Topological Polar Surface Area Defines Substrate Transport by Multidrug Resistance Associated Protein 1 (MRP1/ABCC1)

Janaina Fernandes* and Cerli R. Gattass

Instituto de Biofísica Carlos Chagas Filho, Laboratório de Imunologia Celular, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Bloco G, Cidade Universitária, 21949-900 Rio de Janeiro, Brazil

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Multidrug resistance-associated protein 1 (MRP1/ABCC1) is a very promiscuous transporter. Herein we used topological polar surface area (TPSA), a descriptor defined as the sum of surfaces of polar atoms in a molecule, to analyze drug transport by MRP1. We suggested that compounds with high TPSA are transported while those with low TPSA are not. The conjugation to GSH increases TPSA values favoring transport. A strong correlation between TPSA and transport properties (K_m) was also found.

Introduction

The multidrug resistance phenomenon (MDR) impairs drug efficiency, leading to chemotherapeutic failure and hence causing a strong impact on disease outcome and patient survival. MDR can be mediated by several mechanisms, including the overexpression of efflux pumps belonging to the superfamily of the ATP-binding cassette proteins, whose activity is dependent on ATP hydrolysis. By pumping chemotherapic agents out of the tumor cells, MDR proteins reduce their intracellular accumulation and prevent cell death, leading to resistance.[1] Multidrug resistance protein (MRP1/ABCC1[a]) is one of the encoded plasma membrane transporters thought to be responsible for the resistance of tumor cells to several chemically and functionally unrelated drugs.[2] This protein also has physiological roles in normal tissues such as lung, testis as well as at the blood-brain barrier, where it is expressed at low levels.[3]

For the past 10 years, MRP1 has been described as an ATP-dependent pump for **1** (LTC4) and several other GS-X conjugates.[4−6] The MRP1 protein has also been shown to be capable of transporting glucuronide and sulfate conjugates of an extremely diverse array of endo- and xenobiotics such as 17β-estradiol 17-(β-D-glucuronide) (E217 βG),[7] **2** (aflatoxin B1),[8] fluorescent dyes **3** (calcein),[9] **4** (Fluo-3),[10] and glutathione disulfide **5** (GSSG).[11] The substrate specificity of MRP1 includes several anticancer drugs such as anthracyclines, vinca alkaloids, and etoposide.[12−14]

The mechanisms of transport by MRP1 have been extensively investigated. It has been suggested that MRP1 can transport the drug alone or the drug conjugated to **6** (GSH)[4] or glucuronide or cotransported with these last two.[15,16] However, despite all investigations performed to understand MRP1 functionality and specificity, the reason for its substrate promiscuity remains unclear.

In the past few years, several important tools have been developed to help identify potential drug candidates efficiently and rapidly. Methods of describing absorption and predicting bioavailability of drugs were described. The "rule of five" is a group of four physicochemical properties used to evaluate the probability of a substance to become an effective drug.[17] Additional descriptors were also developed, and one of the most widely used is the polar surface area (PSA) which is defined as the sum of the surface areas of polar atoms in a molecule. In 1996, van de Waterbeemd et al.[18] correlated the PSAs of a series of drugs to membrane transport. Since then, PSA has increasingly served as a useful parameter for the prediction of molecular transport properties, particularly in intestinal absorption and blood−brain barrier penetration.[19−21] In 2000, Ertl et al.[22] used the sum of PSA values of polar fragments of a molecule to define the TPSA index. This index provides results that are practically identical to those of the PSA and has now been adopted by the widely used Pubchem compound database.

Molecules with a PSA greater than 140 Å^2 are believed to have a low capacity for penetrating cell membranes, while those with PSA $\leq$ 60 Å^2 are easily absorbed.[19] If high TPSA accounts for a poor penetration of molecules in a hydrophobic environment, such as biological membranes, it may account for their ready penetration in hydrophilic environments, such as the core of transporter proteins. In this study, we carried out a careful analysis of the chemical properties of substances reported in the literature as MRP1 substrates. The data obtained support the hypothesis that MRP1 pumps out substrates with high TPSA values, i.e., with a high proportion of electronegative elements such as nitrogen and oxygen, while compounds with low TPSA values are not transported. Furthermore, the data are consistent with the suggestion that the conjugation of antitumor drugs to **6** increases their TPSA values, favoring the transport of the GS conjugates.

The hypothesis presented here may represent an important contribution to the process of drug discovery, especially for the design and selection of anti-MRP1 compounds, since it will allow the prediction of which compounds have the potential to be transported by MRP1 independently of their biological activity (antibacterial, antiviral, or antitumoral) or chemical class (antracyclines, taxanes, etc).

Materials and Methods

Bibliographic Data. The bibliographic data were obtained from NCBI Pubmed (http://www.ncbi.nlm.nih.gov/sites/entrez) and from Institute of Scientific Information (ISI) Web of Science bibliographic database (Thomson Scientific Inc.) (http://apps.isiknowledge.com/) under MEC/CAPES/MCT Brasil consortia license.

Chemical Structure Database Search. On the basis of bibliographic data, the compounds reported as MRP1 substrates were submitted to the Pubchem compound database search and in the

* To whom correspondence should be addressed. Phone: 55 (21) 2562-6564. Fax: 55 (21) 2280-8193. E-mail: janainaf@biof.ufrj.br.

[a] Abbreviations: MRP1/ABCC1, multidrug-resistance-associated protein 1; TPSA,topological polar surface area; GST, glutathione-*S*-transferase; GSH, reduced glutathione; GS-X conjugates, glutathione-*S* conjugates.

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Table 1. Values of Topological Polar Surface Area (TPSA) for MRP1 Reported Substrates and Nonsubstrates

compd	TPSA (Å^2)	ref
substrates		
1	216	5
3	232	9
4	221	10
5	318	11
6	159	10
10	121	6
11	150	6
daunorubicin	186	26
doxorubicin	206	12
etoposide	161	14
methotrexate	211	27
mitoxantrone	163	28
paclitaxel	221	26
vincristine	171	13
nonsubstrates		
betulinic acid	58	25
oleanolic acid	58	24
bufalin	67	29
artesunate	101	29
BSO	104	23
7	73	23
8	124	30
9	155	32
13	93	31

compound description section, the data for the TPSA were collected (http://www.ncbi.nlm.nih.gov/sites/entrez). The SciFinder Scholar Software under MEC/CAPES/MCT Brasil consortia license was also used.

Calculation of TPSA. For compounds reported as substrates for MRP1 but whose TPSA values were not available in the Pubchem compound database, the structures were collected from the published reports and/or using SciFinder Scholar Software and TPSA was calculated using the Fast Interactive Calculation of TPSA. This program is written as an add-in module to the Novartis JME Molecular Editor applet (http://www.daylight.com/meetings/emug00/Ertl/tpsa.html).

Statistical Analysis. Data are presented as the mean ± standard deviation. Student's test (Figure 2) was performed using Instat software. A value of $p < 0.05$ was considered statistically significant. The sigmoidal equation $f = a/\{1 + \exp[-(x - x_0)/b]\}$ was fitted to data, where x is the TPSA, using Sigmaplot software, version 11.0 (Systat Inc.). The determination coefficient (r^2) was used to measure the fit of the equation.

Results and Discussion

As shown in Table 1, compounds described as MRP1 substrates, including some anticancer drugs and physiological substrates, cover a wide range of TPSA values (121−318 Å^2). However, compounds characterized as MRP1 inhibitors or nonsubstrates, such as **7** (MK571)[23] and pentacyclic triterpenes,[24,25] have TPSA values ranging from 58 to 155 Å^2. Figure 1 shows that there is a significant difference ($p < 0.0001$) between TPSA values of MRP1 substrates (drugs or physiological substrates) and nonsubstrates (drugs or inhibitors). One hypothesis to explain this difference is the observation that transporter proteins create a hydrophilic environment in the membrane that allows polar substrates to cross the lipid bilayer. Thus, independent of its chemical class, the higher the TPSA of a molecule, the greater is its chance to be transported by MRP1.

However, the transition from nonsubstrates to substrates is not clearly defined, as some compounds with TPSA values ranging from 121 to 155 Å^2 can be transported or not. Thus, although **8** (PAK-104P)[30] (124 Å^2) and **9** (silybin)[32] (155 Å^2) are not transported by MRP1, **10** (LTE4)[6] (121 Å^2) and **11** (LTD4)[6] (150 Å^2) are. This led us to speculate whether in addition to TPSA, molecular geometry might interfere with binding-site recognition and thus be critical for the transport capabilities of some compounds. Indeed, we observed that the structures of **10** and **11** are quite similar to that of **1** (Figure 2), a well-known MRP1 substrate. The data published by Leier et al.[6] showed that the rate of ATP dependent MRP1 transport for the compounds **10** (121 Å^2), **11** (150 Å^2), and **1** (216 Å^2) are respectively 8, 15, and 55 (pmol/mg protein)/min. This suggests that the capacity of molecules with a similar structural pattern to be transported is proportional to their TPSA. Therefore, despite the low TPSA value of **10** and **11** for a substrate, the presence of a pharmacophore with high affinity for MRP1 in **10** and **11** may explain their behavior as a substrate. However, although the TPSA value could point to **9** as a MRP1 substrate, the lack of a pharmacophore with high affinity for MRP1 in its structure might be responsible for its inability to be transported. Therefore, in the edge of low TPSA substrates and high TPSA nonsubstrates, molecular geometry may be the critical determinant to define MRP1 transport.

We analyzed the relationship between affinity data (K_m) and TPSA values for compounds that had K_m values available in the literature (Table 2). A strong sigmoid relationship ($r^2 = 0.9996$) was found between TPSA and K_m (Figure 3), indicating that the affinity of MRP1 to the compounds tends to decrease with decreasing TPSA values. These data reinforced the relationship between TPSA and substrate transport by MRP1 even though, because of a lack of literature data, there is an interval between K_m of 290 and 5000 μM. In addition we also

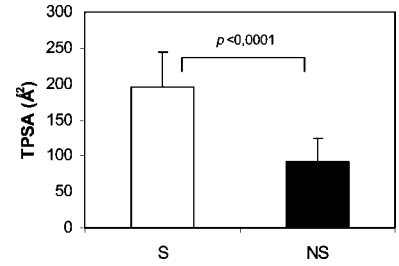

Figure 1. TPSA values for MRP1 substrates (S) and nonsubstrates (NS). Note that there is a significant difference between S and NS values ($P < 0.0001$). Values are expressed as the mean ± SD.

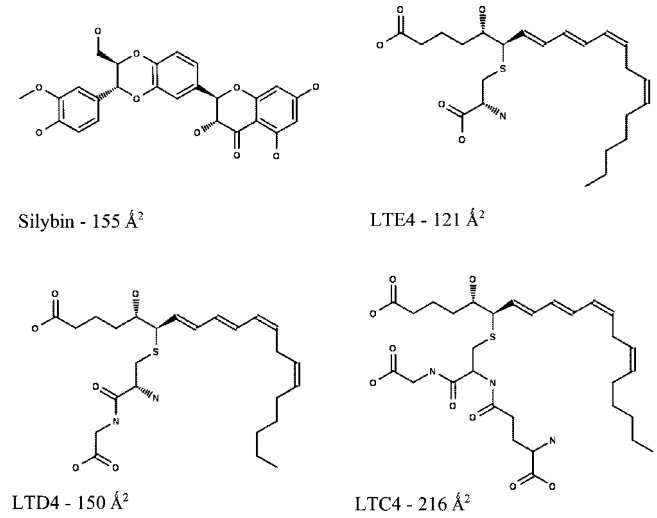

Figure 2. Structures of the compounds placed at the overlap zone and **1**, the substrate with the highest affinity.

Table 2. Affinity data (K_m) and TPSA Values for MRP1 Substrates

compd	K_m (μM)	TPSA (Å^2)	ref
1	0.097	216	6
12	0.19	250	8
arsenic triglutathione	0.32	552[b]	33
chlorambucil-GS	0.37	205[a]	34
melphalan-GS	1.1	231[a]	26
4-hydroxynonenal-GS	1.6	188	35
S-(2,4-dinitrophenyl)glutathione	8.08	245	26
4-nitroquinoline 1-oxide-GS	9.5	183[a]	36
4	12	221	10
5	93	318	11
metolachlor-GS	290	181[a]	15
6	5000	159	6

[a] TPSA fast calculator, [b] SciFinder Scholar software.

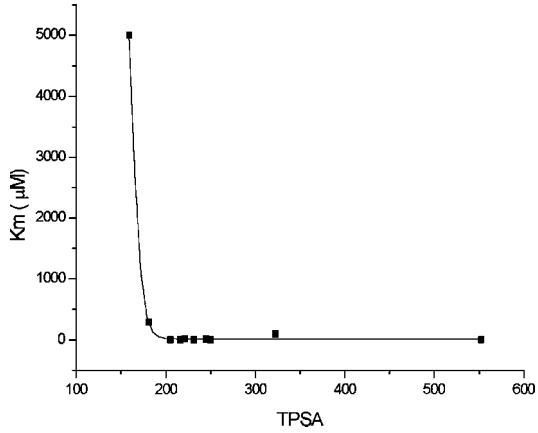

Figure 3. Sigmoid relationship between TPSA and the K_m of the MRP1 substrates.

analyzed the relation between K_m and log *P*. No significant correlation was found between these parameters (data not shown).

Data presented so far indicate that the main requirement for MRP1 transport is the TPSA value. However, previous investigations into the requirements for MPR1 drug transport showed that conjugation to **6** could be required or not required for transport.[33] Indeed, conjugated forms of drugs were found in tumor cells overexpressing MRP1[37] and it has also been shown that formation of glutathione-*S* conjugates of endogenous lipophilic compounds and xenobiotics often precedes their transport across cellular membranes.[34] Evidence that glutathionation can facilitate the transport of anthracyclines[38] and that depletion of **6** reduces drug transport by MRP1 and improves cytotoxicity[39] has also been reported. However, some investigations failed to identify the conjugated forms of several compounds on vesicles[8] and others observed that the synthesis inhibition of **6** did not affect the transport of compounds by MRP1.[40] In addition, it has been proposed that drugs are cotransported by MRP1 instead of being transported as GS-conjugates.[41]

If, as we suggest, the TPSA value is the main determinant for MRP1 transport, substances with high TPSA could be transported alone while those with low TPSA must be conjugated to **6** in order to be transported. It is known that compound **6** possesses a TPSA of 159 Å^2; therefore, conjugation with **6** can significantly increase ($p < 0.0001$) the TPSA value of a compound (Table 3, Figure 4), favoring its transport. Several studies not only showed that MRP1 and GST act as partner to improve drug transport[34,42,43] but also emphasized the importance of GST in cell detoxification.[44] Thus, the commonly transported form of **2**, which has a TPSA of 71 Å^2, is the higher

Table 3. Influence of Glutathionation on the Values of TPSA of Compounds Reported as MRP1 Substrates

	TPSA (Å^2)		
compd	UnC	C	ref
antitumor drugs			
13/**14**[b]	93	252[a]	45
chlorambucil	41	205[a]	47, 34
flutamide	72	237[a]	48
melphalan	67	231[a]	47
thiotepa	9	183[a]	37
4-hydroxynonenal	37	188	35
toxins			
2/**12**[b]	71	250	8
4-nitroquinoline 1-oxide	70	183[a]	36

[a] Values of TPSA calculated with TPSA fast calculator. [b] Unconjugated form/conjugated form.

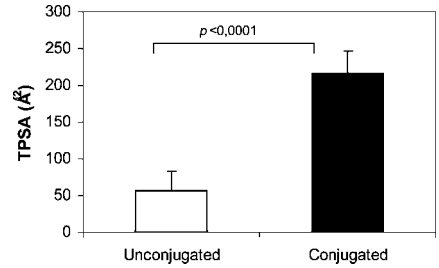

Figure 4. Conjugation to **6** increased TPSA values. The difference in the TPSA values between unconjugated (open bar) and conjugated (closed bar) compounds is statistically significant ($P < 0.0001$). Values are expressed as the mean ± SD.

TPSA **12** (aflatoxin−GS conjugate) (TPSA = 250 Å^2)[8]. Unconjugated **13** (curcumin), with TPSA of 93 Å^2, cannot be transported by MRP1.[30] However, when conjugated to **6** by GST, the resulting **14** (monoglutathionyl curcumin) has a TPSA of 252 Å^2 and becomes a substrate for MRP1.[45] These results indicate that the conjugation with **6** increases the TPSA and thus allows transport of nonsubstrates molecules. Site-directed mutagenesis at the binding site of MRP1 led to the identification of amino acid side chains essential for substrate binding and transport of **6**.[46] Thus, in addition to increasing drug TPSA, the conjugation to **6** seems to increase its chance of binding-site recognition. That this is due to the presence of the high affinity pharmacophore present in **6** remains to be investigated. It is interesting to note that **1**, which possesses the highest affinity for MRP1, is formed by the conjugation of 4-[(2*S*,3*S*)-3-[(1*E*,3*E*,5*Z*,8*Z*)-tetradeca-1,3,5,8-tetraenyl]oxiran-2-yl]butanoic acid (LTA4)[49] and **6**.

Conclusions

Drug discovery researchers have an outstanding challenge to overcome. They have to find a drug that is not a substrate for MRP1 (with low TPSA values); that is not substrate for GST because if it is, the conjugation raises the TPSA value, making the drug suitable for transport; and that does not totally block the pump, which is expressed in normal tissues, to avoid the toxicity induced by pump inhibitors. The analysis of the published data performed in this paper suggests that the promiscuity of MRP1 may be explained by the TPSA of the substrates. In conclusion, it is proposed that (1) MRP1 transports substrates with high TPSA values, (2) conjugation with **6** increases the TPSA to a critical value, making the drug suitable for transport, (3) there is a positive correlation between TPSA values and MRP1 affinity for their substrates, and (4) the presence of a pharmacophore with high affinity may allow a low TPSA compound to be transported by MRP1.

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