REGULATION OF EXPRESSION OF P-GLYCOPROTEIN AND GST BY MULTIDRUG-RESISTANT REVERSORS IN ADM-SENSITIVE AND ADM-RESISTANT HUMAN TUMOR CELL LINES

XIE Zuofu 谢佐福, LIN Xiandong 林贤东, ZHOU Dongmei 周冬梅, LIN Sheng 林 声

Department of Immunopathology, Fujian Provincial Tumor Hospital, Fuzhou 350014, China

ABSTRACT

Objective: To investigate the regulation of p-glycoprotein (PgP) and GST expression from three reversors in ADM-sensitive and ADM-resistant human leukemic cell lines and KB cell lines. Methods: Immunocytochemical(ICC) technique was applied to detect the multidrug-resistant gene products, PgP and GST in K562 cells, K562/ADM cells and KB cells before or after treatment with three resistant reversors, i.e., verapamil(VER), dipyriamole(DPM) and cyclosporin A(CsA). Results: PgP expression was observed in K562/ADM cells but not in K562 cells or KB cells, and GSTPI expression, in KB cells but not in K562 cells or K562/ADM cells. Overexpressions of PgP were induced after treatment with VER, or DPM or CsA for 24 h in K562 cells but not KB cells. DPM-treated K562/ADM cells expressed PgP much lower than DPM-free K562/ADM cells. PgP was not detectable after treatment of K562/ADM cells with CsA for 24 h. Induced GSTPI expression was found after treatment with DPM, but not VER or CsA in K562 cells. No significant difference was observed for GSTPI expresion in KB cells before and after treatment with VER, or DPM, or CsA. Conclusion: The findings suggested that reversal activity of some drug resistant reversors, such as VER, DPM, CsA, may be declined by themselves through induction of PgP, perhaps GST.

Key words: Multidrug resistance, resistant reversors, P-glycoprotein, GST, Tumor cells

Three multidrug resisors, verapamil (VER), dipyriamole (DPM), and cyclosporin a (CsA), have been found to overcome multidrug resistance in various tumor cells mainly through competive combination with anticancer drugs for P-glycoprotein (PgP) from ours and other investigators findings and.[1-3] We reported previously the regulative effect of these reversors on seven kinds of oncoproteins or tumor suppressor oncoproteins expressions.[3] We report here the regulation of PgP and GST expression from these reversors in ADM-sensitive and ADM-resistant human leukemic cell lines and KB cell lines.

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Correspondence to: XIE Zuofu, Department of Immunopathology, Fujian Tumor Hospital, Fu-Ma Road, Fuzhou 350014, China; Phone: (0086- 0591)-3660063 Ext 8517.

MATERIALS AND METHODS

Cell Lines

K562 cell line was obtained from Shanghai Institute for Cell Biology. K562/ADM cell line was established previously by us.[4] KB cell line was purchased from Department of Clinical Pharmacology, Affiliated Hospital, Chinese Military Academy of Medical Science.

Agents

VER, CsA and DPM were purchased from Knoll AG Co. (Germany), Sandoz Pharma Ltd (Switzerland) and Shanghai Tianfeng Pharmaceutical Factory respectively. Monoclonal antibodies for P-glycoprotein(JSB-1), GSTPI(353-10) and other immunocytochemical agents, products of Zymed Co. (USA), were purchased from Fuzhou Maxim Biotech Inc.

S-P Immunocytochemistry (S-P ICC)

ICC was carried out as previously described.[3] Positive immunostaining compound located on membrane of cell(PgP), or cytoplasm(GSTPI) was strong brown but not for background. Immunostaining intensity was defined as follows: +, less than 20% of

numbers of positive cells; ++, 20–60% and +++, more than 60%.

RESULTS

PgP expression was observed in K562/ADM cells but not in K562 cells or KB cells, and GST expression, in KB cells but not in K562 cells or K562/ADM cells. Overexpressions of PgP were induced after treatment with ID50 of VER, DPM or CsA for 24 h in K562 cells but not KB cells. DPM-treated K562/ADM cells expressed PgP much lower than DPM-free K562/ADM cells. PgP was not detectable after treatment of K562/ADM cells with ID50 of CsA for 24 h (Table 1).

Table 1. Expressions of PgP and GSTPI

Cell lines	PgP	GSTPI
K562	–	–
K562+VER	+++	–
K562+DPM	+++	++
K562+CsA	+++	–
K562/ADM	+++	–
K562/ADM+VER	+++	–
K562/ADM+DPM	+	–
K562/ADM+CsA	–	–
KB	–	+
KB+VER	–	+
KB+DPM	–	+
KB+CsA	–	+

Induced GST expression was present in DPM-treated K562 cells but not in VER-treated ones, or in CsA-treated ones. No significant difference was observed for GSTPI expression in KB cells before and after treatment with VER or DPM, or CsA (Table 1) (Figure 1—4).

DISCUSSION

PgP acts as a efflux pump that extrude a wid variety of anticancer drugs, such as ADM, VCR, VP16, etc. This lowers the intracellular accumulation of drugs and develops consequentially that drugs resistance. Expression of PgP has been found in many tumors and some normal tissues.[5,6] Three multidrug resistant reversors, VER, a calcium channel blocker, DPM, an inhibitor of nucleoside transport, and CsA, an inhibitor of immunity, have been previously reported to reverse multidrug resistance through competitive combination with many anticancer drugs for PgP.[3,7,8] Little has been known about effect of VER on expression of PgP. VER has been recently reported to result in a decrease in mdr1 mRNA level at 15-50 μM but at 100 μM VER, which strongly inhibited cell proliferation, a 2-fold increase of mdr1 mRNA levels was observed in the K562/ADM cells,[9] suggesting that the effect of VER on mdr1 mRNA level was associated with an exposed dose of VER. However, VER was also capable of greatly elevating the expression of mdr1 gene product, PgP, even at 19.6 $\mu g.ml^{-1}$ (40μM) of VER, from our findings.

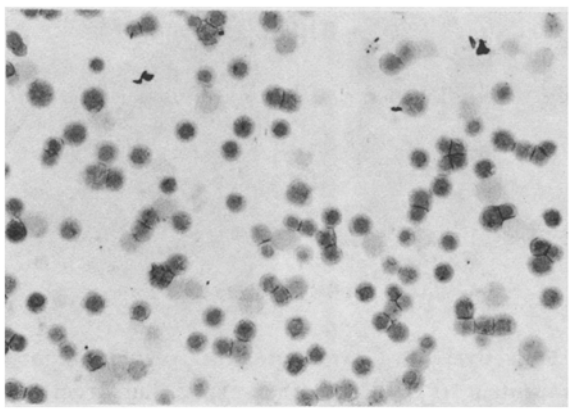

Fig. 1. Overexpression of PgP was induced in VER-treated K562 cells. S-P ICC×400

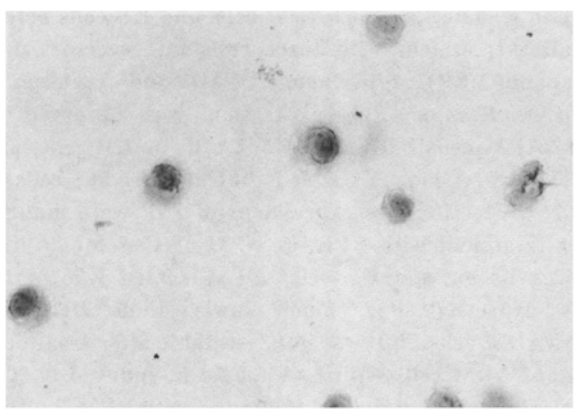

Fig. 2. Overexpression of PgP was induced in DPM-treated K562 cells S-P ICC×200

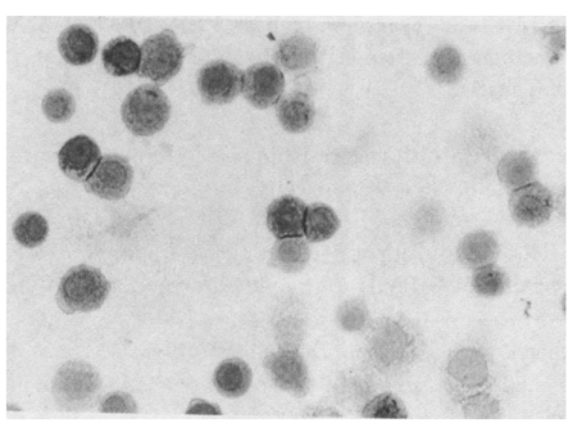

Fig. 3. Overexpression PgP was induced in CsA-treated K562 cells. S-P ICC×200

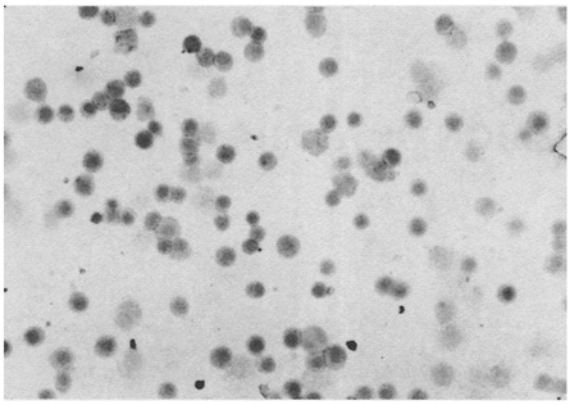

Fig. 4. Expression of GSTPI was induced in DPM-treated K562/ADM cells. S-P ICC×200

Results also showed that DPM and CsA, besides VER, were capable of inducing overexpression of PgP in K562 cells without PgP expression, but reducing PgP expression in K562/ADM cells by DPM and completely inhibiting PgP expression in K562/ADM cells by CsA. Insignificant effect of VER, DPM, CsA on PgP expression level was found in K562/ADM cells.

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