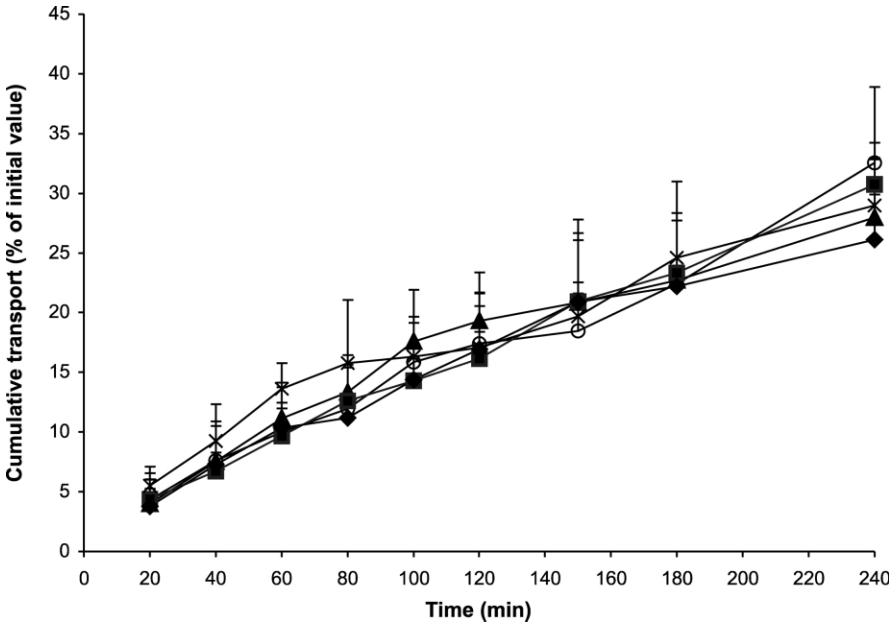

Fig. 2. Cumulative transport of chlorpromazine across Caco-2 cell monolayers in the BL-AP direction as a function of time in the different experimental groups. Key: Chlorpromazine alone (○), chlorpromazine and verapamil (◆), chlorpromazine and progesterone (■), chlorpromazine and ethinyloestradiol (▲), chlorpromazine and a combination of ethinyloestradiol and progesterone (×).

Table 2
Apparent permeability coefficient (P_{app}) values for chlorpromazine across Caco-2 cell monolayers in the different experimental groups

Experimental group	$P_{app} \times 10^{-6}$		
	AP-BL[a,b]	BL-AP[a,b]	(AP-BL)−(BL-AP)[c]
Group 1: chlorpromazine HCl	2.93 ± 0.03[b]	4.25 ± 0.01[a]	−1.32 ± 0.07[b]
Group 2: chlorpromazine HCl and verapamil	4.30 ± 0.3[a]	3.19 ± 0.9[a]	1.11 ± 1.2[a]
Group 3: chlorpromazine HCl and progesterone	2.54 ± 0.8[b]	4.30 ± 1.4[a]	−1.75 ± 1.3[b]
Group 4: chlorpromazine HCl and ethinyloestradiol	4.84 ± 0.5[a]	3.66 ± 0.4[a]	1.17 ± 0.5[a]
Group 5: chlorpromazine HCl, ethinyloestradiol and progesterone	3.05 ± 0.2[b]	3.53 ± 0.8[a]	−0.47 ± 1.1[a,b]

[a] Values with the same letter do not differ statistically significantly (i.e. values with different letters are statistically significantly different, $P < 0.05$).
[b] Each value represents the average of three measurements ± SD.
[c] Difference in the P_{app} values as an indication of a polarised efflux.

The calculated apparent permeability coefficients (P_{app}) of chlorpromazine as calculated from the cumulative transport profiles in the five experimental groups are depicted in Table 2.

The difference in the P_{app} values (AP-BL minus BL-AP) for chlorpromazine is statistically significantly smaller for the control group than for the difference in the P_{app} values (AP-BL minus BL-AP) obtained in the experimental groups where verapamil and ethinyloestradiol were added. This confirms the polarised efflux of chlorpromazine in the BL-AP direction across the cell monolayers.

Both verapamil and ethinyloestradiol increased the P_{app} value of chlorpromazine in the AP-BL direction significantly. Concurrently, the P_{app} value of chlorpromazine in the BL-AP direction decreased when verapamil and ethinyloestradiol were added.

The results from this study suggest that the combination of ethinyloestradiol and progesterone does not affect the efflux of chlorpromazine significantly and therefore cannot explain the increase in chlorpromazine plasma concentration seen in the presence of the combined oral contraceptive. The possibility that progesterone may act at a different site on P-gp to modulate the effects of oestrogens presents a further research challenge.

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