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EFFECT OF DRUG-RESISTANCE REVERSORS ON EXPRESSIONS OF ONCOGENES OR TUMOR SUPPRESSOR ONCOGENES OF HUMAN TUMOR CELL LINES

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MIT method was applied to assay the cytotoxicity of three reversors, verapamil (VER), dipyridamole (DPM) and cyclosporin (CSA) in K562, K562/ADM and KB cell lines. S-P immunocytochemical technique was applied to detect expressions of oncoproteins or tumor suppressor oncoproteins in these tumor cell lines before or after treatment with these reversors. Results showed that three reversors were capable of inhibiting to a certain extent growth of K562 or KB cell lines and reversing greatly adriamycin (ADM)-resistance in K562/ADM cell line. DPM and CsA were observed to inhibit, partly or wholly, expressions of p53, p16, bcl-2, p21 or cerbB-2 oncoproteins. VER showed whole inhibition of expressions of p53, p16, p21 and bcl-2 and partly expression of p53 oncoprotein in K562 cell line. These results suggest that growth inhibition in K562 or KB cell lines by the reversors may be associated with partial or whole inhibition of expressions of p53, p16, p21 or c-erbB-2 oncoproteins. Inhibitions of expressions p53, p16, p21 oncoproteins by VER but not DPM or CsA, may be respossible for reversing activity of VER for ADM-resistance in K562/ADM cell line.

Key words: Neoplasm, Oncoproteins, Drug resistance, Experimental therapy

Failure of chemotherapy results mainly from presence of anticancer-drug resistance of tumor. In the past more than twenty years, tens kinds of reversors or modulators against drug resistance of tumor cell, have been discovered. These reversors are believed to overcome drug resistance of tumor cells through competitive combination with anticancer drugs for p-glycpprotein and glutamine-S transferase (such as buthionine sulfoximine, BSO) or modulation of DNA repair (Such as streptozocin). Oncogenes or tumor suppresser oncogenes were been recently reported to play a great role in induction of drug resistance of tumor cells. Drug resistance was acquired or chemosensitivity was elevated in tumor cells by transfection of oncogenes, such as c-myc, bcl-2 or H-ras into these cells.[1-3] Transfection of wild-type p53 gene elevated the chemosensitivity of human lung cancer cells.[4] However, little has been known and few have been reported at home and abroad about effect of the reversors on expressions of oncogenes or tumor suppressor oncogenes. We report here the effects of three kinds of reversors, VER, dipyridamole (DPM) and CsA on expressions of seven kinds of oncogenes or tumor suppressor oncogenes in K562, K562/ADM and KB cell lines and their relation to reversal efficacy against drug resistance of tumor cells.

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MATERIALS AND METHODS

Materials

Cell lines. K562 cell line was obtained from Shanghai Institute for Cell Biology. K562/ADM cell subline resistant to adriamycin (ADM), derived from K562 cell line, was previously established by us.[5] KB cell line was obtained from Department of Clinical Pharmacology, Affiliated hospital, Chinese Military

Academy of Medical Science. These cell lines grew in medium RPMI 1640, supplemented with 15% fetal calf serum, penicillin (100μ. ml^{-1}) and Streptomycin (100μ. ml^{-1}) 3.8 $\mu g.ml^{-1}$ ADM (resistant dosage) was present in medium for K562/ADM cell line. All cell lines were incubated at 37 ℃ in an atmosphere of 5% CO_2.

Agents. VER, CsA and DPM were purchased from Knoll AG Co. (Germany), Sandoz Pharma Ltd. (Switzerland) and Shanghai Tianfeng Phormaceutical Factory respectively. Monoclonal antibodies for p53 (pAb1801), p21 (NCC-ras001), bcl-2(124), c-myc (9E10) and polyclonal antibodies for p16, C-fos, c-erbB-2, and other agents for immunocytochemistry (ICC) were products of Zymed Co. USA, and purchased from Fuzhou Maxim Biotech Inc.

METHODS

MTT method.[6] For assay of cytotoxicity of the reversors, three kinds of cells in exponential growth cycle were collected and inoculated into 96 well-culture dish, each well containing 2×10^4 cells. The cells in wells were treated with a serial concentration of VER or DPM, or CsA, with triplicate wells for each concentration. Control wells didn't contain reversors or did cell-free medium. Culture dish loaded cells were incubated for 72 hrs at 37 ℃ in an atmosphere of 5% CO_2 . And then added 20 μl MTT (15 $mg.ml^{-1}$) to wells and dish incubated for 6 hrs.

Media in wells were removed before addition of 200 μl DMSD, and absorbance for 570 nm absorptive wavelength was measured in MR 500 Microplate Reader. Experiment was triplecated and ID_{50} for each reversor each cell line was calculated.

S-p ICC was carried out as previously described[7] with some modifications. Cells in exponential growth cycle were exposed to ID_{50} of VER. DPM of CsA. and incubated for 24 hrs at 37 ℃ and then were loaded on slides in Cytospinner, purchased from Instrument Factory, Chinese Military Academy of Medical Institute, with about 1000 cells per slide after centrifugation at 1000 rpm for 2 minutes, and slides were fixed in cool acetone for 10 minutes. The following procedure was carried out as previously described.[7] Positive immunostaining standard was regarded as follows: positive immunostaining compound localized in nucleus or cytoplasm or on membrane of cell and was strong brown but not for background. Immunostaining intensity was defined as follows: +, less than 20% numbers of positive cells; ++ 20 — 60%, and +++, more than 60%.

RESULTS

Growth Inhibition of K562, K562/ADM and KB cell line by Reversors against Drug Resistance

Results from MTT assay showed that VER, DPM and CsA were capable of inhibiting to various extent growth of K562, K562/ADM and KB cell lines in a concentration-dependence fashion (Figure 1-3). For K562, K562/ADM and KB cell lines, ID_{50} of VER were 19.6 ± 2.1, 0.8 ± 0.06 and 15.3 ± 1.6, respectively; DPM, 398.2 ± 39.1, 7.3 ± 1.6 and 239 ± 22.3, respectively; CsA, 7 ± 1.2, 3.9 ± 0.5 and 5.3 ± 0.7, respectively. Sensitivity of growth inhibition of K562/ADM cell line to VER, DPM and CsA were 23-, 52.5- and 1.8- fold respectively over that of K562 cell line.

Expressions of Seven Kinds of Oncogenes Or Tumor Suppresser Oncogenes in K562, K562/ADM and KB Cell Lines

Expression of c-myc oncoprotein was not present in three kinds of cell lines, and cerbB-2, not in K562 and K562/ADM cell lines, and bcl-2, not in KB cell line, and c-fos, not in K562/ADM cell line. p21, p53, p16 expressed to various extent in three kinds of cell lines (Table 1).

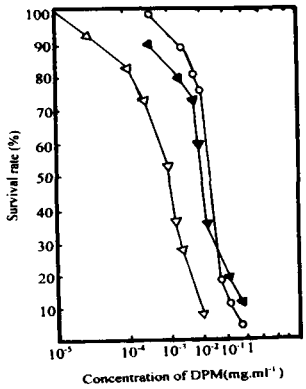

Fig 1. Growth inhibition of tumor cell lines by VER K562/ADM (△), K562 (▲), KB(○).

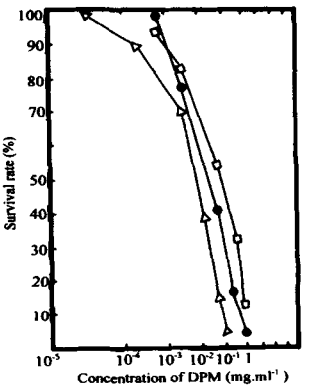

Fig 2. Growth inhibition of tumor cell lines by DPM K562/ADM (△), K562 (□), KB (●)

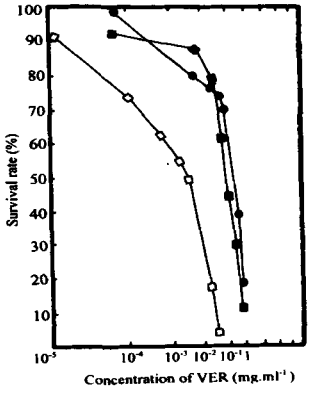

Fig 3. Growth inhibition of tumor cell lines by CsA K562/ADM (□), K562 (●), KB (■).

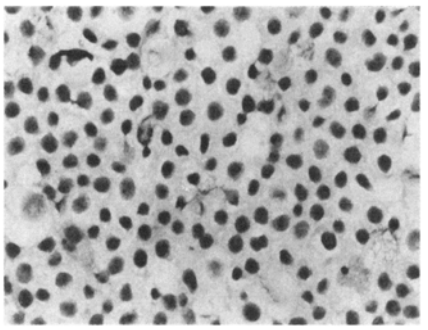

Fig 4. p53 was positive in K562 cell line, S-P ICC, × 400

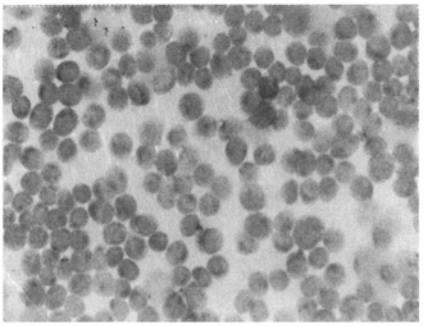

Fig 5. p16 was positive in KB cell line. S-P ICC, × 400.

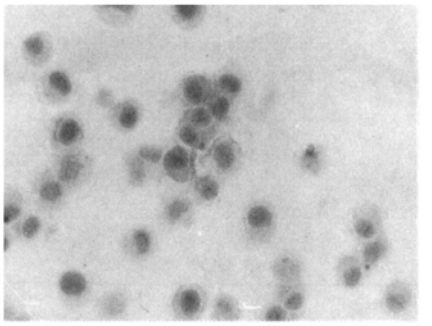

Fig 6. bcl-2 was positive in k562 cell line, S-P ICC, × 400

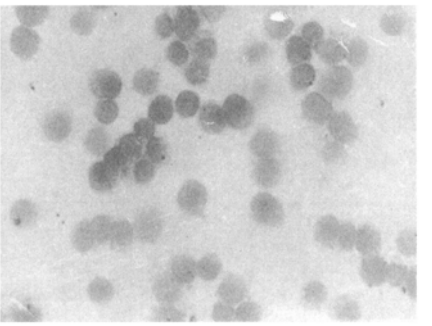

Fig 7. c-erbB-2 was positive in KB cell line, S-P ICC, × 400

Effect of Reversors against Drug Resistance on Expressions of Oncoproteins or Tumor Suppresser Oncoproteins in K562, K562/ADM, KB Call Lines

Expression of p53 was elevated and bcl-2 was induced after K562 cells was exposed to ID_{50} of VER for 24 hrs. Expressions of p53, p16, p21 and c-fos

disappeared after K562 cells were treated with ID_{50} DPM for 24 hrs. Expressions of p53, p16, p21, bc1-2 and c-fos disappeared after K562 cells were treated with ID_{50} of CsA for 24 hrs (Table 1).

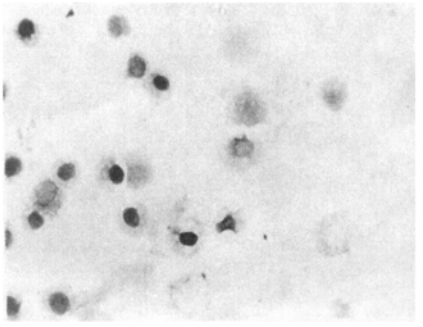

Fig 8. c-fos was strongy positive in k562 cell line exposed to VER, S-P ICC, × 400.

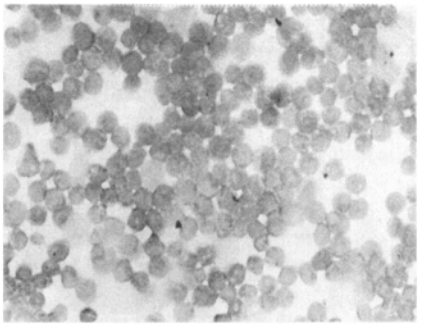

Fig 9. p21 was positive in KB cell line, S-P ICC, × 400

Table 1. Effects of resistance-reversors on expressions of oncoproteins and tumor suppressor oncoprotein

Cell lines + reversor (ID_{50})	p53	p16	c-myc	bc1-2	p21	c-erbB-2	c-fos
K562+VER	++	++	-	-	+	-	++
K562+DPM	-	-	-	++	-	-	-
K562+CsA	-	-	-	-	-	-	-
K562	+++	++	-	+	+	-	++
K56/ADM+VER	-	-	-	-	-	-	-
K56/ADM+DPM	-	-	-	-	-	-	-
K56/ADM+CsA	-	-	-	-	++	-	-
K56/ADM	++	++	-	+	++	-	-
KB + VER	-	+	-	+	+	+	-
KB + DPM	+	+	-	-	-	+	-
KB + CsA	+	+++	-	+	+	+	+
KB	+	+++	-	-	+++	++	+

Expression of p53, p16, bc1-2 and p21 disappeared in K562/ADM cells after the cells were exposed to ID_{50} of CsA-24-hour-exposure on p21 expression in K562/ADM cells was observed. Effect of CsA on expressions of other kinds of oncoproteins or tumor suppresser oncoproteins in K562/ADM cells was the same as VER or DPM (Table 1).

After KB cells were exposed to ID_{50} of VER for 24 hrs, expressions of p16, p21 and cerbB-2 decreased significantly and of p53, c-fos, disappeared, and of bc1-2, was induced. After KB cells were exposed to ID_{50} of DPM for 24 hrs, expressions of p16, cerbB-2 decreased greatly, and of p21, c-fos disappeared. After KB cells were exposed to ID_{50} of CsA for 24 hrs. expression of bcl-2 was induced and of c-erbB-2. decreased obviously (Table 1).

DISCUSSION

Our results showed that VER, DPM and CsA all were capable of inhibiting to various extent growth of K562 and KB cell lines. For growth inhibition, CsA was the strongest, and VER, second-best, and CsA, the weakest.

For study of expression of gene, ICC, an

weakest.

For study of expression of gene, ICC, an important method, can be used to detect directly final products of gene expression and their location in cell and show population and proportion of expressive cells, though it cannot be used to detect directly genes, but PCR, Southern blot, Northern blot or *in situs* hybridization. Our findings using ICC suggested that significant relationship didn't existed between growth inhibition of K562 cell line by VER and expressions of seven kinds of oncoproteins or tumor suppresser oncoproteins, c-myc, bcl-2, p21, c-erbB-2 c-fos, p53 and p16, but between that of KB, for part or whole inhibition of expressions of p53, p16, p21, cebB-2, c-fos. Growth inhibition may be associated with part or whole inhibition of expression of p53, p16, p21, c-803, and that of KB cell line, for p16, p21, c-erbB-2, c-fos. Growth inhibition of K562 cell line by CsA may be associated with whole inhibition of expressions of p53, p16, bcl-2, p21, c-fos, and that of KB cell line, for part inhibition of expressions of p21 and c-erbB-2.

VER was observed to inhibit wholly expression of p53, p16, p231 and bcl-2, partly weakened expression of p53 but not for p21 in K562 cell line. These findings suggested that mechanism of VER reversal against ADM resistance of k562/ADM cell line may be involved in inhibition of expressions of p53, p16, p21 by VER. No relationship was observed between expressions of the seven kinds of oncoproteins or tumor suppresser oncoproteins and reversing effect of DPM or CsA against ADM resistance of K562/ADM cell line.

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