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Bcl-2 has differing effects on the sensitivity of breast cancer cells depending on the antineoplastic drug used

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Abstract

The aim of this paper was to evaluate the role of bcl-2 in the susceptibility of the MCF7 ADR human breast carcinoma line overexpressing the P-170 glycoprotein (P-170) to various drugs. The sensitivity to four multidrug resistance (MDR)-related drugs (doxorubicin (ADR), vincristine (VCR), vinblastine (VBL), actinomycin D (ACTD)) and three MDR-non-related drugs (cisplatin (DDP), bischloroethylnitrosourea (BCNU), 5-fluorouracil (5-FU)) was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay in three bcl-2-overexpressing clones obtained from the MCF7 ADR line. We found that the bcl-2-overexpressing clones show increased resistance to DDP and BCNU, while no difference to 5-FU were observed between the control cells and *bcl-2* transfectants. Surprisingly, bcl-2-overexpressing clones displayed an increased sensitivity compared with the control cells to the MDR-related drugs ADR, VCR, VBL and ACTD. Focusing on DDP and ADR, we found that the increased resistance of the *bcl-2* transfectants to DDP was correlated to their ability to prevent apoptosis, while the enhanced sensitivity to ADR was associated with an increased ADR accumulation and a decreased ADR efflux. Moreover, while bcl-2 overexpression does not induce changes in P-170 glycoprotein expression, it did induce a reduction of the adenosine triphosphate (ATP) levels and basal protein kinase C (PKC) activity, both of which have a crucial role in the regulation of the MDR phenotype. In conclusion, the effect of bcl-2 on antineoplastic sensitivity observed in this study underscores the idea that bcl-2 may have distinct biological effects depending on the anticancer drug used.

Keywords: Breast carcinoma, bcl-2; Antineoplastic drugs; MDR

1. Introduction

Intrinsic and acquired resistance of tumour cells to chemotherapeutic drugs as well as their inability to induce apoptotic cell death, are the main obstacles to effective therapies for human malignancies [1]. Genes which regulate the apoptotic process may play a critical role in determining the sensitivity of tumour cells to chemotherapy [2]. Bcl-2 is the first of a rapidly expanding family of proteins which are implicated in the response to chemotherapy. Indeed, a wide variety of human cancers, with a poor clinical response to chemotherapy, exhibit high levels of bcl-2 expression. The gene *bcl-2* inhibits apoptosis induced by a variety of stimuli including growth factor withdrawal, oncogene activation and antitumour drug treatment [1,3,4]. Overexpression of bcl-2 may affect 'events' downstream of the initiation of toxicity, brought about by a drug, and cause the resistance to drug-induced apoptosis. This has been implied in published studies which have shown that bcl-2 expression provides resistance to a wide variety of cell death stimuli including classical chemotherapeutic drugs and radiation [1].

Furthermore, numerous studies have shown that some tumours and cell lines exposed to a single cyto-

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toxic agent such as the anthracyclines, vinca alkaloids or epipodophyllotoxins frequently develop resistance to a wide range of chemically and functionally unrelated agents [5]. This phenomenon, named multidrug resistance (MDR), has been ascribed to the biochemical properties that are intrinsic to the genotype of tumour cells or to mutations caused by the antitumour agents administered [5]. One of the major changes in the phenotype of MDR cells is a decreased accumulation of cytotoxic drugs, mainly due to an overexpression of a 170 KDa membrane glycoprotein, called P-170, a member of the adenosine triphosphate (ATP)-binding family of membrane transport proteins, which acts as an ATP-dependent efflux pump to extrude anticancer drugs from the cells [6]. The crucial role of ATP in this process is demonstrated by the observation that ATP hydrolysis is required for the drug's transport and that amino acid substitutions in ATP-binding sites affect transport efficiency [7,8]. Elevated protein kinase C (PKC) activity is a common feature of MDR tumour cells, and forced overexpression of PKC or treatment with compounds which activate PKC, can enhance drug resistance or drug efflux indicating that PKC modulation is linked to the cytotoxic effect of the drugs as well as the acquired ability of cells to resist the cytotoxic action [9–12]. Regulation of MDR promoter activity in human carcinoma by PKC has also been demonstrated [13].

We previously demonstrated that the overexpression of bcl-2 in MCF7 ADR cells resistant to ADR results in profound alterations in mitochondrial metabolism. CO_2 production, oxygen consumption, and the activity of the regulatory enzymes of the Krebs cycle are lower in *bcl-2* transfectants than in the control clones [14]. On the basis of these results, the well-established role of ATP in P-170 function and the association of the *bcl-2* gene product with mitochondrial membranes [15] raises the possibility that this protein may interact with intracellular ATP metabolism. To test this hypothesis, we used three bcl-2 and P-170 overexpressing clones to evaluate the role of bcl-2 overexpression in the sensitivity of a breast cancer cell line expressing the MDR-resistant phenotype to some MDR-related and non-related drugs.

2. Materials and methods

2.1. Cell lines and culture conditions

The MCF7 ADR human breast cancer cell line, that is resistant to doxorubicin (ADR), was grown in medium containing ADR and passaged for 2 weeks in drug-free medium prior to each experiment. Three bcl-2-overexpressing clones (MAB25, MAB27 and MAB31) and a control clone (MAN9) were obtained by transfecting the MCF7 ADR cells with a *bcl-2* expression vector or the native vector alone, as previously reported in Ref. [16].

2.2. Western blotting

Western blotting and detection of proteins were performed as previously reported in Ref. [17]. Briefly, 40 μg of total proteins were loaded from each sample on denaturing 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Anti-bcl-2 mAb (Dako S.p.a., Milan, Italy) was used at a 1:200 dilution. To check the amount of proteins transferred to the nitrocellulose membrane, heat shock protein (HSP) was used as a control and detected by an anti-human HSP 72/73 mAb (dil. 1:2000, Ab-1, clone W27, Calbiochem, Cambridge, MA, USA). The relative amount of the transferred proteins was quantified by scanning the autoradiographic films with a gel densitometer scanner (Bio-Rad, Milan, Italy) and normalised according to the related HSP 72/73 amounts.

2.3. Drug preparation and analysis of drug sensitivity

ADR (Pharmacia, Carlo Erba, Milan, Italy), vincristine (VCR) (Eli Lilly, Florence, Italy), vinblastine (VBL) (Eli Lilly), actinomycin D (ACTD) (Merck Sharp & Dohme, Rome, Italy), cisplatin (DDP) (Teva Pharm. Ind., Ltd., Israel), 5-fluorouracil (5-FU) (Teva Pharm.) and bis-chloroethylnitrosourea (BCNU) (Bristol-Myers Syracuse, NY, USA) were freshly prepared before each experiment.

Drug sensitivity was evaluated by using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were plated in quintuplicates in 96-well culture plates (Nunc, Milan, Italy) at a density of 1×10^4 cells/well. 24 h later, the medium was replaced with fresh growth medium containing various concentrations of drugs. After 72 h of growth in the presence of the drug, the cells were assayed for viability. Briefly, MTT labelling reagent (final concentration 0.5 mg/ml) was added to each well and 2 h later adherent cells were lysed with isopropilic alcool. The absorbance of the formazan product was measured with the enzyme linked immunosorbent assay (ELISA) reader (DASIT, Milan, Italy) at a wavelength between 550 and 600 nm. The IC_{50} value is the dose of drug that causes 50% cell viability.

2.4. Determination of ADR intracellular content

Cells were seeded at density of 1×10^6 cells/well in six-well culture dishes. After 24 h, the medium was replaced with fresh growth medium containing [^{14}C] ADR (1 μM). The cells were exposed to the labelled ADR for times ranging from 15 min to 12 h. At the end of labelling, the cells were washed three times with cold phosphate-buffered saline (PBS). The cells were then lysed with 0.2 ml PBS containing 0.5% SDS, and the

radioactivity was counted in a liquid scintillation counter. For drug efflux studies, cells were incubated for 12 h with [^{14}C] ADR (1 μM) and then the medium was replaced with drug-free growth medium. At various time points (ranging from 15 to 60 min), the cells were washed and lysed, and the radioactivity counted as described above.

2.5. Assay of the adenosine triphosphate

The determination of ATP was performed by reverse-phase high performance liquid chromatography (HPLC) according to Fanciulli and colleagues [18] with a LKB 2150 apparatus equipped with a RoSil 5 μm C18 column (Bio Rad). The apparatus was connected to a computer through a Nelson Analytical series 900 interface (Cupertino, CA, USA). The analysis of the chromatograms, as well as the integration of peak areas, was performed with Nelson Analytical Chromatographic Software, version 3.6. Quantitative measurements were carried out by injection of standard solutions of known concentrations.

2.6. Protein kinase C assay

Cytosol and membrane fractions, prepared as previously described in Ref. [19], were adsorbed in a 1.5-ml microcentrifuge tube to 0.2 ml of diethylaminoethyl (DEAE)-Sepharose fast flow equilibrated in buffer A (20 mM HEPES pH 7.5, 10 mM 2-mercaptoethanol, 0.5 mM ethylene glycol tetra acetic acid (EGTA), 0.5 mM ethylene diamine tetra acetic acid (EDTA), washed three times with buffer A, and eluted with buffer A containing 0.2 M NaCl. The eluate was incubated in a 50-μl reaction mixture containing 20 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 40 μg of histone H1, 100 μg/ml of phosphatidylserine, 200 nM phorbol dibutyrate, 500 μM $CaCl_2$ or 200 μM EGTA in place of $CaCl_2$, phosphatidylserine and phorbol dibutyrate. Assays were carried out in triplicate as previously described in Ref. [19].

2.7. Flow cytometric analysis of P-170

The expression of the P-170 glycoprotein was determined by indirect immunofluorescence by means of flow cytofluorimetric analysis (FACScan, Becton Dickinson, Sunnyvale, CA, USA). 1×10^6 cells/sample were incubated with medium (negative control) or a saturating concentration of the primary monoclonal antibody (MRK-16 Kamiya, Thousand Oaks, CA, USA) for 1 h at 4 °C. The cells were then incubated for 1 h at 4 °C with 50 μl of fluorescein isothiocyanate (FITC)-goat anti-mouse antibody (Cappel, West Chester, PA, USA). To exclude non-viable cells, 5 μl of a propidium iodide (PI) solution (1 mg/ml) were added to each sample before the cytofluorimetric analysis. At least 10 000 cells/sample (in triplicate) were acquired. The histograms were analysed using a Becton Dickinson software package. Three independent experiments were performed.

2.8. Detection of apoptosis

Drug-induced apoptosis was detected by flow cytometric analysis of permeabilised PI-stained cells, and by cell morphology of cytocentrifuge preparations stained with May Grunwald/Giemsa as previously reported in Ref. [17]. Exponentially growing cells were treated with different concentrations and exposure times of drugs and the analysis was performed at different times after the end of the treatment. In particular, cells were treated with ADR at doses ranging from 5 to 100 μM for exposure times ranging from 24 to 96 h. The analysis was performed 0, 24 and 48 h after the end of the treatment. Treatment with DDP was performed at doses ranging from 5 to 10 μM for 24 h and the analysis was performed 0 and 24 h after the end of the treatment.

Reactive species of oxygen (ROS) production was assessed as previously reported in Ref. [20].

2.9. Statistical analysis

The two-way ANOVA was used to compare ADR and ATP content, PKC activity and the sensitivity of MCF7 ADR cells, MAN9 control clone and *bcl-2* transfectants to the different drugs.

3. Results

3.1. Drug sensitivity of MCF7 ADR cells and bcl-2 transfectants

We previously demonstrated that MCF7 ADR resistant cells exhibit enhanced energy metabolism compared with MCF7 WT parental sensitive cells [18] and that the overexpression of bcl-2 in the MCF7 ADR cell line results in profound alterations in mitochondrial metabolism [14]. Based on these results and on the finding that P-170 utilises energy from ATP hydrolysis to remove drugs from the resistant cells [21], we determined whether bcl-2 would have differing effects on the sensitivity to MDR-related and MDR-non-related drugs. To this purpose, we used the MCF7 ADR parental line expressing the P-170 glycoprotein, a control clone (MAN9) and three bcl-2-overexpressing clones (MAB25, MAB27 and MAB31) previously derived from the MCF7 ADR line [16] (Fig. 1).

As reported in Table 1, bcl-2 overexpression had different effects on drug sensitivity, depending on the drug used. Interestingly, the three *bcl-2* transfectants showed an increased sensitivity to ADR, VCR, VLB and ACTD

($P<0.01$) when compared with the MCF7 ADR cells and MAN9 clone. Indeed, 40–50% killing of bcl-2-overexpressing cells was achieved by a 20 μM ADR dose, while approximately 35 μM ADR was required for 50% killing of the control clone and the parental line. For VCR, VLB and ACTD 40–50% killing of bcl-2-overexpressing cells was achieved by doses that were approximately half of those required for 50% killing of the control cells. In addition, while bcl-2 overexpression did not affect the sensitivity to 5-FU, it significantly decreased DDP sensitivity ($P<0.01$). In fact, the IC_{50} value was approximately 4 μM for the *bcl-2* transfectants, and 1.3 and 1.4 μM, for the MCF7 ADR line and MAN9 control clone, respectively. In addition, the sensitivity to BCNU was reduced in the *bcl-2* transfectants. An increase of IC_{50} of approximately 40% was observed after bcl-2 overexpression.

3.2. *Apoptosis in MCF7 ADR cells and* bcl-2 *transfectants treated with DDP or ADR*

To evaluate whether the different effects of bcl-2 on MDR-related and non-related drug sensitivity was associated with differing amounts of apoptosis, we chose one drug from each group (ADR and DDP) and flow cytometric analysis of DNA content was performed after exposure to these drugs (Figs. 2 and 3). Apoptosis was evaluated 0 and 24 h after the treatment with DDP for 24 h at the doses of 5, 7.5 and 10 μM. No apoptotic cells were observed at the end of the DDP treatment with all of the doses used in all of the lines tested (data not shown). As reported in Fig. 2a, 24 h after the end of DDP exposure, apoptotic cells were evident only in *neo*-transfected cells treated with the two higher doses (7.5 and 10 μM), the dead cells being approximately 20 and 30% of the total population, respectively. On the contrary, less than 10% of apoptotic cells were observed in the MAB25 and MAB31 clones at the highest dose of DDP used.

Since DDP exerts its cytotoxic activity through the generation of ROS as powerful inducers of apoptotic death [22], we asked whether DDP treatment induced ROS production and whether the overexpression of bcl-2 was able to protect MCF7 ADR cells from apoptosis induced by DDP through inhibition of ROS generation. As reported in Fig. 2 (panels b–d), while a clear increase in the intracellular ROS content was evident after 7.5 μM DDP treatment for 24 h in the MAN9 clone (b), no changes were observed in the relative fluorescence for the MAB25 (c) and MAB31 clones (d).

The ability of ADR to induce apoptosis was evaluated by treating the cells with 50, 75 and 100 μM of the drug for 24 h. The analysis was performed 0, 24 and 48 h after the end of the treatment. No apoptosis was observed in the MCF7 ADR cells, the MAN9 control clone, or the *bcl-2* transfectants at all the doses and times analysed. Fig. 3 shows the percentage of apoptotic cells at increasing ADR doses at a representative time (48 h after the end of treatment). Morphological analysis of the cytospin preparation, performed after ADR and DDP treatment, confirmed the nature of cell death observed and the percentage of apoptotic cells calculated by cytofluorimetric analysis (data not shown).

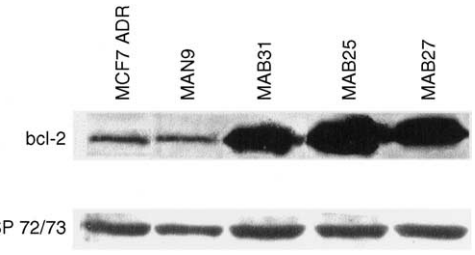

Fig. 1. Bcl-2 protein expression in parental line (MCF7 ADR), a control transfectant clone (MAN9), and three *bcl-2* transfectants (MAB25, MAB27 and MAB31). The relative amount of the transferred bcl-2 protein was compared with the amount of HSP 72/73 protein. A representative western blot is shown.

Table 1
IC_{50} of ADR, VCR, VBL, ACTD, 5-FU, DDP, BCNU in MCF7 ADR, MAN9 control clone and three *bcl-2* transfectants (MAB25, MAB27, MAB31)

Cells	MDR-related drugs				MDR-non-related drugs		
	ADR	VCR	VBL	ACTD	5-FU	DDP	BCNU
MCF7 ADR	35	6.7	2.2	3.3	65	1.3	30
MAN9	34	6.5	2.0	3.1	64	1.4	28
MAB25	20	3.1	1.1	1.6	63	4.0	41
MAB27	22	3.3	0.9	1.4	66	3.8	44
MAB31	21	3.3	1.0	1.8	65	3.8	40

ADR, doxorubicin; VCR, vincristine; VBL, vinblastine; ACTD, actinomycin D; 5-FU, 5-fluorouracil; DDP, cisplatin; BCNU, bischloroethylnitrosourea; MDR, multidrug resistance. IC_{50} values are expressed in μM and were evaluated as reported in Materials and methods. Standard deviations for all of the experiments performed in triplicate was less than 5%.

3.3. *ADR content in MCF7 ADR cells and* bcl-2 *transfectants*

Since MDR resistance is mainly due to alterations of drug influx and efflux, related to P-170 overexpression [6], ADR intracellular accumulation and release were evaluated (Fig. 4). Fig. 4a shows the intracellular [^{14}C] ADR content at different drug exposure times in the MAN9 control clone and MAB25 bcl-2-overexpressing clone, that shows the greatest sensitivity to ADR with respect to the control clone. An increased accumulation of [^{14}C] ADR of approximately 2-fold in the MAB25 cells was evident when compared with that of MAN9 cells after 6 h of drug exposure. The efflux of the drug from the MAN9 and MAB25 clones preincubated for

12 h with [^{14}C] ADR was also compared (Fig. 4b). Following the removal of the radiolabelled drug from the medium, more than 50% of radioactive ADR effluxed from MAN9 cells after 60 min. In contrast, MAB25 cells incubated under the same conditions lost less than 25% of the radiolabelled drug. The statistical analysis using the two-way ANOVA demonstrated that the rate of ADR accumulation was significantly reduced in the control clone with respect to the bcl-2-overexpressing clone ($P=0.02$). The drug efflux in the bcl-2-overexpressing clone differed significantly from that of the MAN9 control clone ($P<0.001$). ADR accumulation and efflux in MCF7 ADR and the other *bcl-2* transfectants was similar to those observed in MAN9 and MAB25, respectively.

To evaluate whether the different kinetics of ADR accumulation and efflux observed could be related to an alteration in P-170 glycoprotein expression, flow cytometric analysis of P-170 protein was performed. Fig. 5 shows P-170 glycoprotein surface expression in the MAN9, MAB25, MAB27 and MAB31 clones. The P-170 glycoprotein was highly expressed in all the both clones, as evident from the similar distribution of the fluorescence intensity.

Based on the finding that P-170 utilises energy from ATP hydrolysis to remove the drug from resistant cells [21], and since many studies strongly suggest a direct involvement of PKC in the MDR phenotype [9–13], we measured the ATP content and the levels of PKC activity in MCF7 ADR cells, MAN9 control clone and three clones overexpressing bcl-2, MAB25, MAB27 and MAB31. As shown in Fig. 6a the quantitative analysis demonstrated a decreased ATP content of approximately 50% in the three *bcl-2* transfectants when compared with MAN9 cells. Fig. 6b shows the percent inhibition of Ca^{++}- and phospholipid-dependent PKC

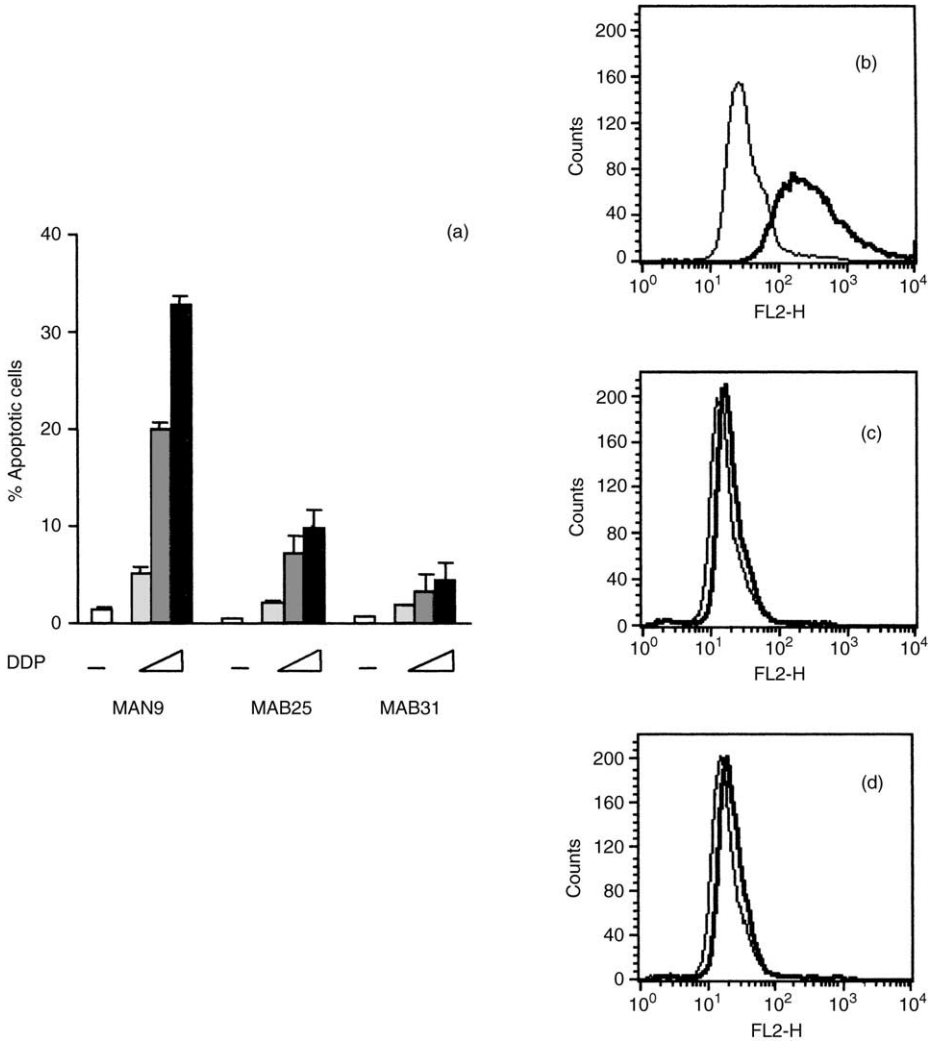

Fig. 2. Panel (a): percentage of apoptotic cells in untreated cells (−) and cells treated for 24 h with cisplatin (DDP) at increasing doses (5, 7.5 and 10 μM). The analysis was performed 24 h after the end of the treatment. Each value represents the mean of three independently performed experiments ± standard deviation (S.D.). Panels (b–d): Reactive oxygen species (ROS) content in MAN9 control clone (b), MAB25 (c) and MAB31 (d) bcl-2-overexpressing clones treated with 7.5 μM DDP for 24 h. The analysis was performed at the end of the treatment.

activity in subcellular fractions of MAB25, MAB27 and MAB31 cells with respect to the PKC activity of MAN9 cells. Cytosolic PKC activity, after elution from DEAE-Sepharose, was reduced by 35, 28 and 22% in MAB25, MAB27 and MAB31 clones, respectively. A decrease of PKC activity (approximately 25%) was also detected in the membrane fractions of both of all the *bcl-2* transfectants compared with control cells.

4. Discussion

We have previously demonstrated that bcl-2 overexpression in MCF7 ADR cells substantially modifies the energy metabolism of the cells, the activity of some regulatory enzymes and inhibits apoptosis induced by lonidamine (LND), a selective inhibitor of the energy metabolism of tumour cells [14].

In this paper, we investigated whether bcl-2 overexpression affects the sensitivity of the MCF7 ADR line to some MDR-related and non-related drugs. The results obtained using bcl-2-overexpressing clones, derived by transfecting the MCF7 ADR human breast cancer line, overexpressing the P-170 glycoprotein, demonstrated that bcl-2 overexpression plays a differing role in modulating drug sensitivity depending on the type of drug used. In particular, bcl-2-overexpressing clones displayed an increased sensitivity to the MDR-related drugs ADR, VCR, VLB and ACTD whereas they show increased resistance to the MDR-non-related drugs DDP and BCNU. In addition, no difference in the response to 5-FU was observed between the control cells and *bcl-2* transfectants. The differing drug responses were independent of the expression of the bcl-x_L and bax proteins, two bcl-2-related proteins that are able to modulate the induction of apoptosis in response to the administration of antineoplastic drugs. In fact, in our experimental model, bcl-x_L and bax proteins are uniformly expressed in *bcl-2*-transfected and control clones (data not shown [14]).

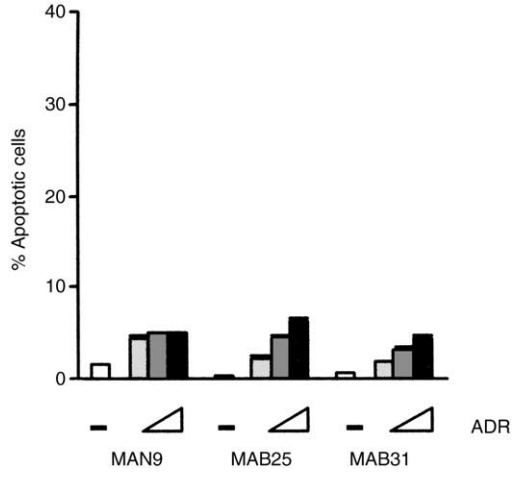

Fig. 3. Percentage of apoptotic cells in untreated cells (−) and cells treated for 24 h with doxorubicin (ADR) at increasing doses (50, 75 and 100 μM). The analysis was performed 48 h after the end of the treatment. Each value represents the mean of three independently performed experiments±standard deviation (S.D.).

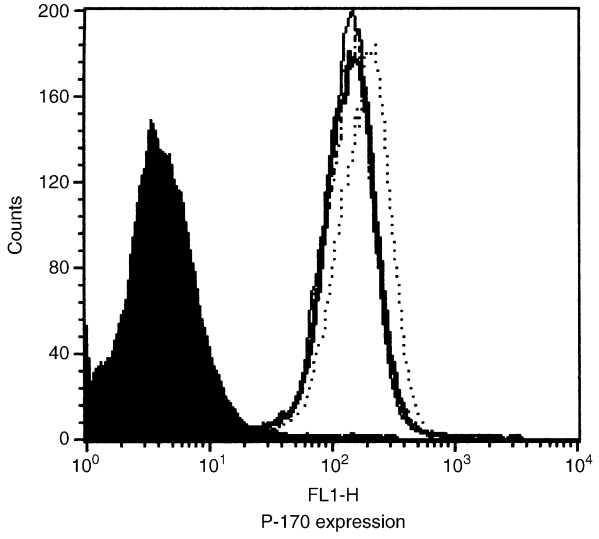

Fig. 5. Cytofluorimetric analysis of P-170 glycoprotein in the MAN9 control clone (bold), MAB25 (thin), MAB27 (dashed) and MAB31 (dotted) bcl-2-overexpressing clones. The black area indicates the negative control (no addition of MRK16 primary antibody to the cells) of the MAN9 cell that is superimposable on the negative control of the *bcl-2* transfectants.

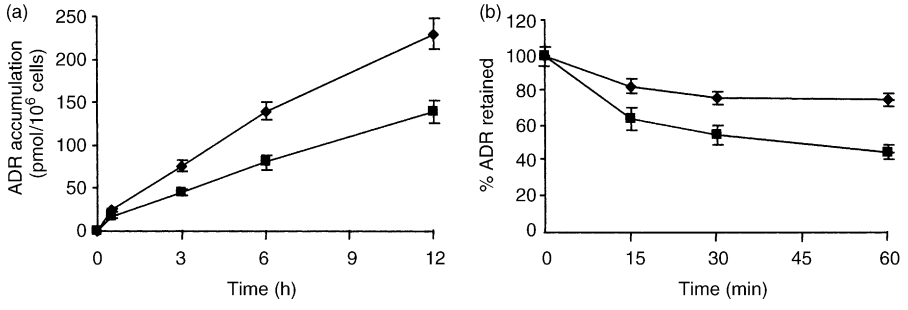

Fig. 4. Analysis of radiolabelled doxorubicin (ADR) accumulation (a) and efflux (b) in a control (MAN9, ■) and a bcl-2-overexpressing clone (MAB25, ◆). The values represent the mean±standard deviation (S.D.) obtained from triplicate determinations.

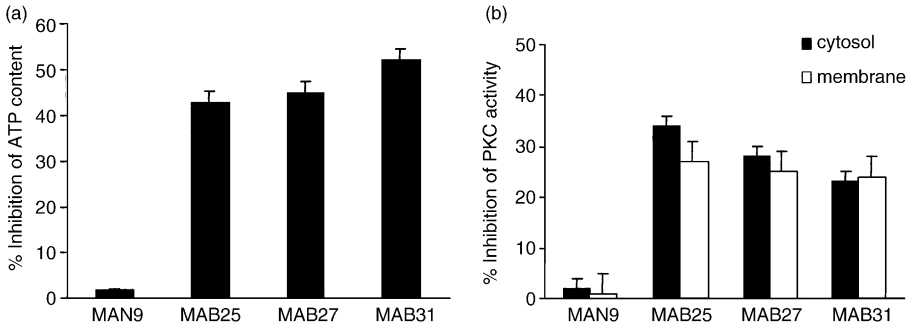

Fig. 6. Inhibition of adenosine triphosphate (ATP) content and protein kinase C (PKC) activity of three bcl-2-overexpressing clones (MAB25, MAB27 and MAB31) compared with control lines. Data are expressed as the percentage of ATP content and PKC activity inhibition with respect to those of MCF7 ADR cells.

The response to ADR and DDP was investigated in more detail. We have evidence that bcl-2 overexpression is able to reduce the apoptosis induced by high doses of DDP. In our model system, high doses of DDP were required to initiate apoptotic cell death, and bcl-2 was able to reduce the DDP-induced apoptosis. Our data are in accordance with results demonstrating that *bcl-2* gene transfer confers DDP resistance in several systems [28]. The increased resistance to BCNU after bcl-2 overexpression is also in accordance with our published data demonstrating the ability of bcl-2 overexpression to increase *in vitro* and *in vivo* resistance of a human glioblastoma line to BCNU [20].

The higher sensitivity to ADR observed in the bcl-2-overexpressing clones correlated with a greater amount of intracellular drug. Since the expression of the P-170 glycoprotein was similar both in the control and transfectants and the overexpression of bcl-2 in the MCF7 ADR cells strongly reduced the ATP content, the increase in ADR content might be ascribed to an impairment of the ATP-driven efflux pump [7]. It is possible that in our model system the low levels of ATP observed in the *bcl-2* transfectants affects P-170 basal activity and P-170 function.

Since it has been demonstrated that the P-170 multidrug transporter closely interacts with PKC and serves as its substrates in MCF7 ADR cells [23], we also evaluated the ability of bcl-2 to influence PKC activity. We found that when MCF7 ADR cells were transfected with *bcl-2*, PKC activity was decreased. We previously demonstrated that transfection with PKC confers increased MDR resistance in MCF7 ADR cells [12]. Thus, studies are ongoing to evaluate whether post-transcriptional regulation of P-170 may differ in parental cells and *bcl-2* transfectants. The sensitivity of the MCF7 ADR line to ADR is not related to the induction of apoptosis. As reported by others [24], we found that the MCF7 ADR line was resistant to ADR-induced apoptosis. No apoptotic cells were observed after ADR treatment despite the lengthy exposures and high doses.

The *bcl-2* protein can be inactivated by mutations, cleavage or serine phosphorylation [25,26]. Even though some antineoplastic drugs are able to affect the function of bcl-2 through its phosphorylation [26], we have evidence that the ability of bcl-2 overexpression to sensitise MCF7 ADR cells to ADR treatment is not related to an ADR effect on bcl-2 phosphorylation. In fact, as demonstrated by Haldar and colleagues in MCF7 WT cells [27], we found that ADR did not induce bcl-2 phosphorylation both in the MCF7 ADR line and in two *bcl-2* transfectants (data not shown).

Even though several studies have demonstrated the ability of bcl-2 to increase resistance to chemotherapeutic treatment, our data underscore the idea that bcl-2 may have distinct biological effects depending on the anticancer drug used.

Other authors found that bcl-2 overexpression sensitises human tumours to chemotherapy [29]. In addition, several reports demonstrated that some chemotherapeutic drugs kill cells through a bcl-2-independent mechanism [25,30]. This could be the mechanism of action of 5-FU in our experimental model. Bcl-2 has also been reported not to influence the sensitivity of a variety of types of haematopoietic or non-haematopoietic cells to apoptosis induced by tumour necrosis factor, H_2O_2 or anticancer drugs such as, DDP, VP16 or methotrexate [31–33].

In conclusion, the effect of bcl-2 on antineoplastic sensitivity observed in this and in other studies underscores the idea that bcl-2 may have distinct biological effects depending on the anticancer drug and on the cell context.

Acknowledgements

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