The Synergistic Inhibitory Effect of STI571 in Combination with Arsenic Trioxide (As_2O_3) on Multidrug-Resistant Leukemic Cells

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OBJECTIVE To study the synergistic effect of STI571, an inhibitor of tyrosine kinase, in combination with arsenic trioxide As_2O_3 on a multidrug-resistant leukemia cell line expressing bcr-abl.

METHODS The cytotoxic effect of STI571 alone or in combination with different concentrations of As_2O_3 on the bcr-abl and mdr1-positive leukemia cell line, K562-n/VCR, was examined by the MTT method.

RESULTS One μmol/L of STI571 alone had no significant cytotoxic effect on K562-n/VCR cells. However the cytotoxic effect increased markedly when combined with As_2O_3 at concentrations of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} mol/L. The IC_{50} of K562-n/VCR cells in As_2O_3 group was 1.879 μmol/L, with . Upon addition of STI571, the IC_{50} decreased to 0.155 μmol/L resulting in a synergistic cytotoxic effect on K562-n/VCR cells that was increased 12.1 times.

CONCLUSION A combination of STI571 with As_2O_3 has a more powerful inhibitory effect on leukemia cells expressing positive bcr-abl and positive mdrl compared to the effect with As_2O_3 alone.

KEYWORDS: STI571, arsenic trioxide, gene, bcr-abl, multidrug resistance.

STI571, a tyrosine kinase inhibitor, is an anticancer gene targeting drug, and has been regarded as the most effective therapeutic agent for patients with Ph positive chronic granulocytic leukemia (CGL). However, results of clinical trials abroad showed that in an advanced stage of CGL, especially for patients in blast crisis, resistance developed in a number of patients who had previously responsed to STI571.[1] To explain the mechanism of resistance to STI571 in CGL patients, and to find a means to overcome that resistance is of great interest in China and abroad.

A method of using a low dose first followed by intermittent, increasing doses of vincristine (VCR) was employed to treat a highly tumorigenic leukemia cell line, K562-n, carried in nude mice. This regimen resulted in the development of a multi-drug resistant cell line K562-n/VCR, which is resistant in various degrees to many chemotherapeutics, such as daunorubicin, homoharringtonine and etoposide, etc.[2] In our study the MTT method was used to examine the inhibitory effect of As_2O_3 in combination with STI571 on

K562-n/VCR cells. Based on our results a new strategy for treating leukemia patients with both bcr-abl and mdrl positive expression may be developed in the future.

MATERIALS AND METHODS

The cell lines

The K562-n cell line, a subline of CGL erythroleukemia K562 cells was developed in our own laboratory. [3,4] K562-n/VCR cells are a multi-drug resistant leukemia cells cultured and aquired by adding increasing levels of VCR to K562-n cells. Compared to K562-n cells, the K562-n/VCR cells can resist a VCR concentration of 297 times. K562-n/VCR cells were cultured and recultured in a culture fluid containing 0.08 μg/ml of VCR at a final concentration. The cells were removed from this culture fluid for 1 to 2 weeks before the experiment was conducted. PCR and immunohistochemical determinations showed that bcr-abl and mdrl expression in K562-n/VCR cells were both positive, whereas bcr-abl expression in K562-n cells was positive but mdrl expression was negative.

The drugs and reagents

STI571 was provided free by the Swiss Novartis Pharmaceutical Co. As_2O_3 was purchased from the Harbin Yida Pharmaceutical Co. Ltd. Calf serum was the product of Hangzhou Sijiqing Bioengineering Materials Co. Ltd. (deactived for 30 min at 56℃). Methyl thiazolyl tetrazolium (MTT) was prepared as a 5 mg/ml solution in saline and then subpackaged and stored below 4℃ . The dimethyl sulfoxide (DMSO) was purchased from the Shanghai Feida Industry and Trade Co. Ltd.

Drug inhibition of the cell line

Suspensions of K562-n and K562-n/VCR cells in an exponential growth phase were adjusted to 1×10^5/ml, and 180 μl of the culture placed into each well of a 96-well culture plate. The cells were incubated at 37℃ under 5%CO_2 and saturated humidity for 24 h. STI571 (10 μl) containing various concentrations was added into each well at the final concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} mol/L. Each concentration was tested in triplicate. After 48 h of incubation, 10 μl of MTT solution (0.5%) was added into each well and the cells incubated again for 4 h followed by centrifugation at 2,500 rpm for 10 min after which the supernant liquid was removed. DMSO (150 μl) was added into each well and the mixture shook for 10 min to ensure that the crystals were completely dissolved. Absorbance (A value) was measured at 492 nm and the IC_{50} calculated along with the cooperation multiple and the survival curve. The survival (%) was equal to the experimental well A/control well A times 100%.

To test the inhibitory effect of As_2O_3 on K562-n/VCR cells, the cells were placed into a 96-well culture plate as described previously and after 24 h of incubation, As_2O_3 and STI571 were added to the cultures. The final concentrations of As_2O_3 were 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} mol/L, and that of STI571 1 μmol/L. Each concentration of As_2O_3 was tested in triplicate after 48 h of incubation and the MTT test and calculation of the IC_{50} were conducted as previously described with and without STI571 ($STI571/IC_{50}$).

RESULTS

Sensitivity of K562-n cells versus K562-n/VCR cells to inhibition by STI571

The IC_{50} of STI571 with K562-n cells was 0.704 μmol/L, and with the K562-n/VCR cells was 3.382 μmol/L. The drug resistance to STI571 was 4.80 times that of K562-n cells which showed that there is a crossed drug resistance of the K562-n/VCR cells to STI571 (Table 1).

The inhibitory effect of the combined treatment of STI571 and As_2O_3 on K562-n/VCR cells

The IC_{50} of As_2O_3 with the K562-n/VCR cells was 1.879 μmol/L. However, in combination with 1 μmol/L of STI571, the IC_{50} decreased to 0.155 μmol/L resulting in an enhancement of inhibition that was 12.12 times compared to the inhibition without STI571 (Table 2).

Table 1. Comparison of the inhibition by STI571 on the K562-n and K562-n/VCR cells

	Survival rate (%)	
STI571 (μmol/L)	K562-n	K562-n/VCR
0.01	95.04 ± 7.00	96.22 ± 9.72
0.1	88.38 ± 4.57	94.28 ± 5.23
1	18.60 ± 2.04	74.52 ± 4.22
10	12.54 ± 1.44	33.91 ± 0.17
100	7.34 ± 1.29	8.15 ± 1.52
IC_{50}	0.704	3.382

DISCUSSION

The treatment of CGL patients with a positive-Ph chromosome has been one of the toughest problems in leukemia therapy. At the present time no available chemotheraputic regimen can improve the natural course of leukemia and the 5-year survival of allogenetic hemopoietic stem cell grafts has been only 40% to 70% . Furthermore, this treatment is not applicable for elderly patients. The mechanism of induction of CGL is believed to be mainly related to the expression product of a bcr-abl fusion gene which has tyrosine kinase activity. This activity results in a blockage of intracellular signal transduction and a disruption of apoptosis. STI571 is an abl-specific tyrosine kinase inhibitor, and in vivo experiments have shown that the drug can effectively inhibit proliferation of leukemic cells with a positive Ph chromosome. However, it was also found in clinical trials that a number of the patients in an advanced stage, especially in an acute transformation phase, who initially had a response to STI571 therapy, soon developed tolerance to the drug. For patients in a chronic stage, about 45% to 50% failed to have a change in their genetic make-up, i.e. they displayed genetic resistance which existed 9 months before treatment. In vitro experiments also have shown that a STI571 resistant cloned strain can be obtained by using a method of gradually increasing the concentration of STI571 in the culture of bcr-abl positive cells over a period of 3 months.[5] However, it has not as yet been concluded whether or not the ST1571 resistance of the bcr-abl positive cells was similar to the typical MDR (multi-drug resistance) mechanism.[6,7]

In our study, we used the MDR K562-n/VCR cell line and the MTT assay to examine the inhibitory effect of STI571. The results indicated that the IC_{50} of STI571 for the K562-n sensitive cells was 0.704 μmol/L, while that for the K562-n/VCR cells was 3.382 μmol/L. The drug-resistance of the K562-n/VCR cells was 4.80 times that of the sensitive cells, suggesting that the amplification of mdr1, the multidrug resistance gene, can increase STI571 resistance of bcr-abl positive leukemic cells.

As_2O_3 is a typical inducer of tumor apoptosis. It has been shown that As_2O_3 also has a definite effect on reversing drug resistance in vitro in MCF-7/ADM cells, the MDR cells of human breast cancer. As_2O_3 also was effective in retinoic acid-resistant acute promyelocytic leukemia (APL). However, it has only a slight effect in the MDR K562-/A02 cell line.[8,9]

To investigate whether treatment with As_2O_3 plus STI571 had a synergistic inhibitory effect on MDR positive bcr-abl leukemic cells, we examined the effect of STI571 combined with As_2O_3 at various concentrations in K562-n/VCR cells. The results indicated that 1 μmol/L of STI571 had a markedly

Table 2. The inhibitory effect of the combined treatment of STI571 and As_2O_3 on K562-n/VCR cells

	Survival rate of cells (%)						
	As_2O_3 dosage (μmol/L, $\bar{x} \pm s$)						
Groups	0.01	0.1	1	10	100	IC_{50}	Synergistic effect
As_2O_3	92.33 ± 2.57	81.66 ± 0.61	79.49 ± 1.87	16.11 ± 0.22	11.75 ± 1.22	1.879	-
As_2O_3+STI571	65.41 ± 2.15	57.80 ± 0.69	48.31± 2.75	11.53 ± 1.03	10.70 ± 0.52	0.155	12.12

Note: The final concentration of STI571 was 1 μmol/L.

inhibitory effect on K562-n cells, but the effect on mdr1 positive K562-n/VCR cells was not significant, suggesting the increased mdr1 expression can decrease the cytotoxic effect of STI571 on the bcr-abl positive leukemic cells. However, 1 μmol/L of STI571 in conjunction with the As_2O_3 at 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} mol/L significantly increased the inhibitory effect in K562-n/VCR cells. The cell-survival rate for the combined application of the 2 drugs using 1 μmol/L STI571 was 70.9% , 70.1% , 60.7% and 71.4% compared to the survival at the above concentrations of As_2O_3 alone. The result also showed that when As_2O_3 at 10^{-4} mol/L was combined with 1 μmol/L of STI571 there was no synergistic effect, possibly because the inhibitory effect of As_2O_3 at that concentration alone was already maximal.

The results of this study may have an impact on the type of therapeutic regimen given to patients with MDR-Ph-positive leukemia.

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