

Bodipy-FL-verapamil: A fluorescent probe for the study of multidrug resistance proteins

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Abstract. Most of the substances used as fluorescent probes to study drug transport and the effect of efflux blockers in multidrug resistant cells have many drawbacks, such as toxicity, unspecific background, accumulation in mitochondria. New fluorescent compounds, among which Bodipy-FL-verapamil (BV), have been therefore proposed as more useful tools. The uptake of BV has been evaluated by cytofluorimetry and fluorescence microscopy using cell lines that overexpress P-glycoprotein (P388/ADR and LLC-PK₁/ADR) or MRP (multidrug resistance-related protein) (PANC-1) and clinical specimens from patients. The effect of specific inhibitors for P-glycoprotein (verapamil and vinblastine) or MRP (MK571 and probenecid) has been also studied. BV intracellular concentrations were significantly lower in the two P-glycoprotein overexpressing cell lines in comparison with the parental lines. In addition, verapamil and vinblastine increased the intracellular concentrations of the dye; MK571 and probenecid, two MRP inhibitors, increased BV levels in PANC-1 cells, that express this protein. These findings were confirmed in clinical specimens from patients. Fluorescence microscopy revealed a faint fluorescence emission in P-glycoprotein or MRP expressing cell lines; however, treatment with specific inhibitors significantly increased the fluorescence. BV is a useful tool for studying multidrug resistance proteins with different techniques such as cytofluorimetry and fluorescence microscopy, but does not discriminate between P-glycoprotein and MRP. In comparison with other classic fluorescent probes, the assay with this dye is extremely rapid, simple, not toxic for cells, devoid of fluorescent background, and can be useful in the clinical settings.

Keywords: Bodipy-FL-verapamil, multidrug resistance, P-glycoprotein, MRP, cytofluorimetry

1. Introduction

Primary or secondary chemoresistance is one of the major problems in cancer chemotherapy. One of the most important and studied mechanisms of chemoresistance is multidrug resistance, that is often mediated by overexpression of a membrane glycoprotein with high molecular weight, termed P-glycoprotein. P-glycoprotein is an active transporter, encoded by the MDR1 gene, that functions as a drug efflux pump that derives energy from ATP hydrolysis, resulting in drug concentrations that are below cytotoxic levels in multidrug resistant cells [1,2]. A number of tu-

mor cells, which do not contain P-glycoprotein but nevertheless show the multidrug resistance phenotype, have been described. Several, but not all of these non P-glycoprotein multidrug resistant cell lines show overexpression of the gene encoding the multidrug resistance-associated protein (MRP), a 180–195 kDa membrane glycoprotein which, like P-glycoprotein, belongs to the ATP-binding cassette superfamily of membrane transport proteins [3,4]. P-glycoprotein and MRP are expressed not only in resistant tumor cells but also in many normal tissues such as the liver, kidney, intestine and the endothelia of the blood brain barrier [31,35,36]. Their physiological role, although not yet completely clear, is related to the extrusion of endogenous toxins, steroid secretion in the adrenal gland, secretion of bile salts in the liver canaliculi and secretory function in the kidney. Induction or inhibition of

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these proteins could be responsible for important pharmacokinetic alterations [16,34].

Different non-functional and functional tests have been performed to accurately detect multidrug resistance. Non-functional tests, based on the quantification of P-glycoprotein expression at protein or mRNA level, are sometimes disappointing, therefore functional assays have been often employed.

Several fluorescent substances have been used in functional studies to study drug transport and the effect of efflux blockers in multidrug resistant cell lines by spectrofluorimetric studies, flow cytometry or fluorescence microscopy. Some of the commonly used drugs for this purpose include anthracyclines, acridines, calcein-AM, rhodamine 123 and others [5, 17,23,29]. Many of these substances have some drawbacks; in particular anthracyclines are perhaps the most widely used, but are extremely toxic for cells. Rhodamine 123 is considered a good indicator for P-glycoprotein activity, however, this dye has been shown to accumulate selectively in mitochondria, and hence changes in the mitochondrial membrane potential could affect the cellular accumulation of the probe [19].

More recently, new fluorochromes have been described, among which BV, a fluorescent conjugate of the drug efflux blocker verapamil that possesses the Bodipy-FL-fluorophore, which has spectral properties similar to those of fluorescein, but is rapidly transported out of multidrug resistant cells. This substance is a substrate for the efflux pump, but does not bind to P-glycoprotein and preferentially accumulates in the lysosomes of normal, drug sensitive cells [12, 24]. Studies have shown that BV is transported by P-glycoprotein and MRP [11].

The aim of our study was therefore to investigate the sensitivity and specificity of the fluorescent probe BV for detection of P-glycoprotein and MRP in tumor and normal cells. Fluorescence cytometry and microscopy were used to evaluate the transport characteristics of the fluorescent dye. The effect of modulators that specifically act on these transporters was also tested. The study was performed on drug-sensitive or -resistant cell lines that overexpress P-glycoprotein or MRP, as well as on clinical specimens from patients.

2. Materials and methods

2.1. Cell lines

The murine lymphoid neoplasm P388 and its counterpart resistant to doxorubicin, P388/ADR were ob-

tained from the NCI Frederick Cancer Research Facility/DCT Tumor Repository, Bethesda, MD and grown in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum.

The pig kidney epithelial cell line LLC-PK₁, obtained from the American Type Culture Collection (ATCC-CRL-1392) was grown in plastic bottles in medium 199 supplemented with 3% fetal bovine serum. The resistant counterpart LLC-PK₁/ADR, obtained by selection with doxorubicin as described previously [14], was grown in medium 199 supplemented with 10% fetal bovine serum.

PANC-1 cells, derived from human pancreatic epithelioid carcinoma, obtained from the American Type Culture Collection (ATCC-CRL-1572), were grown in plastic bottles in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

All cell lines were grown in a 5% CO₂-95% air atmosphere at 37°C.

Cell culture media and supplements were obtained from Sigma-Aldrich S.r.l., Italy.

2.2. Patients

Fourteen consecutive adult leukemic patients, admitted for chemotherapy to the Division of Haematology (University Hospital of Udine, Italy) entered this study. 10 patients had a diagnosis of acute myeloblastic leukemia (AML), 6 at diagnosis, 1 at subsequent relapse and 3 resistant; 4 patients had a diagnosis of acute lymphoblastic leukaemia (ALL). The diagnosis, based on cytological characteristics of the blast cells, was confirmed with immunophenotyping studies.

2.3. Leukemic blast cells

Peripheral blood and marrow samples anticoagulated with heparin were collected during diagnostic procedures. Mononuclear cells were separated by sedimentation on Ficoll Hypaque and washed twice in phosphate-buffered saline (PBS); in all samples over 90% of cells were blasts.

To reduce lymphocyte contamination, for cytometric analysis, only the blast cells electronically gated by using scatter parameters were considered. Cell quality was subsequently checked using a CD3 monoclonal antibody (Dako, Denmark) which, in each sample, reacted with <2% of gated cells.

2.4. Flow cytometric analysis

Flow cytometric analysis of cell lines was performed by an EPICS ELITE ESP, Coulter, while leukaemic cells were analyzed by a FACScan and Lysis II software, Becton Dickinson, both equipped with an argon ion laser excitation source emitting at 488 nm. The Bodipy-FL-fluorophore spectra (λ ex max: 503 nm, λ em max: 512 nm) was very similar to that of fluorescein and hence the fluorescence was collected through the FITC-fluorescence 525 nm band pass filter. Propidium iodide (λ ex max: 540 nm, λ em max: 625 nm) fluorescence was collected with a 575 nm band pass.

At least 20,000 cells were analyzed and gated on the basis of the forward and sideward scatter. The green and red fluorescence amount was measured using log mode amplification. For the measure of intensity of the staining, the fluorescence index (FI) was calculated, which represents the ratio between the mean fluorescence intensity of dyes and the mean fluorescence intensity of untreated cells. In experiments where monoclonal antibody were used, the FI represents the ratio between the mean fluorescence intensity in presence of specific antibody and the mean fluorescence intensity of the isotopic control.

Propidium iodide staining was registered in linear mode amplification, acquiring at least 10000 events.

2.5. Uptake of BV

LLC-PK₁, LLC-PK₁/ADR, P388, P388/ADR and PANC-1 cells (5×10^5 cells/0.5 ml) were incubated in triplicate with increasing concentrations of BV (Molecular Probes, USA) at 37°C for different experimental times in culture medium. After incubation, ice cold PBS was added, and the cells were centrifuged at $150 \times g$ for 5 min at 4°C. The pellet was resuspended in 0.5 ml PBS and immediately subjected to FACS analysis.

Leukemic cells (2.5×10^6) from patients were incubated in RPMI-1640 medium (Biochrom KG, Germany) at 37°C with 5% CO₂ for 15 min with 0.125 μ M BV. After two washes in cold PBS, cells were kept on ice and immediately analyzed by FACS.

2.6. Pretreatment with inhibitors

In some experiments the cells were pretreated at 37°C with P-glycoprotein and MRP inhibitors. Vinblastine (50 μ M) and verapamil (50 μ M) were added 30 min before the addition of the fluorescent probes,

while MK571 (50 μ M) and probenecid (4 mM) were preincubated for 1 h. MK571 was obtained from Biomol Research Lab. Inc, USA, all other substances were from Sigma-Aldrich S.r.l., Italy. Each experiment was performed in triplicate.

2.7. P-gp, MRP1 and MRP2 expression in leukemic blast cells

P-gp, MRP1 and MRP2 expression was evaluated by flow cytometry using the MRK-16 (anti-P-gp), MRPm6 (anti-MRP1) and MRP2 (anti-MRP2) monoclonal antibodies (Kamiya, Seattle, USA) as previously described [26]. To study P-gp, 1×10^6 blast cells were fixed in periodate lysine paraformaldehyde for 15 min, washed twice in PBS and incubated for 30 min in 50 μ l of a PBS-saponine solution (0.02%) containing MRK-16 (2 μ g/ml). After two washes, cells were incubated with 2.5 μ l of fluorescein isothiocyanate (FITC) goat anti-mouse antibody (Dako, Denmark). To study MRP1 and MRP2, 1×10^6 blast cells were fixed and permeabilized in Becton Dickinson lysis solution and, after two washes, incubated with 2 μ l of MRPm6 or cMOAT/MRP2 for one hour at 4°C in a PBS-saponine solution, as recommended by the manufacturer.

Staining was revealed using 2.5 μ l of FITC goat anti-mouse antibody (Dako, Denmark). Control samples were carried out simultaneously by replacing the primary antibodies with the isotopic control (Dako, Denmark).

2.8. Staining with propidium iodide

To determine BV toxicity, after flow cytometric analysis of the uptake of BV, all cell lines were centrifuged at $400 \times g$ for 10 min at 4°C, resuspended in 1 ml of a staining solution containing RNase 1 mg/ml and propidium iodide 0.05 mg/ml (all obtained from Sigma-Aldrich S.r.l., Italy), then analyzed by FACS.

2.9. Fluorescence microscopy

LLC-PK₁, LLC-PK₁/ADR and PANC-1 cells were seeded in Leighton tubes at a cell density of 2×10^4 cells/ml in 1 ml of complete culture medium, and cultured to confluence for 5 days. Indeed, previous studies conducted in our laboratory have evaluated, in LLC-PK₁ cells, the effect of culture time on the uptake of doxorubicin, another P-glycoprotein substrate [13], and demonstrated that the maximum uptake was observed after the development of confluence. P388 and

P388/ADR cells, at a cell density of 1×10^6 cells/ml were also used. Cells were pretreated for 30 min in the presence or absence of verapamil ($50 \mu\text{M}$) and vinblastine ($50 \mu\text{M}$), or for 1 hour with MK571 ($50 \mu\text{M}$) or probenecid (4 mM), and then incubated for 30 additional min with BV ($0.125 \mu\text{M}$). The uptake of the dye was observed under a Zeiss Axioskop microscope equipped with a mercury lamp (Osram HBO, 100 W, Germany) for epifluorescence examination. A $\times 100$ Plan-Neofluar N.A. 1.30 objective at oil immersion was used, under appropriate excitation/emission filter setting conditions (excitation at 480 nm, emission at 510 nm) with fluorescein optics.

2.10. Statistical analysis

Averages \pm SEM were calculated; statistical evaluation of results was carried out using Student's *t* test for independent samples. Values of $p < 0.05$ were considered significant.

3. Results

The uptake of BV was evaluated in drug sensitive and resistant cell lines as well as in clinical specimens from patients. Figure 1a shows results obtained with P388 and P388/ADR cells. A significantly higher ability to extrude the fluorescent dye was evident in the resistant counterpart, which is known to express high levels of P-glycoprotein. Similar results were obtained with the proximal tubular cell line LLC-PK₁ and its resistant counterpart LLC-PK₁/ADR (Fig. 1b).

In P388 cells the uptake of BV was extremely rapid, and a plateau was reached within 15 min; in the resistant cell line, BV concentrations were, as expected, lower, and the equilibrium was reached even earlier, at 5 min (Fig. 2). We next studied the kinetics of BV efflux from P388/ADR cells. As shown in Fig. 3, the efflux was extremely rapid, and the process was almost completed in 15 min.

The effect of reversing agents was evaluated on the intracellular concentrations of BV. The P-glycoprotein inhibitors verapamil and vinblastine were extremely effective in increasing the intracellular concentrations of the fluorescent compound in P388/ADR cells (Fig. 4a), which express high levels of P-glycoprotein, but not in the parental line P388 (data not shown). In addition, vinblastine was also active in reducing BV efflux from preloaded P388/ADR cells (Fig. 3).

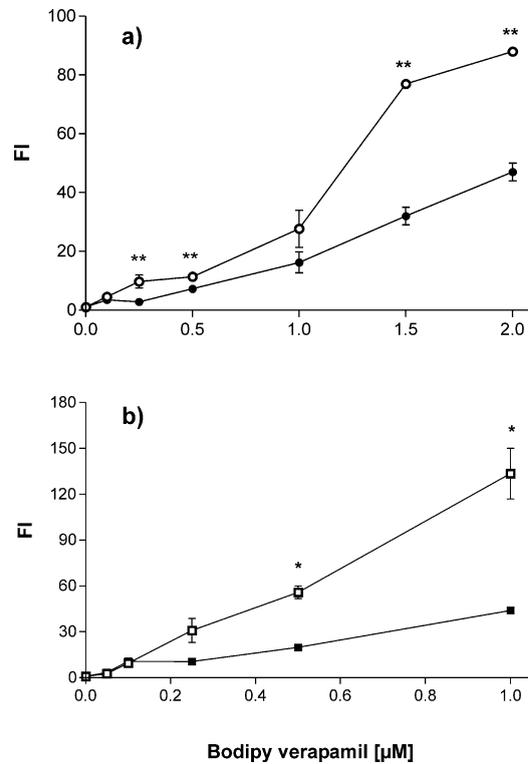


Fig. 1. BV uptake in P388 (○) and P388/ADR (●) (panel a) or LLC-PK₁ (□) and LLC-PK₁/ADR (■) (panel b) cells. Cells were incubated with different concentrations of the fluorescent drug in complete medium for 30 min at 37°C. Each point represents the FI mean \pm SEM of at least three separate experiments. Significantly different from resistant cell line at * $p < 0.05$; ** $p < 0.01$; Student's *t* test for independent data.

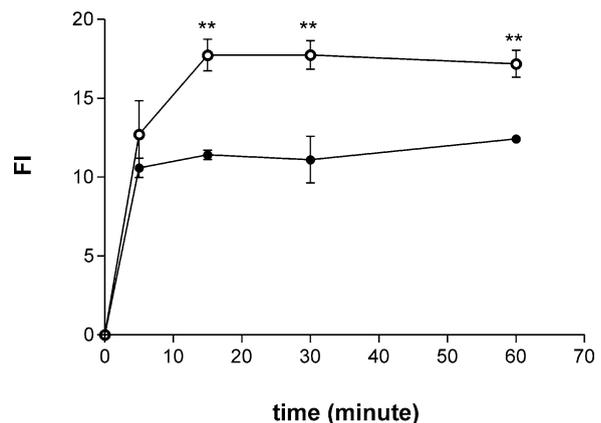


Fig. 2. Time-course of BV uptake in P388 (○) and P388/ADR (●) cells. Cells were incubated with $0.5 \mu\text{M}$ of the fluorescent drug in complete medium for different experimental times at 37°C. Each point represents the FI mean \pm SEM of at least three separate experiments. Significantly different from resistant cell line at * $p < 0.05$; ** $p < 0.01$; Student's *t* test for independent data.

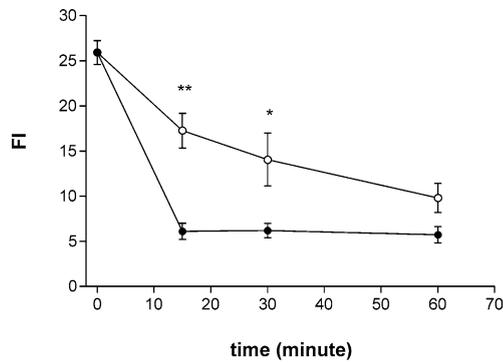


Fig. 3. Kinetics of BV release from P388/ADR cells incubated in complete medium containing the fluorescent dye. After 30 min of incubation with BV $1 \mu\text{M}$, the cells were washed with ice-cold PBS to remove free BV (time 0, total uptake) and incubated at 37°C in drug free medium (●) or in medium containing vinblastine $50 \mu\text{M}$ (○). Each point represents the FI mean \pm SEM of at least three separate experiments. Significantly different from resistant cell line at $*p < 0.05$; $**p < 0.01$; Student's *t* test for independent data.

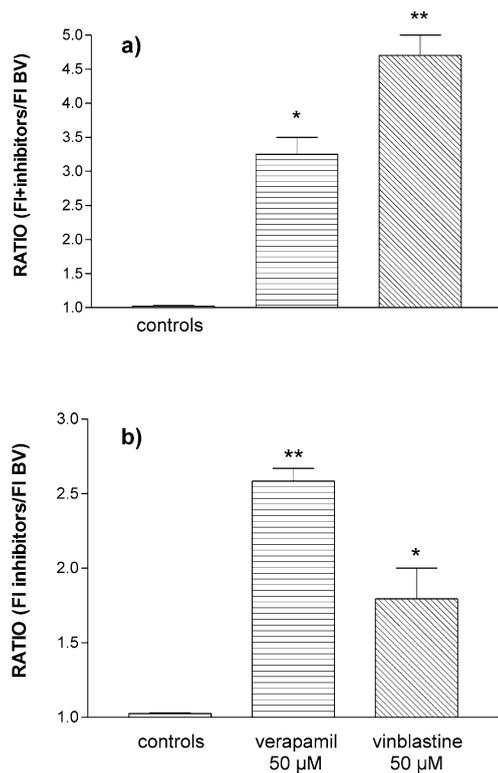


Fig. 4. Effect of verapamil and vinblastine on BV uptake in P388/ADR (panel a) or LLC-PK₁/ADR (panel b) cells. The cells were incubated in medium containing BV $0.25 \mu\text{M}$ after a 30 min pretreatment without or with the inhibitors. Each point represents the ratio between the FI in the presence of inhibitors and the FI of BV alone (mean \pm SEM of at least three separate experiments). Significantly different from controls at $*p < 0.05$; $**p < 0.01$; Student's *t* test for independent data.

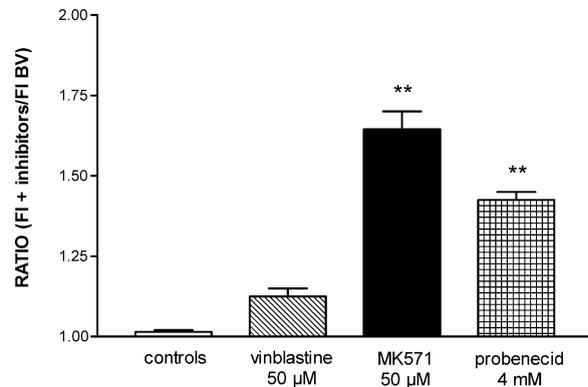


Fig. 5. Effect of vinblastine, MK571 and probenecid, on BV uptake in PANC-1 cells. The cells were incubated in medium containing Bodipy verapamil $0.25 \mu\text{M}$ after a 30 (vinblastine) or 60 min (MK571 and probenecid) pretreatment without or with the inhibitors. Each point represents the ratio between the FI in the presence of inhibitors and the FI of BV alone (mean \pm SEM of at least three separate experiments). Significantly different from controls at $**p < 0.01$; Student's *t* test for independent data.

Similar results were obtained with the two renal cell lines LLC-PK₁ (data not shown) and LLC-PK₁/ADR (Fig. 4b).

PANC-1 cells were used as a cell line that overexpresses MRP; MK571 and probenecid significantly increased the intracellular concentration of BV in these cells, whereas the P-glycoprotein inhibitor vinblastine was almost without effect (Fig. 5).

The toxicity of BV treatment in all cell lines was evaluated by propidium iodide staining. After 30 min treatment with the highest BV concentration ($2 \mu\text{M}$), no difference was observed in the morphology of treated cells (data not shown).

These findings were fully confirmed by direct observation by fluorescence microscopy. When P388/ADR cells were incubated with $0.125 \mu\text{M}$ BV for 30 min, they exhibited an extremely faint fluorescent emission (Fig. 6A) but when cells were pre-incubated with $50 \mu\text{M}$ vinblastine for 30 min before the incubation with BV, the intensity of cell fluorescence strongly increased (Fig. 6B). Similarly, when PANC-1 cells were treated with $0.125 \mu\text{M}$ BV for 30 min, the intensity of cell fluorescence was low (Fig. 6C); on the other hand, pre-incubation of PANC-1 cells with $50 \mu\text{M}$ MK571 for 1 h followed by exposure to BV caused an intense, mostly punctuate cytoplasmic fluorescence (Fig. 6D).

The results obtained in clinical samples are shown in Table 1. For the fourteen specimens tested, BV accumulation and the level of expression of P-glycoprotein,

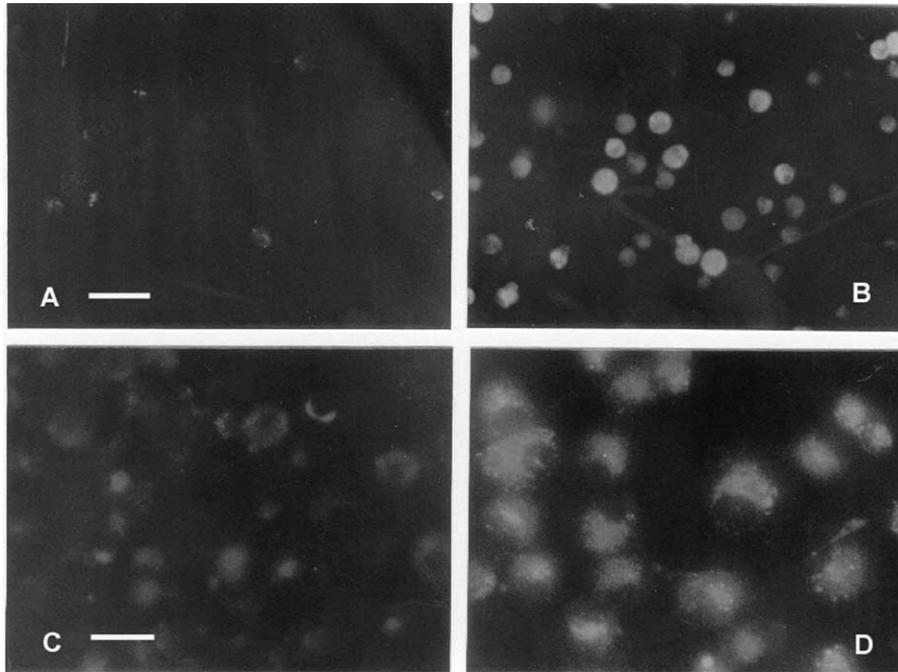


Fig. 6. Fluorescence microscopy of P388/ADR cells (A, B) and PANC-1 cells (C, D) treated with BV in the absence or presence of specific inhibitors of P-glycoprotein or MRP. In (A), P388/ADR cells were incubated with $0.125 \mu\text{M}$ BV for 30 min; in (B), exposure to BV was preceded by incubation with $50 \mu\text{M}$ vinblastine for 30 min. In (C), PANC-1 cells were incubated with $0.125 \mu\text{M}$ BV for 30 min; in (D), a 60 min treatment with MK571 $50 \mu\text{M}$ preceded BV exposure. Bar equals $25 \mu\text{m}$ in A and B and $15 \mu\text{m}$ in C and D.

Table 1
Expression of P-glycoprotein, MRP1, MRP2 and uptake of Bodipy FL verapamil in clinical specimens from patients

Patient no	Diagnosis	Therapy	P-gp (FI)	MRP1 (FI)	MRP2 (FI)	BV (FI)
1	AML t_0		3.2	4.0	1.0	234
2	AML t_0		3.5	3.0	2.8	108
3	AML t_0		4.6	3.3	5.8	175
4	AML t_0		4.4	5.5	3.1	107
5	ALL t_0		3.9	5.4	3.0	249
6	AML res	fludarabine, Ara-C, etoposide, mitoxantrone	7.8	1.0	1.0	141
7	AML res	fludarabine, Ara-C, idarubicin	4.9	2.8	3.2	335
8	ALL t_0		3.1	1.0	1.0	309
9	ALL t_0		2.0	2.2	1.0	511
10	ALL t_0		1.9	1.0	1.0	429
11	AML rel 1	idarubicin, Ara-C, mitoxantrone, 6-thioguanine, 6-mercaptopurine, methotrexate	2.4	2.9	2.0	651
12	AML t_0		3.4	2.6	2.5	455
13	AML res	daunorubicin, Ara-C	2.8	1.0	1.0	149
14	AML t_0		2.7	1.7	2.3	170

AML t_0 : acute myeloblastic leukemia at diagnosis; ALL t_0 : acute lymphoblastic leukemia at diagnosis; AML res: acute myeloblastic leukemia resistant; AML rel 1: acute myeloblastic leukemia first relapse.

MRP1 and MRP2, assessed using specific monoclonal antibody, are reported. The sample with the highest P-gp expression showed the lowest BV uptake (case 6). Five cases showed the highest levels of MRP1 and three of MRP2; also in these cases a lower BV accumulation in comparison with the samples that expressed low levels of these transporters, was observed.

4. Discussion

There is a clinical need for accurate detection tests for multidrug resistant cells, therefore several methods for multidrug resistance detection have been evaluated, and can be classified into non-functional and functional tests. Non-functional tests are based on the quantification of P-glycoprotein expression at protein or mRNA level. However, discrepancies between the detection of P-glycoprotein with monoclonal antibodies and its transport function have been described in leukemic cells and clinical samples [2,32,37]. This discrepancy is presumably related to several reasons, such as P-glycoprotein phosphorylation [8], the prevalence of P-glycoprotein-like and P-glycoprotein mutant forms [21,33] and MRP expression [1,38]. Indeed, in addition to P-glycoprotein, another, structurally related protein, the 190 kDa MRP has been identified as a membrane efflux pump responsible for multidrug resistance [3,10].

To study the accumulation processes of chemotherapeutic drugs in resistant cells, functional assays seem to be extremely useful. These tests are based on the accumulation of fluorescent dyes or of cytotoxic drugs such as rhodamine 123, fluo-3, fura-2, calcein-AM, anthracyclines, and the carbocyanine fluorescent probe JC-1 [17,22,23,28]. Fluorescence analysis is generally performed by flow cytometry or cellular imaging, and, until now, rhodamine 123 has been the most frequently used fluorochrome for clinical diagnosis. Rhodamine 123 is a cationic lipophilic fluorescent dye, very photostable, pH insensitive under physiological conditions and membrane permeable. The dye shows a rapid uptake by mitochondria of living cells [20], and hence changes in mitochondrial membrane potential would affect its cellular accumulation. In addition, a considerable fraction of this dye binds to cell membranes [28], leading to high fluorescence background. Recent data [9] have suggested that in certain cell lines, rhodamine 123 is taken up via a carrier system, and not, as generally assumed, via a passive diffusion process. For this reason, with this dye, better results are obtained from

an assay based on efflux, which is much more time consuming, than influx. The anthracyclines are also widely used but are highly cytotoxic, and daunorubicin displays a weak fluorescence and is quenched when is bound to DNA [25].

During recent years, several groups have tried to define new fluorescent markers in order to study multidrug resistant cells that express P-glycoprotein or MRP.

BV is a fluorescent derivative of verapamil, and possesses the Bodipy fluorophore, which has spectral properties similar to those of fluorescein; this substance is a substrate for P-glycoprotein in multidrug resistant cells, and is concentrated in acidic compartments of drug sensitive cells [6,12,24].

In this study, BV was shown to be transported by P-glycoprotein. Indeed in P388/ADR cells, which express high levels of P-glycoprotein, the uptake of the dye was extremely low, the efflux extremely rapid, and the inhibitors verapamil and vinblastine were effective in increasing its intracellular concentrations. Similar results were obtained in the LLC-PK₁ and LLC-PK₁/ADR cells; the LLC-PK₁ cells are derived from pig normal kidney tubular cells [18] and, similarly to the kidney proximal tubule, express low levels of P-glycoprotein [12,13]; the LLC-PK₁/ADR cells are the resistant counterpart, obtained in our laboratory by means of a chronic treatment with doxorubicin. These cells show an increased expression of P-glycoprotein and provide an interesting model for studying the role of this protein and the consequence of its induction in a normal tissue [14].

In addition we confirmed that BV was transported by MRP in PANC-1 cells [11], a pancreatic cell line that expresses high levels of MRP and is often used to study the role of this transporter. MK571 and probenecid, two specific inhibitors of this protein, increased BV concentrations in these cells. Almost no effect was observed when cells were pretreated with the P-glycoprotein inhibitor vinblastine; this observation is in agreement with the very low levels of P-glycoprotein expression recently shown in these cells [27,30].

A problem encountered with many fluorescent dyes is their cytotoxicity [30], therefore the observation that BV, at the concentrations and for the time employed, was not toxic for cells and did not affect the cell cycle, as assessed by propidium iodide staining, seems of particular interest.

In clinical samples the results, although preliminary, were more difficult to interpret and not so evident as

in cultured cells; however, this is a common problem in patients [7,15]. It should be noted, for example, that cells obtained from patient 13, suffering from AML resistant to chemotherapeutic agents, were characterized by low level of transporters but showed low BV intake suggesting, in this case, a higher sensitivity of the functional method. In clinical practice, the levels of transport proteins are usually evaluated by a wide panel of monoclonal antibody, as well as by functional tests; BV can improve this functional assay, being rapid and sensitive.

5. Conclusion

BV can be successfully employed for studying multidrug resistance with several techniques; good results are obtained by flow cytometry and fluorescence microscopy in different cell lines and in clinical samples obtained from patients with AML and ALL. In particular, the high fluorescence background observed with other fluorescent probes such as rhodamine 123 [28] is not evident with BV. It seems therefore that BV is a useful tool for a simple screening of multidrug resistance caused by P-glycoprotein or MRP overexpression, based on its uptake level in the absence or in the presence of modulators. For its sensitivity and ease of execution, BV seems therefore to be suitable in improving clinical screening of leukemia patients.

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