Review

Does any relationship exist between P-glycoprotein-mediated multidrug resistance and intracellular calcium homeostasis

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Abstract. Multidrug resistance (MDR) of neoplastic tissue represents a real obstacle to the effective chemotherapy of cancer. Several mechanisms of MDR were identified, from which the over-expression and efflux activity of P-glycoprotein (P-gp) – a plasma membrane ATPase (ABCB1 member of ABC transporter family) – represents the most commonly observed reason for neoplastic disease chemotherapy malfunction. The process of P-gp-mediated MDR seems to be related to intracellular calcium homeostasis, at least indirectly, for the following reasons: i. substances blocking calcium influx through L-type of calcium channels like verapamil were often found to antagonize P-gp-mediated MDR; ii. calcium signal abnormalities were observed in cells over-expressing P-gp; iii. cells with P-gp-mediated MDR were often resistant to thapsigargin; iv. several differences in intracellular calcium localization were observed when P-gp-negative and P-gp-positive cells were compared; and v. differences in the contents of several proteins of the endoplasmic reticulum involved in calcium homeostasis were observed to be associated with P-gp over-expression. This current study represents an attempt to summarize the knowledge about the possible relationship between P-gp-mediated MRD and intracellular calcium homeostasis.

Keywords: P-gp-mediated MDR — Calcium homeostasis — Ca^{2+}-antagonists — Calcium binding proteins — Endoplasmic reticulum

Introduction

P-glycoprotein (P-gp) represents a transport ATPase of the plasma membrane that is responsible for the efflux of diverse substances (including cancer chemotherapeutic agents) out of animal cells (Breier et al. 2005). This protein is a member of the ABC (**A**TB-**B**inding **C**assette) transporters family and is characterized by broad substrate specificity for hydrophobic molecules with high membrane affinity (Pawagi et al. 1994). We have proposed that for recognition at P-gp binding sites, a substrate should possess the following characteristic features: (i) flexible structure giving rise to different structural conformers; (ii) molecular weight lower than 1,300 g/mol; and (iii) existence of a non-charged tertiary amino group at neutral pH (Breier et al. 2000). Over-expression of P-gp in neoplastic cells confers a lack of cell sensitivity to P-glycoprotein substrates that represents the most often observed mechanism of multidrug resistance as a real obstacle to effective chemotherapy of cancer (Zhou 2008).

Several authors have focused on whether a relationship exists between processes controlled by intracellular calcium homeostasis and P-gp-mediated multidrug resistance. These investigators were inspired by following data: i. Substances blocking calcium influx through L-type calcium channels like verapamil were often found to antagonize P-gp-mediated MDR (Barancik et al. 1994; Kimura et al. 2004); ii. Calcium signal abnormalities were observed in cells over-expressing P-gp (Witkowski and Miller 1999); iii. Cells with P-gp-mediated MDR were often resistant to thapsigargin (Gutheil et al. 1994; O'Neill et al. 2006; Seres et al. 2008; Wagner-Souza et al. 2003); and iv. Differences in the contents of several proteins of the endoplasmic reticulum involved in calcium homeostasis were detected in P-gp expressing cells (Seres et al. 2008).

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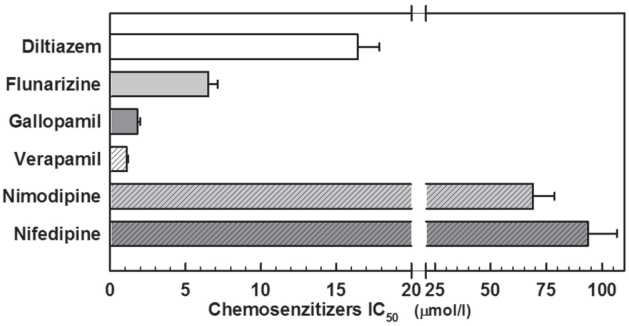

Fig. 1. Values of IC50 characteristic of calcium-channel blockers effects on the P-gp-mediated vincristine resistance of L1210/VCR cells. Drugs were added directly to the cultivation medium for cell cultivation (Barancik et al. 1994). The effects of drugs were measured in the presence of 0.2 mg/l of vincristine that exerts a total cell death effect on parental P-gp-negative cells and did not influence the proliferation activity of P-gp-positive L1210/VCR cells. Calcium blockers in the applied concentration did not significantly influence the proliferation activity of L1210/VCR cells. Data are expressed as mean ± S.E.M. for nine independent values.

The current paper represents a state of the art review of the available knowledge on the multidrug resistance of neoplastic cells (mediated by P-gp) associated with alterations in intracellular calcium homeostasis.

Calcium entry blockers as P-gp antagonizing agents

Substances that are able to antagonize P-glycoprotein-mediated multidrug resistance represent a diverse group of chemicals with different structures and mechanisms of primary pharmacological action, including calcium channel blockers, calmodulin antagonists and many others (Ford 1996). The effectiveness of calcium channel blockers indicated that their MDR-antagonizing effect may be directly related to their action on calcium entry and, consequently, to intracellular calcium concentration regulation. We described the effects of verapamil, galopamil, diltiazem, flunarizine, nimodipine and nifedipine on the reduction of P-glycoprotein-mediated vincristine resistance of L1210/VCR cells (Barancik et al. 1994). All these drugs altered the resistance of cells to vincristine in a concentration-dependent manner with different effectiveness (IC50 values for these actions are summarized in Fig. 1). However, no correlation between the effectiveness of these substances in the reduction of calcium channel activity and P-gp antagonizing activity was observed. Therefore, the idea that the P-gp antagonizing activity of calcium channel blockers is directly related to their effect on calcium entry is improbable. Consistent with the latter idea, stereoisomers of calcium antagonists that differ markedly in their potencies as calcium blockers were equally effective in modulating the drug transport by P-glycoprotein (Hollt et al. 1992). Interestingly, both verapamil and nifedipine stimulated the ATPase activity of P-gp, i.e., they may act also as substrates of P-gp (Shapiro and Ling 1994). The dual function of several substances as both substrates and inhibitors of P-gp was described by Didziapetris et al. (2003) and represents relevant confusion in his attempt to classify the P-gp substrates. Moreover, verapamil was proved to interact with P-gp directly, and P-gp may be specifically labeled by its fluorescent derivatives (Safa 1988). A direct interaction with P-glycoprotein could not be postulated as a common feature of all calcium blockers. The newly synthesized calcium entry blocker, SR33557, with several structural similarities to verapamil failed to label P-gp (i.e., protein band with molecular weigh about 170 kDa) but labeled another protein with a molecular weight of about 65 kDa (Jaffrezou et al. 1991). Labeling of this protein was found to be antagonized by diltiazem and nifedipine but not by verapamil. While verapamil was proved to inhibit P-gp transport activity by direct interaction with a P-gp molecule effect of verapamil on P-gp-mediated MDR could also include alterations in P-gp expression because this drug was proved to significantly reduce the P-gp protein levels (Sulova et al. 2008; Takara et al. 2002). All of the above facts indicated that calcium antagonists are overcoming P-gp-mediated MDR by mechanisms distinct from their inhibitory effect on voltage-dependent calcium channels. These mechanisms include not only direct interaction with the P-gp protein but also interactions with several other proteins that may indirectly alter P-gp transport/activity.

Role of calmodulin and other calcium binding proteins in P-gp mediated MDR

The fact that calmodulin inhibitors like neuroleptics potently reduce P-gp-mediated MDR indicates that calmodulin may also play a role in this phenotype. Consistent with this, calmodulin was proved to interact directly with P-gp (Schlemmer et al. 1995). A more potent interaction was observed in the absence than in presence of calcium, which was associated with calcium-dependent restoration of the P-gp ATPase activity inhibited by calmodulin. We found a statistically significant correlation between the MDR reversal effects of several substances (neuroleptics, local anesthetics and calcium entry blockers) and their affinity to calmodulin using P-gp-positive L1210/VCR cells (Barancik et al. 1994). Moreover, the applied substances were effective against P-gp-mediated MDR via an interaction with something located in the internal space of the cells. Trifluoperazine improved the doxorubicin sensitivity in P-gp-positive L1210/DOXO cells and, moreover, induced an increase of P-glycoprotein

phosphorylation (Ganapathi et al. 1991). In the similar cell model, i.e., L1210/adr, trifluoperazine induced a lower expression of P-gp (Shin et al. 2006). The mechanism of calmodulin inhibitory action on P-gp-mediated MDR is unknown but may be related to the effect of substances on the activity of calmodulin kinase II. Activation of this kinase by substances that induce the increase of the intracellular calcium concentration, like the sodium pump inhibitors (oubain and digoxin) or SERCA inhibitors (artemisinin and parthenolide) potentiated HIF-1α phosphorylation and the induction of P-gp expression (Riganti et al. 2009a; Riganti et al. 2009b). Thus, the interaction of calmodulin inhibitors with calmodulin may oppositely reduce the activity of calmodulin kinase II and consequently cause a decrease in P-gp expression.

Another calcium-binding protein that was described to be related to P-gp-mediated MDR is sorcin (Sugawara et al. 1989). Sorcin was found to be over-expressed in cells expressing P-gp and was closely associated with free ribosomes, rough endoplasmic reticulum, mitochondria, microfilament bundles and perinuclear membranes. Sorcin is a 22 kDa protein that: i. Modulates the cardiac L-type Ca^{2+} current (Fowler et al. 2009), probably by a functional interaction with the alpha1C subunit (Fowler et al. 2008) and ii. Interacts with the calcium/calmodulin-dependent protein kinase and indirectly modulates the ryanodine receptor function (Anthony et al. 2007). A significant correlation between the contents of mRNA encoding sorcin and P-glycoprotein was described in leukemic blast cells of 65 acute myeloid leukemia patients (Tan et al. 2003). The same author described that sorcin over-expression was associated with poor clinical outcomes. However, the over-expression of sorcin alone induces a low level of paclitaxel resistance in human ovarian and breast cancer cells by P-gp-independent mechanisms (Parekh et al. 2002). On the other hand, the knock-down of sorcin induces the up-regulation of MDR1 in HeLa cells (Kawakami et al. 2007). Thus, it could be concluded that the over-expression of sorcin may cause the reduction of cell sensitivity to diverse drugs by mechanisms independent from P-gp. P-gp-mediated MDR and resistance associated with over-expression of sorcin may exist together in the same cells.

Calcium signal abnormalities were observed in cells over-expressing P-gp

P-gp-positive T cells exert smaller elevations of the intracellular calcium ion concentration induced by Con A, anti-CD3 antibodies or ionomycin than P-gp-negative cells (Witkowski and Miller 1999). The latter authors discussed the differences in intracellular calcium signaling by the higher expression of sorcin that may influence intracellular calcium homeostasis. For the measurement of intracellular calcium content, they used indo-1/AM as an intracellular calcium indicator. However, intracellular calcium indicators are known as P-gp substrates (Breier et al. 2005). Low levels of accumulation of calcium indicators in P-gp-positive cells that could be antagonized by P-gp inhibitors were described for: i. Indo-1/AM (Brezden et al. 1994); ii. Fura-2/AM (Fu et al. 1997; Fu et al. 2002); iii. Calcein/AM (Eneroth et al. 2001; Karaszi et al. 2001); iv. Fluo-3/AM (Orlicky et al. 2004; Van Acker et al. 1995). For this reason, the measurement of the intracellular calcium concentration by calcium indicators in P-gp-positive cells may produce incorrect results.

Therefore, to resolve the question if any differences in calcium entry exist between P-gp-negative and -positive cells, we had to measure differences in $^{45}Ca^{2+}$ uptake between sensitive L1210 and resistant L1210/VCR cells (Sulova et al. 2005). The $^{45}Ca^{2+}$ uptake was more pronounced in resistant cells and could not be antagonized by verapamil (Fig. 2). This indicates the fact that calcium entry into P-gp-positive cells is more effective than in P-gp-negative cells. Consistent with this, a higher intracellular calcium concentration in P-gp-positive MCF-7 cells as compared with its P-gp-negative counterparts was described (Mestdagh et al. 1994). The lack of the ability of verapamil to alter calcium uptake to L1210 and L1210/VCR cells indicate that there are other systems responsible for calcium entry besides voltage-dependent calcium channels. Consistent with this, Tarabova and Lacinova (from the Institute of Molecular Physiology and Genetics SAS) measured calcium currents in L1210 and L1210/VCR cells by the whole-cell patch-clamp technique, and they did not find any calcium channel that fulfills the criteria characteristic for a voltage-dependent one. Sensitive L1210 and resistant L1210/VCR cells differ not only in the level of calcium uptake but also in intracellular calcium localization detected by cytochemical precipitation methods using electron microscopy (Sulova et al. 2005). In sensitive cells, calcium precipitates were found to be localized predominantly extracellularly along the surface of cells and within mitochondria, frequently delineating the cristae. In resistant cells, precipitates were also found inside of nuclei, predominantly at the border of heterochromatin clumps and scattered within the cytoplasm, probably corresponding to elements of the endoplasmic reticulum and masking to some extent the image of the mitochondria. Thus, P-gp-positive and P-gp-negative cells differ in the effectiveness of calcium uptake and its intracellular localization. Interestingly, application of high calcium concentrations in extracellular space, which magnified differences in calcium uptake when L1210 and L1210/VCR cells were compared (Sulova et al. 2005), were also improving P-gp expression in clonal parathyroid epithelial cells (Axiotis et al. 1995).

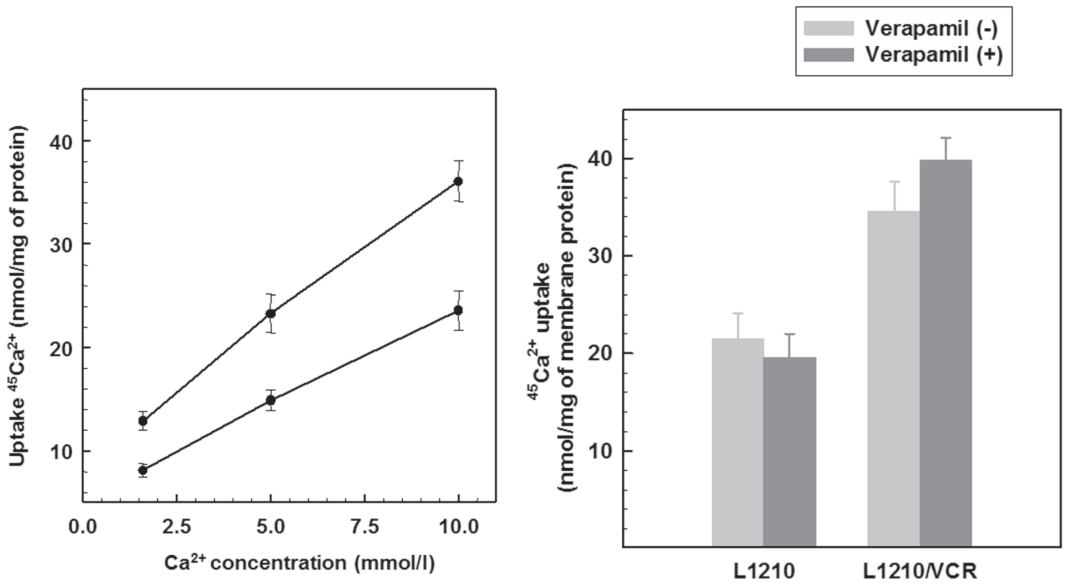

Fig. 2. Differences of $^{45}Ca^{2+}$-uptake by P-gp-negative L1210 and P-gp-positive L1210/VCR cells. Cells were incubated in 20 mmol/l Mops with 10 mmol/l glucose, 117 mmol/l NaCl, 3 mmol/l KCl, and 1 mmol/l $MgSO_4$ supplemented with 1.6, 5.0 and 10.0 mmol/l $CaCl_2$ labeled by [$^{45}Ca^{2+}$] (Amersham, UK) to a specific radioactivity of 24 GBq/mol (final cell density of 3×10^6 cells/ml in 2.5 ml) in 24-well tissue cultivation plates in a humidified atmosphere with 5% CO_2 at 37 °C for 20 min. Then, 200 µl aliquots were removed, filtered on Whatman GF/A glass fiber filters (Whatman) and washed three times with 5 ml ice-cold medium supplemented with 1 mmol/l $LaCl_3$ and 5 mmol/l EGTA. The filters were dried, and the radioactivity was measured in SLT scintillation cocktail on a liquid scintillation counter Beckman LS 6500. The effects of verapamil (10 µmol/l) on Ca^{2+} uptake were also measured. Verapamil at these concentrations induced a total inhibition of P-gp transport activity in L1210/VCR cells measured by calcein/AM as a substrate (Orlicky et al. 2004). Left panel: Concentration dependency of $^{45}Ca^{2+}$ uptake. Symbols: circles – L1210 cells; diamonds – L1210/VCR cells. Right panel: Effect of verapamil on $^{45}Ca^{2+}$ uptake. Data are expressed as mean ± S.E.M. for nine independent values.

Resistance of P-gp positive cells to thapsigargin and changes in protein expressions involved in intracellular calcium homeostasis

Thapsigargin is a well-known inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-pump, SERCA. The inhibitory action of thapsigargin on this transport ATPase will induce intracellular calcium mobilization and calcium depletion from sarco/endoplasmic reticulum as a major intracellular calcium store. Thapsigargin was found to be a P-gp substrate, i.e., it may be eliminated from the cytoplasm in P-gp-positive cells (Gutheil et al. 1994). Thus, the expression of P-gp in the cells may also cause a resistance to thapsigargin. In P-gp-positive cells, thapsigargin did not induce the calcium mobilization effect that was observed in P-gp-negative cells (Wagner-Souza et al. 2003). However, resistance to thapsigargin can involve not only alterations in P-gp expression but also in SERCA isoform expression (Gutheil et al. 1994). L1210/VCR cells were also found to be resistant to thapsigargin (Seres et al. 2008). However, this resistance could not be explained only by the thapsigargin efflux by P-gp for the following reason. The efflux activity of P-gp in L1210/VCR cells may be fully inhibited by the presence of 10 µmol/l of verapamil (Orlicky et al. 2004). However, a total blockade of P-gp activity by verapamil (at this concentration) in L1210/VCR cells did not restore their sensitivity to thapsigargin to the level observed in L1210 cells (Seres et al. 2008). Consistent with this, thapsigargin-induced calcium mobilization could not be achieved even if P-gp was inhibited by verapamil or cyclosporine A (Wagner-Souza et al. 2003). The latter authors showed in the model of the human leukemic cell line K562, selected for its resistance to vincristine, that cross-resistance to thapsigargin is not associated with an elevation of SERCA expression or with the prevalence of thapsigargin less-sensitive SERCA isoforms.

In L1210/VCR cells, the over-expression of P-gp was associated with the depression of SERCA 2 expression (Seres et al. 2008). Ryanodyne receptor expression was found to be unchanged, and IP3 receptor expression was depressed only when L1210/VCR cells were cultivated prior to estimation in the presence of vincristine. The obtained data indicated that L1210 and L1210/VCR cells did not differ from the point of view of expression of calcium-induced calcium release channels. Thus, release of calcium ions from the endoplasmic reticulum should be of similar extent in both cell counterparts. On the other hand, a decrease in SERCA2 expression

in L1210/VCR cells as compared with L1210 cells indicated slower calcium ion uptake to the luminal space of the endoplasmic reticulum. Thus, the predominance of calcium release over calcium uptake should take place in L1210/VCR cells when compared with L1210 cells. This indicated that in P-gp-positive L1210/VCR cells, the calcium intracellular concentration has to be higher than in P-gp-negative L1210 cells. A higher intracellular calcium concentration in P-gp positive MCF-7 cells as compared with its P-gp negative counterparts was also described (Mestdagh et al. 1994). In lymphoid leukemia cells, expression of the SERCA3 isoform may also take place (Papp et al. 2004). Whether depression of SERCA2 content in L1210 cells is partially compensated by an increase in SERCA3 expression remains to be elucidated. However, SERCA3 is known to have a lower affinity for calcium than other isoforms (Wuytack et al. 1995). Thus, the prevalence of SERCA3 over SERCA2 expression has to induce an increase in intracellular calcium concentration (Papp et al. 2004).

Calnexin – a Ca^{2+}-dependent molecular chaperone of the endoplasmic reticulum – was described to be involved in the quality control of newly synthesized P-gp molecules in the endoplasmic reticulum (Loo and Clarke 1994). Only a P-gp molecule with an accurate structure was able to escape association with calnexin in the endoplasmic reticulum and be targeted to the plasma membrane. The interaction of calnexin and the non-mature protein molecule depends on the calcium content in the endoplasmic reticulum. Therefore, substances like thapsigargin that inhibit endoplasmic reticulum calcium pumps consequently induced a lowering in the amount of stored calcium ions in the endoplasmic reticulum and may have influenced the interaction between calnexin and non-matured protein. Consistent with this, thapsigargin induced an escape of the mutant ΔF508-CFTR protein (Cystic Fibrosis Transmembrane conductance Regulator – ABCC7 member of ABC protein family) and its functional cell surface localization (Egan et al. 2002). This phenomenon, however, was not fully proved because other authors were not able to induced CFTR cell surface localization with the SERCA inhibitors curcumin and thapsigargin (Grubb et al. 2006; Loo et al. 2004). Loo and colleagues (2004) also described the inability of both SERCA inhibitors to induce the escape of mutant P-gp from the interaction with calnexin and its functional cell surface localization. All of the above reasons motivated us to measure if there were any differences in the calnexin expression between P-gp-positive L1210 and P-gp-negative L1210/VCR cells. The expression of calnexin was considerably decreased in our resistant cell variant that expressed P-gp (Seres et al. 2008). However, the P-gp protein was present in the material obtained by immuno-precipitation with anti-calnexin antibody of the cell homogenate originating from L1210/VCR cells. Thus, calnexin acts as a P-gp chaperone also in L1210/VCR cells, even if its amount in this cell variant is considerably reduced. These facts indicated that P-gp-positive cell variants may differ from parental cells also in protein maturation, i.e., in protein glycosylation reactions. Interestingly, we described real alterations between the compositions of sugar parts of glycoproteins located on the surface of L1210 and L1210/VCR cells (Fiala et al. 2003; Sulova et al. 2009).

Conclusions

Several facts indicated that some relationship between intracellular calcium homeostasis and P-gp-mediated MDR exists. However, straight connections between intracellular calcium-dependent processes and the regulation of P-gp expression or efflux activity are still unknown. They may involve intracellular calcium concentration regulation, calcium-dependent protein phosphorylation, quality control of newly synthesized protein via calcium dependent molecular chaperons and others. In the end, it could be stressed that P-gp-mediated MDR in neoplastic cells is linked to intracellular calcium homeostasis, but the mechanisms and meaning of this interplay are still unclear.

Acknowledgment. This research was supported in part by the following grants: From Slovak Research and Development Agency: APVV-0084-07, VVCE-0067-07; from Slovak Grant Agency for Science VEGA: 2/7122/27, 2/0155/09.
The authors have no financial interests related to the material in the manuscript nor to the participation in the 2nd ECS Workshop.

References

Anthony D. F., Beattie J., Paul A., Currie S. (2007): Interaction of calcium/calmodulin-dependent protein kinase IIdeltaC with sorcin indirectly modulates ryanodine receptor function in cardiac myocytes. J. Mol. Cell. Cardiol. **43**, 492–503

Axiotis C. A., Bani D., Bianchi S., Pioli P., Tanini A., Brandi M. L. (1995): P-glycoprotein is expressed in parathyroid epithelium and is regulated by calcium. Calcif. Tissue Int. **56**, 170–174

Barancik M., Polekova L., Mrazova T., Breier A., Stankovicova T., Slezak J. (1994): Reversal effects of several Ca^{2+}-entry blockers, neuroleptics and local anaesthetics on P-glycoprotein-mediated vincristine resistance of L1210/VCR mouse leukaemic cell line. Drugs Exp. Clin. Res. **20**, 13–18

Breier A., Barancik M., Sulova Z., Uhrík B. (2005): P-glycoprotein – implications of metabolism of neoplastic cells and cancer therapy. Curr. Cancer Drug Targets **5**, 457–468

Breier A., Drobna Z., Docolomansky P., Barancik M. (2000): Cytotoxic activity of several unrelated drugs on L1210 mouse leukemic cell sublines with P-glycoprotein (PGP) mediated multidrug resistance (MDR) phenotype. A QSAR study. Neoplasma **47**, 100–106

Brezden C. B., Hedley D. W., Rauth A. M. (1994): Constitutive expression of P-glycoprotein as a determinant of loading with fluorescent calcium probes. Cytometry **17**, 343–348

Didziapetris R., Japertas P., Avdeef A., Petrauskas A. (2003): Classification analysis of P-glycoprotein substrate specificity. J. Drug. Target **11**, 391–406

Egan M. E., Glockner-Pagel J., Ambrose C., Cahill P. A., Pappoe L., Balamuth N., Cho E., Canny S., Wagner C. A., Geibel J., Caplan M. J. (2002): Calcium-pump inhibitors induce functional surface expression of Delta F508-CFTR protein in cystic fibrosis epithelial cells. Nat. Med. **8**, 485–492

Eneroth A., Astrom E., Hoogstraate J., Schrenk D., Conrad S., Kauffmann H. M., Gjellan K. (2001): Evaluation of a vincristine resistant Caco-2 cell line for use in a calcein AM extrusion screening assay for P-glycoprotein interaction. Eur. J. Pharm. Sci. **12**, 205–214

Fiala R., Sulova Z., El-Saggan A. H., Uhrík B., Liptaj T., Dovinova I., Hanusovska E., Drobna Z., Barancik M., Breier A. (2003): P-glycoprotein-mediated multidrug resistance phenotype of L1210/VCR cells is associated with decreases of oligo- and/or polysaccharide contents. Biochim. Biophys. Acta **1639**, 213–224

Ford J. M. (1996): Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. Eur. J. Cancer **32A**, 991–1001

Fowler M. R., Colotti G., Chiancone E., Higuchi Y., Seidler T., Smith G. L. (2009): Complex modulation of L-type Ca^{2+} current inactivation by sorcin in isolated rabbit cardiomyocytes. Pflugers Arch. **457**, 1049–1060

Fowler M. R., Colotti G., Chiancone E., Smith G. L., Fearon I. M. (2008): Sorcin modulates cardiac L-type Ca^{2+} current by functional interaction with the alpha1C subunit in rabbits. Exp. Physiol. **93**, 1233–1238

Fu L. W., Pan Q. C., Lin G. Y. (1997): Compared study on Fura-2/AM assay and MTT assay for screening multidrug resistant modulators. Yao Xue Xue Bao **32**, 401–405 (in Chinese)

Fu L. W., Zhang Y. M., Liang Y. J., Yang X. P., Pan Q. C. (2002): The multidrug resistance of tumour cells was reversed by tetrandrine in vitro and in xenografts derived from human breast adenocarcinoma MCF-7/adr cells. Eur. J. Cancer **38**, 418–426

Ganapathi R., Kamath N., Constantinou A., Grabowski D., Ford J., Anderson A. (1991): Effect of the calmodulin inhibitor trifluoperazine on phosphorylation of P-glycoprotein and topoisomerase II: relationship to modulation of subcellular distribution, DNA damage and cytotoxicity of doxorubicin in multidrug resistant L1210 mouse leukemia cells. Biochem. Pharmacol. **41**, R21–R26

Grubb B. R., Gabriel S. E., Mengos A., Gentzsch M., Randell S. H., Van Heeckeren A. M., Knowles M. R., Drumm M. L., Riordan J. R., Boucher R. C. (2006): SERCA pump inhibitors do not correct biosynthetic arrest of deltaF508 CFTR in cystic fibrosis. Am. J. Respir. Cell Mol. Biol. **34**, 355–363

Gutheil J. C., Hart S. R., Belani C. P., Melera P. W., Hussain A. (1994): Alterations in Ca^{2+} transport ATPase and P-glycoprotein expression can mediate resistance to thapsigargin. J. Biol. Chem. **269**, 7976–7981

Hollt V., Kouba M., Dietel M., Vogt G. (1992): Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. Biochem. Pharmacol. **43**, 2601–2608

Jaffrezou J. P., Herbert J. M., Levade T., Gau M. N., Chatelain P., Laurent G. (1991): Reversal of multidrug resistance by calcium channel blocker SR33557 without photoaffinity labeling of P-glycoprotein. J. Biol. Chem. **266**, 19858–19864

Karaszi E., Jakab K., Homolya L., Szakacs G., Hollo Z., Telek B., Kiss A., Rejto L., Nahajevszky S., Sarkadi B., Kappelmayer J. (2001): Calcein assay for multidrug resistance reliably predicts therapy response and survival rate in acute myeloid leukaemia. Br. J. Haematol. **112**, 308–314

Kawakami M., Nakamura T., Okamura N., Komoto C., Markova S., Kobayashi H., Hashimoto N., Okumura K., Sakaeda T. (2007): Knock-down of sorcin induces up-regulation of MDR1 in HeLa cells. Biol. Pharm. Bull. **30**, 1065–1073

Kimura Y., Matsuo M., Takahashi K., Saeki T., Kioka N., Amachi T., Ueda K. (2004): ATP hydrolysis-dependent multidrug efflux transporter: MDR1/P-glycoprotein. Curr. Drug Metab. **5**, 1–10

Loo T. W., Bartlett M. C., Clarke D. M. (2004): Thapsigargin or curcumin does not promote maturation of processing mutants of the ABC transporters, CFTR, and P-glycoprotein. Biochem. Biophys. Res. Commun. **325**, 580–585

Loo T. W., Clarke D. M. (1994): Prolonged association of temperature-sensitive mutants of human P-glycoprotein with calnexin during biogenesis. J. Biol. Chem. **269**, 28683–28689

Mestdagh N., Vandewalle B., Hornez L., Henichart J. P. (1994): Comparative study of intracellular calcium and adenosine 3',5'-cyclic monophosphate levels in human breast carcinoma cells sensitive or resistant to Adriamycin: contribution to reversion of chemoresistance. Biochem. Pharmacol. **48**, 709–716

O'Neill J. P., Velalar C. N., Lee D. I., Zhang B., Nakanishi T., Tang Y., Selaru F., Ross D., Meltzer S. J., Hussain A. (2006): Thapsigargin resistance in human prostate cancer cells. Cancer **107**, 649–659

Orlicky J., Sulova Z., Dovinova I., Fiala R., Zahradnikova A. Jr., Breier A. (2004): Functional fluo-3/AM assay on P-glycoprotein transport activity in L1210/VCR cells by confocal microscopy. Gen. Physiol. Biophys. **23**, 357–366

Papp B., Brouland J. P., Gelebart P., Kovacs T., Chomienne C. (2004): Endoplasmic reticulum calcium transport ATPase expression during differentiation of colon cancer and leukaemia cells. Biochem. Biophys. Res. Commun. **322**, 1223–1236

Parekh H. K., Deng H. B., Choudhary K., Houser S. R., Simpkins H. (2002): Overexpression of sorcin, a calcium-binding protein, induces a low level of paclitaxel resistance in human ovarian and breast cancer cells. Biochem. Pharmacol. **63**, 1149–1158

Pawagi A. B., Wang J., Silverman M., Reithmeier R. A., Deber C. M. (1994): Transmembrane aromatic amino acid distribution in P-glycoprotein. A functional role in broad substrate specificity. J. Mol. Biol. **235**, 554–564

Riganti C., Campia I., Polimeni M., Pescarmona G., Ghigo D., Bosia A. (2009a): Digoxin and ouabain induce P-glycoprotein by activating calmodulin kinase II and hypoxia-inducible factor-1alpha in human colon cancer cells. Toxicol. Appl. Pharmacol. **240,** 385–392

Riganti C., Doublier S., Viarisio D., Miraglia E., Pescarmona G., Ghigo D., Bosia A. (2009b): Artemisinin induces doxorubicin resistance in human colon cancer cells via calcium-dependent activation of HIF-1alpha and P-glycoprotein overexpression. Br. J. Pharmacol. **156**, 1054–1066

Safa A. R. (1988): Photoaffinity labeling of the multidrug-resistance-related P-glycoprotein with photoactive analogs of verapamil. Proc. Natl. Acad. Sci. U.S.A. **85**, 7187–7191

Schlemmer S. R., Yang C. H., Sirotnak F. M. (1995): Functional modulation of multidrug resistance-related P-glycoprotein by Ca^{2+}-calmodulin. J. Biol. Chem. **270**, 11040–11042

Seres M., Polakova E., Krizanova O., Hudecova S., Klymenko S. V., Breier A., Sulova Z. (2008): Overexpression of P-glycoprotein in L1210/VCR cells is associated with changes in several endoplasmic reticulum proteins that may be partially responsible for the lack of thapsigargin sensitivity. Gen. Physiol. Biophys. **27**, 211–221

Shapiro A. B., Ling V. (1994): ATPase activity of purified and reconstituted P-glycoprotein from Chinese hamster ovary cells. J. Biol. Chem. **269**, 3745–3754

Shin S. Y., Choi B. H., Kim J. R., Kim J. H., Lee Y. H. (2006): Suppression of P-glycoprotein expression by antipsychotics trifluoperazine in adriamycin-resistant L1210 mouse leukemia cells. Eur. J. Pharm. Sci. **28**, 300–306

Sugawara I., Mizumoto K., Ohkochi E., Hamada H., Tsuruo T., Mori S. (1989): Immunocytochemical identification and localization of the Mr 22,000 calcium-binding protein (sorcin) in an adriamycin-resistant myelogenous leukemia cell line. Jpn. J. Cancer Res. **80**, 469–474

Sulova Z., Macejova D., Seres M., Sedlak J., Brtko J., Breier A. (2008): Combined treatment of P-gp-positive L1210/VCR cells by verapamil and all-trans retinoic acid induces down-regulation of P-glycoprotein expression and transport activity. Toxicol. In Vitro **22**, 96–105

Sulova Z., Mislovicova D., Gibalova L., Vajcnerova Z., Polakova E., Uhrik B., Tylkova L., Kovarova A., Sedlak J., Breier A. (2009): Vincristine-induced overexpression of P-glycoprotein in L1210 cells is associated with remodeling of cell surface saccharides. J. Proteome Res. **8**, 513–520

Sulova Z., Orlicky J., Fiala R., Dovinova I., Uhrík B., Seres M., Gibalova L., Breier A. (2005): Expression of P-glycoprotein in L1210 cells is linked with rise in sensitivity to Ca^{2+}. Biochem. Biophys. Res. Commun. **335**, 777–784

Takara K., Sakaeda T., Tanigawara Y., Nishiguchi K., Ohmoto N., Horinouchi M., Komada F., Ohnishi N., Yokoyama T., Okumura K. (2002): Effects of 12 Ca^{2+} antagonists on multidrug resistance, MDR1-mediated transport and MDR1 mRNA expression. Eur. J. Pharm. Sci. **16**, 159–165

Tan Y., Li G., Zhao C., Wang J., Zhao H., Xue Y., Han M., Yang C. (2003): Expression of sorcin predicts poor outcome in acute myeloid leukemia. Leuk. Res. **27**, 125–131

Van Acker K. L., De Greef C., Eggermont J., Zhang P., Vandenberghe P., Boogaerts M. A. (1995): Detection of P-glycoprotein with a rapid flow cytometric functional assay using Fluo-3: evaluation of sensitivity, specificity and feasibility in multiparametric analysis. Leukemia **9**, 1398–1406

Wagner-Souza K., Echevarria-Lima J., Rodrigues L. A., Reis M., Rumjanek V. M. (2003): Resistance to thapsigargin-induced intracellular calcium mobilization in a multidrug resistant tumour cell line. Mol. Cell. Biochem. **252**, 109–116

Witkowski J. M., Miller R. A. (1999): Calcium signal abnormalities in murine T lymphocytes that express the multidrug transporter P-glycoprotein. Mech. Ageing Dev. **107**, 165–180

Wuytack F., Dode L., Baba-Aissa F., Raeymaekers L. (1995): The SERCA3-type of organellar Ca^{2+} pumps. Biosci. Rep. **15**, 299–306

Zhou S. F. (2008): Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica **38**, 802–832

Received: October 18, 2009
Final version accepted: October 29, 2009