Carvedilol: a new candidate for reversal of MDR1/P-glycoprotein-mediated multidrug resistance

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In 1983, carvedilol [1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)amino]-2-propanol] was designed and developed as a β-adrenoceptor antagonist with vasodilating activity for efficacious and safe treatment of hypertension and coronary artery disease. Carvedilol belongs to the 'third generation' of β-adrenoceptor antagonists and shows selectivity for the β_1- rather than β_2-adrenoceptor. Carvedilol is also an α_1-blocking agents, with around 2- to 3-fold more selectivity for β_1- than α_1-adrenoceptors. This degree of α_1-blockade is responsible for the moderate vasodilator properties of carvedilol, being different from other β-adrenoceptor antagonists. In addition, carvedilol is a potent antioxidant, with a 10-fold greater activity than vitamin E. Some carvedilol metabolites found in human plasma also exhibit antioxidative activity approximately 50- to 100-fold greater than carvedilol and other antioxidants. These unique properties of carvedilol, i.e. adrenergic (β_1, β_2 and α_1) blockade and antioxidative activity, may be important in preventing progressive deterioration of left ventricular dysfunction and chronic heart failure. Recently, carvedilol has been demonstrated to reverse multidrug resistance (MDR) to anticancer drugs in tumor cells *in vitro* and its reversal effects were comparable with verapamil, which has been used in the first clinical trial for the reversal of MDR. This review introduces the reversal activity and usefulness against MDR, as well as an overview of the pharmacological and pharmacokinetic properties, of carvedilol. *Anti-Cancer Drugs* 15:303–309 © 2004 Lippincott Williams & Wilkins.

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Development of carvedilol

In 1983, carvedilol [1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)amino]-2-propanol] (Fig. 1) was designed and developed as a β-adrenoceptor antagonist with vasodilating activity for efficacious and safe treatment of hypertension and coronary artery disease. This drug contains an oxyisopropanolamine moiety with aromatic substituents linked to both the oxy and amine ends [1–3]. A common structural feature of β-adrenoceptor antagonists is either an arylethanolamine or an aryloxyisopropanolamine moiety, but the compounds differ in the nature of the aryl group, as well as the group(s) linked to the amine moiety. The most important differences between β-adrenoceptor antagonists are related to the degree of selectivity for the β_1- against β_2-adrenoceptor [4–6]. Based on these characteristics, β-adrenoceptor antagonists have been categorized into three classes (Table 1).

The 'first-generation' compounds, such as propranolol and timolol, are non-selective drugs with equal affinity for blocking β_1- and β_2-adrenoceptors, and no important pharmacological effects other than the β-blockade. The 'second-generation' compounds, such as metoprolol, atenolol, ceriprolol, acebutolol and bisoprolol, show selectivity for the β_1-adrenoceptor. Carvedilol belongs to the 'third-generation' compounds including bucindolol, labetalol and nebivolol, and has selectivity (around 7-fold) for β_1- rather than β_2-adrenoceptors, but becomes non-selective at higher target doses. Carvedilol is also an α_1-blocking drug, with around 2- to 3-fold more selectivity for β_1- than α_1-adrenoceptors. This degree of α_1-blockade is responsible for the moderate vasodilator properties of carvedilol, being different from other β-adrenoceptor antagonists.

In addition, carvedilol is a potent antioxidant, with 10-fold greater activity than the antioxidant vitamin E [7–9], and prevents depletion of vitamin E, gluthatione and SH protein induced by oxidative stress. These actions are the main defense mechanisms against tissue injury caused by free radicals and their property derives from the carbazole portion of its chemical structure (Fig. 1). Also, some carvedilol metabolites found in human plasma exhibit antioxidative activity approximately 50- to 100-fold greater than that of carvedilol and other antioxidants to inhibit low-density lipoprotein oxidation by mouse macrophages [10,11]. Collectively, these unique properties of carvedilol, i.e. multiple adrenergic (β_1, β_2 and α_1) blockade and antioxidative activity, might be one of the

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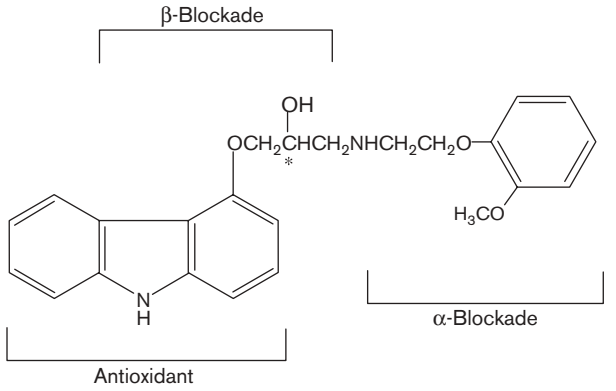
reasons that carvedilol was approved for the treatment of chronic heart failure, although the use of β-adrenoceptor antagonists for chronic heart failure had been questioned because of negative inotropic effects [12].

The pharmacokinetic profiles of carvedilol have been investigated in healthy volunteers and patients with hypertension [1,2,13,14]. Carvedilol is used clinically as a racemic mixture of *R*(+)- and *S*(–)-enantiomers, and demonstrates dose-linear behavior. The absolute oral bioavailability reached 24% probably due to a first-pass effect. After a single oral administration of 50 mg, maximum concentrations of about 70 ng/ml (around 0.2 μM) were achieved within at least 2 h. The plasma concentration of carvedilol at the steady-state was around 160 ng/ml (around 0.4 μM) after repetitive administration at a dose of 50 mg/day. In addition, carvedilol was extensively distributed to the tissues (volume of distribution: around 130 l) and eliminated primarily by hepatic metabolism (total clearance: around 600 ml/min, renal clearance: 4 ml/min). Carvedilol is metabolized by mainly cytochrome P450 2D6 (CYP2D6), with CYP2C9 and CYP1A2, but not CYP3A4, having also been clarified to participate in the metabolism in humans [15]. Detailed information on the pharmacokinetics and pharmacodynamics of carvedilol is available in the literature [1,2,16–19].

Fig. 1



Chemical structure of carvedilol, [1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)amino]-2-propanol], with postulated active sites. An asterisk indicates the point of asymmetry.

Recently, carvedilol has been demonstrated to reverse multidrug resistance (MDR) to anticancer drugs in tumor cells *in vitro* [20,21] and its reversal effects were comparable to those of verapamil, which has been used as a MDR modulator for the first time in clinical trials for the reversal of MDR. In this review, we introduce the reversal activity and usefulness against MDR of carvedilol from the viewpoint of direct interaction with MDR1, β-adrenoceptor selectivity, metabolic process, antioxidative activity, etc.

MDR in cancer chemotherapy

The development of MDR remains a major hurdle to successful cancer chemotherapy [22–24]. Although the cellular basis underlying MDR is not fully understood, several factors have been identified [25–31]. These include down-regulation of uptake, induction of the efflux system (MDR1/P-glycoprotein, etc.), induction of inactivation enzymes (glutathione-S-transferase, etc.), alteration of the targeted molecule (topoisomerase, etc.), changes in DNA repair processes and alteration of the apoptotic mechanism (p53 mutation, bcl-2 over-expression, etc.). The overexpression of MDR1/P-glycoprotein in tumor cells is one of the best-characterized mechanisms of MDR [26,27,32–35]. Recently, the term MDR1 has come to be used instead of human P-glycoprotein, so MDR1 is used in this review.

In 1976, a 170-kDa glycosylated membrane protein (later called MDR1) was discovered through its ability to confer MDR in colchicine-resistant Chinese hamster ovary (CHO) cells by Juliano and Ling [36]. About 10 years later, a gene was isolated from multidrug resistant KB carcinoma cells and designated *MDR1* [37 and references therein]. *MDR1* is located on chromosome 7 at q21.1 in a 600-kb *Nru*I fragment and the *MDR1* coding region is contained in a 120-kb *Xho*I fragment. This gene extends

Table 1 Pharmacological properties of representative β-adrenoceptor antagonists *in vitro*

Generation	Drug	β_1-Blockade	β_2-Blockade	α_1-Blockade	Antioxidative activity
First	propranolol	+ +	+ +	–	+
	timolol	+ + +	+ + +	–	+
Second	acebutolol	+	–	–	?
	atenolol	+ +	–	–	?
	bisoprolol	+	–	–	–
	celiprolol	+	–	–	–
	metoprolol	+	–	–	?
Third	bucindolol	+ +	+	–	–
	carvedilol	+ +	+	+	+ + +
	labetalol	+	+	+	?
	nebivolol	+ +	+	–	–

This table was constructed by referring to [4,5,10,11,86,87]. A question mark '?' indicates inconsistent reports concerning the antioxidative activity.

over more than 100 kb containing 28 introns, 26 of which interrupt the protein-coding sequence. MDR1 mRNA has a size of 4.6 kb, thus its coding region accounts for less than 5% of the total.

MDR1, the product of the *MDR1* gene, belongs to the ATP-binding cassette (ABC) family of transporters, currently numbering 49 members, that share sequence and structural homology (see also http://nutrigene.4t.com/humanabc.htm). MDR1 is a phosphorylated and glycosylated membrane protein of 1279 amino acids, and consists of two homologous halves containing six putative hydrophobic transmembrane segments and an intracellular binding site for ATP [37 and references therein]. This protein has been understood to act as an efflux pump to expel anticancer drugs from cells utilizing the energy of ATP hydrolysis, thus lowering intracellular concentrations. The anticancer drugs that are transported by MDR1 include hydrophobic and/or amphipathic drugs, such as the taxanes (paclitaxel and docetaxel), Vinca alkaloids (vinblastine, vincristine, and vinorelbine), anthracyclines (doxorubicin and daunorubicin), epipodophyllotoxins (etoposide and teniposide), topotecan, actinomycin D and mitomycin C. As a result, MDR1-overexpressing tumor cells show resistance to multiple anticancer drugs. Therefore, the development of MDR1 inhibitors, i.e. MDR modulators, has been carried out industriously with sights set on the reversal of MDR.

Attempts to reverse MDR

The calcium channel blocker verapamil was the first drug found to inhibit MDR1-mediated transport *in vitro* [38]. Tsuruo *et al.* demonstrated a decreased efflux of vincristine in vincristine-resistant P388 leukemia cells in the presence of verapamil [38]. In 1984, Rogan *et al.* started the first clinical trial with verapamil and doxorubicin in patients with ovarian cancer [39]. However, the majority of investigations with these drugs demonstrated disappointing results because low affinities for MDR1 necessitated the use of high doses of modulators, resulting in unacceptable toxicity.

To overcome these problems, second-generation MDR modulators had been developed, such as dexverapamil, valspodar (PSC833) and biricodar (VX-710) [40–43]. The second-generation MDR modulators have been demonstrated to be more effective for reversal of MDR than the first-generation MDR modulators *in vitro* and in animal experiments. However, the second-generation MDR modulators also influence the pharmacokinetics of anticancer drug through the inhibition of MDR1-mediated biliary excretion or intestinal transport. In addition, another mechanism by which they influence the pharmacokinetics of anticancer drug was clarified to be competition for CYP3A4-mediated liver or intestinal metabolism [44,45] and thus the use of second-generation MDR modulators has led to unacceptable toxicity of anticancer drugs in clinical trials. The most common response of clinical researchers to these pharmacokinetic interactions has been to reduce the dose of anticancer drugs. Regrettably, the pharmacokinetic interactions between MDR modulators and anticancer drugs are complicated and unpredictable, and the doses of anticancer drugs co-administered with MDR modulators could not be determined in advance. Thus, there has been no establishment of a safe and effective dose of anticancer drugs. However, it has been noted that MDR1 and CYP3A4 share significant overlap in substrate specificity [46–51] (refer to the next section).

To overcome the limitations of the second-generation MDR modulators, third-generation MDR modulators have been developed using quantitative structure–activity relationship (QSAR) analysis and combinatorial chemistry. The third-generation MDR modulators currently in clinical development include tariquidar (XR9576), zosuquidar (LY335979), R101933 and ONT-093 [52–56]. To date, the results of clinical trials show that the third-generation MDR modulators can be given with full therapeutic doses of anticancer drugs with minimal interference to the pharmacokinetics of anticancer drugs [43]. The preliminary results with the third-generation MDR modulators offer new hope that this goal might be realized. This would be mainly explained by the finding that the third-generation MDR modulators did not affect CYP3A4-mediated metabolism of anticancer drugs at clinically achievable concentrations. Therefore, a key to developing MDR modulators is understanding the overlap in substrate specificity between MDR1 and CYP3A4. Unfortunately, it will presumably take a little more time before the third-generation MDR modulators are released for clinical use. Consequently, drugs marketed for other uses have attracted attention again as candidates for MDR reversal, but the overlap in substrate specificity between MDR1 and CYP3A should be clarified in advance.

Overlap in substrate specificity between MDR1 and CYP3A

MDR1 is expressed not only in resistant tumor cells, but also in normal tissues with an excretory function such as the biliary canalicular membrane of hepatocytes, the luminal membrane of endothelial cells in the blood–brain barrier and blood–testis barrier, the apical membrane of the syncytial trophoblasts of the placenta, the epithelial apical membrane of the intestine, and the renal proximal tubules [57]. From this evidence, MDR1 may be an important barrier to xenobiotics. In addition, pharmacokinetic studies have demonstrated the importance of MDR1 in limiting oral drug bioavailability and the distribution of drugs to tissue when the drugs are MDR1 substrates [37,58–60].

CYP3A is the CYP subfamily responsible for phase I metabolism of more than 50% of drugs administered to humans [61]. CYP3A4 is the most prominent CYP in humans, comprising about 30 and 70% of total CYP in liver and intestine, respectively. CYP3A4 has very broad substrate specificity, encompassing a wide variety of drugs [46]. Many anticancer drugs, such as epipodophyllotoxins and Vinca alkaloids, are substrates for CYP3A4 [62–64].

Like CYP3A4, MDR1 seems to have broad substrate specificity [37,45,59]. Interestingly, a striking overlap of substrates for MDR1 and CYP3A4 has been observed [46–51]. Focusing on these observations and expression sites, Benet and his collaborators proposed that proteins of MDR1 and CYP3A4 act synergistically to present a barrier to absorption from the small intestine and to distribute to the tissues [58,59]. Inhibition of one or both of these proteins can be expected to enhance the bioavailability and to change the distribution of many drugs. That is to say, MDR modulators such as the substrate for CYP3A inhibited not only the MDR1-mediated transport of anticancer drugs, but also CYP3A-mediated metabolism. Therefore, the pharmacokinetics of anticancer drugs in combination with such MDR modulators is complicated and so it is difficult to manage effectively MDR modulators in cancer chemotherapy. Although some compounds interact with MDR1 and CYP3A4 to a similar extent, for the most part, the potency of inhibition for MDR1 does not predict the potency of inhibition for CYP3A and vice versa. Moreover, not all the substrates for CYP3A are substrates for MDR1 and vice versa, e.g. ketoconazole, midazolam and digoxin, etc. [65]. Consequently, it is desirable that MDR modulators only have inhibitory activity for MDR1 not CYP3A4.

Reversal effects of carvedilol on MDR1-mediated MDR

In 1999, Jonsson *et al.* suggested the possibility that carvedilol acts as a MDR modulator in the human breast cancer cell line Hs578T, with no measurable MDR1 expression and its MDR1-overexpressing doxorubicin-resistant subline Hs578T-Dox [20]. They demonstrated that 10 μM of carvedilol only marginally affected the IC_{50} value of doxorubicin, the dose which results in the death of half the number of cells, in Hs578T cells, whereas the IC_{50} value of doxorubicin in Hs578T-Dox cells was reduced 1/20. Also, a decrease in accumulation of calcein, a fluorescence substrate for MDR1, was shown in Hs578T-Dox cells compared to the host Hs578T cells and the effect was reversed by preincubation with 1 or 10 μM carvedilol. However, the concentration of carvedilol examined, i.e. 10 μM, was much higher than the clinically available concentration, and an estimation of its clinical potency was complicated.

In 2003, we examined the reversal activity of carvedilol against MDR1-mediated MDR using a realistic clinical concentration, 1 μM [21]. In human cervical carcinoma HeLa-Ohio (HeLa) cells, the growth curves for vinblastine, paclitaxel and cisplatin were not altered by the addition of 1 μM carvedilol. However, the cytotoxicity of vinblastine and paclitaxel in the MDR1-overexpressing vinblastine-resistant HeLa subline (Hvr100-6) was increased 3.5- and 7.1-fold by 1 μM carvedilol treatment, respectively and this reversal occurred in a carvedilol concentration-dependent manner. In addition, carvedilol slightly modified the cytotoxicity of doxorubicin and daunorubicin in Hvr100-6 cells. In contrast, the IC_{50} values for 5-fluorouracil and cisplatin, which were not substrates for MDR1, were not affected. These findings using a realistic clinical concentration of carvedilol suggested that carvedilol had the clinical potential to reverse MDR1-mediated MDR. We also indicated that carvedilol effectively restored the intracellular accumulation of [^{3}H]vinblastine, [^{3}H]daunorubicin or [^{3}H]digoxin in MDR1-transfected cells (LLC-GA5-COL150 cells) in a concentration-dependent manner [21,66]. Carvedilol was clarified to suppress the MDR1-mediated trans-cellular transport of [^{3}H]daunorubicin or [^{3}H]digoxin in LLC-GA5-COL150 cells, but no alteration of transport was found in host LLC-PK_1 cells. Moreover, the inhibitory effects of carvedilol on the trans-cellular transport of [^{3}H]daunorubicin, [^{3}H]vinblastine or [^{3}H]digoxin were comparable to those of verapamil. These findings suggest that the reversal of MDR by carvedilol was associated with the direct inhibition of MDR1 and the reversal effects of carvedilol were comparable with those of verapamil at the same concentrations *in vitro* [20,21].

Verapamil was the first drug found to inhibit MDR1-mediated transport and clinical trials using verapamil were conducted for the reversal of MDR for the first time [38,39]. In those trials, the serum concentrations of verapamil were only approximately 0.5–1.0 μM [21]. On the other hand, the maximum concentrations of carvedilol after a single oral administration of 50 mg was about 70 ng/ml (around 0.2 μM) and the plasma concentration at the steady state was around 160 ng/ml (around 0.4 μM) after repetitive administration at a dose of 50 mg/day [1,2,13,14]. Since it was previously suggested that carvedilol and verapamil showed similar effects in terms of MDR reversal, carvedilol could be a new candidate modulator of MDR1-mediated MDR in clinical use. However, for controlling the pharmacokinetics and pharmacodynamics of anticancer drugs co-administered with carvedilol, details of the reversal mechanism of carvedilol should be clarified *in vitro*. Then, the effectiveness and usefulness will need to be examined in clinical studies.

Mechanism by which carvedilol reverses MDR1-meditaed MDR

Carvedilol has potent antioxidative activity, as well as β-adrenoceptor antagonistic activity [7–9]. Therefore, other mechanisms of reversing MDR may exist, other than the direct inhibition of MDR1.

We demonstrated that propranolol (β_1- and β_2-adrenoceptor antagonist) and metoprolol and atenolol (both β_1-adrenoceptor selective antagonists) did not affect MDR1-mediated MDR and transport [21], suggesting that the effects of carvedilol (β_1-adrenoceptor selective antagonist with α_1-blockade) on MDR1 were independent of its action on β-adrenoceptors. However, talinolol (β_1-adrenoceptor selective antagonist) has been demonstrated to inhibit the MDR1-mediated transport of digoxin [67]. In addition, celiprolol (β_1-adrenoceptor selective antagonist with slight α_2-agonist action) and acebutolol (β_1-adrenoceptor selective antagonist) have found to be substrates for MDR1 [68–70]. Collectively, the role of β-adrenoceptor antagonists in the inhibitory activity of MDR1 may be dependent on differences in receptor selectivity, not action on β-adrenoceptors.

Concerning the relation between MDR1 and reactive oxygen species, the induction of MDR1 has been reported to be associated with the generation of reactive oxygen species [71–73]. Ziemann *et al.* [72] indicated that the addition of H_2O_2 or a catalase inhibitor induced the expression of mdr1b, the MDR1 ortholog in rats, and its mRNA, and antioxidants markedly suppressed them in primary rat hepatocyte cultures. However, we found no effects of antioxidants, i.e. baicalein, genistein, quercetin, ascorbic acid, hydroquinone, superoxide dismutase and catalase, on MDR1 mRNA expression in HeLa cells and MDR1-overexpressing Hvr100-6 cells [74]. Moreover, the cytotoxicity of anticancer drugs in HeLa and Hvr100-6 cells was not altered by addition of these antioxidants [74]. Jonsson *et al.* [20] also reported that carvedilol did not affect pyrogallol cytotoxicity, which was a superoxide radical generator, and pyrogallol was without any effect on calcein accumulation of MDR1-overexpressing Hs578T-Dox cells, indicating a lack of antioxidative properties affecting MDR1 activity and associated toxicity of the drug [21]. Anyway, the action of antioxidants against MDR1 remains unclear.

Various types of drugs except for anticancer drugs have been clarified to regulate MDR1 expression [75–78]. In addition, some factors affecting MDR1 expression have been identified [79–81], although the transcriptional regulation of the *MDR1* gene was unexpectedly complex and is far from being completely understood. The *MDR1* promoter was found to contain a GC-box for Sp1, an inverted CCAAT element (Y-box) for YB-1 and NF-Y, a p53 element, an AP-1 element, a CAAT element for a complex of NF-κB and c-*fos* proteins, a C/EBP element for NF-IL-6, a heat-shock element (HSE) for heat-shock transcription factor (HSF), an inverted MED-1 for an unknown nuclear protein of about 150–160 kDa, and a steroid xenobiotic receptor (SXR) element for the orphan nuclear receptor SXR (also known as PXR, PAR, PRR or NR1I2). In the latest investigations, some drugs have been found to interact with these transcription factor(s) [82–84]. Moreover, it was reported that the development of MDR1-mediated MDR was a two-step post-transcriptional process mediated by changes in both MDR1 mRNA stability and translation [85]. Consequently, the understanding of these transcriptional processes of MDR1 has led to new possibilities for the reversal of MDR in cancer chemotherapy.

Future directions

From the standpoint of the reversal of MDR using β-adrenoceptor antagonists, the role of β-adrenoceptor antagonists in the inhibitory activity of MDR1 may be dependent on receptor selectivity, not action on β-adrenoceptors. Although the evidence is not complete, the utilization of differences in receptor selectivity for β-adrenoceptor antagonists may lead to a novel course for the development of MDR modulators.

In addition, it is desirable that MDR modulators have inhibitory activity for MDR1 but not CYP3A4, because MDR1 and CYP3A4 share significant overlap in substrate specificity. So, it will be important to design and develop MDR modulators with such specificity. To accomplish this, β-adrenoceptor antagonists including carvedilol, which are mainly metabolized by CYP2D6, will be attractive as novel candidates for the lead compound of a MDR modulator.

A link between MDR1 and reactive oxygen species certainly exists based on the past evidence. Carvedilol has the strongest antioxidative activity among the β-adrenoceptor antagonists (Table 1). Therefore, it is speculated that the antioxidative activity of carvedilol participates in the reversal of MDR. From the evidence of this, the use of antioxidants may achieve a breakthrough in the reversal of MDR.

On the other hand, the transcriptional processes of the *MDR1* gene have gradually been clarified with the advancement of genome science. Moreover, it has been demonstrated that some drugs interacted with factors participating in the transcriptional processes of the *MDR1* gene and thus this transcriptional process must be a hot target for the reversal of MDR. In the near future, these understandings will lead to new possibilities for the reversal of MDR in cancer chemotherapy. Therefore, the effects of β-adrenoceptor antagonists including carvedilol on the transcriptional processes should be elucidated.

Conclusion

It is very important that MDR modulators have a strong and specific inhibitory potency against MDR1, are clinically available drugs at present, and are non-substrates/inhibitors for CYP3A4. So, carvedilol could be a novel candidate modulator of MDR1-mediated MDR. However, its usefulness and effectiveness cannot be satisfied yet for the reversal of MDR in the clinical setting. To be successful in reversing MDR, it will be also necessary to carry out the development of MDR modulators from the viewpoint, which has not been done up to this point.

References

1 Sponer G, Bartsch W, Strein K, Muller-Beckmann B, Bohm E. Pharmacological profile of carvedilol as a beta-blocking agent with vasodilating and hypotensive properties. *J Cardiovasc Pharmacol* 1987; **9**:317–327.

2 McTavish D, Campoli-Richards D, Sorkin EM. Carvedilol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* 1993; **45**:232–258.

3 Dunn CJ, Lea AP, Wagstaff AJ. Carvedilol. A reappraisal of its pharmacological properties and therapeutic use in cardiovascular disorders. *Drugs* 1997; **54**:161–185.

4 Batlouni M, de Albuquerque DC. Beta-adrenergic blocking agents in heart failure. *Arq Bras Cardiol* 2000; **75**:339–360.

5 Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation* 2000; **101**:558–569.

6 Fowler M. Beta-adrenergic blocking drugs in severe heart failure. *Rev Cardiovasc Med* 2002; **3(suppl 3)**:S20–S26.

7 Yue TL, Cheng HY, Lysko PG, *et al.* Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. *J Pharmacol Exp Ther* 1992; **263**:92–98.

8 Cheng J, Kamiya K, Kodama I. Carvedilol: molecular and cellular basis for its multifaceted therapeutic potential. *Cardiovasc Drug Rev* 2001; **19**: 152–171.

9 Nakamura K, Kusano K, Nakamura Y, *et al.* Carvedilol decreases elevated oxidative stress in human failing myocardium. *Circulation* 2002; **105**: 2867–2871.

10 Yue TL, McKenna PJ, Lysko PG, Ruffolo Jr RR, Feuerstein GZ. Carvedilol, a new antihypertensive, prevents oxidation of human low density lipoprotein by macrophages and copper. *Atherosclerosis* 1992; **97**:209–216.

11 Yue TL, Mckenna PJ, Lysko PG, *et al.* SB 211475, a metabolite of carvedilol, a novel antihypertensive agent, is a potent antioxidant. *Eur J Pharmacol* 1994; **251**:237–243.

12 Constant J. A review of why and how we may use beta-blockers in congestive heart failure. *Chest* 1998; **113**:800–808.

13 Neugebauer G, Akpan W, von Mollendorff E, Neubert P, Reiff K. Pharmacokinetics and disposition of carvedilol in humans. *J Cardiovasc Pharmacol* 1987; **10(suppl 11)**:S85–S88.

14 von Mollendorff E, Reiff K, Neugebauer G. Pharmacokinetics and bioavailability of carvedilol, a vasodilating beta-blocker. *Eur J Clin Pharmacol* 1987; **33**:511–513.

15 Oldham HG, Clarke SE. *In vitro* identification of the human cytochrome P450 enzymes involved in the metabolism of *R*(+)- and *S*(–)-carvedilol. *Drug Metab Dispos* 1997; **25**:970–977.

16 Cubeddu LX, Fuenmayor N, Varin F, Villagra VG, Colindres RE, Powell JR. Clinical pharmacology of carvedilol in normal volunteers. *Clin Pharmacol Ther* 1987; **41**:31–44.

17 McPhillips JJ, Schwemer GT, Scott DI, Zinny M, Patterson D. Effects of carvedilol on blood pressure in patients with mild to moderate hypertension. A dose response study. *Drugs* 1988; **36(suppl 6)**:82–91.

18 Abraham WT, Scarpinato L. Higher expectations for management of heart failure: current recommendations. *J Am Board Fam Pract* 2002; **15**:39–49.

19 Cesario DA, Fonarow GC. Beta-blocker therapy for heart failure: the standard of care. *Rev Cardiovasc Med* 2002; **3**:14–21.

20 Jonsson O, Behnam-Motlagh P, Persson M, Henriksson R, Grankvist K. Increase in doxorubicin cytotoxicity by carvedilol inhibition of P-glycoprotein activity. *Biochem Pharmacol* 1999; **58**:1801–1806.

21 Kakumoto M, Sakaeda T, Takara K, *et al.* Effects of carvedilol on MDR1-mediated multidrug resistance: comparison with verapamil. *Cancer Sci* 2003; **94**:81–86.

22 Geney R, Ungureanu M, Li D, Ojima I. Overcoming multidrug resistance in taxane chemotherapy. *Clin Chem Lab Med* 2002; **40**:918–925.

23 Goldman B. Multidrug resistance: can new drugs help chemotherapy score against cancer? *J Natl Cancer Inst* 2003; **95**:255–257.

24 Mattern J. Drug resistance in cancer: a multifactorial problem. *Anticancer Res* 2003; **23**:1769–1772.

25 Shen D, Pastan I, Gottesman MM. Cross-resistance to methotrexate and metals in human cisplatin-resistant cell lines results from a pleiotropic defect in accumulation of these compounds associated with reduced plasma membrane binding proteins. *Cancer Res* 1998; **58**:268–275.

26 Persidis A. Cancer multidrug resistance. *Nat Biotechnol* 1999; **17**:94–95.

27 Ueda K, Yoshida A, Amachi T. Recent progress in P-glycoprotein research. *Anticancer Drug Des* 1999; **14**:115–121.

28 Bush JA, Li G. Cancer chemoresistance: the relationship between p53 and multidrug transporters. *Int J Cancer* 2002; **98**:323–330.

29 Liem AA, Chamberlain MP, Wolf CR, Thompson AM. The role of signal transduction in cancer treatment and drug resistance. *Eur J Surg Oncol* 2002; **28**:679–684.

30 Tsuruo T, Naito M, Tomida A, *et al.* Molecular targeting therapy of cancer: drug resistance, apoptosis and survival signal. *Cancer Sci* 2003; **94**:15–21.

31 Ejendal KF, Hrycyna CA. Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. *Curr Protein Pept Sci* 2002; **3**:503–511.

32 Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2002; **2**:48–58.

33 Stein WD. Reversers of the multidrug resistance transporter P-glycoprotein. *Curr Opin Invest Drugs* 2002; **3**:812–817.

34 Sharma R, Awasthi YC, Yang Y, Sharma A, Singhal SS, Awasthi S. Energy dependent transport of xenobiotics and its relevance to multidrug resistance. *Curr Cancer Drug Targets* 2003; **3**:89–107.

35 Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. *Drug Resist Update* 2003; **6**:71–84.

36 Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976; **455**:152–162.

37 Sakaeda T, Nakamura T, Okumura K. Pharmacogenetics of MDR1 and its impact on the pharmacokinetics and pharmacodynamics of drugs. *Pharmacogenomics* 2003; **4**:397–410.

38 Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* 1981; **41**:1967–1972.

39 Rogan AM, Hamilton TC, Young RC, Klecker Jr RW, Ozols RF. Reversal of adriamycin resistance by verapamil in human ovarian cancer. *Science* 1984; **224**:994–996.

40 Watanabe T, Tsuge H, Oh-Hara T, Naito M, Tsuruo T. Comparative study on reversal efficacy of SDZ PSC 833, cyclosporin A and verapamil on multidrug resistance *in vitro* and *in vivo*. *Acta Oncol* 1995; **34**:235–241.

41 Germann UA, Shlyakhter D, Mason VS, *et al.* Cellular and biochemical characterization of VX-710 as a chemosensitizer: reversal of P-glycoprotein-mediated multidrug resistance *in vitro*. *Anticancer Drugs* 1997; **8**:125–140.

42 Rowinsky EK, Smith L, Wang YM, *et al.* Phase I and pharmacokinetic study of paclitaxel in combination with biricodar, a novel agent that reverses multidrug resistance conferred by overexpression of both MDR1 and MRP. *J Clin Oncol* 1998; **16**:2964–2976.

43 Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting P-glycoprotein. *Cancer Control* 2003; **10**:159–165.

44 Fischer V, Rodriguez-Gascon A, Heitz F, *et al.* The multidrug resistance modulator valspodar (PSC 833) is metabolized by human cytochrome P450 3A. Implications for drug–drug interactions and pharmacological activity of the main metabolite. *Drug Metab Dispos* 1998; **26**:802–811.

45 Achira M, Suzuki H, Ito K, Sugiyama Y. Comparative studies to determine the selective inhibitors for P-glycoprotein and cytochrome P4503A4. *AAPS PharmSci* 1999; **1**:E18.

46 Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinogen* 1995; **13**:129–134.

47 Wacher VJ, Silverman JA, Zhang Y, Benet LZ. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* 1998; **87**:1322–1330.

48 Wandel C, Kim RB, Kajiji S, Guengerich P, Wilkinson GR, Wood AJ. P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. *Cancer Res* 1999; **59**:3944–3948.

49 Kim RB, Wandel C, Leake B, *et al.* Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm Res* 1999; **16**:408–414.

50 Tran CD, Timmins P, Conway BR, Irwin WJ. Investigation of the coordinated functional activities of cytochrome P450 3A4 and P-glycoprotein in limiting the absorption of xenobiotics in Caco-2 cells. *J Pharm Sci* 2002; **91**: 117–128.

51 Yasuda K, Lan LB, Sanglard D, Furuya K, Schuetz JD, Schuetz EG. Interaction of cytochrome P450 3A inhibitors with P-glycoprotein. *J Pharmacol Exp Ther* 2002; **303**:323–332.

52 Dantzig AH, Shepard RL, Cao J, *et al.* Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. *Cancer Res* 1996; **56**:4171–4179.

53 van Zuylen L, Sparreboom A, van der Gaast A, *et al.* The orally administered P-glycoprotein inhibitor R101933 does not alter the plasma pharmacokinetics of docetaxel. *Clin Cancer Res* 2000; **6**:1365–1371.

54 Mistry P, Stewart AJ, Dangerfield W, *et al. In vitro* and *in vivo* reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* 2001; **61**:749–758.

55 van Zuylen L, Sparreboom A, van der Gaast A, *et al.* Disposition of docetaxel in the presence of P-glycoprotein inhibition by intravenous administration of R101933. *Eur J Cancer* 2002; **38**:1090–1099.

56 Mistry P, Folkes A. ONT-093 (Ontogen). *Curr Opin Invest Drugs* 2002; **3**:1666–1671.

57 Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 1987; **84**:7735–7738.

58 Benet LZ, Izumi T, Zhang Y, Silverman JA, Wacher VJ. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *J Control Release* 1999; **62**:25–31.

59 Zhang Y, Benet LZ. The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. *Clin Pharmacokinet* 2001; **40**:159–168.

60 Fromm MF. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. *Adv Drug Deliv Rev* 2002; **54**: 1295–1310.

61 Zhang Y, Guo X, Lin ET, Benet LZ. Overlapping substrate specificities of cytochrome P450 3A and P-glycoprotein for a novel cysteine protease inhibitor. *Drug Metab Dispos* 1998; **26**:360–366.

62 Zhou-Pan XR, Seree E, Zhou XJ, *et al.* Involvement of human liver cytochrome P450 3A in vinblastine metabolism: drug interactions. *Cancer Res* 1993; **53**:5121–5126.

63 Zhou XJ, Zhou-Pan XR, Gauthier T, Placidi M, Maurel P, Rahmani R. Human liver microsomal cytochrome P450 3A isozymes mediated vindesine biotransformation. Metabolic drug interactions. *Biochem Pharmacol* 1993; **45**:853–861.

64 Relling MV, Nemec J, Schuetz EG, Schuetz JD, Gonzalez FJ, Korzekwa KR. *O*-demethylation of epipodophyllotoxins is catalyzed by human cytochrome P450 3A4. *Mol Pharmacol* 1994; **45**:352–358.

65 Achira M, Suzuki H, Ito K, Sugiyama Y. Comparative studies to determine the selective inhibitors for P-glycoprotein and cytochrome P4503A4. *AAPS PharmSci* 1999; **1**:E18.

66 Takara K, Kakumoto M, Tanigawara Y, Funakoshi J, Sakaeda T, Okumura K. Interaction of digoxin with antihypertensive drugs via MDR1. *Life Sci* 2002; **70**:1491–1500.

67 Westphal K, Weinbrenner A, Zschiesche M, *et al.* Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. *Clin Pharmacol Ther* 2000; **68**:345–355.

68 Karlsson J, Kuo SM, Ziemniak J, Artursson P. Transport of celiprolol across human intestinal epithelial (Caco-2) cells: mediation of secretion by multiple transporters including P-glycoprotein. *Br J Pharmacol* 1993; **110**: 1009–1016.

69 Neuhoff S, Langguth P, Dressler C, Andersson TB, Regardh CG, Spahn-Langguth H. Affinities at the verapamil binding site of MDR1-encoded P-glycoprotein: drugs and analogs, stereoisomers and metabolites. *Int J Clin Pharmacol Ther* 2000; **38**:168–179.

70 Lilja JJ, Backman JT, Laitila J, Luurila H, Neuvonen PJ. Itraconazole increases but grapefruit juice greatly decreases plasma concentrations of celiprolol. *Clin Pharmacol Ther* 2003; **73**:192–198.

71 Hirsch-Ernst KI, Ziemann C, Foth H, Kozian D, Schmitz-Salue C, Kahl GF. Induction of mdr1b mRNA and P-glycoprotein expression by tumor necrosis factor alpha in primary rat hepatocyte cultures. *J Cell Physiol* 1998; **176**:506–515.

72 Ziemann C, Burkle A, Kahl GF, Hirsch-Ernst KI. Reactive oxygen species participate in mdr1b mRNA and P-glycoprotein overexpression in primary rat hepatocyte cultures. *Carcinogenesis* 1999; **20**:407–414.

73 Thevenod F, Friedmann JM, Katsen AD, Hauser IA. Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-kappaB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. *J Biol Chem* 2000; **275**:1887–1896.

74 Liu YW, Sakaeda T, Takara K, *et al.* Effects of reactive oxygen species on cell proliferation and death in HeLa cells and its MDR1-overexpressing derivative cell Line. *Biol Pharm Bull* 2003; **26**:278–281.

75 Brouillard F, Tondelier D, Edelman A, Baudouin-Legros M. Drug resistance induced by ouabain via the stimulation of MDR1 gene expression in human carcinomatous pulmonary cells. *Cancer Res* 2001; **61**: 1693–1698.

76 Takara K, Tsujimoto M, Ohnishi N, Yokoyama T. Digoxin up-regulates MDR1 in human colon carcinoma Caco-2 cells. *Biochem Biophys Res Commun* 2002; **292**:190–194.

77 Asghar A, Gorski JC, Haehner-Daniels B, Hall SD. Induction of multidrug resistance-1 and cytochrome P450 mRNAs in human mononuclear cells by rifampin. *Drug Metab Dispos* 2002; **30**:20–26.

78 Dresser GK, Schwarz UI, Wilkinson GR, Kim RB. Coordinate induction of both cytochrome P4503A and MDR1 by St John's wort in healthy subjects. *Clin Pharmacol Ther* 2003; **73**:41–50.

79 Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* 2001; **7**: 584–590.

80 Labialle S, Gayet L, Marthinet E, Rigal D, Baggetto LG. Transcriptional regulators of the human multidrug resistance 1 gene: recent views. *Biochem Pharmacol* 2002; **64**:943–948.

81 Labialle S, Gayet L, Marthinet E, Rigal D, Baggetto LG. Transcriptional regulation of the human MDR1 gene at the level of the inverted MED-1 promoter region. *Ann NY Acad Sci* 2002; **973**:468–471.

82 Dussault I, Lin M, Hollister K, Wang EH, Synold TW, Forman BM. Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J Biol Chem* 2001; **276**:33309–33312.

83 Ekins S, Erickson JA. A pharmacophore for human pregnane X receptor ligands. *Drug Metab Dispos* 2002; **30**:96–99.

84 Takara K, Takagi K, Tsujimoto M, Ohnishi N, Yokoyama T. Digoxin up-regulates multidrug resistance transporter (MDR1) mRNA and simultaneously down-regulates steroid xenobiotic receptor mRNA. *Biochem Biophys Res Commun* 2003; **306**:116–120.

85 Yague E, Armesilla AL, Harrison G, *et al.* P-glycoprotein (MDR1) expression in leukemic cells is regulated at two distinct steps, mRNA stabilization and translational initiation. *J Biol Chem* 2003; **278**:10344–10352.

86 Jenkins RR, Del Signore CM, Sauer P, Skelly C. The effect of beta blocking drugs on lipid peroxidation in rat heart *in vitro*. *Lipids* 1992; **27**:539–542.

87 Gao F, Chen J, Lopez BL, *et al.* Comparison of bisoprolol and carvedilol cardioprotection in a rabbit ischemia and reperfusion model. *Eur J Pharmacol* 2000; **406**:109–116.