Myeloperoxidase: An Enzyme Involved in Intrinsic Vincristine Resistance in Human Myeloblastic Leukemia

By Daniel Schlaifer, Michael R. Cooper, Michel Attal, A. Oliver Sartor, Jane B. Trepel, Guy Laurent, and Charles E. Myers

One of the differences between acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) is their sensitivity to vincristine. Although vincristine plays an important role in chemotherapeutic regimens for ALL, it does not possess clinically significant activity in AML. Horseradish peroxidase, a heme-centered peroxidase, oxidatively degrades Vinca derivatives and thereby abrogates their cytotoxic activity. This finding suggested that myeloperoxidase (MPO), a heme-centered peroxidase characteristically found in AML and not in ALL, might also degrade vincristine.

We first examined the effects of MPO on vincristine in a cell-free system and demonstrated that this enzyme is capable of catalyzing vincristine’s oxidative breakdown. We also observed that vincristine is more rapidly degraded in tissue culture by MPO-positive HL-60 cells than by a MPO-negative HL-60 subclone. The degree of MPO activity in these cell lines correlated in a positive manner with their degree of resistance to vincristine’s cytotoxic activity. Moreover, the differential resistance to vincristine observed between these cell lines could be increased by increasing the concentration of H₂O₂ available to the enzyme. These data support the hypothesis that MPO-mediated oxidation of vincristine accounts in part for this drug’s lack of activity in AML.

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ative human myelomonocytic leukemia cell line (U-937) and the MPO-negative KG-1a acute myelogenous leukemia cell line (both from ATCC) were, respectively, grown in RPMI 1640 medium with 10% FCS and in Iscove’s modified Dulbecco’s medium (IMDM) with 20% FCS under the same conditions.

Characterization of the MPO-Negative and MPO-Positive HL-60 Cell Lines

Ultrastructural studies. For ultrastructural analysis of MPO activity, unfixed fresh cells were incubated in a medium of 20 mg DAB dissolved in 10 mL 0.05 mol/L Ringer’s-Tris buffer containing H$_2$O$_2$ at a final concentration of 0.003% for 1 hour at room temperature in the dark (pH 7.4). In control experiments, either DAB or H$_2$O$_2$ was omitted. After incubation, cells were washed, fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.4, for 2 hours, then put in 0.13 mol/L sodium phosphate buffer and processed for analysis.17

Immunoprecipitation. Cells (1 × 10$^6$ cells/mL) were washed in Dulbecco’s phosphate-buffered saline (PBS) and resuspended to 2 × 10$^6$ cells/mL in methionine-free RPMI 1640 supplemented with [35S]methionine (500 mCi/mL) for 3 hours. Cells were lysed and immunoprecipitated as previously described18 with an anti-MPO antiserum (Calbiochem). Immune complexes were then fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to autoradiography.19

SDS-PAGE. SDS-PAGE was performed by the method of Laemmli.20 The gel consisted of an 11% running gel and of a 3% stacking gel.

MPO activity. MPO activity was determined using the guaiacol method.21 The reaction mixture (3 mL) contained 1 mL of cell sonicates in PBS, pH 7.4, 2 mL of 20 mmol/L guaiacol. The reaction was started by adding 20 µL of 40 mmol/L H$_2$O$_2$ and an increase in absorbance at 470 nm was followed in an LKB Ultraspec spectrophotometer, Model 4053 (LKB Instruments, Gaithersburg, MD). One unit of MPO was defined as the amount of enzyme causing increase of 1 absorbance unit at 470 nm in 1 minute at 20°C under these conditions.

Flow cytometric detection of surface antigen expression and of the multidrug resistance–associated P170-glycoprotein. Expression of specific cell surface markers was evaluated by indirect immunfluorescence using a panel of monoclonal antibodies: MO1 (CD11b), MO2, MY4 (CD14), MY7 (CD13), and MY9 (CD33) from Coulter Cytometry (Hialeah, FL); Leu-M3 (CD14), Leu-M1 (CD15), HPCA-1 (CD34), and HLADR from Becton Dickinson (Mountain View, CA); and MRK16 (against the multidrug resistance–associated P170-glycoprotein, a generous gift from Dr T. Tsuruo, Tokyo, Japan).

Negative and positive controls were used, respectively, a nonreactive IgG2a isotype control and an antitransferrin receptor antibody (CD71) (both from Becton Dickinson). The doxorubicin-resistant MCF-7/ADR$^R$ cell line (a gift from Dr K. Cowan, National Cancer Institute, Bethesda, MD) and the KG-1 a acute myelogenous leukemia cell line were used as a positive control for, respectively, MRK16 and CD34 antibody reactivity. For analysis, 10 million cells were suspended in PBA (PBS with 1% bovine serum albumin [BSA] and 0.1% sodium azide) and incubated with primary antibody at 4°C for 30 minutes. After washing in PBA, cells were resuspended in phycoerythrin-conjugated goat antimouse Ig (TAGO; 1:90 in PBA). After an additional 30 minutes at 4°C, cells were washed and analyzed by using a FACStar flow cytometer (Becton Dickinson Immunocytometry Systems).

High-Performance Liquid Chromatography (HPLC) of VCR

HPLC was performed with a Gilson (Middleton, WI) HPLC system, using two Model 303 pumps, a model 811 dynamic mixer, and a Knauer (Springfield, VA) 8700 variable-wavelength ultraviolet-visible absorbance detector. [3H]VCR was detected by a Flo-One beta radioactive flow detector (Radiomatic Instruments & Chemical Co, Inc, Meriden, CT). Samples were analyzed on a Waters (Milford, MA) Nova-Pak C18 Cartridge column (0.8 × 10 cm) in an RCM-100 radial compression module. Elution was effected by an isocratic mobile phase of acetonitrile and water (5:5, vol/vol) with 0.005 mol/L dibasic ammonium phosphate and 0.005 mol/L 1-pentanesulfonic acid. The flow rate was 2 mL/min and absorbance was monitored at 220 nm. The retention time of VCR under these conditions was 6 minutes.

In Vitro Degradation of VCR by MPO

Incubations of VCR and MPO were performed in 50 mL Falcon (Oxnard, CA) tubes each containing 5 mL of 100 mmol/L acetate buffer pH 6 or pH 7.4, 250 µmol/L of H$_2$O$_2$, 2 U (0.1 mg) of MPO, and 100 µmol/L of VCR. The mixtures were incubated at 37°C for 3 hours in the dark. HRP, 26 U (0.1 mg), was used in place of MPO for comparison. The following controls were performed under the same conditions: VCR alone, VCR plus H$_2$O$_2$ without MPO or HRP, VCR plus MPO or HRP without H$_2$O$_2$. In some experiments, MPO was replaced by other neutrophil enzymes, eg, alkaline phosphatase, acid phosphatase, and esterase, to determine whether they were capable of mediating VCR degradation. In these experiments, the pH of the buffer was adjusted to the pH optimum for each enzyme’s activity. After extraction with Sep-Pak Plus C18 Environmental Cartridges (Waters Corp, Milford, MA), the samples were analyzed by HPLC.

Degradation of VCR by the Cell Lines

[3H]VCR (20 nmol/L) was added to exponentially growing HL-60(A+), and HL-60/IJC114 cell lines (150,000 cells/mL) and incubated for 3 hours at 37°C in the dark. No H$_2$O$_2$ was added. The supernatant was then extracted with a C18 Sep-Pak cartridge and analyzed by HPLC. The cell pellets were processed according to Houghton et al22 and analyzed by HPLC. To address the possibility that hydrogen peroxide could be a limiting factor in our cell experiments, we used in some experiments an enzyme system, the glucose oxidase–mediated oxidation of glucose to gluconic acid, which reduces molecular oxygen directly to hydrogen peroxide. Two milliliters per milliliter and 11 mmol/L of, respectively, glucose oxidase and glucose were used. We then processed the supernatants and the cell pellets as above. Each experiment was performed in triplicate. Another MPO-negative subclone, HL-60/IJC115, was tested in the same conditions.

Cytotoxicity Assays

Cells were adjusted to a concentration of 200,000 cells/mL in complete growth medium and continuously exposed to VCR at different concentrations (0.1 to 10,000 nmol/L). Cell number was determined after 72 hours with an electronic particle counter (model ZBI; Coulter Electronics, Hialeah, FL). Cell viability was tested by trypan blue exclusion. In some experiments, viable cells were determined by counting with a fluorometric method modified from Larson et al.23 Briefly, 500 µL of the cell suspension (100,000 cells) was incubated with 100 µL FDA (7.5 mg/mL) for 30 minutes in the dark before reading the fluorescence with a FACStar flow cytometer. Because the results of the two methods were similar, only the particle counter was used for most of the experiments. The IC$_{50}$ value (concentration of drug that inhibited proliferation by 50%) was determined graphically from semilogarithmic plots of relative proliferation rates as a function of drug concentration.

Cytotoxicity Assays in the Presence of a Hydrogen Peroxide–Generating System

We investigated the effects of 2 nM/L glucose oxidase (a nontoxic concentration) on the survival of MPO-positive and MPO-
negative HL-60 cell lines treated with increasing concentrations of VCR. The concentration of glucose was initially 11 mmol/L in all experiments. Glucose oxidase and glucose were added daily during all experiments. IC<sub>50</sub> values were determined as previously described.

**Degradation of VCR by Cells from Patients With AML**

Bone marrow samples of four patients suffering from AML (2 FAB-M3, 1 FAB-M1, and 1 FAB-M2) were collected before treatment in RPMI 1640 with 100 U/mL heparin and separated by Ficoll-
Hypaque density centrifugation. Marrow cells were washed twice in IMDM, resuspended at the concentration of 2 × 10^5 cells/mL, and cryopreserved in IMDM containing 10% dimethyl sulfoxide (DMSO) and 50% FCS. After thawing, leukemia cells were resuspended in IMDM supplemented with 30% FCS. In each case, most of the cells were MPO positive. The cells were then incubated with 20 nmol/L of [3H]VCR under the same conditions used for the HL-60 cell lines. No H2O2 was added. HPLC analysis was performed in the same conditions as for the HL-60 cells. The MPO-negative myeloid cell lines KG-1a and U-937 were used as negative controls.

RESULTS

Characterization of the MPO-Negative and MPO-Positive HL-60 Cell Lines

HL-60(JC114) cells, which were MPO negative by routine optical examination, were studied for MPO activity in comparison with the MPO-positive HL-60(A+) cell line. Both cell lines had the same cell-doubling time of 36 to 48 hours during the logarithmic phase of growth, as previously reported.

Ultrastructural analysis. There were significantly fewer granules in the HL-60(JC114) cell line compared with the MPO-positive HL-60(A+) cell line. The HL-60(A+) cells contained mainly granules with electron-dense material. When the cells were examined specifically for MPO, very few granules (<1%) were positive in the HL-60(JC114) cells, whereas most of the granules were positive in the HL-60(A+) cells (Fig 1A and B). In addition to this difference in MPO-positive granules, most of the HL-60(JC114) cells contained large lipid droplets.

Immunoprecipitation. MPO was immunoprecipitated as an 88-Kd polypeptide (the size of the MPO precursor polypeptide) but was absent in the HL-60(JC114) subclone (Fig 2).

Guaiacol assay. MPO activity measured in sonicates from HL-60(A+) cells was very high (120.5 ± 2.3 U/mg protein/min) compared with MPO activity in HL-60(JC114) cells (<0.01 U/mg protein/min).

Phenotype of the cell lines. The phenotypes of the HL-60(A+) and the HL-60(JC114) cell lines were determined by flow cytometry and showed in both cases the same positivities: CD13 and CD33 positive, CD14 and CD15 faintly positive, HLA-DR and CD11b negative (Table 1). These results suggest that granulocytic or monocytic differentiation had not occurred during the growth of the cells, so that phenotypic changes could not explain the differences in the MPO content of the two cell lines. CD34 was also negative for both the HL-60(A+) and HL-60(JC114) cell lines (Table 1), suggesting that no undifferentiation occurred in the HL-60(JC114) cells.

VCR Degradation in a Cell-Free System

After 3 hours of incubation at 37°C, 100 μmol/L of VCR was almost completely degraded by 0.1 mg (2 U) of MPO plus H2O2 (Fig 3 and Table 2). There was no difference in the extent of VCR degradation between pH 6 (pKa of MPO) and pH 7.4. Degradation of VCR was not seen when VCR was incubated with H2O2 in the absence of enzyme or when VCR was incubated with the enzyme in the absence of H2O2 (Table 2). Other enzymes found in myeloid blasts, such as esterase and acid and alkaline phosphatases, were unable to degrade VCR (Table 2). These results document that VCR can act as an electron-donating substrate for MPO.

VCR Degradation in Tissue Culture

VCR degradation by MPO-positive and MPO-negative cell lines was assessed by HPLC. Although 75% ± 2% of the [3H]VCR was still present in the supernatant of the MPO-negative HL-60(JC114) cell line, only 58% ± 5% of the [3H]VCR was found in the supernatant of the MPO-positive HL-60(A+) cell line (P < 0.006) (Fig 4 and Table 3). When the cells were analyzed, 78% ± 3% of the [3H]VCR was present in the HL-60(JC114) cells compared with 55% ± 5% in the HL-60(A+) cells (P = .002). Glucose oxidase–dependent H2O2 generation clearly increased the degradation of [3H]VCR by the MPO-positive HL-60(A+) cell line in that

<table>
<thead>
<tr>
<th>Surface Markers</th>
<th>HL-60(JC114)</th>
<th>HL-60(A+)</th>
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<tbody>
<tr>
<td>TR</td>
<td>89 ± 5</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>MO1</td>
<td>2 ± 2</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>MO2</td>
<td>13 ± 4</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Leu-M3</td>
<td>13 ± 4</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Leu-M1</td>
<td>13 ± 3</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>MY4</td>
<td>13 ± 6</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>MY7</td>
<td>34 ± 5</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>MY9</td>
<td>30 ± 3</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>HPCA-1</td>
<td>2 ± 2</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>3 ± 2</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>MRK16</td>
<td>2 ± 2</td>
<td>5 ± 3</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.

* Surface markers: CB11a: MO1; CD13: MY7; CD14: MO2, Leu-M3, and MY4; CD15: Leu-M1; CD33: MY9; CD34: HPCA-1; TR: transferrin receptor (CD71).
the supernatant and the cell pellet showed, respectively, 21% ± 3% and 22% ± 2% of [3H]VCR remaining. In contrast, no change was noted in the MPO-negative HL-60(JC114) cell line, suggesting that hydrogen peroxide availability was a limiting factor in the [3H]VCR degradation in the HL-60(A+) cell line. The same experiment was repeated with another MPO-negative subclone, HL-60(JC115), with similar results (data not shown).

**Cytotoxicity of VCR on the MPO-Negative and MPO-Positive Cell Lines**

The cytotoxicity curves (Fig 5) showed that the MPO-positive HL-60(A+) cell line was more resistant than was the T-lymphoblastic CCRF-CEM cell line. Moreover, the HL-60(A+) cell line was also more resistant to VCR than was the 5% to 10% MPO-positive HL-60(J) cell line or its MPO-negative HL-60(JC114) subclone. The IC50 values are listed in Table 4 and show a significant threefold to fourfold increase for the MPO-positive HL-60(A+) cell line compared with the MPO-negative T-lymphoblastic CCRF-CEM or with the 5% to 10% MPO-positive HL-60(J) cell line or its MPO-negative HL-60(JC114) subclone. The IC50 for the HL-60(JC115) subclone is not significantly different than those of the HL-60(JC114) subclone and the IC50 for the MPO-negative U-937 cell line is similar to the IC50 of the HL-60(J) cell line. In contrast, we found that the MPO-negative KG-1a acute myelogenous cell line was spontaneously very resistant to VCR with an IC50 27 times higher than the 100% MPO-positive HL-60(A+) cell line.

**P170-Glycoprotein Expression by FACS Analysis**

All the HL-60 clones were negative for P170-glycoprotein expression as detected by the MRK16 antibody (Table 1). Therefore, the differences in VCR sensitivity among these cell lines were not due to differences in the expression of the P170-glycoprotein associated with the multidrug-resistant phenotype as determined by FACS analysis. The U-937 and KG-1a cell lines were also P170-glycoprotein negative (data not shown).

### Table 2. VCR Degradation in the Cell-Free System (tripllicate)

<table>
<thead>
<tr>
<th>Products*</th>
<th>% of Degradation†</th>
</tr>
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<tbody>
<tr>
<td>VCR alone</td>
<td>0 ± 3</td>
</tr>
<tr>
<td>VCR + H2O2</td>
<td>2 ± 4</td>
</tr>
<tr>
<td>VCR + MPO without H2O2</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>VCR + MPO + H2O2</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>VCR + HRP + H2O2</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>VCR + esterase</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>VCR + alkaline phosphatase</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>VCR + acid phosphatase</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>VCR + glucose + glucose oxidase</td>
<td>4 ± 4</td>
</tr>
</tbody>
</table>

* The concentrations used were: VCR: 100 μmol/L; H2O2 250 μmol/L; MPO, HRP, esterase, alkaline, and acid phosphatases: 0.1 mg; glucose: 11 mmol/L; and glucose oxidase: 2 mU/mL.

† As determined by the HPLC peak (mean ± SD).

### Effect of the Hydrogen Peroxide-Generating System on the Cytotoxicity of VCR

The results in Figs 1 and 2 document that the difference in MPO expression between HL-60(A+) and HL-60(JC114) is much greater in magnitude than the modest threefold to fourfold difference in the drug sensitivity observed in Fig 5. Because the degradation of VCR by MPO is dependent on the presence of hydrogen peroxide, it is possible that the effectiveness of MPO in HL-60(A+) is limited by the availability of hydrogen peroxide. To test this hypothesis, a hydrogen peroxide-generating system, using the reduction of glucose by glucose oxidase, was used to overcome any limitation of the availability of hydrogen peroxide in the cytotoxicity experiments. The result was an increase in the difference between the sensitivity to VCR seen in HL-60(A+) as compared with that seen in HL-60(JC114) (Fig 6). The MPO-positive HL-60(A+) line had an IC50 greater than 1,000 nmol/L compared with 3.5 to 4 nmol/L for the HL-60(JC114) cells. Thus, increased availability of H2O2 increased the difference between the two cell lines from threefold to fourfold to more than 250-fold. Moreover, the HL-60(A+) cell line was more resistant in the presence of the hydrogen peroxide-generating system (IC50 > 1000 nmol/L) than without (IC50 of 13 nmol/L) (Figs 5 and 6), confirming that hydrogen peroxide availability was a limiting factor in MPO-mediated oxidation of
**MPO AND VCR RESISTANCE IN AML**

Fig 4. Degradation of VCR by the cell lines. [3H]VCR was added to the exponential growing cell lines and incubated for 3 hours at 37°C. The supernatant and cell pellet were then analyzed by HPLC: supernatant of (A) HL-60(JC114) and (B) HL-60(A+); cell pellet of (C) HL-60(JC114) and (D) HL-60(A+).

VCR in HL-60(A+) cells. The hydrogen peroxide-generating system alone was not able to degrade VCR in the cell-free system as measured by HPLC (Table 2).

**VCR Degradation by Cells from Patients With AML**

The ability of cells from four MPO-positive AML patients to degrade [3H]VCR was examined in comparison with MPO-negative AML cell lines and the results are listed in Table 5. [3H]VCR was more efficiently degraded in MPO-positive cells from patients with AML in comparison with the MPO-negative AML cell lines KG-1a and U-937.

**DISCUSSION**

Clinical studies have shown that patients with AML, in contrast to patients with ALL, are generally spontaneously resistant to VCR, but the precise mechanisms involved have remained obscure. It has been shown that, in some cases, the P170-glycoprotein associated with the multidrug-resistant phenotype could be found. Other mechanisms for VCR resistance have been recently reported, including other surface membrane proteins not related to the P170-glycoprotein or an altered tubulin. Our study strongly suggests a new mechanism for VCR resistance involving the degradation of VCR by MPO in presence of hydrogen peroxide.

**Table 3.** [3H]VCR Degradation by the MPO-Positive HL-60(A+) and MPO-Negative HL-60(JC114) Cell Line in the Presence or Absence of the Hydrogen Peroxide-Generating System (triplicate)

<table>
<thead>
<tr>
<th></th>
<th>HL-60(A+) (%)</th>
<th>HL-60(JC114) (%)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without hydrogen peroxide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>58 ± 5</td>
<td>75 ± 2</td>
<td>.006</td>
</tr>
<tr>
<td>Pellet</td>
<td>55 ± 5</td>
<td>78 ± 3</td>
<td>.002</td>
</tr>
<tr>
<td>With hydrogen peroxide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>21 ± 3</td>
<td>74 ± 3</td>
<td>.000006</td>
</tr>
<tr>
<td>Pellet</td>
<td>22 ± 2</td>
<td>81 ± 3</td>
<td>.000001</td>
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</table>

* Percentage of the peak of nondegraded [3H]VCR.
† P value as determined by the t-test.

Fig 5. Effect of continuous exposures of VCR on proliferation of the CCRF-CEM human T-lymphoblastic cell line and of the different human myeloblastic HL-60 cell lines (triplicate).
We first confirmed that MPO, like HRP, is capable of mediating the oxidative destruction of VCR in the presence of hydrogen peroxide in a cell-free system. The concentration of VCR used in these experiments (100 μmol/L) was much higher than the concentrations achieved in patients or the concentrations used in tissue culture experiments. Nonetheless, this large amount of drug was almost completely degraded by 0.1 mg of pure MPO, approximately the amount of MPO contained in 15 × 10⁶ MPO-positive HL-60 cells. This degradation of VCR was specific to peroxidases (MPO and HRP), with other leukocyte enzymes being inactive in this respect.

HL-60 is a myeloblastic cell line that is usually strongly MPO positive. We were fortunate in observing the spontaneous development of an HL-60 clone that had weak (5% to 10% of cells positive) expression of MPO, allowing for the further development of subclones with no detectable MPO. Myeloid differentiation has been associated with a decrease in MPO messenger RNA (mRNA), but the enzymatic activity of the differentiated clone in this instance was only slightly less than that of the parent line. Moreover, the phenotypic analysis of our cell lines showed no evidence for differentiation of the HL-60 subclone. Furthermore, spontaneous emergence of MPO-deficient clones of HL-60 has been described previously during cell culture.

As expected, degradation of VCR occurred more rapidly in tissue culture when the drug was added to MPO-positive HL-60 cells than when it was added to the MPO-negative HL-60 cell line. Of interest, this occurred when no hydrogen peroxide was added to the medium, suggesting that HL-60 cells must produce hydrogen peroxide, as has been recently reported for other cell lines. This degradation was sufficient to deplete VCR from both supernatant and cell pellet, and interestingly enough it was significantly increased by increasing hydrogen peroxide availability, suggesting that hydrogen peroxide is a limiting factor for this degradation. The ability of the MPO-positive HL-60 cell line to survive at higher concentrations of VCR than the MPO-positive clone or the MPO-negative subclone also supports the hypothesis that MPO mediates VCR oxidation and resistance. Further support for this notion comes from the experiments wherein the concentration of hydrogen peroxide available to the cells in tissue culture was augmented by the addition of the glucose-glucose oxidase system. With such augmentation of the hydrogen peroxide concentration, the difference in the IC₅₀ values for VCR between the MPO-positive and MPO-negative cell lines increased from threefold to fourfold to 250-fold.

Two other myeloid cell lines, ie, the MPO-negative human myelomonocytic leukemia cell line U-937 and the MPO-negative KG-1a acute myelogenous leukemia cell line, were tested for VCR sensitivity. Although the U-937 cell line had the same pattern of sensitivity for VCR as the HL-60(A+) cell line, the KG-1a cell line was spontaneously 27 times more resistant than the MPO-positive HL-60(A+) cell line. This was not explained by the expression of the P170-glycoprotein. Therefore, other mechanisms for spontaneous VCR resistance, not mediated by MPO, may be present in the KG-1a cell line and need further studies.

Malignant cells from four MPO-positive patients with AML were able to degrade VCR without the addition of hydrogen peroxide (Table 5), suggesting that this VCR degradation may occur in patients. Further studies with a larger

### Table 5. [³H]VCR Degradation by Cells From MPO-Positive Patients With AML and From MPO-Negative AML Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Supernatant (%)</th>
<th>Pellet (%)</th>
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<tbody>
<tr>
<td>MPO-positive patients with AML:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAB-M3</td>
<td>50</td>
<td>21</td>
</tr>
<tr>
<td>FAB-M3</td>
<td>61</td>
<td>25</td>
</tr>
<tr>
<td>FAB-M1</td>
<td>60</td>
<td>39</td>
</tr>
<tr>
<td>FAB-M2</td>
<td>49</td>
<td>66</td>
</tr>
<tr>
<td>MPO-negative AML cell lines:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KG-1a</td>
<td>76</td>
<td>83</td>
</tr>
<tr>
<td>U-937</td>
<td>73</td>
<td>85</td>
</tr>
</tbody>
</table>

* Percentage of the peak of nondegraded [³H]VCR.
number of MPO-positive and MPO-negative patients with
AML are needed to correlate the MPO content of the malig-
nant blasts and the VCR degradation.

Moreover, because VCR degradation occurred at clinically
relevant concentrations for VCR in the HL-60 cell lines (ie,
1 to 1,000 nmol/L), this phenomenon might provide an
explanation for the poor prognosis of adult or childhood ALL in which MPO activity or mRNA has been detected.

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Myeloperoxidase: an enzyme involved in intrinsic vincristine resistance in human myeloblastic leukemia

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