

Review

The Pharmacology of Cancer Resistance

ROBERT O'CONNOR

The National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland

Abstract. Many tumour cells become resistant to commonly used cytotoxic drugs due to the overexpression of ATP-binding cassette (ABC) transporters. Two proteins, P-gp (MDR-1, ABCB1) and MRP-1 (ABCC1) have been demonstrated to pump a wide selection of the most commonly used cancer drugs and their overexpression correlates broadly with negative treatment response characteristics in many different forms of cancer. Several generations of pharmaceutical inhibitors of P-gp have been examined in preclinical and clinical studies; however, these circumvention trials have largely failed to demonstrate the anticipated increase in therapeutic efficacy. In vitro screening has identified a number of pharmaceuticals which can selectively inhibit pumps such as P-gp, or MRP-1, by virtue of their being substrates for these pumps. The use of low toxicity pharmaceuticals or agents which have anticancer properties as ABC transporter inhibitors may allow a new paradigm of clinically useful drug resistance circumvention. Our increasing understanding of the complex pharmacological interplay of drug transporter proteins indicates that the cellular pharmacokinetics of cancer drug entry into and exit from tumour cells is of prime importance in subsequent drug efficacy and a larger portfolio of pump modulators and targeted efflux inhibition strategies is necessary to effectively overcome multiple drug resistance.

Despite the exquisite sensitivity of many cancer cells to cytotoxic chemotherapeutic agents and an international research effort spanning 60 years, the cytological “sledge hammer” of cytotoxic action is often ultimately ineffective in many forms of cancer treatment. The failure of cancer cells response to such drugs is termed resistance and

tumours may present from the start with a resistance phenotype, being intrinsically resistant, or may initially be sensitive to therapy and later become insensitive to similar doses, termed acquired resistance.

Many different biological factors causing chemotherapy resistance have been characterised (1, 2). Despite the diverse array of genetic and molecular changes that can take place in various cellular drug targets and response intermediates, the fundamentals of whether a drug can reach the required target concentration is of key importance to the life and death of a tumour cell exposed to chemotherapy (3).

A major advance in our understanding of cellular pharmacokinetic resistance mechanisms took place in 1976 with the description of the MDR-1 (P-gp) protein and its characterisation as a cellular efflux pump capable of efficiently removing significant amounts of a wide variety of xenobiotics and drugs from the inside of a cell to the outside (4).

P-gp (MDR-1, and now termed ABCB1) exists as a large transmembrane protein which actively moves a large number of xenobiotics, including many cytotoxic cancer drugs, from one leaflet of the plasma membrane to the other (5). The identification of P-gp helped explain the common observation that cancer cells could be resistant to a range of agents while only having been exposed to any one of a list of such agents, this phenomenon being commonly termed multiple drug resistance (MDR). In due course, it was recognised that P-gp expression did not account for all forms of MDR which had been seen in cell models. A second cellular pump, MRP-1 (ABCC1) was described in 1992 and although it has a fundamentally different mechanism of action, it also pumps many of the important cytotoxic chemotherapy drugs (6). BCRP (MXR, ABCG2) was identified more recently and has been demonstrated to be a smaller transmembrane protein which, unlike the other major drug transporters, dimerises to generate a functional transporter unit (7).

The majority of the commonly used chemotherapy drugs, e.g. taxanes, anthracyclines, vinca alkaloids, as well as newer molecularly-targeted agents, are substrates for one or more of these pumps (8). Several other related proteins have

Correspondence to: Robert O'Connor, Ph.D., The National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland. Tel +353 1 7005691, Fax +353 1 7005484, e-mail: robert.oconnor@dcu.ie

Key Words: Multiple drug resistance, MDR, resistance circumvention, tyrosine kinase inhibitor, MRP-1, drug efflux, drug uptake, review.

been identified in human cells from direct analysis and in searching for a conserved ATPase motif. These include proteins shown to be related in sequence to MRP-1 and therefore classified as part of the same family structure (9, 10). In human cells, the largest family of drug efflux pumps are from the ABCC protein family, *i.e.* proteins with a close sequence homology with the MRP-1 pump, with 9 constituent proteins members described (9). Of the ABCC family, MRP-1 has shown the greatest association with drug resistance in a broad range of human cancer while other members of this protein family appear to have roles in resistance to a narrower, more structurally distinct, range of drugs (10). For example, overexpression of MRP-4 (ABCC4) or MRP-5 (ABCC5) proteins has been shown to confer resistance to certain nucleotide analogues used in the treatment of some forms of leukaemia, although recent data casts doubt on the physiological relevance of this activity (11). The role of other MRP family proteins appears to be largely physiological although further research is needed (12).

More recently still, several new drug transporter proteins have been identified, the members of the solute carrier family (SLC), including organic anion transporters (OATPs) (13, 14). These transporters play a critical role, in combination with the classical protein pumps such as P-gp and MRP-1, in the vectored movement of endogenous and exogenous agents through cells and tissues. Of particular interest to cancer treatment, some members of this group of transporters are important in the uptake of specific cancer drugs. The human copper transporter (CTR-1, SLC31A1) plays a key role in uptake of platinum drugs, *e.g.* cisplatin, and reduced expression of this transporter causes tumour cell resistance (15-17). Similarly, OATP 8 (SLC21A8) has been shown to play an important role in paclitaxel uptake and expression is greatly reduced in several resistant types of cancer (18, 19).

Relevance of Pump Proteins to Cancer Treatment Response

All of the major cancer efflux pumps have been shown to be associated with specific types of treatment resistance in *in vitro* models (20). The clinical picture is, however, much more complex. Several studies have demonstrated the frequent occurrence of drug efflux proteins in cancer tissue, both from chemotherapy-naïve patients and tumours that have been exposed to chemotherapy drugs. In solid tumours and some leukemias, a number of investigators have reported statistically significant correlations between poor treatment response and overexpression of P-gp or MRP-1 in particular types of tumours (21-27). In investigations of specific forms of leukaemia, some authors have reported a prognostic significance for BCRP overexpression in circulating cancer cells (23, 28-30).

Circumvention of Pump-mediated Resistance

P-gp inhibition strategies. Attempts to overcome P-gp-mediated resistance began with observations that certain existing pharmaceuticals such as verapamil and cyclosporine potentiated P-gp substrate cytotoxic action in cellular models overexpressing the pump (20, 31). In *in vivo*, animal-models, evaluations of combinations of such agents with anthracyclines and other P-gp substrates showed significant therapeutic promise (32-34) but follow-on clinical studies have almost universally failed to show any improved treatment efficacy, even with the advent of more specific third generation inhibitors (20, 35). Third generation circumvention agents, such as elacridar and tariquidar, surmounted earlier resistance inhibitor difficulties including poor specificity and metabolic interaction with the cancer drugs being used; however, these agents have also failed to lead to useful increases in chemotherapeutic efficacy (36, 37). The primary drawback with third generation inhibitors appears to be the fundamental problem of body-wide non-specific inhibition of P-gp which reduces the normal processes of cancer drug elimination from the body (37). This fundamentally alters the basic pharmacokinetics of substrate chemotherapy drugs, leading to increased drug exposure and an associated increase in side-effects consistent with an increase in the amount of cancer drug administered. Notwithstanding the pharmacokinetic problems of P-gp inhibition, elegant studies on small numbers of patients using radio-labelled positron emission tomography (PET) P-gp substrate reagents (which permit real-time visualisation of pump-mediated substrate efflux) have shown that these third generation circumvention agents do increase the tumour retention of P-gp substrates in at least some patients (36, 38-40). The pharmacological complexity of P-gp inhibition has led to most pharmaceutical companies changing development emphasis with these drugs to apply them to increased oral bioavailability applications (41). It should also be noted that the majority, but not all (42), of third generation P-gp inhibitors also appear to be potent BCRP inhibitors (43); it is not clear if this property is useful or complicates the therapeutic and/or pharmacokinetic picture. No selective clinical trials of BCRP inhibition as a strategy to improve chemotherapeutic efficacy have been reported in the literature.

Despite 30 years of P-gp research, we still have no clear therapeutic strategy to overcome the actions of this classical drug efflux pump in tumours. There is little evidence in the literature outlining the optimal duration of efflux pump inhibition for maximum therapeutic effect. Research examining the associations of drug concentration and duration of exposure with toxicity in tumour cells using agents such as paclitaxel and doxorubicin suggests that most chemotherapy drugs have an optimal "window" of action during which they exert their useful anticancer properties (44-47). Extrapolating this scenario with the presence of drug

efflux pumps suggests that prolonged, non-specific and irreversible pump inhibition may be counterproductive; by incorporating the concept of optimal cytotoxic drug exposure it may be possible to model optimal pump inhibitor profiles which lead to cancer drugs reaching sufficient concentrations in a tumour cell for a sufficient duration while the normal characteristics of whole body elimination are not interfered with to the extent of increased body-wide toxicity. Clearly, in the case of P-gp at least, blanket, body-wide, long duration inhibition is unlikely to lead to major increases in cancer drug efficacy. The next generation of inhibition strategies may therefore incorporate some aspect of tumour-targeting with an optimal tumour efflux inhibition profile (48).

Interactions of P-gp with newer targeted chemotherapies. More recently, several of the newer molecularly-targeted cancer drugs, such as tyrosine kinase inhibitors, have also been shown to be active substrates for this pump and P-gp may explain how some cancer cells can become resistant to these drugs (8, 49).

The chemotherapeutic treatment of several types of cancer has recently been revolutionised by the development of high specificity small molecule inhibitors of key growth signalling pathways in cancer (50). Such agents are much less toxic than conventional cytotoxic therapies and are given over extended periods to control cancer cell growth. Unfortunately, reports have indicated that a number of the agents, if not all of the currently clinically available inhibitors, are substrates for the P-gp and/or BCRP drug efflux pump (8, 49, 51, 52). This suggests that the classical drug efflux pump mechanism found to protect tumour cells from the actions of cytotoxic drugs may also make them resistant to the actions of molecularly-targeted therapeutics, although other factors also contribute to the overall resistance profile to these drugs.

The nature of pump-mediated efflux means that substrate agents also become inhibitors of pump action when used in appropriate concentrations with other substrate agents and several growth factor receptor small molecule inhibitors can inhibit P-gp-mediated drug efflux in cells overexpressing this pump at concentrations in the physiologically achievable range (53, 54). This property may be of therapeutic use in combination applications of targeted agents with cytotoxic drugs. Conversely, it is probable that prolonged administration of high doses of the targeted agents may interfere with the pharmacokinetics of conventional cytotoxic therapies producing increased side-effects, especially in certain tissues, *e.g.* haematopoietic stem cells, whose high expression rate of some pumps may normally afford partial protection from cytotoxic action (55, 56).

MRP-1 inhibition. MRP-1 (ABCC1) is another large drug efflux pump with an uncharacteristically strong association of overexpression with poor treatment response in a number of common malignancies (21, 57-61). Unlike P-gp and BCRP, no

high-specificity clinically useful inhibitors have been developed. Two agents with MRP-1 inhibitory activity have been investigated in clinical trials. Biricodar has laboratory activity against P-gp, MRP-1 and BCRP; clinical studies have demonstrated an ability to inhibit P-gp at clinically achievable doses but it is unclear if these doses are also sufficient to inhibit MRP-1 (62). Our research team identified that the NSAID, sulindac, could specifically inhibit MRP-1 activity by virtue of its being a substrate/competitive inhibitor for the pump (63, 64). A Phase I clinical evaluation of sulindac in combination with epirubicin indicated that doses from 200 mg to 600 mg sulindac generated levels of sulindac and MRP-1 inhibitory metabolites in excess of those required for *in vitro* competitive inhibition of MRP-1 activity (65). Although MRP-1 is expressed in a number of body tissues, unlike P-gp, this efflux mechanism does not appear to have a critical role in the normal whole-body pharmacokinetic elimination process of chemotherapy drugs (12). Indeed our Phase I study indicated that sulindac did not interfere with the normal toxicity or pharmacokinetic profiles of epirubicin, an MRP-1 substrate chemotherapeutic, while noteworthy treatment improvements were seen. Therefore, MRP-1 may present a more attractive inhibition target since the body-wide toxicological implications of inhibition appear to be inconsequential.

Significance of Drug Pumps in MDR

The interpretation of the true significance of drug efflux pump protein overexpression in tumours is difficult. Circumvention studies have had significant pharmacological problems, but coupled to this, polymorphisms affecting the substrate specificity and activity of efflux pump proteins have been described in human tumours (66-71). The reagents and tissue samples used to provide a metric of efflux pump activity have also sometimes clouded interpretation and may in part explain contradictory results from some groups (20). In addition, although some studies have provided a quantifiable measure of drug pump activity and its inhibition in tumours in a small number of patients (36, 38-40), no studies have been conducted to quantify the overall effect of pump inhibition on tumour levels of substrate cancer drugs. With a significant overlap in substrate specificity, tumours overexpressing more than one pump mechanism may be able to overcome, at least in part, the impact of specific inhibition of a single drug efflux pump.

The recent emergence of the SLC proteins adds a whole new layer of complexity in understanding and predicting the cellular pharmacokinetics and associated drug resistance profiles of tumour cells. For example, as previously mentioned, the human copper transporter (CTR-1, SLC31A1) plays a key role in the uptake of platinum drugs, *e.g.* cisplatin, and OATP 8 (SLC21A8) plays an important role in paclitaxel uptake (15, 18). Some of these transporters

have the potential to play dual roles, being responsible for cellular uptake in some experimental situations while also having a critical role in the movement of drugs through tissues and organs (72, 73).

The preceding text has largely assumed that drug pump expression is static in tumour cells. A limited number of clinical studies have shown that drug efflux protein expression can increase over the course of successive chemotherapy cycles (23-25). *In vitro* studies have indicated that a number of pharmaceutical and biological agents can affect the transcription rate of drug transporter proteins (74-78). Such observations hold out the tentative prospect that future drug resistance strategies may make use of regulators of drug transporter expression, decreasing efflux pump expression and/or increasing uptake transporter expression, one assumes in a tumour-specific manner. Clinical application of such strategies is many years in the future.

Conclusion

The efficacy of many, if not all, small molecule cancer drugs, both the established cytotoxics and newer molecularly-targeted agents, at their most fundamental depends on the balance of drug uptake and efflux. These processes are controlled by a number of transport proteins including the classical drug efflux pumps like P-gp and MRP-1. The identification of new substrates/inhibitors which can usefully modulate the activity of such resistance mechanisms, while at the same time having potentially useful anticancer properties, may be of some therapeutic benefit in due course, particularly as tumour cells can have a significant repertoire of pumps which can reduce their exposure to cancer drugs. Advancing the therapeutic impact of resistance research will, by necessity, require dynamic tumour-selective interventions which control drug uptake and reduce drug efflux in a relatively tumour-specific manner. Making use of such advances will also require better characterisation of the prevalence of the proteins responsible for net drug accumulation (*i.e.* the sum of uptake and efflux) in general forms of cancer and in the individual circumstances of a cancer patient.

Acknowledgements

The NICB is supported by a PRTL I III award from the Higher Education Authority of Ireland.

References

- Dean, M, Fojo T and Bates S: Tumour stem cells and drug resistance. *Nat Rev Cancer* 5: 275-284, 2005.
- Leonessa F and Clarke R: ATP binding cassette transporters and drug resistance in breast cancer. *Endocr Relat Cancer* 10: 43-73, 2003.
- Leonard GD, Polgar O and Bates SE: ABC transporters and inhibitors: new targets, new agents. *Curr Opin Investig Drugs* 3: 1652-1659, 2002.
- Juliano RL and Ling V: A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 455: 152-162, 1976.
- Loo TW and Clarke DM: Recent progress in understanding the mechanism of P-glycoprotein-mediated drug efflux. *J Membr Biol* 206: 173-185, 2005.
- 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* 258: 1650-1654, 1992.
- Sarkadi B, Ozvegy-Laczka C, Nemet K and Varadi A: ABCG2 – a transporter for all seasons. *FEBS Lett* 567: 116-120, 2004.
- Ozvegy-Laczka C, Cserepes J, Elkind NB and Sarkadi B: Tyrosine kinase inhibitor resistance in cancer: role of ABC multidrug transporters. *Drug Resist Updat* 8: 15-26, 2005.
- Kruh GD, Guo Y, Hopper-Borge E, Belinsky MG and Chen ZS: ABCC10, ABCC11 and ABCC12. *Pflugers Arch*, 2006.
- Tian Q, Zhang J, Chan E, Duan W and Zhou SF: Multidrug resistance proteins (MRPs) and implication in drug development. *Drug Development Research* 64: 1-18, 2005.
- Borst P, de Wolf C and van de Wetering K: Multidrug resistance-associated proteins 3, 4 and 5. *Pflugers Arch*, 2006.
- 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: 216-237, 2005.
- Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H and Bruford EA: The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Introduction. *Pflugers Arch* 447: 465-468, 2004.
- Nishimura M and Naito S: Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab Pharmacokinet* 20: 452-477, 2005.
- Zisowsky J, Koegel S, Leyers S, Devarakonda K, Kassack MU, Osmak M and Jaehde U: Relevance of drug uptake and efflux for cisplatin sensitivity of tumor cells. *Biochem Pharmacol* 73(2): 298-307, 2006.
- Samimi G, Varki NM, Wilczynski S, Safaei R, Alberts DS and Howell SB: Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. *Clin Cancer Res* 9: 5853-5859, 2003.
- Holzer AK and Howell SB: The internalization and degradation of human copper transporter 1 following cisplatin exposure. *Cancer Res* 66: 10944-10952, 2006.
- Cui Y, Konig J, Nies AT, Pfannschmidt M, Hergt M, Franke WW, Alt W, Moll R and Keppler D: Detection of the human organic anion transporters SLC21A6 (OATP2) and SLC21A8 (OATP8) in liver and hepatocellular carcinoma. *Lab Invest* 83: 527-538, 2003.
- Smith NF, Acharya MR, Desai N, Figg WD and Sparreboom A: Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. *Cancer Biol Ther* 4: 815-818, 2005.
- Szakacs, G, Paterson JK, Ludwig JA, Booth-Genthe, C and Gottesman MM: Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* 5: 219-234, 2006.

- 21 Larkin A, O'Driscoll L, Kennedy S, Purcell R, Moran E, Crown J, Parkinson M and Clynes M: Investigation of MRP-1 protein and MDR-1 P-glycoprotein expression in invasive breast cancer: a prognostic study. *Int J Cancer* 112: 286-294, 2004.
- 22 Fillpits M, Suchomel RW, Dekan G, Stiglbauer W, Haider K, Depisch D and Pirker R: Expression of the multidrug resistance-associated protein (MRP) gene in colorectal carcinomas. *Br J Cancer* 75: 208-212, 1997.
- 23 van den Heuvel-Eibrink MM, Wiemer EA, Prins A, Meijerink JP, Vossebeld PJ, van der Holt B, Pieters R and Sonneveld P: Increased expression of the breast cancer resistance protein (BCRP) in relapsed or refractory acute myeloid leukemia (AML). *Leukemia* 16: 833-839, 2002.
- 24 van den Heuvel-Eibrink MM, Wiemer EA, de Boevere MJ, van der Holt B, Vossebeld PJ, Pieters R and Sonneveld P: MDR1 gene-related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia. *Blood* 97: 3605-3611, 2001.
- 25 van der Kolk DM, de Vries EG, van Putten WJ, Verdonck LF, Ossenkoppele GJ, Verhoef GE and Vellenga E: P-glycoprotein and multidrug resistance protein activities in relation to treatment outcome in acute myeloid leukemia. *Clin Cancer Res* 6: 3205-3214, 2000.
- 26 Brinkhuis M, Izquierdo MA, Baak JP, van Diest PJ, Kenemans P, Scheffer GL and Scheper RJ: Expression of multidrug resistance-associated markers, their relation to quantitative pathologic tumour characteristics and prognosis in advanced ovarian cancer. *Anal Cell Pathol* 24: 17-23, 2002.
- 27 Diestra JE, Condom E, Del Muro XG, Scheffer GL, Perez J, Zurita AJ, Munoz-Segui J, Vignes F, Scheper RJ, Capella G, Germa-Lluch JR and Izquierdo MA: Expression of multidrug resistance proteins P-glycoprotein, multidrug resistance protein 1, breast cancer resistance protein and lung resistance related protein in locally advanced bladder cancer treated with neoadjuvant chemotherapy: biological and clinical implications. *J Urol* 170: 1383-1387, 2003.
- 28 Swerts K, De Moerloose B, Dhooze C, Laureys G, Benoit Y and Philippe J: Prognostic significance of multidrug resistance-related proteins in childhood acute lymphoblastic leukaemia. *Eur J Cancer* 42: 295-309, 2006.
- 29 Uggla B, Stahl E, Wagsater D, Paul C, Karlsson MG, Sirsjo A and Tidefelt U: BCRP mRNA expression v. clinical outcome in 40 adult AML patients. *Leuk Res* 29: 141-146, 2005.
- 30 Damiani D, Tiribelli M, Calistri E, Geromin A, Chiarvesio A, Michelutti A, Cavallin M and Fanin R: The prognostic value of P-glycoprotein (ABCB) and breast cancer resistance protein (ABCG2) in adults with *de novo* acute myeloid leukemia with normal karyotype. *Haematologica* 91: 825-828, 2006.
- 31 Rogan AM, Hamilton TC, Young RC, Klecker RW Jr and Ozols RF: Reversal of adriamycin resistance by verapamil in human ovarian cancer. *Science* 224: 994-996, 1984.
- 32 Horton JK, Thimmaiah KN, Houghton JA, Horowitz ME and Houghton PJ: Modulation by verapamil of vincristine pharmacokinetics and toxicity in mice bearing human tumor xenografts. *Biochem Pharmacol* 38: 1727-1736, 1989.
- 33 Arvelo F, Poupon MF, Goguel AF, Lizard G, Bourgeois Y, Arriagada R and Le Chevalier T: Response of a multidrug-resistant human small-cell lung cancer xenograft to chemotherapy. *J Cancer Res Clin Oncol* 120: 17-23, 1993.
- 34 Ozols RF, Hamilton TC and Young RC: Modulation of drug resistance in human ovarian cancer: experimental results and clinical applications. *Prog Clin Biol Res* 223: 173-185, 1986.
- 35 Perez-Tomas R: Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. *Curr Med Chem* 13: 1859-1876, 2006.
- 36 Pusztai L, Wagner P, Ibrahim N, Rivera E, Theriault R, Booser D, Symmans FW, Wong F, Blumenschein G, Fleming DR, Rouzier R, Boniface G and Hortobagyi GN: Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. *Cancer* 104: 682-691, 2005.
- 37 Nobili S, Landini I, Giglioni B and Mini E: Pharmacological strategies for overcoming multidrug resistance. *Curr Drug Targets* 7: 861-879, 2006.
- 38 Hendrikse NH: Monitoring interactions at ATP-dependent drug efflux pumps. *Curr Pharm Des* 6: 1653-1668, 2000.
- 39 Hendrikse NH and Vaalburg W: Dynamics of multidrug resistance: P-glycoprotein analyses with positron emission tomography. *Methods* 27: 228-233, 2002.
- 40 Niederhuber JE and Fojo T: Treatment of metastatic disease in patients with neuroendocrine tumors. *Surg Oncol Clin N Am* 15: 511-533, viii, 2006.
- 41 Kuppens IE, Breedveld P, Beijnen JH and Schellens JH: Modulation of oral drug bioavailability: from preclinical mechanism to therapeutic application. *Cancer Invest* 23: 443-464, 2005.
- 42 Shepard RL, Cao J, Starling JJ and Dantzig AH: Modulation of P-glycoprotein but not MRP1- or BCRP-mediated drug resistance by LY335979. *Int J Cancer* 103: 121-125, 2003.
- 43 Maliepaard M, van Gastelen MA, Tohgo A, Hausheer FH, van Waardenburg RC, de Jong LA, Pluim D, Beijnen JH and Schellens JH: Circumvention of breast cancer resistance protein (BCRP)-mediated resistance to camptothecins *in vitro* using non-substrate drugs or the BCRP inhibitor GF120918. *Clin Cancer Res* 7: 935-941, 2001.
- 44 Zheng JH, Chen CT, Au JL and Wientjes MG: Time- and concentration-dependent penetration of doxorubicin in prostate tumors. *AAPS Pharm Sci* 3: E15, 2001.
- 45 Au JL, Jang SH, Zheng J, Chen CT, Song S, Hu L and Wientjes MG: Determinants of drug delivery and transport to solid tumors. *J Control Release* 74: 31-46, 2001.
- 46 Kuh HJ, Jang SH, Wientjes MG and Au JL: Computational model of intracellular pharmacokinetics of paclitaxel. *J Pharmacol Exp Ther* 293: 761-770, 2000.
- 47 Jang SH, Wientjes MG and Au JL: Kinetics of P-glycoprotein-mediated efflux of paclitaxel. *J Pharmacol Exp Ther* 298: 1236-1242, 2001.
- 48 Wang JC, Liu XY, Lu WL, Chang A, Zhang Q, Goh BC and Lee HS: Pharmacokinetics of intravenously administered stealth liposomal doxorubicin modulated with verapamil in rats. *Eur J Pharm Biopharm* 62: 44-51, 2006.
- 49 Ozevgy-Laczka C, Hegedus T, Varady G, Ujhelly O, Schuetz JD, Varadi A, Keri G, Orfi L, Nemet K and Sarkadi B: High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. *Mol Pharmacol* 65: 1485-1495, 2004.
- 50 Marshall J: Clinical implications of the mechanism of epidermal growth factor receptor inhibitors. *Cancer* 107: 1207-1218, 2006.
- 51 Hegedus T, Orfi L, Seprodi A, Varadi A, Sarkadi B and Keri G: Interaction of tyrosine kinase inhibitors with the human multidrug transporter proteins, MDR1 and MRP1. *Biochim Biophys Acta* 1587: 318-325, 2002.

- 52 Elkind NB, Szentpetery Z, Apati A, Ozvegylaczka C, Varady G, Ujhelly O, Szabo K, Homolya L, Varadi A, Buday L, Keri G, Nemet K and Sarkadi B: Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor Iressa (ZD1839, Gefitinib). *Cancer Res* 65: 1770-1777, 2005.
- 53 Mukai M, Che XF, Furukawa T, Sumizawa T, Aoki S, Ren XQ, Haraguchi M, Sugimoto Y, Kobayashi M, Takamatsu H and Akiyama S: Reversal of the resistance to STI571 in human chronic myelogenous leukemia K562 cells. *Cancer Sci* 94: 557-563, 2003.
- 54 Collins D, O'Connor R, O'Donovan N, Clynes M and Crown J: Lapatinib inhibits P-glycoprotein (P-gp) and synergistically enhances taxane cytotoxicity in P-gp+ cell lines. *Ann Oncol* 17: 57, 2006.
- 55 Drach J, Zhao S, Drach D, Korbiling M, Engel H and Andreeff M: Expression of MDR1 by normal bone marrow cells and its implication for leukemic hematopoiesis. *Leuk Lymphoma* 16: 419-424, 1995.
- 56 Drach D, Zhao S, Drach J, Mahadevia R, Gattlinger C, Huber H and Andreeff M: Subpopulations of normal peripheral blood and bone marrow cells express a functional multidrug resistant phenotype. *Blood* 80: 2729-2734, 1992.
- 57 Ohno N, Tani A, Chen ZS, Uozumi K, Hanada S, Akiba S, Ren XQ, Furukawa T, Sumizawa T, Arima T and Akiyama SI: Prognostic significance of multidrug resistance protein in adult T-cell leukemia. *Clin Cancer Res* 7: 3120-3126, 2001.
- 58 Nooter K, Westerman AM, Flens MJ, Zaman GJ, Scheper RJ, van Wingerden KE, Burger H, Oostrum R, Boersma T, Sonneveld P, Gratama JW, Kok T, Eggermont AMM, Bosman FT and Stoter G: Expression of the multidrug resistance-associated protein (MRP) gene in human cancers. *Clin Cancer Res* 1: 1301-1310, 1995.
- 59 Nooter K, Bosman FT, Burger H, van Wingerden KE, Flens MJ, Scheper RJ, Oostrum RG, Boersma AW, van der Gaast A and Stoter G: Expression of the multidrug resistance-associated protein (MRP) gene in primary non-small cell lung cancer. *Ann Oncol* 7: 75-81, 1996.
- 60 Nooter K, Brutel de la Riviere G, Look MP, van Wingerden KE, Henzen-Logmans SC, Scheper RJ, Flens MJ, Klijn JG, Stoter G and Foekens JA: The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. *Br J Cancer* 76: 486-493, 1997.
- 61 Nooter K, Kok T, Bosman FT, van Wingerden KE and Stoter G: Expression of the multidrug resistance protein (MRP) in squamous cell carcinoma of the oesophagus and response to pre-operative chemotherapy. *Eur J Cancer* 34: 81-86, 1998.
- 62 Seiden MV, Swenerton KD, Matulonis U, Campos S, Rose P, Batist G, Ette E, Garg V, Fuller A, Harding MW and Charpentier D: A phase II study of the MDR inhibitor biricodar (INCEL, VX-710) and paclitaxel in women with advanced ovarian cancer refractory to paclitaxel therapy. *Gynecol Oncol* 86: 302-310, 2002.
- 63 O'Connor R, Heenan M, Connolly L, Larkin A and Clynes M: Increased anti-tumour efficacy of doxorubicin when combined with sulindac in a xenograft model of an MRP-1-positive human lung cancer. *Anticancer Res* 24: 457-464, 2004.
- 64 Duffy CP, Elliott CJ, O'Connor RA, Heenan MM, Coyle S, Cleary IM, Kavanagh K, Verhaegen S, O'Loughlin CM, NicAmhlaoibh R and Clynes M: Enhancement of chemotherapeutic drug toxicity to human tumour cells *in vitro* by a subset of non-steroidal anti-inflammatory drugs (NSAIDs). *Eur J Cancer* 34: 1250-1259, 1998.
- 65 O'Connor R, O'Leary M, Ballot J, Collins CD, Kinsella P, Mager DE, Arnold RD, O'Driscoll L, Larkin A, Kennedy S, Fennelly D, Clynes M and Crown J: A phase I clinical and pharmacokinetic study of the multi-drug resistance protein-1 (MRP-1) inhibitor sulindac, in combination with epirubicin in patients with advanced cancer. *Cancer Chemother Pharmacol* 59: 79-87, 2007.
- 66 Choudhuri S and Klaassen CD: Structure, function, expression, genomic organization and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP) and ABCG2 (BCRP) efflux transporters. *Int J Toxicol* 25: 231-259, 2006.
- 67 Conseil G, Deeley RG and Cole SP: Polymorphisms of MRP1 (ABCC1) and related ATP-dependent drug transporters. *Pharmacogenet Genomics* 15: 523-533, 2005.
- 68 Saito S, Iida A, Sekine A, Miura Y, Ogawa C, Kawauchi S, Higuchi S and Nakamura Y: Identification of 779 genetic variations in eight genes encoding members of the ATP-binding cassette, subfamily C (ABCC/MRP/CFTR). *J Hum Genet* 47: 147-171, 2002.
- 69 Jamroz K, Balcerczak, Molewski P, Robey RW, Cebula B, Panczyk M, Kowalczyk M, Szmigielska-Kaplon A, Mirowski M, Bates SE and Robak T: MDR1 (ABCB1) gene polymorphism C3435T is associated with P-glycoprotein activity in B-cell chronic lymphocytic leukemia. *Pharmacol Rep* 58: 720-728, 2006.
- 70 Jamroz K, Balcerczak E, Cebula B, Kowalczyk M, Panczyk M, Janus A, Smolewski P, Mirowski M and Robak T: Multi-drug transporter MDR1 gene polymorphism and prognosis in adult acute lymphoblastic leukemia. *Pharmacol Rep* 57: 882-888, 2005.
- 71 Jamroz K and Robak T: Pharmacogenomics of MDR1/ABCB1 gene: the influence on risk and clinical outcome of haematological malignancies. *Hematology* 9: 91-105, 2004.
- 72 Su Y, Zhang X and Sinko PJ: Human organic anion-transporting polypeptide OATP-A (SLC21A3) acts in concert with P-glycoprotein and multidrug resistance protein 2 in the vectorial transport of Saquinavir in Hep G2 cells. *Mol Pharm* 1: 49-56, 2004.
- 73 Ito K, Suzuki H, Horie T and Sugiyama Y: Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport. *Pharm Res* 22: 1559-1577, 2005.
- 74 Scotto KW: Transcriptional regulation of ABC drug transporters. *Oncogene* 22: 7496-7511, 2003.
- 75 Fernandez C, Buyse M, German-Fattal M and Gimenez F: Influence of the pro-inflammatory cytokines on P-glycoprotein expression and functionality. *J Pharm Pharm Sci* 7: 359-371, 2004.
- 76 Aggarwal BB and Ichikawa H: Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. *Cell Cycle* 4: 1201-1215, 2005.
- 77 Miao ZH, Tang T, Zhang YX, Zhang JS and Ding J: Cytotoxicity, apoptosis induction and downregulation of MDR-1 expression by the anti-topoisomerase II agent, salvicine, in multidrug-resistant tumor cells. *Int J Cancer* 106: 108-115, 2003.
- 78 Cao JG, Tang XQ and Shi SH: Multidrug resistance reversal in human gastric carcinoma cells by neferine. *World J Gastroenterol* 10: 3062-3064, 2004.

Received December 19, 2006
Accepted February 13, 2007