Reversal effect of PI3-K inhibitor LY294002 on P-glycoprotein-mediated multidrug resistance of human leukemia cell line K562/DNR and gastric cancer cell line SGC7901/ADR

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Background and Objective: Phosphatidylinositol-3-kinase/protein kinase B (PI3-K/Akt) signaling pathway plays an important role in cell survival. This study was to explore the reversal effect of PI3-K inhibitor LY294002 on P-glycoprotein (P-gp)-mediated multidrug resistance in human leukemia cell line K562/DNR and gastric cancer cell line SGC7901/ADR. Methods: The cells were divided into simple drug-treated groups and LY294002 pretreated groups: the former groups received treatment of daunorubicin (DNR), adriamycin (ADR), vincristine (VCR) and etoposide (VP-16), respectively; the latter groups received pretreatment of LY294002 before drug treatment. Trypan blue dye exclusion method and MTT assay were used to detect the drug sensitivity of K562/DNR and SGC7901/ADR cells, and the effect of LY294002 on the drug resistance. The expression of P-gp and phosphorylated Akt (p-Akt) in K562/DNR, SGC7901/ADR and their parental cell lines K562 and SGC7901 was detected by Western blot. Intracellular drug accumulation was measured by flow cytometry (FCM). Results: LY294002 pretreatment significantly decreased the 50% inhibition concentration (IC50) of DNR, ADR, VCR and VP-16 for K562/DNR cells, with reverse efficiencies of 72.4%, 64.9%, 60.4% and 52.8%. In SGC7901/ADR cells, the similar result was obtained with a reverse efficiency of 31.0%. LY294002 pretreatment partially inhibited the expression of p-Akt and P-gp, and promoted the intracellular accumulation of DNR and ADR in K562/DNR and SGC7901/ADR cells, respectively. Conclusion: LY294002 could partially reverse multidrug resistance in K562/DNR and SGC7901/ADR cells in vitro via inhibiting PI3-K/Akt pathway.

Multidrug resistance (MDR) of the tumor is the primary cause for the failure of chemotherapy.1-4 P-glycoprotein (P-gp) is the main molecule causing MDR, however, its regulation and control mechanisms remains to be elucidated, and the drug resistance induced by P-gp can not be overcame. Studies showed that the functions of P-gp are regulated by multiple signal proteins.2-4 Phosphatidylinositol-3-kinase (PI3-K)/protein kinase B (Akt) (PI3-K/Akt) pathway plays an important role in the regulation of cell survival. Recent studies have verified that activating PI3-K/Akt pathway in prostate cancer cells can upregulate the expression of P-gp;5 while blocking PI3-K/Akt pathway in lymphoma L1210 cells can suppress transcription of P-gp gene, whereby influencing tumor MDR.6 These studies indicate that PI3-K/Akt is an important signaling molecule in the regulation of P-gp protein transcription and tumor MDR. Nonetheless, the influence of PI3-K/Akt signaling pathway on the functions of P-gp remains to be elucidated.

Materials and Methods

Cell culture. Human myeloid leukemia drug-resistant cell line K562/DNR and its sensitive cell line K562 were granted by Professor T. Ueda (Fukui Medical University, Japan); gastric cancer drug-resistant cell line SGC7901/ADR was granted by the Institute of Gastroenterology, Xijing Hospital, the Fourth Military Medical University; gastric cancer cell line SGC7901 was preserved in our institution. The cells were mixed with RPMI-1640 media containing 10% fetal calf serum and 12 U/mL gentamycin in an incubator with saturated humidity and 5% CO2 at 37°C.

Drugs and reagents. Daunorubicin (DNR) was purchased from St. Louis, MO; adriamycin (ADR) from Zhejiang Hisun Pharmaceutical Co., Ltd.; vincristine (VCR) from Shenzhen Main Luck Pharmaceuticals Inc.; etoposide (VP-16) from QiLu...
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Pharmaceutical Co., Ltd.; LY294002 from Sigma; P-gp mouse monoclonal antibodies from Neomarker; p-Akt and Akt rabbit monoclonal antibody from New England Biolabs; Tubulin mouse monoclonal antibody from BD; enhanced chemiluminescence agent from Pierce.

Detection of drug sensitivity. K562/DNR and K562 cells at logarithmic growth phase were inoculated into 6-well culture plates, cultured with DNR, ADR, VCR and VP16 of various concentrations for 72 h, then counted with Trypan blue dye exclusion method to draw survival curves. SGC7901 and SGC7901/ADR cells were treated with ADR at different concentrations. The 50% inhibition concentration (IC50) was calculated with MTT assay. Absorbance at 570 nm (A570) was measured with enzyme-labeling instrument to calculate cell proliferation inhibition rate: inhibition rate (%) = (A570 of control group - A570 of experimental group) / A570 of control group × 100%. K562 and K562/DNR cells were pretreated with 2.5 μmol/L LY294002, and SGC7901 and SGC7901/ADR cells were pretreated with 25 μmol/L LY294002 to observe the variance of MDR. In the above experiments, triple wells were set to each group.

Detection of P-gp and p-Akt protein expression by western blot. K562, K562/DNR, SGC7901 and SGC7901/ADR cells at logarithmic growth phase were subjected to SDS-PAGE gel electrophoresis and semi-dry transblot. P-gp, p-Akt, Akt and Tubulin monoclonal antibodies (1:500 dilution) were used as first antibodies and horseradish peroxidase-labeled goat anti-mouse IgG or goat anti-rabbit IgG (1:800 dilution) as second antibodies for cell incubation, then cells were subjected to color development according to ECL kit instructions.

Detection of DNR and ADR concentrations with flow cytometry. K562, K562/DNR, SGC7901 and SGC7901/ADR cells were inoculated into 6-well plates and pretreated with LY294002 of various concentrations for 30 min, then incubated with 1 μmol/L DNR or 5 μg/mL ADR at 37°C for 15 min. The fluorescence intensity of the drugs in cells was detected with flow cytometry (FCM).

Statistical analysis. The data was analyzed with t test using SPSS11.0 software. p < 0.05 was recognized as significance.

Results

Cytotoxicity of LY294002 to K562 and SGC7901 cells. LY294002 at a concentration of 2.5 μmol/L or below showed no cytotoxicity to K562 and K562/DNR cells (data not provided); LY294002 at a concentration of 25 μmol/L or below showed no cytotoxicity to SGC7901 and SGC7901/ADR cells (Fig. 1).

Impact of LY294002 on the drug-sensitivity of K562 and SGC7901 cells. LY294002 evidenced reversed the MDR of K562/DNR cells to DNR, ADR, VCR and VP16 (Table 1). Moreover, LY294002 partly reversed the resistance of SGC7901/ADR cells to ADR, and the drug-resistance multiple decreased from 67.26 (15.47/0.23) to 48.86 (10.75/0.22) with relative reverse efficiency of 31.0%. LY294002 showed no effect on the drug-sensitivity of K562 and SGC7901 cells.

Impact of LY294002 on the expression of p-Akt and P-gp. Compared with parental K562 and SGC7901 cells, K562/DNR and SGC7901/ADR cells showed over-expression of P-gp and p-Akt. Pretreatment of 2.5 μmol/L LY294002 in K562/DNR and 25 μmol/L in SGC7901/ADR cells, the expression of p-Akt and P-gp were significantly decreased (p < 0.05). In contrast, the expression of these proteins showed no significant change in K562 and SGC7901 cells (Fig. 2).

Impact of LY294002 on the intracellular accumulation of DNR and ADR in K562/DNR and SGC7901/ADR cells. When K562/DNR cells were incubated with DNR, with the presence of LY294002 of various concentrations, the intracellular concentration of DNR was significantly increased; however, LY294002 had no effect on the intracellular ADR accumulation in K562/DNR and SGC7901/ADR cells (data not provided).

Discussion

MDR mediated by P-gp is one of the main factors influencing the efficacy of chemotherapy on tumors. Although many approaches have been applied to MDR reverse, including chemical drugs, natural herbal medicine, gene therapy and so on, they have
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not been widely used in clinic mainly due to severe adverse events, such as hypotension and arrhythmia, that occur before the effective reverse concentration is reached, furthermore, the cytotoxicity of chemotherapy is significantly exacerbated. Accordingly, the treatment and reverse of tumor MDR relies on elucidating new mechanisms of MDR and identifying novel reverse target sites.

PI3-K/Akt is a common pathway of multiple signal transduction approaches, which can modulate downstream proteins after activation via phosphorylation to realize its biological functions, such as anti-apoptosis, promoting proliferation, and so on. The study on PI3-K/Akt over-activation and tumor MDR has been the highlight in recent years. Lee et al. have demonstrated that the activation of PI3-K pathway can lead to the MDR of prostate cancer via upregulate P-gp expression; while inhibiting the activity of PI3-K, in synergism with chemotherapy, may suppress the MDR of prostate cancer. Barancík et al. have also found that LY294002 can reverse the MDR and promote VCR-induced apoptosis of P-gp-mediated drug-resistant mouse leukemia cell line L1210/VCR. However, to our knowledge, whether targeted small molecule inhibitors of PI3-K/Akt signaling pathway can effectively reverse the MDR of gastric cancer has not been reported yet. Our study further confirmed that LY294002 enhanced intracellular accumulation of ADR in SGC7901/ADR cells by more than 40%, which may be due to the downregulation of P-gp after LY294002 inhibited PI3-K/Akt pathway. All these findings provide new clues for further mechanism elucidation and reverse of tumor MDR.

In conclusion, the cell signal transduction pathway is believed to be a promising research direction for tumor MDR. PI3-K/Akt signaling pathway can be used as a target. Blocking the over-activation of PI3-K/Akt by small molecular inhibitors can enhance the sensitivity of tumor cells to chemotherapy and reverse tumor MDR to improve chemotherapy efficacy.

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References