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Short report

Effects of lovastatin on a human myeloma cell line: increased sensitivity of a multidrug-resistant subline that expresses the 170 kDa P-glycoprotein

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Using a fluorometric microculture cytotoxicity assay for measuring cell viability and proliferation we examine the cytotoxic effect of lovastatin on a drug sensitive myeloma cell line (RPMI 8226) and a multidrug resistant (MDR) clone (8226/Dox40), that was approximately 100-fold less sensitive to doxorubicin. The RPMI 8226 cells were sensitive to lovastatin with an IC_{50} of 15.8 μg/ml. However, the MDR subline exhibited a collateral sensitivity to lovastatin, with an IC_{50} of 1.7 μM, thus having a 9.3-fold greater sensitivity to lovastatin than the parental cell line. The combination of doxorubicin and lovastatin did not show any synergistic or antagonistic effects on any of the cell lines. The increased sensitivity to lovastatin of the P-gp 170-expressing MDR cells 8226/Dox40 might be part of a more general phenomenon that merits further investigation.

Key words: FMCA, lovastatin, MDR, P-glycoprotein.

Introduction

An interesting feature of cancer cells is their increased demand for cholesterol compared with untransformed cells. This has led to the suggestion that lowering the cholesterol serum concentration in cancer patients might be beneficial.[1]

Endogenous cholesterol synthesis is increased in many tumor cells and these cells have in some cases lost their ability to inhibit their own synthesis of cholesterol by negative feedback.[2,3] Several investigators have shown that, using HMG-CoA reductase inhibitors such as lovastatin, cancer growth can be restricted *in vitro* and *in vivo*.[4,5] Anticancer activity has indeed been documented in a few patients in a recent phase I trial at the National Cancer Institute (Bethesda, MD).[6]

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Can lovastatin also be used as an adjunct to chemotherapy? Many chemotherapeutic agents act on cells in the replicative phase, and since inhibition of cholesterol arrest the cell cycle, the combination of a cholesterol synthesis inhibitor and a cytostatic might be counterproductive. However, studies on the use of lovastatin with mitomycin C have unexpectedly shown an additive effect of these drugs.[7]

Using a sensitive semiautomated assay for measuring cell viability and proliferation we examine the cytotoxic effect of lovastatin on a drug sensitive and a multidrug resistant (MDR) cell line, and also study the interactions of lovastatin with the widely used cytostatic agent doxorubicin.

Materials and methods

Cell lines

The myeloma cell line RPMI 8226 and a subline selected for doxorubicin resistance, 8226/Dox40, were kindly provided by Dr WS Dalton (Tucson AZ). The 8226/Dox40 subline has been shown to be MDR and to overexpress P-gp 170.[8] Cell lines were grown in RPMI 1640 supplemented with 10% FCS, glutamine, streptomycin and penicillin.

Drugs and reagents

Fluorescein diacetate (FDA; Sigma, St Louis, MO) was dissolved in dimethyl sulfoxide (DMSO) (Sigma). Doxorubicin (Adriamycin, Farmitalia) was purchased from commercially available sources and lovastatin was kindly provided by MSD (Rathway, NJ). Lovastatin was dissolved in DMSO in a stock solution of 5 mg/ml concentration and kept at

−20°C. Doxorubicin and vincristine were dissolved and diluted as described earlier. Microtiter plates were made, containing 5-fold dilutions of the drugs tested, wells with no drugs and blank wells, where no cells were plated. All platings were made with a Propette (Cetus, CA). Microtiter plates with drugs were prefabricated and kept at −70°C.

In vitro sensitivity testing

The fluorometric microculture cytotoxicity assay (FMCA) was performed essentially as described earlier.[9] Briefly, 20 000 cells from cell cultures were incubated in triplicate in 96-well microtiter plates (Nunc, Roskilde, Denmark) for 72 h under standard culture conditions, with or without drugs. After culture, the medium was removed and the cells washed once in PBS; 100 µl of FDA in PBS was added and the cells incubated for 45 min at 37°C, before fluorescence was read in a Fluoroscan II (Flow). Results for the cell lines are presented as the mean ± SE of 12 experiments. Data are shown as survival index (SI%), defined as the fluorescence of experimental as percent of control wells, with blank values subtracted.

Results

Lovastatin effects on RPMI 8226/s and Dox40

The susceptibility of RPMI 8226/s and Dox40 cells to lovastatin and doxorubicin was determined using FMCA. The 8226/Dox40 cells were approximately 100-fold less sensitive to doxorubicin (Figure 1A), in accordance with earlier reports.[8] The RPMI 8226/s cells were sensitive to lovastatin with an IC_{50} of 15.8 µg/ml. However, the MDR subline 8226/Dox40 was more sensitive to lovastatin, with an IC_{50} of 1.7 µM (Figure 1B). The sensitivity of the P-gp 170-expressing 8226/Dox40 cells was thus 9.3-fold higher than the doxorubicin-sensitive parental cell line and also higher than the lovastatin sensitivity reported for several other cell lines.[10]

Lovastatin effects on sensitivity to doxorubicin

To establish if any interactions could be observed between doxorubicin and lovastatin, a fixed low concentration of lovastatin was added to all wells

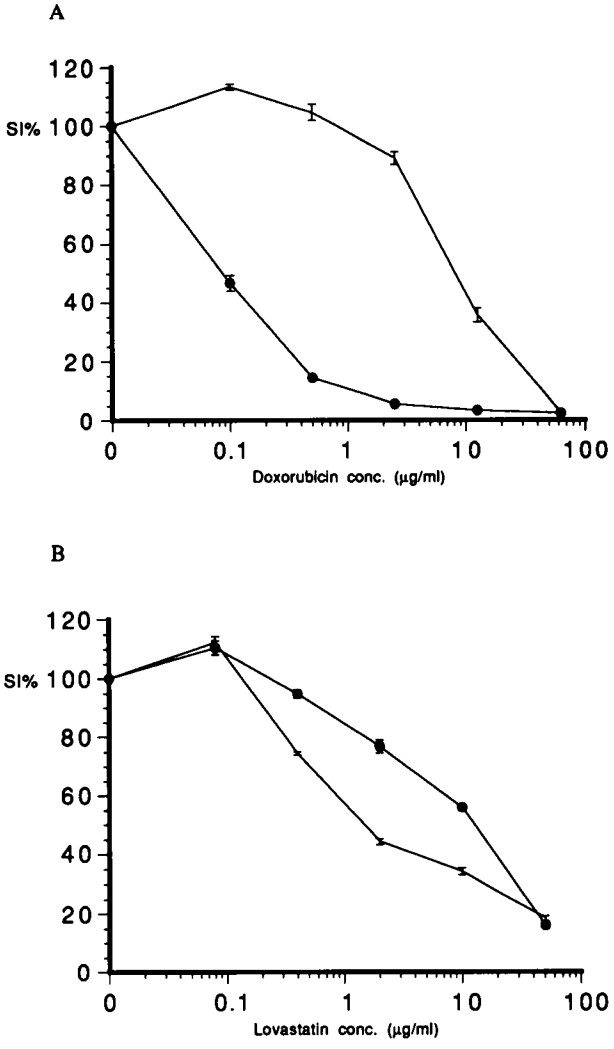

Figure 1. (A) The effects of doxorubicin on the RPMI 8226 myeloma cell line and on a MDR subline (8226/Dox 40), selected for doxorubicin resistance. The results are expressed as SI% and presented as means values ± SE of 12 experiments. (B) The effects of lovastatin on the RPMI 8226 myeloma cell line and on the subline 8226/Dox 40. ●, RPMI 8226/s; –, 8226/Dox 40.

in one set of experiments. A concentration of 0.2 µg/ml was chosen, since it had no effect in itself on the cells. The dose–response curve showed no difference between cells with doxorubicin and lovastatin and doxorubicin alone (Table 1).

Discussion

We have studied the effect of lovastatin on the myeloma cell line RPMI 8226 and a MDR resistant clone 8226/Dox40, expressing P-gp 170. It is clearly demonstrated that the MDR cells are

Table 1. Effect of 0.2 μg/ml lovastatin on doxorubicin (Dox) sensitivity

Cell line	IC_{50}	
	Dox	Dox + lovastatin
RPMI 8226	0.10	0.09
RPMI 8226/Dox 40	9.8	10.2

collaterally sensitive to lovastatin with an approximately 10-fold lower IC_{50} compared with the drug sensitive cells lacking P-gp 170. The phenomenon of collateral sensitivity has earlier been described for several agents, including the resistance-reversal agents verapamil and cyclosporin A, and corticosteroid hormones.[8] The mechanism for this phenomenon is not known. However, it is unlikely that it is mediated by P-gp 170, since lovastatin does not seem to be a substrate of this transporter (e.g. does not increase the effect of doxorubicin on the P-gp 170-expressing cells).

An alternative explanation might be that the MDR cells in parallel to P-gp 170 expression have an increased demand for products of the mevalonate pathway and thus are more sensitive to inhibition of the rate limiting enzyme HMG-CoA reductase. What would be the connection between MDR and cholesterol synthesis? One possible explanation is that altered membrane properties due to increased cholesterol synthesis could decrease drug sensitivity, another that increased production of isoprene intermediates might enhance exocytosis, since several proteins in the excretory pathway are prenylated.[11] Indeed, many MDR cells have been reported to possess an increased capacity for intracellular vesicular trapping of drugs, in addition to overexpression of P-gp 170.[12,13] Further studies on different MDR cells are needed to determine the generality of the discovered collateral sensitivity to lovastatin and to elucidate the underlying mechanisms for this effect.

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