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Selective interactions of verapamil with anthraquinones in adriamycin-sensitive and -resistant murine and human tumour cell lines in vitro

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Summary. Enhancement of the cytotoxicity of adriamycin (ADR) by addition of verpamil (VPM) was selective and, in part, concentration-dependent. In an ADR-resistant murine L5178Y lymphoma subline sensitivity was improved with ADR and 1 μ*M* VPM, whereas the cytotoxic effects of mitoxantrone or esorubicin were not affected by VPM. In human ovarian SK-OV-3 tumour cells ADR cytotoxicity was only enhanced by 6.6 μ*M* VPM and there was no selective advantage against an ADR-resistant subline. Circumvention of ADR resistance by VPM is not a universal phenomenon, appearing least effective against sublines expressing relatively low orders of resistance, which may be those most commonly encountered clinically.

Introduction

Two strategies for overcoming resistance to ADR are being investigated: (1) identification of drugs which display a lack of cross-resistance with ADR [7], and (2) reversal of resistance by selective enhancement of ADR cytotoxicity by the addition of calcium channel blockers [11]. Tsuruo et al. originally demonstrated a 7- to 10-fold enhancement of ADR cytotoxicity achieved by adding VPM using ADR-resistant P388 leukaemia cells in vitro. This has been confirmed using the same model system with ADR or daunomycin plus VPM or related calcium channel blockers [8, 11]. Also, VPM selectively reversed ADR resistance in certain human ovarian cancer cell lines [10]. The mechanism may be related to drug transport alterations [10, 11]. We have investigated the potential of VPM for enhancing ADR cytotoxicity in resistant tumour cells in which drug uptake is not impaired and have determined whether the cytotoxicity of Novantrone and 4'ADR can also be modulated by VPM addition in our L5178Y model system.

Materials and methods

Drugs. ADR and esorubicin (4'-deoxydoxorubicin) were gifts from Farmitalia Carlo Erba Ltd., U. K. and mitoxantrone (Novantrone) was kindly provided by Lederle Laboratories, U. K. VPM hydrochloride was purchased from Abbot Laboratories, U. K. [^{14}C]-Labelled ADR (53.5 mCi per millimole) was kindly donated for these studies by Prof. F. Arcamone, Farmitalia Carlo Erba, Milan, Italy.

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Cell lines. SK-OV-3 human ovarian carcinoma cell line was obtained from the American Tissue Type Collection (HTB77). Details of cell culture of lymphoma L5178Y [5] and SK-OV-3 cells [2] and derivation of resistant sublines are published elsewhere [3, 6]. With drug uptake studies, following a 1-h incubation, excluding light, in Hanks' medium at 37 °C with 10 ng/ml ADR in pre-washed glass tubes or dishes, both L5178Y cell lines accumulated approximately 0.007 pmol/10^6 cells, whilst for the SK-OV-3 lines incubated with 200 ng/ml ADR, the levels achieved were 102 (parental) and 92 (resistant) pmol/mg protein (E. M. Gibby, unpublished data). These levels were not significantly different.

Clonogenic cell survival assays for drug sensitivity estimations. Logarithmically-growing cells were exposed to a range of drug concentrations for 24 h with or without VPM addition. Following drug exposure L5178Y cells were cloned in soft agarose (0.17%) [4], whilst SK-OV-3 cells were cloned in soft agar (0.3%) [1]. Colony formation of each drug treated culture was expressed as a percentage of that in the corresponding control solvent-treated culture and survival curves were constructed.

Results

The addition of VPM at a non-toxic concentration (1 μ*M*) significantly enhanced ADR cytotoxicity in both sensitive (L5178Y-S) and resistant (L5178Y-ADR$_R$) cell lines. The enhancement factor (see Table 1) was significantly higher in the resistant subline, and under these conditions the combination of VPM and ADR effectively overcame ADR resistance.

Similar experiments were carried out using mitoxantrone or 4'ADR, which lack cross-resistance with ADR in our L5178Y-ADR$_R$ cells [6]. The addition of VPM (1 μ*M*) did not significantly affect the cytotoxicities of these drugs in either cell line, with enhancement factors ranging only from 1.1–1.3 (Table 1).

No significant enhancement of ADR cytotoxicity in either SK-OV-3 line was seen using the lower VPM concentration (1 μ*M*) (Fig. 1). However, with 6.6 μ*M*, VPM was slightly cytotoxic, reducing survival by 5%–10% but ADR cytotoxicity was potentiated. This occurred in both cell lines to a similar extent, as indicated by the enhancement

Table 1. Anthraquinone cytotoxicity and verapamil (VPM) interaction in murine L5178Y cell lines

Cell line	Drug	Cytotoxicity data[b] (ng per ml)		Enhancement factor[d]
		IC_{90}[c] drug alone	IC_{90}[c] drug plus 1 μ*M* VPM	
L5178Y	Adriamycin	9.5 (8.7–10.3)	4.8 (4.1–5.3)	2.0
L5178Y-ADR_R[a]	Adriamycin	35.6 (28.5–42.5)	8.0 (7.2–10.0)	4.5
L5178Y	Esorubicin	3.3 (2.9–3.7)	2.6 (2.5–2.9)	1.3
L5178Y-ADR_R	Esorubicin	3.8 (3.2–4.3)	3.4 (2.8–3.6)	1.1
L5178Y	Mitoxantrone	0.48 (0.44–0.60)	0.37 (0.32–0.53)	1.3
L5178Y-ADR_R	Mitoxantrone	0.29 (0.27–0.33)	0.24 (0.21–0.27)	1.2

[a] L5178Y-ADR_R expresses approximate 4-fold order of resistance comparing IC_{90} values for 24 h ADR exposure, from cytotoxicity data

[b] Cytotoxicity was assessed by clonogenic assay. CFE were 35%–80% for L5178Y cells and 50%–80% for L5178Y-ADR_R

[c] IC_{90} is the drug concentration required to reduce survival by 90% of control values. These values were derived from full dose–response curves and represent the mean (range) from either two or three repeat experiments

[d] Enhancement factor was calculated as the $\frac{IC_{90} \text{ value without verapamil}}{IC_{90} \text{ value with verapamil}}$

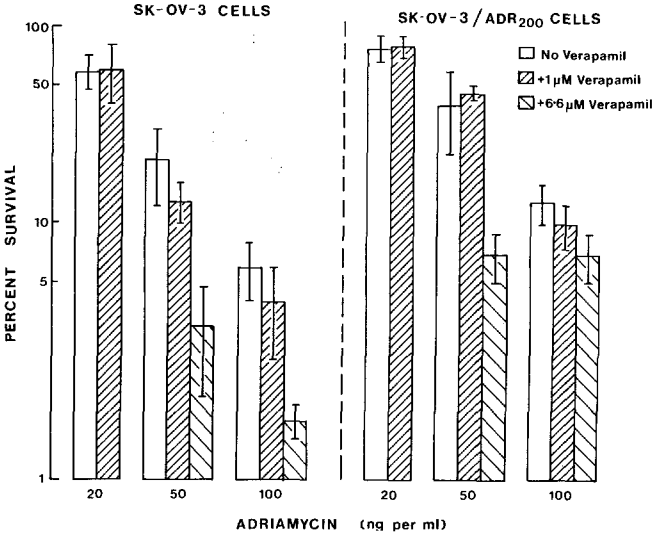

Fig. 1. The cytotoxic effects, judged by clonogenic assay, of a 24-h exposure to adriamycin (ADR) with or without verpamil on parental SK-OV-3 cells and an adriamycin-resistant subline. Each *point* represents the mean of at least three estimations and each *bar*, the SEM. CFE (Colony forming efficiency) of SK-OV-3 cells was 1.2%, and that of SK-OV-3-ADR_R cells, 5%–10%. Cells were maintained and cloned in Dulbecco's modification of Eagle's medium with 10% fetal calf serum. SK-OV-3-ADR_R cells were about 2-fold resistant, from the cytotoxic effects of 50 or 100 ng/ml ADR

factor of 2.5 obtained by comparing IC_{90} values ±6.6 μ*M* VPM. Therefore, there was no evidence in SK-OV-3-ADR_R cells of any selective reversal of resistance by VPM addition.

Discussion

With L5178Y-ADR_R the combination of 1 μ*M* VPM with ADR overcame resistance, a resistance apparently not associated with any reduced accumulation of drug. The enhancement factor of 2 observed in our L5178Y cell line is comparable to that reported with the P388 parental line, although our factor of 4.5 in the L5178Y-ADR_R subline is lower than that of 7–10 described in the P388-resistant cells. This difference may be related to the lower level of ADR resistance expressed in our L5178Y model system. Tsuruo et al. [12] linked the effectiveness of calcium channel blockers on increased cytotoxicity of antitumour agents with the extent of cross-resistance to ADR in the P388 leukaemia system. This may explain the absence of potentiation of ADR cytotoxicity in the SK-OV-3-ADR_R cell line, since the order of resistance expressed was lower. However, enhancement factors of approximately 6 in two human ovarian tumour cell lines exhibiting either a 6- or 150-fold order of ADR resistance have been reported [10]. In this study enhancement factors of 2.5 were only obtained in the SK-OV-3 lines with 6.6 μ*M* VPM. Maximum enhancement factors of 1.5–4.3 with OVCAR ADR-resistant lines, derived from tumours from previously treated patients, were obtained only when VPM concentrations of 3 μg/ml (6.6 μ*M*) were employed [10]. Recently, circumvention of ADR resistance in human glioma cell lines was reported using 13 μ*M* VPM [9]. Thus, it remains questionable whether potentiation of ADR cytotoxicity by VPM can be exploited clinically, since peak plasma concentrations achievable are quoted as 0.25–1 μg/ml or 0.6–2.2 μ*M* [10]. Another explanation proposed for this lower potentiation value in L5178Y-ADR_R cells and the lack of selective action in the SK-OV-3 subline may be related to the fact that drug uptake is not reduced in these lines. In contrast, in both murine and human tumours the promotion by calcium antagonists of anthracycline cytotoxicity has been linked to effects on drug accumulation [10, 11].

We have shown that both Novantrone and 4'ADR lack cross-resistance with ADR and their cytotoxicity is not significantly influenced by VPM addition. In contrast where cross-resistance to Novantrone in the P388 ADR-resistant cells was seen a 6-fold enhancement of cytotoxicity occurred with the addition of 3.5 μ*M* VPM [12]. Thus, VPM appears to be beneficial in overcoming high orders of drug resistance, but it remains to be proven whether this benefit can be established at levels of resistance most frequently encountered clinically. Furthermore, as in our SK-OV-3 ADR_R subline, where VPM addition did not fully restore ADR sensitivity, additional mechanisms for ADR resistance not associated with transport defects are obviously involved and remain to be elucidated.

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