CONCISE REPORT

Relationship of Mitochondrial Membrane Potential to Hemoglobin Synthesis During Friend Cell Maturation

By Willie Wong, Stephen H. Robinson, and Asterios S. Tsiftsoglou

Previous studies have shown that exposure to imidazole dissociates hemoglobin synthesis from other aspects of cell maturation in dimethylsulfoxide (DMSO)-treated mouse erythroleukemia (MEL) cells. In the present study, we have found that imidazole causes hyperpolarization of the mitochondrial membrane in MEL cells exposed to DMSO, in contrast to the depolarization observed with DMSO alone. Like the defect in hemoglobin synthesis, membrane hyperpolarization is reversible upon removal of imidazole and incubation of cells with DMSO alone. These correlations suggest that alterations in the electrostatic properties of the mitochondrial membrane, due directly or indirectly to the effects of imidazole, interfere with heme synthesis but not with other aspects of the maturation process in these developing erythroid cells.

MATERIALS AND METHODS

Cells cultures and chemicals. Cells were of the MEL 745-PC-4 subline derived from the MEL 745 cells originally isolated by Friend et al. Cells were grown in RPMI-1640 (Life Technologies, Bethesda, MD) supplemented with 10% fetal calf serum and antibiotics at 37°C in a humidified atmosphere with 5% CO2. Continuous growth was ensured by maintaining the cells at densities of 3 x 10^6 to 3 x 10^7 cells per milliliter. DMSO (Kodak, Rochester) was added directly to cultures without previous sterilization. Imidazole (Sigma Chemical Co, St Louis) was dissolved at a concentration of 50 mg/mL in distilled water, sterilized by filtration, and stored at −35°C prior to use. The experimental protocol used in this study was similar to that published previously by this laboratory.

Cellular hemoglobin levels were determined spectrophotometrically as described elsewhere.

Determination of benzidine-positive cells and hemoglobin content. The proportion of hemoglobin-containing cells was determined by scoring more than 250 cells on cytocentrifuge slides stained with benzidine/H2O2. Cellular hemoglobin levels were determined spectrophotometrically as described elsewhere.

Heme biosynthesis. Cells were incubated with [2-14C]-glycine (2.5 μCi/mL, 40 to 60 mCi/mmol) in medium for four hours and then washed three times with phosphate-buffered saline. Heme was extracted from the acidic cell lysate with methyl ethyl ketone and its radioactivity was measured in a liquid scintillation counter.

Cytofluorographic assessment of mitochondrial membrane potential. MEL cells treated as indicated in the text were harvested from culture, washed three times with phosphate-buffered saline (pH 7.4), and resuspended at a concentration of 10^6 cells per milliliter. Cells were then incubated with rhodamine-123 (10^-5 mol/L) only for 15 minutes at 37°C and analyzed cytofluorographically as previously described. Imidazole is readily removed from MEL cells by the washing procedure and it is therefore unlikely that this agent directly interferes with the rhodamine-123 assay for mitochondrial membrane polarization.

RESULTS AND DISCUSSION

As shown in Table 1, imidazole markedly inhibits heme biosynthesis and hemoglobin accumulation in both control and DMSO-treated MEL cells. Removal of imidazole from DMSO-treated cultures after 24 hours leads to virtually full resumption of hemoglobin accumulation, 9.80 ± 10.60 μg per 10^6 cells with exposure to DMSO alone. The proportion of MEL cells committed to terminal differentiation is approximately the same (>85%) in cultures exposed to DMSO only or to both DMSO and imidazole for 72 hours (data not shown).

Cytofluorographic analysis with rhodamine-123, staining with which is specifically related to the state of mitochondrial polarization, demonstrated that DMSO induces depolarization, whereas imidazole leads to hyperpolarization of the mitochondrial membrane in MEL cells (Fig 1B and C). Incubation of DMSO-treated cells with imidazole for 24 hours (Fig 1D) significantly prevented DMSO-induced membrane depolarization, as shown by a shift of the curve of rhodamine-123 staining to the right as compared to the curve for cells treated with DMSO alone. Removal of imidazole and reincubation with DMSO alone after 24 hours (Fig 1E) led to a shift to the left once again, i.e., toward depolarization, demonstrating that the membrane hyperpolarization induced by imidazole is reversible, as is the effect of imida-
Table 1. Inhibition of Heme Biosynthesis and Hemoglobin Accumulation by Imidazole in DMSO-Treated MEL Cells Undergoing Terminal Maturation In Vitro

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Benzidine-Positive Cells* (%)</th>
<th>Hemoglobin† (µg/10^6 Cells)</th>
<th>[2-¹⁴C]-Glycine Incorporation Into Heme‡ (cpm/10^7 Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24 h</td>
<td>24-48 h 48-72 h 72-120 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>None None None None</td>
<td>&lt;1</td>
<td>5,275</td>
</tr>
<tr>
<td>None</td>
<td>None None None None</td>
<td>&lt;1</td>
<td>5,750</td>
</tr>
<tr>
<td>None</td>
<td>IM IM IM IM</td>
<td>&lt;1</td>
<td>7,420</td>
</tr>
<tr>
<td>DMSO</td>
<td>DMSO DMSO DMSO DMSO</td>
<td>&gt;95</td>
<td>610</td>
</tr>
<tr>
<td>DMSO + IM</td>
<td>DMSO + IM DMSO + IM DMSO + IM</td>
<td>6</td>
<td>990</td>
</tr>
<tr>
<td>DMSO + IM</td>
<td>DMSO + IM DMSO + IM DMSO + IM</td>
<td>78</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: DMSO, dimethyl sulfoxide (1.5%, vol/vol); IM, imidazole (500 µg/ml); ND, not determined.

*The proportion of benzidine-positive cells was determined on cytoslides stained with benzidine/peroxide. At least 250 cells were scored for each time point.†

†Cells were harvested from culture and washed three times with phosphate-buffered saline, pH 7.4, and cell lysates were assayed for hemoglobin spectrophotometrically as previously described.‡

‡Heme biosynthesis was determined by labeling with [2-¹⁴C]-glycine for four hours and measuring the incorporation of this precursor into extractable heme.

Fig 1. Cytofluorographic analysis of mitochondrial membrane potential in MEL cells exposed to DMSO and/or imidazole (IM). MEL cells treated with no drug or with DMSO (1.5% vol/vol) in the presence or absence of imidazole were washed three times with phosphate-buffered saline, pH 7.4, resuspended at a concentration of 10^6 cells per milliliter, incubated with rhodamine-123 (10⁻⁸ mol/L) for 15 minutes at 37 °C, and then analyzed cytofluorographically. The amount of rhodamine-123 present intracellularly reflects the amount of the dye bound to mitochondria which, in turn, is a function of the degree of mitochondrial transmembrane polarization. The relative fluorescence intensity increases from left to right. The arrows downward and upward indicate the addition and removal of imidazole from the cultures, respectively.

Zole on hemoglobin accumulation (Table 1). Some depolarization of mitochondrial membranes was observed in cultures exposed to DMSO followed by the addition of imidazole (Fig 1D and E); this was probably present in cells that had already passed the point at which imidazole was capable of reversing membrane depolarization. The fact that both the hyperpolarization of the mitochondrial membrane (Fig 1) and the block in hemoglobin synthesis (Table 1) caused by imidazole are reversible on withdrawal of this agent supports the conclusion that these two effects are causally related. It therefore seems likely that imidazole interferes with hemoglobinization of differentiating MEL cells by altering the properties of the mitochondrial membrane. The mitochondria are the sites of both initiation and completion of heme biosynthesis, and iron must be transported into the mitochondrial cristae in order to be incorporated into the protoporphyrin ring. Thus, alterations in the electrostatic properties of the mitochondrial membrane could explain the marked diminution in iron transport and heme synthesis that are produced by imidazole in differentiating MEL cells. This could be a direct effect of imidazole's interaction with the mitochondrial membrane or an indirect effect mediated, for example, through alterations in the transport of cations such as calcium within the cell. It also remains possible that the defect in heme synthesis and the membrane hyperpolarization are independent effects of imidazole.

Imidazole has also been reported to decrease the accumulation of globin mRNA in differentiating MEL cells. Although this could be due to an independent effect of imidazole, eg, on transcription, it seems possible that it is a secondary effect of the inhibition of heme synthesis in the mitochondria of these cells. Since hemoglobin synthesis is often thought of as an intrinsic aspect of erythroid cell differentiation, it is of considerable interest that the effects of imidazole on heme and hemoglobin synthesis and mitochondrial membrane polarization take place while other aspects of erythroid cell maturation proceed in an apparently normal fashion.
REFERENCES


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