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

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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.[1] Many of them are ubiquitous, and are expressed in prokaryotes and archaea as well as in inferior and superior eukaryotes. A companion review[2] 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.[3–7] 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.[8]

## 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[9] 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[10] (for example, MRP1 and MRP3). Moreover, some transporters, such as $Na^+$ phosphate transporter (NPT)1, found at the apical membrane of some cells but at the basolateral membrane of others,

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**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 | **fluoroquinolones** | | |
| | | | | | | | levofloxacin | kidney | 32 |
| | | | | | | | sparfloxacin | intestine | 30 |
| | | | | | | | grepafloxacin | liver | 78 |
| | | | | | | | ofloxacin, ciprofloxacin | transfected cells | 52 |
| | | | | | | | HSR-903 | brain | 34 |
| | | | | | | | **macrolides** | | |
| | | | | | | | erythromycin | intestine | 31,52 |
| | | | | | | | | transfected cells, liver | 78 |
| | | | | | | | clarithromycin | kidney, transfected cells | 33,52 |
| | | | | | | | roxithromycin, josamycin, azithromycin, spiramycin | transfected cells | 52 |
| | | | | | | | **β-lactams: lipophilic cephalosporins** | | |
| | | | | | | | cefoperazone, ceftriaxone, cefazolin (ceftazidime, cefradine, cefotetan) | cancer cells | 79 |
| | | | | | | | dicloxacillin | transfected renal cells | 80 |
| | | | | | | | **tetracyclines** | transfected cells | 81 |
| | | | | | | | tetracycline | | |
| | | | | | | | **streptogramins** | intestine | 82 |
| | | | | | | | pristinamycin | | |
| | | | | | | | **trimethoprim** | transfected renal cells | 80 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 3.A.1.208. conjugate-transporter-2 | ATP hydrolysis; (glutathione as co-factor for some of them) | MRP1 | phospholipids leukotrienes conjugates glucurono-and glutathione-conjugates | anthracyclines vincristine methotrexate | probenecid gemfibrozil MK-571 | **fluoroquinolones** difloxacin | cancer cells | 83 |
| | | | | | | | | ofloxacin | transfected cells | 84 |
| | | | | | | | | **rifamycins** rifampicin | cancer cells | 85 |
| | | | | | | | | **macrolides** erythromycin | cancer cells | 84 |
| | | | | MRP2 | bilirubin-conjugates leukotrienes conjugates glucurono- and glutathione-conjugates | anthracyclines vincristine methotrexate | probenecid | **fluoroquinolones** HSR-903 | liver | 34 |
| | | | | | | | | grepafloxacin | liver | 28 |
| | | | | | | | | **β-lactams** cefodizime | liver | 27 |
| | | | | | | | | rifampicin | hepatic cells | 86 |
| | | | | MRP3 | glycocholate, bile salts glucurono-conjugates | methotrexate etoposide purine and nucleotide analogues | probenecid | **rifampicin** | hepatic cells | 86 |
| | | | | MRP5 | cyclic nucleotides | | probenecid sildenafil | **rifampicin** | hepatic cells | 86 |
| | 2.A.1. MFS | 2.A.1.13. monocarboxylate porter family | monocarboxylate uptake/efflux port | MCT1 | lactate, pyruvate, mevalonate | pravastatin | mersalyl acid | **β-lactams** cefdinir | intestine | 22 |
| | | | | | | | | carindacillin (prodrug of carbenicillin) | intestine | 23 |
| | | 2.A.1.14. anion:cation symporter family | anion:cation symport | NPT1 | phosphate | foscarnet | phosphono-formic acid | **β-lactams anionic > zwitterionic** cloxacillin, cefoperazone, cefpiramide, nafcillin, dicloxacillin, apalcillin, penicillin G, cefixime, (ceftizoxime, cefalexin, ampicillin, cefradine, cyclacillin, cefalothin, cefaloridine) | liver | 11 |
| | | | | | | | | faropenem | kidney | 12 |

**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 |
|---|---|---|---|---|---|---|---|---|---|
| | 2.A.1.19 organic cation transporter family | ion uniport or ion:$H^+$ symport | OAT1 | bile salt prostaglandins cyclic nucleotides | anionic drugs steroids NSAID diuretics | probenecid | **(fluoro)quinolones** nalidixic acid | kidney | 14 |
| | | | | | | | ofloxacin | kidney | 69 |
| | | | | | | | cinoxacin | kidney | 17 |
| | | | | | | | **β-lactams** cefaloridine, cefalothin, cefazolin, cefalexin, carbenicillin | kidney | 17 |
| | | | | | | | cefoperazone, ceftriaxone, cefadroxil, cefamandole | kidney | 87 |
| | | | OAT3 | anionic neuro-tranmsmitters | anionic drugs cimetidine | probenecid indocyanine green | **β-lactams** penicillin G | brain | 88 |
| | | | | | | | cefazolin, cefoperazone, cefalothin, cefaloridine, cefadroxil, cefamandole | kidney | 87 |
| | | | OAT4 | prostaglandins | | probenecid | cefazolin, cefoperazone, cefalothin, cefaloridine, cefadroxil, cefamandole | kidney | 87 |
| | | | OCT1 | neuro-transmitters vitamins | cationic drugs | tetraethyl-ammonium | **fluoroquinolones** ofloxacin | kidney (rat) | 69 |
| | | cation:$Na^+$ symport | OctN2 | carnitine | quinidine verapamil | quinidine verapamil | **β-lactams (quaternary ammonium)** 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 | **rifampicin** | liver | 20 |
| 2.A.17 Proton-dependent oligopeptide transporter family | 2.A.17.4. | peptide:$H^+$ symport | PEPT1 | peptides | protease inhibitors quinapril | sulfonylureas | **β-lactams** ceftibuten, cyclacillin, cefadroxil, cefamandole, cefradine, cefaclor, cefuroxime axetil, cefixime, cefalothin, cefalexin, ampicillin | intestine | 24 |
| | | | PEPT2 | peptides | valaciclovir | sulfonylureas | **β-lactams (amino group on the phenyl ring)** cefalexin; cefadroxil, cefradine | kidney | 89 |
| | | | | | | | cefadroxil, cyclacillin | kidney | 25 |

MCT, monocarboxylate transporter; MDR, multiple drug resistance; MRP, multidrug resistance-associated protein; NPT, $Na^+$ 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,[90] 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.

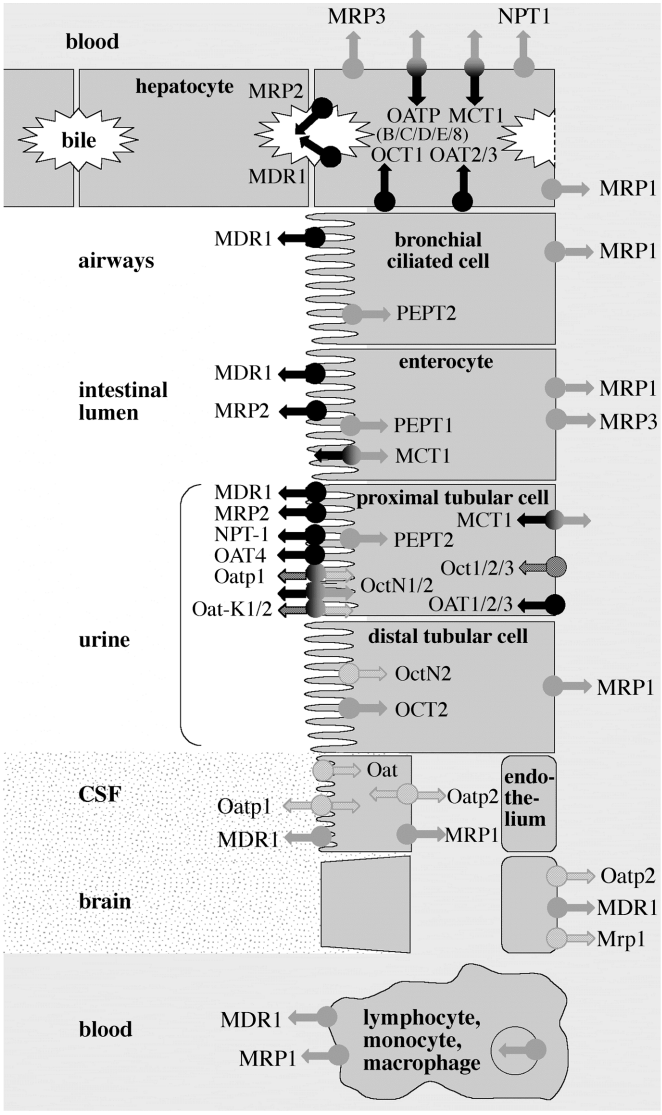


**Figure 1.** Schematic representation of the main transporters potentially involved in antibiotic movement at the level of epithelial cells in the main organs (liver, bronchial tree, intestine, kidney), the blood–brain barrier and in leucocytes (polymorphonuclear leucocytes are not considered here since 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 β-lactams, which are substrates for this transporter, may be secreted at the level of the kidney but be reabsorbed at that of the liver.[11,12]

Influx transporters located at the basolateral membrane will increase the drug concentration within the epithelial cells. If these are bordering the external medium,[13–15] 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 β-lactams.[16,17] 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.[18,19]

Bidirectional transporters have also been found and these can take various roles depending on their localization.[20–23]

## Modulation of the absorption and elimination of antibiotics

The role of drug transporters in the modulation of antibiotic pharmacokinetics has been mainly studied for β-lactams, fluoroquinolones and, to a lesser extent, macrolides. β-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,[24] see also details in Table 1—or monocarboxylate transporter (MCT)—as is the case for carindacillin, the oral prodrug of carbenicillin.[23] In the same way, OctN2 and PEPT2 have been shown to facilitate the reabsorption of β-lactams from the renal tubular filtrate,[25,26] 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).[26] A critical role of drug transporters in the elimination of β-lactams through the renal and hepatobiliary tracts has also been suggested as implying that transporters are located at the basolateral and apical levels.[17,27] 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 glucuronoconjugates.[28,29] Similarly, clearance of both macrolides and fluoroquinolones can be accelerated by the action of MDR1 or MRP2, in the intestine, kidney, liver or CNS.[29–34]

## 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[35–37] (and Figure 1). This is probably most important for β-lactams and fluoroquinolones. Indeed a parallel has been observed between the propensity of fluoroquinolones to induce seizures[38] and their rate of efflux from

the CNS.[39] 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.[40]

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),[2] 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,[41] as demonstrated with MRP2 for grepafloxacin.[29] This has led to the concept of 'Phase III' elimination of drugs.[42] 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.[43] The subsequent change in their activity may shed a new light on the specific mechanisms of some drug–antibiotic interactions.[44] For instance, rifampicin reduces the blood level of several drugs by inducing both cytochrome P450 and MDR expression,[45] whereas erythromycin increases that of digoxin, by inhibiting the activity of both proteins.[46]

## Modulation of cellular accumulation of antibiotics

The intracellular concentration of antibiotics is considered to be an important determinant in their activity against intracellular organisms.[47,48] Monocytes, macrophages and lymphocytes have been shown to express MRP and MDR transporters[49–51] (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.[52] In contrast, gemfibrozil, an inhibitor of organic anion transporters, significantly improves the activity of fluoroquinolones against the same bacteria.[53] 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.[54,55] We do not know, however, to what extent the cellular pools correspond to active proteins.[56–58] Modification of intracellular accumulation may also be associated with corresponding changes in toxicity. A well-known example is given by the β-lactams that are substrates for the renal organic anion transporter (OAT)—such as cefaloridine. These are more nephrotoxic than other cephalosporins,[16] related to their increased accumulation in proximal tubular cells.[17] 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.[59,60] 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,[61–63] macrolides,[64] fluoroquinolones[65] or chloramphenicol.[66]

## 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 β-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),[25] 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.[67] Despite this, one recent, successful example might be HSR-903.[34,68]

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 β-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.[69,70] 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.[71,72] This has been partially achieved with preferential inhibitors of MDR or MRP, for instance,[72–75] some of which are currently being evaluated for their potential use in therapy.[76,77] 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

pharmacokinetics, pharmacodynamics and toxicodynamics of antibiotics.

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## References

**1.** Saier, M. H., Jr (2000). A functional-phylogenetic classification system for transmembrane solute transporters. *Microbiology and Molecular Biology Reviews* **64**, 354–411.

**2.** Van Bambeke, F., Glupczynski, Y., Plésiat, P., Pechère,J. C. & Tulkens, P. M. (2003). Antibiotic efflux pumps in prokaryotic cells: occurrence, impact on resistance and strategies for the future of antimicrobial therapy. *Journal of Antimicrobial Chemotherapy* **51**, 1055–65.

**3.** Zheleznova, E. E., Markham, P., Edgar, R., Bibi, E., Neyfakh, A. A. & Brennan, R. G. (2000). A structure-based mechanism for drug binding by multidrug transporters. *Trends in Biochemical Sciences* **25**, 39–43.

**4.** Breier, A., Drobna, Z., Docolomansky, P. & Barancik, M. (2000). Cytotoxic activity of several unrelated drugs on L1210 mouse leukemic cell sublines with P-glycoprotein (PGP) mediated multidrug resistance (MDR) phenotype. A QSAR study. *Neoplasma* **47**, 100–6.

**5.** Ekins, S., Kim, R. B., Leake, B. F., Dantzig, A. H., Schuetz, E. G., Lan, L. B. *et al.* (2002). Application of three-dimensional quantitative structure–activity relationships of P-glycoprotein inhibitors and substrates. *Molecular Pharmacology* **61**, 974–81.

**6.** Salerno, M., Przewloka, T., Fokt, I., Priebe, W. & Garnier-Suillerot, A. (2002). Preferential efflux by P-glycoprotein, but not MRP1, of compounds containing a free electron donor amine. *Biochemical Pharmacology* **63**, 1471–9.

**7.** Stouch, T. R. & Gudmundsson, O. (2002). Progress in understanding the structure–activity relationships of P-glycoprotein. *Advanced Drug Delivery Reviews* **54**, 315–28.

**8.** Johnstone, R. W., Ruefli, A. A. & Smyth, M. J. (2000). Multiple physiological functions for multidrug transporter P-glycoprotein? *Trends in Biochemical Sciences* **25**, 1–6.

**9.** Rao, V. V., Dahlheimer, J. L., Bardgett, M. E., Snyder, A. Z., Finch, R. A., Sartorelli, A. C. *et al.* (1999). Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood–cerebrospinal-fluid drug-permeability barrier. *Proceedings of the National Academy of Sciences, USA* **96**, 3900–5.

**10.** Borst, P., Evers, R., Kool, M. & Wijnholds, J. (1999). The multidrug resistance protein family. *Biochimica et Biophysica Acta* **1461**, 347–57.

**11.** Yabuuchi, H., Tamai, I., Morita, K., Kouda, T., Miyamoto, K., Takeda, E. *et al.* (1998). Hepatic sinusoidal membrane transport of anionic drugs mediated by anion transporter Npt1. *Journal of Pharmacology and Experimental Therapeutics* **286**, 1391–6.

**12.** Uchino, H., Tamai, I., Yabuuchi, H., China, K., Miyamoto, K., Takeda, E. *et al.* (2000). Faropenem transport across the renal epithelial luminal membrane via inorganic phosphate transporter Npt1. *Antimicrobial Agents and Chermotherapy* **44**, 574–7.

**13.** Sekine, T., Cha, S. H., Tsuda, M., Apiwattanakul, N., Nakajima, N., Kanai, Y. *et al.* (1998). Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Letters* **429**, 179–82.

**14.** Sekine, T., Watanabe, N., Hosoyamada, M., Kanai, Y. & Endou, H. (1997). Expression cloning and characterization of a novel multispecific organic anion transporter. *Journal of Biological Chemistry* **272**, 18526–9.

**15.** Urakami, Y., Okuda, M., Masuda, S., Saito, H. & Inui, K. I. (1998). Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *Journal of Pharmacology and Experimental Therapeutics* **287**, 800–5.

**16.** Takeda, M., Tojo, A., Sekine, T., Hosoyamada, M., Kanai, Y. & Endou, H. (1999). Role of organic anion transporter 1 (OAT1) in cephaloridine (CER)-induced nephrotoxicity. *Kidney International* **56**, 2128–36.

**17.** Jariyawat, S., Sekine, T., Takeda, M., Apiwattanakul, N., Kanai, Y., Sophasan, S. *et al.* (1999). The interaction and transport of beta-lactam antibiotics with the cloned rat renal organic anion transporter 1. *Journal of Pharmacology and Experimental Therapeutics* **290**, 672–7.

**18.** Liang, R., Fei, Y. J., Prasad, P. D., Ramamoorthy, S., Han, H., Yang-Feng, T. L. *et al.* (1995). Human intestinal H+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. *Journal of Biological Chemistry* **270**, 6456–63.

**19.** Liu, W., Liang, R., Ramamoorthy, S., Fei, Y. J., Ganapathy, M. E., Hediger, M. A. *et al.* (1995). Molecular cloning of PEPT 2, a new member of the H+/peptide cotransporter family, from human kidney. *Biochimica et Biophysica Acta* **1235**, 461–6.

**20.** van Montfoort, J. E., Stieger, B., Meijer, D. K., Weinmann, H. J., Meier, P. J. & Fattinger, K. E. (1999). Hepatic uptake of the magnetic resonance imaging contrast agent gadoxetate by the organic anion transporting polypeptide Oatp1. *Journal of Pharmacology and Experimental Therapeutics* **290**, 153–7.

**21.** Masuda, S., Takeuchi, A., Saito, H., Hashimoto, Y. & Inui, K. (1999). Functional analysis of rat renal organic anion transporter OAT-K1: bidirectional methotrexate transport in apical membrane. *FEBS Letters* **459**, 128–32.

**22.** Tsuji, A., Tamai, I., Nakanishi, M., Terasaki, T. & Hamano, S. (1993). Intestinal brush-border transport of the oral cephalosporin antibiotic, cefdinir, mediated by dipeptide and monocarboxylic acid transport systems in rabbits. *Journal of Pharmacy and Pharmacology* **45**, 996–8.

**23.** Li, Y. H., Tanno, M., Itoh, T. & Yamada, H. (1999). Role of the monocarboxylic acid transport system in the intestinal absorption of

an orally active beta-lactam prodrug: carindacillin as a model. *International Journal of Pharmaceutics* **191**, 151–9.

**24.** Bretschneider, B., Brandsch, M. & Neubert, R. (1999). Intestinal transport of beta-lactam antibiotics: analysis of the affinity at the H+/peptide symporter (PEPT1), the uptake into Caco-2 cell monolayers and the transepithelial flux. *Pharmaceutical Research* **16**, 55–61.

**25.** Ganapathy, M. E., Brandsch, M., Prasad, P. D., Ganapathy, V. & Leibach, F. H. (1995). Differential recognition of beta-lactam antibiotics by intestinal and renal peptide transporters, PEPT 1 and PEPT 2. *Journal of Biological Chemistry* **270**, 25672–7.

**26.** Ganapathy, M. E., Huang, W., Rajan, D. P., Carter, A. L., Sugawara, M., Iseki, K. *et al.* (2000). Beta-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *Journal of Biological Chemistry* **275**, 1699–707.

**27.** Sathirakul, K., Suzuki, H., Yamada, T., Hanano, M. & Sugiyama, Y. (1994). Multiple transport systems for organic anions across the bile canalicular membrane. *Journal of Pharmacology and Experimental Therapeutics* **268**, 65–73.

**28.** Sasabe, H., Terasaki, T., Tsuji, A. & Sugiyama, Y. (1997). Carrier-mediated hepatic uptake of quinolone antibiotics in the rat. *Journal of Pharmacology and Experimental Therapeutics* **282**, 162–71.

**29.** Sasabe, H., Tsuji, A. & Sugiyama, Y. (1998). Carrier-mediated mechanism for the biliary excretion of the quinolone antibiotic grepafloxacin and its glucuronide in rats. *Journal of Pharmacology and Experimental Therapeutics* **284**, 1033–9.

**30.** Cormet-Boyaka, E., Huneau, J. F., Mordrelle, A., Boyaka, P. N., Carbon, C., Rubinstein, E. *et al.* (1998). Secretion of sparfloxacin from the human intestinal Caco-2 cell line is altered by P-glycoprotein inhibitors. *Antimicrobial Agents and Chermotherapy* **42**, 2607–11.

**31.** Takano, M., Hasegawa, R., Fukuda, T., Yumoto, R., Nagai, J. & Murakami, T. (1998). Interaction with P-glycoprotein and transport of erythromycin, midazolam and ketoconazole in Caco-2 cells. *European Journal of Pharmacology* **358**, 289–94.

**32.** Ito, T., Yano, I., Tanaka, K. & Inui, K. I. (1997). Transport of quinolone antibacterial drugs by human P-glycoprotein expressed in a kidney epithelial cell line, LLC-PK1. *Journal of Pharmacology and Experimental Therapeutics* **282**, 955–60.

**33.** Wakasugi, H., Yano, I., Ito, T., Hashida, T., Futami, T., Nohara, R. *et al.* (1998). Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. *Clinical Pharmacology and Therapeutics* **64**, 123–8.

**34.** Murata, M., Tamai, I., Kato, H., Nagata, O. & Tsuji, A. (1999). Efflux transport of a new quinolone antibacterial agent, HSR-903, across the blood–brain barrier. *Journal of Pharmacology and Experimental Therapeutics* **290**, 51–7.

**35.** Schinkel, A. H. (1999). P-Glycoprotein, a gatekeeper in the blood–brain barrier. *Advanced Drug Delivery Reviews* **36**, 179–94.

**36.** Sugiyama, Y., Kusuhara, H. & Suzuki, H. (1999). Kinetic and biochemical analysis of carrier-mediated efflux of drugs through the blood–brain and blood–cerebrospinal fluid barriers: importance in the drug delivery to the brain. *Journal of Controlled Release* **62**, 179–86.

**37.** Terasaki, T. & Hosoya, K. (1999). The blood–brain barrier efflux transporters as a detoxifying system for the brain. *Advanced Drug Delivery Reviews* **36**, 195–209.

**38.** De Sarro, A., Cecchetti, V., Fravolini, V., Naccari, F., Tabarrini, O. & De Sarro, G. (1999). Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice. *Antimicrobial Agents and Chermotherapy* **43**, 1729–36.

**39.** Ooie, T., Terasaki, T., Suzuki, H. & Sugiyama, Y. (1997). Kinetic evidence for active efflux transport across the blood–brain barrier of quinolone antibiotics. *Journal of Pharmacology and Experimental Therapeutics* **283**, 293–304.

**40.** Suzuki, H., Terasaki, T. & Sugiyama, Y. (1997). Role of efflux transport across the blood–brain barrier and blood–cerebrospinal fluid on the disposition of xenobiotics in the central nervous system. *Advanced Drug Delivery Reviews* **25**, 257–85.

**41.** Benet, L. Z., Izumi, T., Zhang, Y., Silverman, J. A. & Wacher, V. J. (1999). Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *Journal of Controlled Release* **62**, 25–31.

**42.** Ishikawa, T. (1992). The ATP-dependent glutathione S-conjugate export pump. *Trends in Biochemical Sciences* **17**, 463–8.

**43.** Synold, T. W., Dussault, I. & Forman, B. M. (2001). The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nature Medicine* **7**, 584–90.

**44.** Leveque, D. & Jehl, F. (1995). P-glycoprotein and pharmacokinetics. *Anticancer Research* **15**, 331–6.

**45.** Hebert, M. F., Roberts, J. P., Prueksaritanont, T. & Benet, L. Z. (1992). Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clinical Pharmacology and Therapeutics* **52**, 453–7.

**46.** Kim, R. B., Wandel, C., Leake, B., Cvetkovic, M., Fromm, M. F., Dempsey, P. J. *et al.* (1999). Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharmaceutical Research* **16**, 408–14.

**47.** Tulkens, P. M. (1990). Intracellular pharmacokinetics and localization of antibiotics as predictors of their efficacy against intraphagocytic infections. *Scandinavian Journal of Infectious Diseases*: *Supplementum* **74**, 209–17.

**48.** Nix, D. E., Goodwin, S. D., Peloquin, C. A., Rotella, D. L. & Schentag, J. J. (1991). Antibiotic tissue penetration and its relevance: impact of tissue penetration on infection response. *Antimicrobial Agents and Chermotherapy* **35**, 1953–9.

**49.** Puddu, P., Fais, S., Luciani, F., Gherardi, G., Dupuis, M. L., Romagnoli, G. *et al.* (1999). Interferon-gamma up-regulates expression and activity of P-glycoprotein in human peripheral blood monocyte-derived macrophages. *Laboratory Investigation* **79**, 1299–309.

**50.** Ichibangase, Y., Yamamoto, M., Yasuda, M., Houki, N. & Nobunaga, M. (1998). Induction of drug resistance to gold sodium thiomalate in a monocyte cell line, THP-1. *Clinical Rheumatology* **17**, 214–8.

**51.** Jones, K., Bray, P. G., Khoo, S. H., Davey, R. A., Meaden, E. R., Ward, S. A. *et al.* (2001). P-Glycoprotein and transporter MRP1 reduce HIV protease inhibitor uptake in CD4 cells: potential for accelerated viral drug resistance? *AIDS* **15**, 1353–8.

**52.** Nichterlein, T., Kretschmar, M., Schadt, A., Meyer, A., Wildfeuer, A., Laufen, H. *et al.* (1998). Reduced intracellular activity of antibiotics against *Listeria monocytogenes* in multidrug resistant cells. *International Journal of Antimicrobial Agents* **10**, 119–25.

**53.** Rudin, D. E., Gao, P. X., Cao, C. X., Neu, H. C. & Silverstein, S. C. (1992). Gemfibrozil enhances the listeriacidal effects of fluoroquinolone antibiotics in J774 macrophages. *Journal of Experimental Medicine* **176**, 1439–47.

**54.** Kim, H., Barroso, M., Samanta, R., Greenberger, L. & Sztul, E. (1997). Experimentally induced changes in the endocytic traffic of P-glycoprotein alter drug resistance of cancer cells. *American Journal of Physiology* **273**, C687–702.

**55.** Oh, Y. K. & Straubinger, R. M. (1997). Cellular retention of liposome-delivered anionic compounds modulated by a probenecid-sensitive anion transporter. *Pharmaceutical Research* **14**, 1203–9.

**56.** Molinari, A., Cianfriglia, M., Meschini, S., Calcabrini, A. & Arancia, G. (1994). P-glycoprotein expression in the Golgi apparatus of multidrug-resistant cells. *International Journal of Cancer* **59**, 789–95.

**57.** Molinari, A., Calcabrini, A., Meschini, S., Stringaro, A., Del Bufalo, D., Cianfriglia, M. *et al*. (1998). Detection of P-glycoprotein in the Golgi apparatus of drug-untreated human melanoma cells. *International Journal of Cancer* **75**, 885–93.

**58.** Larsen, A. K., Escargueil, A. E. & Skladanowski, A. (2000). Resistance mechanisms associated with altered intracellular distribution of anticancer agents. *Pharmacology and Therapeutics* **85**, 217–29.

**59.** Schuetz, E. G., Schinkel, A. H., Relling, M. V. & Schuetz, J. D. (1996). P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and humans. *Proceedings of the National Academy of Sciences, USA* **93**, 4001–5.

**60.** Schuetz, E. G., Yasuda, K., Arimori, K. & Schuetz, J. D. (1998). Human MDR1 and mouse mdr1a P-glycoprotein alter the cellular retention and disposition of erythromycin, but not of retinoic acid or benzo(a)pyrene. *Archives of Biochemistry and Biophysics* **350**, 340–7.

**61.** Nakagawa, T., Yamane, H., Shibata, S. & Nakai, Y. (1997). Gentamicin ototoxicity induced apoptosis of the vestibular hair cells of guinea pigs. *European Archives of Oto-rhino-laryngology* **254**, 9–14.

**62.** Nakagawa, T., Yamane, H., Takayama, M., Sunami, K. & Nakai, Y. (1998). Dose-dependent response of vestibular hair cells of guinea pigs following streptomycin ototoxiation. *Acta Otolaryngologica* **118**, 530–3.

**63.** El Mouedden, M., Laurent, G., Mingeot-Leclercq, M. P., Taper, H. S., Cumps, J. & Tulkens, P. M. (2000). Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. *Antimicrobial Agents and Chermotherapy* **44**, 665–75.

**64.** Aoshiba, K., Nagai, A. & Konno, K. (1995). Erythromycin shortens neutrophil survival by accelerating apoptosis. *Antimicrobial Agents and Chermotherapy* **39**, 872–7.

**65.** Aranha, O., Zhu, L., Alhasan, S., Wood, D. P., Jr, Kuo, T. H. & Sarkar, F. H. (2002). Role of mitochondria in ciprofloxacin induced apoptosis in bladder cancer cells. *Journal of Urology* **167**, 1288–94.

**66.** Turton, J. A., Yallop, D., Andrews, C. M., Fagg, R., York, M. & Williams, T. C. (1999). Haemotoxicity of chloramphenicol succinate in the CD-1 mouse and Wistar Hanover rat. *Human and Experimental Toxicology* **18**, 566–76.

**67.** Tamai, I., Yamashita, J., Kido, Y., Ohnari, A., Sai, Y., Shima, Y. *et al*. (2000). Limited distribution of new quinolone antibacterial agents into brain caused by multiple efflux transporters at the blood–brain barrier. *Journal of Pharmacology and Experimental Therapeutics* **295**, 146–52.

**68.** Tsuji, A. (1999). Tissue selective drug delivery utilizing carrier-mediated transport systems. *Journal of Controlled Release* **62**, 239–44.

**69.** Foote, E. F. & Halstenson, C. E. (1998). Effects of probenecid and cimetidine on renal disposition of ofloxacin in rats. *Antimicrobial Agents and Chermotherapy* **42**, 456–8.

**70.** Bakos, E., Evers, R., Sinko, E., Varadi, A., Borst, P. & Sarkadi, B. (2000). Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. *Molecular Pharmacology* **57**, 760–8.

**71.** Ford, J. M. & Hait, W. N. (1990). Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacological Reviews* **42**, 155–99.

**72.** Krishna, R. & Mayer, L. D. (2000). Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *European Journal of Pharmacological Science* **11**, 265–83.

**73.** Hyafil, F., Vergely, C., Du, V. P. & Grand-Perret, T. (1993). *In vitro* and *in vivo* reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Research* **53**, 4595–602.

**74.** Gekeler, V., Ise, W., Sanders, K. H., Ulrich, W. R. & Beck, J. (1995). The leukotriene LTD4 receptor antagonist MK571 specifically modulates MRP associated multidrug resistance. *Biochemical and Biophysical Research Communications* **208**, 345–52.

**75.** Dantzig, A. H., Shepard, R. L., Cao, J., Law, K. L., Ehlhardt, W. J., Baughman, T. M. *et al*. (1996). Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. *Cancer Research* **56**, 4171–9.

**76.** Dantzig, A. H., Law, K. L., Cao, J. & Starling, J. J. (2001). Reversal of multidrug resistance by the P-glycoprotein modulator, LY335979, from the bench to the clinic. *Current Medicinal Chemistry* **8**, 39–50.

**77.** Kruijtzer, C. M., Beijnen, J. H., Rosing, H., Bokkel Huinink, W. W., Schot, M., Jewell, R. C. *et al*. (2002). Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. *Journal of Clinical Oncology* **20**, 2943–50.

**78.** Zhao, Y. L., Cai, S. H., Wang, L., Kitaichi, K., Tatsumi, Y., Nadai, M. *et al*. (2002). Possible involvement of P-glycoprotein in the biliary excretion of grepafloxacin. *Clinical and Experimental Pharmacology and Physiology* **29**, 167–72.

**79.** Gosland, M. P., Lum, B. L. & Sikic, B. I. (1989). Reversal by cefoperazone of resistance to etoposide, doxorubicin, and vinblastine in multidrug resistant human sarcoma cells. *Cancer Research* **49**, 6901–5.

**80.** Susanto, M. & Benet, L. Z. (2002). Can the enhanced renal clearance of antibiotics in cystic fibrosis patients be explained by P-glycoprotein transport? *Pharmaceutical Research* **19**, 457–62.

**81.** George, A. M., Davey, M. W. & Mir, A. A. (1996). Functional expression of the human MDR1 gene in *Escherichia coli*. *Archives of Biochemistry and Biophysics* **333**, 66–74.

**82.** Phung-Ba, V., Warnery, A., Scherman, D. & Wils, P. (1995). Interaction of pristinamycin IA with P-glycoprotein in human intestinal epithelial cells. *European Journal of Pharmacology* **288**, 187–92.

**83.** Gollapudi, S., Thadepalli, F., Kim, C. H. & Gupta, S. (1995). Difloxacin reverses multidrug resistance in HL-60/AR cells that overexpress the multidrug resistance-related protein (MRP) gene. *Oncology Research* **7**, 213–25.

**84.** Terashi, K., Oka, M., Soda, H., Fukuda, M., Kawabata, S., Nakatomi, K. *et al.* (2000). Interactions of ofloxacin and erythromycin with the multidrug resistance protein (MRP) in MRP-overexpressing human leukemia cells. *Antimicrobial Agents and Chermotherapy* **44**, 1697–700.

**85.** Courtois, A., Payen, L., Vernhet, L., de Vries, E. G., Guillouzo, A. & Fardel, O. (1999). Inhibition of multidrug resistance-associated protein (MRP) activity by rifampicin in human multidrug-resistant lung tumor cells. *Cancer Letters* **139**, 97–104.

**86.** Schrenk, D., Baus, P. R., Ermel, N., Klein, C., Vorderstemann, B. & Kauffmann, H. M. (2001). Up-regulation of transporters of the MRP family by drugs and toxins. *Toxicology Letters* **120**, 51–7.

**87.** Takeda, M., Babu, E., Narikawa, S. & Endou, H. (2002). Interaction of human organic anion transporters with various cephalosporin antibiotics. *European Journal of Pharmacology* **438**, 137–42.

**88.** Kusuhara, H., Sekine, T., Utsunomiya-Tate, N., Tsuda, M., Kojima, R., Cha, S. H. *et al.* (1999). Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. *Journal of Biological Chemistry* **274**, 13675–80.

**89.** Terada, T., Saito, H., Mukai, M. & Inui, K. (1997). Recognition of beta-lactam antibiotics by rat peptide transporters, PEPT1 and PEPT2, in LLC-PK1 cells. *American Journal of Physiology* **273**, F706–11.

**90.** Saier, M. H., Jr (2002). [Online.] http://www-biology.ucsd.edu/~msaier/transport/ (last accessed 15 February 2003).