Reversal Effect of Arsenic Sensitivity in Human Leukemia Cell Line K562 and K562/ADM Using Realgar Transforming Solution

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The success of arsenic trioxide (ATO) in treatment of acute promyelocytic leukemia (APL) attracts a great deal of attention to researchers to explore its activity of anti-leukemia. However, ATO has unavailable effect on chronic myeloid leukemia (CML), especially multidrug resistant (MDR)-CML, unless using high concentration. Realgar (As4S4) has been employed in Chinese traditional medicine for 1500 years. Research evidences confirmed realgar has similar effect on treating with APL as ATO, but the problem of large dose and long period in the CML/MDR-CML treatment still exist. By using a microbial leaching process with Acidithiobacillus ferrooxidans, we obtained realgar transforming solution (RTS) which showed significantly higher extent in inhibiting CML cell line K562 and MDR-CML cell line K562/ADM, and then trigger apoptosis. Both K562 and K562/ADM showed arsenic-dose-dependent effect on RTS. Interestingly, the overexpression of MDR1 mRNA and P-glycoprotein (P-gp) in K562/ADM cells were down-regulated by RTS, where there are no obvious effects on ATO and realgar and arsenic can be subsequently accumulated in K562/ADM cells efficiently. The intracellular accumulation of arsenic in K562/ADM cells treated with RTS for 4 h was 2-fold and 16-folds higher than those treated with realgar or ATO. Meanwhile, Western blot analysis of AQP9, the main transporter of arsenic, was increased by RTS treatment particularly in K562/ADM. Thus, these results suggested that the effect from a certain arsenical or a variety of arsenicals in RTS might be a promising candidate both for treating CML/MDR-CML alone and as combinations with currently used anti-CML/MDR-CML drug, although arsenical forms in RTS are undefined.

Key words realgar transforming solution; arsenic sensitivity; multi-drug resistance; arsenic uptake; aquaglyceroporin 9

Arsenic appears to be extremely effective as a chemotherapeutic agent in treating certain types of cancers. In particular, arsenic trioxide (As2O3, ATO) is an effective drug in treating acute promyelocytic leukemia (APL), via induction of apoptosis and differentiation.1,2) The anti-APL activity of ATO attracts a great deal of attention to researchers to explore broad-spectrum anti-leukemia activity of ATO.

Human chronic myelogenous leukemia (CML) is one of a type of leukemia, which malignancy of pluripotent hematopoietic cells that is caused by the dysregulated activity of the tyrosine kinase that is encoded by the chimeric oncogene. CML cells are highly resistant to chemotherapeutic drug, especially multidrug-resistant (MDR) CML,3) At present, failure to respond to chemotherapy is the key contributing factor for the high mortality resulting from leukemia, therefore, anticancer drugs that active against CML/MDR-CML are urgently needed. However, research evidence proved CML/MDR-CML cells at relatively high ATO concentrations in treating on CML/MDR-CML cells than APL cells when trigger apoptosis.4,5) These results indicated the limitations of ATO in the treatment of CML/MDR-CML. Thus, it prompts researchers to explore other arsenic derivatives, which should have not only similar effect as ATO in APL, but also the ability to overcome CML/MDR-CML.

Realgar (As4S3), a traditional Chinese medicine, was chosen as such a candidate for its good therapeutic reputation and perceived low toxicity in traditional medicine. It has been used alone or in combination with other traditional drugs for more than 1500 years to treat several diseases, such as syphilis, psoriasis, malaria, parasitic infections and cancer.6) Research evidence revealed that realgar and realgar-containing formulations, similar to the ATO, even have more effective and low toxicity in induce apoptosis or differentiation in APL cells.2) Although realgar has less adverse effect than ATO on APL, both of them need relative high concentrations in treating on CML/MDR-CML cells than APL cells when trigger apoptosis.3,7) For instance, report confirmed at a large dose (0.75–3.75 g once a day) of realgar and long period (2–9 weeks) of treatment was necessary for the clinical complete release (CR) on CML.4) The high concentration of arsenic treatment could limit its clinical application.

Both CML/MDR-CML are highly resistant to many arsenicals, including ATO and realgar. Generally, the membrane transport protein and drug resistant protein could be as the main factors in drug resistant. Research reported that ATO and realgar could effectively accumulated by increasing aquaglyceroporin 9 (AQP9) expression in APL cell lines and then trigger differentiation or apoptosis, but no effect on CML cell line K562 cells.8,9) In mammals, AQP9 was shown to transport multiple forms of arsenic.10) Studies on multiple cultured cell lines revealed increased AQP9 expression, resulting in increased arsenic accumulation and toxicity.9,11) Thus, membrane transport protein AQP9 must play an important role during the treatment of CML/MDR-CML.

Multidrug-resistant (MDR) influences drug sensitivity as well. It may be caused by a variety of factors, one of the most common causes of MDR is through the overexpression of P-glycoprotein (P-gp).12) P-gp is a glycoprotein which responsible for complicated cross resistance of the cell line

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to chemotherapeutic drug, and is constitutively expressed in K562/ADM cell line.\(^\text{13}\) Therefore, regulating P-gp expression in cells could be contributed to multidrug-resistant. Meanwhile, both ATO and realgar alone has obviously unavailable effect on down-regulate of P-gp in K562/ADM cell.\(^\text{15}\)

ATO and realgar showed effect in the cause of leukemia treatment. Therefore, we hypothesized that the anti-leukemia effect might be more effective from the combination of a variety of arsenicals. In a previous study, we recovered arsenic from realgar using bioleaching technology from biohydro-metallurgy with indigenous acidophilic microorganisms.\(^\text{14,15}\) Recent studies have confirmed the antitumor activities, both in vivo and in vitro cancerous models, of realgar extracted using *Acidithiobacillus ferrooxidans*.\(^\text{16,17}\) The realgar trans forming solution (RTS) treatment significantly increased the potency of antitumor effect of realgar with highly selective affinity to tumor tissue.\(^\text{16}\)

This work focused on the potency of the bioleaching product RTS for leukemia therapy, its anti-CML activities were examined on K562 and K562/ADM cells. We evaluated the capacity of RTS in the reversion of arsenic sensitivity in vitro and its potential mechanism.

**MATERIALS AND METHODS**

**Cell Lines and Microorganisms** Two cell lines, the chronic myeloid leukemia (CML) cell line K562, and P-glycoprotein (P-gp) expression of multidrug-resistant human chronic myeloid leukemia (MDR-CML) cell line K562/ADM, which was highly resistant to adriamycin, and cross-resistant to daunorubicin and doxorubicin.\(^\text{13}\) All cell lines were obtained from the Laboratory Center for Medical Sciences, Lanzhou University (Lanzhou, China) and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37°C in a humidified 5% CO\(_2\) incubator.

*Acidithiobacillus ferrooxidans* BY3 (CCTCC-M 204057) was isolated from acid mine effluent Northwestern China and cultured in 9K medium\(^\text{8}\): 3.0 g (NH\(_4\))\(_2\)SO\(_4\), 0.1 g KCl, 0.5 g K\(_2\)HPO\(_4\), 0.5 g MgSO\(_4\)·7H\(_2\)O, 0.01 g Ca(NO\(_3\))\(_2\), and 1.7 g FeSO\(_4\)·7H\(_2\)O per 1000 mL H\(_2\)O, pH 1.8.

**Reagents and Antibodies** Realgar transforming solution (RTS) was obtained following the process described previously.\(^\text{16,17}\) In brief, 100 mL iron-free 9K medium and 1 g Fe\(^{3+}\)/L as additional energy source was added to 1.5 g realgar powder in 250 mL flasks. *A. ferrooxidans* BY3 was then inoculated at 20% (v/v), after a culture of *A. ferrooxidans* in 9K medium to reach an exponential growth phase. After 30 d of incubation with *A. ferrooxidans*, the RTS supernatant was harvested by centrifuging at 10000 rpm for 10 min to remove *A. ferrooxidans*, and adjusted to pH 7.0 with 1 M NaOH and 2 M Na\(_2\)EDTA (ethylenediaminetetraacetic acid). Then, the supernatant was filtered through a 0.22 μm membrane to remove bacteria. Arsenic trioxide for injection (ATO, Yierda\(^\text{®}\)) with arsenic concentration of 10 mg/mL was purchased from Yida Medicinal Ltd. (Harbin, China). Realgar was obtained from Shimen county of Hunan province and purified by using conventional methods, which is called “refining powder with water” based on Chinese Pharmacopoeia. Samples were mixed with nitric acid (Merck, Germany) and hydrogen peroxide (AR) to digestion. After treating by Microwave Digestion System, diluted samples were mixed with thiourea, and then the total arsenic concentration of each of the three arsenicals was measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Jobin-Yvon Ultimate 2R).\(^\text{14,16,17}\) P-gp and AQP9 antibody were purchased from ZhongShan Golden Bridge (Beijing, China) and Saier Biotechnology Inc. (Tianjin, China) respectively. All the reagents for reverse transcription-polymerase chain reaction (RT-PCR) were purchased from TaKaRa Ltd. (Dalian, China) and Biomed-tech Ltd. (Beijing, China).

**Cell Culture and Drug Treatment** Cells were seeded into 96 well dishes at 10\(^3\) cells per well and incubated with a range of arsenic concentration (0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 μg/mL) for realgar, ATO or RTS for 24 h. Ten microliter methyl thiazoly l tetrazolium (MTT) was added into each well to make the final MTT concentration 0.5 mg/mL. After 4 h incubation, dimethyl sulfoxide (DMSO) was added and absorbance measured at OD570.

**Cell Cycle Distribution** After incubation with realgar, ATO or RTS at 0.2 μg/mL arsenic for 24 h, cells were washed twice with cold phosphate buffered saline (PBS), and suspended in 70% ice-cold ethanol. All the samples were kept in −20°C at least overnight before analysis. After washing with PBS, RNaseA (1 mg/mL, Sigma Chemical Co.) and propidium iodine (PI, ≥95% purity, 50 μg/mL, Sigma Chemical Co.) were added to each sample. Cells were filtered through a 30 μm pore size nylon mesh before cell cycle analysis with flow cytometry (FCM). The cells with DNA content less than G1 phase were taken as apoptotic cells.

**Measurement of MDR1 mRNA by RT-PCR** K562/ADM cells incubated with realgar, ATO or RTS at 0.2 μg/mL arsenic for 24 h. The total RNA was extracted using RNA kit (EASY spin cell RNA kit, Biomed-tech, Beijing), and reversed transcribed into cDNA using the One Step SYBR\(^\text{®}\) PrimeScript\(^\text{®}\) RT-PCR Kit (TaKaRa, Dalian, China) according to manufacturer’s instruction. The cycling conditions were as follows: denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 20 s, 58°C for 20 s, and 70°C for 20 s. All samples were quantified in triplicates. The primers were as follows: MDR1: forward 5′-GAC TGT CAG CTG CTG TCT GGG CA-3′, reverse 5′-GCC AAG ACC TCT TCA GCC CT-3′; β-actin, forward 5′-GGT CGG CGC CCC AGG CAC CA-3′, reverse 5′-CTC CCT ATT GTC ACG CAC GAT TTC-3′.

The expression level of MDR1 in K562/ADM after arsenic treatment was analyzed using the delta-delta threshold cycle (ΔΔCt) method (Bio-Rad CFX Manager), with β-actin as the internal reference gene to control for RNA quantity. Each ΔCt value was determined by subtracting β-actin mRNA Ct value from the target gene Ct value. The ΔΔCt was calculated by subtracting the ΔCt value of the untreated control from the ΔCt value of the treated samples. 2\(^\text{ΔΔCt}\) represented the average relative amount of mRNA for target gene.

**Protein P-gp Expression** Cells incubated with realgar, ATO or RTS at 0.2 μg/mL arsenic for 24 h and were washed twice with ice-cold PBS, added to RIPA buffer (50 mm Tris–HCl, pH 7.4; 1% NP-40; 0.25% Na-deoxycholate; 150 mm NaCl; 1 mm EDTA; 1 mm phenylmethylsulfonyl fluoride (PMSF)) and protease inhibitor (Cocktail, EDTA free, Sigma, U.S.A.). The cells were then broken down by sonication and through freezing and thawing cycles repeatedly. Samples were centrifuged at 10000×g at 4°C, 15 min. Protein concentration
of each sample was measured (BCA kit, Beyotime, Jiangsu, China) and adjusted to lysis buffer and 5× sample buffer (Beyotime). Samples were boiled at 95°C for 10 min and kept at 37°C, and loaded equally (30 µg/35 µL) in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (separation gel, 120 V, 2 h; spacer gel, 80 V, 0.5 h). The protein was electrophoretically transferred to a nitrocellulose (NC) membrane at 4°C for 2.5 h. The membrane was blocked with 5% low-fat milk for 2 h, incubated overnight with rabbit anti-P-gp antibody (1:300, ZhongShan Golden Bridge, Beijing, China) at 4°C and washed with phosphate buffered saline Tween 20 (PBST) three times (10 min/each). Anti-rabbit secondary antibody was added (1:8000, ZhongShan Golden Bridge, Beijing) and probed at room temperature for 2 h, then washed with PBST. Immunoblotting was evaluated after chemiluminescence development (Beyotime). The band intensity was quantified with ImageJ software.

**Arsenic Uptake** After incubation with realgar, ATO or RTS for 0, 2, 4, 8, 16, 32 h, cells were washed with PBS, the cell number of each sample was counted and the cells were resuspended in 1 mL of concentrated nitric acid for 4 h and then mixed with 20 µL H2O2 and bathed in boiling water overnight. The cell suspension was further homogenized by ultrasonification. The total arsenic concentration was calculated using the following equation after measurement of optical density (OD) of each sample with ICP-AES:

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\text{arsenic concentration (µg/cell)} = \text{arsenic concentration (OD)} \times \text{dilution volume / cell number}
\]

**RT-PCR for AQP9** The total RNA was extracted using RNA Kit (EASY spin cell RNA kit, Biomed-tech, Beijing), and reverse transcribed into cDNA using the One Step SYBR® PrimeScript® RT-PCR Kit (TaKaRa, Dalian) according to manufacturer’s instruction. RT-PCR conditions were 95°C for 2 min, 40 cycles of 95°C for 10 s and 60°C for 30 s. The data were collected and analyzed using Bio-Rad CFX Manager. All samples were quantified in triplicates. The primers were as follows:

**Fig. 1.** MTT Analysis of ATO, Realgar and RTS on Cell Growth at Various Concentrations on K562 and K562/ADM Cells after 24 h
(a) K562/ADM. (b) K562. The results were expressed as means±S.D. from three independent trials. *p<0.05 realgar treatment compared to RTS treatment. \#p<0.05 ATO treatment compared to RTS treatment.

**Fig. 2.** Effect of 0.2 µg/mL (Arsenic) of ATO, Realgar and RTS on Cell Cycle Distribution of K562 and K562/ADM Cells Assessed by Flow Cytometry after 24 h Treatment
Untreated cells acted as control. Result is one representative experiment from three independent trials.
β-Actin, forward, 5′-TTC CAG CCT TCC TTC CTG G-3′; reverse, 5′-GAT ACT CCT CAT AAT CGT CT TGC-3′; AQP9, forward, 5′-TCT CGG GTT CTA AGT CGT CGC-3′; reverse, 5′-TTC CTG GAG ATG TCT GGT AA-3′.

The expression level of AQP9 in K562 and K562/ADM after arsenic treatment was analyzed using the ΔΔCt method (Bio-Rad CFX Manager), with β-actin as the internal reference gene to control for RNA quantity. ΔΔCt represented the average relative amount of mRNA for target gene.

Western Blotting for AQP9 After 4h incubation with realgar, ATO or RTS at 0.2 µg/mL arsenic, all the cells were collected. The method was as same as P-gp protein measurement. The membrane was subsequently incubated with a rabbit anti-human-AQP9 antibody (1:500, Saier Biotechnology, Tianjin, China) and secondary antibody (1:8000, ZhongShan Golden Bridge, Beijing). Immunoblotting was evaluated after chemiluminescence development (Beyotime, Jiangsu). The band intensity was quantified with ImageJ software.

Statistical Analysis Results are presented as the mean value±standard deviation (S.D.), and were subjected to the one-way analysis of variance (ANOVA) test. p values lower than 0.05 were considered to be statistically significant.

RESULTS

Enhancement of Arsenic Sensitivity of K562 and K562/ADM Cells by RTS The sensitivities of cell cultures to the treatments, measured as percentage of viable cells, were different between the agents, but all demonstrated a arsenic-dose-dependent increase in cytotoxic effect. We first examined and compared the effects of RTS with that of ATO and realgar on the growth of a drug-resist leukemia cell line K562/ADM. For a control we used the isogenetic leukemia cell line K562. As shown in Figs. 1a and b, in both cell lines, RTS was more potent than both ATO and realgar at all concentration of arsenic tested. More interestingly, RTS was more effective against K562/ADM cells than K562 cells. Thus at 0.2 µg/mL arsenic concentration, RTS caused 50% cell death in K562/ADM cells but only 30% in K562 cells. Based on this data, 0.2 µg/mL arsenic concentration was then chosen for assessing the effect on cell cycle, protein and RNA expressions.

Effects of RTS on Cell Cycle Distribution Apoptosis-induction has been suggested as one of the major mechanisms involved in the anticancer activity of arsenic compounds.19,20 We therefore examined whether RTS is more effective than ATO or realgar in inducing apoptosis in K562/ADM drug-resistant leukemia cells.

The FCM analysis summarized in Fig. 2 showed that whereas RTS treatment has a minor reduction of cells in the G0/G1 phase, it produced higher sub-G1 cell population (11.8% for K562 and 15.9% for K562/ADM) than that
produced by either ATO or realgar. The result is consistent with the notion that apoptosis might account at least partially for the effectiveness of RTS in inhibiting the growth of both leukemia and drug-resistant leukemia cells.

Reduction of MDR1 mRNA and P-gp Expression by RTS in K562/ADM Cells ATO and realgar treatment for 24 h did not change the P-gp protein expression in K562/ADM cells. In contrast, a reduction of both MDR1 mRNA (Fig. 3a) and P-gp expression (Fig. 3b) with the reduction being more significant with RTS.

Elevation of Arsenic Uptake in Multi-Drug Resistant K562/ADM Cells by RTS One possible mechanism to explain the potency of RTS as compared to ATO or realgar on per arsenic unit basis is that RTS may improve the bioavailability or uptake of arsenic. To examine this possibility, we analyzed the intracellular content of arsenic in both leukemia cell lines 4 h after treating with RTS, ATO or realgar. As shown in Fig. 4, the intracellular accumulation of arsenic in K562/ADM treated with RTS for 4 h was 2 to 16 folds higher than those treated with realgar or ATO.

Increased AQP9 Expression by RTS AQP9, one of the aquaporin water channel family that is permeable to uncharged small molecules like glycerol, has been found to be the transmembrane transporter of arsenic compounds.9,10,21 Accordingly, in both K562 and K562/ADM cell lines, AQP9 expression may have a role in regulating the cellular sensitivity to arsenic. We therefore examined and compared the effects of RTS, ATO and realgar on the expression of AQP9 at both mRNA and protein levels in both leukemia cell lines. As the result showed in Fig. 5, AQP9 expression in K562 cells was insensitive to ATO or realgar treatment, which is in agreement with the previous reports.8,21 On the contrary, AQP9 expression was increased by RTS (Fig. 5b). In K562/ADM cells, RTS significantly induced the expression of AQP9, and mRNA expression assessed by RT-PCR further supported these findings (Fig. 5a). In K562 cells, the mRNA expression was higher in cells treated with RTS than with the other agents (p<0.05). In K562/ADM cells, RTS significantly increased the AQP9 mRNA level to 10-fold of those in other treatments (p<0.05).

DISCUSSION

CML/MDR-CML cells a highly resistant to chemotherapeutic drug, including ATO and realgar in arsenicals. In contrary, according to our results, both K562 and K562/ADM were sensitive to RTS treatment. RTS is significantly more potent than realgar or ATO, on per unit arsenic basis in killing both K562 and K562/ADM cells (Figs. 1a,b). More importantly, the cytotoxic effect of RTS on K562/ADM is greater than that on K562 cells. The cells exhibited an arsenic-dose-dependent effect to RTS confirmed arsenic is the main active ingredient in it. Thus, treatment of RTS at 0.2 µg arsenic/mL for 24 h resulted in 50% of growth inhibition of K562/ADM cells as compared to 30% for K562 cells (Figs. 1a,b). Consistent with the more potent cytotoxic effect of RTS as compared to ATO and realgar, flow cytometry analysis of cell cycle distribution showed much higher sub-G1 population indicating more apoptotic cells generated (Fig. 2). As shown in Fig. 2, RTS exhibited more potent activity in cell apoptosis inductivity, and more sub-G1 population was observed for K562/ADM cells than for K562 cells.

The principle mechanism for the multi-drug resistance of K562/ADM cells is their constitutive expression of MDR1 gene.5,22 The MDR1 gene is a term that describes the cross-resistance of cells against a range of drugs with different structures and targets. This gene encodes an efflux transporter P-gp that limits a wide variety of drugs from penetrating cells and depositing them into the extracellular space. The P-gp overexpress of K562/ADM cells may act as a drug-resistant mechanism via escape apoptosis triggered by chemotherapeutic agents.22 After 4 h incubation, neither ATO nor realgar has effect on suppressing P-gp expression (Fig. 3b) due to K562/ADM cells is not cross-resistant to arsenicals. In contrary, K562/ADM cells with RTS obviously suppressed the expression of MDR1 gene at mRNA expression (Fig. 3a) and P-gp level (Fig. 3b). These findings showed that the RTS displayed a forceful suppression on mdr1/P-gp expression in K562/ADM cells, and this suggests that RTS exerts greater induction of apoptosis in drug resistant cells via down-regulating P-gp expression enforce apoptosis death at a low dosage. It likely to K562/ADM recovered drug sensitivity through bioleaching effectively improved arsenic accumulation and bioavailability of realgar.

The level of arsenic accumulation in individual cells determines how the cells respond to arsenic-containing drugs.19,23 In the past, the leukemia cell lines K562, K562/ADM, and HL-60 were tested. These demonstrated higher responses to RTS than other cancer cell lines (A549, MGC-803, PC12) (data not shown). The intracellular accumulation of arsenic in K562/ADM treated with RTS for 4 h was 2 to 16 folds higher than those treated with realgar or ATO. Interestingly, the difference in arsenic accumulation between RTS-treated and realgar-treated K562 cells was not as great as that between RTS-treated and realgar-treated K562/ADM cells (Fig. 4). These data suggested us different arsenicals in ATO, realgar and RTS showed different accumulation level in CML cells. In Fig. 4, the intracellular accumulation of arsenic in K562 cells treated with realgar was higher than those treated with ATO, however, the cell viability was almost similar (Fig. 1). Comparing with RTS, the reason we supposed may be the intracellular accumulation of effective arsenic need to achieve to a certain level and then regulating gene/protein expression or triggering apoptosis. On the other hand, the effective accumulation of RTS in K562/ADM cells is consistent with the notion that higher accumulation of arsenic correlates with higher cytotoxicity.

In tumor cells, membrane transporters play a key role in switching the cells from drug-sensitivity to drug-resistance.12 In contrary, this change is frequently associated with a decreased cellular accumulation of antitumor drugs, which then results in multi-drug resistance of tumor cells.24,25 AQP9 is a member of the aquaporin gene family for membrane transporters involved in arsenic uptake by mammalian cells.10,11 Previous studies indicated that increased AQP9 expression in several cultured cell lines was associated with arsenic accumulation and cytotoxicity.5,25 The arsenic containing TCM ‘Realgar-Indigo naturalis’ was shown to be very effective in treating APL cell lines but not CML cell lines through increased AQP9 expression, indicating that the efficiency of arsenic in realgar could be improved.18 In the present study, we found that RTS treatment significantly up-regulated AQP9 in K562 and K562/ADM cells, both at protein (Fig. 5b) and mRNA level (Figs. 5a-1,
Moreover, it can be noted that the effect of RTS on AQP9 expression, both at protein and mRNA level are much greater, almost 10-fold, for K562/ADM cells than that for K562 cells (Figs. 5a, b). In contrary, ATO and realgar do not have efficacy on CML cells, even inhibited AQP9 protein expression. RTS can be efficiently utilized by cells. The potent cytotoxic effect of RTS on K562/ADM can be explained by this enhanced arsenic accumulation.

Arsenic in RTS may have higher bioavailability results in enhanced expression of AQP9, consecutively lead to increased arsenic uptake whereas the suppression of MDR1 and P-gp should attenuate the excretion of arsenic species in K562/ADM cells particularly. The fact that RTS selectively enhanced AQP9 expression and suppression of MDR1 in K562/ADM clearly contributed to the increased arsenic bioavailability in this drug-resistant leukemia cell line.

By using bioleaching approach, RTS from realgar exhibited a great activity to CML cells. It is imply that bioleaching processing could play a key role in this transformation. Bioleaching is a well-established biotechnological technique to recovery of heavy metals by applying microorganisms from their insoluble sulfide minerals. The bacteria, i.e. Acidithiobacillus spp., act as an oxidizer for ferrous ion which is produced in the dissolution of metal sulfides. In a previous study, we have succeeded in recovery of arsenic from realgar, one of the typical metal sulfides, by using pure and mixed indigenous Acidithiobacillus spp. By taking advantage of an arsenic-resistant strain of indigenous A. ferrooxidans BY-3, we applied this bioleaching method to develop a bio-arsenic aqueous solution RTS from realgar. RTS treatment displayed obviously effect on K562/ADM. Although the effect from certain arsenical or a variety of arsenicals is undefined, this...
phenomenon indicates arsenic from RTS may able to have more pharmaceutical potency (Fig. 6).

In summary, the bioleaching product RTS displayed significant activity against CML, especially in treating with MDR-resistant leukemia K562/ADM cell line, by increasing selective affinity and bioavailability via regulate P-gp and AQP9, consequently recovery of arsenic sensitivity and trigger apoptosis. Although arsenic is the main active ingredient in RTS, due to the limitation of research conditions, the forms of arsenscals in RTS remain undefined. The studies reported here may be highly relevant in light of RTS serve as a potential clinical candidate for anti-CML therapeutic drug. Certainly, more research is warranted.

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