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HPLC ASSAY FOR INTRACELLULAR ACCUMULATION OF VERAPAMIL IN VER-RESISTANT HUMAN LEUKEMIC CELL SUSLINES AND THEIR PARENTAL CELL LINES

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Objective: To establish a HPLC method using fluorometric detection for quantitatively determinating intracellular accumulation of verapamil (VER). Methods: Chromatography column was packed with spherisorb ODS (250 ×4.6 mm, 10 μm). The mobile phase consisted of the mixture of methanol:NaAC (0.01 mol/L): diethylamine (65:35:0.25). The detect wavelength was 280/310 nm (Ex/Em). Results: The standard curve showed a good correlation between concentration and peak area within the range of 5–50 ng/ml. RSD was 0.86%, and recovery radio of loading sample, 100%. The detection limit for cell sample was 0.2–148 ng/ml. Intracellular accumulation of VER was observed to decrease from a 13-fold to 5-fold in K562/ADM cells, and from a 3.5-fold to 4.3-fold in K562/VER cells and from a 2.1-fold to 6.5-fold in K562/ADM/VER cells, compared with the relevant control cells. Conclusion: HPLC method was proved to be sensitive and specific for using to quantitatively determine the intracellular accumulation of VER.

Key words: HPLC, Verapamil, Intracellular accumulation, Leukemic cells.

Verapamil (VER), a calcium channel blocker, has been found to effectively reverse multidrug resistance in tumor cells through competitive combination with some anticancer drugs for p-glycoprotein (pgp). The intracellular accumulation of VER in K562/ADM cells resistant to ADM was 30% of the parental cells, K562 cells, and in CHO/VCR cells resistant to vincristine (VCR) with VER hypersensitivity and elevated pgp level, significantly decreased, compared with the parental cells, CHO cells. Intracellular accumulation of VER has been previously reported to be evaluated with [3H] labeled VER and silica gel thin layer chromatography[1] or scintillation counting,[2] and VER content of preparation, with ultraviolet spectrophotometer.[3] We has recently developed a novel assay, HPLC assay, for evaluating intracellular accumulation of VER in VER-resistant human leukemic cells, K562/VER, K562/ADM/VER, and their parental cells, K562, K562/ADM, and report this study as follows.

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MATERIALS AND MTTHODS

Cell Line and Culture

K562 cell line, was obtained from Shanghai Institute for cell biology, K562/ADM cell line resistant to ADM, selected previously by us, and K562/VER cell line and K562/ADM/VER cell line resistant to VER, established in the presence of increasing concentration of VER previously in our department. K562 cells grew in the RPMI 1640 media containing of 15% fetal bovine serum, and 100 μg/ml of penicillin, and 100 μg/ml of streptomycin. K562/ADM cell grew in the RPMI 1640 media containing of 3.8 μg/ml of ADM, and K562/VER, in the media, containing of 130 μg/ml VER, and K562/ADM/VER,

in the media containing of both 4.2 μg/ml VER and 3.8 μg/ml ADM.

Agents and Instruments

VER for standard measure (Sigma), VER HCL (Knoll AG Co., Germany), LC–4A HPLC system, RF-530 Fluoromontitor and Data Acquisition Instrument (Shimadzu, Japan).

HPLC Assay for Intracellular Accumulation of VER

Chromatography condition chromatograph column was spherisorb ODS (250×4.6 mm, 10 μm), and mobile phase methanol:0.01 mol/L of NaAC (dissolved into 3.3% of glacid acetic acid:diethyamine (65:35:0.25), and rate of flow, 1.0 ml/min. Fluorescence wavelength for VER was 280/310 nm (Ex/Em).

Cells in exponential growth cycle were pretreated with 0.5–12 μg/ml VER for 2 h, followed by washing cells with PBS buffer for three times, and regulating total cell numbers up to 1×10^6 for each VER treated does tube. The cells were centrifugalized at 2000 rpm for five min, followed by removing supernatant and then extracting cell pellets with ether for two times, for detection of fluorointensity.

RESULTS

VER Absorption Peak

Specific absorption peak was observed in VER standard solution but control solution (PBS) (Figure 1). A good linear correlation between VER concentration and peak area was obtained after series of sample injection, 5–50 μml of VER (1 μg/ml). Regression equation was y=188.37+3999.48X and correlation coefficient r=0.99988.

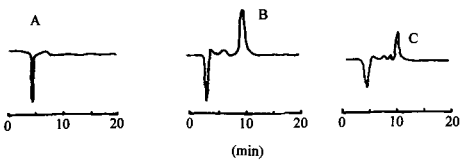

Fig. 1. VER specific absorption peak
A. Blank control (PBS);
B. VER standard solution;
C. Sample (VER-treated cells).

Degree of Precision and Recovery Rate

RSD was obtained to be 0.86% from repeated sample injection of 5μml of VER (2 $\mu g/ml^{-1}$) standard solution and recovery ratio, 100% from comparison of VER treated cell tubes with control tubes (PBS).

Assay for Intracellular Accumulation of VER

The detection rang for cell sample was 0.2–148 μg/ml. Intracellular accumulation of VER was observed to decrease from a 13-fold to 5-fold in K562/ADM cells, and from a 3.5-fold to 4.3-fold in K562/VER cells and from a 2.1-fold to 6.5-fold in K562/ADM/VER cells, compared with the relevant control cells (Table 1).

Table 1. Intracellular accumulation of VER (ng/ml)

Cell line	Concentration of VER exposure (μg/ml)					Decreasc times of accumulation
	0.5	1	3	6	12	
K562	0.5±0.85	2.6±0.71	121.5±4.22	134.5±5.43	148.0±3.35	
K562/ADM	0.5±0.03	4.9±0.35	24.2±0.12	26.8±0.64	29.1±0.57	5.2– 13 (a)
K562/VER	1.8±0.42	7.5±0.31	30.1±0.73	32.4±0.52	34.8±0.33	3.5– 43 (b)
K562/ADM/VER	0.2±0.01	2.3±0.35	3.7±0.21	4.2±0.67	5.2±0.44	2.1– 6.5 ©

a: Comparison between k562/ADM cells and K562 cells.

b: Comparison between K562/VER cells and k562 cells.

c: Comparison between K562/ADM/VER cells and K562/ADM cells.

DISCUSSION

HPLC method using fluorometric detection for quantitative assay of intracellular accumulation of VER has not been previously reported. This method was proved to be very sensitive and specific due to specific absorption peak at 280/310 nm (Ex/Em) for VER without interference from other compounds and a good linear correlation between concentration and peak area and 100% of recovery ratio from our experiment. It is also simple and secure, compared with the method used popularly at present, isotope labeled VER which is very complicated and involved isotope label and its purity by assending chromatography and radioactivity detection possible for radiocontaminations.

Sample detection showed that intracellular accumulation of VER showed significant decrease in VER-resistant cells, compared with their parent cells, and significant decrease in K562/ADM cells, compared with K562 cells that was concordant with other investigator's findings from [3H] labeled VER and silica gel thin layer chromatography,[2] suggesting that this method may be used to quantitatively determine the intracellular accumulation of VER.

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