P-Glycoprotein Is More Efficient at Limiting Uptake than Inducing Efflux of Colchicine and Vinblastine in HL-60 Cells

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Purpose. To investigate the role of the P-glycoprotein (P-gp) drug efflux pump in the intracellular disposition of colchicine and vinblastine.

Methods. Uptake and efflux kinetics were studied *in vitro* in human lymphocytes and in HL-60 cells with or without the P-gp modulator, verapamil.

Results. In human lymphocytes, colchicine was slowly taken up (uptake half-life was 18.9 ± 1.1 hr.) and verapamil increased colchicine uptake by 37%, whereas it did not modify colchicine efflux from cells. In HL-60 cells, colchicine uptake was non-linear and slower than that of vinblastine, the colchicine uptake half-life (11.1 ± 0.5 hr.) being 25-fold longer than that of vinblastine at 25 nM. Verapamil did not significantly modify colchicine uptake half-life, but increased its intracellular accumulation by 23% and that of vinblastine by 81%. Immunoflow cytometry showed that P-gp expression in HL-60 cells increased significantly from 24 hr. following colchicine or vinblastine exposure. The significant increase in colchicine uptake induced by verapamil at 24 hr. was correlated with this enhanced P-gp expression. The drug efflux half-life was 11.5-fold higher for colchicine (23 ± 0.9 hr) than vinblastine, indicating a much slower elimination of colchicine from cells that could be related to its longer dissociation half-life from the tubulin receptor. Verapamil treatment did not modulate either colchicine or vinblastine efflux kinetics, suggesting that the intracellular drugs are not available to the transmembrane P-gp binding sites.

Conclusions. P-gp may not be the main reason for the slowness of colchicine uptake. It may be more efficient at controlling entry of colchicine and vinblastine through the plasma membrane than at mediating their efflux from HL-60 cells.

KEY WORDS: colchicine; vinblastine; verapamil; P-glycoprotein; flow cytometry.

INTRODUCTION

Colchicine, an alkaloid obtained from *Colchicum autumnale,* is widely used in the treatment of acute gouty arthritis and in the prophylaxis of familial Mediterranean fever or inflammatory diseases. The anti-inflammatory effects of colchicine result from modulation of leucocyte activities such as lymphocyte chemotaxis (1). Thus, leucocytes have been reported to be an active pharmacologic compartment of colchicine where intracellular colchicine kinetics superpose those of its biological effects. Maximal colchicine concentration is only reached in leucocytes 48 hr. after oral administration of 1 mg colchicine to healthy volunteers; that is the time generally needed for clinical improvement of inflammation (2). The extremely slow colchicine uptake raises the question of the existence of a cellular barrier, such as P-glycoprotein (P-gp).

P-gp is an ATP-dependent transmembrane glycoprotein, a product of the human mdr1 gene, which reduces the intracellular accumulation of structurally and functionally unrelated compounds including anthracyclines, vinca alkaloids such as vinblastine, or colchicine, by extruding them out of cells (3). Overexpression of P-gp in human cancer cell lines is responsible for classical multidrug resistance (MDR) in cancer chemotherapy. The presence of P-gp in normal tissues, including the apical membrane of mucosal intestinal cells, the brush border of renal proximal tubules and the biliary membrane of hepatocytes (4), suggests that P-gp could also modulate the absorption or excretion of drugs. Moreover, Speeg *et al.* (5–6) reported that colchicine is actively secreted into urine and bile by P-gp. Though P-gp has been shown to contribute to colchicine elimination *in vivo,* the influence of P-gp on colchicine distribution and specially in one effector compartment, i.e. human leucocytes where P-gp expression has been reported (7), remains unknown. The hypothesis of a P-gp-mediated effect on blocking colchicine entrance or mediating its efflux could be an explanation for the slowness of colchicine uptake and of its delayed pharmacological activity.

Because of the limited availability of human lymphocytes, we first of all compared colchicine uptake and efflux kinetics with or without verapamil (a well known inhibitor of P-gp (8)) in human lymphocytes and in an HL-60 human promyelocytic cell line characterized by cell-surface basal expression of P-gp (9). As we found comparable kinetic data, we then used HL-60 cells for more complete uptake and efflux kinetic studies of colchicine at concentration levels close to those used in human therapy (10). Results for colchicine were compared with those of vinblastine, another microtubule disrupting agent for which cellular uptake has been described as rapid, like most lipophilic compounds.

MATERIALS AND METHODS

Materials

^{3}[H]-Colchicine (Ring C, ^{3}H-methoxy, 66 Ci/mmol) was obtained from New England Nuclear (Paris, France). ^{3}[H]-Vinblastine sulphate (13.5 Ci/mmol) was from Amersham (Les Ulis, France). Verapamil was purchased from Sigma (Saint Quentin Fallavier, France).

Cell Culture

Lymphocytes were obtained from healthy human donors and separated from the other blood cells by standard Ficoll

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ABBREVIATIONS: P-gp, P-glycoprotein; MDR, multidrug resistance; HBSS, Hank's balanced salt solution; PBS, phosphate-buffered saline; AUC, area under curve.

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gradient centrifugation (9). Lymphocytes and the HL-60 human promyelocytic leukemia cell line were suspended in RPMI 1640 supplemented with 10% fetal calf serum and 2 mM glutamine in a 5% CO_2 humidified atmosphere in air. HL-60 cells were seeded at a density of 10^6 cells/ml every three days and cell viability checked by trypan blue exclusion was > 95%.

Colchicine and Vinblastine Uptake Kinetics

10^6 lymphocytes/ml were incubated with 6.25 nM ^{3}[H]-colchicine at 37°C in the absence or presence of 10 μM verapamil. Verapamil was added 30 min. before drug uptake and maintained at 10 μM in the culture medium throughout the experiment. Aliquots of cell culture were removed at 1.5, 4, 5.5, 29, 48, 54 hr. after colchicine incubation. Each aliquot was divided into two parts, one for protein assay and the other for intracellular drug concentration measurement. Cells were washed three times with Hank's balanced salt solution (HBSS) at +4°C to remove the extracellular drug, and intracellular radioactivity was determined in duplicate. The protein assay was performed in duplicate by the method of Lowry *et al.* (12). To study drug kinetics in HL-60 cells, 10^6 cells/ml were incubated with different extracellular ^{3}[H]-colchicine concentrations (3.12 to 62.5 nM) or with 25 nM ^{3}[H]-vinblastine at 37°C in the absence or presence of 10 μM verapamil, an extracellular concentration that did not affect the growth of HL-60 cells (11). Intracellular drug concentration was determined as described in lymphocytes uptake studies.

Colchicine and Vinblastine Efflux Kinetics

10^6 lymphocytes/ml were pre-incubated with 6.25 nM ^{3}[H]-colchicine for 48 hr. in order to attain the maximal intracellular drug concentration. Cells were washed three times with drug-free medium (+4°C) and resuspended in a drug-free prewarmed medium at 37°C to allow intracelllular drug efflux in the absence or presence of 10 μM verapamil. Aliquots of the resuspended culture were removed at 0, 2.5, 5.5, 23.5, and 29.5 hr. Drug efflux was maintained by replacing, at each experimental time point, the extracellular medium with drug-free prewarmed culture medium. Intracellular colchicine concentrations were measured as described in the uptake studies.

To study drug efflux in HL-60 cells, 10^6 cells/ml were pre-incubated with 25 nM ^{3}[H]-colchicine for 48 hr. or with 25 nM ^{3}[H]-vinblastine for 4 hr. Aliquots of the resuspended culture were removed at 0, 1, 4, 8, 24, 32, 48, 56, 72 hr. for colchicine or at 0, 15, 30, 45, 60, 120, 180, 240 min. for vinblastine.

P-glycoprotein Expression Analysis

Expression of P-gp on HL-60 cell surfaces was determined by flow cytometry before (control) and after the addition of 25 nM colchicine or 25 nM vinblastine for 4, 8, 24, 48 and 72 hr. At each time point, cells were washed with ice-cold HBSS and 10^6 cells were incubated with UIC2 monoclonal anti-Pgp antibody (50 μg/ml) (UIC2, Immunotech, Marseille, France) for 30 min at +4°C. They were then washed three times with phosphate-buffered saline (PBS) containing 0.5% of human AB serum. Antibody binding was revealed following incubation with fluorescein isothiocyanate-conjugated goat anti-mouse Ig $(Fab')_2$ (Nordic Immunology, Tilburg, The Netherlands) for 30 min. at +4°C. After washing with PBS at 4°C, immunofluorescence analysis was performed on a FACScan (Becton Dickinson, Immunocytometry System, San Jose, CA). Mean fluorescence intensity of positive cells was converted into the number of antigen binding site using the Quantum Simply Cellular Microbeads Kit (Sigma, St Louis, Mo, U.S.A.).

Pharmacokinetic Analysis

Colchicine and vinblastine uptake kinetics were fitted to a closed two-compartment model, where compartments 1 and 2 represent the extracellular and intracellular drug, respectively, using the equation:

$$C = C_{ss}\,(1\text{-}e^{-kt})$$

where **C** is the intracellular drug concentration, $\mathbf{C_{ss}}$ the intracellular drug concentration at steady-state and **k** the drug uptake first order rate constant. Drug uptake half-life ($t_{1/2\ uptake}$) was calculated by:

$$t_{1/2\ uptake} = 0.693/k$$

The nonlinear regression program MK-MODEL (version 4, Biosoft, Cambridge, UK) was used to estimate C_{ss} and k. The area under the curve (AUC) of the uptake kinetics was calculated by the trapezoidal method using the computer program INPLOT (GraphPAD software, ISI Sorrento Valley, CA). Drug efflux half-life was calculated using least-squares linear regression.

Statistical Analysis

Uptake and efflux kinetics were performed at least in quadruplicate. Data are expressed as means ± S.D. Student's t-test was used to compare the AUC in the absence or presence of verapamil. Bonferroni's test was used to analyse the effect of verapamil on the intracellular drug concentration at each experimental drug uptake time point. Student's t-test was used to examine the increase in P-gp expression induced by colchicine and vinblastine.

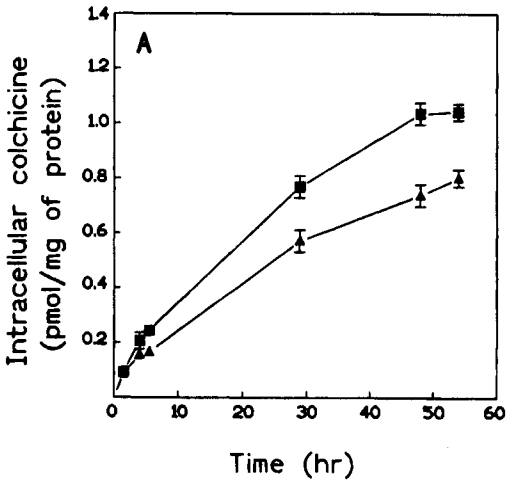
RESULTS

Colchicine Uptake and Efflux Kinetics in Human Lymphocytes

^{3}[H]-colchicine uptake by lymphocytes was slow as the $t_{1/2\ uptake}$ was 18.9 ± 1.5 hr. and the $AUC_{0\text{-}54\ hr}$ was 27.3 ± 1.8 pmol.h/mg of protein. Verapamil at 10 μM significantly increased colchicine uptake by 37% as shown by the $AUC_{0\text{-}54\ hr}$ and slightly decreased the colchicine $t_{1/2\ uptake}$ by 8% (Fig. 1A). Intracellular colchicine efflux kinetics were monoexponential with a half-life of 15.6 ± 0.8 hr. and were not significantly different in the presence of 10 μM verapamil (17 ± 1.1 hr) (Fig.1B).

Colchicine and Vinblastine Uptake Kinetics in HL-60 Cells

^{3}[H]-colchicine uptake in HL-60 cells is shown in Fig. 2. For extracellular colchicine concentrations ranging from 3.12 to 62.5 nM, colchicine uptake was a slow process, since the intracellular colchicine concentration at steady-state was



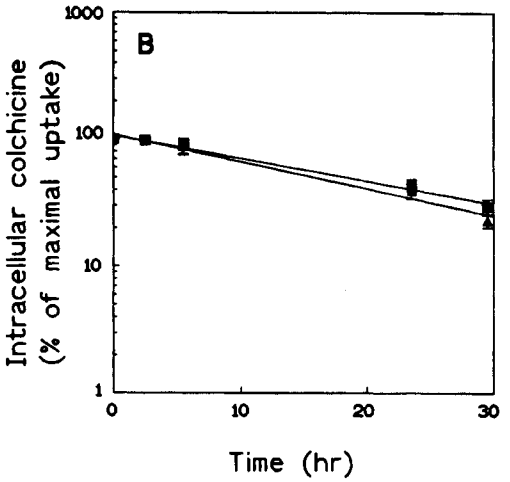


Fig. 1. Influence of verapamil of [^{3}H]-colchicine uptake and efflux kinetics in human lymphocytes. (A) Cells were incubated with 6.25 nM [^{3}H]-colchicine in the absence (▲) or presence (■) of 10 μM verapamil pre-incubated with cells 30 min before colchicine exposure. (B) Cells were pre-incubated with 6.25 nM [^{3}H]-colchicine for 48 hr. Efflux was performed in the absence (▲) or presence (■) of 10 μM verapamil. Results are means ± S.D. of three different experiments.

reached only 30 hr. after colchicine exposure. However, colchicine $t_{1/2\ uptake}$ decreased 2.7-fold when the extracellular colchicine concentration increased from 3.12 to 62.5 nM (Table I), suggesting a more rapid uptake into HL-60 cells at higher extracellular concentration. Moreover, the C_{ss} and $AUC_{0-72\ hr}$ of the colchicine uptake kinetics increased linearly up to an extracellular colchicine concentration of 25 nM (Fig. 3).

^{3}[H]-vinblastine uptake at 25 nM in HL-60 cells is shown in Fig. 4(B). Uptake of vinblastine was more rapid than that of colchicine and reached a plateau at 120 min. Vinblastine $t_{1/2\ uptake}$ was about 26 min. and the intracellular vinblastine concentration at steady-state was about three-fold higher than for colchicine (25 and 8 pmol/mg of protein for vinblastine and colchicine, respectively).

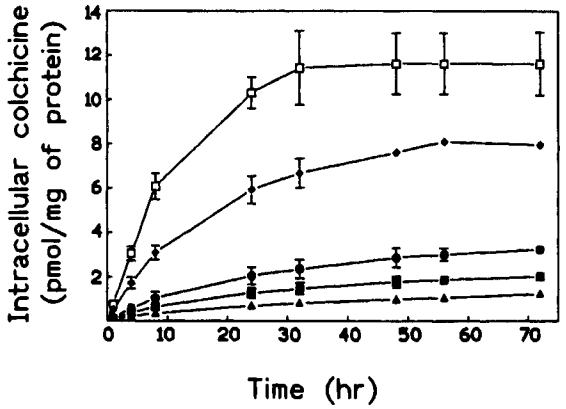


Fig. 2. Time course for [^{3}H]-colchicine uptake by HL-60 cells. Cells were incubated with [^{3}H]-colchicine at various extracellular concentrations (3.12 nM (▲), 6.25 nM (■), 10 nM (●), 25 nM (◆), and 62.5 nM (□). Intracellular radioactivity was determined and values are means ± S.D. of 4 different experiments.

Effects of Verapamil on Colchicine and Vinblastine Uptake by HL-60 Cells

Verapamil at 10 μM significantly increased the intracellular colchicine concentration by 23% as shown by the AUC_{0-72h} ($p < 0.05$) (Fig. 4 (A)). However, the difference in colchicine uptake was only significant after 24 hr. of colchicine exposure ($p < 0.05$). Colchicine $t_{1/2\ uptake}$ was not significantly different in the absence or presence of verapamil (11.1 ± 0.5 hr. and 11.3 ± 1.0 hr. respectively).

Vinblastine uptake was also significantly enhanced by verapamil as soon as the 5th min. of drug exposure, resulting in a final 81% enhancement in the AUC ($p < 0.01$) (Fig.4 (B)), i.e. a 3.5-fold higher increase than for colchicine. Verapamil also decreased the $t_{1/2\ uptake}$ from 26 min. to 12 min.

Effect of Verapamil on Colchicine and Vinblastine Efflux from HL-60 Cells

Intracellular colchicine efflux kinetics were monoexponential with a half-life of 23.0 ± 0.9 hr. By 72 hr. after the start of efflux, 90% of intracellular colchicine had been removed. The colchicine efflux half-life was not significantly altered by adding 10 μM verapamil (22.9 ± 0.3 hr). Like colchicine, vinblastine efflux kinetics were monoexponential with a half-life of 120 ± 8 min. that was not significantly altered by the addition of verapamil (113 ± 10 min.) (Fig. 5).

Table I. Pharmacokinetic Parameters of Colchicine Uptake by HL-60 Cells

Extracellular colchicine concentration (nM)	$t_{1/2\ uptake}$ (hr)	C_{ss} (pmol/mg of protein)
3.12	21.2±5.1	1.18±0.12
6.25	19.8±5.9	2.20±0.14
10.0	13.9±0.8	3.31±0.07
25.0	11.1±0.5	8.25±0.14
62.5	8.0±0.6	11.82±1.10

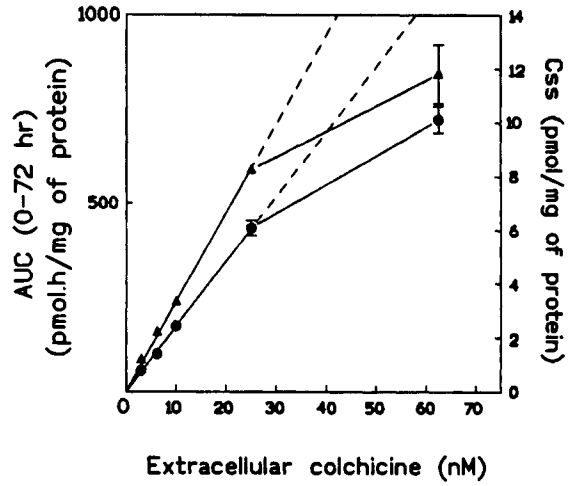


Fig. 3. Relationships between the area under the curve (AUC 0-72 hr) of the colchicine uptake or the intracellular colchicine concentration at steady-state (Css) and the extracellular colchicine concentration. AUC (●) and Css (▲) were calculated as described in Materials and Methods. Values are means ± S.D. of the 4 uptake kinetics shown in Figure 2.

P-glycoprotein Expression Analysis

Quantitative flow cytometry showed that HL-60 cells expressed about 3800 P-gp ABS at the cell surface before drug exposure (Fig. 6). Neither colchicine nor vinblastine, at an extracellular concentration of 25 nM, modified P-gp expression during the first 8 hr. of drug incubation. However, P-gp expression was significantly increased by both drugs after 24 hr. and reached maximal values at 72 hr. with the increase representing 57% and 118% of basal values for colchicine and vinblastine, respectively.

DISCUSSION

The pharmacological effects of colchicine in leucocytes are directly linked to the drug intracellular concentration which reaches steady-state very slowly, i.e. in 48 hr. (9). No explanation can be given but previous studies have shown that colchicine can both induce P-gp-mediated multidrug resistance (13) and bind P-gp (14). The aim of this study was to determine whether P-gp can modify the rate and amount of colchicine uptake and efflux in human lymphocytes and in an HL-60 promyelocytic cell line. If the uptake and efflux drug kinetics of the two cell lines were similar with colchicine, this would justify the use of HL-60 cells as a lymphocytic model. In fact, we found the kinetics to be similar, and due to the problems involved in preparing large batches of isolated lymphocytes from donors, we therefore used HL-60 cells for the further larger-scale experiments.

Our results show that the kinetic properties of colchicine uptake depend on the extracellular colchicine concentration. At extracellular concentration > 25 nM, which corresponds to toxic plasma concentrations, colchicine uptake was not linear, suggesting the existence of saturation processes in the cellular uptake of colchicine through the cell membrane. A recent study had already shown that colchicine uptake in lymphocytes is lower at 4° than at 37°C suggesting that an active and rather simple diffusion process characterizes colchicine uptake by

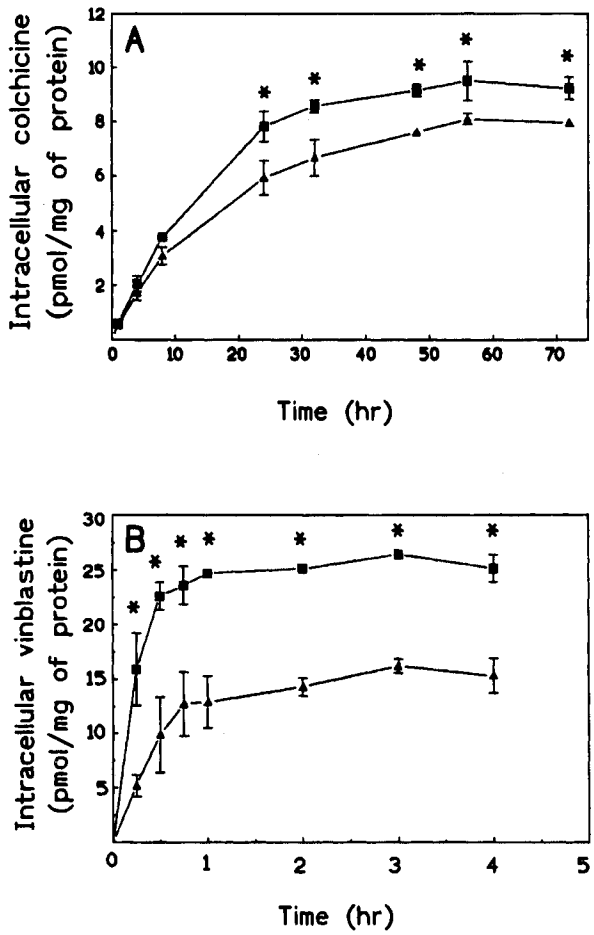


Fig. 4. Influence of verapamil on [^{3}H]-colchicine and [^{3}H]-vinblastine uptake by HL-60 cells. Cells were pre-incubated without (▲) or with 10 μM verapamil (■) for 30 min. Cells were then incubated with 25 nM [^{3}H]-colchicine (A) or 25 nM [^{3}H]-vinblastine (B). Verapamil was maintened during drug uptake. Results are means ± S.D. of three different experiments. *: $p < 0.05$ vs control (Bonferroni's test).

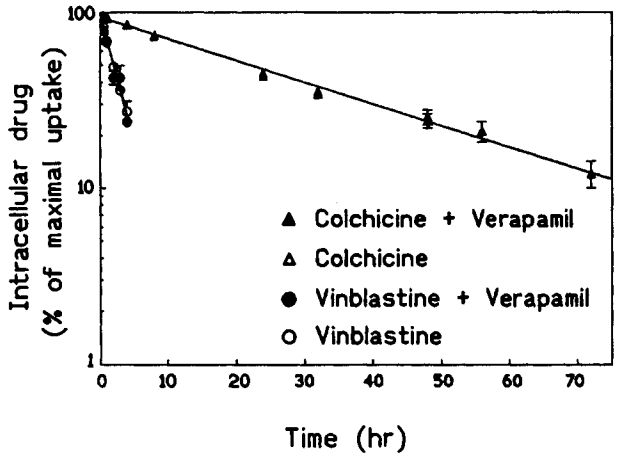


Fig. 5. Infuence of verapamil on [^{3}H]-colchicine and [^{3}H]-vinblastine efflux from HL-60 cells. Cells were pre-incubated with 25 nM [^{3}H]-colchicine for 48 hr or with 25 nM [^{3}H]-vinblastine for 4 hr. Efflux was performed in absence or presence of 10 μM verapamil. Results are means ± S.D. of three independent experiments.

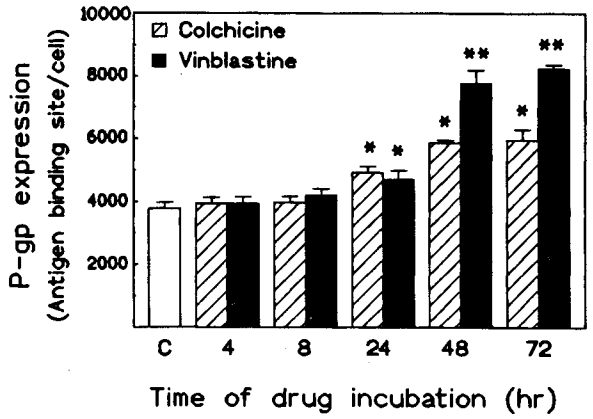


Fig. 6. Induction of P-gp by colchicine and vinblastine. Cells were incubated without (Control = C) or with 25 nM colchicine or 25 nM vinblastine for 4, 8, 24, 48, and 72 hr. At each experimental time point, P-gp expression was determined by quantitative flow cytometry using UIC2 anti-Pgp antibody. Values are means ± S.D. of three independent experiments. *: $p < 0.05$, **: $p < 0.01$ vs control (Student's t-test).

these cells (9). On the other hand, this difference may be the result of temperature-dependent changes in membrane fluidity affecting diffusion of colchicine into the cell membrane. In contrast, at extracellular concentrations ranging from 3.12 to 25 nM, which are close to colchicine plasma concentrations at therapeutic dosage, we found that colchicine uptake was linear. Moreover, the rate of colchicine uptake was dose-dependent, as the $t_{1/2\ uptake}$ decreased by 2.65-fold with increasing extracellular colchicine concentration. This dose-dependent effect on the rate and amount of colchicine uptake has not often been reported in *in vitro* drug uptake studies. Like vinblastine, which is rapidly taken up by HL-60 cells, several other drugs such as cyclosporine, daunorubicin and zidovudine, which interact with P-gp, are also characterized by rapid uptake by leukocytes (9).

Although colchicine and vinblastine are both lipophilic compounds characterized by a similar octanol/water partition coefficient (log P = 1.28 and log P = 1.68, respectively) (15–16), it is intriguing that colchicine uptake was considerably slower than vinblastine at the same extracellular concentration. Since P-gp has been shown to decrease the intracellular concentration of these two drugs by pumping them out of the cells, the slowness of colchicine uptake could be explained by a more pronounced interaction of colchicine with P-gp than vinblastine. The role of P-gp in colchicine and vinblastine uptake was studied by using verapamil, which is known to inhibit P-gp (10). Verapamil treatment did not alter colchicine uptake half-life, suggesting that P-gp could not be mainly responsible for the slowness of colchicine uptake. The only effect was a slight increase in the colchicine level inside the cells, which reached significance only after 24 hr. of colchicine exposure. Moreover, the verapamil effect was 3.5-fold more pronounced for vinblastine and its $t_{1/2\ uptake}$ was enhanced, suggesting that P-gp inhibition has a more marked effect on vinblastine uptake than on that of colchicine.

These results could be explained by differences in binding affinities of colchicine and vinblastine to P-gp. Many competition studies have shown that vinblastine and verapamil may bind to an identical site or may interact with overlapping binding sites on P-gp (17). Furthermore, the absence of competition between vinblastine, verapamil and colchicine has suggested the existence of two distinct binding sites on P-gp, one for vinblastine and another for colchicine, or the existence of a common binding site with a higher affinity for vinblastine than for colchicine (14). If there is one binding site for these three drugs, our results are consistent with the fact that colchicine may have a lower affinity for P-gp than vinblastine and suggest that the capacity of P-gp to limit the cellular acccumulation of drugs depends on their affinity for P-gp. If there are two binding sites on P-gp, one for vinblastine and verapamil and another for colchicine, it would be interesting to know how verapamil can increase colchicine accumulation. These results raise the question of the possible allosteric regulation of colchicine binding to P-gp by verapamil, as has already been described for vinblastine and dihydropyridine (18). Another possibility is that verapamil could be a less efficient inhibitor of colchicine binding to P-gp than vinblastine.

The hypothesis of a lower affinity of colchicine for P-gp than vinblastine or a less interactive effect of verapamil on displacement of colchicine from its binding site is also supported by measurement of membrane P-gp expression by immunocytometry. Previous studies have shown that many drugs, including doxorubicin or vinblastine, are able to increase P-gp expression in HL-60 cell lines (8–11). To determine whether cell-surface P-gp density was modified during colchicine and vinblastine uptake, quantitative flow cytometry was performed using the monoclonal antibody UIC2, which recognizes an extracellular epitope of P-gp. Flow cytometry analysis showed that neither colchicine nor vinblastine modified cell-surface P-gp expression during the first 8 hr. of their uptake into HL-60 cells. Since vinblastine uptake has been studied for 4 hr., the increase in vinblastine accumulation in HL-60 cells induced by verapamil could be related to the inhibition of the basal expression of P-gp. In contrast, colchicine uptake was not significantly increased by verapamil until after 8 hr. of incubation, suggesting that P-gp expression was not high enough to modify colchicine uptake and the too low affinity of colchicine for P-gp was not compensated by a high concentration of transmembrane P-gp. However, P-gp expression was induced by both colchicine and vinblastine after 24 hr. of drug exposure, which corresponds to the experimental time point at which verapamil significantly increased colchicine uptake. Thus, colchicine could modulate its own uptake in HL-60 cells by increasing P-gp expression over time. The observations that the up-regulation of P-gp expression begins at 24 hr. suggest that transcriptional events are involved rather than insertion of P-gp into the cell membrane from pre-formed intracellular stores. Our data are similar to those of Vollrath *et al.* (19) who showed that ip colchicine increased mdr mRNA expression with maximal value at 24 hr. and maximal P-gp expression at 72 hr. after colchicine administration.

It is important to note that colchicine-induced P-gp expression was maximal from 48 hr. of colchicine exposure, i.e. the incubation time we chose for study of intracellular colchicine efflux. Efflux of colchicine and vinblastine from HL-60 cells occurred when the intracellular drug concentration was at steady-state. Colchicine efflux was much slower than that of vinblastine with an efflux half-life 10-fold longer. Verapamil treatment, which could have increased efflux half-life for both drugs, did not modulate either colchicine or vinblastine efflux, as if P-gp could not contribute to their extrusion from the

intracellular compartment. Thus, P-gp was more active at limiting colchicine and vinblastine accumulation than at expelling them after their intracellular distribution. Two questions are raised by these findings: [1] why is vinblastine efflux markedly more rapid than that of colchicine and [2] why does verapamil not modify efflux kinetics especially for vinblastine which was more sensitive to P-gp in the uptake experiments than colchicine? The first question could be explained by the binding properties of both colchicine and vinblastine with intracellular receptors. Whereas colchicine binding to tubulin is slow and weakly reversible (20) with a dissociation half-life ranging from 10.7 to 38.5 hr. (21), vinblastine binding is rapid and highly reversible (22). Recently, Chappey *et al.* (9) have shown that efflux of colchicine from human lymphocytes induced by colchicine-specific Fab fragments was rate-limited by the dissociation of the colchicine-tubulin complex, and that the colchicine efflux half-life was close to the dissociation half-life of this complex. Thus, the differences observed in the colchicine and vinblastine efflux studies could be explained by the much slower dissociation of colchicine from intracellular tubulin than that of vinblastine. Similarly, the much slower penetration of colchicine into HL-60 cells could also be related to its binding properties to tubulin. In fact, under this hypothesis, uptake and efflux kinetics of colchicine and vinblastine would be rate-dependent on their binding kinetics to intracellular tubulin.

The question of the inability of verapamil to modify the efflux of both drugs in our model raises the problem of the access of colchicine and vinblastine for the plasma membrane P-gp or of verapamil for the binding sites of P-gp in HL-60 cells. Several studies have reported that these drugs, which are substrates for P-gp, are removed following direct interactions at the plasma membrane level (3). These drugs are active in the uptake study because both lipophilic colchicine and vinblastine can access the intramembrane hydrophobic domain of P-gp binding sites. In contrast, intracellular colchicine and vinblastine are probably not available for P-gp-mediated efflux because of their binding to tubulin or their trapping within intracellular P-gp vesicles. Recently, P-gp expression has been found in intracellular compartments, such as the Golgi apparatus (23) and the nucleus (24), and was shown to be associated with a decrease in doxorubicin distribution in a non-exchangeable intracellular pool. This intracellular localization of P-gp could also be responsible for a decrease in the access of antitumor drugs to their intracellular targets and represents another drug resistance mechanism (25). Our results suggest that a similar intracellular P-gp expression in HL-60 cells could be responsible for the decrease in colchicine and vinblastine access to the cell-surface P-gp by sequestrating them in intracellular microcompartments. Another possible explanation concerns cell-surface P-gp density. Consoli *et al.* (26) showed that verapamil decreased doxorubicin efflux from a HL-60 doxorubicin-resistant subline, which expresses high levels of P-gp, but not from the parental HL-60 cell line which expresses lower levels of P-gp, suggesting that efflux of drug from the intracellular space could depend on cell-surface P-gp density. The final hypothesis concerns the allosteric interaction of verapamil with the P-gp binding sites which can be intramembranar or facing the cytosol. Our data support the hypothesis that verapamil may not have access on an inner P-gp binding site which prevents it decreasing the efflux of colchicine or vinblastine.

In conclusion, plasma membrane P-gp does not explain the slowness of colchicine uptake, but its inhibition by verapamil allows a more marked uptake specially of vinblastine which has probably more affinity for P-gp than colchicine. In contrast, P-gp does not significantly participate in the efflux of either drug. These findings suggest that P-gp is more efficient at controlling drug entry through the plasma membrane than at eliminating them. These events mean that P-gp can be considered a more efficient permeability controller than effluxer system, whose efficiency is principally dependent on the access of intracellular molecules to the pump binding sites. In other terms, when HL-60 cells are exposed to colchicine or vinblastine, P-gp is more efficient at protecting rather than detoxifying them.

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