studies of 96 h infusion of doxorubicin in advanced breast cancer patients. *Eur J Cancer Clin Oncol* 1989, **25**, 505–511.
20. Legha SS, Benjamin RS, MacKay B, *et al.* Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. *Ann Intern Med* 1982, **96**, 133–139.
21. Powis G. Anticancer drug pharmacodynamics. *Cancer Chemother Pharmacol* 1986, **14**, 177–183.
22. Moore MJ, Erlichman C. Therapeutic drug monitoring in oncology. Problems and potential in antineoplastic therapy. *Clin Pharmacokin* 1987, **13**, 205–227.
23. Ackland SP, Ratain MJ, Vogelzang NJ, *et al.* Pharmacokinetics and pharmacodynamics of long-term continuous infusion doxorubicin. *Clin Pharmacol Ther* 1989, **45**, 340–347.
24. Calvert AH, Newell DR, Gumbrell LA, *et al.* Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989, **7**, 1748–1756.
25. Bennett CL, Sinkule JA, Schilsky RL, Senekjian E, Choi KE. Phase I clinical and pharmacologic study of 72-hour continuous infusion of etoposide in patients with advanced cancer. *Cancer Res* 1987, **47**, 617–623.
26. Santini J, Milano G, Thyss A, *et al.* 5-FU therapeutic monitoring with dose adjustment leads to an imkproved therapuetic index in head and neck cancer. *Br J Cancer* 1989, **59**, 287–290.

Acknowledgements—The help and the cooperation of the nursing staff of the "Unité de Pharmacologie Clinique" is greatly appreciated. This work was supported by a grant from Lederle Laboratories, France.

Eur J Cancer, Vol. 27, No. 6, pp. 739–744, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Bepridil in Combination with Anthracyclines to Reverse Anthracycline Resistance in Cancer Patients

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The use of calcium antagonists as multidrug resistance reversing agents is limited by acute cardiac toxicity which, for verapamil, becomes prohibitive when concentrations in plasma approach those required *in vitro* for its action. A new calcium antagonist, bepridil, is as active as verapamil in reversing drug resistance *in vitro*. In addition, bepridil has some more favourable pharmacological properties compared with verapamil and other calcium antagonists. 14 patients with progressive advanced cancer, resistant to doxorubicin or epirubicin, were treated with the same anthracycline in combination with bepridil. Bepridil was administered in a continuous 36 h infusion at 22 mg/kg/36 h, with a dose scheme which should result in a steady state plasma concentration of approximately 5 μmol/l, able to reverse anthracycline resistance *in vitro*. Pharmacokinetic studies demonstrated a median bepridil plasma concentration of 5.3 μmol/l (range 2.6–19.3 μmol/l), at the time of administration of the anthracycline. No acute cardiac toxicity was observed and apparently bepridil did not induce an increase or change in anthracycline toxicity. However, 2 patients developed overt chronic heart failure after treatment discontinuation, which caused 1 patient's death, and a significant reduction in left ventricular ejection fraction was seen in 4 patients. This chronic cardiac toxicity could be related to the total anthracycline dose received. 5 patients attained short lasting minor responses, 3 had stable disease and 6 progressed. Immunohistochemical studies in 7 tumours failed to reveal P-glycoprotein expression. Further trials with escalating doses of bepridil in combination with multiple drug resistance related anticancer agents are warranted.
Eur J Cancer, Vol. 27, No. 6, pp. 739–744, 1991

INTRODUCTION

THE OCCURRENCE of drug resistance is considered as a major cause of chemotherapy failure in solid tumours. Several mechanisms of drug resistance have been discovered in *in vitro* systems and in animal models, but their significance in human cancer awaits confirmation. Anthracyclines are among the most effective antineoplastic drugs in current use. *In vitro* studies have revealed that anthracyclines display crossresistance to a group of structurally and functionally unrelated cytotoxic agents. This phenomenon, called multidrug resistance (MDR), is related to a decreased intracellular drug accumulation and changes in intracellular distribution of drugs [1–3]. The overexpression of a 170–180 kD P-glycoprotein [4], is thought to be responsible for an energy dependent outward transport of xenobiotics and cytotoxic drugs derived from them [5, 6]. It has been shown that P-glycoprotein mediated resistance to anthracyclines can be reversed *in vitro* by several substances, including a number of calcium channel blockers [7]. The results of clinical studies in

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Revised 4 Mar. 1991; accepted 12 Mar. 1991.

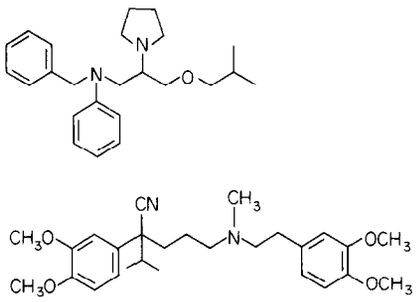

Fig. 1. Structural formulas of bepridil (β-[(2-methylpropoxy)-methyl]-N-phenyl-N-(phenylmethyl)-1-pyrrolidine ethanamine) (upper) and verapamil (lower). Both drugs are lipophylic, have two aromatic rings and a tertiary nitrogen atom. The conformation of these structures is suggested to be important for the resistance reversing capacities of calcium channel blockers [11].

patients with solid tumours in which doxorubicin was combined with the calcium channel blocker verapamil were disappointing [8, 9], the cause probably being the impossibility of adequate verapamil dose escalation due to its prohibitive cardiac toxicity sustained at plasma levels of approximately 2–3 μmol/l [8–10]. Nevertheless, some responses were reported with verapamil added to a doxorubicin and vincristine containing regimen in patients with multiple myeloma resistant to this combination. The responding patients exhibited P-glycoprotein expression on their tumour cells [10]. Several other calcium channel blockers have been proposed for *in vivo* reversal of drug resistance [11]. Among these, bepridil (Fig. 1) seems to be quite promising [11]. This drug was shown to have similar potency as verapamil to reverse doxorubicin resistance *in vitro* [12]; moreover, bepridil significantly inhibited binding of photoaffinity analogs of verapamil to P-glycoprotein [13]. In comparison to other calcium antagonists, such as verapamil, bepridil has a larger volume of distribution and a longer half-life of elimination, indicating extensive tissue uptake or specific tissue binding and low hepatic clearance [14, 15]. Moreover, the concentrations required *in vitro* to reverse resistance (5 μmol/l) could be reached without significant side-effects in plasma of patients with coronary artery disease during an 1 h infusion (1 mg/kg/h) after a bolus injection of 3 mg/kg [16]. Because of the anti-MDR activity and the favourable clinical pharmacological properties of bepridil, we initiated a clinical phase II study with a combination of bepridil with anthracyclines, aimed at reversing resistance in patients by achieving steady state plasma bepridil concentrations that had been reported to reverse drug resistance in *in vitro* systems [12].

MATERIALS AND METHODS

Patients

14 patients with documented intrinsic resistance to doxorubicin or epirubicin were entered in this study. Intrinsic resistance was defined as progressive disease on doxorubicin or epirubicin-containing regimens. Patient characteristics are summarised in Table 1. Median age was 43 (range 19–69) years. Median number of previously administered anthracycline containing cycles was 3.5 (range 2–8). 11 patients received prior doxorubicin therapy and 3 patients epirubicin, as a single agent or in combination regimens (Table 1). 2 patients (numbers 10 and 14) received both agents. Median cumulative dose of prior anthracycline treatment was 232.5 mg/m^2 (range 100–300) for doxorubicin and 370 mg/m^2 (range 240–435) for epirubicin. Tumour types were: breast cancer in 5 patients, soft tissue sarcoma in 6, pulmonary blastoma in 1, ovarian cancer in 1 and medullary thyroid cancer in 1. Eligibility criteria included: clear tumour progression on doxorubicin-containing or epirubicin-containing regimens, measurable disease, a life expectancy of at least 2 months, performance scale < 3 (WHO scale), age 70 years or less, treatment-free period of at least 4 weeks, white blood cell (WBC) count ≥ 4000/μl, platelet count ≥ 125 000/μl and bilirubin ≤ 20 μmol/l. Patients were excluded from this study if there was cumulative doxorubicin dose 350 mg/m^2 (epirubicin 500 mg/m^2) or higher, heart rate ≤ 50/min, concurrent use of β-blocking agents, radioisotope left ventricular ejection fraction (LVEF) ≤ 50%, or a decrease > 10% during previous therapy with anthracyclines, electrocardiogram (ECG) abnormalities or history of cardiac disease. Written informed consent was obtained from each patient.

Treatment

Before treatment a central venous catheter was introduced. Bepridil (bepridil monohydrochloride monohydrate) has a half-life of distribution of 2–3 h, a half-life of elimination (multiple dosing) of 42 (S.D. 12) h and a volume of distribution of 8 l/kg [14]. Based on these pharmacokinetic parameters, bepridil was administered as a 36 h continuous infusion preceded by a bolus injection in order to achieve steady state plasma levels of approximately 5 μmol/l after 24 h of infusion (time of doxorubicin administration). On the first day patients received an intravenous bolus of 5 mg/kg, administered as a 30 min infusion (10 mg/kg/h), followed by a continuous infusion at 1 mg/kg/h during 12 h. After 12 h the dose was lowered to 0.21 mg/kg/h, which was maintained for 24 h (5 mg/kg/24 h). The anthracycline was administered as a 30 min infusion, 24 h after initiation of bepridil, at the same dose and infusion scheme used in the treatment to which anthracycline resistance had been previously documented. Cardiovascular monitoring, performed in the initial 4 patients in an intensive care unit, included continuous checking of heart rate and rhythm, blood pressure every hour, ECG every 2 hours, and LVEF before every cycle. Cycles were repeated every 3 weeks. Patients were evaluated for antitumour response after 2 cycles. WHO criteria were used for toxicity and response assessment [17]. Complete blood cell counts and biochemical studies (renal and liver function tests) were obtained weekly. Tumour parameters, performance status, chest X-ray, ECG and LVEF were recorded or performed prior to each cycle.

Pharmacokinetic studies

Pharmacokinetic studies of doxorubicin and bepridil were performed in 8 and 10 patients, respectively. Blood samples for bepridil pharmacokinetics (5 ml citrate tubes) were drawn just prior its administration, at the end of the bolus infusion, at the end of the 12 h loading dose, just before and after anthracycline infusion and at 2, 4, 6, 8, 10 and 12 (discontinuation of bepridil administration) hours thereafter. Blood samples for pharmacokinetics of doxorubicin and 5 major metabolites (10 ml Sarstedt Monovette tubes) were taken just before and after doxorubicin infusion and at time points 0.25, 0.5, 1, 2, 4, 8, 24 and 48 h after doxorubicin administration. Tubes were immediately placed on ice and centrifuged within 1 h. In patients 1, 2 and 3 pharmacokinetic studies were performed during consecutive cycles; in subsequent patients pharmacokinetic studies were

Table 1. Patients' characteristics and treatment outcome

Patient (age, sex)	Diagnosis	Prior CT (cycles)	Cumulative dose of anthracyclines (mg/m^2)	Dose (mg/m^2)	Cycles	Response
1 (65, M)	Medullary thyroid cancer	MITO (3) DOX (3)	45 MITO 240	90	3	SD
2 (41, M)	STS	IFO DOX (4)	190	50	3	MR (2)
3 (49, F)	Breast	CMF, DOX/MMC (4)	240	60	3	SD
4 (42, F)	Breast	CMF, MTX 5-FU DOX/MMC (2)	100	50	2	SD
5 (46, M)	STS	CYVADIC (2)	100	50	3	MR (2)
6 (22, M)	STS	CYVADIC (3) DOX (1)	225	75	1	PD
7 (27, M)	STS	CYVADIC (5) IFO, DOX (1)	300	75	1	PD
8 (19, F)	Pulmonary blastoma	IVA (6), CDDP/DOX (2)	120	60	2	PD
9 (39,F)	Breast	CMF, EPI (3)	370 EPI	100 EPI	2	MR (1)
10 (53, F)	Breast	EPI (3) FAC (5)	250	50	2	PD
11 (43, F)	Ovary	CDDP/CP EPI (3)	435 EPI	120 EPI	4	MR (3)
12 (69, F)	STS	IFO, DOX (4)	200	50	2	PD
13 (60, M)	STS	DOX (2)	180	90	1	PD
14 (44, F)	Breast	DOX (4), EPI (3)	240 EPI	90 EPI	2	MR (1)

STS = soft tissue sarcoma, MITO = mitoxantrone; CYVADIC = cyclophosphamide (CP), vincristine, doxorubicin (DOX), dacarbazine (DTIC); CMF = cyclophosphamide, methotrexate (MTX), 5-fluorouracil (5-FU); IVA = ifosfamide (IFO), vincristine, actinomycin D; MMC = mitomycin; CDDP = cisplatin; EPI = epirubicin; FAC = 5-fluorouracil, doxorubicin, cyclophosphamide; SD = stable disease; PD = progressive disease; MR = minor response (no. of cycles after which response was observed).

performed only during the first cycle. Doxorubicin concentrations were determined by high performance liquid chromatography [18]. Bepridil concentrations were determined using gas chromatography [19]. In patient 13 a subcutaneous metastasis was removed immediately before doxorubicin administration, and bepridil tissue concentration was assayed.

Tissue handling and immunocytochemistry/histochemistry

In order to detect expression of P-glycoprotein we used an immunohistochemical method described in detail elsewhere [20]. Briefly, surgical specimens of the 6 patients with soft tissue sarcoma and the pulmonary blastoma were snap frozen in liquid nitrogen and stored until use. Cryostat sections (5 μm) were fixed in cold acetone (10 min 0°C) and air dried. Three monoclonal antibodies (Mab) directed against different epitopes of the P-glycoprotein molecule were used: C-219, kindly provided by Centocor; MRK-16, a gift of Dr Tsuruo; and JSB-1, which was raised in our laboratory [21]. As immunohistochemical method an avidine-biotin complex (ABC) immunoperoxidase method (Histostain-SP kit, Zymed, San Francisco) was used. The slides were developed with amino-ethyl carbazole (AEC), counterstained with haematoxylin and mounted with Aquamount. Cytospin preparations of chemosensitive human lung carcinoma cells (SW-1573) and two doxorubicin-resistant sublines (SW-1573/2R160 and SW 1573-2R500) were used as negative and positive controls, respectively. The use of an unrelated (mouse anti-immunoglobulin) antibody was also included in all the experiments as negative control. This method allows detection of P-glycoprotein in cultured tumour cells with 4–6 fold resistance to anthracyclines [22].

RESULTS

Median number of cycles administered to the 14 patients entered in the study was 2 (range 1–4). Cumulative doxorubicin

Table 2. Plasma pharmacokinetics of doxorubicin and all measured metabolites with or without bepridil

Doxorubicin and metabolites	No bepridil*		With bepridil (n = 8)	
	$t_{\frac{1}{2},\,final}$ (h)†	AUC (μmol. min/l)	$t_{\frac{1}{2},\,final}$ (h)†	AUC (μmol. min/l)
Doxorubicin	28.3 (2.8)	133 (26)	30.3 (6.1)	129 (20)
Aol		46 (16)		42 (17)
7d-Aolon		18 (9)		9 (5)
Aolon		2 (2)		0.6 (0.6)
7d-Aon		6 (3)		3 (2)
Aon		2.8 (2.6)		
Total		207.8		183.6

*From [23].
†Normalised to 50 mg/m².
$t_{\frac{1}{2},\,final}$ = half-life of elimination, Aol = doxorubicinol, 7d-Aolon = 7-deoxy doxorubicinol aglycone, Aolon = doxorubicinol aglycone, 7d-Aon = 7-deoxy doxorubicin aglycone, Aon = doxorubicin aglycone. The standard error of the mean is given in parentheses.

and epirubicin doses at therapy termination were 300 mg/m² (range 200–510) and 570 mg/m² (range 420–915), respectively.

Toxicity

Main side-effects were leukopenia and nausea and vomiting: leukopenia was severe (WHO grade 3 or 4) in 6 of 12 evaluable patients. No significant difference in myelotoxicity was observed in patients who received single agent anthracycline in previous cycles. Vomiting was observed in 8 of 14 evaluable patients. In 2 patients in whom bepridil was given in a peripheral vein, severe thrombophlebitis developed. No hepatic, renal or pulmonary toxicities were encountered and no instances of diarrhoea were seen. Severe signs of acute cardiac toxicity were not recorded. A transient and asymptomatic drop in blood pressure was recorded only in 1 patient. 2 patients died of chronic heart failure 4 months (patient 1) and 6 weeks (patient 14) after the last cycle, respectively. The first patient, whose disease remained stable until death (10 months), received 510 mg/m² total dose of doxorubicin; the ejection fraction decreased from 73% pretreatment value to 66%, 1 month before death. Lung radioisotope scan failed to show any sign of pulmonary embolism. Anthracycline induced myocardial damage could not be ruled out in this patient, although it was unlikely since the heart failure was mainly right-sided. Patient 14 received 280 mg/m² of doxorubicin and 420 mg/m² of epirubicin total dose. LVEF dropped from 69% pretreatment to 36% at the end of treatment. Necropsy revealed clear signs of anthracycline myocardiotoxicity, with vacuolisation of myocardial fibers. In another 3 patients a reduction of LVEF of 10% or more was recorded (patients 3, 6 and 11), without signs of decompensation. In patient 6 the decrease in LVEF had most likely been due to pericardial effusion caused by disease progression.

Response to treatment

All patients were evaluable for response (Table 1). No major objective responses were observed. However, reductions of below 50% in dimensions of disease sites were achieved in 5 patients (2 visceral sites and 3 lymph-nodal sites), lasting less than one cycle duration in all the cases but one (2 cycle duration). 3 patients had stable disease (5 weeks, 6 weeks and 10 months) and 6 patients had progressive disease, which was already evident after the first cycle in 3.

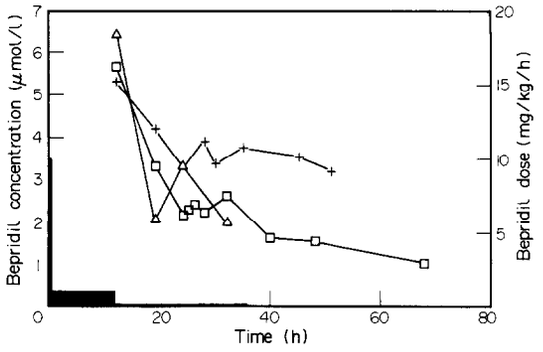

Fig. 2. Plasma bepridil pharmacokinetics of patient 1 during 3 subsequent cycles. □–□ cycle 1, +–+ cycle 2 and △–△ cycle 3. Solid bars represent dose of bepridil and scheme of adminstration. The anthracycline was administered as a 30 min infusion, 24 h after initiation of bepridil infusion.

Pharmacokinetics

Pharmacokinetic data of doxorubicin and its metabolites are shown in Table 2. Total area under the curve (AUC) normalised to 50 mg/m² was 183.6 μmol·min/l for doxorubicin and five metabolites (Aol, 7d-Aolon, Aolon, 7d-Aon and Aon). Elimination half-life (S.E.) was 30.3 (6.1) h. No significant differences were noted when these results were compared to the pharmacokinetic profile in patients receiving doxorubicin alone [23]. A representative pharmacokinetic profile of bepridil (patient 1) during three subsequent cycles is shown in Fig. 2. Median bepridil plasma concentrations were 8.32 μmol/l (range 5.96–26.2) after the bolus injection and 6.41 μmol/l (range 3.65–26.85) after 12 h continuous infusion. Bepridil plasma concentrations at the time of doxorubicin administration (after 24 h of bepridil infusion) are shown in Fig. 3. Median bepridil concentration at that time was 5.3 μmol/l (range 2.60–19.33). Plasma concentrations tended to decrease after the 12 h loading dose, but remained stable after 24 h of administration. In 1

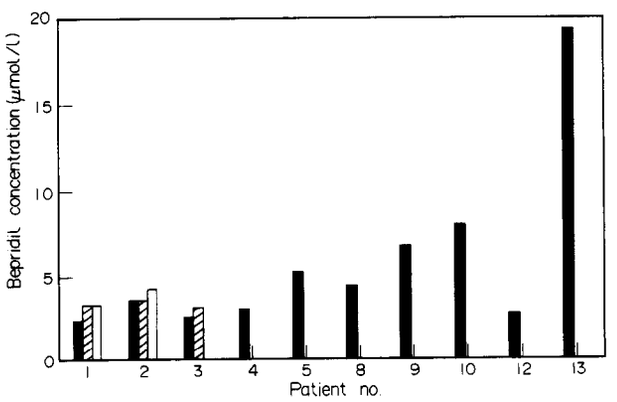

Fig. 3. Plasma bepridil concentration in 10 patients at the start of doxorubicin or epirubicin administration (24 h after initiation of bepridil). ■ cycle 1, ▨ cycle 2 and □ cycle 3.

patient (number 13) plasma levels were 4–5 times higher than in the other patients (Fig. 3). Bepridil tumour concentration found in a subcutaneous metastasis removed from this patient, just prior to doxorubicin administration, was 120 μmol/kg, which is 6–7 times higher as compared to the plasma concentration at that time in the same patient. In this patient no liver function disturbances or use of other drugs could explain the finding. In this patient doxorubicin kinetics were not significantly different from those of the other patients.

P-glycoprotein immunohistochemistry

P-glycoprotein immunohistochemistry was performed in 7 cases. All the samples were removed before the patients entered this study: 4 before any anthracycline treatment and 3 just before commencement of the study. In none of the 7 tumours (patients 2, 5, 6, 7, 8, 12 and 13) P-glycoprotein expression was detected.

DISCUSSION

The present study shows that concentrations of bepridil, able to reverse anthracycline resistance *in vitro* [12], can be achieved in plasma when bepridil is given in combination with doxorubicin or epirubicin in anthracycline-resistant patients. Although no partial or complete responses were attained, 5 minor responses of short duration were observed. It might be speculated that either the optimal dose of bepridil, or the dose of the anthracycline, or doses of both drugs were not delivered in the present study, and that higher doses could achieve major responses.

The dose of bepridil administered in the present study was at least 5 times higher than the highest dose given intravenously to cardiac patients [15, 16, 24]. In addition, patients with angina pectoris or arrhythmias usually receive bepridil orally at a dose of 100–600 mg/day [24–27], resulting in steady state plasma levels of about 0.5–2 μmol/l [14, 19, 27]. At these doses the drug is definitely safe in the treatment of angina pectoris [26, 27], but severe ventricular arrhythmia have been reported in patients with atrial fibrillation [25]. The plasma concentrations of bepridil as given in this study are at least twice as high as those obtained with the maximal tolerable dose of verapamil, at which major acute cardiac toxicity is observed [8–10]. No significant acute cardiac toxicity was observed with the dose of bepridil used in this study (22 mg/kg/36 h) and this suggests the possibility of further increasing the dose of bepridil. The design of our study was such that the dose of anthracyclines was the same given in the prior treatment irrespective of whether the drug was given alone or in combination with other agents. Although this might have caused underdosing in some patients, the dose of the anthracycline was not increased because we wanted to make sure that the achieved effect was due to the combination with bepridil and not to an increase of anthracycline dose; in fact a dose-response relationship has been described for anthracyclines [28].

We did not observe a significant increase or change in anthracycline-related side-effects with this combination, as also reported in other studies of resistance modifiers combined with anthracyclines [8, 9, 29]. Therefore, the high degree of leukopenia observed is likely to be related to the prior therapy status of most patients. On the other hand, tumour bearing mice treated with vincristine and high dose verapamil (plasma level 10 μmol/l), experienced increased neurological and intestinal toxicities and significant changes in vincristine uptake in liver, kidneys and small intestine [30], organs known to express P-glycoprotein [20]. In our study 2 patients died of chronic cardiac insufficiency after treatment discontinuation; in one case a clear anthracycline-induced myocardial damage was demonstrated at necropsy, while the cause of cardiac insufficiency remained unclear in the other patient. A potentiating effect of bepridil on anthracycline chronic cardiotoxicity is difficult to exclude, although this was never reported in the studies with verapamil [8–10]. The high cumulative doses of anthracyclines received by both patients could, *per se*, explain the outcome. As cumulative doses of anthracyclines given to the studied patient population approached critical levels, monitoring of LVEF was closely performed; significant reductions of LVEF were in fact observed as expected, including the patient who developed chronic heart failure and died, despite discontinuation of treatment.

We did not observe significant changes in plasma pharmacokinetics of doxorubicin as compared to a previous study in patients receiving doxorubicin alone [23]. Although the plasma levels of total bepridil were in the range calculated, parameters like drug binding to plasma proteins need to be taken into consideration, as bepridil [14] and verapamil [31] are in fact 99% and 90% plasma protein bound, respectively. The reversing property of both verapamil and bepridil decreases dramatically in cell culture in the presence of high bovine serum albumin concentrations or human plasma [2]; plasma protein binding in humans does not seem, however, to interfere with tissue distribution of these calcium channel blockers, given the short half-lives of distribution (2–3 h) and high volumes of distribution [14, 32]. This property is explained in part by the lipophylic nature of the drugs [11]. In addition, bepridil has a much longer elimination half-life than verapamil (19–48 vs. 4–5 h, respectively) [14, 15, 31, 32], which might be explained by a slow biotransformation of bepridil by the liver [32] and/or an extensive uptake in poorly perfused tissues [15]. In our study a very high tumour concentration was observed in tumour tissue from a patient after 24 h of bepridil infusion. The same patient displayed plasma bepridil concentrations 4–5 fold higher than in the other patients studied. The interpatient variability in plasma concentrations has been reported in other pharmacokinetic studies with bepridil and verapamil [8, 14, 15, 27, 31, 32] and might be related to binding to the plasma protein α-1-acid glycoprotein [14, 32, 33]. α-1-acid glycoprotein, an acute phase protein, significantly increases in a number of diseases, including malignant disease, but also varies across the normal population [14, 32, 33]. P-glycoprotein immunohistochemistry was negative in all cases tested, including 2 soft tissue sarcoma patients who achieved minor responses. Unlike our study, responding patients with myeloma expressed P-glycoprotein on their tumour cells [10]. Nevertheless, very low levels of *mdr*1 gene expression, detectable only after polymerase chain reaction amplification, might be responsible for clinical drug resistance in soft tissue sarcoma [34]. Expression of the *mdr*1 gene has been demonstrated in breast cancer [35] and ovarian cancer [36], but its relevance is still unclear.

Our study suggests that bepridil is an attractive alternative to verapamil in the attempt to reverse MDR in patients. Development of chronic cardiomyopathy appears to be a crucial problem in this type of study with anthracyclines. Recent investigations suggest that vinca alkaloid resistance in both P-glycoprotein and non-P-glycoprotein mediated MDR can be reversed more efficiently by calcium antagonists *in vitro* as compared to anthra-

cycline resistance [30, 37, 38]. In addition, no critical cumulative dose is known for vinca alkaloids. Further studies of vinca alkaloids in combination with escalating doses of bepridil are now underway at our institution.

1. Fojo A, Akiyama SI, Gottesman MM, *et al.* Reduced drug accumulation in multiple drug resistant human KB carcinoma cell lines. *Cancer Res* 1985, **45**, 3002–3007.
2. Broxterman HJ, Kuiper CM, Schuurhuis GJ, *et al.* Daunomycin accumulation in resistant tumor cells as a screening model for resistance modifying drugs: role of protein binding. *Cancer Lett* 1987, **35**, 87–95.
3. Schuurhuis GJ, Broxterman HJ, Cervantes A, *et al.* Quantitative determination of factors contributing to doxorubicin resistance in multidrug-resistant cells. *J Natl Cancer Inst* 1989, **81**, 1887–1892.
4. Kartner N, Rordan JR, Ling V. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science* 1983, **221**, 1285–1288.
5. Horio M, Gottesman MM, Pastan I. ATP-dependent transport of vinblastine in vesicles from human multidrug resistant cells. *Proc Natl Acad Sci USA* 1988, **85**, 3580–3584.
6. Broxterman HJ, Pinedo HM, Kuiper CM, *et al.* Induction by verapamil of a rapid increase in ATP consumption in multidrug resistant cells. *Faseb J* 1988, **2**, 2278–2282.
7. Tsuruo T. Reversal of acquired resistance to vinca alkaloids and anthracycline antibiotics. *Cancer Treat Rep* 1983, **67**, 889–894.
8. Ozols RF, Cunnion RE, Klecker RW, *et al.* Verapamil and adriamycin in the treatment of drug resistant ovarian cancer patients. *J Clin Oncol* 1987, **5**, 641–647.
9. Presant CA, Kennedy PS, Wiseman C, *et al.* Verapamil reversal of clinical doxorubicin resistance in human cancer. *Am J Clin Oncol* 1986, **9**, 355–357.
10. Dalton WS, Grogan TM, Meltzer PS, *et al.* Drug resistance in multiple myeloma and non-Hodgkin lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. *J Clin Oncol* 1989, **7**, 415–424.
11. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990, **42**, 155–198.
12. Schuurhuis GJ, Broxterman HJ, van der Hoeven JJM, *et al.* Potentiation of doxorubicin cytotoxicity by the calcium antagonist bepridil in anthracycline resistant and sensitive cell lines. *Cancer Chemother Pharmacol* 1987, **20**, 285–290.
13. Safa AR. Photoaffinity labeling of the multidrug-resistance-related P-glycoprotein with photoactive analogs of verapamil. *Proc Natl Acad Sci USA* 1988, **85**, 7187–7191.
14. Benet LZ. Pharmacokinetics and metabolism of bepridil. *Am J Cardiol* 1985, **55**, 8C–13C.
15. Lesko LJ, Benotti JR, Alpert JS, *et al.* Pharmacokinetics of intravenous bepridil in patients with coronary disease. *J Pharmacol Sci* 1986, **27**, 693–699.
16. Remme WJ, Kruyssen DACM, Van Hoogenhuyze DCA, Hofman B, Krauss H, Storm CJ. Acute hemodynamic and antiischemic properties of intravenous bepridil in coronary artery disease. *Am J Cardiol* 1989, **63**, 670–675.
17. WHO. *Handbook for Reporting Results of Cancer Treatment.* WHO Offset Publication 48, Geneva, WHO, 1979.
18. Maessen PM, Mross K, Pinedo HM, *et al.* An improved method for the determination of 4'-epi-doxorubicin and seven metabolites in plasma by HPLC. *J Chromatogr* 1987, **417**, 339–344.
19. Vink JH, van Hal HJM, Pognat JF, *et al.* Determination of the anti-ischaemic drug bepridil in human plasma using gas chromatography. *J Chromatogr* 1983, **272**, 87–94.
20. Van der Valk P, van Kalken CK, Ketelaars H, *et al.* Distribution of multidrug resistance-associated P-glycoprotein in normal and neoplastic human tissues. *Ann Oncol* 1990, **1**, 56–64.
21. Scheper RJ, Bulte JWM, Brakkee JGP, *et al.* Monoclonal antibody JSB-1 detects a highly conserved epitope on the P-glycoprotein associated with multidrug-resistance. *Int J Cancer* 1988, **42**, 389–394.
22. Van Kalken CK, Van der Valk P, Hadisaputro MMN, *et al.* Differentiation dependent expression of P-glycoprotein in the normal and neoplastic human kidney. *Ann Oncol* 1991, **2**, 55–62.
23. Mross K, Maessen P, van der Vijgh WJF, *et al.* Pharmacokinetics and metabolism of epidoxorubicin and doxorubicin in humans. *J Clin Oncol* 1988, **6**, 517–526.
24. Roy D, Montigny M, Klein GJ. Electrophysiologic effects and long-term efficacy of bepridil for recurrent supraventricular tachycardias. *Am J Cardiol* 1987, **59**, 89–92.
25. Perelman MS, McKenna WJ, Rowland E, Krikler DM. A comparison of bepridil with amiodarone in the treatment of established atrial fibrillation. *Br Heart J* 1987, **58**, 339–344.
26. Sharma MK, Voyles W, Prasad R, *et al.* Long-term bepridil monotherapy for angina pectoris. *Am J Cardiol* 1988, **61**, 1210–1213.
27. Nielsen HK, Krusell LR, Husted SE, Pluymers RJ. Bepridil versus verapamil in stable angina pectoris—a controlled clinical trial. *Int J Cardiol* 1989, **23**, 357–364.
28. Jones RB, Holland JF, Bhardwaj S, Norton L, Wilfinger C, Strashun A. A phase I/II study of intensive dose adriamycin for advanced breast cancer. *J Clin Oncol* 1987, **5**, 172–177.
29. Miller RL, Bukowski RM, Budd T, *et al.* Clinical modulations of doxorubicin resistance by the calmodulin-inhibitor trifluoroperazine: a phase I/II trial. *J Clin Oncol* 1988, **6**, 880–888.
30. Horton JK, Thimmaiah KN, Houghton JA, *et al.* Modulation by verapamil of vincristine pharmacokinetics and toxicity in mice bearing human tumor xenografts. *Biochem Pharmacol* 1989, **38**, 1727–1736.
31. Hamann SR, Blouin RA, McAllister RG. Clinical pharmacokinetics of verapamil. *Clin Pharmacokinet* 1984, **9**, 26–31.
32. Singlas E, Martre H, Taburet AM. Clinical pharmacology of calcium inhibitors. *Arch Mal Coeur* 1985, **78**, 15–22.
33. Chatterjee M, Robson CN, Harris AL. Reversal of multidrug resistance by verapamil and modulation by α-1-acid glycoprotein in wild-type and multidrug resistant chinese hamster ovary cell lines. *Cancer Res* 1991, **50**, 2818–2822.
34. Noonan KE, Beck C, Holzmayer TA, *et al.* Quantitative analysis of MDR1 (multidrug resistance) gene expression in human tumors by polymerase chain reaction. *Proc Natl Acad Sci USA* 1990, **87**, 7160–7164.
35. Goldstein LJ, Galski H, Fojo A, *et al.* Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst* 1989, **81**, 116–124.
36. Bourrhis J, Goldstein LJ, Rioy G, Pastan I, Gottesman MM, Bernard J. Expression of a human multidrug resistance gene in ovarian carcinomas. *Cancer Res* 1989, **49**, 5062–5065.
37. Kuiper CM, Broxterman HJ, Baas F, *et al.* Drug transport variants without P-glycoprotein overexpression from a human squamous lung cancer cell line after selection with doxorubicin. *J Cell Pharmacol* 1990, **1**, 40–46.
38. Mikisch GH, Roehrich K, Koessig J, *et al.* Mechanisms and modulation of multidrug resistance in primary human renal cell carcinoma. *J Urol* 1990, **144**, 755–759.

Acknowledgement—We thank the Organon scientific development group for supply and analysis of bepridil. This study was supported by a research grant from the Bristol-Myers Squibb company. C.K. van Kalken is recipient of a Margot Mattheijssen-van der Voort fellowship.