

## Antibiotic efflux pumps in eukaryotic cells: occurrence and impact on antibiotic cellular pharmacokinetics, pharmacodynamics and toxicodynamics

F. Van Bambeke\*, J.-M. Michot and P. M. Tulkens

Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, 73.70 avenue Mounier 73, B-1200 Brussels, Belgium

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Active efflux is now recognized as a key element in drug disposition and activity. Original observations were first limited to a few compounds examined in specific situations, such as anthracyclines in the context of resistance of cancer cells, and tetracyclines in the context of bacterial resistance. However, the combination of systematic surveys involving commonly used drugs and genome sequencing has identified ~20 families of drug transporters.<sup>1</sup> Many of them are ubiquitous, and are expressed in prokaryotes and archaea as well as in inferior and superior eukaryotes. A companion review<sup>2</sup> deals with antibiotic transporters in prokaryotes, where we examine their role and impact on intrinsic antimicrobial activity and resistance. We concentrate here on eukaryotic cells in general, and on animals (including man) in particular, to show how transporters need to be taken into account for a proper understanding as to how antibiotics are handled *in vivo*.

### Why are antibiotics transported in eukaryotic cells?

In general, drug transporters show broad specificity, recognizing a large number of compounds with unrelated pharmacological properties. This is because substrate recognition is based on physico-chemical properties, such as hydrophobicity, aromaticity, hydrogen binding capacity and an ionizable character (within a given spatial environment) rather than on defined chemical properties, as in classical enzyme–substrate or ligand–receptor recognition.<sup>3–7</sup> It is therefore no surprise that antibiotics are recognized by many transporters. More broadly, transporters act as a general means for cells to protect themselves from undesirable invasion by amphiphilic compounds, which freely diffuse across membranes. They may also serve to facilitate the transmembrane transport of endogenous molecules, such as phospholipids (by acting as flippases), cytokines, metabolic intermediates or nutrients.

Finally, they may serve as an influx mechanism for polar compounds or act as true transport functions across epithelial barriers in pluri-cellular organisms. In all of these situations, antibiotics, like other drugs, really appear as opportunistic substrates.<sup>8</sup>

### Occurrence and general properties of antibiotic transporters

Table 1 gives a summary of the main characteristics of the transporters that have been described as interacting with antibiotics in eukaryotic cells. It also gives, for each type of transporter, the best characterized non-antibiotic drug substrates and, when known, the non-drug substrates, often tentatively identified as the physiological substrates. Figure 1 shows the distribution of these transporters among the main cell types. Whereas some transporters are considered ubiquitous [for example, multiple drug resistance (MDR)1 and multidrug resistance-associated protein (MRP)1], many others show quite specific distribution. Moreover, the function of these transporters depends on their orientation. Accordingly, drug movement must be analysed in terms of influx or efflux not only at the level of a single cell, but also at that of the whole organism.

Efflux-oriented transport is mainly facilitated by the so-called multidrug transporters. If localized at the brush border membrane of polarized cells (for example, MDR1 and MRP2), they will cause accelerated clearance, although MDR1 in choroid cells<sup>9</sup> is responsible for increased concentration of its substrates in the CNS. Conversely, they will cause retention of the drug in the organism if they are located at the basolateral surface of polarized cells<sup>10</sup> (for example, MRP1 and MRP3). Moreover, some transporters, such as Na<sup>+</sup> phosphate transporter (NPT)1, found at the apical membrane of some cells but at the basolateral membrane of others,

\*Corresponding author. Tel +32-2-764-73-78; Fax +32-2-764-73-73; E-mail: vanbambeke@facm.ucl.ac.be

**Table 1.** Transporters involved in the transmembrane passage of antibiotics in eukaryotic cells

Superfamily	Family	Source of energy	Transporter	Physiological substrates	Typical examples of non-antibiotic substrates	Typical examples of inhibitors	Examples of recognized antibiotics	Organ where transport has been demonstrated	References
3.A.1. ABC	3.A.1.201. multidrug resistance exporter	ATP hydrolysis	MDR1	phospholipids cytokines	anthracyclines vincristine methotrexate	verapamil cyclosporin A GF120918 LY335979	<b>fluoroquinolones</b> levoﬂoxacin sparﬂoxacin grepafloxacin ofloxacin, ciproﬂoxacin HSR-903 <b>macrolides</b> erythromycin  clarithromycin  roxithromycin, josamycin, azithromycin, spiramycin <b>β-lactams:</b> <b>lipophilic</b> <b>cephalosporins</b> cefoperazone, ceftriaxone, cefazolin (ceftazidime, cefradine, cefotetan) dicloxacillin  <b>tetracyclines</b> tetracycline <b>streptogramins</b> pristinamycin <b>trimethoprim</b>	kidney intestine liver transfected cells brain  intestine transfected cells, liver kidney, transfected cells transfected cells  cancer cells  transfected renal cells transfected cells intestine  transfected renal cells	32 30 78 52 34  31,52 78 33,52  52  79  80 81 82 80



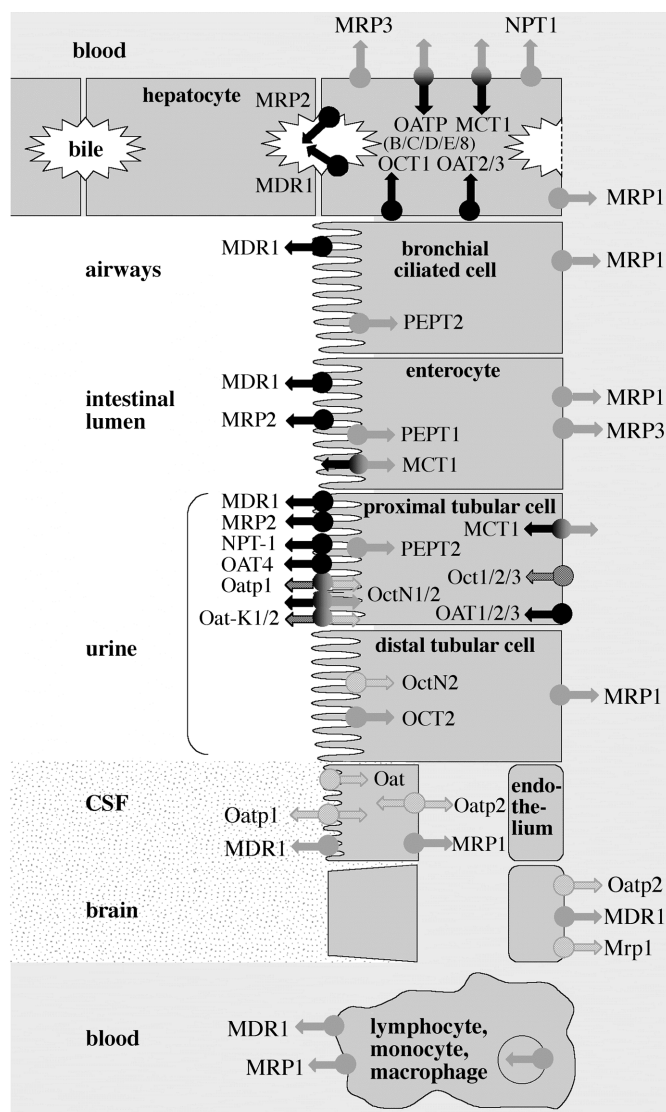
**Table 1.** (continued)

Superfamily	Family	Source of energy	Transporter	Physiological substrates	Typical examples of non-antibiotic substrates	Typical examples of inhibitors	Examples of recognized antibiotics	Organ where transport has been demonstrated	References	
1070	2.A.1.19 organic cation transporter family	ion uniport or ion:H <sup>+</sup> symport	OAT1	bile salt prostaglandins cyclic nucleotides	anionic drugs steroids NSAID diuretics	probenecid	<b>(fluoro)quinolones</b> nalidixic acid ofloxacin cinoxacin	kidney kidney kidney	14 69 17	
							<b>β-lactams</b> cefaloridine, cefalothin, cefazolin, cefalexin, carbenicillin cefoperazone, ceftriaxone, cefadroxil, cefamandole	kidney	17	
			OAT3	anionic neuro-transmitters	anionic drugs cimetidine	probenecid indocyanine green	<b>β-lactams</b> penicillin G cefazolin, cefoperazone, cefalothin, cefadroxil, cefamandole	brain kidney	88 87	
			OAT4	prostaglandins		probenecid	cefazolin, cefoperazone, cefalothin, cefaloridine, cefadroxil, cefamandole	kidney	87	
			OCT1	neuro-transmitters vitamins carnitine	cationic drugs	tetraethyl-ammonium	<b>fluoroquinolones</b> ofloxacin	kidney (rat)	69	
			OctN2	cation:Na <sup>+</sup> symport	quinidine verapamil	quinidine verapamil	<b>β-lactams (quaternary ammonium)</b> cefaloridine, cefepime, cefluprenam (cefoselis, cyclacillin)	kidney	26	

2.A.60. Organo anion transporter family transporter	2.A.60.1.	anion uniport or anion:anion antiport	Oatp1	bile salts steroid hormones	digoxin	indocyanine green	<b>rifampicin</b>	liver	20
2.A.17 Proton- dependent oligopeptide transporter family	2.A.17.4.	peptide:H <sup>+</sup> symport	PEPT1	peptides	protease inhibitors quinapril	sulfonylureas	<b>β-lactams</b> ceftibuten, cyclacillin, cefadroxil, cefamandole, cefradine, cefaclor, cefuroxime axetil, cefixime, cefalothin, cefalexin, ampicillin	intestine	24
			PEPT2	peptides	valaciclovir	sulfonylureas	<b>β-lactams</b> <b>(amino group on the phenyl ring)</b> cefalexin; cefadroxil, cefradine cefadroxil, cyclacillin	kidney	89
								kidney	25

MCT, monocarboxylate transporter; MDR, multiple drug resistance; MRP, multidrug resistance-associated protein; NPT, Na<sup>+</sup> phosphate transporter; OAT, organic anion transporter; OCT, organic cation transporter; PEPT, peptide transporter.

Nomenclature is based on that proposed by Saier (see the corresponding website,<sup>90</sup> which is regularly updated). It consists of five components, where the first and second components correspond to the transporter class and subclass, based on the mechanism of transport, the third and fourth components correspond to the family and subfamily, based on the phylogeny, and the last component (not given here because it differs for each transporter inside a family) corresponds to the range of substrates and the polarity of the transporter.



**Figure 1.** Schematic representation of the main transporters potentially involved in antibiotic movement at the level of epithelial cells in the main organs (liver, bronchial tree, intestine, kidney), the blood–brain barrier and in leucocytes (polymorphonuclear leucocytes are not considered here since the role of drug transporters in these cells is unclear). Black arrows denote transport towards extracorporeal compartments such as urine, bile, intestine and airways (i.e. transporters involved in drug elimination from the body). Grey arrows indicate uptake processes from extracorporeal fluids into cells (i.e. allowing drugs to accumulate in tissues), or from cells to body fluids [i.e. causing the drug to be transported from one body fluid to another (for example from blood to CSF)]. The level of expression of each transporter may differ between species (arrows with a chequerboard background indicate transporters evidenced, so far, in animals only). The direction of transport of bidirectional transporters may differ according to the cell type.

will have opposite effects on drug clearance, depending on the organ in which they are found. This explains, for instance, why  $\beta$ -lactams, which are substrates for this transporter, may be secreted at the level of the kidney but be reabsorbed at that of the liver.<sup>11,12</sup>

Influx transporters located at the basolateral membrane will increase the drug concentration within the epithelial cells. If these are bordering the external medium,<sup>13–15</sup> increased clearance can be obtained provided the drug can diffuse out of these cells. An excellent example is organic anion transporter (OAT)1, which is responsible for the tubular secretion of  $\beta$ -lactams.<sup>16,17</sup> Conversely, an inwards transporter localized at the brush border membrane of epithelial cells can indirectly increase the systemic concentration of its substrates by driving them into these cells, from where they can diffuse into the blood.<sup>18,19</sup>

Bidirectional transporters have also been found and these can take various roles depending on their localization.<sup>20–23</sup>

### Modulation of the absorption and elimination of antibiotics

The role of drug transporters in the modulation of antibiotic pharmacokinetics has been mainly studied for  $\beta$ -lactams, fluoroquinolones and, to a lesser extent, macrolides.  $\beta$ -Lactams are known as generally being poorly reabsorbed with, however, a few notable exceptions. These concern derivatives that have been shown to be substrates for either peptide transporter (PEPT)1—oral cephalosporins or ampicillin,<sup>24</sup> see also details in Table 1—or monocarboxylate transporter (MCT)—as is the case for carindacillin, the oral prodrug of carbenicillin.<sup>23</sup> In the same way, OctN2 and PEPT2 have been shown to facilitate the reabsorption of  $\beta$ -lactams from the renal tubular filtrate,<sup>25,26</sup> thereby prolonging their plasma half-life. OctN2 recognizes derivatives with a quaternary ammonium substituent (such as cefaloridine), whereas PEPT2 transports cephalosporins with an amino group in the substituent of the cephem nucleus (such as cefadroxil).<sup>26</sup> A critical role of drug transporters in the elimination of  $\beta$ -lactams through the renal and hepatobiliary tracts has also been suggested as implying that transporters are located at the basolateral and apical levels.<sup>17,27</sup> A concerted action, implying pairs of transporters localized at both the basolateral and apical poles of the hepatocytes, has also been proposed for the fluoroquinolone grepafloxacin (although fluoroquinolones are most probably able to diffuse freely across membranes) and its glucuronconjugates.<sup>28,29</sup> Similarly, clearance of both macrolides and fluoroquinolones can be accelerated by the action of MDR1 or MRP2, in the intestine, kidney, liver or CNS.<sup>29–34</sup>

### Barrier effects

Transporters identified at the blood–brain and the blood–CSF barriers probably play a key role in clearing the CNS of drugs and other toxins<sup>35–37</sup> (and Figure 1). This is probably most important for  $\beta$ -lactams and fluoroquinolones. Indeed a parallel has been observed between the propensity of fluoroquinolones to induce seizures<sup>38</sup> and their rate of efflux from

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the CNS.<sup>39</sup> Active efflux may be detrimental for treatment of meningitis and other infections of the CNS. For example, failures with cefalothin have been attributed to the active efflux of this molecule.<sup>40</sup>

Drug-inactivating mechanisms and drug transporters may also combine to cause more efficient barrier effects. This concept, which is well known in the case of resistant bacteria (see companion review),<sup>2</sup> is now increasingly recognized in mammals, where intestinal and liver transporters cooperate with cytochrome P450-based metabolism to decrease quickly and effectively the amount of active molecules present in the body. Thus, Phase I metabolism adds polar functions to drug molecules, which are further transformed into bioconjugates by Phase II enzymes. The increased polarity of metabolites favours their recognition by efflux pumps,<sup>41</sup> as demonstrated with MRP2 for grepafloxacin.<sup>29</sup> This has led to the concept of 'Phase III' elimination of drugs.<sup>42</sup> Interestingly, the orphan nuclear receptor SXR, which is activated upon exposure to substrates common to cytochromes P450 and MDR, can co-regulate the expression of these two clearance systems.<sup>43</sup> The subsequent change in their activity may shed a new light on the specific mechanisms of some drug–antibiotic interactions.<sup>44</sup> For instance, rifampicin reduces the blood level of several drugs by inducing both cytochrome P450 and MDR expression,<sup>45</sup> whereas erythromycin increases that of digoxin, by inhibiting the activity of both proteins.<sup>46</sup>

### Modulation of cellular accumulation of antibiotics

The intracellular concentration of antibiotics is considered to be an important determinant in their activity against intracellular organisms.<sup>47,48</sup> Monocytes, macrophages and lymphocytes have been shown to express MRP and MDR transporters<sup>49–51</sup> (Table 1), which have the potential to decrease cellular antibiotic concentrations and to impair their activity. This has been seen for fluoroquinolones, macrolides, streptogramins, lincosamides and rifampicin in cells infected by *Listeria monocytogenes* with also an overexpression of MDR1.<sup>52</sup> In contrast, gemfibrozil, an inhibitor of organic anion transporters, significantly improves the activity of fluoroquinolones against the same bacteria.<sup>53</sup> The impact of efflux pumps on antibiotic activity is, however, more difficult to predict when considering bacteria localized in the phagosomal (*Legionella pneumophila*, *Mycobacteria* spp.), or lysosomal (*Staphylococcus aureus*, *Salmonella* spp.) compartments. Efflux pumps are also found in intracellular structures and could therefore modify the subcellular distribution of their substrates.<sup>54,55</sup> We do not know, however, to what extent the cellular pools correspond to active proteins.<sup>56–58</sup> Modification of intracellular accumulation may also be associated with corresponding changes in toxicity. A well-known example is given by the  $\beta$ -lactams that are substrates

for the renal organic anion transporter (OAT)—such as cefaloridine. These are more nephrotoxic than other cephalosporins,<sup>16</sup> related to their increased accumulation in proximal tubular cells.<sup>17</sup> On the other hand, a lower hepatic concentration of rifampicin or erythromycin, through the activity of MDR1, lowers their ability to modulate cytochrome P450 activity.<sup>59,60</sup> In a wider context, multidrug transporters are also thought to play a protective role against apoptosis induced by several drugs, an effect that, however, could be due to mechanisms other than drug efflux itself (see 8 for review). It is noteworthy in this context that several antibiotic classes may be apoptogenic, for example, aminoglycosides,<sup>61–63</sup> macrolides,<sup>64</sup> fluoroquinolones<sup>65</sup> or chloramphenicol.<sup>66</sup>

### Strategies for the future

The role of transporters in the modulation of antibiotic pharmacokinetics should be taken into account in the future selection of drugs. In relation to the examples discussed in this paper, a prime example is the design of  $\beta$ -lactams with increased oral absorption and decreased elimination. It is unfortunate, however, that the substrate specificities of the intestinal PEPT1 and the renal PEPT2 transporters are not exactly the same (as shown in Table 1),<sup>25</sup> which may make it difficult to obtain molecules optimized with respect to both transporters. Another area of interest would be the selection of fluoroquinolones with decreased penetration into or retention within the CNS. Structure–activity relationships in this context and design of improved compounds appear, however, difficult, due to the multiplicity of transporters interacting with a given drug.<sup>67</sup> Despite this, one recent, successful example might be HSR-903.<sup>34,68</sup>

Inhibition of transporters may also prove useful. An historical example is probenecid, used for a long time as a sparing drug against the renal elimination of  $\beta$ -lactams and fluoroquinolones. We know today that this effect is mediated, at least in part, by the inhibitory effect probenecid exerts towards OAT and MRP2.<sup>69,70</sup> Similar effects on pharmacokinetics or cellular retention have been observed with gemfibrozil, and several other drugs (for example, verapamil and cyclosporin A), which are now known to be modulators of drug transport. The next step should be the design of new chemical entities able to inhibit selectively a given class of transporters, without exerting other pharmacological activities.<sup>71,72</sup> This has been partially achieved with preferential inhibitors of MDR or MRP, for instance,<sup>72–75</sup> some of which are currently being evaluated for their potential use in therapy.<sup>76,77</sup> A major unknown in this area is, however, the detrimental effects impairment of transporters may have on the handling of their natural substrates. Thus there is still room for further research aimed at a better understanding of the complex relationships between transporters and the

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pharmacokinetics, pharmacodynamics and toxicodynamics of antibiotics.

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