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Verapamil Upregulates Sensitivity of Human Colon and Breast Cancer Cells to LAK-cytotoxicity *in vitro*

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Pretreatment of human colon cancer LoVo-H cells and human breast cancer ZR-75 1A cells with low doses of verapamil, a Ca^{2+} channel blocker, for 48 h has a slight growth stimulatory effect and substantially increases cell sensitivity to lymphokine-activated killer (LAK) mediated cytotoxicity in the standard ^{51}Cr release assay. The role of intracellular Ca^{2+} levels in determining verapamil effect is demonstrated by cytochemical evidence of intracellular Ca^{2+} lowering in verapamil-treated cells and by the reversal by the Ca^{2+} ionophore A-23187 of verapamil-induced sensitivity to LAK-mediated cytotoxicity.
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INTRODUCTION

HUMAN TUMOUR CELLS are heterogeneous in their sensitivity to natural killer (NK) and lymphokine activated killer (LAK) cytotoxicity *in vitro*. Despite the initial promising results, interleukin 2 (IL-2) with LAK as adoptive immunotherapy is ineffective in a substantial proportion of patients with advanced cancer [1]. Therefore, attempts to upregulate the intrinsic tumour cell sensitivity to lytic effectors is of clinical interest.

A major obstacle to the development of such therapeutic strategies is that the molecular mechanisms of cancer cell killing by cytotoxic cells have not been completely elucidated. While a common mechanism has been proposed for cell-mediated killing, tumour cell determinants which regulate sensitivity or resistance to NK or LAK remain mostly unknown [2].

It is conceivable that physical properties of tumour cell membrane might affect tumour cell sensitivity to NK or LAK effectors. Notably, sensitivity of hepatocarcinoma cells to NK appears to correlate with membrane potential of tumour cells [3].

Verapamil is a Ca^{2+} channel blocker which can induce changes in membrane cell polarity [4]. We have studied the effect of verapamil on the sensitivity of human cancer cells to LAK-mediated cytotoxicity on two human tumour cell-lines. The results of our study show that 48 h treatment with 100 nmol/l verapamil induces a drastic increase in sensitivity of human colon and breast cancer cell-lines to LAK-mediated cytotoxicity.

MATERIALS AND METHODS

Cell culture

LoVo-H cells, kindly provided by G. Parmiani, (Istituto Nazionale Tumori, Milan, Italy) and ZR-751A cells, kindly provided by S. Iacobelli (Università di Chieti, Chieti, Italy) were respectively grown in Ham's F-12 and Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/l l-glutamine, 10 UI/ml insulin, 20 mmol/l Hepes and antibiotics (Flow).

Cell growth was evaluated with haemocytometric cell count and cell viability was assessed by the trypan blue exclusion assay.

LAK generation from human PBL

Peripheral blood lymphocytes (PBL) after Ficoll (Flow) separation, were incubated in RPMI 1640 (Flow) medium supplemented with 10% FBS. 1000 U/ml IL-2 (Cetus, Emeryville, U.S.A.) was subsequently added to the medium, and PBL were incubated at 37°C for 18 h. IL-2 treatment of human PBL under these conditions induces LAK activity, mostly due to activated NK cells [5].

LAK-cytotoxicity determined by ^{51}Cr release assay

ZR-751A and LoVo-H cells were seeded in 24-well dishes and exposed for 48 h to 100 nmol/l VP. Incubation with ^{51}Cr (Amersham) was performed for 30 min and, after washing tumour target cells with phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}, LAK cells were added at different effector/target ratios. Tumour cells and lymphocytes were incubated for 4 h and specific ^{51}Cr release (SR) was calculated as follows: SR = (cpm EP - cpm RS) × 100 ÷ cpm TC - cpm RS, where: EP = experimental point, RS = spontaneous release (from well in the absence of effectors), and TC = total count from the well.

RESULTS

Pretreatment with verapamil

Verapamil at 100 nmol/l induced a proliferative stimulus on LoVo-H and ZR-75 1A cells, the effect being more evident in ZR-75 1A cells. Figure 1 shows the effects of verapamil on tumour cell growth 48 hours after the beginning of treatment.

Increased sensitivity to LAK-mediated cytoxicity

Untreated LoVo-H and ZR-75 1A cells were almost completely resistant to LAK-mediated cytotoxicity, while cells treated for 48 h with verapamil became sensitive to LAK cells, as assessed in the ^{51}Cr release assay (Fig. 2).

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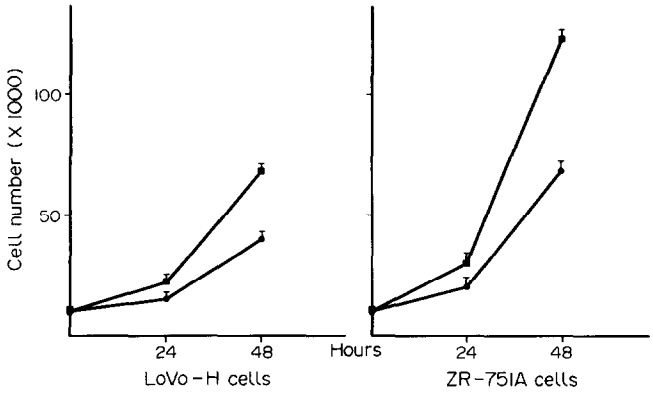

Fig. 1. Effects of 100 nmol/l verapamil on LoVo-H and ZR-75 1A cell growth. • = control and ■ = verapamil-treated cells.

In addition, a substantial LAK cytotoxicity was also observed on verapamil-treated cells also at low effector/target ratios.

Effect of verapamil on LAK-sensitivity of tumour cells

Verapamil which blocks the Ca^{2+} channel, induced a substantial decrease in intracellular Ca^{2+} levels, in both LoVo-Dx and ZR-75 1A cells, whereas the Ca^{2+} ionophore A-23187 increased intracellular Ca^{2+} levels in verapamil-treated cells (data not shown). We therefore evaluated verapamil antagonisation effect of the Ca^{2+} ionophore A-23187 on tumour cell sensitivity to LAK-cytotoxicity. In fact, pretreatment with low doses of A-23187 completely abrogated the promoting effects of verapamil on LAK-sensitivity of both cell-lines (Fig. 3). Interestingly, the ionophore alone had no univocal effects on the two cell-lines: it appeared, in fact, to have no effect on LAK-sensitivity of LoVo-H and to reduce LAK-sensitivity of ZR-75 1A. We did not find

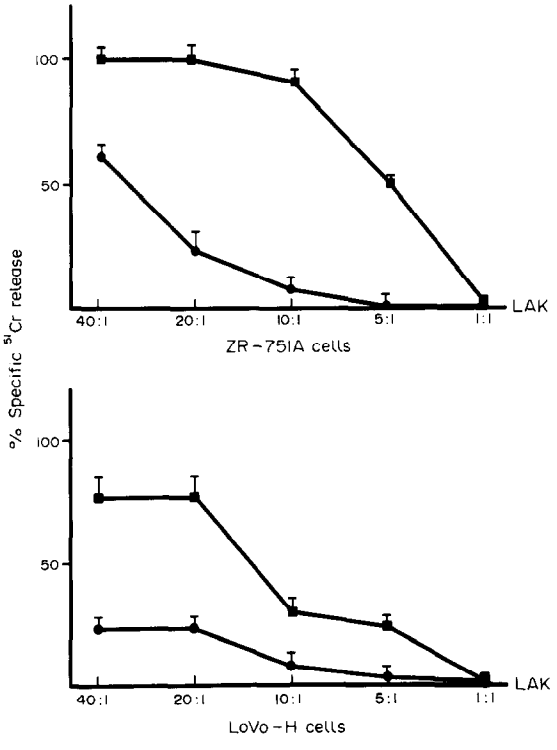

Fig. 2. Effect of 48 h treatment with 100 nmol/l verapamil on LoVo-H and ZR-75 1A sensitivity to LAK-cytotoxicity, evaluated at different effector/target ratios. • = control and ■ = verapamil-treated cells.

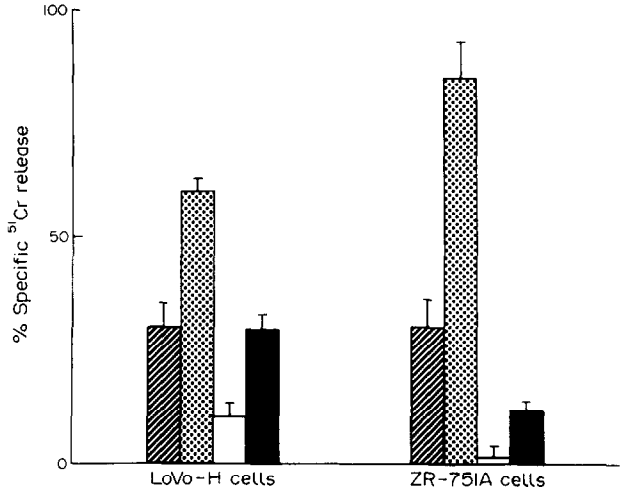

Fig. 3. A-23187, a calcium ionophore, antagonises the effect of verapamil on the sensitivity of LoVo-H and ZR-75 1A cells to LAK-mediated cytotoxicity. ▨ = Control, ▩ = verapamil, □ = verapamil + A-23187 cells, ■ = A-23187 cells alone.

a direct correlation between the effect of verapamil and A-23187 on LAK-sensitivity and tumour cell growth modulation: the ionophore alone induced growth stimulation on both cell-lines, while antagonising verapamil-induced growth stimulation on LoVo-H cells but not on ZR-75 1A cells (Fig. 4).

DISCUSSION

As cancer cells are heterogeneous in their sensitivity to LAK-mediated cytotoxicity, the identification of agents which could upregulate the intrinsic responsiveness of tumour cell to cytotoxic effectors would be of major clinical benefit. In this report we show that LAK-sensitivity of two human cancer cell-lines can be greatly enhanced by 48 h treatment with 100 nmol/l of verapamil.

It has been widely reported that verapamil can abrogate the multidrug-resistant phenotype (MDR) of human cancer cells which express the 170 kD membrane protein product of *mdr*-1 gene [6]. Clinical investigations are in progress to determine a possible effect of verapamil in the treatment of patients with drug resistant tumours [7]. While the reversal of MDR phenotype of cancer cells is produced at VP concentrations which might be

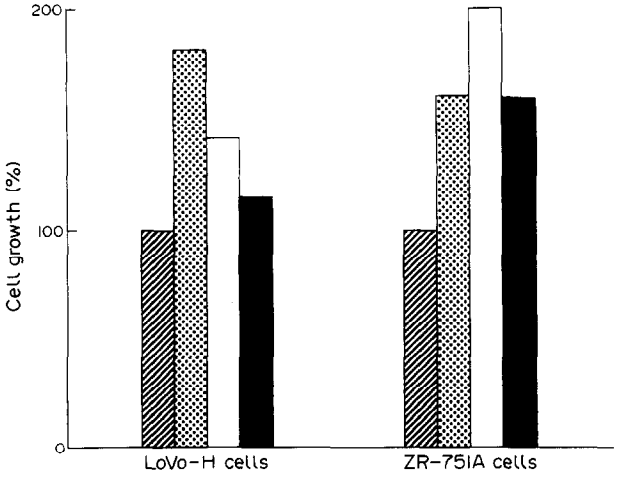

Fig. 4. Effects of A-23187 on verapamil-induced growth stimulus of LoVo-H and ZR-75 1A cells.

toxic *in vivo*, the effect on LAK-sensitivity reported here is achieved at lower doses of VP, which might be much less toxic *in vivo*.

Our preliminary observation that the Ca^{2+} ionofore A-23187 can reverse verapamil's effect on LAK-sensitivity of both cancer cell-lines suggests involvement of changes in intracellular Ca^{2+} levels in the determination of these effects. We are presently trying to identify the molecular mechanisms of verapamil's effects on tumour cell sensitivity to LAK cells.

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Quantitative and Qualitative Cosmetic Evaluation after Conservative Treatment for Breast Cancer

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148 consecutive patients treated by two different types of conservative surgery were objectively and subjectively evaluated for cosmetic outcome. In 73 patients, tumorectomy, axillary dissection, external radiotherapy (45 Gy) plus iridium implant (15 Gy) were performed, while in the other group of 73 patients a more extensive surgical approach was carried out: quadrantectomy, axillary dissection plus external radiotherapy (50 + 10 Gy). The appearance of the patients' breasts was analysed for symmetry by computer, and differences in symmetry were correlated with tumour location and breast size. A subjective assessment was given by a 3-member panel and the results were correlated with objective measurements. In addition, patients were asked to fill out a self-assessment questionnaire on the aesthetic result of the operated breast. Better results were generally noted in the group of patients treated by more conservative surgery. Substantial differences in the aesthetic outcome were noted between the patient's own evaluation, the computer's measurement of symmetry and the assessment of the panel of observers.
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INTRODUCTION

LOCAL CONTROL of disease is the primary objective of conservative treatment of breast cancer. In addition improvement of the patient's quality of life by achievement of an acceptable cosmetic outcome is the basis of the philosophy of breast preservation. In fact, unsatisfactory results can contribute to psychological morbidity owing to body image alteration and its effects on social functioning. The most important clinical trials [1–4] comparing mastectomy to conservative therapy have shown no differences in local recurrence rates and distant disease-free and overall survival.

So far, studies on cosmetic outcome have been small or overly subjective [6, 7]. Deformities produced by conservative surgery (quadrantectomy, lumpectomy or wide excision) are difficult to evaluate objectively and subjective assessment of cosmetic outcomes has been extremely variable [8, 9]. Asymmetry of the treated breast may occur as a result of deformities such as malposition or distortion of the nipple–areola complex, tissue deficiency, breast retraction and shrinkage. Measurement of such a symmetry may be a more objective method of evaluating the deformities and, indirectly, the cosmetic results after conservative treatment for breast cancer. [9, 10].

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