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Cellular redox state and its relationship to the inhibition of clonal cell growth and the induction of apoptosis during all-trans retinoic acid exposure in acute myeloblastic leukemia cells

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ABSTRACT

Background and Objectives. **All-trans retinoic acid (ATRA) induces growth arrest and apoptosis in acute myeloblastic leukemia (AML) cells. Since cellular redox state regulates these events, we were interested in studying whether it has any role in the responsiveness of AML cells to ATRA.**

Design and Methods. **Two human AML cell lines, the ATRA-sensitive OU-AML-3, and the ATRA-resistant OU-AML-7, were used as models. Clonogenic cell culture assay, annexin V method, and measurement of mitochondrial membrane potential were used for the determination of cell growth and apoptosis. Peroxide formation was analyzed by flow cytometry, glutathione and γ-glutamylcysteine synthetase (γ-GCS) activity was determined spectrophotometrically, and the expression of manganese superoxide dismutase (MnSOD) by Western blotting.**

Results. **ATRA inhibited clonogenic cell growth and induced apoptosis particularly in OU-AML-3 cells. The OU-AML-7 cells had a higher basal level of glutathione and γ-GCS activity than the OU-AML-3 cells. ATRA enhanced the generation of peroxides after 24h exposure, which was more prominent in the sensitive than the resistant cell line and was not preventable by N-acetyl-L-cysteine. ATRA also increased the activity of γ-GCS, which was associated with increased intracellular glutathione in the resistant cell line, while the glutathione level was maintained in the sensitive cell line. During ATRA exposure, MnSOD was induced in the sensitive cell line, but not until after 72h. Buthionine sulfoximine significantly increased the inhibitory effect of ATRA on colony formation in both cell lines, but only marginally enhanced the effect of ATRA on the induction of apoptosis.**

Interpretation and Conclusions. **The balance between oxidative and antioxidative actions of ATRA, as well as the basal redox state of the cells seem to have a definite influence on the responsiveness of AML cells to ATRA.**

Key words: AML, apoptosis, ATRA, MnSOD, glutathione, γ-GCS, BSO, NAC

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There is growing evidence to suggest that the cellular redox state regulates cell survival and apoptosis.[1,2] Reactive oxygen species (ROS) have been found to inhibit the growth of malignant cells[3,4] and/or to induce apoptosis.[5-7] On the other hand, a high cellular antioxidative capacity, including a high glutathione level,[8,9] a high γ-glutamyl-cysteine synthetase (γ-GCS) level, a rate-limiting enzyme in glutathione synthesis,[10-12] and a high manganese superoxide dismutase (MnSOD) level,[13,14] have been shown to protect cancer cells against the toxic effects of chemotherapy agents.

Retinoids (RAs) are vitamin A derivatives whose antiproliferative properties have been used for a long time in cancer prevention and therapy,[15] while the ability of RAs to induce apoptosis is a recent finding.[16,17] In hematologic malignancies, all-trans retinoic acid (ATRA), one of the natural RAs, has been shown to enhance the differentiation of malignant promyelocytic leukemia (APL) cells[18-21] and to inhibit blast cell growth in AML subtypes other than APL.[22-25] ATRA is also able to induce apoptosis in AML blast cells.[26] Recent findings from our laboratory indicated that ATRA-induced apoptosis in AML is associated with mitochondrial dysfunction and caspase activation.[27]

RAs are able to generate ROS in target cells. The differentiation-inducing effects of RAs, particularly in myeloid hematopoiesis, are associated with superoxide production as analyzed by the nitroblue tetrazolium test,[19-21,28-30] while RA-induced apoptosis has been related to peroxide generation[31-33] as analyzed by dichlorohydrofluorescein-diacetate (DCHF-DA). The antiproliferative effects of RAs, on the other hand, are coupled with antioxidative capacity, but the possible modulatory effects of RAs on intracellular antioxidants are unclear. The role of the intracellular redox state in RA resistance also remains obscure.

Based on the evidence that the cellular redox state has an influence on the survival of malignant cells, we suggested that it could also play a role in the responsiveness of AML cells to ATRA. This issue was investigated in two recently characterized AML cell lines, OU-AML-3 and OU-AML-7, which differ from each other in their responsiveness to ATRA.[27,34] The suggested role of ATRA as an oxidant as well as its possible effects on the antioxidative glutathione system and an important mitochondrial protector, MnSOD, were the specific targets of investigation.

Design and Methods

Cell lines and cell cultures

The human AML cell lines OU-AML-3 and OU-AML-7 have been established and characterized by the authors at the University of Oulu.[34] Both cell lines are derived from patients with AML of FAB subtype M4. Neither cell line shows signs of differentiation after ATRA exposure as determined by morphology or the nitroblue tetrazolium test.[26] The OU-AML cell lines were grown in a suspension with a density of 1×10^6 cells/mL in α-minimal essential medium (α-MEM) (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) in an atmosphere of 95% air and 5% CO_2 at 37°C. The medium was changed 2-3 times per week in basic growth conditions. For the present studies, fresh medium was added 24h before each experiment to ensure the exponential growth of the cells. All the exposures were conducted in suspension, whereafter the cells were washed and harvested for analyses. Corresponding control cells were prepared similarly in the absence of the agent. When the effect of the agent on clonogenic cell growth was investigated, the exposed cells were washed and plated into methylcellulose as described previously.[26] Roughly, 1×10^3 viable cells (trypan blue-negative cells)/well were cultured in a volume of 0.1 mL of basic growth medium in 96-well microtiter plates in triplicate, and colonies of more than 20 cells were counted on day 3 using an inverted microscope.

ATRA and other agents used in cell culture

ATRA was purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). It was dissolved immediately before use in 100% ethanol and diluted in α-MEM to the desired concentration. The final concentration of ethanol present in the cultures did not affect cell growth. All the experiments with ATRA were performed in subdued light, and the tubes and culture plates containing ATRA were covered with aluminium foil. Based on results of our previous studies, the concentration of ATRA used was 1 µmol/L.[26,27] In selected experiments, the cells were treated with 0.5 mM buthionine sulfoximine (BSO) and 10 mM N-acetyl-L-cysteine (NAC) (both from Sigma) with or without ATRA. The choice of the abovementioned concentrations of BSO and NAC was based on results of previous studies[32,33] and our own titrations. BSO inhibits the γ-glutamylcysteine synthetase (γ-GCS) enzyme and causes glutathione depletion.[35] NAC is able to elevate glutathione levels by providing cysteine for glutathione synthesis.

Annexin V-assay for the detection of apoptosis

Fluorescein iso-thiocyanate (FITC)-conjugated annexin V (Boehringer Mannheim, Mannheim, Germany) with propidium iodide was used in flow cytometry to detect apoptotic cells. Annexin V has been shown to interact strongly and specifically with phosphatidylserine (PS) residues, and it can be used to detect apoptosis by targeting for the loss of plasma membrane symmetry.[36] For the analyses, cells were harvested from the suspension cultures, washed twice with phosphate-buffered saline (PBS) and incubated in annexin V-Fluos labeling solution (2% annexin-V-Fluos and 1.0 µg/mL propidium iodide in 10 mM Hepes/NaOH, pH 7.4; 140 mM NaCl; 5 mM $CaCl_2$) for 15 min at room temperature in the dark). After the incubation, the cells were washed twice with Hepes buffer and analyzed by using a FACSort flow cytometer (Becton Dickinson).

Detection of peroxides by flow cytometry

AML cells (5×10^5 cells/mL) were cultured with or without exposure to the agent for various periods up to 72h. After the exposure, the cell suspension was adjusted to a density of 0.75×10^6 cells/mL and incubated in 10% FBS and α-MEM for 25 min at 37°C in a humidified atmosphere of 5% CO_2 in the dark with 5 µM DCFH-DA (Molecular Probes, Inc., Eugene, OR, USA). At the end of the incubation period, the cells were washed twice in cold PBS, suspended in a total volume of 350 µL and analyzed by using a FACSort flow cytometer (Becton-Dickinson).

Determination of glutathione and γ-GCS

Total glutathione content was determined spectrophotometrically following the reduction of 5,5'-dithiobis(2-nitrobenzoic) acid by NADPH in the presence of glutathione reductase.[37] The glutathione content is expressed as nmol/1.5×10^6 cells. The activity of γ-GCS was determined using a modification of the previously described HPLC method,[38] which measures the end product γ-glutamylcysteine following conjugation with monobromobimane. Cells were lysed by repeated freezing and thawing into 100 mM Tris-HCl buffer (pH 8.2) containing 50 mM KCl, 20 mM $MgCl_2$ and 2 mM EDTA. The lysates were centrifuged at 15,000 g for 15 min at 4°C and the supernatants were filtered through Micro Bio-Spin Chromatography Columns (Bio-Rad, Hercules, CA, USA). Aliquots of the supernatant were added to the reaction mixture (100 mM Tris-HCl, pH 8.2, 50 mM KCl, 20 mM $MgCl_2$, 6 mM DTT, 3 mM L-cysteine, 15 mM glutamic acid and 10 mM ATP) and incubated at 37°C for 30 min. A volume of 50 µL of incubation mixture was then removed and added to 50 µL of 30 mM monobromobimane in 50 mM N-ethylmorpholine, pH 8.4. After derivatization for 5 min in the dark at room temperature, the reaction was stopped with 10 µL of 100% trichloroacetic acid. After centrifugation, 1 µL of supernatant was injected into a Waters Novapak C-18 HPLC column (4 mm, 3.9×150 mm) with an isocratic mobile phase consisting of 4% acetonitrile, 0.25% acetic acid and 0.25% perchloric acid (pH 3.7). Fluorescent product γ-glutamylcysteine was detected with a Shimadzu RF-10 AxL spectrofluorometer (excitation and emission wavelengths 394 and 480 nm, respectively). The product was quantified by comparison to γ-glutamylcysteine (Bachem, Budendorf, Switzerland) standards derivatized and analyzed as above. The protein concentration was determined from filtered cell lysates using a Bio-Rad DC protein assay kit.

Detection of MnSOD by Western blotting

The cells were harvested and washed twice with ice-cold PBS. For MnSOD detection, mitochondrial protein fractions were isolated as described previously,[34]

and then 30 µg of the protein were applied to 12% SDS-PAGE (Bio-Rad Laboratories) and transferred onto a Hybond nitrocellulose membrane (Amersham). After blocking for 30 min with 8% dried fat-free milk in Tris-buffered saline-Tween (TBS-T) (20 mM Tris-HCl pH 7.6, 137 mM NaCl, 0.1% Tween-20), the membranes were incubated for 1 hour with the primary antibody. The specific protein-antibody complex was detected by using a horseradish peroxidase-conjugated secondary antibody (Amersham) and an enhanced chemiluminescence (ECL) detection kit (Amersham). The rabbit antibody to recombinant human MnSOD was a gift from JD Crapo (Jewish Medical Center, Denver, CO, USA). The antibody was diluted in the blocking solution at 1:10,000. The β-actin expression of the cells was detected with a monoclonal anti-actin antibody (1:30,000). The Western blotting signals were quantified using a 300A Computing Densitometer and Image Quant Software v 3.0 Fast Scan (Molecular Dynamics, Sunnyvale, CA, USA).

Cytofluorimetric analysis of mitochondrial membrane potential ($\Delta\psi m$)

The variation of $\Delta\psi$m during exposure to ATRA, BSO or NAC was studied using 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1; Molecular Probes, Inc., Eugene, OR, USA).[39,40] After the exposure, the cell suspension was adjusted to a density of 0.75×10^6 cells/mL and incubated in 10% FBS and α-MEM for 25 min at 37°C in a humidified atmosphere of 5% CO_2 in the dark with 10 mg/mL of JC-1. At the end of the incubation period, the cells were washed twice in cold PBS, suspended in a total volume of 350 µL and analyzed by using a FACSort flow cytometer (Becton-Dickinson).

Results

Inhibition of clonogenic cell growth and induction of cell death by ATRA

The clonogenic cell culture assay (repeated three times) showed that the number of colonies decreased from 156±12 (mean±SD) to 19±6 in the case of OU-AML-3 and from 198±11 to 93±7 in the case of OU-AML-7 cells after 72h exposure (Figure 1). The percentages of annexin V-positive cells (analysis was done twice) increased to 57 and 83 % in the OU-AML-3 cells during 72h exposure, the corresponding values in the OU-AML-7 cells being 15 and 20 % (Figure 2A). ATRA-induced apoptosis was also confirmed by morphology (data not shown). The number of necrotic cells (annexin V-positive/PI-positive cells) was constantly less than 10% of annexin V-positive cells, which means that most of the dead cells were apoptotic, i.e. annexinV-positive/PI-negative (see Figure 2B). Flow cytometric data of the present study are in agreement with those of our previous study performed using the same AML cell lines.[27]

Generation of peroxides by ATRA

Fluorescence indicative of peroxide generation in DCFH-DA assay was observed in both cell lines in basal conditions. OU-AML-3 cells exposed to ATRA for 24h showed a 1.6±0.1-fold increase in the generation of peroxides, the corresponding change in OU-AML-7 being 1.3±0.2 (n=3). Representative histograms of both cell lines are shown in Figure 3.

Induction of γ-GCS during ATRA exposure in both cell lines

The basal activity of γ-GCS was lower in the OU-AML-3 cells than in the OU-AML-7 ones, being 1.58±0.2 and 2.28±0.2 nmol/min/mg of protein (n=6), respectively. γ-GCS activity was induced in both cell lines after 24h exposure, and the induction was up to 160±8% (n=3) in the OU-AML-3 cells and to 139±3% (n=3) in the OU-AML-7 cells during 72h exposure (Figure 4).

Glutathione levels during ATRA exposure

The basal glutathione content was lower in the sensitive OU-AML-3 cells than in the resistant OU-AML-7 cells. It was 5.2±1.9 in the OU-AML-3 and 6.0±2.0 nmol/L/1.5×10^6 cells (n=6) in the OU-AML-7 cells. Glutathione increased to 133±23% in the OU-AML-7 cells after 24h exposure and to 169±32% (n=3) after 72h exposure. At the same time, glutathione content of the OU-AML-3 cells remained unchanged, the corresponding values being 92±17% and 106±35 % (n=3) (Figure 5).

Effects of glutathione modulators, BSO and NAC, on AML cells with or without ATRA

Glutathione was depleted to 8% by 0.5 mM BSO during 24h exposure and remained low for the next 48 hours in the OU-AML-3 cells, while in the OU-AML-7 cells the concentration decreased to 43% after 24h exposure and further to 12% after 72h exposure (Figure 5). BSO also induced more pronounced generation of ROS than ATRA in both cell lines (Figure 3). In spite of these changes, the effect of BSO on phosphatidylserine externalization and $\Delta\psi$m in the

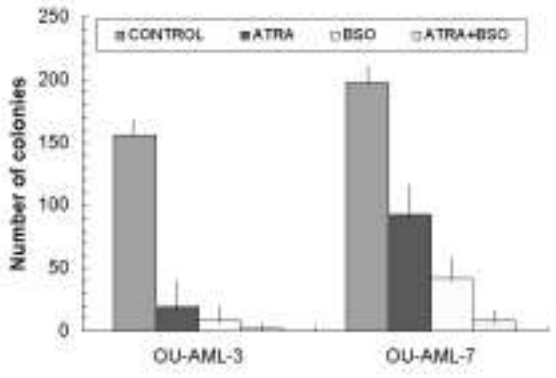

Figure 1. Inhibition of colony formation by AML cells exposed to 1 µM all-trans retinoic acid (ATRA), 0.5mM buthionine sulfoximine (BSO), and their combination for 72h. The results are presented as number of colonies / plated 1,000 cells (mean ± SD of three independent experiments). Control = no ATRA and / or BSO.

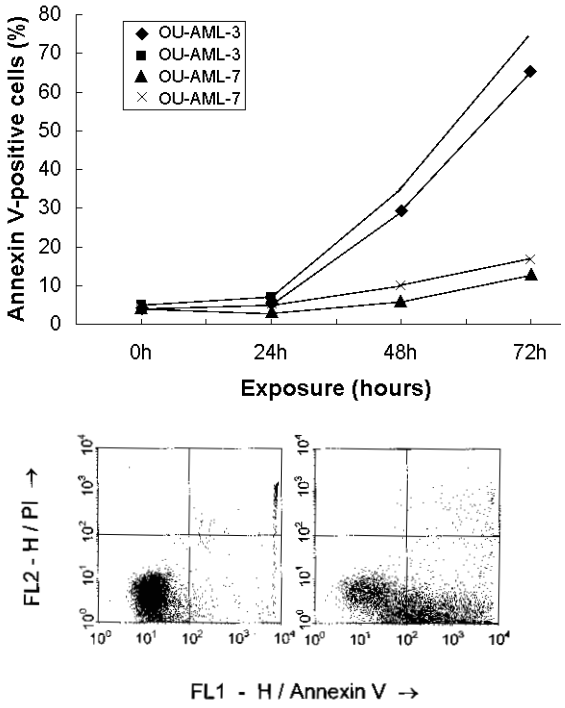

Figure 2. Detection of cell death by annexin V assay. AML cells were incubated in the presence of 1 µM ATRA for up to 72h. A. The percentage of annexin V-positive cells (two independent experiments) is presented at various time points. B. Representative histogram of an annexin V assay, in which the cells were double-stained with annexin V-Fluos and propidium iodide (PI). The left column shows OU-AML-3 cells in the absence of ATRA and the right column similar cells after exposure to ATRA. Apoptotic (annexin-positive / PI-negative) cells are shown in the lower right quadrant and necrotic (annexin-positive / PI-positive) cells in the upper right quadrant of each column.

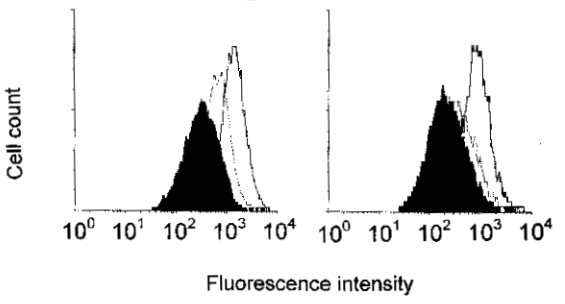

Figure 3. Representative histograms showing an increase in peroxide generation as measured by DCHF-DA fluorescence. The left-side (black) histograms show cells without exposure, the middle ones show cells in the presence of 1 µM ATRA, and the right-side histograms show cells exposed to 0.5 mM BSO for 24h. The left hand graph represents the OU-AML-3 cell line and the right the OU-AML-7 cell line.

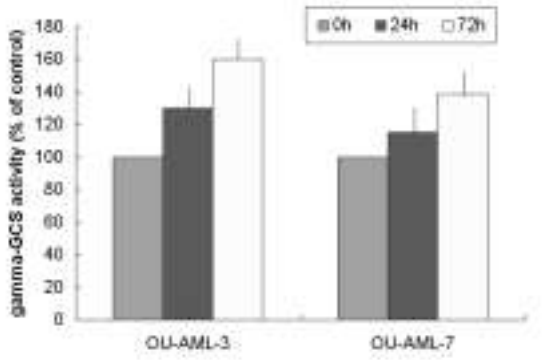

Figure 4. Activity of γ-GCS in AML cells. AML cells were exposed to 1 µM ATRA for 24 and 72h, whereafter the γ-GCS activity was determined as described in *Design and Methods*. The results are presented as percentages compared to corresponding controls (mean±SD of three independent experiments).

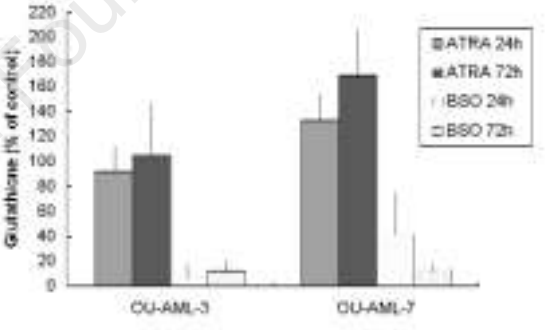

Figure 5. Measurement of glutathione in AML cells. AML cells were exposed to 1 µM ATRA and 0.5 mM BSO for 24 and 72h, whereafter the glutathione content was determined as described in *Design and Methods*. The results are presented as percentages compared to corresponding controls (mean±SD of three independent experiments).

OU-AML-3 cells was marginal with or without ATRA when analyzed by annexin V (Table 1) and JC-1 (Figure 6A) fluorocytometrically. In addition, NAC had no effect on $\Delta\psi$m (Figure 6B) or ATRA-induced ROS generation and apoptosis (data not shown) in the OU-AML-3 cells. BSO was, however, able to inhibit clonogenic cell growth more effectively than ATRA in both cell lines, and this effect was more evident in the OU-AML-3 than in the OU-AML-7 cells (Figure 1). In addition, the most prominent inhibitory effect on clonogenic cell growth was obtained by combining ATRA and BSO (Figure 1).

Effect of ATRA on MnSOD expression

The basal MnSOD level was lower in the OU-AML-3 than the OU-AML-7 cells, and also relatively low com-

Table 1. Percentage of annexin V-positive cells after 72h exposure of AML cells to 1 µM all-trans retinoic acid (ATRA), 0.5 mM buthionine sulfoximine (BSO), or their combination (mean ± SD of three independent experiments).

Exposure agent	*OU-AML-3*	*OU-AML-7*
Control	12±1	10±2
ATRA	53±14	20±10
BSO	19±3	13±2
ATRA+BSO	59±18	24±13

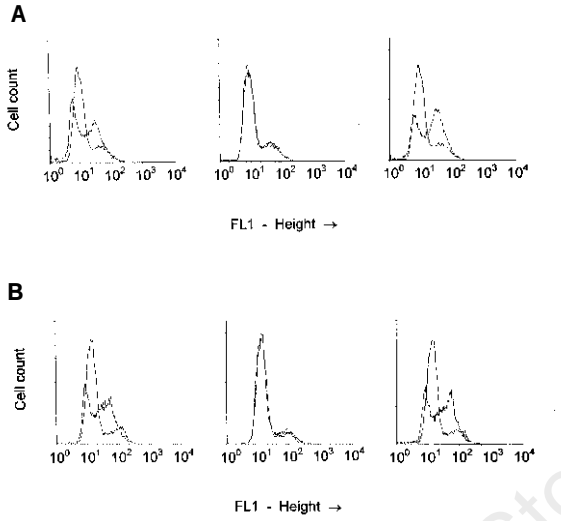

Figure 6. Representative histograms showing changes in mitochondrial membrane potential (Δψm). A. OU-AML-3 cells exposed to 1 µM ATRA (left graph), 0.5 mM BSO (middle graph), and their combination (right graph) for 72h. B. OU-AML-3 cells exposed to 1 µM ATRA (the left graph), 10 mM N-acetyl-L-cysteine (the middle graph), and their combination (the right graph) for 72h. The decrease in Δψm is shown as an increase in monomers.

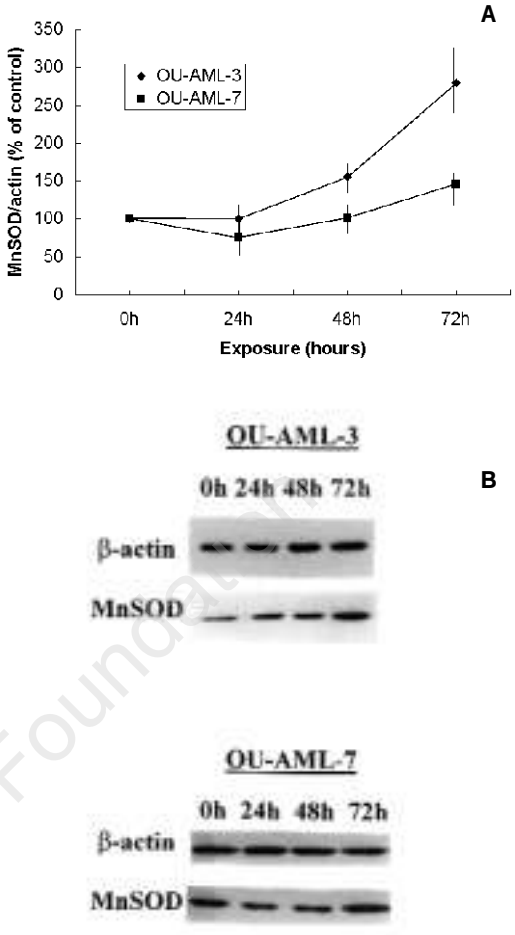

Figure 7. MnSOD protein analyzed by Western blotting. AML cells were incubated in the presence of 1 µM ATRA for up to 72h. The mitochondrial fraction (30 µg) was loaded on to SDS-polyacrylamide gel and visualized by enhanced chemiluminescence and a specific polyclonal non-commercial anti-MnSOD antibody. A. Expression of MnSOD in ratio to β-actin (mean ±SD of three independent experiments). B. Representative gel electrophoresis of MnSOD expression.

pared to in other malignant cell lines, such as the lung carcinoma and mesothelioma cell lines available in our laboratory. During 72h exposure to ATRA, MnSOD was induced more evidently in the sensitive OU-AML-3 than the resistant OU-AML-7 cells (Figure 7).

Discussion

In the present study, ATRA was demonstrated to manifest both oxidant and antioxidant properties in AML cells with definite consequences for cell growth and cell death. Basal glutathione and γ-GCS also had an influence on the responsiveness of the AML cells to ATRA.

ATRA acted as an oxidant by enhancing peroxide generation in AML cell lines, more evidently in the sensitive than in the resistant cell line. Peroxide formation has also been seen previously during RA exposure, in embryonic stem cells,[31] in lymphatic cells,[33] and in HL-60 cell line cells.[32] In these studies, peroxide formation has been coupled with the induction of apoptosis, since NAC, by providing cysteine for glutathione synthesis, has been shown to be able to delay the apoptotic effect of RAs, which suggests a possible role of glutathione depletion in ROS generation. Surprisingly, however, RAs appear to have no effect on glutathione levels in cells in which the oxidative function of RAs has been suggested to affect apoptosis.[33, 41] In the present study, in spite of the similar concentration of NAC, NAC was not able to delay or inhibit ATRA's effect on peroxide formation, phosphatidylserine externalization or Δψm in the sensitive OU-AML-3 cells. Based on this, we suggested that ATRA cannot cause glutathione depletion, although it induces oxidative stress, in these AML cells. In fact, this was confirmed by the glutathione measurement. Glutathione levels were maintained in the sensitive cell line cells, but they were increased even further in the resistant cell line cells during ATRA exposure.

One important reason for the maintenance of intracellular glutathione during oxidative stress might be the high basal activity of γ-GCS, a rate-limiting enzyme in glutathione synthesis. This was observed in the present study, in which γ-GCS activity was higher in the resistant OU-AML-7 cells in which the glutathione content was also higher. Previously, high γ-GCS has been shown to be associated with increased drug resistance, at least in the case of doxorubicin,[12] melphalan[11, 42] and cisplatin.[10] High glutathione has also been connected with drug resistance in cancer.[8,9,43-46] On the basis of the present study, we suggest that ATRA might have direct antioxidative properties, since the induction of γ-GCS activity was observed without depletion of glutathione in both AML cell lines. This is usually a sign of adaption to chronic oxidative stress.[47]

Generation of reactive oxygen species has been found previously in myeloid malignancies during RA exposure. ATRA is able to induce superoxide formation in APL, an effect which is coupled with differentiation.[20-21,29-30] In other AML subtypes, however, this has been found to be very exceptional.[20] Previously, we have also noted that the AML cell lines which do not represent APL do not produce superoxide as analyzed by the nitroblue tetrazolium test.[26] According to the present study, generation of peroxides might, in contrast, have an important role in mediating the antiproliferative effects of RAs in AML. As an indication of this, we found that clonogenic cell growth, i.e. colony formation in methylcellulose, was also inhibited by ATRA more efficiently in the cell line which generated more peroxides. In addition, the inhibitory effect of BSO, which caused more pronounced peroxide generation than ATRA, was even more efficient than that of ATRA in both cell lines. Furthermore, the most effective inhibition of clonal cell growth was caused by a combination of these two drugs. These results are consistent with the previous findings showing that ROS may either increase or inhibit cell proliferation, depending on the dosage and duration of exposure and the cell type investigated.[2,48]

As the present study shows, other unknown mechanisms apart from ROS are associated with apoptosis caused by ATRA, since BSO, which caused more pronounced generation of peroxides and depletion of glutathione than ATRA, was not able to reinforce significantly the effects of ATRA, when measured by phosphatidylserine externalization or a change in $\Delta\psi m$.

ATRA-induced apoptosis in these AML cell lines is related to mitochondrial membrane disruption, the efflux of cytochrome c to cytosol and caspase-3 activation.[27] Since MnSOD is one of the most important mitochondrial protectors,[49-51] we also wanted to analyze its possible role in ATRA-induced apoptosis. Furthermore, there are no studies so far on the effects of RAs on MnSOD. The basal MnSOD levels in both cell lines were low when compared to those in human mesothelioma cell line cells, for instance, which represent a malignant cell type with high MnSOD activity.[52] High basal MnSOD has been connected with drug resistance.[13,52-53] On the other hand, the induction of MnSOD has been observed to protect or sensitize cells against subsequent oxidant stress.[13,14,54–58] The induction of MnSOD was more evident in the sensitive OU-AML-3 cell line than the resistant OU-AML-7 one. Thus, if anything, this induction might sensitize AML cells to the oxidative effect of ATRA. The present study does not indicate any evident protective role of MnSOD against ATRA in AML cells.

We can conclude that while ATRA acts as an oxidant in AML cells by enhancing peroxide generation, it also has obvious antioxidative properties by enhancing γ-GCS and affecting the glutathione content of the cells. The balance between these two actions of ATRA as well as the basal redox state of the cells seem to have a distinct influence on the responsiveness of AML cells to ATRA.

Contributes and Acknowledgments

PM performed and analyzed the flow cytometer data: annexin V assay, detection of peroxidase, detection of mitochondrial membrane potential. He made the first draft, literature search, and gave comments on the final version. He also participated in the interpretation of data. TG is the medical student in our research group; he analyzed the glutathione, did a literature search, and gave comments on the final version. TS analyzed MnSOD expression, participated in the interpretation of data and gave comments on the draft and final version. MS was responsible for the glutathione measurements, cell cultures and production of figures and gave comments on the draft and final version. E-RS is the chief of the hematology laboraory and was responsible for the flow cytometer methodology, interpretation of data and gave comments on the final version. A-LL was responsible for gamma-glutamylcysteine synthetase activity methodology and measurements. She also gave comments on the final version. VK gave ideas, made contributions to the study design and revision and gave critical comments on redox balance measurements. She also participated in the interpretation of data and revision of the final version. PK had the ideas for the study design, made cell cultures, intepreted original data, revised the drafts and wrote the final version to be submitted.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

- ATRA might have a role in the treatment of AML (other than acute promyelocytic leukemia) due to its growth inhinbitory effect and ability to induce apoptosis.
- The growth inhibitory effect can be reinforced by glutathione depletors, i.e. by BSO.
- The redox balance of target cells should be taken into account when thinking of combining ATRA with other agents in AML, since ATRA may also increase antioxidative capacity of the cells.

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