JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1021–1028

Received November 10, 2000
Accepted March 28, 2001
ISSN 0022-3573

Designing multidrug-resistance modulators circumventing the reverse pH gradient in tumours

Madeleine Castaing, Alain Loiseau and Michele Dani

Abstract

Multidrug-resistant tumours often exhibit a reverse pH gradient (acid outside), as they have an acid extracellular pH (pHe) and a neutral alkaline intracellular pH (pHi). This study was designed to test the hypothesis that the ability of lipophilic drugs to mediate multidrug resistance (MDR) reversal by interacting with the membrane phospholipids may be correlated with pH in resistant tumours. The permeation properties of five MDR modulators were therefore studied at 37°C by quantifying their ability to induce the leakage of Sulfan blue through unilamellar anionic liposomes, over the range pH 6.5–7.7, and in the absence of any membrane potential (pHe = pHi). The dye leakage induced by two calcium blockers (diltiazem and verapamil) and two antiparasitic agents (thioacridine derivative and mepacrine) was found to significantly increase with the pH of the medium ($P < 0.001$), whereas that induced by a non-ionic detergent (Triton X-100) showed almost no pH-dependent variations. This process was a cooperative one ($0.8 <$ Hill coefficient < 8.5) and the permeation doses inducing 50% dye leakage (PD50) ranged from 1.6 to 36.0 mM. The permeation ability of the MDR modulators (log(1/PD50)) significantly increased with their octanol–buffer distributions (logD) (slope $= 0.35 \pm 0.06$; y intercept $= 1.65 \pm 0.14$; $P < 0.0001$) and significantly decreased with their net electric charge (z) (slope $= -0.48 \pm 0.07$; y intercept $= 2.85 \pm 0.08$; $P < 0.0001$). A highly significant multiple correlation was found to exist between the variations of log(1/PD50) with those of logD and z (dlog(1/PD50)/dlogD $= 0.21 \pm 0.05$; dlog(1/PD50)/dz $= -0.34 \pm 0.07$; y intercept $= 2.27 \pm 0.17$; $P < 0.000001$). The results provide evidence that in resistant tumours (acid pHe and neutral alkaline pHi), the MDR reversal might be enhanced by favourable drug–membrane interactions if the modulators are designed in the form of highly lipophilic (logP $\cong 4$) mono-basic drugs with a near neutral pK_a ($pK_a \cong 7$–8).

GERCTOP-ESA6009, Faculté de Pharmacie, Marseille, France

Madeleine Castaing, Michele Dani

INSERM-IFR2, Faculté de Médecine Xavier-Bichat, Paris, France

Alain Loiseau

Correspondence: M. Castaing, GERCTOP-ESA6009, 27 Boulevard Jean Moulin, Faculté de Pharmacie, 13385 Marseille Cedex 05, France. E-mail: gerctop@pharmacie.univ-mrs.fr

Acknowledgement and Funding: This work was supported by a grant from the Conseil Général des Bouches-du-Rhône, France. We are grateful to Professors M. Santelli and J. Barbe for encouraging the progress of this work. We also thank Dr C. Santelli-Rouvier for helpful comments and advice, and Dr A. Iliadis, Dr P. Loiseau, Dr P. Brouant and A. Mahamoud for help during the course of this work.

Introduction

Chemotherapeutic strategies are based on the fact that tumour cells are known to be approximately 5-fold more sensitive to anticancer drugs than healthy cells (Simon et al 1994). During chemotherapy, however, tumour cells often lose this sensitivity. After being exposed to a single drug, they develop resistance to a broad range of structurally and functionally unrelated drugs (Sharom 1997). This acquired multidrug resistance (MDR) is a major challenge to those seeking to develop efficient chemotherapeutic tools to deal with malignant tumours.

One of the main types of MDR results from the overexpression of a plasma membrane P-glycoprotein (P-gp). This protein acts as an efflux pump, extruding cytotoxic agents from the tumour cell (Beck 1987). Data in the literature have

shown that the intracellular pH (pHi) is equally or slightly more alkaline than normal in a number of solid tumour xenografts. Concomitantly, the extracellular pH (pHe) is more acid than normal (Keizer & Joenje 1989; Griffiths 1991; Negendank 1992; Gillies et al 1994; McCoy et al 1995, Raghunand et al 1999), due to the poor vascularization of tumours (Vaupel et al 1989). In some cases, the reverse pH gradient (acid outside) generated increases in magnitude with the tumour size (Raghunand et al 1999). It has therefore been suggested that P-gp may alter the passive drug partitioning by modulating the pHi or the membrane potential (ΔVm) (Roepe et al 1993; Hoffman et al 1996; Robinson & Roepe 1996; Roepe et al 1996; Wadkins & Roepe 1997). Since most of the chemotherapeutic drugs to which MDR cells are resistant are weak bases, a high pHi or a low pHe and/or a low ΔVm can be expected to decrease the charge-dependent cytoplasmic sequestration of these compounds.

A variety of substances, particularly the calcium-channel blocker verapamil, have been found to promote the reversal of MDR (Sharom 1997). Given the structural heterogeneity of these chemosensitizing compounds (modulators), several mechanisms have been suggested to explain their anti-MDR action (Higgins & Gottesman 1992; Gottesman & Pastan 1993; Seydel et al 1994; Eytan et al 1996; Sharom 1997). Among these mechanisms, it has been speculated that the ability of the MDR modulators to interact with the membrane phospholipids might play a role in MDR reversal (Seydel et al 1994). Since most of the MDR modulators show high lipophilic levels, they may affect the membrane permeability and fluidity as well as the structural organization of the lipids surrounding embedded proteins, thus changing their functional modes (Pajeva et al 1996). The results of several recent studies have proved that drug–membrane interactions play a key role in the reversal of MDR (Callaghan & Riordan 1995; Drori et al 1995; Pajeva et al 1996). Since the MDR phenotype results in alterations of the intra- and the extracellular pH of tumours, it seems likely that these drug–membrane interactions might depend on the pH.

To assess the pH-dependence of MDR modulator efficiency in terms of the drug–membrane interactions, the ability of five modulators to induce dye leakage from anionic liposomes was determined over the range pH 6.5–7.7. The effects of the net electric charge (z) of the drugs were also studied by comparing the dye leakage induced by mono- and di-basic amines with that induced by the amphiphile Triton X-100, a non-ionic polyoxyethylene detergent. Triton X-100 has been found to reverse MDR (Cano-Gauchi & Riordan 1987; Drori et al 1995) and to behave similar to a P-gp substrate (Doige et al 1993; Loe & Sharom 1993; Sharom 1997). Since two of the compounds under investigation exhibit fluorescence, the method based on carboxyfluorescein leakage could not apply. Sulfan blue was therefore used here as a membrane permeation indicator (Castaing et al 2000).

Materials and Methods

Chemicals

L-α-Phosphatidyl choline prepared from fresh egg yolk (EPC), L-α-phosphatic acid prepared from egg yolk lecithin (EPA), diltiazem hydrochloride and, t-octylphenoxypolyethoxyethanol; (Triton X-100) were purchased from Sigma (St Louis, MO). Mepacrine dihydrochloride hydrate and the dye anhydro-4–4′-bis-(diethylamino)triphenyl-methanol-2″,4″-disulfonic acid monosodium salt (Sulfan blue) were purchased from Aldrich (Steinheim, Germany). Verapamil hydrochloride was a gift from Knoll France Laboratory (Levallois-Perret, France) and cholesterol was obtained from Fluka (Buchs, Switzerland). Diethyl ether, $Na_2HPO_4.2H_2O$ and $NaH_2PO_4.H_2O$ were purchased from Merck (Darmstadt, Germany), Sephadex PD-10 columns (G-25M) were from Pharmacia (Uppsala, Sweden), and polycarbonate porous membranes were from Nucleopore Corporation (Pleasanton, CA).

Thioacridine ether (Figure 1) was prepared from the corresponding thioacridinone (Hevér et al 1998). Verapamil hydrochloride, diltiazem hydrochloride, thioacridine dihydrochloride and Triton X-100 were dissolved in water. Mepacrine dihydrochloride was dissolved in 100 mM phosphate buffer (pH 6.5, 6.9, 7.4 or 7.7).

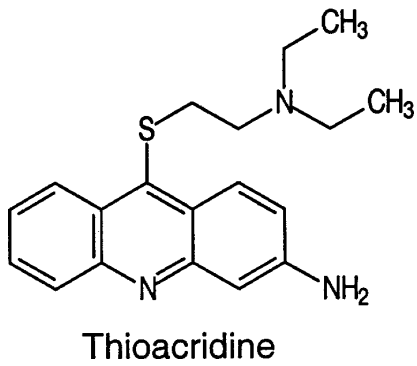

Figure 1 Chemical structure of thioacridine ether derivative.

Preparation of EPC/EPA/cholesterol liposomes

Large unilamellar vesicles (LUV) containing EPC, EPA and cholesterol in an 8:1:1 molar ratio were prepared by reverse-phase evaporation, as previously described (Castaing et al 2000).

Permeability measurements

The absorbance of Sulfan blue at 640 nm was determined by recording the visible absorption spectra of the samples with a Uvikon 933 spectrophotometer (Milan, Italy). Permeability measurements were performed on 1 mL LUV suspensions as previously described (Castaing et al 2000). The leakage of Sulfan blue entrapped was induced by 0–27 mM compound (or 0–4.0 mol compound (mol lipid)$^{-1}$, i.e. approx. 0–27.2 μmol compound m^{-2} surface membrane), through negatively charged LUV membranes. The leakage was quantified after incubation for 3 min at pH 6.5, 6.9, 7.4 and 7.7 (37°C), in the absence of membrane potential (pHe = pHi). Results were plotted as the percentage of total dye leaked as a function of drug concentration.

Data analysis

The variations in the percentage of total dye leaked with different drug concentrations were fitted to the dose–response curves described by:

$$\text{Total dye leaked (\%)} = \frac{100 \times [\text{Drug}]^h}{(\text{PD50}^h + [\text{Drug}]^h)} \quad (1)$$

where PD50 is the drug dose inducing 50 % dye leakage from the liposomes and h is the Hill coefficient, that is the parameter characterizing the cooperativity of the permeation process.

Hydrophobicity of the drugs

The Pallas 2.0 software program of Compudrug Chemistry Ltd (Budapest, Hungary) was used to calculate the octanol–buffer partition coefficient (P), the octanol–buffer distribution coefficient (D) and the ionization constant (pK_a) of Triton X-100 and of the thioacridine derivative at pH 6.5, 6.9, 7.4 and 7.7 ($\mu = 0.22$). In the case of diltiazem, verapamil and mepacrine, the experimental P and pK_a values given by Craig (1990) were taken into account. Using these values, the pH-dependent D values of the compounds were calculated according to the equations given by Bowden (1990) for mono- and di-basic drugs (Equations 2 and 3, respectively):

$$\log D = \log P - \log(1 + 10^{pKa - pH}) \quad (2)$$

$$\log D = \log P - \log(1 + 10^{pK_{a1} - pH} + 10^{pK_{a2} + pK_{a2} - 2\,pH}) \quad (3)$$

where $pK_{a1} > pK_{a2}$

Net electric charge of the drugs

With the exception of Triton X-100, the drugs studied were weak bases existing in various states of ionization at the pH investigated. The net electric charge (z) of the various drugs (i.e. the mean net electric charge per drug molecule) was therefore calculated at the four experimental pH values according to the following equation:

$$z = (1/C_T) \times \Sigma C_i z_i \quad (4)$$

where C_T is the total concentration of the drug in the aqueous phase, and C_i is the concentration of species i, with valence z_i.

Statistical analysis

Data are expressed as mean $\pm$ s.d. At each pH, the variations in the percentage of total dye leaked with different drug concentrations were fitted to non-linear regressions (Equation 1). The pH dependence of the permeation properties of the drugs (log(1/PD50) vs pH and h vs pH) were fitted to linear regressions as well as the variations of the permeation doses of the drugs with their octanol–buffer distribution (log(1/PD50) vs logD) and with their net electric charge (log(1/PD50) vs z). Besides, the variations of the permeation doses of the drugs with these two latter parameters (log(1/PD50) vs logD and z) were fitted to a bilinear regression. All the above regressions were calculated using the least-square method. Values of $P < 0.05$ were considered significant.

Results

Drug permeation properties (PD50 and h) depending on the pH

The leakage of Sulfan blue induced by five drugs through the membrane of negatively charged liposomes (EPC/EPA/cholesterol) was quantified after a 3-min incubation at pH 6.5, 6.9, 7.4 and 7.7 (37°C). The PD50 and h values determined for the various drugs are reported in Table 1. Except for the non-ionic detergent Triton X-100, all these drugs induced a significant pH-dependent leakage ($P < 0.01$) when added at concentrations ranging from 0 to 27 mM. At the PD50 of the com-

Table 1 Permeation dose (PD50), Hill coefficient (h), ionization constant (pK_a), net electric charge (z) and octanol–water distribution (logD) of MDR modulators.

Compound	pH	PD50 (mM)	h	pK_a[a]	z	logD[a]
Non-ionic detergent						
Triton X-100	6.5	1.6±0.03	8.5±0.24	Non-ionizable	0.00	2.98[b]
	6.9	1.7±0.06	7.6±0.68		0.00	2.98[b]
	7.4	1.6±0.04	7.3±2.32		0.00	2.98[b]
	7.7	1.6±0.13	6.5±0.06		0.00	2.98[b]
Calcium-channel blockers						
Diltiazem	6.5	8.9±0.39	3.9±0.34	7.70	0.94	1.47
	6.9	5.9±0.34	4.7±0.50		0.86	1.84
	7.4	3.3±0.33	5.6±0.64		0.67	2.22
Verapamil	7.7	2.8±0.18	5.7±0.40		0.50	2.40
	6.5	3.7±0.08	2.8±0.17	8.92	1.00	1.37
	6.9	2.8±0.21	3.5±0.06		0.99	1.77
	7.4	1.7±0.25	4.2±0.50		0.97	2.26
	7.7	1.7±0.09	5.8±0.96		0.94	2.54
Antiparasitic agents						
Thioacridine	6.5	22.6±1.24	0.8±0.01	7.11[b]; 9.29[b]	1.80	0.27[b]
	6.9	10.6±0.04	0.9±0.17		1.62	0.96[b]
	7.4	6.3±0.49	1.8±0.18		1.33	1.70[b]
Mepacrine	7.7	4.4±0.33	2.3±0.21		1.18	2.07[b]
	6.5	Inactive	Inactive	7.73; 10.18	1.94	1.25
	6.9	36.0±5.67	1.1±0.14		1.87	2.01
	7.4	4.5±0.64	1.6±0.07		1.68	2.90
	7.7	2.7±0.40	1.4±0.01		1.51	3.38

The parameters PD50 and h are expressed as mean ± s.d. of the permeation parameters obtained from the study of two large unilamellar vesicle preparations. [a]Calculated from the data given by Craig (1990), except when noted. [b]Calculated from the data predicted by the Pallas 2.0 software program.

pounds, the lipid–drug molar ratios ranged from 0.2 (mecaprine at pH 6.9) to 4.1 (Triton X-100 at pH 6.5).

When induced by Triton X-100, the membrane permeation was a highly cooperative process ($6.5 < h < 8.5$), whereas the permeation induced by the thioacridine derivative was almost non-cooperative at pH 6.5 and 6.9 ($h = 0.8$–0.9), although some cooperativity was found to exist at pH 7.4 and 7.7 ($h = 1.8$–2.3) (Table 1). Except for mepacrine, the cooperativity of the permeation process varied significantly with the pH ($P < 0.01$), depending on the drug involved.

Correlation between the permeation properties (PD50) of the drugs and the octanol–buffer distribution (logD)

The induction of dye leakage through lipid membranes is likely to depend on the lipophilicity of the drug. The octanol–buffer distribution (logD) of diltiazem, verapamil and mepacrine were therefore calculated at each pH level, using the experimental octanol–buffer partition coefficient (logP) and pK_a values given by Craig (1990) and the equations given by Bowden (1990) for mono- and di-basic drugs (see Equations 2 and 3). In the case of Triton X-100 and the thioacridine derivative, the Pallas 2.0 software program was used to calculate these parameters.

The log(1/PD50) vs logD linear regression (slope = 0.35 ± 0.06; y intercept = 1.65 ± 0.14; $F_{(1,38)} = 35.2$, $s_{residual} = 0.283$; $r = 0.703$; $P < 0.0001$) was highly significant. Approximately 50% of the permeation properties of the drugs observed at various pH levels could be explained in terms of their lipophilicity ($r^2 = 0.494$). This result indicates that the higher the octanol–buffer distribution (logD) of the drug occurring at alkaline pH levels, the greater its ability to induce dye leakage through membranes.

Correlation between the permeation properties (PD50) of the drugs and the net electric charge

It is generally agreed that numerous MDR modulators are cationic compounds. However, it has by now been clearly established that amphiphiles such as Triton X-

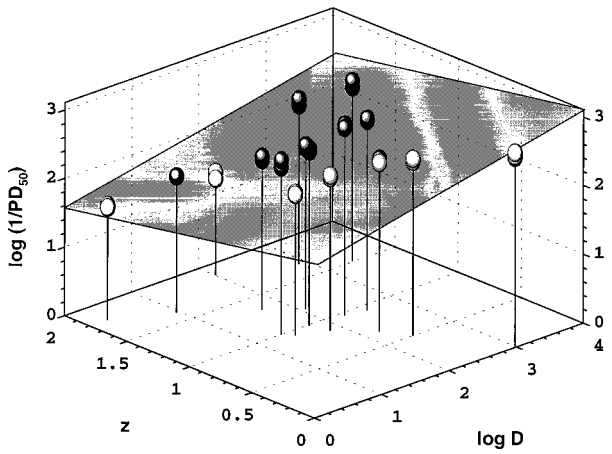

Figure 2 Bilinear dependence of the membrane permeation ability (log(1/PD50)) of the modulators on their octanol–buffer distribution (logD) and their net electric charge (z); each point is the result obtained from the study of one large unilamellar vesicle preparation. The experimental data above (filled circles) and below (open circles) the plane of the multiple regression are plotted.

100 can have chemosensitizing effects (Sharom 1997). The net electric charge of the compounds studied here was therefore calculated at each pH using either their experimental pK_a (Craig 1990) or their calculated pK_a value (Pallas 2.0 software program) (Table 1).

The log(1/PD50) vs z linear regression (slope = -0.48 ± 0.07; y intercept = 2.85 ± 0.08; $F_{(1,38)} = 48.3$, $s_{residual} = 0.260$; $r = -0.757$; $P < 0.0001$) was highly significant. Approximately 58 % of the permeation properties of the drugs observed at various pH levels could be explained in terms of their net electric charge ($r^2 = 0.573$). This result indicates that the higher the net electric charge of the drug occurring at acid pH levels, the lower its ability to induce dye leakage through membranes.

Multiple correlation between the permeation properties (PD50) of the drugs, and the octanol–buffer distribution (logD) and net electric charge

The results presented above clearly indicate that dye leakage through membranes was prevented at acid pH levels by both the increase in the ionization (z) of the drug and the concomitant decrease in its lipophilicity (logD).

Multiple correlation was therefore performed between the permeation properties (log(1/PD50) of the drugs and their lipophilicity (logD) and net electric charge. The results are expressed by the following equation:

$$\log(1/PD50) = 0.21 \pm 0.05\ \log D - 0.34 \pm 0.07\ z + 2.27 \pm 0.17$$

This bilinear dependence of log(1/PD50) on logD and z ($F_{(2,35)} = 40.5$, $s_{residual} = 0.221$; $r = 0.836$; $P < 0.000001$) was highly significant. Nearly 70 % of the permeation properties of the drugs observed at the various pH levels could be explained in terms of the concomitant changes in their octanol–buffer distribution (logD) and their net electric charge ($r^2 = 0.681$). On comparing this value of 70 % with those obtained taking the changes in logD (50 %) and in z (58 %) separately, it was concluded that the multiple correlation model describes the pH-induced changes in the permeation properties of the drugs more accurately than simple correlation models.

Figure 2 shows the multiple correlation between log(1/PD50) and logD and z. It clearly illustrates that the higher the octanol–buffer distribution (logD) and the lower the net electric charge of the drug occurring at alkaline pH levels, the higher its ability to induce dye leakage through membranes, whereas the lower the octanol–buffer distribution (logD) and the higher the net electric charge of the drug occurring at acid pH levels, the lower its ability to induce dye leakage through membranes.

Discussion

The results of the present study show that the five MDR modulators (Klohs et al 1986; Inaba & Maruyama 1988; Sharom 1997; Hevér et al 1998), bearing very different electric charges, induced either pH-dependent or pH-independent dye leakage by interacting with the membrane of anionic liposomes. At pH 6.5, which may be that prevailing in the extracellular media of some solid tumours (Gillies et al 1994), the permeation efficiency (1/PD50) of diltiazem and verapamil (monobasic amines) was 2–3 times lower than at pH 7.7, and that of mepacrine and the thioacridine derivative (dibasic amines) was between 5 and more than 13 times lower, whereas the permeation efficiency of Triton X-100 (non-ionic detergent), a P-gp substrate (Sharom 1997), was independent of the pH, over the range investigated (pH 6.5–7.7). In this study, Triton X-100 was chosen as a control substance since its net electric charge is zero at any given pH.

The question arises as to whether the pH-dependence of the MDR modulator efficiency observed here in anionic liposomes may be extrapolated to whole cells. In a study on the effects of the membrane potential and the pHi on the cellular retention of chemotherapeutic drugs,

Robinson & Roepe (1996) have stressed that the effects in the liposomes may not be exactly analogous to the behaviour of whole cells for several reasons. Firstly, the drugs having a rather high distribution coefficient (logD) and the relative percentage volume of a LUV membrane being orders of magnitude greater than that of a plasma membrane, the partitioning in LUV vs cells is very different. Secondly, many studies with LUV have used high concentrations of drugs at which unusual behaviour may occur (aggregation). However, results from LUV studies helped these authors to choose the mechanism underlying their results on whole cells. Indeed, the conclusions drawn from the studies in liposomes often suggest answers to various aspects of the behaviour of whole cells (Eytan et al 1996). Also, Pajeva et al (1996) found a significant correlation between the strength of various MDR modulators to interact with artificial membranes and their effectiveness to reverse the MDR in whole cells. In this case again, the effects observed in the liposomes could be extrapolated with accuracy to those in whole cells. The limited data available in the literature on the pH-dependence of MDR modulator efficiency suggest that in the present study this may also be the case. Studies on verapamil have shown that its ability to reverse MDR was approximately 4 times lower at pH 7.3 than at pH 7.8 in leukaemic cells (Boer et al 1994) and 2 times lower at pH 6.8 than at pH 7.5 in intestinal carcinoma cells (Zacherl et al 1994). The results obtained here with verapamil are in reasonably good agreement with the data published by these authors using MDR cell lines.

The results of the present study showed the existence of a highly significant bilinear dependence of the permeation properties (log(1/PD50)) of the drugs on the increase in their octanol–buffer distribution (logD) and the decrease in their net electric charge. This multiple correlation model was found to explain the pH-induced changes in the permeation properties of the drugs more completely than simple correlation models (70 vs 50–58%). This model indicates that when the pH acidifies, as occurs in the interstitial fluid of solid tumours, the ability of the drugs to interact with membranes strongly enough to induce dye leakage is reduced by the concomitant decrease in their hydrophobicity (logD) and increase in their net electric charge. At the molecular level, these pH-induced changes in the drug–membrane interactions leading to dye leakage result from the complex interplay between several physicochemical parameters (pH, pK_a, logD and the electrostatic surface potential; Ψ). In the present study, the negative Ψ of the membrane brought about a redistribution of the protons, cations and anions in the vicinity of the lipid–buffer interface (Tocanne & Teissié 1990; Romsicki & Sharon 1999). This decreased the interfacial pH ($pH_{interfacial} < pH_{bulk}$) and increased the interfacial pK of the drugs ($pK_{interfacial} > pK_{bulk}$). These variations additionally decreased the hydrophobicity (logD) of the drugs at the lipid–buffer interfaces, to an extent depending on the number of their basic amine groups (see Equations 2 and 3), and also increased the net electric charge. Note also that over the pH range investigated here, changes in the magnitude of Ψ may have accompanied the changes in the lipid ionization state of phosphatidic acid (Tocanne & Teissié 1990). The Ψ can therefore be expected to be less negative at acid than at alkaline pH levels. Consequently, the Ψ-induced decrease in the hydrophobicity (logD) of the drugs and the Ψ-induced increase in their net electric charge may have been of lower magnitude at acid than at alkaline pH. The dye leakage through bilayer membranes occurring in response to a drug involves both interactions between the drug and its receptors at the membrane interface (first stage), and interactions between the drug and the phospholipids located in the core of the membrane (second stage). The first stage frequently results from electrostatic interactions occurring between cationic drug molecules and negatively charged phospholipid head groups. At acid pH levels this stage is therefore favoured (the PD50 decreases) by the enhancement of the drug ionization (which is indirectly amplified by the effect of Ψ) and inhibited (PD50 increases) by the concomitant decrease in the concentration of the anionic phospholipid head groups at the membrane interface. The second stage can be expected to depend on the hydrophobicity and the electric charge of the drug molecules (Taylor 1990). At acid pH levels, this stage in the permeation process is not favoured (the PD50 increases) by the enhanced drug ionization, both directly through the decrease of the un-ionized form of the drug present in the aqueous phase, which is the only species able to partition into the organic phase (Taylor 1990) and indirectly through the effects of the negative Ψ on the interfacial pH and pK_a, which further decreases the hydrophobicity of the drug (logD).

To sum up, the overall outcome of the molecular events described seems to be that in resistant tumours (acid pHe, neutral alkaline pHi), the passive entry and the retention of MDR modulators within the cell after their interactions with the plasma membrane, might be easier: for drugs having one basic amine group rather than two; for basic drugs having a near neutral pK_a rather than a more alkaline pK_a; and for drugs having a high rather than a low level of hydrophobicity.

It was concluded from this study that the dye leakage

induced by MDR modulators through anionic membranes, and probably also their ability to reverse MDR in leukaemic cells, decrease greatly at acid pH levels. This decrease is partly counteracted when the compounds are mono-basic drugs with a near neutral pK_a ($pK_a = 7–8$) and a high level of lipophilicity ($logP \cong 4$). This study therefore provides a basis for developing new MDR modulators that may efficiently circumvent the reverse pH gradient (acid outside) existing in resistant tumour cells.

References

Beck, W. T. (1987) The cell biology of multiple drug resistance. *Biochem. Pharmacol.* **36**: 2879–2887

Boer, R., Hass, S., Schödl, A. (1994) Influence of dexniguldipine-HCl on rhodamine-123 accumulation in a multidrug-resistant leukaemia cell line: comparison with other chemosensitisers. *Eur. J. Cancer* **30A**: 1117–1123

Bowden, K. (1990) Electronic effects. In: Ramsden, C. A. (ed.) *Comprehensive Medicinal Chemistry*, Vol 4. Pergamon Press, Oxford, pp 205–239

Callaghan, R., Riordan, J. R. (1995) Collateral sensitivity of multidrug resistant cells to narcotic analgesics is due to effects on the plasma membrane. *Biochim. Biophys. Acta* **1236**: 155–162

Cano-Gauci, D. F., Riordan, J. R. (1987) Action of calcium antagonists on multidrug resistant cells. Specific cytotoxicity independent of increased cancer drug accumulation. *Biochem. Pharmacol.* **36**: 2115–2123

Castaing, M., Brouant, P., Loiseau, A., Santelli-Rouvier, C., Santelli, M., Alibert-Franco, S., Abdallah, M., Barbe, J. (2000) Membrane permeation by MDR-modulators and non-modulators: effects of hydrophobicity and electric charge. *J. Pharm. Pharmacol.* **52**: 289–296

Craig, P. N. (1990) Drug compendium. In: Drayton, C. J. (ed.) *Comprehensive Medicinal Chemistry*, Vol 6, Pergamon Press, Oxford, pp 237–991

Doige, C. A., Yu, X., Sharom, F. J. (1993) The effects of lipids and detergents on ATPase-active P-glycoprotein. *Biochim. Biophys. Acta* **1146**: 65–72

Drori, S., Eytan, G. D., Assaraf, Y. G. (1995) Potentiation of anticancer-drug cytotoxicity by multidrug-resistance chemosensitizers involves alterations in membrane fluidity leading to increased membrane permeability. *Eur. J. Biochem.* **228**: 1020–1029

Eytan, G. D., Regev, R., Oren, G., Assaraf, Y. G. (1996) The role of passive transbilayer drug movement in multidrug resistance and its modulation. *J. Biol. Chem.* **271**: 12897–12902

Gillies, R. J., Liu, Z., Bhujwalla, Z. (1994) ^{31}P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. *Am. J. Physiol.* **267**: C195–203

Gottesman, M. M., Pastan, I. (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* **62**: 385–427

Griffiths, J. R. (1991) Are cancer cells acidic? *Br. J. Cancer* **64**: 425–427

Hevér, A., Santelli-Rouvier, C., Brouant, P., El Khyari, S., Molnar, J., Barra, Y., Barbe, J. (1998) Effect of new thioacridine derivatives on P-gp function and on mdr1 gene expression. *Anticancer Res.* **18**: 3053–3058

Higgins, C. F., Gottesman, M. M. (1992) Is the multidrug transporter a flippase? *Trends Biochem. Sci.* **17**: 18–21

Hoffman, M. M., Wei, L.-Y., Roepe, P. D. (1996) Are altered pHi and membrane potential in hu MDR 1 transfectants sufficient to cause MDR protein-mediated multidrug resistance? *J. Gen. Physiol.* **108**: 295–313

Inaba, M., Maruyama, E. (1988) Reversal of resistance to vincristine in P388 leukemia by various polycyclic clinical drugs, with a special emphasis on quinacrine. *Cancer Res.* **48**: 2064–2067

Keizer, H. G., Joenje, H. (1989) Increased cytosolic pH in multidrug-resistant human lung tumor cells: effect of verapamil. *J. Natl Cancer Inst.* **81**: 706–709

Klohs, W. D., Steinkampf, R. W., Havlick, M. J., Jackson, R. C. (1986) Resistance to anthrapyrazoles and anthracyclines in multiresistant P388 murine leukemia cells: reversal by calcium blockers and calmodulin antagonists. *Cancer Res.* **46**: 4352–4356

Loe, D. W., Sharom, F. J. (1993) Interaction of multidrug-resistant Chinese hamster ovary cells with amphiphiles. *Br. J. Cancer* **68**: 342–351

McCoy, C. L., Parkins, C. S., Chaplin, D. J., Griffiths, J. R., Rodrigues, L. M., Stubbs, M. (1995) The effect of blood flow modification on intra- and extracellular pH measured by ^{31}P magnetic resonance spectroscopy in murine tumours. *Br. J. Cancer* **72**: 905–911

Negendank, W. (1992) Studies of human tumors by MRS: a review. *NMR Biomed.* **5**: 303–324

Pajeva, I. K., Wiese, M., Cordes, H. P., Seydel, J. K. (1996) Membrane interactions of some catamphiphilic drugs and relation to their multidrug-resistance-reversing ability. *J. Cancer Res. Clin. Oncol.* **122**: 27–40

Raghunand, N., Altbach, M. I., van Sluis, R., Baggett, B., Taylor, C. W., Bhujwalla, Z. M., Gillies, R. J. (1999) Plasmalemmal pH-gradients in drug-sensitive and drug-resistant MCF-7 human breast carcinoma xenografts measured by ^{31}P magnetic resonance spectroscopy. *Biochem. Pharmacol.* **57**: 309–312

Robinson, L. J., Roepe, P. D. (1996) Effects of membrane potential versus pHi on the cellular retention of doxorubicin analyzed via a comparison between cystic fibrosis transmembrane regulator (CFTR) and multidrug resistance (MDR) transfectants. *Biochem. Pharmacol.* **52**: 1081–1095

Roepe, P. D., Wei, L. Y., Cruz, J., Carlson, D. (1993) Lower electrical membrane potential and altered pHi homeostasis in multidrug-resistant (MDR) cells: further characterization of a series of MDR cell lines expressing different levels of P-glycoprotein. *Biochemistry* **32**: 11042–11056

Roepe, P. D., Wei, L. Y., Hoffman, M. M., Fritz, F. (1996) Altered drug translocation mediated by the MDR protein: direct, indirect or both? *J. Bioenerg. Biomembr.* **28**: 541–555

Romsicki, Y., Sharon, F. J. (1999) The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporter. *Biochemistry* **38**: 6887–6896

Seydel, J. K., Coats, E. A., Cordes, H. P., Wiese, M. (1994) Drug membrane interaction and the importance for drug transport, distribution, accumulation, efficacy and resistance. *Arch. Pharm. (Weinheim)* **327**: 601–610

Sharom, F. J. (1997) The P-glycoprotein efflux pump: how does it transport drugs? *J. Membrane Biol.* **160**: 161–175

Simon, S., Roy, D., Schindler, M. (1994) Intracellular pH and the control of multidrug resistance. *Proc. Natl Acad. Sci. USA* **91**: 1128–1132

Taylor, P. J. (1990) Hydrophobic properties of drugs. In: Ramsden, C. A. (ed.) *Comprehensive Medicinal Chemistry*, Vol 4. Pergamon Press, Oxford, pp 241–294

Tocanne, J. F., Teissié, J. (1990) Ionization of phospholipids and phospholipid-supported interfacial lateral diffusion of protons in membrane model systems. *Biochim. Biophys. Acta* **1031**: 111–142

Vaupel, P., Kallinowski, F., Okunieff, P. (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res.* **49**: 6449–6465

Wadkins, R. M., Roepe, P. D. (1997) Biophysical aspects of P-glycoprotein-mediated multidrug resistance. *Int. Rev. Cytol.* **171**: 121–165

Zacherl, J., Hamilton, G., Thalhammer, T., Riegler, M., Cosentini, E. P., Ellinger, A., Bischof, G., Schweitzer, M., Teleky, B., Koperna, T., Wenzl, E. (1994) Inhibition of P-glycoprotein-mediated vinblastine transport across HCT-8 intestinal carcinoma monolayers by verapamil, cyclosporine A and SDZ PSC 833 in dependence on extracellular pH. *Cancer Chemother. Pharmacol.* **34**: 125–132