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Relation between the ability of some compounds to modulate the MRP1-mediated efflux of glutathione and to inhibit the MRPl-mediated efflux of daunorubicin

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Abstract

Much effort has been recently directed to identify the transport-modulating agents in order to overcome the P-gp- and MRP1-mediated drug resistance. Contrary to what is observed for P-gp, very few compounds have been shown to reverse multi-drug resistance (MDR) mediated by MRP1. On the other hand, despite of critical role of GSH in transporting the MRP1 substrates, not much is known about GSH interactions with MRP1. In this work, three compounds that were shown to inhibit the MRP1-mediated efflux of daunorubicin (DNR) have been studied. Depending on their nature the selected compounds have different effects, e.g. at 40 μM, verapamil inhibits 50% of DNR efflux whereas GSH efflux is increased about two-fold. PAK-104P has shown the same effect, i.e. the inhibition of the MRP1-mediated efflux of DNR is accompanied by a stimulation of GSH efflux. However, the PAK-104P concentration required to obtain the same effect is about 40 times smaller that in the case of verapamil. MK571 has been shown to inhibit the efflux of both DNR and GSH. Based on these observations and those reported earlier, a working model is proposed.
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Keywords: P-gp; MRP1; GLC4/ADR; Daunorubicin; Glutathione; Verapamil; PAK-104P; MK571

1. Introduction

Treatment of cancer patients with chemotherapeutic drugs is often unsuccessful due to the drug-resistant tumors. A critical problem arises when cancer cells develop multi-drug resistance (MDR). Although, many different mechanisms may be involved in MDR and in cultured tumor cells, it frequently appears to be associated with increased expression of the ATP-binding cassette transporter proteins, P-glycoprotein (P-gp) and/or multi-drug resistance protein (MRPl) [1–5]. In the cells which overexpress P-gp or MRPl, drug accumulation is reduced. This supports the notion that the multi-drug phenotype caused by both proteins involves increased drug extrusion. However, contrary to P-gp case, the MRPl-mediated active transport of unmodified chemotherapeutic drugs, such as vincristine and daunorubicin, requires the presence of glutathione (GSH) as well as ATP [6–9].

Substantial effort has been undertaken to identify the agents capable of reversing resistance mediated by MRPl and P-gp because of their potential clinical importance [10–13]. In this context, the ability of several molecules to inhibit the MRPl-mediated efflux of substrates has been checked. Surprisingly, it was observed that some of them such as verapamil stimulate GSH efflux [13–16].

The aim of this work was to study the relation between the ability of a molecule to inhibit or to stimulate the efflux of GSH and its ability to inhibit the efflux of a well-known substrate, daunorubicin (DNR). Three molecules were selected to clarify their effects on MRPl-mediated drug resistance: verapamil, PAK-104P and MK571. We have investigated the ability of these drugs to modulate direct transport of GSH and DNR in living GLC4/ADR cells that overexpress MRPl. The data have shown that: (i) verapamil,

Abbreviations: MDR, multi-drug resistance; P-gp, P-glycoprotein; MRP1, multi-drug resistance protein; GSH, glutathione; DNR, daunorubicin; PAK-104P, 2-[4-(diphenylmethyl)-1-piperazinyl] ethyl-5-(*trans*-4,6-dimethyl-1,3,2-dioxaphosphorinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinecarboxylate P oxide; MK571, 3-([{3-(2-[7-chloro-2-quinolinyl] ethenyl)phenyl}-{(3-dimethylamino-3-oxopropyl)-thio}-methyl]thio)propanoic acid; LTC4, leukotriene C4; DNP-SG, dinitrophenyl-*S*-glutathione

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PAK-104P and MK571 inhibit DNR transport in GLC4/ADR cells in a non-competitive manner and (ii) verapamil and PAK-104P stimulate whereas MK571 inhibits GSH export. Based on these observations and other data reported in the literature, a working model is proposed.

2. Materials and methods

2.1. Cell culture

GLC4 cells are small-cell lung cancer. GLC4 and MRP1-expressing GLC4/ADR cells [17], were cultured in RPMI 1640 (Sigma Chemical Co, St Louis, MO) medium supplemented with 10% fetal calf serum (Invitrogen, Cergy-Pontoise, France) at 37 °C in a humidified incubator with 5% CO_2. The resistant GLC4/ADR cells were cultured with 1.2 μM doxorubicin, respectively, until one to four weeks before the experiments. For the measurements, in order to have cells in the exponential growth phase, culture was initiated at 5×10^5 cells/mL and cells were used 24 h later, they were then at a density of about 8×10^5 cells/mL.

2.2. Drugs and chemicals

Purified daunorubicin was kindly provided by laboratoire Pharmacia-Upjohn. Concentrations were determined by diluting stock solutions to approximately 10^{-5} M with $\varepsilon_{480} = 11500\ M^{-1}\ cm^{-1}$. Stock solutions were prepared just before use. Verapamil was from Sigma. 2-[4-(Diphenylmethyl)-l-piperazinyl]ethyl-5-(*trans*-4,6-dimethyl-l,3,2-dioxapho-sphorinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinecarboxylate P oxide (PAK-104P) was a gift from Dr. Shudo, Dr. Iwasaki and Dr. Akiyama (Nissan chemical industries, ltd., Japan). 3-([{3-(2-[7-Chloro-2-quinolinyl] ethenyl) phenyl}-{(3-dimethylamino-3-oxo-propyl)-thio}-methyl] thio) propanoic acid (MK571) was provided by Dr. R.N. Young (Merck-frosst centre for therapeutic research, Pointe claire-dorval, Quebec, Canada). Triton X-100 (TX) was from Sigma and was dissolved in water. Reduced glutathione, glutathione disulfide (GSSG), glutathione transferase from equine liver (GSH_T), L-buthionine-(*S*,*R*) sulphoximine (BSO), and avicin were from Sigma. Monochlorobimane was from Molecular Probes (Eugene, OR). Before the experiments, the cells were counted, centrifuged and resuspended in HEPES buffer solutions containing 20 mM HEPES plus 132 mM NaCl, 3.5 mM KC1, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$ at pH 7.3 with or without 5 mM glucose. All other reagents were of the highest quality available. Deionized double-distilled water was used throughout the experiments.

2.3. GSH measurements

In order to quantify free GSH either inside the cells or released in the extracellular medium, an enzymatic technique was used [9]. The non-fluorescent monochlorobimane is conjugated to GSH by glutathione *S*-transferase to yield a fluorescent adduct [18]. We have used this property to develop a very rapid and sensitive fluorometric method for GSH measurement [9]. Briefly, a 10 mM stock solution of monochlorobimane was prepared in ethanol, and aliquots were stored at −80 °C in the dark. The non-enzymatic reaction that occurred between GSH and monochlorobimane was very slow. However, when glutathione *S*-transferase was added, the increase of the fluorescent signal characteristic of monochlorobimane–GSH derivative formation was very fast. The initial rate of monochlorobimane–GSH formation was determined as the increase of the fluorescent signal between 100 and 150 s after the addition of glutathione *S*-transferase to monochlorobimane and GSH. The monochlorobimane and glutathione *S*-transferase concentrations were kept constant equal to 100 μM and 0.5 u/mL, respectively. The fluorescence signal recorded over a short time (50 s), which was used as a measure of the initial rate of monochlorobimane–GSH formation, is directly proportional to the concentration of GSH at least within the range 0–20 μM (this corresponds to the concentrations expected when 10^6 cells/mL were lysed with the intracellular GSH concentrations being within the 0–20 mM range). We have checked that GSSG did not give rise to any modification of the fluorescence signal.

For the intracellular GSH determination, 2×10^6 cells/mL suspended in 2 mL of buffer were disrupted by sonication on ice (3 × 10 s, power 2). The rate of monochlorobimane–GSH formation was followed after addition of monochlorobimane 100 μM and GSH_T 0.5 u/mL as described above.

For the determination of GSH released by the cells, they were re-suspended in HEPES buffer ($l0^6$/mL) in the absence or in the presence of the appropriate concentration of inhibitor. After specified time intervals, 2-mL aliquots containing 2×10^6 cells were centrifuged, the GSH concentration present in the extracellular medium, and

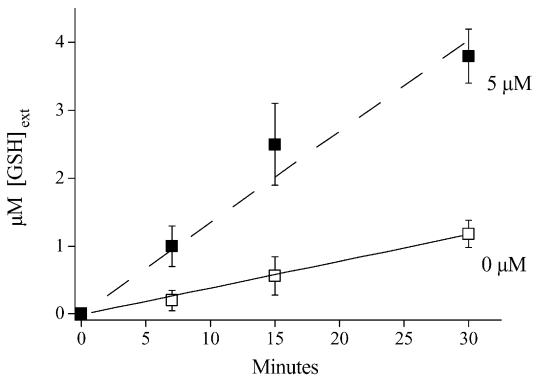

Fig. 1. Determination of the rate of MRP1-mediated efflux of GSH. The extracellular GSH concentration has been plotted as a function of the time of incubation of cells in HEPES buffer in the absence (□) and in the presence (■) of 5 μM PAK-104P.

therefore released from the cells, and the GSH present in the pellet were determined. Extracellular concentration of GSH was not affected by 250 μM acivicin, indicating negligible activity of γ-glutamyltransferase in membrane of GLC4/ADR cells. As an example of the methodology, Fig. 1 shows a typical plot of the variation of the extracellular GSH concentration as a function of time in the absence and in the presence of inhibitor, respectively. During the first 30 min, the plot was linear and the slope yielded V_a (GSH), the rate of MRP1-mediated efflux of GSH. Thereafter, V_a^0 (GSH) and V_a^i (GSH) will represent the rate of MRP1-mediated efflux of GSH in the absence and in the presence of inhibitor, respectively.

2.4. Cellular daunorubicin accumulation

The details and validation of our experimental set-up for measuring the kinetics of the active transport of anthracyclines by tumor cells have been extensively described and discussed elsewhere [19–22]. It is based on the continuous spectrofluorometric monitoring (Perkin Elmer LS50B spectrofluorometer) of the decrease in the fluorescence signal of anthracycline at 590 nm (λ_{ex} = 480 nm) after incubation with the cells in a 1 cm quartz cuvette. The decrease in fluorescence intensity occurring during the drug incubation with the cells is due to the quenching of fluorescence after intercalation of anthracycline between the base pairs of DNA. It was already shown that this approach allows the accurate measurement of the free cytosolic concentration of anthracyclines under steady-state conditions, their initial rates of uptake and the kinetics of an active efflux [19–22].

2.5. Determination of the MRP1-mediated efflux of daunorubicin

Cells (1×10^6/mL; 2 mL per cuvette) were preincubated for 30 min in HEPES buffer with sodium azide without glucose (energy-deprived cells). Depletion of ATP in these cells, checked with the luciferin–luciferase test [23] reached 90%. The cells remained viable throughout the experiment, as checked with trypan blue and calcein vital stain (not shown). After addition of anthracycline, the decrease in the fluorescence signal intensity was monitored until the steady state was reached. Since pH of the buffer was equal to intracellular pH, at the steady state, the extracellular free drug concentration (C_e) was equal to the cytosolic free drug concentration (C_i). Then, inhibitor at the appropriate concentration was added about 2 min before the addition of glucose, which led to the restoration of the control ATP levels within 2 min and to an increase in the fluorescence signal due to the efflux of anthracycline. ATP-dependent anthracycline efflux was determined from the slope of the tangent for the curve $F = f(t)$, where F is the fluorescence intensity at the time of glucose addition. Since under these conditions, at the moment of glucose addition $C_i = C_e$, the passive influx and efflux were equal, the net initial efflux V_a (DNR) represented only the rate of MRP1-mediated active efflux [19,22] (Fig. 2).

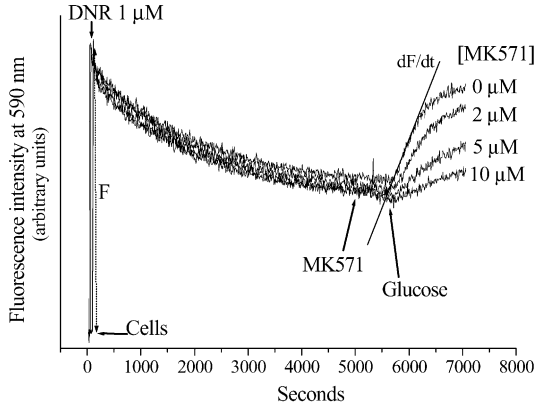

Fig. 2. Incorporation of DNR in energy-depleted GLC4/ADR cells and determination of the rate of active efflux (V_a) from dF/dt after the addition of MK571 0, 2, 5 and 10 μM and glucose 5 mM.

3. Results

3.1. Effect of verapamil on the MRP1-mediated efflux of GSH and DNR

Fig. 3 shows the plots of V_a^i (GSH)/V_a^0 (GSH) and V_a^i (DNR)/V_a^0 (DNR) as a function of the verapamil concentration. V_a^0 (GSH) and V_a^0 (DNR), the rate of the GSH and DNR efflux in the absence of inhibitor, were equal to 1.2×10^{-18} and 0.6×10^{-18} mole/cell/s, respectively. In these experiments, the concentration of DNR added to the cells was 1 μM and according to the experimental procedure used, the free intracellular DNR concentration at steady state before the addition of glucose was ~0.5 μM [22]. The

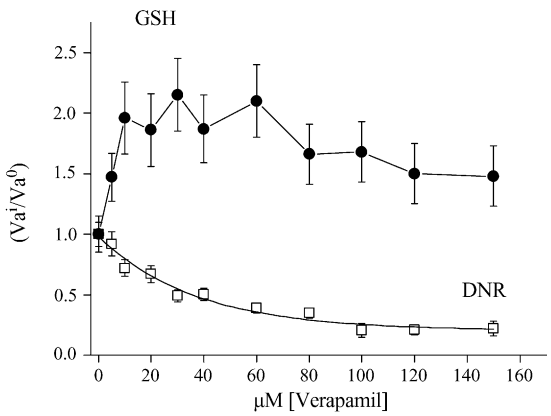

Fig. 3. Variation of V_a (GSH), the rate of MRP1-mediated efflux of GSH and V_a (DNR), the rate of MRP1-mediated efflux of DNR, in the presence of verapamil. The ratios V_a^i/V_a^0 have been plotted as a function of the verapamil concentration for GSH (●) and DNR (□). Experimental conditions: 10^6 cell/mL, [DNR] = 1 μM, and HEPES buffer. Data are mean ± S.D. from three to five independent experiments on different days.

V_a^i (DNR)/V_a^0 (DNR) ratio decreased as a function of the verapamil concentration added, indicating an inhibition of the MRP1-mediated efflux of DNR. Fifty percent of the inhibition was obtained at 40 ± 5 μM of verapamil. However, the variation of V_a^i (GSH)/V_a^0 (GSH) as a function of the verapamil concentration was bimodal; the MRP1-mediated efflux of GSH was stimulated with about two-fold increase at ~40 μM, but only 1.5-fold at concentration higher than 80 μM.

3.2. Effect of PAK-104P on the MRP1-mediated efflux of GSH and DNR

Fig. 4 shows the plots of V_a^i (GSH)/V_a^0 (GSH) and of V_a^i (DNR)/V_a^0 (DNR) as a function of the PAK-104P concentration. The V_a^i (DNR)/V_a^0 (DNR) ratio decreased as a function of the PAK-104P concentration added, indicating as previously shown [24], an inhibition of the MRP1-mediated efflux of DNR. Fifty percent of the inhibition was obtained with 1.0 ± 0.3 μM of PAK-104P. However, the variation of V_a^i (GSH)/V_a^0 (GSH) versus PAK-104P concentration was bimodal; the MRP1-mediated efflux of GSH was highly stimulated at low inhibitor concentrations about four-fold increase at 5 μM, while at higher concentrations an inhibition of the efflux was observed.

3.3. Effect of MK571 on the MRP1-mediated efflux of GSH and DNR

Fig. 5 shows the plots of V_a^i (GSH)/V_a^0 (GSH) and of V_a^i (DNR)/V_a^0 (DNR) versus MK571 concentration; both ratios decreased as the MK571 concentration increased indicating an inhibition of DNR and GSH efflux from the cells. The DNR concentrations used were 1 and 5 μM. As shown in Fig. 6, for both concentrations 50% of the inhibition was obtained at the same MK571 concentration of 3.0 ± 0.5 μM. This result suggests a non-competitive mechanism for the MK571 inhibition of the DNR efflux.

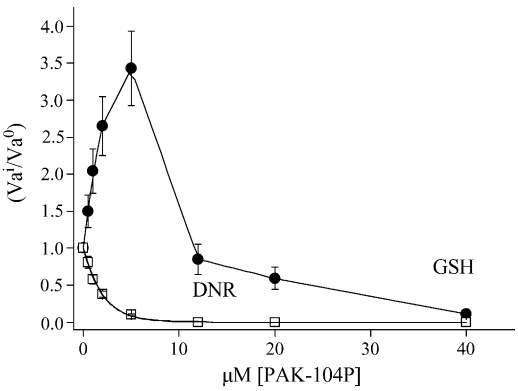

Fig. 4. Variation of V_a (GSH), the rate of MRP1-mediated efflux of GSH, and V_a (DNR), the rate of MRP1-mediated efflux of DNR, in the presence of PAK-104P. The ratios V_a^i/V_a^0 have been plotted as a function of the PAK-104P concentration for GSH (●) and DNR (□). Experimental conditions: 10^6 cell/mL, [DNR] = 1 μM, and HEPES buffer. Data are mean ± S.D. from three to five independent experiments on different days.

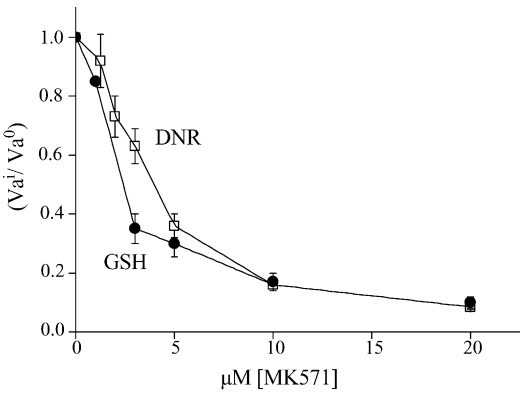

Fig. 5. Variation of V_a (GSH), the rate of MRP1-mediated efflux of GSH, and V_a (DNR), the rate of MRP1-mediated efflux of DNR, in the presence of MK571. The ratios V_a^i/V_a^0 have been plotted as a function of the MK571 concentration for GSH (●) and DNR (□). Experimental conditions: 10^6 cell/mL, [DNR] = 1 μM, and HEPES buffer. Data are mean ± S.D. from three to five independent experiments on different days.

3.4. Control experiments

Verapamil, PAK-104P, and MK571 were dissolved in ethanol. The volume of ethanol added to the cell suspension never exceeded 1/100 of the total sample volume. The ethanol added did not affect the rates of MRP1-mediated efflux of DNR and GSH. To check whether verapamil, PAK-104P and MK571 affect the intracellular concentration of GSH, the concentration of GSH was measured during the cell incubation with various concentrations of verapamil ranging from 0 to 100 μM, PAK-104P ranging from 0 to 40 μM and MK571 ranging from 0 to 20 μM. As the incubation of cells with studied inhibitors never exceeded 20 min, the intracellular GSH concentration was determined after 20 min of incubation. A small decrease of $[GSH]_I$ was observed, but it never exceeded 20%. The intracellular GSH concentration in GLC4/ADR cells has been measured to be 13 ± 3 mM [9,30]. In the experiments described above, the intracellular GSH con-

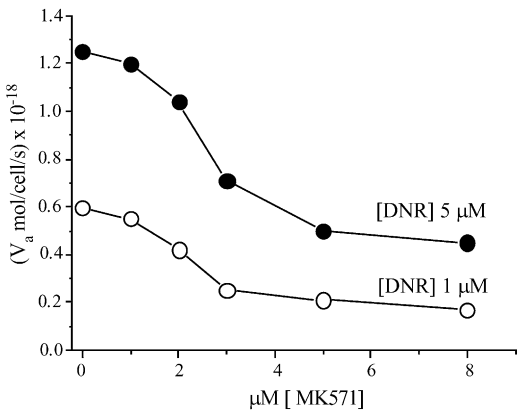

Fig. 6. Variation of V_a (DNR) the rate of MRPl-mediated efflux of DNR in the presence of MK571. Experimental conditions: 10^6 cell/mL, HEPES buffer, and [DNR] = 1 μM (○) or 5 μM (●). Data points are from a representative experiment.

centration was never lower than 8 mM, and therefore the rate of MRP1-mediated GSH efflux was always very close to the V_M value, i.e. 1.2×10^{-18} mole/(cell s) [9]. As it was previously shown, the rate of GSH efflux starts to decrease for the intracellular GSH concentrations below 6 mM [9].

We have also checked that up to 5 μM DNR has no impact on the GSH efflux (data not shown).

4. Discussion

In recent years, much effort has been directed to the identification of transport-modulating agents in order to circumvent P-gp- and MRP1-mediated drug resistance. Numerous compounds have been shown to revert MDR mediated by P-gp, even if the clinical use of these compounds is far from being trivial. However, for MRP1 very few inhibitors are known.

Here, we have studied three compounds that were already shown to inhibit the MRP1-mediated efflux of the very well-known antitumor drug DNR. We have shown that these compounds depending on their nature have different effects: at 40 μM verapamil inhibits 50% of DNR efflux whereas GSH efflux is increased about two-fold. PAK-104P has the same effect, i.e. the inhibition of the MRPl-mediated efflux of DNR is accompanied by a stimulation of the GSH efflux; however, the concentration required yielding these effects is much smaller than that in the case of verapamil, being equal to about 1 μM. However, MK571 inhibited both the efflux of DNR and GSH. In order to clearly highlight these effects, the ratio V_a^i (DNR)/V_a^0 (DNR) has been plotted as a function of the ratio V_a^i (GSH)/V_a^0 (GSH) for the three inhibitors (Fig. 7). As can be seen for verapamil and PAK-104P, the experimental points are on a same line despite the fact that the range of concentrations is not the same. This strongly suggests that

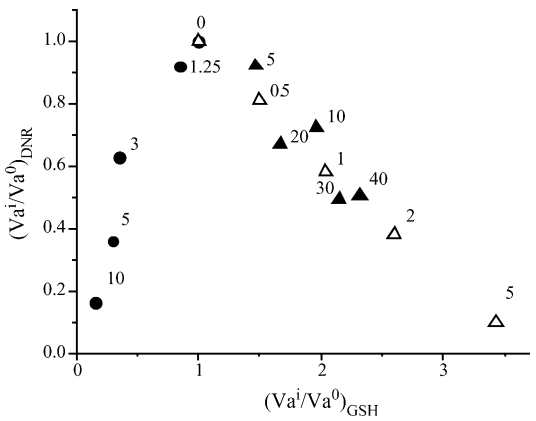

Fig. 7. Simultaneous modification of V_a (DNR) and V_a (GSH) in the presence of different inhibitors. The ratio V_a^i (DNR)/V_a^0 (DNR) has been plotted as a function of the ratio V_a^i (GSH)/V_a^0 (GSH) determined in the presence of various concentrations of inhibitors: MK571 (●), verapamil (▲) or PAK-104P (△). The concentration (μM) used for each experiment is indicated for each point.

verapamil and PAK-104P are acting upon MRP1 in a similar way while MK571 is working in a completely different manner. Unlike the most of MRP1 substrates, GSH appears to play a role in facilitating the transport process, possibly by conjugation process or most probably by co-transport with other molecules. However, despite the critical role played by GSH in transporting of MRP1 substrates, little is known about mutual GSH and MRP1 relations. Several studies suggest that substrates such as DNR on the one hand and GSH on the other hand have different binding sites within MRP1 protein [25]. Let us call site D for DNR and site G for binding of GSH. Several studies suggest that these two sites interact with each other with the most evident support derived from the fact that DNR can be pumped out only in the presence of GSH.

According to the data presented above, one could divide the molecules able to modulate the MRP1 transport of substrates into two classes; class I would contain the compounds that inhibit the MRP1 transport of all the substrates independently on their nature and in addition, the concentration required to inhibit 50% of the transport would not depend on the nature of the substrate. MK571 belongs to this class. In contrast, compounds of class II could modulate the MRP1-mediated transport of substrates, but depending on the nature of the substrate, stimulation or an inhibition of the transport would be observed. Verapamil and PAK-104P belong to this class; they stimulate the MRP1-mediated efflux of GSH whereas they inhibit the MRP1-mediated transport of molecules such as DNR.

It is now well established that compounds such as LTC4 and DNP-SG do not require the presence of GSH to be transported by MRP1 [6,26–28]. We propose that these compounds together with GSH itself constitute class G, while those compounds, which need GSH to be transported, constitute class D, which includes DNR, vincristine, and rhodamine.

Based on these results and the literature data (vide infra), we propose the following working model (see Fig. 8). Let us assume that MRP1 is composed of two interlocked wheels G and D. Wheel G binds GSH and LTC4, i.e. the G-class substrates. Wheel G is the power unit which turns when energy is provided by the hydrolysis of ATP and when one of the G-class substrates is bound to the G site. Wheel D is inert and can turn only when it is connected to wheel G. In the presence of saturating amount of GSH and DNR, both wheels are able to turn and expel one molecule of GSH and one molecule of DNR, respectively [9]. Inhibitors of class I such as MK571 block completely the system because they act directly on wheel G preventing a turning of wheel D. Inhibitors of class II such as PAK-104P and verapamil disconnect the two wheels: it follows that wheel G, which has not to drag the D wheel anymore, turns more rapidly while wheel D cannot.

The following observations made on (1) the role of cellular GSH depletion, (2) the MRPl-ATPase activity

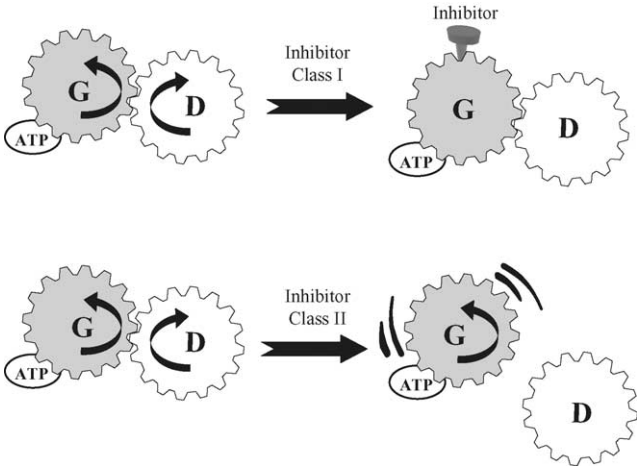

Fig. 8. Working model: MRP1 is composed of two interlocked wheels G and D. Wheel G can bind class-G substrates such as GSH and LTC4. Wheel G is the power unit which turns when energy is provided by ATP hydrolysis and when one of the G-class substrates is bound to the G site. Wheel D is inert and can turn only when it is connected to wheel G. Inhibitor of class I, such as MK571, block completely the system because they act directly on wheel G preventing it to turn and therefore also wheel D. Inhibitors of class II, such as PAK-104P and verapamil, disconnect the two wheels; it follows that wheel G, which has not to drag the D wheel, turns more rapidly while wheel D does not turn.

and (3) the MRP1-mediated transport of different substrates are in agreement with this model.

Concerning point 1, it is now well recognized that cellular depletion of GSH abolishes the MRP1-mediated efflux of neutral and basic drugs. The millimolar levels of cellular GSH is necessary for MRP1-mediated efflux of cytotoxic agents, such as anthracyclines [29,30] and probably of all the compounds of class D.

Concerning point 2, the MRP1-ATPase activity of reconstituted MRP1 is stimulated by LTC4 with 50% maximal stimulation achieved at concentration of 150 nM [31]. On the other hand, Hooijberg et al. [32] have shown that GSH stimulates the ATPase activity of MRP1 in a natural plasma membrane environment. This stimulation is dose dependent up to 5 mM. At the opposite, the MRP1 substrates vincristine and daunorubicin do not induce MRP1-ATPase activity. In addition, the effect of GSH on the MRP1-ATPase activity is not increased by daunorubicin or vincristine. Thus, the compounds of the G-class stimulate ATPase activity because they activate the power unit whereas those of class D do not.

Concerning point 3, it has been shown that the stoichiometry of the DNR:GSH co-transport is 1:1 when for both substrates, the V_M is reached [9]. This gives a good support of our model, according to which when wheel G makes one turn wheel D does the same. The transport of compounds of class G as well as those of class D in the presence of GSH is inhibited by the LTD4 receptor antagonist MK571 (class I inhibitor) at concentration close to 1 μM; this has been shown for instance for LTC4, GSH and DNR [26,33]. The inhibitors of class II inhibit the transport of the substrates of class D but stimulate the transport of GSH that belongs to class G. In this context, the effect of verapamil has already been largely studied by the group of Cole [13,14]. Class II inhibitors should also stimulate efflux of glutathione conjugate substrates, but to our knowledge up to now there is no clear evidence to support or to eliminate this assumption. In addition, we show here that PAK-104P has similar effect while being 10-fold more efficient that verapamil. To clarify the effects of verapamil on MRP1-mediated drug resistance, Loe et al. [13] have investigated the ability of this drug to inhibit direct transport of the well-characterized MRP1 substrate LTC4. They have determined that verapamil inhibits LTC4 transport into inside-out MRP1-enriched membrane vesicles, but only in the presence of GSH. This could be due to the fact that verapamil does increase the affinity of GSH for the G site and therefore compete more efficiently with LTC4 for the site. Indeed, Leslie et al. [16] have shown that in the presence of 30 μM verapamil the K_m value for GSH decreased 10-fold.

It is interesting to consider the data of photolabelling [25] in order to assess whether GSH interacts directly with MRP1 and, if so, to identify the regions of MRP1 involved in GSH binding. Karwatsky et al. [25] have synthesized a radiolabeled, iodinated azido-derivative of GSH (iodoarylazido glutathione, IAAGSH) to produce photoaffinity label for MRPl. The results have shown that IAAGSH specifically interacts with MRPl at physiologically relevant sites, and that the binding is inhibited by MRPl substrates leukotriene C-4 and by MRPl inhibitor MK571. Interestingly, verapamil enhances IAAGSH photolabeling of MRPl in agreement with observations that this drug enhances GSH transport.

Recently Westlake et al. [34] have identified cytoplasmic loop 3 as the GSH binding site. Loop 3 could correspond to what we have named wheel G.

In summary, with the model proposed in which MRPl is composed of two interlocked wheels G, the power unit, and D, the inert wheel, it is possible to explain that some molecules inhibit the MRPl-mediated efflux of both DNR and GSH (e.g. MK571), whereas other molecules (PAK-104P, verapamil) inhibit the MRPl-mediated efflux of DNR while stimulating the MRPl-mediated efflux of GSH.

Acknowledgements

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