P-glycoprotein (Pgp; Mr=170,000) is encoded by a family of genes—Multidrug resistance gene. The Pgp has been demonstrated to mediate resistance to multiple structurally dissimilar drugs, which functions as an energy-dependent efflux pump so that a cell with high level of mdr expression can more effectively eliminate cytotoxic drugs. In this report, a simplified method for analysis of clinical samples and assess the level of gene expression was set up. Furthermore, by using $^{32}$P labelled mdr-1 cDNA as the probe and RNA dot blotting the mdr-mRNAs from 5 cases of myeloblastic leukemia cells were analysed. It was shown that the level of mdr-1 expression in different myeloleukemic cells was various and reduced in one case after remission. The established method for mRNA analysis could be generalized for evaluating the level of mRNA in clinical samples.

Key words: mdr, RNA; Dot blotting, Myeloblastic leukemia

Chemotherapy is one of the most important strategy for treating tumors, but a difference of sensitivity of chemotherapy agents have been found in many patients. The study on cytotoxic resistant cell lines in vitro has demonstrated that there is a kind of high expressional membrane glycoprotein which made the cells have cross resistance in many drugs of completely different structure and function. The molecular weight of the protein is 170 kDa, known as p170, which is encoded by mdr-1 gene. The cells of high expression of p170 are resistant to many cytotoxic drugs. Such as anthracyclines, actinomycin D, Vincristine and Epipodophyllotoxins etc. The analysis of the mdr-1 mRNA of white blood cell in AML (acute myeloblastic leukemia) by a simple method was reported.

MATERIALS AND METHODS

Patients

Case 1  femal, age 26, when pregnancy 7 months she was diagnosed as acute promyelocytic leukemia (APML). Partial remission was achieved by inducing therapy of retinoic acid. Complete remission was achieved by treatment of HAD+ retinoic acid (AAP). The analysis of mdr-1 mRNA was made before treatment, and after partial remission.

Case 2  male, age 22, CML (chronic
myeloblastic leukemia), conversion into chronic phase after chemotherapy.

Case 3 male, APML (acute promyelocytic leukemia), no reaction to chemotherapy, died of cerebral haemorrhage.

Case 4 male, age 63, APML, no reaction to chemotherapy, died of cerebral haemorrhage.

Case 5 female, age 27, APML, complete remission by chemotherapy after 3 courses treatment.

Blood samples were taken before chemotherapy in the last 4 cases.

Isolation of White Blood Cell

In 10 ml anticoagulant blood 5 ml of 3% Ficoll (Mr = 250K) were added, after mixing, stand for 2 hours at room temperature, taking the supernatant, and spinning at 3500 rpm for 5 min, the pellet was washed with normal saline 2 times and stored in liquid nitrogen.

Extraction of Total RNA in WBC

The freezed WBC was melted in water bath, and suspended in the denatured solution (4mol/L guanidinc isothiocyanate (BRL), 25 mmol/L sodium citriate, pH7.0, 0.5%(w/v) sodium N-Lauroyl Sarcosine, 0.1mol/L B-mercaptoethanol), The volume was taken on base of 10^6 cells per 100 μl, the mixture was homogenized 20 times with a Loose Dounce Homogenizer. The 1/10 volume of NaOAc buffer (2mol/L pH4.0) was added, after mixing, equal volume of phenol saturated with water and 1/5 volume of chloroform isoayl alcohol (49,1). The mixture was shaked and incubated at 4 c for 15 min, spun at 10k for 20 min. The aqueous phase was taken off, after adding equal volume of isopropyl alcohol, it was mixed and standed -20°C for one hour. The resulting mixture was spun again at 1k, 4°C. for 20 min. The pellet was dissolved in the denatured solution of 1/10 volume of initial homogenate. Precipitating with isopropyl alcohol again, the pellet was washed with cold 75% alcohol and dried in vacuum. The RNA preparation was dissolved in 0.5% SDS and incubated at 65°C for 10 min, stored in the liquid nitrogen. The concentration of the RNA was assayed by a UV-spectrometer. The ratio of A260/280 should be about 2.0.

Preparation of mdr-1 Gene Probe

About 600 bp of mdr-1 cDNA was recombined into EcoRI site of PUC-9 named PCHP-1 which was transformed with E. Coli JM101. The amplified plasmid DNA was separated and purified by alkaline denaturation and polyethene glycol precipitation. The insert was recovered and labelled with 32P by random primer extension.

RNA Dot Blotting

The total RNA was denatured in 7.4% formaldehyde 10×SSC, at 55°C for 15 min. The denatured RNA was dotted on nitrocellulose membrane in various dilution, the filter was baked at 80°C for 2 hours, Prehybridized (5×SSC, 0.1% Sarksoy1, 0.02% SDS, 20 mmol/L Tris-HCl pH8.0, denatured Herring Sperm DNA 100 μg/ml, 5×Denhardt, 50% deionized formamide) at 37°C for 1—2 hours. Then the 32P labelled probe was added (20—40 ng/ml) and incubated at 37°C overnight. Washing strengency was 2×SSC, 0.1% SDS, 15 min, once; 1×SSC, 0.1% SDS, 15 min once; and 0.1×SSC, 0.1% SDS at