Analysis of Hemin-induced Protection of Human Hemopoietic Cells from the Cytotoxic Effects of Anthracyclines

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ABSTRACT

Experiments were performed with K562 erythroleukemia cells to further characterize the observation that hemin protects hemopoietic cells from the cytotoxic effects of anthracycline drugs. The present studies demonstrate that this protective effect of hemin applies only to anthracyclines and not to other classes of antineoplastic agents. Hemin interferes with the cellular accumulation of various anthracyclines, as measured by cytofluorography, and prevents binding of anthracyclines to isolated cell nuclei. Exposure of K562 cells to hemin retards the anthracycline-induced arrest of cells at the G1-M interval of the cell cycle and permits cells to undergo continuing division as demonstrated by clonal growth in plasma clot cultures. Furthermore, hemin decreases the ability of anthracyclines to unwind simian virus 40 supercoiled DNA in vitro. The protective effect of hemin fails to occur if cells have been preincubated with this agent for 72 h before they are exposed to Adriamycin in the absence of hemin. The findings suggest that hemin prevents anthracycline-induced cytotoxicity by acting at several levels. These effects may be mediated by direct interactions of hemin with DNA and perhaps other cellular constituents or by molecular complex formation between hemin and anthracyclines at intracellular sites.

INTRODUCTION

Recent studies from this laboratory (1) have shown that hemin, a natural product which is an inducer of globin gene activation in murine and human erythroleukemia cells (2) and is thus a potential physiological regulator of hemopoietic cell differentiation (3), protects hemopoietic cells from the cytotoxic effects of anthracycline drugs, such as Adriamycin and daunomycin. This protective effect of hemin is more or less specific for hemopoietic cells, such as mouse erythroleukemia cells, human K562 and HEL-1 erythroleukemia cells, human HL-60 promyelocytic leukemia cells, human U937 histiocytic leukemia cells, and burst-forming units-erythroid (BFU-E) from normal human bone marrow, but not nonhemopoietic cells, such as human breast MCF-7 adenocarcinoma cells, mouse 3T3 cells, monkey VERO kidney cells, and human colon-205 carcinoma cells (1). In the present study we have assessed whether hemin's protective effect on hemopoietic cells is unique to the toxicity of anthracyclines as compared to other antineoplastic agents, whether pretreatment with hemin alters the susceptibility of cells to Adriamycin cytotoxicity, whether hemin inhibits anthracycline uptake into whole cells and isolated cell nuclei, and whether hemin alters the effects of anthracyclines on the cell cycle and the physical properties of DNA.

MATERIALS AND METHODS

Chemicals and Cells. Hemin was purchased from Eastman Kodak, Rochester, NY, and dissolved in slightly alkaline solution. This did not alter the pH of the final culture medium. Adriamycin and daunomycin were obtained from Sigma Chemical Co., St. Louis, MO. The anthracyclines aclacinomycin, mussetamycin, pyrromycin, and marcellomycin were kindly donated by Bristol Myers Laboratory, Syracuse, NY. These agents were dissolved in 100% ethanol and then diluted so that the final concentration of ethanol was no more than 0.1%. At this concentration ethanol caused no detectable effect on growth or differentiation of K562 cells. [3H]Daunomycin (specific activity, 1 to 5 Ci/mmol) was purchased from New England Nuclear Corp., Boston, MA. SV-40 viral DNA was purchased from BRL Laboratories, Bethesda, MD.

Cells used throughout this study were human K562 erythroleukemia cells originally developed by Lozzio and Lozzio (4). These cells were seeded at a concentration of 1 x 10⁶ cells/ml in RPMI-1640 supplemented with 10% fetal calf serum and kept in exponential growth by replenishing the cultures with fresh medium every 48 h. Cell growth was determined at various times by measuring the number of cells with a Model ZBI Coulter Counter.

Cytofluorographic Assessment of Cellular Anthracycline Levels. Cells were treated with no drug or with an anthracycline in the presence or absence of 30 μM hemin. At various times cells were harvested from culture, washed 3 times with PBS, pH 7.4, resuspended at 1 x 10⁶ cells/ml, and analyzed cytofluorographically at 488 nm (average wavelength of maximal absorption of anthracyclines) as described previously (5).

Binding of [3H]Daunomycin to Isolated Nuclei. K562 cells were harvested from culture, washed 3 times with ice-cold Dulbecco's PBS, pH 7.4, and then resuspended (5 to 10 x 10⁶ cells/ml) in solution containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and 3 mM CaCl₂, pH 7.0. Cellular disruption was achieved by passing the cells 10 to 15 times through an 18-gauge 3/8-in spinal needle (Randall Faichney Corp., Boston, MA). Nuclei were then isolated by centrifugation at 350 x g for 5 min. Cytoplastic contamination of the nuclear preparation was checked by phase microscopy. For studies of [3H]-daunomycin binding, nuclei were incubated in PBS, pH 7.4, with [3H]daunomycin (0.5 μCi/ml, 1 x 10⁻⁷ M) in the presence or absence of 30 μM hemin. At various times of incubation, 0.2-ml aliquots of the suspension (containing 2 x 10⁶ nuclei) were layered over 24% sucrose/sodium Ringer's solution and centrifuged for 2 min at 7000 rpm to remove unbound [3H]daunomycin. Radioactivity in the pellet was counted after solubilization with 1% sodium dodecyl sulfate.

Cytofluorographic Analysis of the Cell Cycle. Exponentially growing K562 cells were treated with no drug, 30 μM hemin, 3 x 10⁻⁸ M Adriamycin, or both Adriamycin and hemin. After 24 h of incubation, some cells that had been treated with either Adriamycin or hemin were washed 3 times with PBS and exposed simultaneously to both Adriamycin and hemin, and incubation continued at 37°C for 2 more h. Cells were harvested, washed with PBS, and examined by cytofluorographic analysis of DNA using propidium iodide, a fluorescent DNA binding dye, as previously reported (5).

Electrophoretic Analysis of DNA Unwinding. Five μg of SV-40 viral DNA were incubated in 10 mM Tris-HCl:1 mM EDTA, pH 8.0, with no drug or with varying concentrations of daunomycin and a constant concentration of hemin. SV-40 DNA was also incubated with varying concentrations of hemin in the presence of a constant concentration of daunomycin. After incubation at 37°C for 30 min, SV-40 DNA was analyzed electrophoretically on a 1.0% agarose gel. DNA was stained with ethidium bromide and visualized under UV light. The effect of

3 The abbreviations used are: SV-40, simian virus 40; PBS, phosphate-buffered saline.
RESULTS

Effect of Hemin on Cytotoxicity of Different Antineoplastic Drugs. To determine whether the protective effect of hemin is specific for Adriamycin or applies to other chemotherapeutic agents as well, K562 cells were exposed to Adriamycin, daunomycin, or mussetamycin, and growth was determined in the presence and absence of 30 \( \mu \)M hemin. As shown in Table 1, cotreatment with hemin led to decreased cytotoxicity of Adriamycin, daunomycin, and mussetamycin. K562 cells were also cultured with nonanthracycline antineoplastic agents in the presence or absence of hemin. As shown in Fig. 1A, culture with bleomycin, 1-\( \beta \)-D-arabinofuranosylcytosine, 5-fluorouracil, mitomycin C, or methotrexate caused pronounced inhibition of K562 cell growth. The addition of 30 \( \mu \)M hemin had no protective effect against the cytotoxic action of any of these drugs (Fig. 1B). Thus, the hemin protective effect appears to be specific for drugs of the anthracycline class.

Effect of Pretreatment with Hemin on Adriamycin Cytotoxicity. K562 cells were preexposed to hemin for 72 h, then removed from the hemin-containing medium, and incubated with Adriamycin in the absence of hemin. As shown in Fig. 2, preexposure to hemin for 72 h did not protect the cells from Adriamycin's cytotoxic effects. Thus, hemin does not cause persistent changes in K562 cells which protect them from the cytotoxic action of Adriamycin.

Effect of Hemin on Cellular Uptake of Anthracyclines. In an earlier study (1) we observed that hemin inhibits the cellular accumulation of Adriamycin and daunomycin, as measured by cytofluorography or uptake of labeled drug. To determine whether this also occurs with other members of the anthracycline class, we exposed cells to a variety of anthracyclines in the presence or absence of hemin and measured the cellular content of these agents cytofluorographically. As shown in Fig. 3, treatment of cells with hemin suppressed the cellular accumulation of Adriamycin, marcellomycin, pyrromycin, and mussetamycin. There was limited uptake of mussetamycin and virtually no uptake of aclacinomycin in the absence of hemin. The limited uptake of mussetamycin is apparently sufficient to cause substantial cell killing, as demonstrated in Table 1. Aclacinomycin has been found to act synergistically with hemin in promoting erythroid maturation and hemoglobin synthesis in K562 cells (6); this effect also occurs with mussetamycin but not with the other anthracyclines, perhaps reflecting different modes of transport and/or action for these drugs.

Effect of Hemin on the Association of Anthracyclines with K562 Cell Nuclei. Anthracyclines bind to the cell nucleus because of their affinity for DNA (7). To determine whether hemin interferes with this process, nuclei isolated from K562 cells were incubated with \(^3\)H-daunomycin in the presence or absence of 30 \( \mu \)M hemin. As shown in Fig. 4, binding of \(^3\)H-daunomycin to the nucleus occurred very rapidly. The addition of hemin decreased nuclear binding of \(^3\)H-daunomycin by about 40%. Alternatively, hemin may have promoted increased efflux of daunomycin from the nuclei.

Effect of Hemin on Adriamycin-induced Effects on the Cell Cycle. To further determine whether hemin counteracts the effects of Adriamycin on K562 cells, we exposed cells to both Adriamycin and hemin under different conditions and analyzed their position in the cell cycle. As shown in Fig. 5, cells exposed

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Table 1: Effect of the anthracyclines Adriamycin, daunomycin, and mussetamycin on growth of K562 cells in the presence or absence of 30 \( \mu \)M hemin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Anthracycline concentration (( \mu )M)</th>
<th>Cell growth (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100.0</td>
</tr>
<tr>
<td>Hemin</td>
<td>3 ( \times ) 10(^{-5})</td>
<td>85.0</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>5 ( \times ) 10(^{-4})</td>
<td>56.6</td>
</tr>
<tr>
<td>Adriamycin + hemin</td>
<td>2 ( \times ) 10(^{-4})</td>
<td>47.3</td>
</tr>
<tr>
<td>Adriamycin + hemin</td>
<td>5 ( \times ) 10(^{-4})</td>
<td>84.6</td>
</tr>
<tr>
<td>Adriamycin + hemin</td>
<td>2 ( \times ) 10(^{-4})</td>
<td>83.5</td>
</tr>
<tr>
<td>Mussetamycin</td>
<td>5 ( \times ) 10(^{-4})</td>
<td>71.3</td>
</tr>
<tr>
<td>Mussetamycin + hemin</td>
<td>5 ( \times ) 10(^{-4})</td>
<td>35.3</td>
</tr>
<tr>
<td>Mussetamycin + hemin</td>
<td>2 ( \times ) 10(^{-4})</td>
<td>95.6</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>5 ( \times ) 10(^{-4})</td>
<td>73.5</td>
</tr>
<tr>
<td>Daunomycin + hemin</td>
<td>3 ( \times ) 10(^{-4})</td>
<td>32.5</td>
</tr>
<tr>
<td>Daunomycin + hemin</td>
<td>5 ( \times ) 10(^{-4})</td>
<td>80.0</td>
</tr>
</tbody>
</table>

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Fig. 1. Effect of hemin on cytotoxicity induced by nonanthracycline chemotherapeutic drugs. Exponentially growing K562 cells were seeded in culture at a concentration of 1 \( \times \) 10\(^{6}\) cells/ml and incubated with the following agents in the absence (A) or presence (B) of 30 \( \mu \)M hemin: no drug (C); bleomycin (1.0 \( \mu \)g/ml) (D); 5-fluorouracil (5.0 \( \mu \)g/ml) (E); mitomycin C (1.0 \( \mu \)g/ml) (F); methotrexate (1.0 \( \mu \)g/ml) (G); or 1-\( \beta \)-D-arabinofuranosylcytosine (10.0 \( \mu \)g/ml) (H). Points, mean of duplicate determinations.

Fig. 2. Effect of pretreatment with hemin on Adriamycin-induced cytotoxicity. Exponentially growing K562 cells were exposed to no drug, 30 \( \mu \)M hemin, 3 \( \times \) 10\(^{6}\) M Adriamycin, or both agents. At the time indicated (\( t \)), a portion of the hemin-treated culture was washed 3 times with medium and then exposed to Adriamycin (ADR) alone. Points, mean of duplicate determinations.
cells were harvested, washed 3 times with PBS (pH 7.4), resuspended in the same buffer at a concentration of $1 \times 10^9$ cells/ml, and analyzed with a cytofluorograph.

Adriamycin ($1 \times 10^{-7}$ M); MUS, mussetamycin ($5 \times 10^{-8}$ M); ACL, aclacinomycin ($5 \times 10^{-8}$ M); ADR, Adriamycin ($1 \times 10^{-7}$ M).

Relative fluorescence intensity increases from left to right. Cells incubated with anthracyclines in the presence (—) or absence (—) of hemin. He, hemin (30 μM); MARC, marcellomycin ($5 \times 10^{-4}$ M); PYR, pyrromycin ($1 \times 10^{-7}$ M); MUS, musstetamycin ($5 \times 10^{-4}$ M); ACL, aclacinomycin ($5 \times 10^{-8}$ M); ADR, Adriamycin ($1 \times 10^{-7}$ M).

Although there was still an increased proportion of cells in $G_1$ compared to cells treated with Adriamycin alone (Fig. 5B), this observation that hemin does not completely protect K562 cells from Adriamycin cytotoxicity (Fig. 5, A and D, respectively). This is consistent with the observation that hemin does not completely protect K562 cells from Adriamycin cytotoxicity (Fig. 5B; Table 1). None of the cells remained in $G_2$ and $S$, although these cultures also contained a small proportion of broken cells and cells in $G_1$ and $S$ (Fig. 5B). Treatment with both Adriamycin and hemin increased the proportion of cells in $G_2$ and $M$ (Fig. 5E), consistent with the observation that hemin cannot rescue cells that have been pretreated with Adriamycin or hemin for more than 12 h (1). Cells pretreated with hemin and then switched to both Adriamycin and hemin exhibited a cell cycle pattern similar to that of cells exposed to Adriamycin and hemin from the outset (Fig. 5F); thus, hemin pretreatment offered no additional protection, as compared to the addition of hemin simultaneously with Adriamycin, as anticipated from the observation that pretreatment with hemin fails to protect K562 cells from subsequent Adriamycin cytotoxicity (Fig. 2).

Table 2: Effect of hemin on Adriamycin-induced killing of clonogenic K562 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (M)</th>
<th>No. of colonies from 200 cells plated</th>
<th>Plating efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemin</td>
<td>$3 \times 10^{-8}$</td>
<td>24</td>
<td>102.0 ± 26.1 $^*$</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>$3 \times 10^{-8}$</td>
<td>24</td>
<td>96.7 ± 25.8</td>
</tr>
<tr>
<td>Hemin + Adriamycin</td>
<td>$3 \times 10^{-4}$ + $3 \times 10^{-8}$</td>
<td>24</td>
<td>41.3 ± 13.3</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>96</td>
<td>58.7 ± 1.1</td>
</tr>
<tr>
<td>Hemin</td>
<td>$3 \times 10^{-8}$</td>
<td>96</td>
<td>39.0 ± 4.4</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>$3 \times 10^{-8}$</td>
<td>96</td>
<td>0.0 ± 0.6</td>
</tr>
<tr>
<td>Hemin + Adriamycin</td>
<td>$3 \times 10^{-8}$ + $3 \times 10^{-8}$</td>
<td>96</td>
<td>29.0 ± 9.0</td>
</tr>
</tbody>
</table>

$^*$ Mean ± SE from three experiments.

Fig. 4. Effect of hemin on the association of $[\text{H}]$daunomycin with K562 cell nuclei. Nuclei isolated from K562 cells were incubated in buffer with $[\text{H}]$-daunomycin ($0.5 \mu\text{C/ml, }1 \times 10^{-7}$ M) in the presence or absence of 30 μM hemin. At various times of incubation, 0.2-ml aliquots containing $2 \times 10^6$ nuclei were overlaid on sucrose/sodium Ringer's solution and centrifuged for 2 min at 7000 rpm to remove unbound $[\text{H}]$daunomycin. The pellet was assayed for radioactivity after solubilization with 1% sodium dodecyl sulfate. Points, mean of 3 to 4 determinations; bars, SE.

Fig. 5. Effect of hemin on Adriamycin-induced alterations in the cell cycle. Exponentially growing K562 cells were treated for 24 h with no drug, $3 \times 10^{-4}$ M Adriamycin, 30 μM hemin, or both Adriamycin and hemin, as shown in A, B, D, and E, respectively. In other experiments cells were first incubated with Adriamycin or hemin for 24 h, then washed 3 times with PBS, and exposed to both Adriamycin and hemin (C and F, respectively). After 12 h of incubation, the cells were harvested and washed with PBS prior to cytofluorographic DNA analysis using propidium iodide, a fluorescent DNA binding dye, as a probe.
The simultaneous addition of hemin permits roughly 50% of the clonogenic cells present in these cultures to survive and form colonies. The surviving clonogenic cells must have been able to progress through the entire cell cycle in order to produce daughter cells, supporting the finding in Fig. 5E that the addition of hemin to Adriamycin leads to a decreased proportion of cells held up at G2-M. The fact that plating efficiency was uniformly lower in cells from 96- as compared to 24-h cultures presumably reflects the inimical effect of increasing time in culture on the clonogenicity of K562 cells.

Effect of Hemin on Anthracycline-induced Alterations in DNA. Earlier studies have shown that anthracyclines act in large part by causing DNA damage (7, 8). These drugs alter the physical state of DNA by causing unwinding of the DNA helix and inhibiting the B to Z transition in synthetic polynucleotides (9). We performed studies with SV-40 circular DNA to determine whether hemin would modify the direct effects of anthracyclines on the physical state of DNA, independent of its effect on cellular and nuclear uptake of these drugs. SV-40 circular DNA exists in two forms, the relaxed (I) and the supercoiled (II), which migrate differently on agarose gel electrophoresis. The rate of migration of each form is a function of both the size of the molecule and the degree of unwinding. Agents that cause unwinding of supercoiled DNA decrease its mobility. Thus, Form I migrates more slowly than Form II in an agarose gel. As shown in Fig. 6, incubation of SV-40 DNA with increasing concentrations of daunomycin caused a concentration-dependent unwinding of supercoiled DNA, as evidenced by progressive small decrements in the electrophoretic mobility of Form II DNA. The addition of hemin prevented this unwinding effect. Increasing concentrations of hemin (Lanes i, j, and k) caused incremental restoration of the normal pattern of migration of the supercoiled form of DNA.

DISCUSSION

Earlier studies from this laboratory (1) demonstrated that low concentrations of hemin protect cells from the cytotoxic effects of anthracycline drugs and that this protective effect occurs preferentially with hemopoietic cells. The observations reported in this paper show that the protective effect of hemin is specific for drugs of the anthracycline class, since hemin had no protective effect when cells were treated with a variety of other antineoplastic agents (Fig. 1). The findings also demonstrate that hemin protects K562 erythroleukemia cells from effects exerted by anthracyclines at the level of the cell nucleus, including binding of these drugs to isolated nuclei, alterations in cell cycle characteristics, and changes in the physical state of DNA. Although hemin can still rescue K562 cells that have been preincubated with Adriamycin for up to 12 h (1), preincubation with hemin for as long as 3 days fails to prevent the toxic effects of Adriamycin when hemin is then omitted from the culture medium (Fig. 2). Presumably this is because the hemin is readily washed from the cells before addition of the anthracycline.

It is of interest to compare the modifying influence of hemin on Adriamycin’s effect on total cell survival, as assayed by cell counts (Table 1), and on the survival of clonogenic cells capable of giving rise to colonies in plasma clots (Table 2). Adriamycin treatment led to an approximately 50% decline in total cell number, and the addition of hemin reduced this decline to about 15% (Table 1) (1). On the other hand, Adriamycin caused a complete loss of clonogenic cells, and this was reduced to a 50 to 60% loss by the addition of hemin (Table 2). Although these experiments are not strictly comparable in terms of the concentrations of Adriamycin or the times of incubation, this comparison suggests that Adriamycin alone destroys some cells outright, while causing cytostatic changes in virtually all of the others, rendering them incapable of continuing cell division. The addition of hemin apparently increases the number of cells in a stathmokinetic state but also returns almost 50% of the cell population to a condition permitting ongoing cell replication. These findings are consistent with the cell cycle profiles in Fig. 5 which show that, after treatment with both Adriamycin and hemin, there is still an increased number of cells held up at G2-M as compared to normal cells (Fig. 5, E and A, respectively), but also an increased proportion of cells returned to G1 as compared to cells treated with Adriamycin alone (Fig. 5, E and B, respectively). That the latter are now capable of proceeding through the entire cell cycle and undergoing cell division is clear from the colony assays shown in Table 2.

Taken together, the observations that hemin prevents the cellular uptake of anthracyclines (1) (Fig. 3), retards [3H]daunomycin uptake by separated cell nuclei (Fig. 4), and interferes with the effects of daunomycin on the unwinding of isolated DNA (Fig. 6) suggest the following explanations for the protective effect of hemin against anthracycline cytotoxicity: (a) interference by hemin with anthracycline binding to or action on the cell membrane and other structures (e.g., DNA) within the cell; and/or (b) direct molecular interaction of hemin with drugs of the anthracycline class, preventing their access to or promoting their efflux from the cellular milieu.

Preliminary studies from this laboratory (10) suggest that hemin interacts directly with specific regions of DNA, perhaps helping to explain hemin’s ability to activate transcription of genomic sequences concerned with globin mRNA production. It is possible that changes in DNA induced by its interaction with hemin interfere with the direct effects of anthracyclines on DNA, as observed in the experiments with isolated SV-40 circular DNA (Fig. 6). These changes may prevent anthracyclines from binding to DNA, thus displacing these drugs to the...
Hemin-induced cell protection from anthracyclines

exterior of the cell or the cell nucleus, explaining the findings in Figs. 3 and 4.

Preliminary experiments along a different line (11) have suggested that anthracyclines form direct chemical complexes with hemin or with the hemoprotein cytochrome c. Such complex formation with hemin in the incubation medium would readily explain the decreased uptake of anthracyclines by whole cells (Fig. 3) and isolated nuclei (Fig. 4) and probably also the decreased effect of these drugs on isolated DNA (Fig. 6). However, if complex formation between hemin and anthracyclines is an explanation for the hemin-protective effect, two observations suggest that this is most important intracellularly. (a) KS62 cells can be preincubated with Adriamycin for up to 12 h and still be rescued from Adriamycin cytotoxicity by addition of hemin to the culture medium (1). This suggests that hemin must combine with Adriamycin already present in intracellular sites before this cytotoxic drug has had the opportunity to exert irreversible effects within the cell. (b) Hemin preferentially protects hemopoietic as compared to nonhemopoietic cells from anthracycline toxicity (1). Perhaps only hemopoietic cells in which hemin has a physiological function have hemin receptors on the cell membrane (12), and only these cells, which permit physiological entry of hemin, can be rescued from anthracycline toxicity by this agent.

Elucidation of the mechanisms by which hemin protects hemopoietic cells from the toxic effects of anthracycline drugs should provide us with insights into mechanisms by which these agents disrupt cellular behavior. For example, direct complex formation between anthracyclines and natural heme-containing cellular constituents, such as the mitochondrial cytochromes, could account for one aspect of the cytotoxicity of these drugs, their effects on aerobic metabolism, and the generation of reactive oxidative species. Moreover, these studies should provide new information about the possible regulatory role of hemin in hemopoietic cells. Hemin is known to have numerous effects on transcriptional and posttranscriptional processes, but its role as a physiological regulator of differentiation remains unclear. Finally, it is possible that this preferential protective effect of hemin on hemopoietic cells could be exploited clinically to reduce bone marrow toxicity in patients undergoing anthracycline therapy for nonhemopoietic neoplasms; however, such an application of these findings must be preceded by much additional work in both in vitro and in vivo systems.

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REFERENCES

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