in vivo *21*: 237-244 (2007)

Review

Comparison of Multidrug Resistant Efflux Pumps of Cancer and Bacterial Cells with Respect to the Same Inhibitory Agents

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Abstract. *Bacteria and cancer cells develop resistance to more than one agent as a consequence of being exposed to ineffective levels of the agent for a prolonged period of time. The resistance of these cells is mediated by over-expressed efflux pumps that have the ability to extrude a large variety of unrelated chemicals. This review discusses the main types of multidrug resistant (MDR) efflux systems of bacteria and cancer cells, and shows the similarity of specific efflux systems between them with respect to given agents that inhibit efflux, thus rendering these cells once more susceptible to agents to which they had developed MDR.*

Despite the huge range of available antibiotics, numbering in thousands, mortality caused by bacteria continues to escalate in both economically favoured and disadvantaged countries. Globally, respiratory infections caused by non-mycobacteria and *Mycobacterium tuberculosis* kill over four million people world-wide and those bacteria that cause diarrhoeal disease kill an additional 1.75 million per year (1). Globally, over 20% of all deaths result from bacterial infections (2). The rates of bacterial infections and ensuing mortality are expected to rise dramatically due to the emergence of HIV infections world-wide and subsequent development of AIDS. Although bacterial infections are of great concern, they are not the major health threat encountered globally. According to the WHO Global Report on Cancer 2000 (3), 6 million deaths were caused by cancer world-wide. More disturbing is the prediction made by the WHO, namely that by the year 2020 at least 15 million will die from cancer (3).

Why are global bacterial infections on the rise despite the availability of a large and ever-increasing gamut of antibiotics? The advent of AIDS and the predisposition of this viral infection to co-infection by bacteria is, of course, part of the problem. But as of the time writing, it is minor in comparison to the problem of emerging resistance of bacterial pathogens. As an example, the sensitivity of *Staphylococcus aureus* to the first natural antibiotic, penicillin, was essentially 100% in the early 1950's. However, within the span of one decade, resistance to the beta-lactam methicillin was evident; today, 60 to 80% of all clinical isolates of *Staphylococcus aureus* are resistant to methicillin (oxacillin) (4). Acquisition of methicillin resistant *S. aureus* (MRSA), once thought to be nosocomially mediated, is now known to have part of its origin in the community (5-8). The period of time for global development of resistance to oxacillin by *Staphylococcus aureus* was exceeded by the organism's resistance to fluoroquinolones, namely within a few years (9), making this antibiotic relatively useless today. The problem of antibiotic resistance of *Staphylococcus aureus* has become more acute now that resistance to the last drug of defence, vancomycin, is increasing (10).

Although antibiotic resistance is now common for all important pathogenic bacteria (11), multidrug resistance (MDR) of *Mycobacterium tuberculosis* (9, 10, 12), Gram-negative bacteria (13), enterococci (14), streptococci (15) and the like have seriously affected the selection of therapeutic modality.

Bacterial Efflux Pumps

All living cells contain genes that code for proteins which make up efflux pumps that have the capacity to extrude

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Key Words: Multidrug resistance (MDR), efflux pumps, MDR bacteria, MDR cancer cells, inhibitory agents, review.

noxious agents present in the environment, or extrude toxins that are produced by the cell which require immediate removal and extrusion (16).

The efflux pumps of bacteria that are responsible for multi-drug resistance are part of the Superfamily ATP binding cassette of ABC transporters. The classification of this superfamily is as follows: the Major Facilitator Superfamily (MFS); the multi-drug/toxic extrusion (MATE) family; the Small Multi-drug Resistance (SMR) family; and the Resistance Nodulation Division (RND) family. The efflux pumps of each family are further characterized by the type of energy which drives them, the number of proteins that make up the pump, the number of domains of the transporter protein of the efflux pump spanning the plasma membrane, the type or types of substrates extruded by the pump, the type of agents that inhibit the extrusion of the substrate by competition for the active site of the transporter and the type of agents that inhibit the extrusion of the substrate on a non-competitive basis. As is evident from these characteristics, efflux pumps function very much as enzymes, and therefore factors such as the substrate extrusion, competitive inhibitors, non-competitive inhibitors, temperature, concentrations of substrate should be defined and quantified along Michaelis-Menten lines. Unfortunately, at the time of writing, the application of basic enzymological rules has yet to be applied for the characterization of efflux pump activity.

MDR phenotypic resistance produced by over-expression of efflux pumps of clinical isolates has been identified for *Staphylococcus aureus* (17), *E. coli* (18), Enterobacteriaceae (19) and *Pseudomonas aeruginosa* (20). It is only a matter of time before MDR of clinical isolates is shown to be primarily due to over-expressed efflux pumps. Assuming that this prediction comes to pass, it is important to understand the nature of efflux pumps of Gram-negative and positive bacteria, how over-expression of these efflux pumps develops in patients and what the agents are that may be used as lead compounds inasmuch as they have the capacity to inhibit the activity of specific efflux pumps.

Simple one protein efflux pumps. As a rule, Gram-positive bacteria contain efflux pumps consisting of one distinct transporter protein that is inhibited by agents that inhibit P-glycoprotein of cancer cells (21-23). *Staphylococcus aureus* is now known to have a number of efflux pumps that belong to different families of the ABC transporter superfamily. The pump extruding fluoroquinolones is the NorA and belongs to the SMR family; the pump that extrudes acriflavin, benzalkonium, chlorhexidine (detergents) is the QacA and belongs to the MF superfamily; the one that extrudes aminoglycosides and cationic drugs is the NorM, belonging to the MATE family. These efflux pumps that are over-expressed in clinical isolates of *Staphylococcus aureus* can be inhibited by reserpine (24-25), and phenothiazines (24, 25) but not by verapamil (24), the common inhibitor of efflux pumps of cancer cells. Further characterization of efflux pumps of *Staphylococcus aureus* as a consequence of differential responses to a variety of agents (27, 28) indicates that some pumps may be of a mixed type (28). It seems certain that additional efflux pumps will be identified in clinical isolates of *Staphylococcus aureus*.

Tri-partite efflux pumps. Gram-negative bacterial isolates that exhibit an MDR phenotype have received considerable attention due to problematic therapy of these infections. The major cause for the MDR phenotype is due to over-expressed efflux pumps that are part of the RND family of transporters. These efflux pumps have the ability to recognize and extrude a large variety of unrelated antibiotics from the periplasmic space of the cell envelope, or from the cytoplasm, and hence prevent these agents from reaching their intended targets. All RND efflux pumps consist of three proteins: the membrane fusion protein, located in the periplasmic space of the cell envelope performing an ancillary function in the extrusion of the antibiotic; the transporter protein, which performs the actual transport of the agent from the periplasm/cytoplasm; and the TolC protein, which is an outer membrane protein creating the channel through which the agent being transported reaches the environment in which the bacterium is found. The energy required for the operation of the efflux pump is provided by the proton motive force created by the proton gradient resulting from electron transport. The demonstration of the need for the proton gradient is provided by the exposure of MDR Gram-negative bacteria to the uncoupler of the proton motive force, carbonyl cyanide m-chlorophenyl hydrazone (CCCP). When CCCP is added to the medium, the ability of the efflux pump to extrude the fluorescent substrate ethidium bromide (EB) is lost and EB rapidly accumulates within the cell and intercalates between the nucleic bases of the DNA. However, because the proton gradient results from electron transport activity, prolonged exposure of the MDR bacteria to 4˚C reduces the generation of protons, and when this occurs the ability of the efflux pump to extrude EB is highly curtailed (29). Agents that inhibit the binding of calcium to calcium-dependent ATPase will also inhibit the efflux pump of Gram-negative bacteria (30, 31), as well as those of *Mycobacterium tuberculosis* that are over expressed (32). The inhibition of ATPase by these inhibitors of calcium binding/transport will therefore reduce the generation of protons and therefore reduce the energy provided by the proton gradient.

Gram-negative bacteria contain many distinct tripartite efflux pumps. As an example, although the main efflux pump of *E. coli* is coded by the operon acrAB/TolC, there

are at least 9 others whose operons have also been fully defined (30). Because as much as 35% of the Gram-negative bacterial genome is devoted to the coding of efflux pump proteins (33), there may be as many as 28 or more such transporter systems in Gram-negative bacteria (33). However, are these efflux pumps active at the same time, or is there a sequence of efflux pumps that are activated when the bacterium is placed under environmental stress such as that produced by the presence of antibiotics? The main efflux pump of *E. coli* (acrABTolC) is coded by three genes that make up one efflux pump operon: acrA codes for the fusion protein, acrB codes for the transporter protein and TolC codes for the outer membrane channel protein, TolC. When the acrAB operon is deleted, the efflux pump coded by the acrEFTolC operon takes over (30). However, when acrAB-deleted *E. coli* strains are exposed to increasing concentrations of tetracycline, the acrEF efflux pump is increased 80-fold over that present in the unexposed parental strain (30). The remaining 8 efflux pumps of the acrAB deleted strains are also increased as much as four-fold. Although similar exposure of the intact acrAB wild-type strain to increasing concentrations of tetracycline does increase the activities of all 9 efflux pumps, the increases are far lower than those observed with the acrAB-deleted strain. This supports the contention that it is the acrAB system which is the major RND efflux pump of *E. coli* and that, when this pump is deleted, the necessary extrusion of tetracycline for the survival of the organism is accomplished by a huge over-expression of the acrEF and, to lesser extent, of the remaining efflux pumps. That there is differential activity between over-expressed acrAB and acrEF efflux pumps is supported by the inhibition of the over-expressed acrAB system by Phe-Arg-napthylamide (MC-207,110; PAN), whereas this agent does not inhibit the over-expressed acrEF pump (30). Other Gram-negative bacteria have been fully characterized for their RND tri-partite efflux pumps and the reader is directed to some excellent reviews (34-36).

ABC Transporters as Efflux Pumps of Cancer Cells

Over 200 proteins involved in the transport of substrates across biological membranes are members of the ABC superfamily of proteins, also known as the traffic ATPases (37, 38). A typical ABC transporter protein consists of four units. Two are membrane-bound domains (TM), both with six trans-membrane segments, and two are nucleotide-binding domains (NBD), which bind and hydrolyze ATP. Two sequence motifs located 100-200 amino acids apart in each NBD, designated Walker A and Walker B, are conserved among all ABC transporter superfamily members, as well as in numerous other ATP-binding proteins (39). In addition, unique to ABC proteins, there is a third, highly conserved amino acid sequence (ALSGGQ) located between the Walker A and B motifs, referred to as the ABC signature motif (or C motif). The precise function of this sequence has not yet been determined although it has been directly implicated in the recognition, binding and hydrolysis of ATP (38).

The most common member of ABC transporter is the 170 kDa multidrug resistance protein 1 (MDR1) or P-glycoprotein (P-gp) (encoded by *ABCB*1) (39). The isolation of a second distantly related ABC protein, the 190-kDa multidrug resistance related protein 1 (MRP1) (encoded by *ABCC*1) facilitated the discovery of eight more genes within the same ABC subfamily, of which at least six: MRP2 (encoded by *ABCC*2), MRP3 (encoded by *ABCC*3), MRP4 (encoded by *ABCC*4), MRP5 (encoded by *ABCC*5), MRP6 (*ABCC*6) and MRP7 (encoded by *ABCC*10) are potentially involved in mediating drug resistance (40-44). Two additional members, MRP8 (*ABCC*11) and MRP9 (*ABCC*12), have been reported more recently (45, 46).

A third drug transporter, also distantly related to P-glycoprotein and the MRPs, is the breast cancer resistance protein (BCRP) (encoded by *ABCG*2) originally isolated from a multi-drug resistant breast cancer cell line co-selected with doxorubicin and verapamil (47, 48).

Although the lung resistance protein (LRP) is not an ABC transporter, it is frequently included in discussions of drug resistance as it is expressed at high levels in drug-resistant cell lines and some tumors (49, 50). LRP is a major vault protein found in the cytoplasm and on the nuclear membrane. Vaults are large ribonucleoprotein particles that are present in all eukaryotic cells and might confer drug resistance by redistributing drugs away from intracellular targets.

In addition to their role in drug resistance, there is substantial evidence that these efflux pumps have overlapping functions in tissue defense. Collectively, these proteins are capable of transporting a vast and chemically diverse array of toxicants, including bulky lipophilic cationic, anionic, and neutrally charged drugs and toxins as well as conjugated organic anions that encompass dietary and environmental carcinogens, pesticides, metals, metalloids, and lipid peroxidation products. P-glycoprotein, MRP1, MRP2 and BCRP/ABCG2 are expressed in tissues important for absorption (*e.g.*, lung and gut) and metabolism and elimination (liver and kidney) (38). In addition, these transporters have an important role in maintaining the barrier function of sanctuary site tissues (*e.g.*, blood-brain barrier, blood-cerebral spinal fluid barrier, blood-testis barrier and the maternal-foetal barrier or placenta). Thus, these ABC transporters are increasingly recognized for their ability to modulate the absorption, distribution, metabolism, excretion and toxicity of xenobiotics (38).

The clinical relevance of these proteins in multidrug resistance is not yet established. However, expression of

MRP1 and P-glycoprotein has been reported in a variety of haematological and solid tumors, suggesting a significant role for these transport proteins in clinical drug resistance (51).

Comparison Between Efflux Pumps of Cancer Cells and those of Bacteria

Tumor inducing effects of some bacteria, *e.g. Bartonella*, *Helicobacter* and the plant bacterium *Agrobacterium*, are known (52) and probably mediate their effects *via* similar processes. The transformation of eukaryotic cells to a type IV secretion system by bacteria may involve the simultaneous transmission of DNA and protein from bacteria to eukaryotic cells. Because secretion involves a variety of transport systems, the inheritance of a type IV secretion system by the transformed eukaryotic cells suggests that the genes transferred from bacteria may code for efflux pumps that are able to function in a new cellular environment.

Apart from the aforementioned connection between prokaryotic and eukaryotic cells there is a relationship in the drug resistance of bacteria and cancer cells that is based on similarity or partial homology of membrane transporters responsible for extruding the various drugs from the bacterial or cancer cells (53).

The emergence of multidrug resistance of cancer cells is associated with the over-expression of para-glycoproteins which are ABC transporters responsible for extrusion of cytotoxic drugs from cancer cells.

During the course of treatment, the intrinsic para-glycoprotein 1 is over expressed and confers resistance not only to the agent that was employed for therapy, but also to numerous dissimilar anticancer agents, as well as xenobiotics (54). The response of a cancer cell (55) that is exposed to one noxious agent is identical to that which takes place when a bacterium is exposed to a noxious agent (30, 32), namely, each over-expresses an efflux pump that extrudes the noxious agent thereby preventing access to its intended target. However, whereas it takes weeks to induce MDR in Gram-negative bacteria (30), and a full year to induce an over-expressed efflux pump in *Mycobacteria* (32), it takes at least two years for cancer cells treated with various anticancer drugs to develop MDR (55, 56). In one study (56) the sensitive parental MCF-7 cells were subjected to various anticancer agents, *e.g.* paclitaxel and vincristine, by stepwise increases in drug concentrations resulting in resistant sub-lines that presented with MDR1 and MRP1 phenotypic resistance, as well as another form of resistance associated with decreased casapase-3 activity. These phenotypic forms of resistance could be modulated by phenothiazines and disiloxan compounds (56). The use of real-time quantitative PCR demonstrated changes in the expression levels of *MDR*1, *MRP*1, *BCRP*, *bcl-2* and *bax* genes. Whereas gene and protein analysis demonstrated the over expression of Pgp, the apoptotic *bax* gene expression was down regulated (57).

MDR phenotypes of cancer cells can be obviated with a variety of agents, many of which are inhibitors of calcium binding and transport. As an example, heterocyclic compounds such as phenothiazines and their derivatives decrease the extrusion of rhodamine 123 by resistant mouse lymphoma and MDR/COLO 320 cells (58). Two of the phenothiazine derivatives, namely perphenazine and prochlorperazine dimaleate, proved to be very effective inhibitors of the rhodamine efflux pump. Other phenothiazine derivatives, namely promethazine hydrochloride, oxomemazine, methotrimeprazine maleate, trifluoropromazine hydrochloride and trimeprazine, also increased intracellular drug accumulation in both resistant cell lines, however, they exerted additional cytotoxic effects. The differences observed between the effects of the test compounds on intracellular drug accumulation could be due to differences in the chemical structure of the phenothiazine, which may be crucial for drug-cell membrane interactions. By inhibiting efflux, the phenothiazines also increase the amount of these compounds that reach and bind irreversibly by intercalation to regions of the DNA rich in guanosine and cytosine nucleosides (59). When such binding takes place, DNA-based activities are inhibited and cell death is imminent. Phenothiazines are also bactericidal *via* an identical mechanism (60). Moreover, phenothiazines potentiate the activity of inhibitors of over-expressed efflux pumps of cancer cells. The mouse lymphoma cells containing the human *MDR*-1 could be rendered more susceptible to agents to which they were initially resistant by use ofcombinations of the phenothiazine, triflourperazine, and verapamil than by either agent alone (59). Phenothiazines also potentiate the activity of antibiotics to which the bacterium is sensitive (61) even when the bacterium is initially resistant (62). The activity of phenothiazines takes place initially on the plasma membrane of eukaryotic cells much in the same manner as that taking place in neurological cells. This effect is characterized by the inhibition of the membrane potential by these agents, hence they are called "membrane stabilizers" (63). All anti-psychotic drugs have this "membrane stabilizing" property and hence it is not surprising to find that a variety of non-phenothiazine neuroleptics can also block the activity of the P-glycoprotein (64). *MDR*-1 gene transfected L121C MDR, L5178 MDR and the KB-V-1 cells selected for resistance can be made susceptible to daunorubicin when treated with antipsychotic drugs.

During recent years, plants have been shown to be a promising source of agents with inhibitory activity against MDR cancer cells by directly affecting the para-

glycoprotein that constitutes the efflux mechanism. Among the first such studies was the demonstration that a methanol extract of *Carpobrotus edulis*, a common plant found along the Portuguese coast, could inhibit a verapamil-sensitive efflux pump of L5178 mouse T-cell lymphoma cell line thereby rendering these multi-drug resistant cells susceptible to anticancer drugs (65). This same material could enhance the killing of phagocytosed bacteria (51) and intracellular *Mycobacterium tuberculosis* (66). Other plant extracts, such those from *Peschiera fuchsiaefolia* (67) and Curcuminoids purified from turmeric powder (68), have been shown to render cancer cells susceptible to agents to which they were initially resistant. The ability to reverse the resistance of cancer cells is not limited to the agents discussed above. Derivatives of silicon (69, 70), of piperazines (71, 72) and sila compounds (72) can obviate MDR of cancer cells. Piperazine derivatives have antibacterial properties (73-75) and some have been shown to reverse the MDR phenotype of bacteria (74). As piperazine derivatives of a fluoroquinolone enhance the activity of the antibiotic (76) there is no doubt that these derivatives will soon prove their significance in the therapy of MDR Gram-positive and Gram-negative infections.

As mentioned earlier, bacteria have been shown to induce a variety of cancers. Gram-negative bacteria that contain plasmids carrying MDR genes may also be implicated. The ability of a compound to eliminate a plasmid carrying antibiotic resistance gene is important in clinical and veterinary medicine. To this end, much work has shown that heterocyclic compounds have the ability to cure Gram-negative bacteria of plasmids *in vitro* (77-85) and since children infected with recurrent pylonephritis caused by plasmids carrying gentamycin-resistance genes can be cured with phenothiazine derivatives (86), the potential of heterocyclic compounds for the prevention of cancer induced by plasmid-carrying bacteria is very significant.

Conclusion

Phenotypic expression of MDR is problematic for the therapy of bacterial infections as well as cancer. The development of MDR in both types of diseases is the result of exposure of the bacterium or eukaryotic cancer cell to a concentration of drug that allows the cells to survive. Survival in the presence of continuous exposure to a given agent induces the bacterium or cancer cell to synthesise more efflux pump units thus adding additional survival advantage to the cell. With respect to bacteria, the demonstration of this adaptive response is possible *in vitro* within a period of weeks for Gram-negative bacteria, as long as one year for *Mycobacterium tuberculosis* and considerably longer for cancer cells. These *in vitro* situations are considered to mimic what takes place with the patient who receives a given antibiotic for a long period of time, each time at a dose level that allows replication of the cell and hence its survival. The demonstration of the active extrusion of a large variety of agents by MDR phenotypic cells has fostered a search for agents that can inhibit efflux and hence render ineffective antibiotics effective again. Although many of the agents that inhibit efflux systems can also inhibit the replication of bacteria and cancer cells, they normally do so at concentrations which are either toxic or are beyond clinical reach (10). Fortunately, for most of these agents, such as the phenothiazines, amounts of agent that are well below the levels found in the plasma of patients treated with the agent are sufficient to enhance the activity of antibiotics to which the bacterium or cancer cell is resistant. Nevertheless, because even these small doses of phenothiazines can cause moderate to severe side-effects (10), we must consider the possibility that their derivatives may be as effective as the parent compound and yet be devoid of any toxicity or serious side-effects. This approach has been followed in recent years and appears promising. For the time being, we must continue to study the intrinsic efflux systems of all infectious agents and cancer cells at the molecular, genetic and physiological levels, purify and physically characterize the components of the relevant MDR efflux pumps, identify the active site(s) to which inhibitors of these components bind, and thus employing these agents as lead compounds (87) create new derivatives that are effective and yet devoid of side-effects. Although the will and technology exists, and we anticipate success in the very near future, the matter of flux may be more complicated than currently understood as evident from the recent studies of Liang et al. (89). These latter studies clearly demonstrate that the manipulation of K+ fluxes with antibodies and the H+ pump with omeprazole results in opposite effects on cisplatin resistance in KB and BEL-7404 cell lines and conclude that K+ and H+ homeostasis are not critical factors in cisplatin resistance.

Acknowledgements

We wish to thank the COST B16 action of the European Commission/European Science Foundation for the many discussions held on the subject of this article. This work was partially supported by grants EU-FSE/FEDER-POCTI-37579/FCB/2001 and EU-FSE/FEDER-POCI/SAU-MMO/59370/2004 provided by the Fundação para a Ciência e a Tecnologia (FCT) of Portugal.

References

1 Cars O and Nordberg P: Antibiotic Resistance: The Faceless Threat. The Global Threat of Antibiotic Resistance. Exploring Roads Toward Concerted Action. A Multi-disciplinary Meeting at the Dag Hammerskjold Foundation; Uppsala, Sweden, 5-7 May, 2004. Background Document.

2 W.H.O. Shaping the Future. World Health Report 2003, World Health Organization, 1211 Geneva 27, Switzerland.

3 W.H.O. World Health Organization Report on Global Cancer 2003. World Health Organization, 1211 Geneva 27, Switzerland.

4 Eveillard M, Schmit JL, Biendo M, Canarelli B, Daoudi F, Laurans G, Rousseau F, Thomas D and Eb F: Evaluation of the efficacy of a multiresistant bacteria control programme in a teaching hospital, studying the evolution of methicillin- resistant *Staphylococcus aureus* incidence. Pathol Biol (Paris) *50(9)*: 538-543, 2002.

5 Miklasevics E, Haeggman S, Balode A, Sanchez B, Martinsons A, Olsson-Liljequist B and Dumpis U: Report on the first PVL-positive community acquired MRSA strain in Latvia. Euro Surveill *9(11)*: 29-30, 2004.

6 Eveillard M, Lancien E, de Lassence A, Branger C, Barnaud G, Benlolo JA and Joly-Guillou ML: Impact of the reinforcement of a Methicillin-Resistant *Staphylococcus aureus* Control Programme: a 3-year evaluation by several indicators in a French University Hospital. Eur J Epidemiol Aug 17, 2006 (EPub ahead of print) PMID: 16915525 (PubMed).

7 Halem M, Trent J, Green J and Kerdel F: Community-acquired methicillin resistant *Staphylococcus aureus* skin infection. Semin Cutan Med Surg *25(2)*: 68-71, 2006.

8 Huijsdens XW, van Santen-Verheuvel MG, Spalburg E, Heck ME, Pluister GN, Eijkelkamp BA, de Neeling AJ and Wannet WJ: Multiple cases of familial transmission of community-acquired methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol *44(8)*: 2994-2996, 2006.

9 Uete T, Matsuo K and Uete G: Cross-resistance of clinical isolates of methicillin-resistant *Staphylococcus aureus* to aminoglycosides and fluoroquinolones. Jpn J Antibiot *45(8)*: 943-948, 1992.

10 Amaral L, Viveiros M and Kristiansen JE: "Non-antibiotics": alternative therapy for the management of MDRTB and MRSA in economically disadvantaged countries. Curr Drug Targets *7(7)*: 887-891, 2006.

11 Kristiansen JE and Amaral L: The potential management of resistant infections with non-antibiotics. J Antimicrob Chemother *40(3)*: 319-327, 1997.

12 Amaral L, Viveiros M and Molnar J: Antimicrobial activity of phenothiazines. In Vivo *18(6)*: 725-731, 2004.

13 Al Naiemi N, Heddema ER, Bart A, de Jonge E, Vandenbroucke-Grauls CM, Savelkoul PH and Duim B: Emergence of multidrug-resistant Gram-negative bacteria during selective decontamination of the digestive tract on an intensive care unit. J Antimicrob Chemother *58(4)*: 853-856, 2006.

14 Jonas BM, Murray BE and Weinstock GM: Characterization of emeA, a NorA homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob Agents Chemother *45(12)*: 3574-3579, 2001.

15 Beekmann SE, Heilmann KP, Richter SS, Garcia-de-Lomas J and Doern GV: The GRASP Study Group. Antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and group A beta-haemolytic streptococci in 2002-2003. Results of the multinational GRASP Surveillance Program. Int J Antimicrob Agents *25(2)*: 148-156, 2005.

16 Piddock LJ: Multidrug-resistance efflux pumps – not just for resistance. Nat Rev Microbiol *4(8)*: 629-636, 2006.

17 Gotoh N: Antibiotic resistance caused by membrane impermeability and multidrug efflux systems. Nippon Rinsho *59(4)*: 712-718, 2001.

18 Kern WV, Steinke P, Schumacher A, Schuster S, von Baum H and Bohnert JA: Effect of 1-(1-naphthylmethyl)-piperazine, a novel putative efflux pump inhibitor, on antimicrobial drug susceptibility in clinical isolates of *Escherichia coli*. J Antimicrob Chemother *57(2)*: 339-343, 2006.

19 Schumacher A, Steinke P, Bohnert JA, Akova M, Jonas D and Kern WV: Effect of 1-(1-naphthylmethyl)-piperazine, a novel putative efflux pump inhibitor, on antimicrobial drug susceptibility in clinical isolates of Enterobacteriaceae other than *Escherichia coli*. J Antimicrob Chemother *57(2)*: 344-348, 2006.

20 Kriengkauykiat J, Porter E, Lomovskaya O and Wong-Beringer A: Use of an efflux pump inhibitor to determine the prevalence of efflux pump-mediated fluoroquinolone resistance and multidrug resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother *49(2)*: 565-570, 2005.

21 Seral C, Carryn S, Tulkens PM and Van Bambeke F: Influence of P-glycoprotein and MRP efflux pump inhibitors on the intracellular activity of azithromycin and ciprofloxacin in macrophages infected by *Listeria monocytogenes* or *Staphylococcus aureus*. J Antimicrob Chemother *51(5)*: 1167-1173, 2003.

22 Gibbons S, Oluwatuyi M and Kaatz GW: A novel inhibitor of multidrug efflux pumps in *Staphylococcus aureus*. J Antimicrob Chemother *51(1)*: 13-17, 2003.

23 Guz NR, Stermitz FR, Johnson JB, Beeson TD, Willen S, Hsiang J and Lewis K: Flavonolignan and flavone inhibitors of a *Staphylococcus aureus* multidrug resistance pump: structure-activity relationships. J Med Chem *44(2)*: 261-258, 2001.

24 Kristiansen MM, Leandro C, Ordway D, Martins M, Viveiros M, Pacheco T, Molnar J, Kristiansen JE and Amaral L: Thioridazine reduces resistance of methicillin-resistant *Staphylococcus aureus* by inhibiting a reserpine-sensitive efflux pump. In Vivo *20(3)*: 361-366, 2006.

25 Kaatz GW, Moudgal VV and Seo SM: Identification and characterization of a novel efflux-related multidrug resistance phenotype in *Staphylococcus aureus*. J Antimicrob Chemother *50(6)*: 833-838, 2002.

26 Truong-Bolduc QC, Zhang X and Hooper DC: Characterization of NorR protein, a multifunctional regulator of norA expression in *Staphylococcus aureus*. J Bacteriol *185(10)*: 3127-3138, 2003.

27 Kaatz GW, DeMarco CE and Seo SM: MepR, a repressor of the *Staphylococcus aureus* MATE family multidrug efflux pump MepA, is a substrate-responsive regulatory protein. Antimicrob Agents Chemother *50(4)*: 1276-1281, 2006.

28 Kaatz GW, Moudgal VV, Seo SM, Hansen JB and Kristiansen JE: Phenylpiperidine selective serotonin reuptake inhibitors interfere with multidrug efflux pump activity in *Staphylococcus aureus*. Int J Antimicrob Agents *22(3)*: 254-261, 2003.

29 Martins M, Santos B, Martins A, Viveiros M, Couto I, Cruz A, the Management Committee Members of COST B16 of the European Commission/European Science Foundation, Pagès JM, Molnar J, Fanning S and Amaral L: An instrument-free method for the demonstration of efflux pump activity of bacteria. In Vivo *20*: 657-664, 2006.

30 Viveiros M, Jesus A, Brito M, Leandro C, Martins M, Ordway D, Molnar AM, Molnar J and Amaral L: Inducement and reversal of tetracycline resistance in *Escherichia coli* K-12 and the expression of proton gradient dependent multidrug efflux pump genes. Antimicrobial Agents Chemother *49(8)*: 3578-3582, 2005.
31 Molnar J, Hever A, Fakla I, Fischer J, Ocsovski I and Aszalos A: Inhibition of the transport function of membrane proteins by some substituted phenothiazines in *E. coli* and multidrug resistant tumor cells. Anticancer Res *17(1A)*: 481-486, 1997.
32 Viveiros M, Portugal I, Bettencourt R, Victor TC, Jordaan AM, Leandro C, Ordway D and Amaral L: Isoniazid-induced transient high-level resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother *46(9)*: 2804-2810, 2002.
33 Worley MJ, Stojiljkovic I and Heffron F: The identification of exported proteins with gene fusions to invasin. Mol Microbiol *29(6)*: 1471-1480, 1998.
34 Piddock LJ: Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev *19(2)*: 382-402, 2006.
35 Pagès JM, Masi M and Barbe J: Inhibitors of efflux pumps in Gram-negative bacteria. Trends Mol Med *11(8)*: 382-389, 2005.
36 Li XZ and Nikaido H: Efflux-mediated drug resistance in bacteria. Drugs *64(2)*: 159-204, 2004.
37 Sharom FJ: The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol *160(3)*: 161-175, 1997.
38 Leslie EM, Deeley RG and Cole SP: Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2 and BCRP (ABCG2) in tissue defense. Toxicol Appl Pharmacol *204(3)*: 210-237, 2005.
39 Walker JE, Saraste M, Runswick MJ and Gay NJ: Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J *1(8)*: 945-951, 1982.
40 Dean M, Rzhetsky A and Allikmets R: The human ATP binding cassette (ABC) transporter superfamily. Genome Res *11(7)*: 1156-1166, 2001.
41 Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM and Deeley RG: Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science (Wash. DC), *258(5088)*: 1650-1654, 1992.
42 Ambudkar SV, Dey S and Hrycyna CA: Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol *39*: 361-398, 1999.
43 Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJT, Juijn JA, Baas F and Borst P: Analysis of expression of *cMOAT* (*MRP*2), *MRP*3, *MRP*4, and *MRP*5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res *57(16)*: 3537-3547, 1997.
44 Borst P, Evers R, Kool M and Wijnholds J: A family of drug transporters: The multidrug resistance-associated proteins. J Natl Cancer Inst *92(16)*: 1295-1302, 2000.
45 Bera TK, Lee S, Salvatore G, Lee B and Pastan I: MRP8, a new member of ABC transporter superfamily, identified by EST database mining and gene prediction program, is highly expressed in breast cancer. Mol Med *7(8)*: 509-516, 2001.
46 Bera TK, Iavarone C, Kumar V, Lee S, Lee B and Pastan I: MRP9, an unusual truncated member of the ABC transporter superfamily, is highly expressed in breast cancer. Proc Natl Acad Sci USA *99(10)*: 6997-7002, 2002.
47 Bates SE, Robey R, Miyake K, Rao K, Ross DD and Litman T: The role of half-transporters in multidrug resistance. J Bioenerg Biomembr *33(6)*: 503-511, 2001.
48 Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK and Ross DD: A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc. Natl Acad Sci *95(26)*: 15665-15670, 1998.
49 Dalton WS and Scheper RJ: Lung-resistance protein: determining its role in multidrug resistance. J Natl Cancer Inst *91(19)*: 1604-1605, 1999.
50 Scheffer GL, Schroeijers AB, Izquierdo MA, Wiemer EA and Scheper RJ: Lung resistance-related protein/major vault protein and vaults in multidrug-resistant cancer. Curr Opin Oncol *12(6)*: 550-556, 2000.
51 Cole SPC and Deeley RG: Multidrug resistance II: MRP and related proteins. *In*: Bertino JR (ed.), Encyclopedia of Cancer. Elsevier Science, USA, pp. 255-267, 2002.
52 Gyémánt N, Molnár A, Spengler G, Mándi Y, Szabo M and Molnar J: Bacterial models for tumor development. Acta Microbiol Immunol Hung *51(3)*: 321-332, 2004.
53 Gerlach JH, Endicott JA, Juranka PF, Henderson G, Sarangi F, Deuchars KL and Ling V: Homology between P glycoprotein and bacterial haemolysin transport protein suggests a model for multidrug-resistance. Nature *324(6096)*: 485-489, 1986.
54 Molnar J, Hevér A, Fakla I, Fischer J, Ocsovszky and Aszalos A: Inhibition of the transport function of membrane proteins by some substituted phenothiazines in *E. coli* and multidrug resistant tumor cells. Anticancer Res *17(1A)*: 481-486, 1997.
55 Demirel KM, Darcansoy IÖ, Gündüz U, Ugur UA, Arpaci F and Molnár J: Development of rational *in vitro* models for drug resistance in breast cancer and modulation of MDR by selected compounds. Anticancer Res *26*: 4559-4568, 2006.
56 Demirel KM, Darcansoy IÖ, Gündüz U, Ugur UA and Molnár J: Modulation of multidrug resistance in paclitaxel and vincristine resistant MCF-7 cell lines. The FEBS Journal *273*: 278, 2006.
57 Demirel KM, Darcansoy IÖ, Gündüz U, Doleschall Z, Csuka O, Bak M and Molnár J: Analysis of multidrug resistance (MDR) related expression profiles in MCF-7 cell lines. Proc. of 19th Congress of European Association of Cancer Res, 1-4 July, 2006.
58 Pajak B, Molnar J, Engi H and Orzechowski A: Preliminary studies on phenothiazine-mediated reversal of multidrug resistance in mouse lymphoma and COLO 320 cells. In Vivo *19(6)*: 1101-1114, 2005.
59 Molnár J, Szabo D, Mándi Y, Mucsi I, Fischer J, Varga A, König S and Motohashi N: Multidrug resistance Reversal in mouse lymphoma cells by heterocyclic compounds. Anticancer Res *18(4C)*: 3033-3038, 1998.
60 Amaral L, Viveiros M and Molnar J: Antimicrobial activity of phenothiazines. In Vivo *18(6)*: 725-732, 2004.
61 Amaral L, Kristiansen JE and Lorian V: Synergistic effect of chlorpromazine on the activity of some antibiotics. J Antimicrob Chemother *30*: 556-558, 1992.
62 Viveiros M and Amaral L: Enhancement of antibiotic activity against poly-drug resistant *Mycobacterium tuberculosis* by phenothiazines. Int J Antimicrob Agents *17(3)*: 225-228, 2001.
63 Lind K and Kristiansen JE: Effect of some psychotropic drugs and a barbiturate on mycoplasmas. Int J Antimicrob Agents *14(3)*: 235-238, 2000.

64 Szabo D, Szabo GJ, Ocsovszki I, Aszalos A and Molnar J: Antipsychotic drugs reverse multidrug resistance of tumor cell lines and human AML cells *ex vivo*. Cancer Lett *139(1)*: 115-119, 1999.

65 Ordway D, Hohmann J, Viveiros M, Viveiros A, Molnar J, Leandro C, Arroz MJ, Gracio MA and Amaral L: *Carpobrotus edulis* methanol extract inhibits the MDR efflux pumps, enhances killing of phagocytosed *S. aureus* and promotes immune modulation. Phytother Res *17(5)*: 512-519, 2003.

66 Martins M, Viveiros M, Schelz Z and Amaral L: Methanol extract of *Carpobrotus edulis* enhances killing of phagocytosed Mycobacterium avium. Unpublished Observations.

67 Meschini S, Marra M, Condello M, Calcabrini A, Federici E, Dupuis ML, Cianfriglia M and Arancia G: Voacamine, an alkaloid extracted from *Peschiera fuchsiaefolia*, inhibits P-glycoprotein action in multidrug-resistant tumor cells. Int J Oncol *27(6)*: 1597-1603, 2005.

68 Chearwae W, Wu CP, Chu HY, Lee TR, Ambudkar SV and Limtrakul P: Curcuminoids purified from turmeric powder modulate the function of human multidrug resistance protein 1 (*ABCC*1). Cancer Chemother Pharmacol *57(3)*: 376-388, 2006.

69 Molnar J, Mucsi I, Nacsa J, Hevér A, Gyémánt N, Ugocsai K, Hegyes P, Kiessig St, Gaál D, Lage H and Varga A: New silicon compounds as resistance modifiers against multidrug-resistant cancer cells. Anticancer Res *24(2B)*: 865-872, 2004.

70 Zalatnai A and Molnar J: Effect of SILA-409, a new organosilicon multidrug resistance modifier on human pancreatic cancer xenografts. In Vivo *20(1)*: 137-140, 2006.

71 Gillet R, Jeannesson P, Sefraoui H, Arnould-Guerin ML, Kirkiacharian S, Jardillier JC and Pieri F: Piperazine derivatives of butyric acid as differentiating agents in human leukemic cells. Cancer Chemother Pharmacol *41(3)*: 252-255, 1998.

72 Williams CL, Phelps SH and Porter RA: Expression of Ca^{2+}/calmodulin-dependent protein kinase types II and IV, and reduced DNA synthesis due to the Ca^{2+}/calmodulin-dependent protein kinase inhibitor KN-62 (1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenyl piperazine) in small cell lung carcinoma. Biochem Pharmacol *51(5)*: 707-715, 1996.

73 Narendra Sharath Chandra JN, Sadashiva CT, Kavitha CV and Rangappa KS: Synthesis and *in vitro* antimicrobial studies of medicinally important novel N-alkyl and N-sulfonyl derivatives of 1-[bis(4-fluorophenyl)-methyl]piperazine. Bioorg Med Chem *14(19)*: 6621-6627, 2006.

74 Foroumadi A, Ghodsi S, Emami S, Najjari S, Samadi N, Faramarzi MA, Beikmohammadi L, Shirazi FH and Shafiee A: Synthesis and antibacterial activity of new fluoroquinolones containing a substituted N-(phenethyl)piperazine moiety. Bioorg Med Chem Lett *16(13)*: 3499-3503, 2006.

75 Lohray BB, Lohray VB, Srivastava BK, Gupta S, Solanki M, Pandya P and Kapadnis P: Novel 4-N-substituted aryl pent-2-ene-1,4-dione derivatives of piperazinyloxazolidinones as antibacterials. Bioorg Med Chem Lett *16(6)*: 1557-1561, 2006.

76 Martins M, Schelz Z, Viveiros M, Molnar J and Amaral L: Piperazine derivatives reverse MDR phenotypes of *E. coli* that over-express 9 efflux pumps. Unpublished observations.

77 Molnar J, Kiraly J and Mandi Y: The antibacterial action of R factor inhibiting activity by chlorpromazine. Experientia *31(4)*: 444-445, 1975.

78 Molnar J, Foldeak S, Nakamura MJ, Rausch H, Domonkos K and Szabo M: Antiplasmid activity: Loss of bacterial resistance to antibiotics. APMIS Suppl *30*: 24-31, 1992.

79 Spengler G, Molnar A, Schelz Z, Amaral L, Sharples D and Molnar J: The mechanism of plasmid curing in bacteria. Curr Drug Targets *7(7)*: 823-841, 2006.

80 Wolfart K, Spengler G, Kawase M, Motohashi N, Molnar J, Viveiros M and Amaral L: Synergistic interaction between proton pump inhibitors and resistance modifiers: promoting effects of antibiotics and plasmid curing. In Vivo *20(3)*: 367-372, 2006.

81 Sharples D, Spengler G, Molnar J, Antal Z, Molnar A, Kiss JT, Szabo JA, Hilgeroth A, Gallo S, Mahamoud A and Barbe J: The interaction between resistance modifiers such as pyrido[3,2-g]quinoline, aza-oxafluorene and pregnane derivatives with DNA, plasmid DNA and tRNA. Eur J Med Chem *40(2)*: 195-202, 2005.

82 Molnar J, Molnar A, Spengler G and Mandi Y: Infectious plasmid resistance and efflux pump mediated resistance. Acta Microbiol Immunol Hung *51(3)*: 333-349, 2004.

83 Spengler G, Miczak A, Hajdu E, Kawase M, Amaral L and Molnar J: Enhancement of plasmid curing by 9-aminoacridine and two phenothiazines in the presence of proton pump inhibitor 1-(2-benzoxazolyl)-3,3,3-trifluoro-2-propanone. Int J Antimicrob Agents *22(3)*: 223-227, 2003.

84 Molnar A, Amaral L and Molnar J: Antiplasmid effect of promethazine in mixed bacterial cultures. Int J Antimicrob Agents *22(3)*: 217-222, 2003.

85 Motohashi N, Kawase M, Saito S, Miskolci C, Berek L and Molnar J: Plasmid elimination and immunomodulation by 3-benzazepines *in vitro*. Anticancer Res *19(6B)*: 5075-5078, 1999.

86 Molnár J, Haszon I, Bodrogi T, Martonyi E and Turi S: Synergistic effect of promethazine with gentamycin in frequently reccuring pyelonephritis. Int Urol Nephrol *22(5)*: 405-411, 1990.

87 Wainwright M and Amaral L: The phenothiazinium chromophore and the evolution of antimalarial drugs. Trop Med Int Health *10(6)*: 501-511, 2005.

88 Liang XJ, Taylor B, Cardarelli C, Yin JJ, Annereau JP, Garfield S, Wincovitch S, Szakacs G, Gottesman MM and Aszalos A: Different roles for K+ channels in cisplatin-resistant cell lines argue against a critical role for these channels in cisplatin resistance. Anticancer Res *25(6B)*: 4113-4122, 2005.

Received September 8, 2006
Revised October 23, 2006
Accepted October 31, 2006