Leukemia & Lymphoma, February 2005; 46(2): 257–263

Taylor & Francis
Taylor & Francis Group

Biological effects of Atra and Arsenic Trioxide on short term cultures of non-M3 leukemic blasts

MARIA COSENZA, MONICA CIVALLERO, STEFANO SACCHI, RAFFAELLA MARCHESELLI, & SAMANTHA POZZI

Program of Innovative Therapy in Oncology and Hematology, Department of Oncology and Hematology, University of Modena, Policlinico, Italy

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

Abstract
The efficacy of All-Trans Retinoic Acid (Atra) and Arsenic Trioxide (As_2O_3) in the treatment of Acute Promyelocytic Leukemia (APL) is well known. Further, these drugs inhibit cell growth and induce apoptosis in several cell lines, but few data are reported on leukemic blasts.
The aim of this study was to evaluate the biological effects on non-M3 Acute Myeloid Leukemia (AML) cells. Blasts of six patients, after exposition to Atra and As_2O_3 were tested for growth inhibition, induction of apoptosis and change in cell cycle distribution, evaluating cell viability, percentage of apoptotic cells and of blasts positive for Ki-67 and BrdU. In the present study we demonstrated that either Atra or As_2O_3 inhibit leukemic cells proliferation by induction of apoptosis. The effects are time and dose dependent. We did not observe additive or synergistic effects with the combination of the drugs. Further, our results showed that Atra and As_2O_3 have effects on cell cycle distribution reducing S-phase and proliferating cells. These results should be taken in to account preparing future laboratory and clinical experimental protocols that associate these drugs with antineoplastic agents with different cell cycle specificity.

Keywords: *Atra, arsenic trioxide, non-M3 leukemic blasts, growth inhibition, apoptosis, cell cycle*

Introduction

All-Trans Retinoic Acid (Atra) is effective for induction of complete remission (CR) in Acute Promyelocytic Leukemia (APL) [1–4]. Atra acts to circumvent the differentiation block of APL cells by targeting PML-RARα chimeric receptor and activating the gene expression network necessary for cell differentiation [5]. The drug acts by inducing apoptosis and also down-regulates the expression of bcl-2 [6]; this gene is a modulator of apoptosis that antagonizes apoptotic cell death. *In vivo*, Atra exerts antitumoral effects in combination with IFN-alpha, in other malignancies [7–8] and inhibits cell growth in different cell lines [9–13]. Studies *in vitro* have also shown effects of Atra alone or in combination with chemotherapeutic agents in non-M3 Acute Myeloid Leukemia (AML) cells [14–17].

Recently, Arsenic Trioxide (As_2O_3), an ancient drug used in traditional Chinese Medicine attracted wide interest for its ability to induce CR in most patients with APL [18–21]. APL is uniquely sensitive to As_2O_3, which degrades the PML-RARα fusion protein characteristic of the disease, promoting differentiation. As_2O_3 may have a similar effect on fusion proteins in other malignancies [22]. *In vitro* studies have shown in APL cells that As_2O_3 exerts dose-dependent dual effects: it triggers apoptosis at higher concentrations with a strong down-regulation of the bcl-2 [23] and induces a partial differentiation at lower concentrations [24]. As_2O_3 shows these activities not only in untreated patients but also in patients resistant to chemotherapy and Atra [25]. As_2O_3 has also been shown to induce apoptosis and inhibit cell growth in malignant lymphoid cell lines [26–28], in cells from patients with myeloma [29], in chronic myelogenous leukemia cells [30] and in cells from patients with non-M3 AML [31]. These data suggest the ability of As_2O_3 to induce apoptosis

Correspondence: S. Sacchi, Program of Innovative Therapy in Oncology and Hematology, Department of Oncology and Hematology, University of Modena, Centro Oncologico Modenese, Policlinico, 41100 Modena, Italy. Tel: +39-059-4222175. Fax: +39-059-4223306. E-mail address: ssacchi@unimo.it

Received for publication 9 July 2004.

ISSN 1042-8194 print/ISSN 1029-2403 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/10428190400014991

RIGHTSLINK

not only in APL. Several reports showed evidence that the activation of caspases family are involved in As_2O_3-induced apoptosis [26,32]. *In vitro* studies on arsenic resistant NB4 cells showed that Atra and As_2O_3 synergize for induction of differentiation and apoptosis and cells resistant to one agent are sensitive to the other [33].

The aim of this study was to evaluate, *in vitro*, the biologic effects of Atra and As_2O_3 on myeloid blasts from patients with non-M3 AML. Therefore, we have investigated cellular growth, apoptosis and cell cycle distribution induced by these drugs, utilized either alone or in combination.

Materials and methods

Patients and samples

After informed consent, peripheral blood samples were obtained from six patients with non-M3 AML at the time of diagnosis. Four patients had M1 and two M2 AML. Peripheral blood mononuclear cells (MNCs) were separated by centrifugation on Ficoll's solution (Nycomed Pharma). After isolation, leukemic blast were more than 90%. Leukemic cells were cryopreserved at $-80°$ C for 24 h and then in liquid nitrogen in the presence of 50% heat-inactivated foetal bovine serum (FBS, Gibco, Life Technologies), 10% dimethyl sulfoxide (Carlo Erba) and RPMI 1640 (Gibco, Life Technologies).

Reagents

Atra was purchased by Sigma Aldrich. It was prepared just before use in 100% ethanol and diluted into RPMI 1640 at the desired concentration. The final concentration of ethanol was less than 0.01% in the samples. All experiments involving Atra were performed in subdued light, and the tubes and culture plates containing Atra were covered with aluminium foil. Atra was stored at $-20°C$ in the dark.

Arsenic trioxide was purchased from Sigma Aldrich. A stock solution was made with phosphate-buffered saline (PBS) (Gibco, Life Technologies) at concentration of 10^{-2} mol/l.

Cultures of leukemic cells

For culture experiments, the cells were quickly thawed, washed twice with RPMI 1640 and cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM glutamine (Gibco, Life Technologies), 100 UI/ml each streptomycin and penicillin (Gibco, Life Technologies). The myeloid blast were quickly thawed and then incubated at 37°C in 5% CO_2 for 24 h and used in short term cultures. Cell viability after thawing was always more than 80%, as assessed by tripan-blue staining (Gibco, Life Technologies).

Cell proliferation inhibition

The leukemic cells were counted at the start of the experiment and after 24 h and 48 h. The cells were mixed with 2% tripan-blue staining and both total and viable cells were counted in triplicate in a haemacytometer.

Apoptosis study

Cell morphology

For morphological observation, the myeloid blasts were centrifuged onto slides by cytospin, stained with May-Grunwald Giemsa and examined under light microscope. Apoptosis is distinguishable from cellular necrosis by a unique series of ultrastructural changes, including chromosomal and cytoplasmic condensation, nuclear fragmentation, membrane blebbing and the formation of membrane-bound apoptotic bodies.

Terminal deoxynucleotidyltransferase mediated dUTP nick-end labeling (TUNEL) assay

DNA degradation was estimated by labeling DNA strand breaks with terminal deoxynucleotidyltransferase (TdT).

TUNEL assay was performed with the use of the in situ Cell Death Detection Kit, AP according to manufacturer's instruction (Roche, Molecular Biochemicals). Fast red tables (Roche, Molecular Biochemicals) were used as the chromogenic substrate. Cells were analysed by fluorescence microscope.

Cell cycle study

Immunocytochemical detection of Ki-67

The leukemic cells after 48h of exposure with drugs and the cells from control cultures, were washed with PBS, centrifuged onto slides by cytospin at 4500 r.p.m. for 5 min and air dried. The cells were fixed in acetone for 10 min at 4°C after 18–24 h. The immunocytochemical detection of Ki-67 was performed with the APAAP method [34]; the slides were counterstained with Gill's hematoxylin for 5 min and examined under light microscope.

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

RIGHTSLINK

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

Immunocytochemical detection of BrdU

The leukemic cells after 48 h of exposure with drugs as well as controls were pulse labeled for 30 min with 10 μM BrdU (Bromodeoxiuridin) (Sigma Chemical) at 37°C in 5% CO_2; then the cells were washed with PBS, centrifuged onto slides and air dried. After 18–24 h, the cells were then fixed in 70% ethanol for 30 min at 4°C. The immunocytochemical detection of BrdU was performed with the APAAP method. The rabbit mouse antibody (BrdU) was diluted 1 : 50 in Tris-buffered saline (TBS) 0.05 ml pH 7.6.

Statistical analysis

All experiments were set up in triplicate, and the results were expressed as the mean $\pm$ SD of the six samples. Statistical analysis was performed by Bonferroni test, Student's *t*-test and linear regression.

Results

Effects of Atra and As_2O_3 on growth inhibition

The myeloid blasts were plated in 24-well plate (Nunc) at a density of 1×10^{-6} cell ml^{-1}. After 24 h the leukemic cells were treated with: (1) Atra and As_2O_3 alone at various concentrations (10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M); (2) with a combination of both drugs at a concentration of 10^{-6} M. In the combination studies, the blasts were treated (a) with simultaneous addition of both drugs; (b) with either Atra or As_2O_3 for 6 h before the addition of the second drug. In the control cultures, medium diluted ethanol and PBS were utilized instead of Atra and As_2O_3 solution.

Experiments with Atra alone or in combination with As_2O_3 were performed using a yellow light to avoid photodecomposition of Atra.

Atra and As_2O_3 alone. The treatment with the drugs at a concentration of 10^{-9} M and 10^{-8} M did not have a statistically significant inhibitory effect on cellular growth in comparison with the control (P = ns). Utilizing concentrations of Atra at 10^{-7} M, 10^{-6} M, 10^{-5} M and of As_2O_3 10^{-6} M, 10^{-5} M we observed an important inhibition that was statistically significant ($P < 0.001$), reaching the 80% with the concentration of 10^{-5} M after 48 h. Statistical analysis showed that the inhibition of proliferation is time ($r^2 \geqslant 0.90$; $P < 0.001$) and dose dependent ($P < 0.001$). The results of the experiments are reported in Figure 1a,b.

Combination treatments. Simultaneous and the sequential treatment either with Atra or As_2O_3 followed 6 h later by As_2O_3 or Atra at concentration of 10^{-6} M, showed after 48 h of exposition, a percentage of inhibition similar to that obtained with single drugs at the same dose (P = ns). Thus, we did not observe additive or synergistic effect between the two drugs. The results of the experiments are reported in Figure 1c.

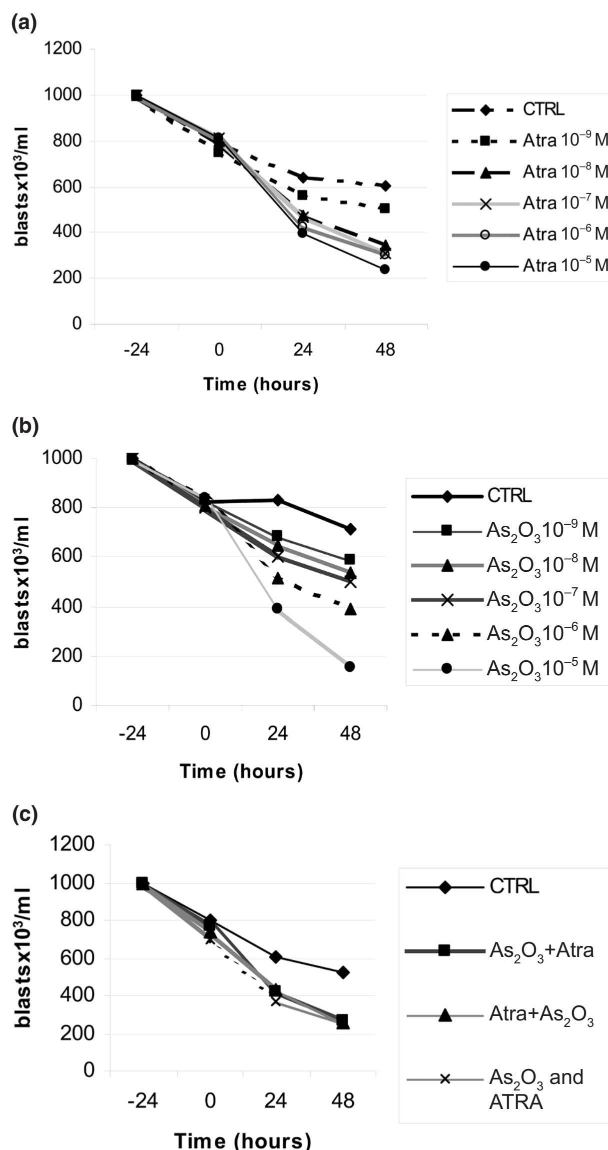

Figure 1. Inhibitory effect on cellular proliferation of Atra (a), As_2O_3 (b) at various concentrations and in combination at the concentration of 10^{-6} M (c). Results are expressed as mean of six experiments performed in triplicate. SD is not reported. CTRL: control; As_2O_3 + Atra, Atra + As_2O_3: addition of the second drugs after 6 h; As_2O_3 and Atra: simultaneous addition.

Effects of Atra and As_2O_3 on apoptosis

Leukemic blasts were exposed to single drugs and combinations for 48 h and then were centrifuged onto slides by cytospin. Apoptotic cells were defined

RIGHTSLINK

by apoptotic morphology and by typical labeling with fluorescent antibodies. On each slide, 200–400 cells were counted under light or fluorescent microscope and the percentage of apoptotic cells of the total cells number was calculated.

Morphological analysis by May-Grunwald Giemsa staining

In the controls, spontaneous apoptosis varied from 20–30%. Both drugs and combinations determined an increase of apoptotic cells.

Statistically significant differences were observed only with Atra and As_2O_3 at concentrations of 10^{-6} M and 10^{-5} M ($P < 0.001$). Because these results are similar to those obtained by TUNEL method, the percentage of apoptotic cells observed after May-Grunwald Giemsa staining are not reported

Morphological analysis by TUNEL

We have utilized the TUNEL method that consists of attaching the fluorescein isothiocyanate-conjugated dUTP to DNA fragments by TdT enzyme.

Atra and As_2O_3 alone. The treatment with the drugs at the concentration of 10^{-9} M did not have a significative effect (P = ns) on the apoptosis in comparison with the controls.

The percentage of apoptotic blasts after treatment with Atra and As_2O_3 alone at 10^{-6} M was 47 and 54%, respectively; at 10^{-5} M the percentage was 80 and 90%, respectively, (Figure 2a,b). Statistical differences in comparison with controls were highly statistically significant ($P < 0.001$).

Atra and As_2O_3 in combination. Simultaneous and sequential treatment with Atra or As_2O_3 followed 6 h later by As_2O_3 or Atra at concentration of 10^{-6} M, showed a percentage of apoptotic cells similar to that obtained with single drugs at the same concentration (P = ns). Thus, we did not observe additive or synergistic effect between the two drugs (Figure 2c).

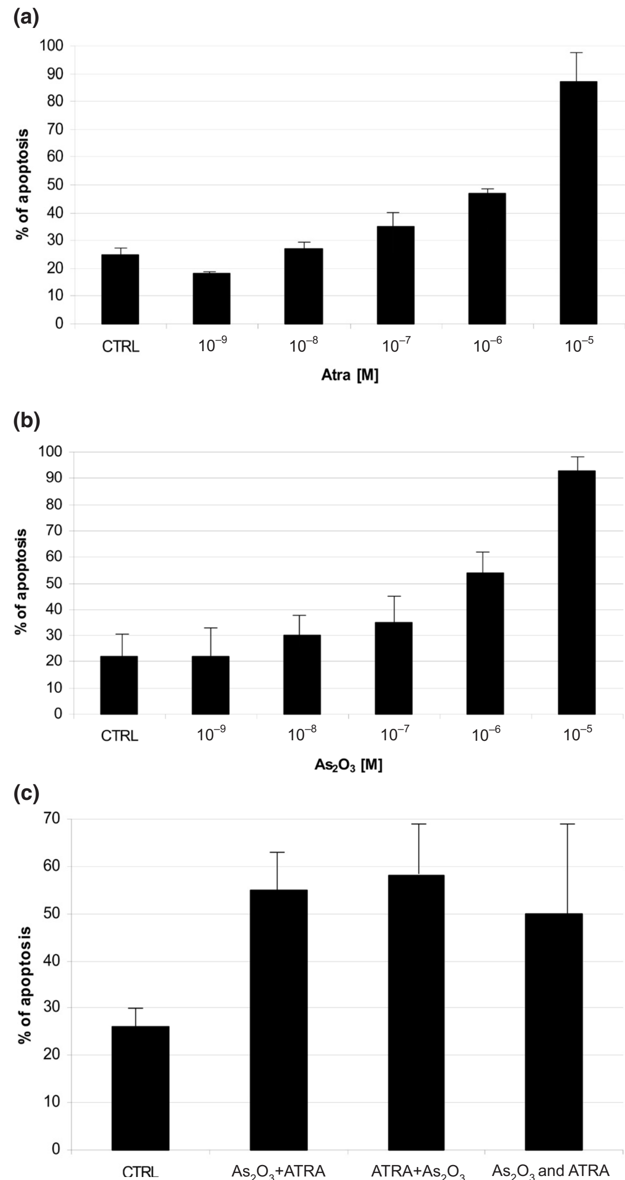

Figure 2. Percentage of apoptotic cells evaluated with TUNEL assay of Atra (a), As_2O_3 (b) at various concentrations and in combination at concentration of 10^{-6} M (c). Results are expressed as mean ± SD of six experiments performed in triplicate. CTRL: control; As_2O_3 + Atra, Atra + As_2O_3: addition of the second drugs after 6 h; As_2O_3 and Atra: simultaneous addition.

Effects of Atra and As_2O_3 on cell cycle

The effects of Atra and As_2O_3 alone and in combination on the cell cycle were investigated by expression of cell cycle related protein Ki-67 and by incorporation of BrdU. The analysis of the cellular cycle has been made after 24 and 48 h of treatments. After 24 h, the reduction of positive cells in comparison with controls was minimal (P = ns) and the data are not reported. After 48 h, a statistically significant decrease ($P < 0.001$) in the percentage of both Ki-67 positive cells and S-phase cells was observed in all the samples analyzed treated with Atra and As_2O_3 at concentration of 10^{-7} M, 10^{-6} M and 10^{-5} M (Figure 3a,b).

The results with combinations of drugs showed a percentage of positive cells similar to that obtained with single drugs at the same concentration (P = ns). Thus we did not observe additive or synergistic effect between the two drugs (Figure 3c).

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

RIGHTSLINK

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

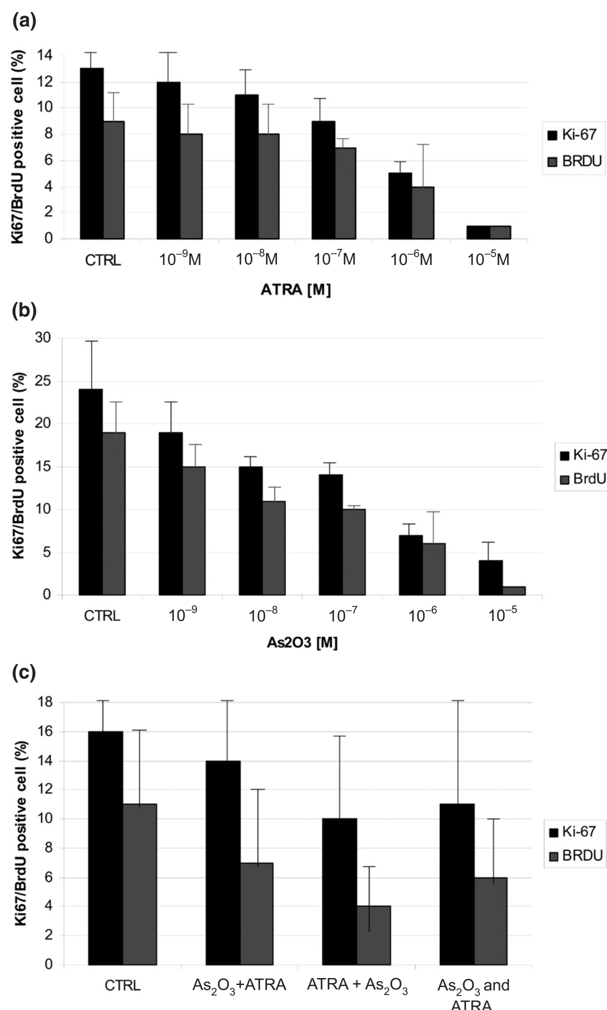

Figure 3. Percentage of Ki-67 and BrdU positive cells after 48 h of treatment with Atra (a), As_2O_3 (b) at various concentrations and in combination at the concentration of 10^{-6} M (c). Results are expressed as mean ± SD of four experiments performed in triplicate. ■ Ki-67, ■ BrdU. CTRL: control; As_2O_3 + Atra, Atra + As_2O_3: addition of the second drugs after 6 h; As_2O_3 and Atra: simultaneous addition.

Discussion

Acute myeloid leukemia is a neoplastic pathology characterized from an uncontrolled cellular proliferation. In basic and clinical cancer research one of the most important goals is to identify treatments able to reduce cancer cell growth.

It is known that anticancer drugs work mostly by inducing apoptosis in target cells or by inducing them to differentiate. Atra was first introduced to clinical use for the treatment of APL and now its effects are well know. The drug acts by induction of differentiation and apoptosis. Some clinical trials with Atra associated with chemotherapeutic agents in AML patients showed conflicting results in terms of efficacy [35,36] and an increased toxicity, when associated with Interferon-alpha for treating Chronic Myeloid Leukemia patients [37].

Recently, As_2O_3 was shown to induce complete remission in a high percentage of patients with APL in relapse after treatment with Atra and chemotherapy [18,20,21]. Atra and As_2O_3 exert antitumoral effects in other malignancies than APL and inhibit cell growth in different cell lines [9–14,26–30]. In NB4 cell line, Atra and As_2O_3 synergize for induction of differentiation and apoptosis and that cell resistant to one agent are sensitive to the other [33].

Until now, there have been few reports [14–17,31] about the effect of Atra and As_2O_3 on leukemic cells derived from non-M3 AML patients. Therefore, the aim of the study was to evaluate, *in vitro*, the biologic effects of Atra and As_2O_3 alone and variously combined on myeloid blast from non-M3 AML patients.

In the present study, we demonstrated that either Atra or As_2O_3 inhibit leukemic cells proliferation by induction of apoptosis, although the specific apoptotic pathway is not yet clear. Increased apoptosis could be determined by down-regulation of bcl-2 [6], that is over expressed in leukemic blasts [38,39]. Other observed mechanisms by which Atra and As_2O_3 may induce apoptosis are JNK activation [40], cell cycle arrest [41,42] and expression of TRAIL (tumor necrosis factor related apoptosis-inducing ligand) [43]. These effects are dependant on exposure time and drug concentrations. Atra and As_2O_3 at the 10^{-5} M induced more than 80% morphological apoptosis after 48 h of incubation. This concentration might be too toxic if applied *in vivo* [44] and cause also suppression of normal myeloid hematopoiesis. Concentration of 10^{-6} M maintain strong effect on apoptosis induction while being clinically obtainable and tolerable [18,45]. Smaller concentration (10^{-9} M) can also cause a inhibition of proliferation and apoptotic cell death, but required a longer exposure [17]. Atra and As_2O_3 synergistic apoptotic effect has been observed either in primary APL cell or NB4 cell starting from 60 h of drug exposure [46]. We did not observe additive or synergistic effect in inducing growth inhibition or apoptosis with the combination of the drugs but we determined the percentage of apoptosis only after 24 and 48 h.

Further, our results showed that these drugs have effects on cell cycle distribution, reducing S-phase and proliferating cells. This implies that these drugs are able to arrest leukemic cells in earlier cell cycle phase. Thus, Atra or As_2O_3 cause growth inhibition by induction of apoptosis and/or by determining specific change in cell cycle progression.

RIGHTS LINK

These results should be taken in to account when preparing future laboratory and clinical experimental protocols that associate these drugs with antineoplastic agents with different cell cycle specificity.

Acknowledgements

This work was supported in part by grants from MIUR (to MD Sacchi and BS Civallero) and from the "Angela Serra Association for Cancer Research" (to BS Consenza, BS Marcheselli and MD Pozzi).

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

References

[1] Huang ME, Ye YC, Chen SR, Chai Jr, Lu JX, Zhoa L, Gu LJ, Wang ZY. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood 1988;72(2):567–572.

[2] Castaigne S, Chomienne C, Daniel MT, Ballerini P, Berger R, Fenaux P, Degos L. All-trans-retinoic acid as a differentiating therapy for acute promyelocytic leukemia. Clinical results. Blood 1990;76(9):1704–1709.

[3] Warrell RP, Frankel SR, Miller WH, Scheinberg DA, Itri ML, Hittelman WN, Vyas R, Andreeff M, Tafuri A, Jacubowski A, et al. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). New England Journal of Medicine 1991;324(20): 1385–1393.

[4] Tallman MS, Andersen JW, Appelbaum ASC, Appelbaum FR. All-trans-retinoic acid in acute promyelocytic leukemia. New England Journal of Medicine 1997;337(15):1021–1028.

[5] Chen Z, Tong JH, Dong S, Zhu J, Wang ZY, Chen SJ. Retinoic acid regulatory pathways, chromosomal translocations and acute promyelocytic leukemia. Genes Chromosomes and Cancer 1996;15(3):147–156.

[6] Andreeff M, Jiang S, Zhang X, Konopleva M, Estrov Z, Snell VE, Xie Z, Okcu MF, Sanchez-Williams G, Dong J, et al. Expression of Bcl-2-related genes in normal and AML progenitors: changes induced by chemotherapy and retinoic acid. Leukemia 1999;13(11):1881–1892.

[7] Athanasiadis I, Kies MS, Miller M, Ganzenko N, Joob A, Marymont M, Rademaker A, Gradishar WJ. Phase II study of all-trans-retinoic acid and alfa-interferon in patients with advanced non-small cell lung cancer. Clinical Cancer Research 1995;1(9):973–979.

[8] Bailey J, Pluda JM, Foli A, Saville MW, Bauza S, Adamson PC, Murphy RF, Cohen RB, Broder S, Yarchoan R. Phase I/II study of intermittent all-trans-retinoic acid, alone and in combination with interferon alfa-2a in patients with epidemic Kaposi's sarcoma. Journal of Clinical Oncology 1995;13(8):1966–1974.

[9] Krupitza G, Hulla W, Dittrich E, Kallay E, Harant H, Huber H, Grunt T, Dittrich C. Retinoic acid induced death of ovarian carcinoma cells correlates with c-myc stimulation. International Journal of Cancer 1995;61(5):649–657.

[10] Massad LS, Turyk M, Bitterman EP, Wilbanks GD. Interferon-alfa and all-trans-retinoic acid reversibly inhibit the in vitro proliferation of cell lines derived from cervical cancers. Gynecologic Oncology 1996;60(3):428–434.

[11] Moasser MM, Motzer RJ, Khoo KS, Lyn P, Murphy BA, Bosl GJ, Dmitrovsky E. All-trans-retinoic acid for treating germ cell tumors: in vitro activity and results of a phase II trial. Cancer 1995;76(4):680–686.

[12] Ponzoni M, Bocca P, Chiesa V, Decensi A, Pistoia V, Raffaghello L, Rozzo C, Montaldo PG. Differential effects of *N*-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: apoptosis versus differentiation. Cancer Research 1995;55(4):853–861.

[13] Mangiarotti R, Danova M, Alberici R, Pellicciari C. All-trans retinoic acid (ATRA) induced apoptosis is preceded by G1 arrest in human MCF-7 breast cancer cells. British Journal of Cancer 1998;77(2):186–191.

[14] Lehmann S, Bengtzen S, Broberg U, Paul C. Effects of retinoids on cell toxicity and apoptosis in leukemic blast cells from patients with non-M3 AML. Leukemia Research 2000;24(1):19–25.

[15] Lishner M, Curtis JE, Minkin S, McCulloch EA. Interaction between retinoic acid and cytosine arabinoside affecting the blast cells of acute myeloblastic leukemia. Leukemia 1989;3(11):784–788.

[16] Yang GS, Minden MD, McCulloch EA. Regulation by retinoic acid and hydrocortisone of the anthracycline sensitivity of blast cells of acute myeloblastic leukemia. Leukemia 1994;8(12):2065–2075.

[17] Zheng A, Savolainen ER, Koistinen P. All-trans retinoic acid induces apoptosis in acute myeloblastic leukaemia cells. Apoptosis 1997;2(3):319–329.

[18] Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, et al. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 1997;89(9):3354–3360.

[19] Wang Zhen-Hi. Arsenic compound as anticancer agents. Cancer Chemotherapy and Pharmacology 2001;48(Suppl 1):72–76.

[20] Chen Z, Chen GQ, Shen ZY, Chen SY, Wang ZY. Treatments of acute promyelocytic leukemia with arsenic trioxide compound: in vitro and in vivo. Seminars in Hematology 2001;38(1):26–36.

[21] Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, Wu W, Zhang FQ, Chen Y, Zhou L, et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood 1999;94(10):3315–3324.

[22] Davison K, Mann KK, Miller WH Jr. Arsenic trioxide: mechanism of action. Seminars in Hematology 2002;39 (2 Suppl 1): 3–7.

[23] Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY. In vitro studies on cellular and molecular mechanism of Arsenic Trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with downregulation of Bcl-2 of PML-RAR(/PML proteins. Blood 1996;88(3):1052–1061.

[24] Chen GQ, Shi XG, Tang W, Xiong XM, Zhu J, Cai X. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 1997;89(9):3345–3353.

[25] Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, Corso D, DeBlasio A, Gabrilove J, Scheinberg DA, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. New England Journal of Medicine 1998;339(19):1341–1348.

[26] Akao Y, Mizoguchi H, Kojima S, Naoe T, Ochichi N, Yagi N. Arsenic induces apoptosis in B-cell leukemic cell lines in vitro: activations of caspases and downregulation of bcl-2 protein. British Journal of Hematology 1998;102(4):1055–1060.

RIGHTSLINK

[27] Konig A, Wrazel L, Warrel RP Jr, Rivi R, Pandolfi PP, Jakubowski A, Gabrilove JL. Comparative activity of melarsoprol and arsenic trioxide in chronic B-cell leukemia cell lines. Blood 1997;90(2):562 – 570.

[28] Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y, Tang W, Shi GY, Sun YP, Dai J, et al. Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinical achievable concentrations. Journal of National Cancer Institute 1999;91(9):772 – 778.

[29] Rousselot P, Labaume S, Marolleau JP, Larghero J, Noguera MH, Brouet JC, Fermand JP. Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and in plasma cells from myeloma patients. Cancer Research 1999;59(5):1041 – 1048.

[30] Puccetti E, Guller S, Orleth A, Bruggenolte N, Hoelzer D, Ottmann OG, Ruthardt M. BCR-ABL mediates arsenic trioxide-induced apoptosis independently of its aberrant kinase activity. Cancer Research 2000;60(13):3409 – 3413.

[31] Lehmann S, Bengtzen S, Paul A, Christensson B, Paul C. Effect of arsenic trioxide (As_2O_3) on leukemic cells from patients with non-M3 acute myelogenous leukemia: studies of cytotoxicity, apoptosis and the pattern of resistance. European Journal of Haematology 2001;66(6):357 – 364.

[32] Huang XJ, Wiernik PH, Klein RS, Gallagher RE. Arsenic trioxide induces apoptosis of myeloid leukemia cells by activation of caspases. Medical Oncology 1999;16(1):58 – 64.

[33] Gianni M, Koken MH, Chelbi-Alix MK, Benoit G, Lanotte M, Chen Z, de The H. Combined arsenic trioxide and retinoic acid treatment enhances differentiation and apoptosis in arsenic resistant NB4 cells. Blood 1998;91(11):4300 – 4310.

[34] Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KA, Stein H, Mason DY. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). Journal of Histochemistry and Cytochemistry 1984;32(2):219 – 229.

[35] Estey EH, Thall PF, Pierce S, Cortes J, Beran M, Kantarjian H, Keating MJ, Andreeff M, Freireich E. Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin ± all-trans retinoic acid ± granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndrome. Blood 1999;93(8):2478 – 2484.

[36] Venditti A, Tamburini A, Buccisano F, Scimo MT, Del Poeta G, Maurillo L, Cox MC, Abruzzese E, Tribalto M, Masi M, Amadori S. A phase-II trial of all trans retinoic acid and low-dose cytosine arabinoside for the treatment of high-risk myelodysplastic syndromes. Annals of Hematology 2000;79(3):138 – 142.

[37] Sacchi S, Kantarjian HM, Freireich EJ, O'Brien S, Cortes J, Rios MB, Kornblau S, Giles FJ, Koller C, Gajewski J, Talpaz M. Unexpected high incidence of severe toxicities associated with alpha interferon, low-dose cytosine arabinoside and all-trans retinoic acid in patients with chronic myelogenous leukemia. Leukemia and Lymphoma 1999;35(5 – 6):483 – 489

[38] Bensi L, Longo R, Vecchi A, Messora C, Garagnani L, Bernardi S, Tamassia MG, Sacchi S. Bcl-2 oncoprotein expression in acute myeloid leukemia. Haematologica 1995;80(2):98 – 102.

[39] Delia D, Aiello A, Soligo D, Fontanella E, Melani C, Pezzella F, Pierotti MA, Della Porta G. bcl-2 proto-oncogene expression in normal and neoplastic human myeloid cells. Blood 1992;79(5):1291 – 1298.

[40] Davison K, Mann KK, Waxman S. JNK activation is a mediator of arsenic trioxide-induced apoptosis in acute promyelocytic leukemia cells. Blood 2004;103(9):3496 – 3502.

[41] Cai X, Yu Y, Huang Y, Zhang L, Jia PM. Arsenic trioxide-induced mitotic arrest and apoptosis in acute promyelocytic leukemia cells. Leukemia 2003;17:1333 – 1337.

[42] Park JW, Choi YJ, Jang MA, Baek SH, Lim JH, Passaniti T, Know TK. Arsenic trioxide induces G2/M growth arrest and apoptosis after Caspase-3 activation and bcl-2 phospolylation in promonocytic U937 cells. Biochemical and Byophysical Research Communications 2001;286:726 – 738.

[43] Altucci L, Rossin A, Raffelsberger W, Reitmair A, Chomienne C, Groenemeyer H. Retionic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. Nature Medicine 2001;7(6):680 – 686.

[44] Lotan R. Effect of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochimica Biophysica Acta 1980;605(1):33 – 91.

[45] Rojewski MT, Baldus C, Knauf W, Thiel E, Shrezenmeier H. Dual effects of arsenic trioxide (As_2O_3) on non-acute promyelocytic leukemia myeloid cell lines: induction of apoptosis and inhibition of proliferation. British Journal of Haematology 2002;116(3):555 – 563.

[46] Shen ZX, Shi ZZ, Fang J, Gu BW, Li JM, Zhu YM, Shi JY, Zheng PZ, Yan H, Liu YF, et al. All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. Proc Natl Acad Sci USA. 2004;101(15):5328 – 5335.

Leuk Lymphoma Downloaded from informahealthcare.com by QUT Queensland University of Tech on 11/06/14
For personal use only.

RIGHTSLINK