The Histochemical Journal **33:** 259–266, 2001.

Expression and function of P-glycoprotein and absence of Multidrug Resistance-related Protein in rat and beige mouse peritoneal mast cells

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Received 16 February 2001 and in revised form 22 May 2001

Summary

To clarify the function of the multidrug transporter P-glycoprotein in mast cells we used the green fluorescent compound Bodipy-FL-verapamil, which is a substrate of P-glycoprotein. This compound is also transported by Multidrug Resistance-related Protein (MRP), another membrane transport protein expressed in many tumour resistant cells as well as in normal cells. When rat peritoneal mast cells were incubated with Bodipy-verapamil, a rapid uptake of this compound was observed. Pretreatment with modulators of P-glycoprotein activity, such as verapamil and vinblastine, increased Bodipy-verapamil intracellular concentrations. In addition, Bodipy-verapamil efflux from these cells was rapid and also inhibited by verapamil and vinblastine. In contrast, no effect was observed when cells were treated with agents, such as probenecid and indomethacin, that are known inhibitors of MRP. Methylamine and monensin, substances that modify the pH values in the granules, were able to lower the concentrations of Bodipy-verapamil. Microscopical observations, conducted in both rat and beige mouse mast cells, demonstrated that the fluorochrome accumulated in the cytoplasmic secretory granules. RT–PCR performed on rat peritoneal mast cells revealed the presence of *MDR1a* and *MDR1b* mRNAs; on the contrary, MRP mRNA was not expressed. Mast cells were further treated with the fluorescent probe LysoSensor Blue, a weak base that becomes fluorescent when inside acidic organelles. This substance accumulated in mast cell granular structures and its fluorescence was reduced either by treatment with P-glycoprotein modulators or with agents that disrupt pH gradients. In conclusion, these data further confirm the presence of an active P-glycoprotein, but not of MRP, in rat peritoneal mast cells. These findings, coupled with previous ultrastructural data, lend further support to the assumption that this protein is located on the mast cell perigranular membrane. The functional role of P-glycoprotein in these cells is at present unclear, but a possible involvement in the transport of molecules from the granules to the cytosol can be hypothesized. Alternatively, this protein might be indirectly implicated in changes of pH values inside secretory granules.

Introduction

Mast cells are professional secretory cells that store synthetic products in large granules filling their cytoplasm. Cytoplasmic granules are unique membrane-bound structures, with an acidic internal pH of 5.55 ± 0.06 (De Young *et al*. 1987), composed of a proteoglycan matrix consisting of high molecular weight heparin proteoglycans and highly sulphated chondroitin sulphate proteoglycans, in which are embedded other components such as histamine and neutral proteases.

P-glycoprotein (P-gp) and Multidrug Resistance-related Protein (MRP) are transmembrane multidrug transporters, members of the ABC (ATP Binding Cassette) protein superfamily. Humans have two P-gp genes, *MDR1* and *MDR3*. In mice and rats three different genes can be separated, *mdr1* (also called *mdr1b*), *mdr2* and *mdr3* (also called *mdr1a*). Both P-gp and MRP are typically overexpressed in tumour cells but have been identified also in various normal tissues, like the epithelial cells of the large intestine, of the proximal renal tubules and intrahepatic bile canaliculi (Thiebaut *et al*. 1987, 1989, Cordon-Cardo *et al*. 1990, Roelofsen *et al*. 1997). P-gp expression, in particular, has also been demonstrated in a number of haematopoietic cells (Gupta *et al*. 1992, Klimecki *et al*. 1994, Ludescher *et al*. 1998) and in a mast cell line derived from normal mouse liver (Wein & Gupta 1996); it has been suggested that this protein may play a role in the secretion of certain cytokines. Thevenod *et al*. (1994) and Barg *et al*. (1999) have recognized a 65 kDa-*mdr*-like P-gp in rat pancreatic zymogen granule membranes and in mouse pancreatic β-cell granules that seem to work as a Cl^- channel or to regulate Cl^- conductance. This 65 kDa protein could represent a truncated or alternatively spliced form of *mdr1* gene or a separate member of the ABC transporter superfamily with structural homology to *mdr1* (Thevenod *et al*. 1994). Recently, we have identified P-gp-like immuno-reactive material at the membrane of secretory

granules in rat peritoneal mast cells, both by immunofluorescence and immunogold electron microscopy (Crivellato *et al*. 1997). Hence, the possibility that the P-gp itself or a P-gp-related protein may be involved in the transport of substances into or out of mast cell granules has prompted us to investigate further on this issue.

Conflicting data have been reported on the presence of MRP in mast cells. Bartosz *et al*. (1998) have shown that MRP1 is expressed in HMC-1 human mast cells and mediates the ATP dependent export of leukotriene C4; in contrast, Nguyen & Gupta (1997) could not demonstrate the presence of MRP in the murine hepatic mast cell line MC-9.

Bodipy-FL-verapamil (BV), a green fluorescent derivative of verapamil, has been shown to be a substrate for the efflux pump activity of both P-gp (Lelong *et al*. 1991) and MRP (manufacturer's literature). This substance was used in the present study to evaluate the specific extrusion function of these proteins in mast cells. On the other hand, an increasing body of evidence suggests that P-gp might exert some biological effects not necessarily as a result of direct efflux pump activity but also indirectly, through perturbation of ion exchange and alterations in pH gradients (Valverde *et al*. 1992, Sardini *et al*. 1994, Higgins 1995, Wine & Luckie 1996, Roepe & Martiney 1999, Idriss *et al*. 2000). In particular, P-gp has been proposed to function as a Cl^- channel or to regulate a Cl^- transporter, being possibly implicated in cell and organelle volume regulation (Thevenod *et al*. 1994, Wu *et al*. 1996, Vanoye *et al*. 1997, Barg *et al*. 1999). In the present study, the fluorescent molecule Lyso-Sensor Blue (LSB) was used in order to evaluate intragranular pH changes, indirectly induced by P-gp activity. LSB is a weak base that is selectively concentrated in acidic organelles as a result of its protonation; indeed, with its low pKa (5.1), this substance is almost non-fluorescent except when inside acidic compartments, where protonation relieves the fluorescence quenching of the dye by the weakly basic side chain. Both fluorescent compounds have been used in the presence or absence of substances known to interfere either with the P-gp- and MRP-dependent transport of molecules across the membranes or with the pH of acidic compartments. Experiments have been performed using mast cells obtained from the peritoneal fluid of both rats and beige mice. Beige mice represent an homologue of the Chédiak–Higashi syndrome, an autosomal recessive disease characterized cytologically by markedly enlarged organelles in a variety of cell types (Chi & Lagunoff 1975): in particular, mast cell granules are enlarged and few in number, and hence represent a useful tool for microscopical investigations concerning the transport of fluorescent molecules inside and outside granules.

Materials and methods

Mast cell collection

Wistar rats (200–400 g) from a local conventional breeding colony and beige (C57BL/6j-bg^j/bg^j) mice, obtained by breeding adult animals purchased from Harlan Nossan, Italy, were used. The animals were housed in groups of three (rats) or 10 (mice) at 20 °C on a 12 h light/dark cycle; all animals had free access to both food and water. Rats and mice were anaesthetised with ether, then killed by cervical dislocation prior to intraperitoneal injection of 10–20 ml of buffered saline solution at 37 °C. The abdomen of the animals was massaged for 30 s, and then the peritoneal cavity was exposed through a ventral incision. The peritoneal fluid was collected and cells were suspended in balanced salt solution containing 154 mM NaCl, 2.7 mM KCl, 0.68 mM $CaCl_2$, 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 1 g/l bovine serum albumin, adjusted to pH 7.2 before use. Cells were sedimented by centrifugation at 150 *g* for 10 min. The supernatant fraction was removed and cells were resuspended in buffered medium. For biochemical studies, rat mast cells were purified from peritoneal fluid according to Lagunoff & Rickard 1987; the purity of the mast cells in the final preparation, determined by staining with toluidine blue, was more than 90%. For microscopical studies, mixed peritoneal cells were used.

Biochemical studies

Quadruplicate 1 ml aliquots of the cell suspension, at a cell density of 10^5/ml, were allowed to equilibrate at 37 °C for 10 min in a metabolic shaker with gentle mechanical agitation, then BV and other tested substances were added, and the incubation continued at 37 °C for 15 min. To determine the kinetics of BV uptake, the reaction was terminated after fixed periods of time by the addition of 10 ml ice-cold buffer and cells were separated from supernatants by centrifugation.

The cell pellet was resuspended in 2 ml 0.3 N HCl in 50% ethanol, mixed thoroughly in a vortex mixer, and centrifuged at 700 *g* for 10 min at 4 °C. The BV content in the supernatant fraction was determined fluorimetrically on a Perkin Elmer 650-10S fluorescence spectrophotometer (excitation 480 nm, emission 510 nm). Standard curves of BV dissolved in 0.3 N HCl–50% ethanol were used for computation of BV content. Quadruplicate 200 µl aliquots of mast cell suspension at a cell density of 10^5 cells/ml, were incubated in PBS for 15 min at 37 °C in a metabolic shaker with 2 µM LSB in a 96-well microplate with or without various inhibitors. At the end of the incubation period, the fluorescence was read with a Packard Fluorocount (excitation 360 nm, emission 425 nm). Results were expressed as fluorescence intensity.

Emission spectra of LSB were obtained at different pH values with an excitation of 373 nm. Emission spectra of buffered solutions containing both LSB and other tested substances were also recorded with a Perkin Elmer 650-10S fluorescence spectrophotometer.

The protein concentrations of samples were determined by the method of Lowry *et al*. (1951).

Statistical analysis

Averages ± SE of the means were calculated for data from three to six samples from typical experiments; statistical evaluation of results was carried out using Student's *t*-test for

independent samples. Values of $p < 0.05$ were considered significant.

Fluorescence microscopy studies

Peritoneal mast cells obtained from Wistar rats and beige (C57BL/6J-bg^{j}/bg^{j}) mice, were incubated with 0.5–2 μM BV or 2 μM LSB for 20 min at 37 °C. Experiments were conducted in the presence or absence of substances known to block either P-gp activity (verapamil, vinblastine) or MRP activity (probenecid, indomethacin), or to interfere with the pH of acidic compartments (monensin, methylamine).

For fluorescence microscopy examination, small aliquots of cells were placed over a glass slide and covered with a coverslip. A Zeiss Axioskop microscope was used. The instrument contained two illumination sources, a tungsten bulb for bright-field observation and a mercury lamp (Osram HBO 100 W, Germany) for epifluorescence examination. The fluorescence setting was equipped with fluorescein optics for BV examination (excitation 480 nm, emission 510 nm). LSB fluorescence was observed under appropriate excitation/emission filter setting conditions (excitation 375 nm, emmission 425 nm). Mast cells were studied using an oil immersion ×100 Plan-Neofluar objective (N.A. 1.30), by alternate bright field and fluorescence observations. At least 100 cells were examined for each experiment.

Chemicals

BV and LSB were obtained from Molecular Probes, Eugene OR, USA. Verapamil, vinblastine, probenecid, indomethacin, methylamine and monensin were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade.

RT–PCR

Total RNA was extracted from 10×10^7 cells using the one step guanidinium thiocyanate–phenol chloroform method (Chomczynski & Sacchi 1987). mRNA was purified using poly A$^+$ tract Isolation System IV (Promega). 100–500 ng of mRNA was reverse transcribed using Superscript II RNAse H- (Gibco BRL). Different aliquots (0.5–1 μl) of the cDNA were used in PCR reaction in a final volume of 10 μl.

Synthetic oligonucleotide primers (Boehringer Mannheim, Italia) were used to investigate the presence of *MDR1b*, *MDR1a*, and MRP mRNA transcripts. *MDR*1b sense, 5′-GACGGACAGGACATCAGGACC-3′, antisense 5′-GCAAACACTGGTTGTATG-CAC-3′ (rat sequence accession # M81855, 823 bp fragment); *MDR1a* sense 5′-TTGATGCAAACGTGCCACC-3′, antisense 5′-GGAACAACTGATAAGAGCAG-3′ (mouse sequence accession # M33581, 533 bp fragment). MRP sense1 5′-CGGAAACCATCCACGACCCTAATCC-3′, antisense1 5′-TCCTCATTCGCATCCACCTTGG-3′; (human sequence accession # L05628, 292 bp fragment); MRP sense2 5′-TTCCCATTTCAACGAGACCTTGCTG-3′, antisense2 5′-TCCGCCCCACGATGCCGAC-CT-3′ (human sequence accession # L05628, 530 bp fragment).

PCR was carried out using *Taq* polymerase. The amplification was performed using a DNA thermal cycler PTC 100 MJ Research. After denaturing at 94 °C for 5 min the reaction was conducted for 30 cycles (94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min and final step at 72 °C for 10 min). PCR products were subjected to electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining. cDNA from P388/ADR cells, a leukaemic murine cell line expressing high levels of P-gp, and from rat liver, that expresses high levels of MRP, were used as positive controls. As an internal control primers amplifying a fragment of β-actin were used. Negative controls containing water instead of cDNA were also included in each experiment.

The DNA marker was $\phi \times 174$DNA/HaeIII (Promega Corp.).

Results

Spectrofluorimetric studies

Figure 1 illustrates the time course of BV uptake by purified rat peritoneal mast cells; the uptake of the fluorescent compound is completed in about 15 min. The effect of various substances on this process is presented in Figure 2: methylamine and monensin decrease the amount of BV uptake, whereas verapamil and vinblastine increase this process and probenecid and indomethacin had no significant effect. The efflux of BV from rat peritoneal mast cells incubated with 1 μM of the fluorescent compound is rapid and almost-complete in 60 min. Vinblastine and verapamil significantly reduce this efflux. The fluorescence index of mast cells treated

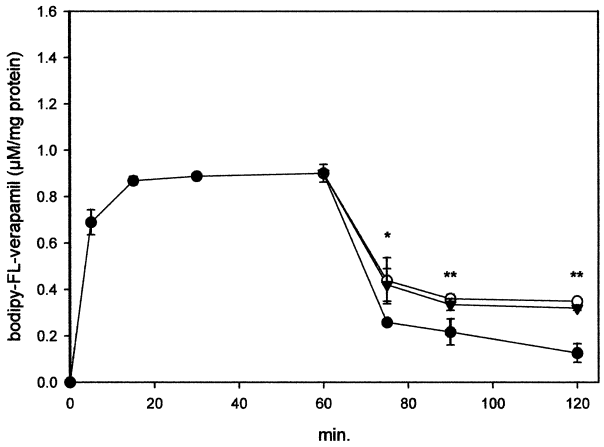

Figure 1. BV uptake and efflux in rat peritoneal mast cells. Cells were incubated with 1 μM BV at 37 °C, for up to 60 min. Cells were then washed with ice cold PBS to remove free BV and incubated in fresh PBS (●) or PBS containing 100 μM vinblastine (○) or 100 μM verapamil (▼). BV remaining in the cells was measured at determined times. Each point represents mean ± SE of data from four determinations from a typical experiment. $*p < 0.05$; $**p < 0.01$ Student's test for independent data.

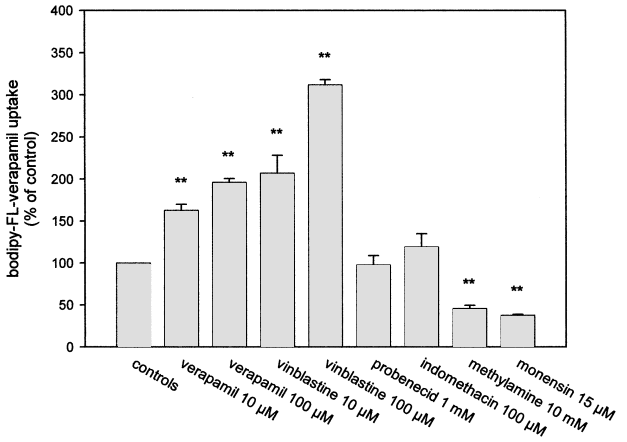

Figure 2. Effect of P-gp inhibitors, MRP inhibitors and lysosomotropic amines on BV uptake in rat peritoneal mast cells. The cells were preincubated in buffer containing the substances at 37 °C for 30 min, then in buffer containing BV 1 μM and the inhibitors for an additional 20 min. Each point represents mean ± SE of data from four determinations from a typical experiment. Control value: 0.88 ± 0.003 μM/mg protein. $**p < 0.01$ Student's test for independent data.

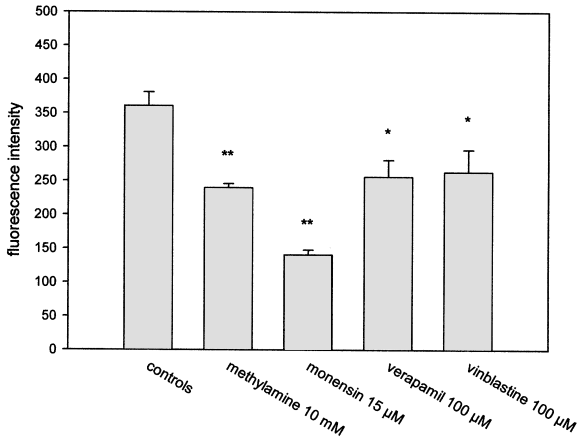

Figure 3. Effect of methylamine, monensin, verapamil and vinblastine on LSB uptake. The cells were preincubated in PBS containing the inhibitors for 30 min at 37 °C, then in PBS containing 2 μM LSB and the inhibitors for 20 additional minutes. Each point represents mean ± SE of data from four determinations from a typical experiment. $**p < 0.01$ Student's test for independent data.

with LSB (2 μM) is shown in Figure 3. The intracellular fluorescence is significantly reduced by methylamine, monensin, vinblastine and verapamil.

The emission spectra of LSB in buffered solutions at different pH values are shown in Figure 4. Addition of verapamil, vinblastine, monensin and methilamine did not modify the emission spectra of this substance (data not shown).

Fluorescence microscopy studies

High doses (2 μM) of BV led to accumulation of the fluorescent compound within secretory granules both in rat and beige mouse mast cells (Figure 5a,b). At low concentration (0.5 μM), BV did not elicit any fluorescent response in mast

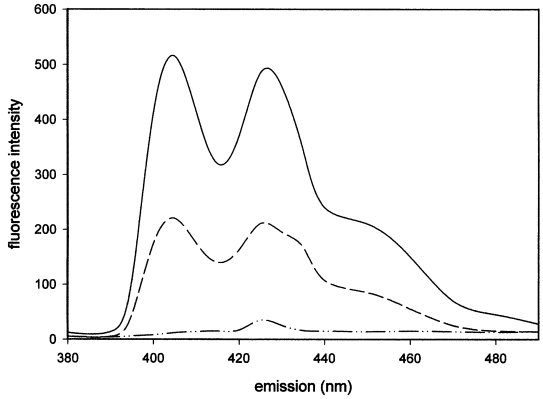

Figure 4. Emission spectra of LSB 2 μM (excitation 373 nm) in PBS at different pH values (– · · –) pH 7.5; (– – –) pH 4.5; (——) pH 3.0.

cell granules (Figure 5d) but, when co-incubated with verapamil (100 μM) or vinblastine (100 μM), a clear granular fluorescent signal could be observed (Figure 5e). Co-incubation with methylamine (10 mM) (Figure 5c) or monensin (15 μM) (not shown) consistently reduced BV fluorescence. Mast cell treatment with probenecid (1 mM) or indomethacin (100 μM) did not induce any microscopical modification of BV fluorescence intensity and pattern (data not shown).

LSB also exhibited a granular fluorescence emission (Figure 5f,g). Equilibration of the pH gradient through mast cell granules was performed by addition of weak bases or carboxylic ionophores. Co-incubation with methylamine (10 mM) (not shown) or monensin (15 μM) (Figure 5h) strongly reduced LSB fluorescence in mast cells. Co-incubation with verapamil (100 μM) (Figure 5i) or vinblastine (100 μM) (not shown) led to a significant decrease of LSB fluorescence intensity both in the rat and beige mouse mast cell granules.

RT–PCR

The mRNA levels of *MDR1b, MDR1a*, MRP and *β-actin* as control were analysed by RT–PCR technique. All samples were normalized to actin, which was not expected to change under treatment conditions, to ensure uniform sample loading. The mast cells expressed *MDR1a* as well as *MDR1b* mRNAs (Figure 6). On the contrary, these cells did not express MRP mRNA (Figure 6).

Discussion

In previous studies we demonstrated the presence of P-gp in rat peritoneal mast cells both in a Western blot assay and by immunocytochemistry (Crivellato *et al*. 1997, Candussio *et al*. 1999). In particular, immunofluorescence and immunogold electron microscopy, using the specific monoclonal antibody JSB-1, revealed immuno-reactive material at specific subcellular sites in mast cells, namely the membrane of secretory granules and, to a much smaller extent, the plasma membrane. These studies are in keeping with those reported

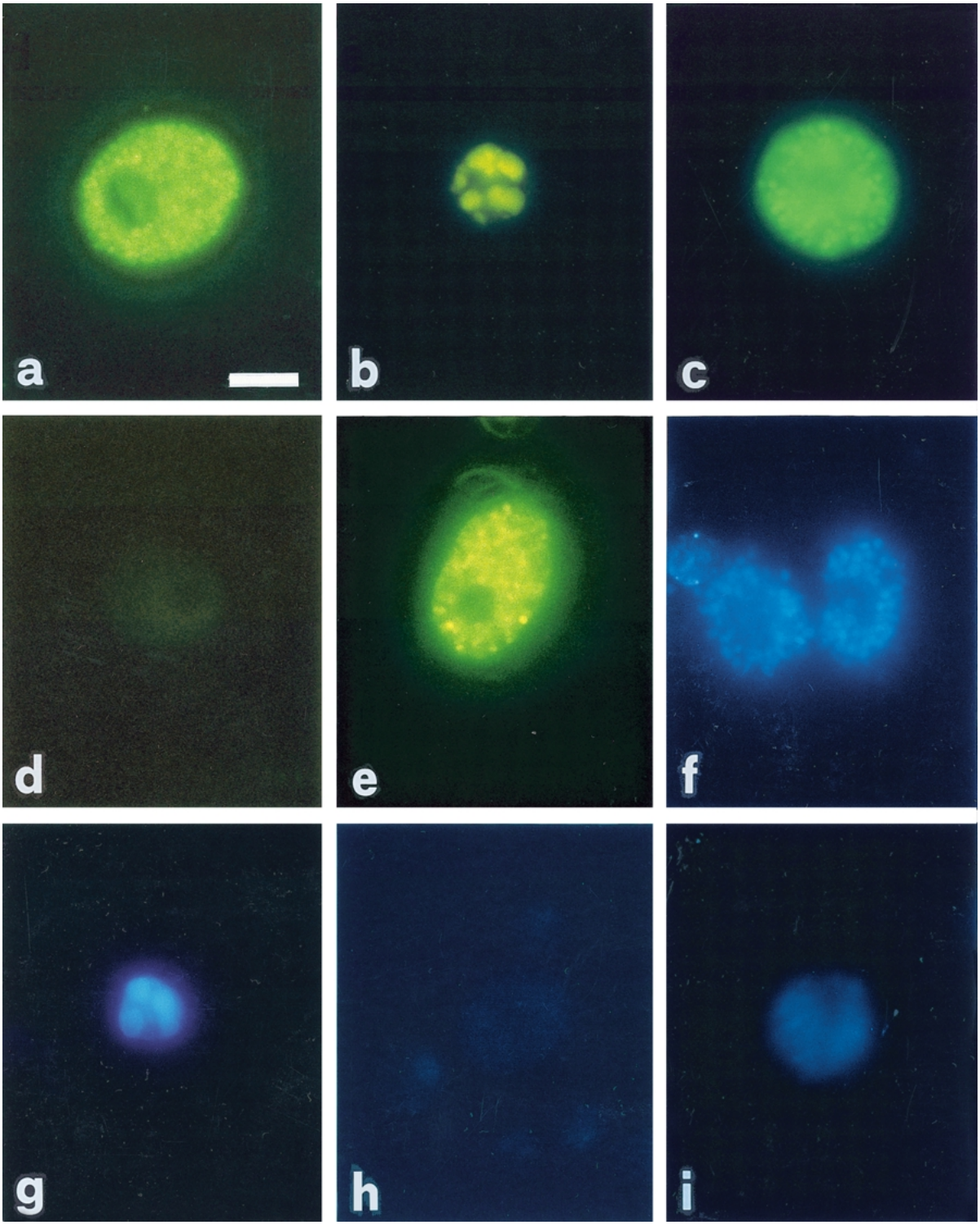

Figure 5. Intracellular localization of BV (a–e) and LSB (f–i) in rat and beige mouse peritoneal mast cells monitored by fluorescent microscopy. (a) and (b) show a rat and a beige mouse mast cell, respectively, which were incubated with 2 μM BV for 20 min. In (c), a rat mast cell was incubated with 10 mM methylamine for 30 min, then with 2 μM BV plus 10 mM methylamine for additional 20 min; this led to reduction of BV fluorescent signal. (d) shows a rat mast cell exposed to 0.5 μM BV for 20 min; no fluorescent response can be elicited. In (e), a rat mast cell was treated with 100 μM vinblastine for 30 min, followed by additional 20 min incubation with 0.5 μM BV and 100 μM vinblastine; a clear granular fluorescence is observable. (f) and (g) show rat and beige mouse mast cells, respectively, as they appear after 20 min exposure to 2 μM LSB. The rat mast cell in (h) was incubated with 15 μM monensin for 30 min and then with 2 μM LSB plus 15 μM monensin for additional 20 min; this cell shows a strongly reduced fluorescence. In (i), a rat mast cell was treated with 100 μM verapamil for 30 min, followed by incubation with 2 μM LSB plus 100 μM verapamil for additional 20 min, leading to a decrease of granular fluorescence. Bar (a)–(i) = 10 μm.

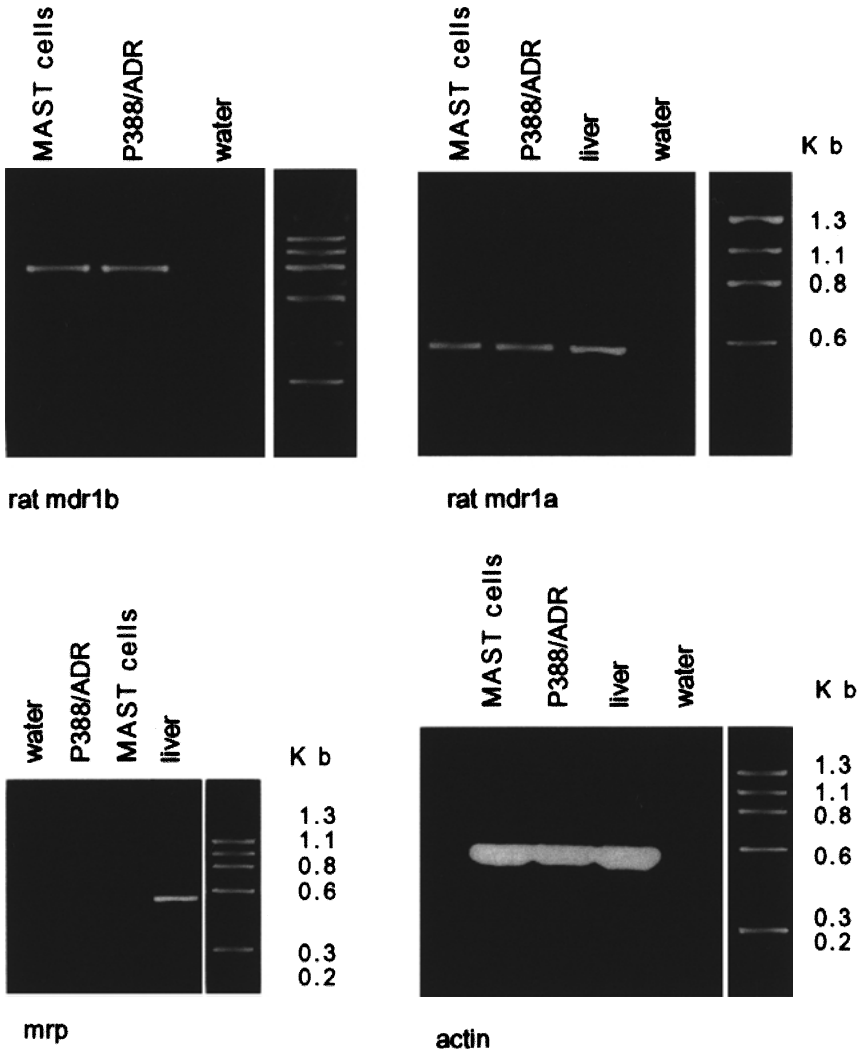

Figure 6. RT–PCR analysis of *MDR1a*, *MDR1b* and MRP expression in rat peritoneal mast cells. The size of the amplified product was determined from mobility of the DNA size markers indicated in the corresponding lane.

by Thevenod and co-workers (Thevenod *et al*. 1994, Barg *et al*. 1999) who recognized a 65-kDa-*mdr*-like P-gp at the membrane of both pancreatic zymogen granules and β cell granules.

In the present study we have used the fluorescent compound BV to test P-gp function as a transport pump in mast cells. BV is a fluorescent conjugate of the drug efflux blocker verapamil that possesses the Bodipy fluorophore, which has spectral properties similar to those of fluorescein (Lelong *et al*. 1991). This substance is a substrate for the efflux pump but does not bind to P-gp, and has been used as a fluorescent marker to trace the transport of P-gp substrates in different drug sensitive or resistant cell lines (Lelong *et al*. 1991, Simmons *et al*. 1995, 1997, Crivellato *et al*. 1999). BV has been shown to accumulate in acidic compartment (Lelong *et al*. 1991, Crivellato *et al*. 1999). Mast cell secretory storage granules actually seem to share some common properties with acidic compartments. This can be inferred by decreased intragranular BV concentration when mast cells are treated with substances, such as methylamine and monensin, that dissipate pH gradients across biological membranes. As shown in this study, BV rapidly enters mast cells. The efflux is also rapid and significantly reduced by verapamil and vinblastine. Both substances increase the intracellular concentrations of BV. Parallel microscopic examination reveals that BV accumulates within secretory granules both in rat and beige mouse peritoneal mast cells. The fluorescence signal increases when cells are pretreated with verapamil or vinblastine, suggesting, therefore, that this may be due to an inhibition of the perigranular efflux transport protein.

P-gp has recently been implicated in the molecular mechanisms of ion conductance and cell volume control (Valverde *et al*. 1992, Sardini *et al*. 1994, Higgins 1995, Wine & Luckie 1996, Idriss *et al*. 2000). In particular, P-gp has been proposed to function as a Cl^- channel or to regulate a Cl^- transporter, thus being able to alter Cl^- fluxes and influence pH values through an indirect mechanism (Thevenod *et al*. 1994, Wu *et al*. 1996, Vanoye *et al*. 1997, Roepe & Martiney 1999). It is of great relevance that P-gp presents close structural homology with the human Cystic Fibrosis Transmembrane Regulator (CFTR), another member of the ABC superfamily of transporters. This protein is a plasma membrane Cl^- channel

that is disfunctional during cystic fibrosis (Ibriss *et al*. 2000). In the light of these data, the subcellular distribution of LSB in mast cells has been studied. This substance is a weak base that is likely to cross biological membranes and enter cells by passive diffusion. It is selectively concentrated in acidic organelles, as a result of its protonation. This reagent exhibits a pH-dependent increase in fluorescence intensity upon acidification and is useful for investigating the acidification of lysosomes and alterations of lysosomal function and trafficking (Crivellato *et al*. 2000). When mast cells are incubated with LSB, a granular fluorescence emission is evident. Granular fluorescence is significantly reduced, both in microscopic and spectrofluorimetric studies, by substances like methylamine, a lysosomotropic amine which decreases the pH gradient between the cytoplasm and the acidic vescicles, or monensin, an inhibitor of the sodium/proton exchanger, which dissipates pH gradients across all membranes (Pressman & Fahim 1982). Modulators of P-gp activity, such as verapamil and vinblastine, also decrease LSB fluorescence in mast cell granules. Reduction of fluorescence intensity may be interpreted as an effect of both verapamil and vinblastine on P-gp activity which, in turn, would influence intragranular pH values and LSB protonation. Vinblastine is a weak base, with a pKa ranging from 5.4 to 7.4. Theoretically, this substance might decrease LSB fluorescence by directly increasing intragranular pH values through self-protonation. On the other hand, verapamil is an amphiphatic compound, which would not be able to affect *per se* the H^+ concentration in the secretory granules. Moreover verapamil has been reported to block whole-cell currents from P-gp-expressing cells (Idriss *et al*. 2000). Therefore, the reduced LSB fluorescence that accompanies verapamil treatment, could be explained by a specific functional inhibition of P-gp activity.

Conflicting data have been reported in the literature regarding the presence of MRP in mast cells. Authors have indeed shown that this protein transports leukotriene C4 (Leier *et al*. 1994a,b). This molecule is synthesized via conjugation of leukotriene A4 with glutathione in a limited number of competent cells including mast cells, and it has been shown that bone marrow derived mast cells of mice homozygous for the MRP mutant allele have a decreased secretion of leukotriene C4 (Wijnholds *et al*. 1997). In addition, Bartosz *et al*. (1998) have shown that, in the HMC-1 human mast cell line, MRP1 is expressed and mediates the ATP-dependent export of leukotriene C4. Nguyen & Gupta (1997) however could not demonstrate the presence of MRP in the mast cell stabilized line MC-9, using flow cytometry with anti MRP antibody; accordingly, PCR and Northern blot analysis did not evidence the presence of the corresponding mRNA.

In the present study we have used two specific MRP inhibitors, probenecid and indomethacin, to verify the possible presence of MRP in mast cells. Combined biochemical and microscopic studies did not show any effect of the two inhibitors on BV uptake in mast cells. These data were confirmed by RT–PCR. No MRP mRNA could indeed be detected in rat peritoneal mast cells, confirming, therefore, the observations of Nguyen & Gupta (1997), and not those of Bartosz *et al*. (1998). On the contrary, mast cells expressed *MDR1a* as well as *MDR1b* mRNAs.

In conclusion, these data confirm the presence of a functional P-gp in rat peritoneal mast cells and lend further support for its localization at the membrane of secretory granules. Conversely, the other multidrug transport protein MRP can not be detected in this experimental model. The functional role of P-gp in rat peritoneal mast cells is at present unclear, but a possible involvement either in the mechanisms of extruding molecules from the granules or in influencing intragranular pH values can be hypothesized.

Acknowledgements

This research was supported by grants from the Ministero Università e Ricerca Scientifica e Tecnologica 60% and Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale (Farmacotossicologia di antracicline antitumorali: meccanismi e basi molecolari per nuove strategie terapeutiche).

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