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ARTICLE

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A predictive model for the selective accumulation of chemicals in tumor cells

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Abstract Cationic lipophilic dyes can accumulate in mitochondria, and especially in mitochondria of tumor cells. We investigated the chemical properties and the processes allowing selective uptake into tumor cells using the Fick–Nernst–Planck equation. The model simulates uptake into cytoplasm and mitochondria and is valid for neutral molecules and ions, and thus also for weak electrolytes. The differential equation system was analytically solved for the steady-state and the dynamic case. The parameterization was for a generic human cell, with a 60 mV more negative potential at the inner mitochondrial membrane of generic tumor cells. The chemical input data were the lipophilicity (log K_{OW}), the acid/base dissociation constant (pK_a) and the electric charge (z). Accumulation in mitochondria occurred for polar acids with pK_a between 5 and 9 owing to the ion trap, and for lipophilic bases with p$K_a > 11$ or permanent cations owing to electrical attraction. Selective accumulation in tumor cells was found for monovalent cations or strong bases with log K_{OW} of the cation between −2 and 2, with the optimum near 0. The results are in agreement with experimental results for rhodamine 123, a series of cationic triarylmethane dyes, F16 and MKT-077, an anticancer drug targeting tumor mitochondria.

Keywords Cancer · Cation · Mitochondria · Nernst · Rhodamine 123 · Tumor

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Introduction

Chen and coworkers observed that plasma and mitochondrial membrane potentials of tumor cells were typically higher than those of normal cells. Moreover they suggested this phenomenon could provide the basis for selective *mitochondrial targeting* of anticancer drugs (Davis et al. 1985; Chen 1988). Subsequently a mitochondrially localizing drug, MKT-077, reached phase I clinical trials (Britten et al. 2000).

More recently Kandela et al. (2002) validated experimentally the concept of mitochondrial targeting as a therapeutic strategy for chemotherapy of tumor cells. The authors used cationic triarylmethane (TAM+) dyes and demonstrated the selective phototoxicity of certain dyes to cancer cells. Very lipophilic TAM+ dyes attacked both healthy cells and cancer cells. More hydrophilic dyes selectively targeted the tumor cells. However, the authors acknowledged that the relationships between the structure of chemical compounds and their specific effects on cancer cells are not well understood: "The lack of a robust model to describe the relationship between molecular structure and tumor selectivity has prevented mitochondrial targeting from becoming a more dependable therapeutic strategy" (Kandela et al. 2002).

Although modeling of the principles and molecular properties responsible for the distribution of molecules inside generic animal cells has been described (Horobin 2001), quantitation of uptake was not provided. Interestingly however, there is a formal similarity between the selective targeting of cancer cells and the design of selective and systemic herbicides.

Models predicting the necessary molecular properties for transport inside plants to the target site have been developed by Briggs et al. (1987), Kleier (1988), Trapp (2000) and others. The charge and the pH of the cells and their subcompartments play a crucial role. For example, it has been found that weak acids are preferable as herbicides, owing to their accumulation and

transport in the basic sap (pH 8) of the phloem (Hsu and Kleier 1996). The theory for plant cells was described by Trapp (2004) and is applied here to the problem of targeting human tumor cells.

Methods

Model for uptake of molecules into cells

The underlying principles of chemical uptake into plant cells have been reviewed by Trapp (2004) and are valid for all types of cells. The main modification made here is that the vacuole compartment of plant cells is replaced by mitochondria. This is because animal cells lack vacuoles, and because the increased mitochondrial potential of tumor cells, compared with that of healthy cells, might turn out to be their Achilles heel.

Figure 1 shows the processes involved in the uptake of a weak base into a simplified version of a single cell. The cell is separated into two compartments: cytoplasm (the cell sap including lipids) and mitochondria. The cell is surrounded by a biomembrane, the plasmalemma. Owing to the proton pump located in the inner membrane of the mitochondrion, a charge at the inner mitochondrial membrane of about −160 mV is established when this pump is active (see, for instance, standard monographs, e.g., Alberts et al. 2002). The interior of the mitochondrion consequently becomes alkaline (pH 8). The cytoplasm is negatively charged relative to the external milieu (or "outside") at approximately −70 mV, and the cytoplasmic pH is 7–7.5. A base may coexist in several forms under these conditions: as undissociated BOH; as the cation B^+; as a complex, e.g., B^+Cl^-; or as a zwitterion. The speciation depends on pH, the chemical nature of the base, the ion composition, and on the *mass action law* (Appelo and Postma 1999). The total flux across membranes is the sum of the fluxes of all molecular species. We will consider here only the undissociated (neutral) and the dissociated (ionic) forms, but the extension to complexes and other molecular species is possible and has been made (Trapp 2004).

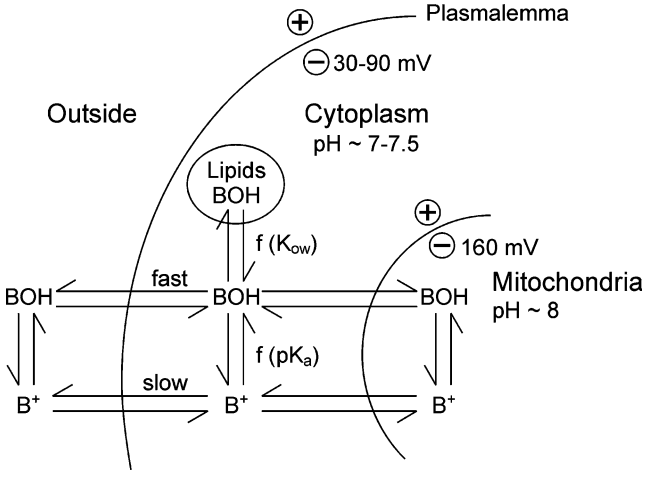

Fig. 1 The cell. Compartments, molecular species, pH and charges in a cell shown for a weak base

Neutral molecules

The diffusive flux of neutral molecules across membranes is driven by the chemical potential, and is described by Fick's first law of diffusion:

$$J = P(a_o - a_i), \tag{1}$$

where J is the unit flux from the outside (o) to the inside (i) of the membrane (kilograms per meter squared per second), P is the permeability of the membrane (meters per second), and a is the activity of the compound (kilograms per meter cubed).

Electrolytes

The flux of ions across electrically charged membranes is driven by the chemical and the electrical potential. This is described by the Nernst–Planck equation. With the assumption of a linear potential gradient across the interface of the membrane, a net current flow of zero and with each ion flux is at steady state, an analytical solution (the constant field approach of Goldman 1943 and Hodgin and Katz 1949) for the flux of the ion is (Briggs et al. 1961)

$$J = P\frac{N}{e^N - 1}\left(a_o - a_i e^N\right), \tag{2}$$

where $N = zEF/RT$, z is the electric charge (synonym valency, for acids −, for bases +), F is the Faraday constant (96 484.56 C/mol) (Kuchling 1981), E is the membrane potential (volts), R is the universal gas constant (8.314 J/mol/K) and T is the absolute temperature (kelvin).

Weak electrolytes

Under physiologically relevant conditions, molecules of organic electrolytes may exist as ions or in a neutral form. The activity ratio between both is calculated by the Henderson–Hasselbalch equation (Henderson 1908):

$$\log\frac{a_d}{a_n} = i(\mathrm{p}H - \mathrm{p}K_a), \tag{3}$$

where a is the activity, d is the index for dissociated (synonym ionic), n is the index for for neutral, i is 1 for acids and −1 for bases; pK_a is the negative logarithm ($\log_{10}$) of the dissociation constant.

The total (measurable) concentration C_t of the compound comprises the neutral (n) and dissociated (d) molecules; both kinds can be in solution or sorbed. The activity a of the molecules in solution is the driving force for diffusive exchange and is related to the total concentration C_t by (Trapp 2004)

$$f_n = a_n/C_t = \frac{1}{W/\gamma_n + K_n/\gamma_n + W \times 10^{i(\mathrm{pH}-\mathrm{p}K_a)}/\gamma_d + K_d/\gamma_d}, \quad (4)$$

where W is the volumetric water fraction, γ is the activity coefficient, with $a = \gamma C$, and K_n and K_d are the sorption coefficients of the neutral and the dissociated molecule.

Per definition $a_d = a_n \times 10^{i(\mathrm{pH}-\mathrm{p}K_a)}$; therefore, $f_d = a_d/C_t = f_n 10^{i(\mathrm{pH}-\mathrm{p}K_a)}$.

The total flux of the compound across the membrane is the sum of the fluxes of the neutral molecule and the ion. Combining Fick's first law of diffusion for the neutral molecule with the Nernst–Planck equation for the ion gives for the total flux

$$J = P_n\left(a_{n,o} - a_{n,i}\right) + P_d \frac{N}{e^N - 1}\left(a_{d,o} - a_{d,i}e^N\right). \quad (5)$$

Under steady-state conditions, the net flux is zero, and the concentration ratio between inside and outside the membrane is

$$\frac{C_i}{C_o} = \frac{f_{n,o} \times P_n + f_{d,o} \times P_d \times N/(e^N - 1)}{f_{n,i} \times P_n + f_{d,i} \times P_d \times e^N \times N/(e^N - 1)} = K_{io}. \quad (6)$$

Active transport across membranes (e.g., by transporter proteins) is not considered.

Model for uptake into mitochondria

Mitochondria are organelles located inside the cytoplasm. The calculation of the uptake of molecules into mitochondria therefore first requires the solution of the equations for uptake from outside into the cytoplasm, and then for uptake from the cytoplasm into mitochondria.

Steady-state solution

In the absence of degradative processes, the steady-state concentration ratio between cytoplasm and outside is identical to Eq. 6, where o is outside the cell and i is the cytoplasm. For mitochondria, the concentration ratio with respect to the cytoplasm is calculated with the same equation, but o is then cytoplasm and i is mitochondria. The ratio mitochondria to outside is the product of both results.

Dynamic solution

If o denotes the outside of the cell, c the inside, m the mitochondria, and J the corresponding unit fluxes across surface area A, then the change of mass in the cytoplasm m_c is equal to the flux from outside minus the flux to the outside minus the flux to mitochondria plus the flux from mitochondria

$$\frac{dm_c}{dt} = A_c \times J_{o,c} - A_c \times J_{c,o} - A_m \times J_{c,m} + A_m \times J_{m,c} \quad \text{cytoplasm} \quad (7)$$

and the change of mass in the mitochondria m_m is equal to the flux to mitochondria minus the flux to cytoplasm

$$\frac{dm_m}{dt} = A_m \times J_{c,m} - A_m \times J_{m,c} \quad \text{mitochondria} \quad (8)$$

Concentration $C = m/V$, where V is the volume, and therefore

$$\frac{dC_c}{dt} = A_c \times J_{o,c}/V_c - A_c \times J_{c,o}/V_c - A_m \times J_{c,m}/V_c + A_m \times J_{m,c}/V_c \quad (9)$$

and

$$\frac{dC_m}{dt} = A_m \times J_{c,m}/V_m - A_m \times J_{m,c}/V_m. \quad (10)$$

This coupled linear differential equation system was solved analytically with the solution given by Nazaroff and Alvarez-Cohen (2001). All equations were implemented as a spreadsheet version, which is available from the corresponding author.

Model parameterization

Cell data

Generic data for cytoplasm and mitochondria of cells are listed in Table 1. The data used for tumor cells were identical, except for a 60 mV more negative electrical potential at the mitochondrial membrane.

Table 1 Parameter data for generic and tumor cells

Parameter	Symbol	Value	Unit
Diameter of cell[a]		10^{-5}	m
Diameter of mitochondria[a]		10^{-6}	m
pH outside	$\mathrm{pH_o}$	7	–
pH of cytoplasm	$\mathrm{pH_c}$	7.5	–
pH of mitochondria	$\mathrm{pH_m}$	8	–
Water content of cytoplasm	W_c	0.95	m^3/m^3
Lipid content of cytoplasm	L_c	0.05	g/g
Water content of mitochondria	W_m	1	m^3/m^3
Lipid content of mitochondria	L_m	0	g/g
Ionic strength of cytoplasm	I_c	0.3	mol
Ionic strength of mitochondria	I_m	0.3	mol
Membrane potential of plasmalemma	E_c	−0.07	V
Membrane potential of mitochondria	E_m	−0.16	V
Membrane potential of mitochondria of tumor cells	E_t	−0.22	V

[a]The volume and surface area were calculated from the diameter assuming a sphere

Chemical data

The chemical data needed as inputs in Eqs. 6, 7, 8, 9 and 10 are the dissociation constant pK_a and the electric charge z, plus activity coefficients γ, permeabilities P and sorption coefficients K of both the neutral and the dissociated compound.

The permeability P depends on (Schönherr and Riederer 1989)

$$P = DK/\Delta x, \tag{11}$$

where D is the diffusion coefficient, K is the partition coefficient, and Δx is the membrane thickness. With D of organic compounds in biomembranes of about 10^{-14} m^2/s, Δx of about 50 nm, and K approximately K_{OW} (Riederer 1995) the log–linear relationship is

$$\log P = \log K_{OW} - 6.7. \tag{12}$$

The equation is valid for neutral molecules and is similar to the empirical relation derived by Grayson and Kleier (1990), $\log P = 1.2 \log K_{OW} - 7.5$, which was found by inverse modeling of phloem transport. Kleier (1988) assumed that his equation is also valid for ions when log K_{OW} of the dissociated molecule is entered into the equation. He suggested the relation $\log K_{OW}(\text{neutral}) = \log K_{OW}(\text{ion}) + 3.7$. Measurements by Briggs et al. (1987) gave only a 3.235 log-units lowered log K_{OW} of the ions ($n = 4$, anions). As a compromise, we use subsequently $\log K_{OW}(\text{neutral}) = \log K_{OW}(\text{ion}) + 3.5$. Raven (1975) found a permeability ratio of P(neutral) to P(ion) of approximately 1 000, whereas LeBlanc (cited in Raven 1975) suggested a ratio of 10 000. Using Eq. 12 with a 3.5 log-units lowered log K_{OW} of the ions yields a permeability ratio of 3 162, which is within this range. Since measured data are rare, this approximation is used subsequently. If not stated otherwise, all log K_{OW} values are for the neutral molecular species.

K_n and K_d, the sorption coefficients of the neutral and the dissociated molecule, were estimated by assuming that lipids sorb like *n*-octanol, i.e., $K = L \times 1.22 \times K_{OW}$, where K_{OW} is either for the neutral or for the dissociated molecule, L is the lipid fraction (grams per gram), and 1.22 is a unit conversion factor (Trapp and Matthies 1995, erratum).

The activity coefficient γ_n of all neutral molecules, $z = 0$, was calculated from the ionic strength I (moles) with the Setchenov equation to be 1.23 at $I = 0.3$ mol. The activity of ions, γ_d, was calculated with the Davies approximation of the modified Debye–Hückel equation (Appelo and Postma 1999) and was 0.74 for monovalent ions, $|z| = +1$, at $I = 0.3$ mol. For conditions outside the cell, no corrections for the ionic strength were made, and activities were set approximately equal to concentrations ($\gamma = 1$).

Results

Sensitivity studies

In this section, the chemical input data—lipophilicity log K_{OW}, acid/base dissociation constant pK_a, and valency z—are varied to find molecular properties and to identify processes that may lead to selective accumulation of chemicals in mitochondria of tumor cells.

Task 1: which chemicals can selectively accumulate in mitrochondria, and why?

All calculations in this section were made for the steady-state ($t = \infty$).

Neutral compounds. The model provides no mechanism for a selective accumulation of neutral compounds in mitochondria. The opposite holds: owing to the higher lipid content in other regions of the cytoplasm, lipophilic neutral compounds (high log K_{OW}) will preferably accumulate elsewhere in the cytoplasm.

Acids. Figure 2 shows the concentration ratio to outside C/C_o for cytoplasm and mitochondria of a polar acid ($z = -1$, log $K_{OW} = 0$) with varying pK_a. An accumulation in cytoplasm and mitochondria occurs for pK_a values near but below the pH of cytoplasm ($pH_c = 7.5$) and mitochondria ($pH_m = 8$). This accumulation is due to the "ion trap" effect, which occurs when the molecule is neutral outside ($pH_o = 7$), but dissociates inside. Since the membrane permeability of the ion is slower (Eq. 12), the ion is trapped. This effect was first detected by Raven (1975) for plant cells and later verified with intact barley plants (Briggs et al. 1987). The phenomenon has also been observed in cultured animal cells, and the same mechanism was proposed (Rashid and Horobin 1991).

Bases. Figure 3 shows the concentration ratio to outside C/C_o for cytoplasm and mitochondria for a polar base ($z = +1$, log $K_{OW} = 0$) with varying pK_a. At $pK_a > pH_m$, the opposite ion trap occurs, because the base is then neutral inside and dissociates outside, and by this is excluded from cytoplasm and mitochondria. The ion trap of bases was experimentally verified for plants (Inoue et al., 1998). At even higher pK_a values,

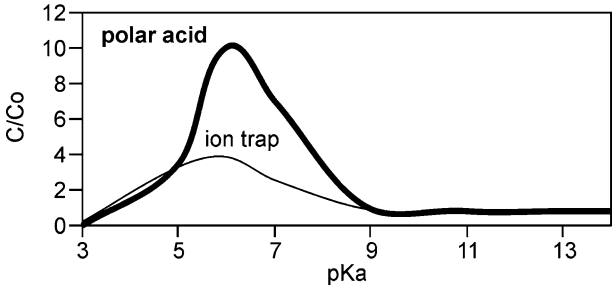

Fig. 2 Accumulation of polar acid. Steady-state concentration ratio to outside C/C_o for cytoplasm (*thin line*) and mitochondria (*solid line*) shown for a polar acid ($z = -1$, log $K_{OW} = 0$)

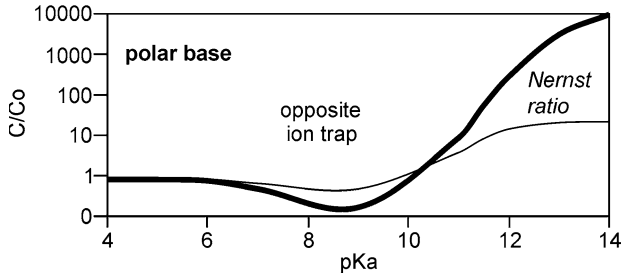

Fig. 3 Accumulation of polar base. Steady-state concentration ratio to outside C/C_o for cytoplasm (*thin line*) and mitochondria (*solid line*) shown for a polar base (log $K_{OW}=0$, $z=+1$)

when the base is almost completely dissociated, a strong accumulation occurs, owing to the electrical attraction of the positive cation by the negative membrane potential of cytoplasm ($E_c=-70$ mV) and mitochondria ($E_m=-160$ mV).

The theoretical accumulation due to the electrical potential is determined by the Nernst ratio (Nernst 1889):

$$\frac{C_i}{C_o}=e^{\frac{-zEF}{RT}}. \qquad (13)$$

If $E=E_c+E_m=-0.23$ V, $C_i/C_o=9001$ for the accumulation in mitochondria alone due to electrical attraction of monovalent cations. The calculated value at the right side of Fig. 3 is even higher, because the reduced activity of the ion inside the cell is taken into account. The selective accumulation in mitochondria decreases (both for acids and bases) with increasing lipophilicity of the chemical, because sorption to lipids leads to higher accumulation in the cytoplasm. Figure 4 depicts this for a very lipophilic strong base (log $K_{OW}=6$, $z=+1$).

Task 2: which chemicals can accumulate selectively in tumor cells, and why?

In this section, simulation results for mitochondria of normal cells ($E_m=-160$ mV) and mitochondria of tumor cells with 60 mV lower membrane potential (Chen 1988) ($E_t=-220$ mV) are compared in an effort to identify those chemicals that can accumulate in higher amounts in the mitochondria of tumor cells.

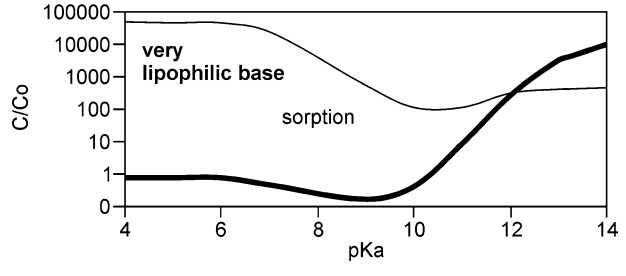

Fig. 4 Very lipophilic base. Steady-state concentration ratio to outside C/C_o for cytoplasm (*thin line*) and mitochondria (*solid line*) shown for a very lipophilic base (log $K_{OW}=6$, $z=+1$)

Although polar acids have a potential to accumulate in mitochondria, acids will not accumulate to higher amounts, but to similar or lower amounts in tumor mitochondria than in normal mitochondria. The reason is that for anions, the valency z is less than 0, and the Nernst ratio (Eq. 13) of the chemical distribution between tumor and normal mitochondria will always be 1 or less. Negatively charged anions are repulsed by the negative charge of the mitochondria—which is more negative for tumor cells.

The preferable accumulation of strong bases or permanent cations in tumor mitochondria—although high according to the Nernst ratio—may be kinetically limited. The concentration ratio between tumor cells and normal cells after 1 h, 1 day, and at $t=\infty$ of a cation with varying log K_{OW} is calculated with Eqs. 9 and 10 and is shown in Fig. 5. For a potential difference of $E_t-E_m=-0.06$ V, the Nernst ratio is 10.75 if $z=+1$. This equilibrium value is reached for a permanent cation ($pK_a\rightarrow\infty$) of any lipophilicity at $t=\infty$, and for more lipophilic cations [log K_{OW}(ion) >0] for $t<1$ h. More polar cations [log K_{OW}(ion) <-1.5], however, will not reach the equilibrium at $t<1$ day.

Task 3: which chemicals will only accumulate in mitochondria of tumor cells, and why?

The model predicts a higher accumulation of cations in tumor mitochondria than in normal mitochondria. However, the targeting of tumor cells is more precise if an accumulation occurred exclusively in mitochondria of tumor cells, while the accumulation in the cytoplasm of normal cells is low. As depicted in Fig. 6, for cations with log K_{OW} values (of the ion) in the range between -2 and 2, this condition is fulfilled. Below, the uptake into mitochondria is kinetically limited; above, sorption to lipids in the cytoplasm dominates.

Summary: which chemicals fulfill all criteria ?

If the results from the sensitivity study of tasks 1–3 are taken together, only very specific chemicals can fulfill the three criteria. These are strong bases or permanent

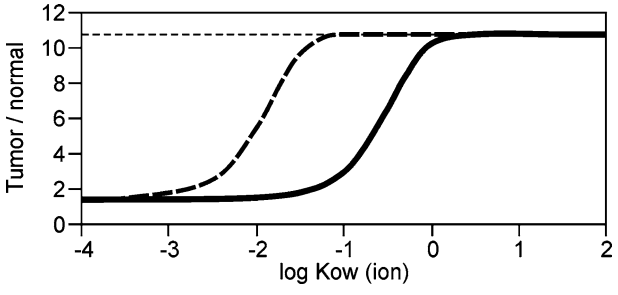

Fig. 5 Kinetic limitation. Concentration ratio between the mitochondria of a tumor cell and the mitochondria of a normal cell for a permanent cation ($z=+1$, p $K_a\rightarrow\infty$) with varying lipophilicity (log K_{OW} of ion given) after $t=1$ h (*dashed solid line*), $t=1$ day (*solid line*) and $t=\infty$ (*dashed thin line*)

cations with intermediate lipophilicity (log K_{OW} of the ion between −2 and 2, optimum at 0). Compounds with these properties are the candidates with the highest potential for selective anticancer agents.

Multidrug resistance of tumor cells

Anticancer-drug-based therapy of various human malignancies is often hampered by multidrug resistance, owing to enzymes acting as extrusion pumps, expelling cytotoxic agents (Eytan et al. 1997). This process was mimicked in the model simulations by increasing the permeability of the plasmalemma for fluxes outwards. The model predicts that the extrusion pump is mainly relevant for more polar compounds. This can be explained by the fact that more lipophilic compounds have higher membrane permeability. The tumor cell pumps out the molecules at the same (enzymatically limited) rate, but the influx increases with lipophilicity. Thus, more lipophilic compounds would have a higher potential to target multi-drug-resistant tumor cells. Eyten et al. (1997) also found experimentally that a fast transmembrane movement of anticancer drugs might overcome the multi-drug-resistance phenomenon. However, excessively lipophilic compounds will target other lipophilic structures inside the cytoplasm rather than mitochondria. Figure 6 shows additionally the concentration ratio between tumor mitochondria and normal cytoplasm for an increase of P (outwards) of 10^{-7} m/s for all chemicals. As a result, the optimum log K_{OW} for selective accumulation of chemicals in the tumor cells is less pronounced and shifted to slightly more lipophilic cations (log K_{OW} of the ion near 1).

Discussion

Comparison of the model with experimental findings

Several structurally different chemicals with the common properties of lipophilicity and monovalent positive charge, the so-called delocalized lipophilic cations, have been identified to selectively target the mitochondria of tumor cells (Modica-Napolitano and Aprille 2001). The most widely investigated dye is perhaps rhodamine 123, which stains mitochondria almost exclusively, and which accumulates more extensively and is retained longer in tumor cells (Davis et al. 1985). It has been suggested that the deprotonation of rhodamine 123 would occur above pH 11, which means a pK_a of at least 12 (Ferguson et al. 1999). The K_{OW} of the cation is 0.24 (Kandela et al. 2002), or log K_{OW}(ion) = −0.62, which is close to the optimum lipophilicity that was found using the model to calculate mitochondria-specific uptake of cations (Fig. 6). Fantin et al. (2002) screened the activity of 16 000 compounds on cancer cell lines. Only one compound, named F16, selectively acted on tumor cells. Mitochondria were the major F16-concentrating organelle of the cell. Uptake and accumulation depended on the membrane potential. F16 is a delocalized lipophilic cation with pyridinium and indole structure. The log K_{OW} of F16 was estimated, using the procedures described by Hansch and Leo (1995), to be −1.3. Consider also the mitochondrially localizing anticancer drug MKT-077, a cationic rhodacyanine, which has reached phase I clinical trials (Britten et al. 2000). A log K_{ow} value of the ion of −1.6 has been determined for this compound (Tatsuta et al. 1999). Again, this falls into the region near the optimum log K_{ow} shown in Fig. 6.

Kandela et al. (2002) used a series of TMA+ dyes with varying lipophilicity to determine the effect of the molecular structure on selective toxicity towards tumor cells. TMA+ dyes are quaternary ammonium compounds and hence do not give rise to free bases at high pH; however, electrically neutral pseudobases can be formed; for a brief review see Horobin and Kiernan (2002). The TMA+ dyes had differing alkyl substitutions at the N atom, thus varying their lipophilicity. Only the less lipophilic dyes (log K_{OW} of the ion between −0.62 and 0.34) exhibited selective tumor cell toxicity; more lipophilic dyes attacked normal cells, too. Table 2 summarizes the experimental results. As can be seen, the range of log K_{OW} needed for selective toxicity found

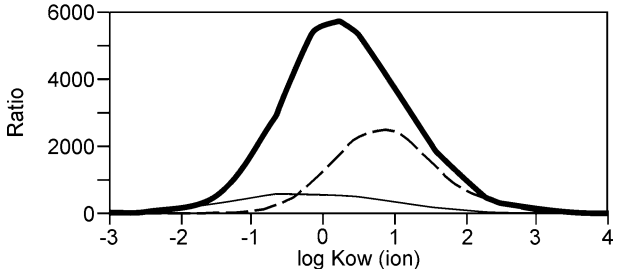

Fig. 6 Selective accumulation in tumor mitochondria. Concentration ratio between tumor mitochondria and cytoplasm (C_t/C_c) (*solid line*) and between normal mitochondria and cytoplasm (C_m/C_c) (*thin line*) for permanent cations ($z = +1$, p $K_a \rightarrow \infty$) with varying lipophilicity (log K_{OW} of the ion is shown) after short-term exposure ($t = 1$ h); (*dashed solid line*) C_t/C_c for a tumor cell with multidrug resistance

Table 2 Experimental results on selective tumor cell toxicity of triarylmethane dyes of varying lipophilicity (Kandela et al. 2002) compared with electric charge z and K_{OW} values

Name[a]	z	K_{OW} (ion)	log K_{OW}(ion)	Selective tumor toxicity?
EV+	+1	237	2.37	No
VPBBO+	+1	180	2.26	No
VBR+	+1	39	1.59	No
CV+	+1	2.21	0.34	Yes
I	+1	2.15	0.33	Yes
II	+1	1.82	0.26	Yes
III	+1	1.63	0.21	Yes
IV	+1	0.77	−0.11	Yes
V	+1	0.59	−0.23	Yes
VI	+1	0.21	−0.68	Yes

[a]Chemical structures given in Kandela et al. (2002)

experimentally is almost identical to the range identified by the use of the model. Whilst this is not proof of either the experimental or the model approach, it certainly generates confidence in both sets of results.

Kandela et al. (2003) presented a simple model based on the ratio between the concentration of a cation located inside the mitochondria (calculated with the Nernst ratio) and the concentration in any lipophilic compartment inside the cell (calculated with log K_{OW}). The model predicts the accumulation of cations of varying lipophilicity in tumor mitochondria by a log–linear relation declining with log K_{OW}. Although the approach is different from our model, there is no contradiction. The equation is applied in the range of log K_{OW}(ion) from −0.68 to 2.37. In this range, our more complex model also predicts a monotonous decrease of the accumulation in mitochondria with increasing log K_{OW}.

Uncertainties of the model

Although the model can explain several experimental findings, there is no reason to be euphoric. The model is grossly simplified, which means it neglects the existence of most cell structures and processes. Figure 7 depicts an uncertainty study for a strong base ($z = +1$, p$K_a = 14$). The absolute accumulation in mitochondria, using the original parameterization (Table 1), is lower than for a permanent cation (Fig. 6), because despite the pK_a being high, the opposite ion trap still occurs to some extent. Three variations of parameters have been made to identify uncertainties.

1. Mitochondria lipids. The model was parameterized for a generic cell. We idealized such that mitochondria contain no lipids ($L_m = 0$). If the mitochondria contain lipids, but less than the cytoplasm ($L_m < L_c$), the optimum region does not change. The curve in Fig. 7 shows the simulation result for $L_m = 2\%$. This increases the concentration ratio C_t/C_c (tumor mitochondria to cytoplasm) for log K_{OW}(ion) > 0.
2. Membrane permeability of ions P_d. This relevant input parameter was estimated by regression from log K_{OW}. The log K_{OW} value is a parameter that is easily available for many chemicals, but it is not always a good predictor for the sorption of organic cations (Hunziker et al. 2001), and thus also not for their membrane permeability. Changes of the membrane permeability of the dissociated molecular species can have a large influence on the result. Cations might pass through the membrane more rapidly by following hydrophilic pathways, or by facilitated or active transport. However, the model is robust in this respect: when the membrane permeability of the cation increases, compared with the estimated permeability, the opposite ion trap will always decrease. This is shown in Fig. 7 for a tenfold increase of P_d. The simulated concentration in mitochondria is higher and closer to the Nernst ratio, and the selective accumulation in tumor cells is more pronounced and starts at lower pK_a and log K_{OW} values.
3. Mitochondrial membrane surface area. The inner mitochondrial membrane is folded and moves wildly (Chen 1989). This could increase the uptake of compounds. The third parameter varied is therefore the area of the inner membrane, which was increased by a factor 10. This leads to better accumulation of the more polar cations [log K_{OW}(ion) < 0].

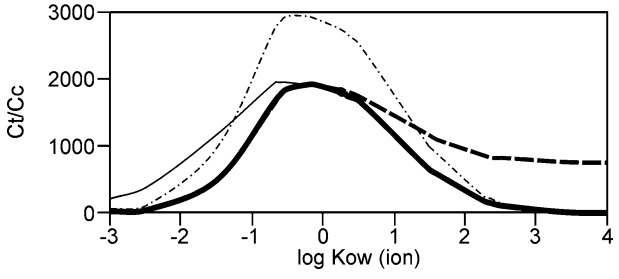

Fig. 7 Uncertainty analysis. Concentration ratio between tumor mitochondria and cytoplasm C_t/C_c for strong bases ($z = +1$, p$K_a = 14$) with varying lipophilicity (log K_{OW} of the ion is shown) after short-term exposure ($t = 1$ h). Variation of parameters: (original) the *solid line* is all parameters as in Fig. 6 except p K_a; the *dashed solid line* is 2% lipid content of mitochondria; the *dashed thin line* is a tenfold increase in membrane permeability of the cation; the *thin line* is a tenfold increase in the area of the inner mitochondrial membrane

In summary, the model behaved quite robustly towards changes. The variations increased mitochondrial accumulation of either more lipophilic cations [log K_{OW}(cation) > 0], or more polar cations [log K_{OW}(cation) < 0], or a more pronounced optimum region was found, but the optimum remained in all cases near log K_{OW}(cation) = 0.

Conclusions

Despite all uncertainties, the model does identify molecular properties and processes, which lead to a selective accumulation of chemicals in tumor mitochondria. Although the absolute concentrations as calculated may well be inaccurate, the processes exist and can be exploited. The hope is that this model, quantitatively specifying the relationship between molecular structure and tumor selectivity, can be used for developing future therapeutic strategies. Furthermore, we expect the model to be of general validity, which would allow the expansion to many other medicinal and biological problems.

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