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Verapamil sensitisation to alkaloids on colchicine-selected human colon adenocarcinoma cells

Multidrug resistance phenotype (MDR), the main cause of failure in cancer chemotherapy, is characterised by the extrusion of the antineoplastic drugs from the cells by means of the membrane bound P-glycoprotein (P-gly) or P-170, that acts as an extracting pump (2). Therefore, the main strategy to overcome the multidrug resistance associated with P-gly focuses on inhibiting the drug efflux caused by this protein. Verapamil reverses drug resistance by competitively inhibiting the binding of P-gly and the cytotoxic drugs, allowing for the drug to accumulate intracellularly in a dose dependent manner (5).

The aim of this study is to investigate if a multidrug resistant colon carcinoma cell line that has been selected with colchicine shows verapamil-increased sensitisation to several cytotoxic alkaloids with the same mechanism of action.

Drugs used were colchicine (CCH) (MERCK, Darmstadt), vincristine sulphate (VCR), vinblastine sulphate (VBL), (LILLY, Madrid) and verapamil (VRP) (KNOLL AG, Ludwigshafen). Human colon adenocarcinoma cells (HCA-2/1^{cch}), selected from the parental line (6) by continuous exposure to CCH (8), were grown in RPMI-1640 medium supplemented with hepes buffer 1 M (15 ml/l), sodium bicarbonate 7.5 % (28 ml/l), 10 % heat inactivated calf serum and 1 % antibiotic-antimycotic solution 100 X (PSF, Gibco); at 37 °C in 5 % CO_2/air atmosphere (9).

Clonogenic assays were used to study antineoplastic drug activity and verapamil effect on cellular survival (8). From an exponentially growing culture in drug free medium, 300 cells were seeded per Petri dish. The following day, cells were exposed to different drug doses for 1 hour and were then incubated for 7-10 days until colony formation. The effect of verapamil as a drug sensitiser was performed by the exposure of colon cells to different drugs in the presence of 10 µg/ml verapamil for 1 hour (1).

The Wilk-Shapiro rankit-plot test was used to assess the normal distribution of the data. Additional statistical analyses were made with the student's *t*-test. A significance level of 95 % ($p<0.05$) was adopted in all cases.

Table I shows the effect of verapamil on the sensitisation of the selected cell subline (HCA-2/1^{cch}) to different alkaloids. In all cases, the surviving fraction decreased when 10 µg/ml verapamil was added together with the drug. The highest sensitisation value obtained was 90.51 % for verapamil increased sensitivity to 0.5 µg/ml CCH, the drug present during the selection. Verapamil increased sensitivity to 8.0 µg/ml VBL by 60 % and by 63.58 % for 25.0 µg/ml VCR.

We have previously reported that this cell line expresses P-glycoprotein and shows cross-resistance to CCH, VBL and VCR (8). Verapamil (10 µg/ml) partially reversed the resistance to the assayed drugs. The present results are similar to findings (3) reporting that verapamil (1-5 µg/ml) produces increased sensitisation for VBL and CCH in the AuxB1 and F4-6RADR cell lines. While other authors (4) found no sensitisation for VBL with 1.5-10 µg/ml of verapamil in KB-C1 cells. PIRKER *et al.* (7) have demonstrated that 5 µg/ml verapamil is sufficient to completely reverse the resistance to CCH in these cells. Therefore, it appears that a verapamil dose between 5-50 µg/ml is sufficient to produce a significant sensitising

Table I. *Different sensitisation levels by verapamil on human colon adenocarcinoma cells (HCA-2/1^{cch}) exposed to different alkaloids.*

Drug	Dose (µg/ml)	Verapamil (µg/ml) 0	Verapamil (µg/ml) 10	% of sensitisation
Colchicine	0.1	0.990 ± 0.027	0.868 ± 0.068	12.32**
	0.5	0.959 ± 0.026	0.091 ± 0.035	90.51****
	1	0.622 ± 0.076	0.082 ± 0.033	86.82****
	2	0.340 ± 0.040	0.055 ± 0.028	83.82***
	3	0.059 ± 0.017	0.031 ± 0.026	47.46*
Vinblastine	3	0.545 ± 0.029	0.384 ± 0.026	29.54**
	5	0.189 ± 0.063	0.087 ± 0.026	53.97**
	8	0.015 ± 0.002	0.006 ± 0.002	60.00**
Vincristine	1	0.826 ± 0.091	0.644 ± 0.077	22.03*
	10	0.704 ± 0.053	0.452 ± 0.064	35.79****
	15	0.570 ± 0.035	0.281 ± 0.030	50.70***
	20	0.430 ± 0.028	0.181 ± 0.025	57.91***
	25	0.302 ± 0.035	0.110 ± 0.041	63.58*
	30	0.185 ± 0.031	0.075 ± 0.013	59.46*

Data represent mean surviving fraction ± SD of three independent experiments in triplicate. *p<0.05; **p<0.01; ***p<0.005; ****p<0.001; (Student's *t*-test).

effect for the majority of cell lines (11), however, an early study (10) in conflict with our findings reported that verapamil (10 µg/ml) did not sensitise the multidrug resistant EMT6/AR1.0 cell subline nor the EMT6/P parental cell line.

The data obtained in this study suggest that verapamil may sensitise these colon adenocarcinoma cells to drugs with the same mechanisms of action as that of the drug presence during selection, although with different efficacy.

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M. J. Ruiz Gómez, A. Souviron, L. Gil and M. Martínez Morillo

Departamento de Radiología y Medicina Física, Facultad de Medicina, Universidad de Málaga, Teatinos s/n, 29071 Málaga (Spain)

Correspondence to M. Martínez Morillo (Tel.: +34 952 131 631; Fax: +34 952 131630; e-mail: mmorillo@uma.es).