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Increase in Doxorubicin Cytotoxicity by Carvedilol Inhibition of P-Glycoprotein Activity

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ABSTRACT. Acquired resistance to chemotherapy is a major problem during cancer treatment. One mechanism for drug resistance is overexpression of the *MDR1* (multidrug resistance) gene encoding for the transmembrane efflux pump, P-glycoprotein (P-gp). The calcium channel blocker verapamil has been shown to reverse cellular drug resistance by inhibiting P-gp drug efflux. This study evaluated whether the new antihypertensive drug carvedilol influenced doxorubicin (Dox) cytotoxicity and P-gp activity in a P-gp-expressing cell line compared to a non-expressing subline. Verapamil (10 μmol/L), and even more markedly, carvedilol (10 μmol/L) increased cellular uptake of P-gp-transported calcein of a P-gp-expressing breast cancer cell line (Hs578T-Dox). In the subline (Hs578T) not expressing P-gp, no effects of carvedilol or verapamil on calcein uptake were seen. Carvedilol and verapamil (10 μmol/L) reduced the LD_{50} (dose which results in the death of half the number of cells) of the Hs578T-Dox subline from 200 mg/L to approx. 10 mg/L Dox, whereas the LD_{50} of the Hs578T subline was only marginally affected. Carvedilol (10 μmol/L) reduced P-gp activity approximately twice as effectively as verapamil at an equimolar concentration. Carvedilol did not affect pyrogallol cytotoxicity and pyrogallol was without effect on calcein accumulation of the Hs578T-Dox cell line, indicating the lack of antioxidative properties affecting P-gp activity and associated toxicity of the drug. The results suggest that carvedilol has the clinical potential to reverse tumour MDR involving the efflux protein P-gp. BIOCHEM PHARMACOL **58**;11:1801–1806, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. carvedilol; doxorubicin; MDR; vasoactive drugs; P-glycoprotein; verapamil

Acquired resistance to chemotherapy with ensuing cross-resistance to other cytotoxic drugs is a major clinical problem during cancer treatment [1–3]. The two major cellular mechanisms for drug resistance are the overexpression of the *MDR1*§ gene encoding for the transmembrane efflux pump, P-gp, and the presence of MRP, which functions to maintain the intracellular drug concentration below cytotoxic levels by ATP-dependent extrusion of the drug. Measurement of the formation of free calcein from calcein AM in the tumour cells has been shown to be useful to differentiate the cell membrane drug transport activity of both *MDR1* and MRP and has several advantages over the utilisation of other fluorescent dyes such as rhodamine123 [4]. Several vasoactive drugs and especially the calcium channel blocking agent, verapamil, have been shown to reverse cellular drug resistance by inhibiting the P-gp drug efflux in several *in vitro* as well as *in vivo* studies [5–8]. Unfortunately, toxic side effects of the vasoactive drugs have hampered attempts to achieve optimal drug concentrations to overcome resistance in patients [9, 10]. Other pharmaceuticals with quite different mechanisms of action have also been shown to modify resistance during cancer treatment [11–14]. Carvedilol, an antihypertensive drug with β-adrenoceptor antagonist activity has also been shown to mediate vasodilatation mainly through α-adrenergic blockade [reviewed in 15]. Carvedilol also affects calcium channels [16, 17] and displays antioxidative properties [18–21]. These properties are of potential interest for carvedilol-treated patients, who are also treated with irradiation or with cytostatic drugs that are assumed to act through free radicals.

We therefore found it of interest to investigate whether carvedilol might influence the cytotoxicity of doxorubicin. Furthermore, we studied whether carvedilol could reverse P-gp drug efflux as measured by fluorescent calcein accumulation on a P-gp-expressing cell line and evaluated carvedilol with respect to protection against extracellular free radical production employing the autoxidation of pyrogallol.

MATERIAL AND METHODS

Cell Culture

We used two human breast cancer cell sublines: Hs578T [22], with no previously measurable P-gp expression, and

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§ *Abbreviations:* calcein AM, calcein acetoxymethylester; Dox, doxorubicin; FDA, fluorescein diacetate; *MDR1* gene, multidrug resistance gene; MRP, multidrug resistance-associated protein; NEM, *N*-ethylmaleimide; P-gp, P-glycoprotein; and TBST, TRIS-buffered saline with Tween.

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Hs578T-Dox, which is adapted to growth in the presence of 20 mg/L Dox. The Hs578T-Dox subline has previously shown a high level of P-gp expression as determined by immunohistochemistry [23]. The cell lines were cultured as monolayers on plastic Petri dishes. The nutrient consisted of Eagle's minimum essential medium supplemented with 10% foetal bovine serum, 200 μmol/L L-glutamine, and 100 μg/L gentamicin. The cells were incubated at 37° in a humidified atmosphere of 5% CO_2–95% air.

Fluorometric Microculture Cytotoxicity Assay

To quantify the effects of carvedilol and verapamil on doxorubicin cytotoxicity, FDA was used in a semiautomated fluorometric method [24]. FDA is non-fluorescent and membrane-permeable and is cleaved to fluorescent fluorescein by unspecific esterases of the cell cytoplasm. The esterase activity is dependent on the cell viability and integrity of the membrane. FDA crosses the membrane of intact cells only and is cleaved to fluorescein, which is retained intracellularly. The amount of fluorescence will thus correlate to the number of living cells. The FDA fluorescence excitation/emission was measured at 485/538 nm. Cells from both sublines were harvested and plated at 2×10^4 cells/well in a volume of 100 μL 96-well microtiter plates using a multichannel pipette. The plates were first incubated at 37° for 24 hr with culture medium only. The medium was changed and the drugs diluted in culture medium to the desired drug concentration were added in triplicate and the cells then cultured for 48 hr under the conditions described above. The culture plates were then centrifuged (300–400 *g* for 5 min), the medium removed by flicking the plate, and the wells washed once with 200 μL PBS. To each well was then added 100 μL of PBS containing 10 μg/mL FDA. The plates were then incubated for 60 min at 37° followed by fluorescence determination.

Western Blot Analysis of P-Glycoprotein

A membrane fraction of cells were prepared for SDS-PAGE according to Gruber and co-workers [25] with some modifications. Cells ($40–60 \times 10^6$) were homogenised in 0.5 mL lysis buffer (10 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, and 10 mmol/L Tris-HCl, pH 7.4) containing 2 mmol/L phenylmethyl-sulfonyl fluoride (PMSF), 5 μg/mL leupeptin, 5 μg/mL pepstatin, and 210 μg/mL aprotinin (Sigma). Following centrifugation (10 min, 1300 *g*, +4°), the supernatants were transferred to new tubes and centrifuged for 30 min at 3000 *g*. The supernatants were discarded and the pellets were suspended in 50–100 μL buffer (250 mmol/L sucrose, 10 mmol/L Tris-HCl, 1 mmol/L PMSF, pH 7.4). Determination of protein concentration was performed according to standard methods with BSA as standard. Prior to application on gel, the samples were diluted three times with sample buffer (40 mmol/L boric acid, 5 mmol/L EDTA, 705 mmol/L 2-mercaptoethanol, 146 mmol/L sucrose, 10% SDS, and 42 mmol/L Tris-HCl, pH 8.6), and denatured on ice for 2 hr. A total of 30 μg protein from control samples of K562 cells [26] and 100 μg protein from the other cell lines was applied to each gel lane. Membrane proteins were separated on 6% polyacrylamide gradient gels at 100 V for 3 hr at room temperature. After electrophoresis, the proteins were transferred to a 0.2 μm polyvinylidene difluoride (PVDF) membrane (Trans-Blot® Transfer Medium, Bio-Rad) by electroblotting overnight at 20 V in 50 mmol/L NaH_2PO_4, pH 6.5. The membranes were then blocked in a suspension of 5% dry milk in TBST (137 mmol/L NaCl, 0.05% Tween 20, and 20 mmol/L Tris-HCl, pH 7.6) for 1 hr at 37°. After washing in TBST (1×10 min, 3×15 min), the membranes were incubated for 15 hr with a polyclonal antibody (MDR (ab-1); Oncogene Research Products) raised against a synthetic peptide in the C-terminal cytoplasm domain of P-glycoprotein, diluted 1:500 in TBST. Following washing as described above, the membranes were incubated for 2 hr at room temperature with horseradish peroxidase-labelled antirabbit anti-rabbit immunoglobulin G (Amersham Life Science) diluted 1:2000 in TBST. Detection of bands was performed using enhanced chemiluminescence (ECL) and Western blotting detection reagents (Amersham) as described by the supplier. Autoradiograms were obtained by exposing the membranes to Hyperfilm-ECL (Amersham).

Calcein Accumulation Assay

Calcein AM has proved to be a functional probe for measurement of P-gp transport activity, and by using appropriate inhibitors, the functions of *MDR1* and MRP can be differentiated [4]. Cells expressing *MDR1* or MRP activity rapidly remove the non-fluorescent probe calcein AM, resulting in decreased accumulation of the fluorescent dye calcein in the cytoplasm compartment. Cells from both cell lines were harvested and plated in a volume of 100 μL at 5×10^4 cells/well in 96-well microtiter plates using a multichannel pipette. The plates were first incubated at 37° for 24 hr with culture medium only. The cells were then washed twice with PBS containing 5 mmol/L glucose, and calcein AM (50 mg/L final concentration) with or without carvedilol or verapamil was then added to the medium and further incubated for 0–120 min. The fluorescence with excitation at 495 nm and emission at 515 nm was immediately read on a Perkin Elmer LS50B luminescence spectrometer. To test the possible interference on calcein accumulation by the presence of MRP, the effects on calcein uptake of 50 μmol/L indomethacin, 1 μmol/L NEM, and 50 μmol/L ethacrynic acid were tested. At these concentrations, the substances strongly inhibit MRP activity whereas the concentrations are too low to affect P-gp activity [4].

Cytotoxicity and Drug Efflux Pump Sensitivity to Superoxide Radicals

Pyrogallol (1,2,3-benzenetriol) rapidly autoxidises in aqueous solution. At physiological pH, the autoxidation is

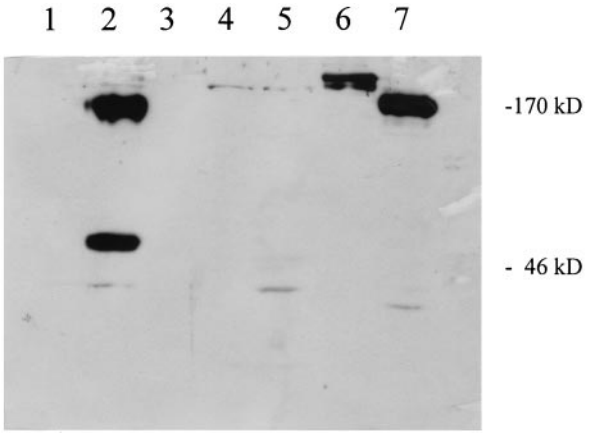

FIG. 1. Estimation of the transmembrane efflux pump, P-glycoprotein (P-gp) in two breast cancer sublines by immunoblot detection using the MDR (Ab-1) polyclonal antibody. The amount of protein loaded was 100 μg for all cell lines except for the positive control (K562), lane 7, to which 30 μg protein was loaded. Lane 1: Hs578T; lane 2: Hs578T-Dox; lane 3: the human breast cancer cell line MDA-231 not grown in doxorubicin; lane 4: MDA-231 adapted to growth in doxorubicin; lane 5: the human mesothelioma cell line P31; lane 6: the BT4C rat malignant glioma line; and lane 7: the human erythroleukemic cell line K562 was used as positive control.

almost totally inhibited by superoxide dismutase but not by catalase, indicating an almost total dependence on the participation of the superoxide anion radical, but no involvement of hydrogen peroxide in the autoxidation mechanism [27]. Cells from the Hs578T-Dox cell line were handled as in the described fluorometric microculture cytotoxicity assay and calcein accumulation assay protocols, except that they were cultured with 100 μmol/L pyrogallol for 5 hr 30 min prior to addition of FDA. A dose–response curve was initially performed to find a suitable dose of pyrogallol.

Drugs

Carvedilol (SmithKline Beecham Pharmaceuticals) was dissolved in ethanol/medium (1% ethanol [v/v] final conc.) to a concentration of 1–10 μmol/L. Verapamil hydrochloride (CAS 52-53-9, Knoll AG) was dissolved in sodium chloride/medium to a concentration of 1–10 μmol/L. Pyrogallol (CAS 87-66-1) was from Merck KgaA. Doxorubicin was from Farmitalia Carlo Erba. FDA (from Sigma) was dissolved in DMSO/PBS (1% DMSO [v/v] final conc.) to a concentration of 10 mg/L. Calcein AM (Molecular Probes) was dissolved in DMSO/PBS to a concentration of 50 mg/L. Indomethacin, NEM, and ethacrynic acid were from Sigma.

RESULTS

Western Blot Analysis of P-Glycoprotein

Western blot analysis for detection of the P-gp protein content of Hs578T-Dox as well as the Hs578T subline were performed. Figure 1 shows that P-gp protein was markedly expressed in the Hs578T-Dox but not the Hs578T subline. An extra band at approx. 50 kD appeared in the Hs578T-Dox lane (lane 2). This band was not present in the positive control lane (lane 7) with the *MDR1*-expressing cell line K562.

Effects of Carvedilol and Verapamil on Doxorubicin Cytotoxicity

The Hs578T subline was approx. 200 times as sensitive to doxorubicin cytotoxicity compared to Hs578T-Dox. The LD_{50} for Hs578T-Dox was approx. 200 mg/L and 1 mg/L for Hs578T (Figs. 2 and 3). Carvedilol (10 μmol/L) and verapamil (10 μmol/L) reduced the LD_{50} for doxorubicin to approx. 10 mg/L for Hs578T-Dox (Fig. 2). Verapamil (1 μmol/L) also increased the cytotoxicity of doxorubicin at most tested doxorubicin concentrations, whereas carvedilol at the same concentration seemed less efficient in increasing doxorubicin cytotoxicity at lower (10 and 30 mg/L) doxorubicin concentrations. Carvedilol or verapamil, on the other hand, only marginally affected the cytotoxicity to Hs578T cells (Fig. 3).

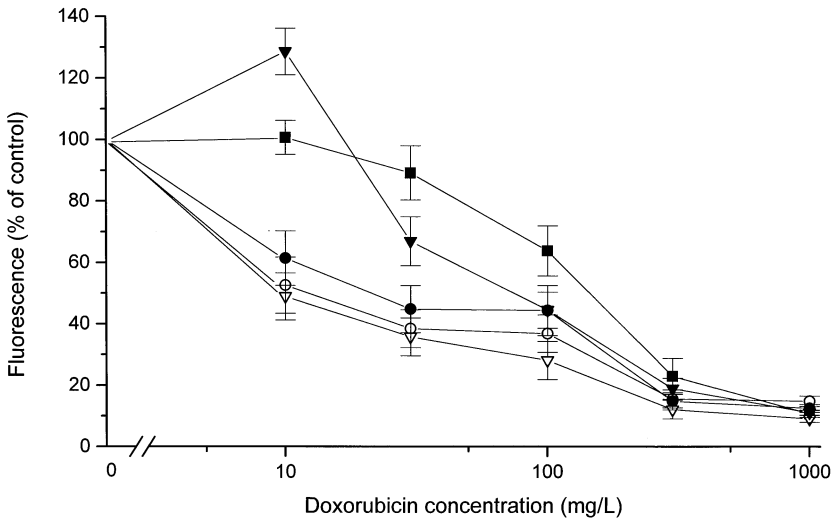

FIG. 2. Mean survival (percentage of untreated control) of the breast cancer subline (Hs578T-Dox) expressing P-gp activity, exposed for 48 hr to various concentrations of doxorubicin with or without carvedilol or verapamil. In the fluorometric microculture cytotoxicity assay, fluorescein diacetate was added and the incubation continued for 60 min followed by fluorescence determination. Results are expressed as means ± SE (N = 12). ■ = doxorubicin alone; ▼ = carvedilol 1 μmol/L; ▽ = carvedilol 10 μmol/L; ● = verapamil 1 μmol/L; ○ = verapamil 10 μmol/L.

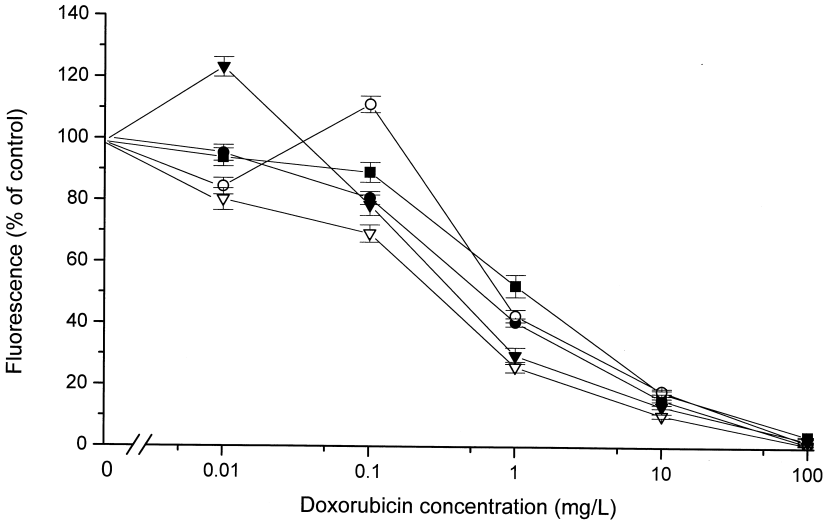

FIG. 3. Mean survival (percentage of untreated control) of the subline (Hs578T) not expressing P-gp activity, exposed for 48 hr to various concentrations of doxorubicin with or without carvedilol or verapamil. In the fluorometric microculture cytotoxicity assay, fluorescein diacetate was added and the incubation continued for 60 min followed by fluorescence determination. Results are expressed as means ± SE (N = 9). ■ = doxorubicin alone; ▼ = carvedilol 1 μmol/L; ▽ = carvedilol 10 μmol/L; ● = verapamil 1 μmol/L; ○ = verapamil 10 μmol/L.

Effects of Carvedilol on Calcein Accumulation

In the Hs578T-Dox subline, calcein accumulation slowly increased in the untreated control cells during a 120-min incubation (Fig. 4). Preincubation with 1 μmol/L or 10 μmol/L carvedilol or with verapamil for 120 min markedly increased calcein uptake. At 1 μmol/L, calcein accumulation was equal for verapamil and carvedilol, but at 10 μmol/L the calcein uptake was approximately doubled with carvedilol compared to verapamil, i.e. the P-gp activity inhibition was twice as effective with equimolar carvedilol compared to verapamil (Fig. 4). In Hs578T cells, P-gp efflux activity was low in untreated control and calcein fluorescence was only slightly stimulated by the presence of carvedilol or verapamil (Fig. 5).

Effects of Carvedilol on Pyrogallol-Affected Cell Growth and Calcein Fluorescence of Hs578T-Dox Cells

A dose–response curve was initially performed to find a suitable dose of pyrogallol. The superoxide radical generator pyrogallol (100 μmol/L; 5 hr 30 min) reduced the fluorescence (viability) of the Hs578T-Dox to 84.6 ± 3.9% of untreated control ($n = 12$). The addition of verapamil and carvedilol (1 and 10 μmol/L) reduced viability to 89.7 ± 3.4, 82.1 ± 4.0, 86.4 ± 3.8, and 90.1 ± 4.5%, respectively. These changes were not statistically significant (one-way ANOVA analysis) when compared with the effect of pyrogallol alone. Calcein accumulation by Hs578T-Dox cells was not affected when the cells were incubated with 100 μmol/L pyrogallol for 6 hr (results not shown).

DISCUSSION

At higher concentrations, the antihypertensive drug carvedilol, in a more pronounced fashion than verapamil, increased the sensitivity to doxorubicin and enhanced calcein accumulation by reducing the efflux activity of P-gp in the P-gp-expressing subline Hs578T-Dox. These effects of carvedilol were not seen in Hs578T, the subline without P-gp activity. Interestingly, however, a reduced cytotoxic effect seemed to exist with 1 μmol/L carvedilol at the lowest tested doxorubicin concentrations of the Hs578T-

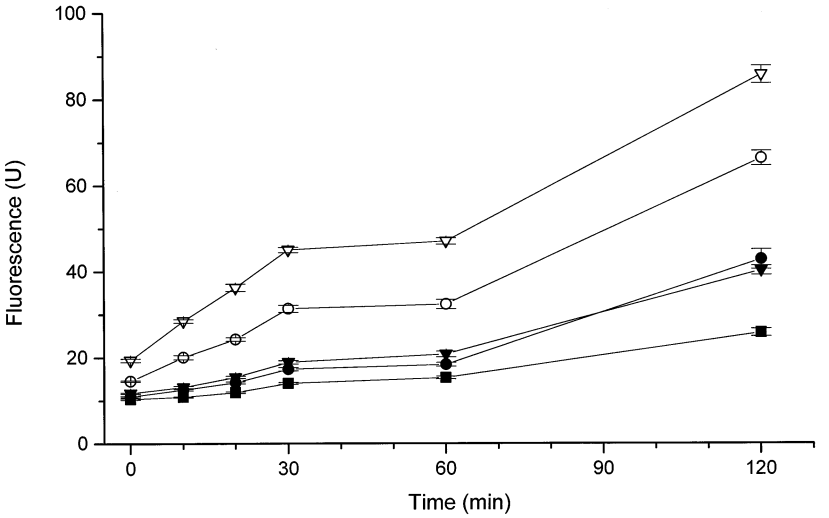

FIG. 4. Accumulation of calcein (fluorescence) in the subline (Hs578T-Dox) expressing P-gp activity, exposed to calcein AM (50 mg/L) with or without carvedilol or verapamil for 0–120 min. Results are expressed as means ± SE (N = 24–32). ■ = untreated control; ▼ = carvedilol 1 μmol/L; ▽ = carvedilol 10 μmol/L; ● = verapamil 1 μmol/L; ○ = verapamil 10 μmol/L.

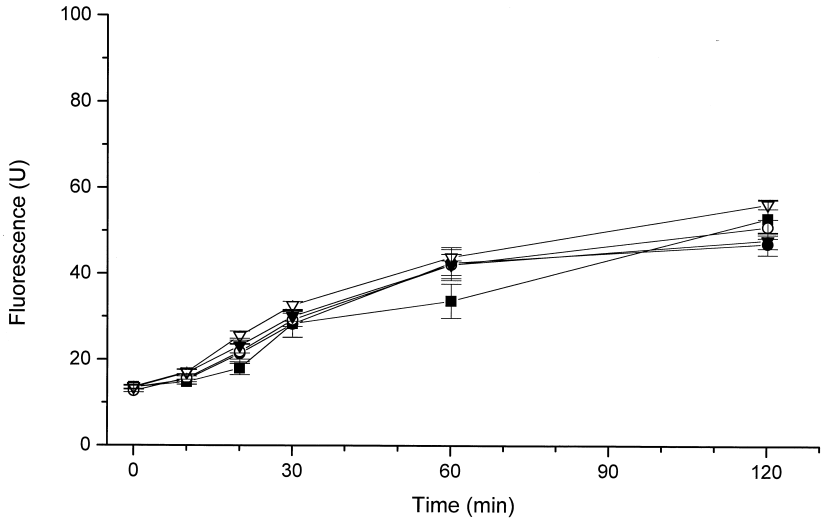

FIG. 5. Accumulation of calcein (fluorescence) in the subline (Hs578T) not expressing P-gp activity, exposed to calcein AM (50 mg/L) with or without carvedilol or verapamil for 0–120 min. Results are expressed as means ± SE (N = 24–32). ■ = untreated control; ▼ = carvedilol 1 μmol/L; ▽ = carvedilol 10 μmol/L; ● = verapamil 1 μmol/L; ○ = verapamil 10 μmol/L.

Dox subline. This could be of clinical importance, as the effect is seen at a pharmacologically relevant carvedilol blood concentration.

Several studies on the ability of calcium channel blockers, especially verapamil, to reverse the multidrug resistance of tumour or tumour cells to antineoplastic drugs have been published [8]. The main limiting problem in the clinic is the toxic side effects of the calcium channel blockers at therapeutic (reversing) concentrations.

Carvedilol, an antihypertensive drug, has until now not been investigated for its ability to reverse multidrug resistance nor have there been any reports on the interaction of carvedilol with cancer chemotherapy or irradiation. Carvedilol has a favourable adverse event profile compared to verapamil [15]. In a study conducted on 212 chronic stable angina patients, a twice daily dose of 25 mg carvedilol was as efficient in its anti-anginal and anti-ischaemic effects when compared to 120 mg verapamil administered twice a day. The adverse effects of carvedilol, particularly those of the gastrointestinal variety, were less than with verapamil [28]. However, antioxidant and free radical scavenging effects have been attributed to carvedilol in other investigations [18]. Two reports on neuro- and myocardial protection due to a proposed antioxidant or radical scavenging effect have been published. Doxorubicin, the anthracycline used in this report, represents a group of cytostatic drugs that are especially interesting to study in this context, as free radical production is one of the proposed mechanisms of action [29].

As we found that carvedilol increased the sensitivity of the Hs578T-Dox cell line to Dox-induced cytoxicity, we also evaluated whether overexpression of the *MDR1* gene, encoding for the transmembrane efflux pump P-gp of the two cell lines, was affected by carvedilol or verapamil pretreatment. We found that the accumulation of calcein was increased following pretreatment of the Hs578T-Dox cell line with 1 or 10 μmol/L carvedilol. The increase in calcein accumulation of the Hs578T-Dox cell line was approximately doubled with 10 μmol/L carvedilol compared to 10 μmol/L verapamil. Only minute effects of carvedilol (or verapamil) on doxorubicin cytotoxicity or calcein accumulation were found with the Hs578T cell line. Neither indomethacin, NEM, nor ethacrynic acid affected calcein accumulation of the Hs578T-Dox cells to any extent, indicating the absence of MRP activity [4]. Thus, the lowered calcein accumulation of the Hs578T-Dox subline is probably due to P-gp activity only.

As the antioxidative effects of carvedilol could be another mechanism for protection against the effects of cytotoxic drugs that exert their action through generation of free oxygen radicals, we tested whether carvedilol could also reduce pyrogallol-induced cytotoxicity or calcein accumulation of the Hs578T-Dox cell line. We observed that carvedilol did not affect pyrogallol cytotoxicity and that pyrogallol was without effect on calcein accumulation of the Hs578T-Dox cell line, indicating the lack of antioxidative properties affecting P-gp activity and associated toxicity of the drug.

In conclusion, we found that the antihypertensive drug, carvedilol, increased the sensitivity to doxorubicin cytotoxicity by reducing P-gp activity of a multidrug-resistant cell line. The effect is probably not due to its proposed free radical scavenging or antioxidative effects. These results indicate that carvedilol at lower doses than verapamil, and thus with reduced adverse reactions, has the potential to be tested for reversal of multidrug resistance of tumours depending on elevated activity of the efflux protein P-gp.

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References

1. Djurhuus R, Multiresistens mot cytostatika-biokjemi og klinisk betydning. *Farmakoterapi* **4:** 140–151, 1991.
2. Lum BL, Gosland MP, Kaubisch S and Sikic BI, Molecular

targets in oncology: Implications of the multidrug resistance gene. *Pharmacotherapy* **13:** 88–109, 1993.
3. Bates SE, Regis JI, Robey RW, Zhan Z, Scala S and Meadows BJ, Chemoresistance in the clinic: Overview 1994. *Bull Cancer* **9**(Suppl 2): 55s–61s, 1994.
4. Holló Z, Homolya L, Hegedus T and Sarkadi B, Transport properties of the multidrug resistance-associated protein (MRP) in human tumour cells. *FEBS Lett* **383:** 99–104, 1996.
5. Tsuruo T, Iida H, Tsukagoshi S and 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* **41:** 1967–1972, 1981.
6. Rogan AM, Hamilton TC, Young RC, Klecker RW and Ozols RF, Reversal of adriamycin resistance by verapamil in human ovarian cancer. *Science* **224:** 994–996, 1984.
7. Jirtle RL, Chemical modification of tumour blood flow. *Int J Hyperthermia* **4:** 355–371, 1988.
8. Goldstein LJ, Clinical reversal of drug resistance. *Curr Probl Cancer* **19:** 65–124, 1995.
9. Ford JM and Hait WN, Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* **42:** 155–199, 1990.
10. Raderer M and Scheithauer W, Clinical trials of agents that reverse multidrug resistance. *Cancer* **72:** 3553–3563, 1993.
11. Zamora JM, Pearce HL and Beck WT, Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. *Mol Pharmacol* **33:** 454–462, 1988.
12. Peterson HI, Modification of tumour blood flow—a review. *Int J Radiat Biol* **60:** 201–210, 1991.
13. Jonsson Ö, Henriksson R and Grankvist K, Interactions of cardiovascular drugs with the cytotoxicity induced by epirubicin and irradiation *in vitro*. *Int J Oncol* **3:** 655–659, 1993.
14. Sandström PE, Jonsson Ö, Grankvist K and Henriksson R, Identification of potassium flux pathways and their role in the cytotoxicity of estramustine in human malignant glioma, prostatic carcinoma and pulmonary carcinoma cell lines. *Eur J Cancer* **30A:** 1822–1826, 1994.
15. Dunn CJ, Lea AP and Wagstaff AJ, Carvedilol, a reappraisal of its pharmacological properties and therapeutic use in cardiovascular disorders. *Drugs* **54:** 161–185, 1997.
16. Morita K, Hamano S, Yoshizumi M and Oka M, Inhibitory action of carvedilol, a novel α,β-adrenoceptor antagonist, on catecholamine secretion and calcium influx in cultured bovine adrenal chromaffin cells. *Biochem Pharmacol* **38:** 4461–4465, 1989.
17. McTavish D, Campoli-Richards D and Sorkin EM, Carvedilol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* **45:** 232–258, 1993.
18. Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, Lysko KA, Davis LL and Feuerstein G, Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. *J Pharmacol Exp Ther* **263:** 92–98, 1992.
19. Lysko PG, Lysko KA, Yue TL, Webb CL, Gu JL and Feuerstein G, Neuroprotective effects of carvedilol, a new antihypertensive agent, in cultured rat cerebellar neurones and in gerbil global brain ischemia. *Stroke* **23:** 1635–1636, 1992.
20. Feuerstein GZ and Yue TL, Myocardial protection by the novel vasodilating beta-blocker, carvedilol: Potential relevance of anti-oxidant activity. *J Hypertens Suppl* **11:** S41–48, 1993.
21. Yue TL, McKenna PJ, Gu JL, Cheng HY, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new antihypertensive agent, prevents lipid peroxidation and oxidative injury to endothelial cells. *Hypertension* **22:** 922–928, 1993.
22. Hackett AJ, Smith HS, Springer EL, Owens RB, Nelson-Rees WA, Riggs JL and Gardner MB, Two syngeneic cell lines from human breast tissue: The aneuploid mammary epithelial (Hs578T) and the diploid myoepithelial (Hs578Bst) cell lines. *J Natl Cancer Inst* **58:** 1975–1806, 1977.
23. De la Torre M, Hao X-Y, Larsson R, Nygren P, Tsuruo T, Mannervik B and Bergh J, Characterisation of four doxorubicin adapted human breast cancer cell lines with respect to chemotherapeutic drug sensitivity, drug resistance associated membrane proteins and glutathione transferases. *Anticancer Res* **13:** 1425–1430, 1993.
24. Larsson R, Nygren P, Ekberg M and Slater L, Chemotherapeutic drug sensitivity testing of human leukemia cells *in vitro* using a semiautomated fluorometric assay. *Leukemia* **4:** 567–571, 1990.
25. Gruber A, Areström I, Albertioni F, Björkholm M, Peterson C and Vitols S, Multidrug resistance (*Mdr1*) gene expression in peripheral blasts from patients with acute leukemia only rarely increases during disease progression after combination chemotherapy. *Leuk Lymphoma* **18:** 435–442, 1995.
26. Gruber A, Larsson R, Nygren P, Bjorkholm M and Peterson C, A non-P-glycoprotein-mediated mechanism of vincristine transport which is affected by resistance modifiers and present in chemosensitive cells. *Leukemia* **8:** 985–989, 1994.
27. Marklund SL, Westman NG, Lundgren E and Roos G, Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Res* **42:** 1955–1961, 1982.
28. Hauf-Zachariou U, Blackwood RA, Gunawardena KA, O'Donnell JG, Garnham S and Pfarr E, Carvedilol versus verapamil in chronic stable angina: A multicentre trial. *Eur J Clin Pharmacol* **52:** 95–100, 1997.
29. Grankvist K and Henriksson R, Doxorubicin and epirubicin iron-induced generation of free radicals *in vitro*. A comparative study. *Biosci Rep* **7:** 653–658, 1987.