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Membrane Permeation by Multidrug-resistance-modulators and Non-modulators: Effects of Hydrophobicity and Electric Charge

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Abstract

This study was designed to test the hypothesis that lipophilic cationic drugs with only roughly similar structures mediate the reversal of multidrug-resistance (MDR) by interacting with membrane phospholipids. The permeation properties of MDR-modulators and non-modulators were studied by quantifying their ability to induce the leakage of Sulphan blue through the membrane of negatively charged unilamellar liposomes.

Of the 22 compounds under investigation, only those bearing a net positive electric charge per molecule (z) $\geq 0{\cdot}2$ induced dye leakage. All these efficient drugs are well-known MDR-modulators: calcium-channel blockers (propranolol, verapamil, diltiazem and dipyridamole), calmodulin antagonists (clomipramine and thioridazine) and antiparasitic agents (mepacrine, thioacridine derivatives and quinine). The non-modulators tested, including antineoplasic agents and steroids, did not induce any membrane permeation. The permeation process was a co-operative one ($1{\cdot}1 < \text{Hill coefficient} < 4{\cdot}1$) and the permeation doses inducing 50% dye leakage (PD50) were $1{\cdot}9-11{\cdot}2$ mM. The permeation ability of the MDR-modulators (log(1/PD50)) increased significantly with octanol-buffer distributions per unit net electric charge ((logD)/z).

The results provide evidence that a complex interplay occurs between the electric charge and the lipophilicity of the MDR-modulators when a dye leakage is induced through model membranes, and probably also when the MDR is reversed in leukaemic cells.

Clinical resistance to chemotherapeutic drugs is one of the main problems which arise in the treatment of cancer. One form of drug resistance, termed multidrug-resistance (MDR), has been defined as the ability of cells exposed to a single drug to develop resistance to a broad range of structurally and functionally unrelated drugs (Ford & Hait 1990; Ford 1995, 1996; Ford et al 1996). The MDR process is generally associated with either amplification or over-expression of the mdr 1 gene, which encodes a cell-surface P-glycoprotein (P-gp). This protein acts as an energy-dependent efflux pump, extruding cytotoxic agents (anthracyclines, vinca alkaloids and other compounds) from the tumour cell, thus preventing them from accumulating at the intracellular level and abolishing the cytotoxic effects (Beck 1987; Ford & Hait 1990).

Since the first report on the pharmacological reversal of MDR by the calcium-channel blocker verapamil (Tsuruo et al 1981), a variety of substances has been found to inhibit P-gp-mediated drug efflux and thus to reverse MDR to chemotherapeutic agents. These substances include calcium-channel blockers, calmodulin antagonists, cyclic peptides, steroids, hormonal analogues and miscellaneous other compounds (Ford 1996).

Given the considerable structural heterogeneity of the chemosensitizing compounds (modulators) that have been found to alter MDR, numerous biochemical mechanisms have been suggested to explain their anti-MDR action (Ford & Hait 1990).

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Most of these modulators show similar high levels of lipophilicity and have a basic nitrogen atom (Zamora et al 1988). It has therefore been speculated that interactions between these compounds and membrane phospholipids might contribute to the mechanism underlying MDR modulation (Seydel 1991; Seydel et al 1992, 1994). As pointed out by Pajeva et al (1996), drug-membrane interactions might lead to the reversal of MDR either directly (by changing the membrane permeability and fluidity) or indirectly (via the inhibition of P-gp phosphorylation resulting from the inhibition of protein kinase C or via changes in the conformation and functional modes of membrane-integrated proteins resulting from changes in the structural organization of the surrounding lipids). The results of several recent studies have proved that drug–membrane interactions play a key role in the reversal of MDR (Callaghan et al 1993; Callaghan & Riordan 1995; Drori et al 1995; Ecker et al 1996; Leibovici et al 1996; Pajeva et al 1996; Wiese & Pajeva 1997).

To assess the strength of the drug–membrane interactions, the ability of 10 MDR-modulators and 12 non-modulators to induce dye leakage from negatively charged liposomes was quantified. Because many of the compounds under study exhibited fluorescence, the method based on carboxyfluorescein leakage could not be applied. Instead, Sulphan blue was used for the first time here as a membrane permeation indicator. This appeared to be a highly suitable dye for studying fluorescent compounds absorbing light in the visible range below 600 nm. The results obtained here indicate that cationic drugs bearing a low net electric charge ($z < 0{\cdot}10$), non-electrolytes and anionic compounds tested were unable to induce membrane permeation. In addition, the permeation properties of the calcium-channel blockers, calmodulin antagonists and antiparasitic agents studied were found to be controlled by the combined effects of the drug's hydrophobicity and its net electric charge. The results are discussed in terms of the physicochemical and electric characteristics of the modulators.

Materials and Methods

Chemicals

L-α-phosphatidyl choline (EPC) prepared from fresh egg yolk, L-α-phosphatic acid (EPA) prepared from egg yolk lecithin, clomipramine hydrochloride, *S*-(−)-propranolol, diltiazem hydrochloride, fenofibrate, captopril, dipyridamole, cyclophosphamide monohydrate, 6α-methyl-17α-hydroxy-progesterone acetate, chlorambucil, betamethasone 21-phosphate sodium, cefuroxime sodium, quinine hydrochloride, mephenesin and dimethylsulphoxide (DMSO) were purchased from Sigma (St Louis, MO). Thioridazine hydrochloride, mepacrine dihydrochloride hydrate and the dye anhydro-4-4′-bis(diethylamino)triphenyl-methanol-2″,4″-disulphonic acid monosodium salt (Sulphan blue or Patent blue VF) 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). Absolute ethanol for spectroscopy, diethyl ether, benzene, chloroform, $Na_2HPO_4.2H_2O$ and $NaH_2PO_4.H_2O$ were purchased from Merck (Darmstadt, Germany), Sephadex PD-10 columns (G-25M), from Pharmacia (Uppsala, Sweden) and polycarbonate porous membranes, from Nucleopore Corporation (Pleasanton, CA). Thioacridine ethers (Figure 1) were prepared from the corresponding thioacridinones (Hevér et al 1998). Thioacridine 1 dihydrochloride, thioacridine 2 dihydrochloride, and the basic thioacridines 3, 4, 5 and 6 were used in the present experiments.

Thioridazine, clomipramine, *S*-(−)-propranolol, verapamil, diltiazem, captopril, cefuroxime, quinine and thioacridines 1 and 2 were dissolved in water. Betamethasone, mephenesin, mepacrine, thioacridine 3, thioacridine 4 and thioacridine 6 were dissolved in 100 mM phosphate buffer (pH 7·0). Dipyridamole, cyclophosphamide and chlorambucil were dissolved in absolute ethanol. Fenofibrate was dissolved in benzene, 6α-methyl-17α-hydroxy-progesterone acetate was dissolved in chloroform and thioacridine 5 was dissolved in DMSO.

Preparation of EPC/EPA/cholesterol liposomes

Large unilamellar vesicles (LUV) were prepared according to the method of Szoka & Papahadjopoulos (1978) using 40 μmol lipid mixture comprising L-α-phosphatidyl choline (EPC), L-α-phosphatic acid (EPA) and cholesterol in an 8 : 1 : 1 molar ratio per mL internal buffer (100 mM phosphate, 1·2 mM Sulphan blue pH 7·0 at 37°C). After vesicle formation by reversed-phase evaporation under reduced nitrogen pressure, removal of the external dye was carried out by passage through two Sephadex G-25 columns eluted with the external vesicular buffer (100 mM phosphate pH 7·0 at 37°C). The suspension was then filtered successively through polycarbonate membranes of 1 and

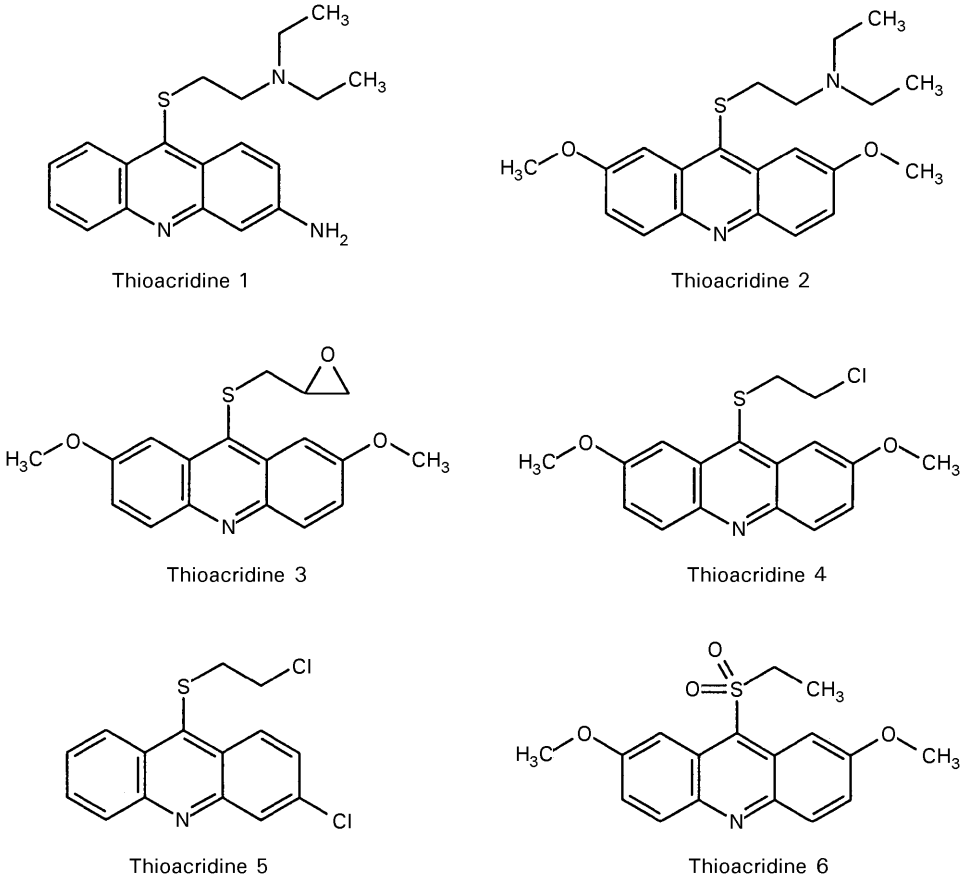

Figure 1. Chemical structure of thioacridine ether derivatives.

0·4 μm pore size, diluted 1 : 3 with the external vesicular buffer, and stored at 4°C until use.

Permeability measurements

The absorbance of Sulphan blue at 640 nm was determined by recording the visible absorption spectra of the samples with a Hitachi U-2000 spectrophotometer (Tokyo, Japan). Permeability measurements were performed on 1 mL LUV. Following the addition of the drug (or the solvent), the sample was stirred for 3 min at 37°C using a Salvis SBK shaker bath (Reussbühl-Luzern, Switzerland). This induced membrane perturbation and leakage of Sulphan blue from the vesicles. The external dye was then removed by passage through a Sephadex PD-10 column (G-25), and the sample was diluted to a final volume of 2 mL for spectrophotometric measurements. The absorbance of the dye remaining entrapped inside the liposomes was quantified by recording the visible difference spectrum of each sample against a LUV blank (i.e., a LUV suspension without dye molecules entrapped). Results were plotted as the percentage of total dye leaked as a function of the drug concentrations. Control experiments demonstrated that the organic solvent concentrations used in these experiments had no detectable effect on the parameters measured.

Data analysis

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

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

where PD50 is the drug dose inducing 50% dye leakage from the liposomes and h is the Hill coefficient (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, of the drugs, and their octanol-buffer distribution coefficient, D. The distribution coefficient is

defined as the ratio between the sum of the concentrations of all drug species in the organic phase (octanol) and the aqueous phase (pH 7·0 and $\mu = 0{\cdot}22$). The ionization constants (pKa) predicted by this software program were used to calculate the net electric charge (z) of the thioacridine derivatives 1 to 6.

Net electric charge of the drugs

Since most of the drugs studied here are weak acids or weak bases, they exist 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 (pH 7·0) according to the following equation:

$$z = (1/C_T) \cdot \sum C_i \cdot z_i \qquad (2)$$

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 means ± s.e.m. Linear and non-linear regressions were calculated using the least-square method. Results are considered significant at $P < 0{\cdot}05$.

Results

Drug permeation properties (PD50 and h)

In the present study, Sulphan blue was chosen as a membrane permeability indicator for two reasons: firstly, its main absorption band (640 nm) does not overlap with that of the drugs which also absorbed light and fluoresced in the visible range (mepacrine, dipyridamole and the thioacridine derivatives) and secondly, it is a hydrophilic anionic chromophore which at the pH investigated could not interact with the phospholipid head groups of the membrane.

The leakage of Sulphan blue induced by 22 drugs through the membrane of negatively charged liposomes (EPC/EPA/cholesterol) was quantified following a 3-min incubation at 37°C (pH 7·0). Of these drugs, only those known to act as MDR-modulators were found to induce dye leakage when added at concentrations ranging from 0 to 16 mM (lipid : drug molar ratios of 1 : 0 to 1 : 2·4) (Table 1).

The co-operativity (h) of the permeation process varied depending on the drug involved: the Hill coefficient was almost equal to unity (absence of co-operativity) for the thioacridine 1 derivative ($h = 1{\cdot}1 \pm 0{\cdot}01$) and reached a value of $4{\cdot}1 \pm 0{\cdot}06$ for diltiazem (Table 1).

It is generally agreed that MDR-modulators are cationic compounds. The net electric charge (z) of all the compounds studied here was therefore calculated (pH 7·0) using either their experimental pKa (Craig 1990) or their calculated pKa value (Pallas 2.0 software program) (Table 1). As shown in Table 1, dye leakage from the liposomes was induced only by the cationic drugs bearing a net electric charge $z \geq 0{\cdot}20$. The cationic drugs with a net electric charge $z < 0{\cdot}10$, the non-electrolytes and the anionic drugs all failed to induce any membrane permeation.

Correlation between the permeation properties (PD50) of the drugs and the octanol-buffer distribution coefficient per net electric charge ((logD)/z)

The induction of dye leakage through bilayer membranes by a drug can be expected to involve two stages: the interaction between the drug and its receptors at the membrane interface and the interaction between the drug and the phospholipids in the core of the membrane. The first stage is likely to result from electrostatic interactions between the cationic drug molecules and the negatively charged phospholipid head groups. The second stage can be expected to depend on the hydrophobicity and the electric charge of the drug molecules (Taylor 1990). The latter stage in the permeation process is unfavoured by the ionization of the drug, both directly and indirectly. The direct effect of the ionization decreases 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). As a result, the octanol-buffer drug distribution, D, has a lower value than the octanol-buffer drug partition, P. The indirect effect results from the physicochemical properties of the organic/buffer interface: the properties of lipid/buffer interfaces can differ considerably from those of octanol/buffer interfaces, especially when the membranes bear electric charges (Tocanne & Teissié 1990; Romsicki & Sharon 1999). In the present study, the negative electrostatic surface potential of the membrane brought about a redistribution of the protons, cations and anions in the vicinity of the lipid/buffer interface. This decreased the interfacial pH ($pH_{interfacial} < pH_{bulk}$) and increased the interfacial pK of the drugs ($pK_{interfacial} > pK_{bulk}$). It is therefore likely, that the partition of the drug may have further decreased, depending inversely on the net electric charge of the drugs (1/z). To estimate the respective effects

Table 1. Permeation dose, Hill coefficient, ionization constants and net electric charge of the MDR-modulators and non-modulators.

Compounds	PD50 (mM)	h	pKa[a]	z
Calcium-channel blockers				
Propranolol	11·2 ± 0·52	3·3 ± 0·19	9·45	1·00
Verapamil	2·1 ± 0·09	3·3 ± 0·32	8·92	0·99
Diltiazem	4·5 ± 0·16	4·1 ± 0·06	7·70	0·83
Dipyridamole	1·9 ± 0·03	1·5 ± 0·01	6·40	0·20
Calmodulin antagonists				
Clomipramine	4·3 ± 0·27	1·6 ± 0·01	9·38	1·00
Thioridazine	2·4 ± 0·09	3·4 ± 0·16	9·50	1·00
Antiparasitic agents				
Mepacrine	11·1 ± 0·19	1·3 ± 0·04	7·73; 10·18	1·84
Thioacridine 1	9·2 ± 0·67	1·1 ± 0·01	7·11[b]; 9·29[b]	1·56
Thioacridine 2	8·4 ± 0·08	2·5 ± 0·22	5·69[b]; 9·29[b]	1·04
Quinine	6·5 ± 0·01	2·8 ± 0·19	4·1; 8·5	0·97
Antineoplasic agents				
Cyclophosphamide	inactive	inactive	non-ionizable	0·00
Chlorambucil	inactive	inactive	5·75	−0·95
Steroid				
Bethamethasone	inactive	inactive	non-ionizable	0·00
Medroxy-progesterone acetate	inactive	inactive	non-ionizable	0·00
Other compounds				
Thioacridine 3	inactive	inactive	5·98[b]	0·09
Thioacridine 4	inactive	inactive	5·91[b]	0·08
Thioacridine 5	inactive	inactive	4·76[b]	0·01
Thioacridine 6	inactive	inactive	2·76[b]	0·00
Fenofibrate	inactive	inactive	non-ionizable	0·00
Mephenesin	inactive	inactive	non-ionizable	0·00
Captopril	inactive	inactive	3·7; 9·8	−1·00
Cefuroxime	inactive	inactive	2·5	−1·00

The leakage of Sulphan blue entrapped was induced by 0–16 mM compound (or 0–2·4 mol compound/mol lipid; i.e., about 0–16·3 μmol compound/m^2 surface membrane) through negatively charged LUV membranes. The leakage was quantified following a 3-min incubation at 37°C (pH 7·0). The permeation parameters (permeation dose, PD50; Hill coefficient, h) were determined according to equation 1 in Materials and Methods. Results are expressed as means ± s.e.m. of the permeation parameters obtained from the study of 2 or 3 LUV preparations. The ionization constants (pKa) of the compounds are [a]experimental or [b]predicted values, and the mean net electric charges (z) were calculated (pH 7·0) according to equation 2 in Materials and Methods, and to the pKa values given. [a]Data taken from Craig (1990), except when noted. [b]Data predicted by the Pallas 2.0 software program.

of the above parameters, the permeation properties of the compounds (PD50) were correlated with their octanol-buffer partition coefficient (logP), with their octanol-buffer distributions (logD), and with the ratio between their octanol-buffer distribution and their net electric charge (z) (i.e., with their octanol-buffer distribution per net electric charge, (logD)/z).

The octanol-buffer partition coefficient of the drugs, and their octanol-buffer distributions, were calculated using the Pallas 2.0 software program (Table 2). Where these data were available in the literature, the experimental logP values (Craig 1990) were similar to the predicted ones, except in the case of verapamil (3·79 vs 5·52 predicted). Also, the experimental logD values (Bouzoubaa et al 1984; Zamora et al 1988; Betageri & Dipali 1993) were in agreement with the predicted ones, except in the case of quinine. The predicted value for this compound (logD = −0·06) was obviously inaccurate compared with the experimental value (logD = 2·1 at pH 7·4) determined by Zamora et al (1988).

The log(1/PD50) vs logP linear regression ($r = 0{\cdot}091$ and $P = 0{\cdot}80$) and the log(1/PD50) vs

Table 2. Octanol-buffer partition coefficient (logP) and octanol-buffer distributions (logD) of the MDR-modulators.

Compounds	logP[a]	logD[a]
Calcium-channel blockers		
Propranolol	3·00	1·03
Verapamil	5·52	4·21
Diltiazem	2·58	1·36
Dipyridamole	1·71	1·71
Calmodulin antagonists		
Clomipramine	5·16	2·97
Thioridazine	5·13	2·59
Antiparasitic agents		
Mepacrine	6·16	1·15
Thioacridine 1	3·77	1·15
Thioacridine 2	4·83	2·54
Quinine	2·63	−0·06

[a]Data predicted by the Pallas 2.0 software program.

logD regression ($r = 0{\cdot}581$ and $P = 0{\cdot}08$) were not significant. The latter, however, since it took the ionization of the drug in the aqueous phase into account, explained 35% of the experimental variations in the permeation properties of the drugs. In addition, the log(1/PD50) vs (logD)/z linear regression (slope $= 0{\cdot}09 \pm 0{\cdot}03$, y-intercept $2{\cdot}07 \pm 0{\cdot}09$, $F_{(1,8)} = 11{\cdot}4$, $s_{residual} = 0{\cdot}203$, $r = 0{\cdot}768$ and $P < 0{\cdot}01$) was highly significant. In this case, about 60% of the permeation properties of the drugs could be explained ($r^2 = 0{\cdot}589$). This result clearly indicates that the indirect effect of the drug ionization at the membrane/buffer interface is an important factor which should be taken into account when dealing with trans-membrane processes. It should also be noted that upon replacing the calculated logP by the experimental one obtained with verapamil (logP = 3·79), the log(1/PD50) vs logP regression was still not significant, although it was improved ($r = 0{\cdot}272$ and $P = 0{\cdot}45$). Upon replacing the calculated logD by the experimental one obtained with quinine (logD = 2·1), the log(1/PD50) vs logD regression was still not significant ($r = 0{\cdot}612$ and $P = 0{\cdot}06$), whereas the log(1/PD50) vs (logD)/z regression was still significant at $P < 0{\cdot}01$ ($F_{(1,8)} = 12{\cdot}5$, $s_{residual} = 0{\cdot}198$, $r = 0{\cdot}781$).

Figure 2 shows the variation of the permeation properties of the membrane-active compounds with their octanol-buffer distribution per net electric charge. This figure clearly illustrates the fact that two compounds with different net electric charges and hydrophobicity levels (e.g. verapamil: logD = 4·21, z = 0·99; dipyridamole: logD = 1·71, z = 0·20), may have similar membrane permeation effects (similar PD50 value).

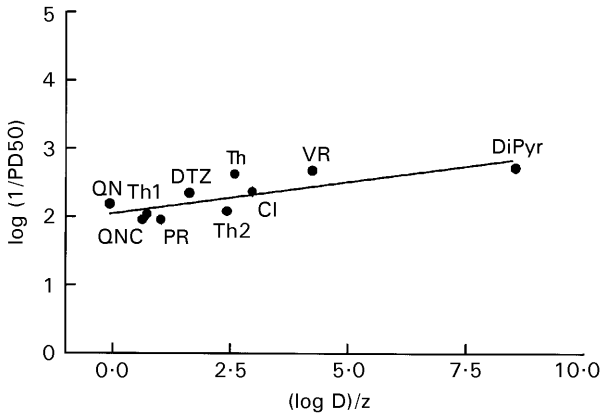

Figure 2. Dependence of the membrane permeation ability (log(1/PD50)) of the modulators on their octanol-buffer distribution per net electric charge (logD)/z. Results are expressed as means ± s.e.m. of the PD50 values obtained from the study of 2 or 3 LUV preparations. The octanol-buffer distribution per net electric charge (logD)/z was calculated according to the data given in Tables 1 and 2. QNC, mepacrine; PR, propranolol, Th1, thioacridine 1 derivative; Th2, thioacridine 2 derivative; QN, quinine; DTZ, diltiazem; Cl, clomipramine; Th, thioridazine; VR, verapamil; DiPyr, dipyridamole.

Discussion

In this study, the potential interactions between 22 drugs and the membranes of negatively charged liposomes were investigated. Of these compounds, only those previously found to act as MDR-modulators (Tsuruo 1983; Ramu et al 1984; Klohs et al 1986; Inaba & Maruyama 1988; Bennis et al 1997; Hevér et al 1998) were found here to affect the lipid bilayer of model liposomes sufficiently strongly for the passage of the entrapped polar dye, Sulphan blue, to occur.

The dye leakage approach has proved to be a useful tool in the field of pharmacology (Menassa & Sandorfy 1986; Castaing et al 1991; Sikkema et al 1994; Callaghan & Riordan 1995; Drori et al 1995). Drug–membrane interactions are generally quantified by measuring carboxyfluorescein leakage through membranes. This fluorophore could not be used here because mepacrine, dipyridamole and the thioacridines are also fluorescent compounds. Therefore, Sulphan blue was used here for the first time as it allows the study of any compound absorbing light below 600 nm. Like carboxyfluorescein, it is a hydrophilic anion which does not interact with the negatively charged phospholipid head groups of the membrane. The method is characterized by the following aspects: leakage of Sulphan blue from liposomes cannot be monitored continuously; whatever the leakage extent (0–100%), liposomes must be separated from released solute to quantify the dye amount remaining entrapped; due to the low sensitivity of spectrophotometry (at least 100 times less than spectrofluorimetry (Fersht 1985)), the effect is observed at high liposome concentrations, and therefore at high drug concentrations (millimolar range).

To assess the physiological relevance of the Sulphan blue method, a lipophilic cryptand was studied in preliminary experiments. Its PD50 amounted to 1·25 mM when tested in the present system, whereas it inhibits the Na,K-ATPase in kidney cells at concentrations of 2–10 μM (Loiseau et al 1997). Thus, the drug concentrations inducing Sulphan blue leakage can sometimes be 100 to more than 500 times higher than those required to induce biological effects in-vitro. As stressed above, this discrepancy is mainly accounted for by the low sensitivity of spectrophotometric measurements. And indeed, Pajeva et al (1996) have shown that membrane interactions with verapamil

occurred at lipid:drug molar ratios varying from 1 : 0·05 to 1 : 0·40. In the present study, this ratio amounted to 1 : 0·31 at the PD50 of verapamil, a result in agreement with the data of these authors. This clearly underlines the fact that the only meaningful parameter one need consider for pertinent comparisons of drug–membrane interactions in different model systems is the lipid:drug molar ratio. All the compounds inducing dye leakage here (PD50 = 1·9–11·2 mM) can therefore be expected to reverse MDR in in-vitro systems via drug–membrane interactions only at concentrations of 5–100 μM.

Of those compounds inducing membrane permeation here, verapamil and mepacrine have previously been found to interact with membranes (Callaghan & Riordan 1995; Drori et al 1995; Webb et al 1995; Pajeva et al 1996). Verapamil has been found to induce the leakage of carboxyfluorescein through model membranes in the micromolar range (high sensitivity of spectrofluorimetry) (Callaghan & Riordan 1995; Drori et al 1995) and to cross the membrane of liposomes (Webb et al 1995). Pajeva et al (1996) have also reported that the membrane interaction with verapamil was moderate compared with its interaction with mepacrine. From the present experiments, it can be estimated that to induce 50% dye leakage, the quantity of verapamil in the membrane must be much higher than that of mepacrine; this finding is in agreement with the data of Pajeva et al (1996).

With the exception of thioacridine 1, the permeation induced here by the MDR-modulators was found to be a co-operative process, based on the changes in the modulator concentrations ($1{\cdot}1 < h < 4{\cdot}1$). This indicates that the binding of one ligand molecule somehow accelerates the binding of the subsequent ones. An alternative interpretation is that the phospholipid organization in the bilayer may be destabilized when several ligand molecules interact concomitantly at the same site on the membrane. Co-operative processes of this kind have previously been found to exist in the field of liposome (Castaing et al 1991) and cell (Cano-Gauci & Riordan 1987) permeation by structurally unrelated compounds. According to Koshland's induced-fit theory (Korolkovas 1970a), which was put forward to explain the mode of drug action, deep conformational perturbations may occur as the result of the binding of a ligand to a receptor. These changes, which in some instances consist of an allosteric transition, are responsible for the observed biological effect.

As a general rule, the action of non-structurally related drugs results from their physicochemical properties (Korolkovas 1970b). This is precisely so for the MDR-modulators inducing membrane permeation here. All of them were highly lipophilic cationic compounds, and a significant correlation was found to exist between their permeation properties and their octanol-buffer distribution per net unit electric charge (Figure 2). This correlation shows that the electric charge plays a very special role in the permeation process, favouring the electrostatic interaction between the modulator and the anionic groups of the membrane interface in the first stage, and counteracting the drug penetration into the membrane in the second stage. As a result, compounds having different lipophilicities and electric charges can be expected to have similar permeation efficiencies.

It can be concluded from our study that hydrophobicity and electric charge are important properties enabling compounds to induce membrane permeation and reverse MDR in tumour cells by means of their interaction with the membrane phospolipids. The permeation of negatively charged liposomes with entrapped Sulphan blue is proposed as a suitable tool for screening compounds for their potential MDR-reversing activity, especially in cases where carboxyfluorescein cannot be used.

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