Arsenic Trioxide Induces Erythroid Differentiation and Apoptosis of K562 Human Leukemia Cells through the Down-Regulation of Bcl-2

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Background: Arsenic trioxide (As_2O_3) has been identified as an effective drug for the treatment of acute promyelocytic leukemia (APL). However, the role of As_2O_3 during the erythroid differentiation of human leukemic cells remains unknown. In this study, we investigated the in vitro effects of As_2O_3 on the erythroid differentiation of the K562 cell line and also on the expression and regulation of the apoptotic modulators of this process.

Methods: The K562 cells were cultured in the presence of 0.1, 0.5 and 1.0 μM As_2O_3, or they were cultured in the presence of 1.0 and 10 μM all trans retinoic acid (ATRA). The expression of glycophorin A before and after treatment with As_2O_3 or with ATRA in the K562 cells was assessed by flow cytometry and western blotting. The expressions of Bcl-2 and caspase-3 were determined by western blotting.

Results: The viability of the K562 cells was not decreased after treating with 0.1 and 0.5 μM of As_2O_3, but the viability was significantly reduced at a dose of 1.0 μM. Caspase 3 activation was not observed at 0.1 and 0.5 μM of As_2O_3 until 12 days, but Caspase 3 was activated by 1.0 μM of As_2O_3 from day 3. The expression of glycophorin A was increased in dose dependent manner by As_2O_3 treatment, but this was not changed in the ATRA treated K562 cells. The expression of Bcl-2 was increased by 0.1 and 0.5 μM of As_2O_3, but it was abruptly reduced by 1.0 μM of As_2O_3.

Conclusion: These results suggest that As_2O_3 induces the erythroid differentiation of K562 cells and that 1.0 μM of As_2O_3 induces apoptosis through the down-regulation of Bcl-2. ***(Korean J Hematol 2005; 40:93-100.)***

Key Words: Arsenic trioxide, Differentiation, Apoptosis, ATRA, K562

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INTRODUCTION

Although arsenic compounds are known poisons, they have been used in traditional oriental medicine for centuries. Recently arsenic compounds, such as arsenic trioxide (As_2O_3) and arsenic disulfide, have proven to be effective in the treatment of relapsed acute promyelocytic leukemia (APL). Moreover, toxicities were found to be acceptable, neither bone marrow depression nor other severe clinical side effects were observed. The mechanisms of action were shown to exert dose dependent- dual effects in APL cells, i.e., by triggering apoptosis and inducing partial differentiation.[1-3] These effects were associated with the modulation and degradation of the t(15:17)-specific fusion protein PML-RARα.[4,5] However, recent reports suggest that the apoptotic effect of As_2O_3 is not specific for APL cells, and that it can be observed in non-APL leukemia and lymphoma cell lines[6-9] as well as in other tumor cell lines.[10,11] As_2O_3 can induce the activation of caspases,[12,13] the downregulation of Bcl-2,[14,15] the modulation of p53,[16] as well as the uncoupling of the mitochondrial potential.[1,17]

The induction of tumor cell differentiation represents an attractive strategy for the treatment of a wide range of malignancies. Differentiation of HL-60 promyelocytic leukemia cells towards neutrophils or monocytes have been shown to induce apoptotic cell death. This differentiation-induced apoptosis was found to be coupled by the downregulation of Bcl-2.[18,19] Because little is known about As_2O_3 in tumor models of erythroid differentiation, we investigated the in vitro effect of As_2O_3 on the erythroid differentiation of K562 cell line using glycophorin A, a marker for erythroid differentiation and upon the expressions and regulations of the Bcl-2 and caspase 3.

MATERIALS AND METHODS

1. Cell culture

The K562 human leukemia cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were grown in RPMI 1640 (GIBCO-BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; GIBCO-BRL) and 1% penicillin-streptomycin. To induce differentiation, cells were cultured in the presence of 0.1 μM, 0.5 μM, and 1 μM As_2O_3 (Sigma, St. Louis, MO, USA), or 1 μM and 10 μM all trans retinoic acid (ATRA). Fresh medium containing the differentiation inducers was added to the cultures every 72 hours. Cell viability was assessed at 3, 6, 9, and 12 days after culture by the trypan blue exclusion assay.

2. Reagents

As_2O_3 and ATRA were purchased from the Sigma Chemical Co. (St. Louise, MO, USA). Monoclonal mouse antibody for glycophorin A was obtained from Biogenesis Ltd (Poole, UK) and PE-conjugated anti-human glycophorin A was obtained from BD Pharmingen (Mountain view, CA, USA). Monoclonal mouse anti-human bcl-2 and polyclonal rabbit anti-caspase 3 were obtained from BD Pharmingen.

3. Flow Cytometric Analysis

Flow cytometry was performed on a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA). The expression of glycophorin A before and after treatment with As_2O_3 or ATRA in K562 cells was assessed by PE-conjugated anti-human glycophorin A. PE- conjugated anti-mouse IgG1 was used as a negative control and 10,000 events were counted for each analysis.

4. Western Blot Analysis

Expression of glycophorin A, Bcl-2, and caspase-3 were determined by western blot analysis. Cells were lysed in ice-cold PBS containing 1% Nonidet

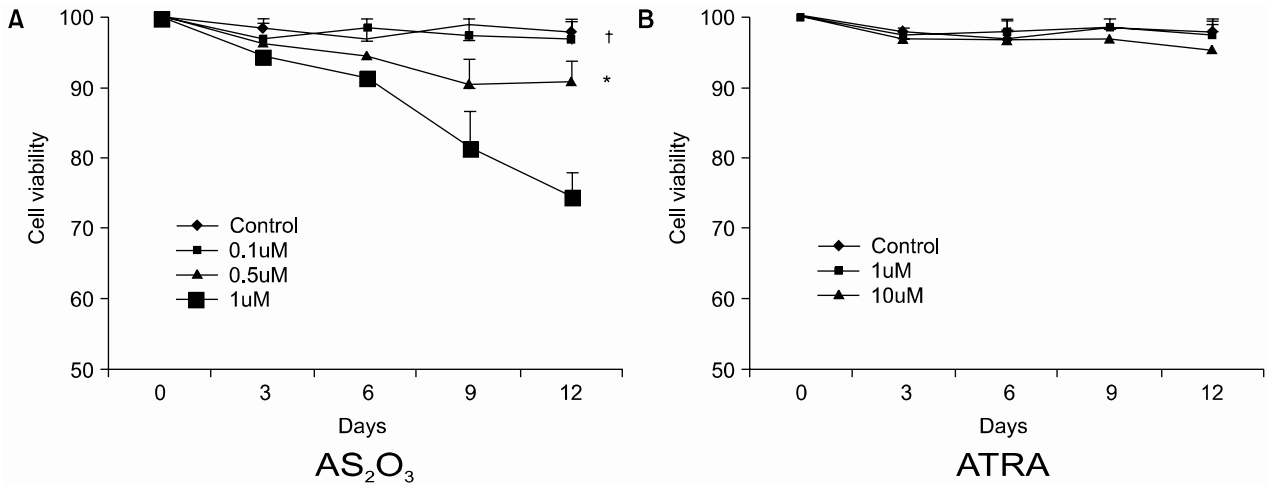

Fig. 1. Viability of K562 cells after treatment with As_2O_3 or ATRA. (A) The percentage of viable K562 cells after treatment with 1 μM of As_2O_3 began to decrease significantly 9 days after treatment. (B) The viabilities of K562 cells were 97%, 91%, and 74.5% by day 12 of treatment with 0.1 μM, 0.5 μM, and 1 μM of As_2O_3, respectively ($^*P<0.05$, $^\dagger P<0.05$). The viability of K562 cells was not changed by adding 1 μM and 10 μM of ATRA until day 12.

P-40, 0.5% sodium deoxycholate, 0.1% SDS, 2.0 μg/mL aprotinin and protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany) by passing them through a 21 gauge needle. Lysed cells were centrifuged at 12,000 rpm to remove cellular debris, and the protein concentrations of the extracts were determined by colorimetric bicinchoninic acid analysis (Pierce, Rockford, IL, USA). 30 μg of proteins were separated by 10% SDS-PAGE, and the proteins were electrophoretically transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 8% non-fat dry milk in TTBS (50 μM Tris-Hcl (pH 7.5), 150 μM NaCl, 0.1% (v/v) Tween 20) for 1 hour at room temperature and incubated in primary antibody diluted to 1 : 1,000 in TTBS/ 8% non-fat dry milk for 4 hours at room temperature. The membrane was then washed three times with TTBS for 15 minutes and subsequently incubated in HRP-conjugated goat anti-rabbit IgG (DAKO, Glostrup, Denmark) or HRP-conjugated goat anti-mouse IgG (Bio-Rad, Hercules, CA, USA) diluted to 1 : 3,000 in TTBS/ 8% non-fat dry milk for 1 hour at room temperature. The membrane was then washed three times, as described above and developed using the ECL detection system (Amersham, Arlington Heights, IL, USA).

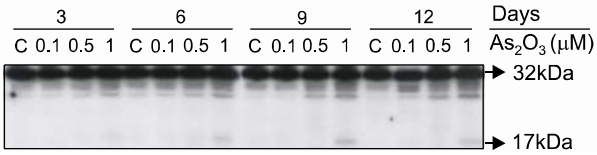

Fig. 2. Caspase 3 activation in K562 cells after treatment with As_2O_3. Activation of cleaved caspase-3 (17kDa) was observed in K562 cells treated with 1 μM of As_2O_3. Caspase-3 was not activated in K562 cells treated with a low dose As_2O_3 (0.1 μM and 0.5 μM).

5. Statistical Analysis

All of the assays were conducted in triplicate. The results are expressed as means±SD. Statistical analysis for the viability assay was determined by students t-test. Values of P less than 0.05 were considered statistically significant.

RESULT

1. Viability of K562 cells after treatment with As_2O_3 or ATRA

In the first set of experiments, we investigated the viability of K562 cells after treatment with

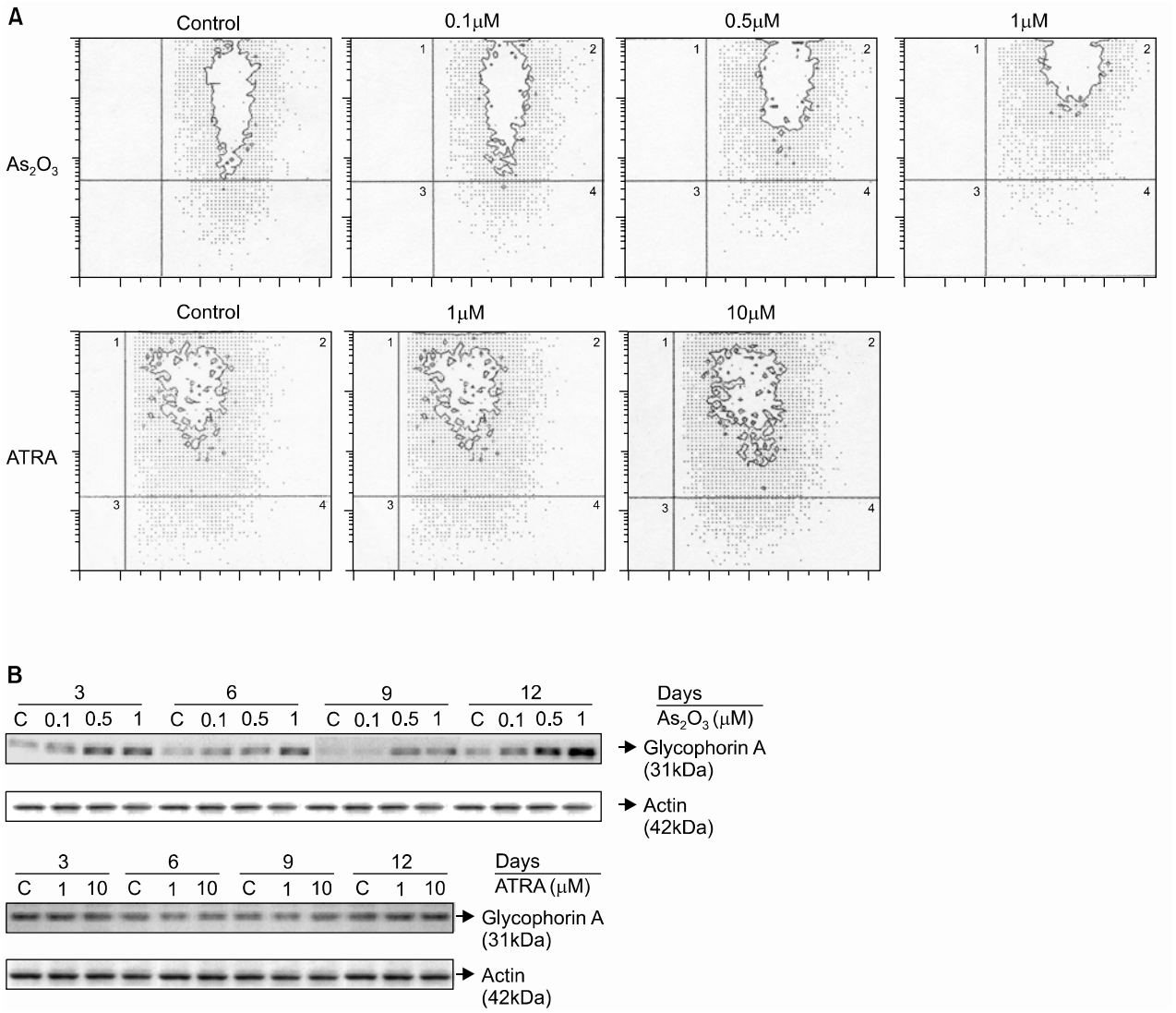

Fig. 3. Expression of glycophorin A in K562 cells after treatment with As_2O_3 or ATRA by using the flow cytometry and western blot analysis. (A) Flow cytometric analysis showed that the intensity of glycophorin A expression was increased in a dose dependent fashion in K562 cells after 3 days incubation with As_2O_3 but was unchanged in K562 cells incubated with ATRA. (B) Western blot analysis showed that treatment with As_2O_3 induced upregulation of glycophorin A in a dose dependent fashion, whereas glycophorin A remained unaltered when treated with ATRA.

As_2O_3 or ATRA by using the trypan blue exclusion assay (Fig. 1). The percentage of viable K562 cells after treatment with 1.0 μM of As_2O_3 began to decrease significantly 9 days after treatment. The viabilities of the K562 cells were 97%, 91%, and 74.5% by day 12 of treatment with 0.1 μM, 0.5 μM, and 1.0 μM of As_2O_3, respectively ($P < 0.05$). The viability of K562 cells was not changed by adding 1.0 μM and 10 μM of ATRA until day 12.

2. Induction of apoptosis by As_2O_3

To determine whether the cytotoxic effect induced by As_2O_3 is induced by apoptosis, we examined the expression of caspase-3, which plays an essential role in the induction of apoptosis, during the treatment of K562 cells with As_2O_3 or ATRA by a western blot analysis (Fig. 2). After

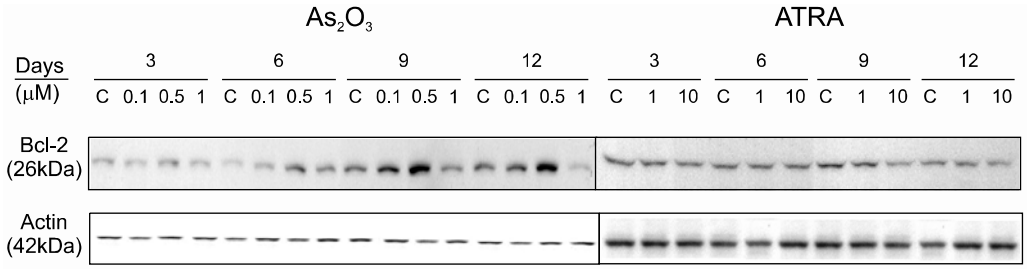

Fig. 4. Expression of Bcl-2 in K562 cells after treatment with As_2O_3 or ATRA. The expression of Bcl-2 was increased in a time and dose dependent fashion in K562 cells treated with 0.1 μM or 0.5 μM of As_2O_3 but abruptly decreased in K562 cells from 9 days after incubation with 1.0 μM of As_2O_3. Bcl-2 expression was not changed in K562 cells incubated with ATRA.

treatment, cleaved caspase-3 (17kDa) activation was observed in K562 cells treated with 1.0 μM of As2O3. Caspase-3 was not activated in K562 cells treated with 0.1 μM and 0.5 μM of As_2O_3 or ATRA (1 μM and 10 μM).

3. Erythroid differentiation of K562 cells

We next assessed the expression of glycophorin A in K562 cells after treatment with As_2O_3 or ATRA by flow cytometry and western blot. Flow cytometric analysis showed that glycophorin A expression was increased in a dose dependent fashion in K562 cells after 3 days of incubation with As_2O_3, but was unchanged in K562 cells incubated with ATRA (Fig. 3A). Western blot analysis showed that treatment with As_2O_3 induced the upregulation of glycophorin A in a dose dependent fashion, whereas glycophorin A remained unaltered when treated with ATRA (Fig. 3B).

4. Expressions of Bcl-2

Although, ATRA resistant K562 cells can undergo erythroid differentiation in the presence of 0.1 μM and 0.5 μM of As_2O_3, apoptosis was observed only at 1.0 μM of As_2O_3. To investigate whether abnormal expressions of apoptotic modulators are involved during erythroid differentiation and apoptosis, we examined the expressions of Bcl-2 in K562 cells after treatment with As_2O_3 or ATRA. The expression of Bcl-2 was increased in a time and dose dependent fashion in K562 cells treated with 0.1 μM or 0.5 μM of As_2O_3 but abruptly decreased in K562 cells treated with 1.0 μM of As_2O_3. Bcl-2 expression was unchanged in K562 cells incubated with ATRA (Fig. 4).

DISCUSSION

As_2O_3 at a dose of 1$\sim$2 μM was shown to have therapeutic effect against APL cells, even in patients resistant against ATRA or conventional chemotherapy, with minimal toxicity by induction of apoptosis of APL cells[3,10,20,21]. The success of As_2O_3 in inducing high rates of complete remission in patients who have relapsed with this aggressive, acute disease provides an impetus for uncovering its specific mechanisms of action that underlie these dramatic clinical responses. Although there has been considerable focus on PML- RARα and PML in terms of response to arsenic, recent investigation has yielded considerable data on the mechanisms by which arsenic acts on other cellular pathways. Arsenic acts on cells through a variety of mechanisms, influencing numerous signal transduction pathways, and this results in a vast range of cellular effects that include apoptosis induction, growth inhibition, promotion or inhibition of differentiation, and angiogenesis inhibition.[22] These cellular effects suggest a rationale for the evaluation of As_2O_3 as a possible therapy in other hematologic malignancies. Therefore, we investigated the in vitro effect of As_2O_3 on the K562 erythroid leukemia cell line.

In this study, we examined the in vitro effect of As_2O_3 on the erythroid differentiation of K562 cells and upon the expressions and regulations of the apoptotic modulators of this process. We used low doses of As_2O_3 (0.1～1 μM) because 1～2 μM of the plasma concentration of As_2O_3 is thought to be a safe range in human.[3] K562 cells undergo erythroid differentiation after treatment with As_2O_3. We found that the erythroid differentiation of K562 cells was observed from 0.1 μM of As_2O_3 but significant loss of cell viability by activation of an apoptotic process was only observed at 1.0 μM of As_2O_3. Activation of cleaved caspase-3 was also observed in K562 cells treated with 1.0 μM of As_2O_3. Caspase-3 was not activated in K562 cells treated with 0.1 μM and 0.5 μM of As_2O_3. Moreover, the apoptosis of K562 cells was associated with the diminished expression of Bcl-2 protein. The expression of Bcl-2 increased in a time and dose dependent fashion in K562 cells treated with 0.1 μM and 0.5 μM of As_2O_3 but abruptly decreased in K562 cells treated with 1.0 μM of As_2O_3. As_2O_3 has been shown to exert dose-dependent dual effects in APL cells, i.e., by triggering apoptosis and inducing partial differentiation.[1-3,23] Low dose As_2O_3 (0.1～0.5 μM)-induced differentiation might be mediated directly or indirectly by RARa-related signaling pathways in APL cells. On the other hand, high dose As_2O_3 (1.0 μM)- induced apoptosis involves collapse of the mitochondrial transmembrane potential, triggers the release of pro-apoptotic factor from mitochondria to cytoplasm, which is followed by caspase activation and the degradation of specific substrates.[1] Although As_2O_3 induced differentiation has been selectively observed in APL cells previously, in this study, we demonstrate the As_2O_3 induced erythroid differentiation of K562 erythroid leukemic cells. 0.1～0.5 μM of As_2O_3 induced glycophorin A expression without a loss of cell viability in K562 cells. These results suggest that low dose As_2O_3 induces erythroid differentiation in K562 cells, and that the activation of Bcl-2 was found to inhibit apoptosis. Benito et al.[18] demonstrated the erythroid differentiation of K562 and HEL cells after treatment with retinoic acid, hemin, or TGF-β. The erythroid differentiation of both HEL and K562 cells led to progressive loss of cell viability by activating an apoptotic process. In our study, the apoptosis of K562 cells was associated with the diminished expression of Bcl-2 protein. The expression of Bcl-2 increased in a time and dose dependent fashion in K562 cells treated with 0.1 μM and 0.5 μM of As_2O_3 but abruptly decreased in K562 cells treated with 1.0 μM of As_2O_3. These results indicate that apoptosis and erythroid differentiation proceed simultaneously, but that they can be uncoupled by the expression of Bcl-2. This data is in agreement with that reported for HL-60 myeloid leukemic cells, in that the overexpression of Bcl-2 cells did not affect their maturation, but prevented the apoptosis induced by myeloid differentiation.[19,24,25] Thus, the dose dependent dual effects in K562 cells treated with As_2O_3 can be explained by a decreased Bcl-2 expression. These results suggest that 0.1～0.5 μM of As_2O_3 induced erythroid differentiation in K562 cells and up-regulate Bcl-2 inhibited apoptosis. On the other hand, 1.0 μM of As_2O_3 potentially down-regulates Bcl-2 gene expression. There are some arguments for the change of Bcl-2 expression in K562 cells treated with As_2O_3. Zhu et al.[26] reported Bcl-2 expression did not change in K562 cells after incubation with 1.0 μM of As_2O_3 for 2 days but other investigators showed down-regulation of the Bcl-2 after incubation for 4 days with 2.0 μM of As_2O_3.[27] In our data, we found definite down-regulation of the Bcl-2 expression from 9 days after incubation with 1.0 μM of As_2O_3.

Our study suggests that As_2O_3 can induce K562 erythroid leukemic cell differentiation and apoptosis. 0.1～0.5 μM of As_2O_3 induced the erythroid differentiation of K562 cells but only 1.0 μM of As_2O_3 induced apoptosis. Dose dependent dual effects in K562 cells treated with As_2O_3 can be explained partly by a decreased Bcl-2 expression,

although the precise roles and mechanisms involved require further investigation.

요 약

배경: 삼산화비소(Arsenic trioxide, As_2O_3)는 급성전골수성백혈병의 효과적인 치료제로 알려져 있다. 뿐만 아니라 As_2O_3는 급성전골수성백혈병 이외의 골수성백혈병에서도 백혈병 세포의 세포고사를 유도하여 백혈병 세포의 증식을 억제한다. 그러나 As_2O_3를 이용한 기존의 연구들은 주로 골수구계로의 분화 및 세포고사에 관한 연구가 대부분이었으며 백혈병 세포의 적혈구계 분화에 대한 As_2O_3의 역할에 대하여는 아직 연구가 미진한 상태이다.

방법: 본 연구에서는 As_2O_3를 이용하여 적혈구계 급성골수성백혈병에서 기원한 K562 세포주의 분화를 유도하였으며 이 과정에서 세포고사에 관여하는 Bcl-2 유전자 발현의 변화를 검색하였다. 분화 유도를 위해서 K562 세포에 0.1 μM, 0.5 μM과 1 μM의 As_2O_3 또는 1 μM과 10 μM의 all trans retinoic acid (ATRA)를 처리하여 배양하였다. K562 세포에 As_2O_3 또는 ATRA 투여 전후에 glycophorin A의 발현 여부를 flow cytometry와 western blot을 이용하여 확인하였으며, Bcl-2와 caspase 3의 발현을 western blot을 이용하여 확인하였다.

결과: K562 세포의 활성도는 0.1과 0.5 μM의 As_2O_3를 투여한 경우에는 변화가 없었으나 1 μM을 투여한 경우에는 의미 있게 감소하였다. Caspase 3 역시 0.1과 0.5 μM의 As_2O_3를 투여한 경우에는 배양 12일까지도 변화가 없었으나 1 μM을 투여한 경우에는 배양 3일부터 발현이 증가하였다. Glycophorin A는 As_2O_3를 투여한 경우 용량에 비례하여 발현이 증가하였으나 ATRA를 투여한 경우에는 glycophorin A의 발현은 변화가 없었다. Bcl-2의 발현은 0.1과 0.5 μM의 As_2O_3를 투여한 경우에는 변화가 없었으나 1 μM을 투여한 경우에는 발현이 감소하였다.

결론: As_2O_3는 저용량의 투여에 의하여도 K562 세포의 적혈구계 분화를 유도하였으며 1 μM을 투여한 경우에는 K562 세포의 apoptosis를 유도하였다. As_2O_3에 의한 K562 세포의 세포고사는 Bcl-2의 발현 감소와 관계가 있었다. 본 연구의 결과는 As_2O_3가 골수세포의 적혈구계 분화 및 세포고사에 관여함을 보여주며 향후 이에 관한 지속적인 연구가 필요하리라 생각한다.

REFERENCES

1. Cai X, Shen YL, Zhu Q, et al. Arsenic trioxide- induced apoptosis and differentiation are associated respectively with mitochondrial transmembrane poten- tial collapse and retinoic acid signaling pathways in acute promyelocytic leukemia. Leukemia 2000;14: 262-70.
2. Chen GQ, Zhu J, Shi XG, et al. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with down-regulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 1996;88:1052-61.
3. Chen GQ, Shi XG, Tang W, et al. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytoc leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 1997;89:3345-53.
4. de The H, Chomienne C, Lanotte D, Degos L, Dejean A. The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. Nature 1990;347:558-61.
5. Kakizuka A, Miller WH Jr, Umesono K, et al. Chromosomal translocation t(15;17) in acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell 1991;66:663-74.
6. Rousselot P, Labaume S, Marolleau JP, et al. Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and in plasma cells from myeloma patients. Cancer Res 1999;59:1041-8.
7. Zhang W, Ohnishi K, Shigeno K, et al. The induction of apoptosis and cell cycle arrest by arsenic trioxide in lymphoid neoplasms. Leukemia 1998;12:1383-91.
8. Walter R, Schoedon G, Bachli E, et al. Establishment and characterization of an arsenic-sensitive monoblastic leukemia cell line (SigM5). Brit J of Haematol 2000; 109:396-404.
9. Rojewski MT, Baldus C, Knauf W, Thiel E, Schrezenmeier H. Dual effects of arsenic trioxide (As_2O_3) on non-acute promyelocytic leukaemia myeloid cell lines: induction of apoptosis and inhibition of proliferation. Br J Haematol 2002;116:555-63.
10. Chen F, Lu Y, Zhang Z, et al. Opposite effect of NF-kappa and c-Jun-N-terminal kinase on p53-independent GADD45 induction by arsenite. J Bio Chem 2001;276:11414-9.
11. Zhang TC, Cao EH, Li JF, Ma W, Qin JF. Induction of apoptosis and inhibition of human gastric cancer MGC-803 cell growth by arsenic trioxide. Eur J

Cancer 1999;35:1258-63.
12. Seol JG, Park WH, Kim ES, et al. Potential role of caspase-3 and -9 in arsenic trioxide-mediated apoptosis in PCI-1 head and neck cancer cells. Int J Oncol 2001;18:249-55.
13. Kitamura K, Minami Y, Yamamoto K, et al. Involvement of CD95-independent caspase 8 activation in arsenic trioxide-induced apoptosis. Leukemia 2000; 14:1743-50.
14. Perkins C, Kim CN, Fang G, Bhalla KN. Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x(L). Blood 2000;95:1014-22.
15. Mahieux R, Pise-Masison C, Gessain A, et al. Arsenic trioxide induces apoptosis in human T-cell leukemia virus type 1- and type 2-infected cells by a caspase-3-dependent mechanism involving Bcl-2 cleavage. Blood 2001;98;3762-9.
16. Jiang XH, Wong BC, Yuen ST, et al. Arsenic trioxide induces apoptosis in human gastric cancer cells through up-regulation of p53 and activation of caspase-3. Int J Cancer 2001;91:173-9.
17. Woo SH, Park IC, Park MJ, et al. Arsenic trioxide induces apoptosis through a reactive oxygen species- dependent pathway and loss of mitochondrial membrane potential in HeLa cells. Int J Oncol 2002; 21: 57-63.
18. Benito A, Silva M, Grillot D, Nunez G, Fernandez-Luna JL. Apoptosis induced by erythroid differentiation of human leukemic cell lines is inhibited by Bcl-XL. Blood 1996;87:3837-48.
19. Benito A, Grillot D, Nunez G, Fernandez-Luna JL. Regulation and function of Bcl-2 during differentiation-induced cell death in HL-60 promyelocytic cells. Am J Pathol 1995;146;481-90.
20. Soignet SL, Maslak P, Wang ZG, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med 1998;339:1341-8.
21. Soignet SL, Frankel SR, Douer D, et al. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 2001;19:3852-60.
22. Miller WH Jr, Schipper HM, Lee JS, Singer J, Waxman S. Mechanisms of action of arsenic trioxide. Cancer Res 2002;62:3893-903.
23. Gianni M, Kohen MHM, Chelbi-Alix MK, et al. Combined arsenic and retinoic acid treatment enhances differentiation and apoptosis in arsenic-resistant NB4 cells. Blood 1998;91:4300-10.
24. Naumovki L, Cleary ML. Bcl-2 inhibits apoptosis associated with terminal differentiation of HL-60 myeloid leukemic cells. Blood 1994;83:2261-7.
25. Park JR, Robertson K, Hickstein DD, Tsai S, Hocken berry DM, Collins SJ. Dysregulated Bcl-2 expression inhibits apoptosis but not differentiation of retinoic acid-induced HL-60 granulocytes. Blood 1994;84:440-5.
26. Zhu J, Okumura H, Ohtake S, Nakamura S, Nakao S. The molecular mechanism of arsenic trioxide-induced apoptosis and oncosis in leukemia/lymphoma cell lines. Acta Hematol 2003;110:1-10.
27. Zhang Y, Shen WL. Bcl-2 antisense oligodeoxynucleotide increases the sensitivity of leukemic cells to arsenic trioxide. Cell Biol Int 2003;27:953-8.