Tiazofurin Induction of Mouse Erythroleukemia Cell Hemoglobin Production in the Absence of Commitment or Changes in Protooncogene Expression

By Matthew L. Sherman, Timothy D. Shafman, Michael S. Colman, and Donald W. Kufe

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193), is a synthetic nucleoside inhibitor of inosine monophosphate dehydrogenase and blocks guanine nucleotide biosynthesis. In the present study, we examined the effects of tiazofurin on mouse erythroleukemia (MEL) cell differentiation and protooncogene expression. Tiazofurin induced hemoglobin production in MEL cells in a concentration-dependent manner, as measured by an increase in benzidine staining. Northern blot analysis of MEL cells treated with 7 μmol/L tiazofurin demonstrated accumulation of both α- and β-globin RNA transcripts. This induction of differentiation was blocked by the presence of exogenous guanosine (100 μmol/L). In contrast to the down-regulation of c-myc and c-myb RNA in MEL cells induced by dimethyl sulfoxide (DMSO) or hexamethylene bisacetamide (HMBA), there was no detectable change in levels of these transcripts after tiazofurin treatment. Furthermore, MEL cells induced by tiazofurin did not commit to terminal differentiation. These results suggest a role for guanine nucleotides, at least in part, in the regulation of MEL cell differentiation.

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RESULTS

We monitored the effects of varying concentrations of tiazofurin on MEL cell proliferation. Although treatment with 1 μmol/L tiazofurin had no effect on cell growth, increasing concentrations were associated with decreases in viability.

![Graph showing the effects of tiazofurin on MEL cell proliferation.](image)

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MEL cell proliferation (Fig 1). Cytostasis was achieved with 10 μmol/L tiazofurin (Fig 1). We also determined the effects of varying concentrations of tiazofurin on induction of MEL cell differentiation. Tiazofurin at 1 μmol/L had no detectable effect on the appearance of benzidine-positive cells. However, higher concentrations of this agent induced MEL cell hemoglobin synthesis in a dose-dependent manner (Table 1). Thus, treatment with 10 μmol/L tiazofurin resulted in maximal induction of benzidine positive cells (39.8%). Cell viability following exposure to 10 μmol/L tiazofurin remained >95% as determined by trypan blue exclusion, whereas higher concentrations were partially cytotoxic to MEL cells. MEL cells were also treated with tiazofurin in the presence of guanosine to determine if hemoglobin production and loss of proliferation were blocked by repletion of guanine nucleotides. There was no detectable MEL cell hemoglobin production or loss of proliferative capacity induced by tiazofurin in the presence of 100 μmol/L guanosine (Table 1).

Northern blot analysis of MEL cytoplasmic RNA hybridized to globin DNA probes is shown in Fig 2. Treatment of MEL cells with tiazofurin for various times up to 96 hours resulted in an increase in both α- and β-globin RNA transcripts (Fig 2A). In addition, this effect was inhibited by the addition of exogenous guanosine to tiazofurin-treated MEL cells (Fig 2B).

We next studied the effects of tiazofurin on MEL cell protooncogene expression. Northern blot analysis of MEL cytoplasmic RNA collected over 96 hours of drug exposure is shown in Fig 3. In contrast to induction of MEL cell differentiation by DMSO or HMBA, treatment with 7 μmol/L tiazofurin had no detectable effect on levels of c-myc or c-myb RNA.

Previous studies have demonstrated that certain inducers such as DMSO commit MEL cells to terminal differentiation. Control, DMSO-treated, and tiazofurin-treated MEL cells were grown in methylcellulose to determine the percentage of committed cells. As listed in Table 2, treatment with DMSO for three days before plating in methylcellulose was associated with 65% benzidine-positive colonies. In contrast, treatment with tiazofurin had no detectable effect on c-myc or c-myb RNA.

Table 1. Effect of Tiazofurin on MEL Cell Differentiation

<table>
<thead>
<tr>
<th>Addition</th>
<th>Cell Count (x 10^5/mL)</th>
<th>Benzidine Positive (%)</th>
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<tbody>
<tr>
<td>None</td>
<td>4.4*</td>
<td>0</td>
</tr>
<tr>
<td>DMSO, 1.8%</td>
<td>5.0*</td>
<td>88.5 ± 1.1</td>
</tr>
<tr>
<td>Tiazofurin (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.67*</td>
<td>39.8 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>1.3*</td>
<td>35.5 ± 4.7</td>
</tr>
<tr>
<td>5</td>
<td>3.3*</td>
<td>22.2 ± 1.8</td>
</tr>
<tr>
<td>1</td>
<td>4.0*</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>0.77†</td>
<td>0</td>
</tr>
<tr>
<td>Tiazofurin 7 μmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanosine 100 μmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanosine/tiazofurin</td>
<td></td>
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</tbody>
</table>

*MEL cells were seeded at 8 x 10^5/mL on day 0. Cell counts and benzidine staining were determined after four days. Concentrations of tiazofurin >10 μmol/L were cytotoxic.

†Cell counts and benzidine staining were determined after three days of drug treatment.

Fig 2. Effects of tiazofurin and/or guanosine on globin RNA levels. (A) MEL cells were treated with 7 μmol/L tiazofurin for the indicated times. Cytoplasmic RNA (15 μg/lane) was purified as described, separated by agarose-formaldehyde gel electrophoresis, and transferred onto a nitrocellulose filter. Hybridizations were performed using the indicated nick-translated (2 x 10^6 cpm/μg DNA) 32P-labeled DNA probes. (B) MEL cells were treated with 7 μmol/L tiazofurin and/or 100 μmol/L guanosine for 72 hours.
EFFECTS OF TIAZOFURIN ON MEL CELLS

Fig 3. Effects of tiazofurin on the induction of c-myc and c-myb RNA during MEL cell differentiation. MEL cells were treated with 7 μmol/L tiazofurin for the indicated times. Cytoplasmic RNA was purified as previously described and hybridized to nick-translated probes after transfer to nitrocellulose.

tiazofurin had little if any effect on terminal differentiation of MEL cells.

DISCUSSION

Treatment of MEL cells with agents such as DMSO and HMBA induces a coordinated program of molecular events including expression of globin RNA, heme synthesis, inhibition of growth, and a loss of self-renewal capacity. Other events associated with MEL cell differentiation include changes in c-myc and c-myb gene expression. In contrast, compounds such as hemin fail to induce the entire differentiation program. For example, although hemin increases levels of globin mRNA and hemoglobin, this agent has no effect on growth rates of MEL cells and fails to induce commitment to terminal differentiation. Furthermore, hemin treatment of MEL cells has no detectable effect on commitment to terminal MEL cell differentiation. The identification of tiazofurin as a compound which dissociates hemoglobin accumulation from changes in c-myc expression and commitment should provide an opportunity to examine further the molecular mechanisms responsible for induction of the differentiated MEL phenotype.

Table 2. Effect of Tiazofurin on Commitment to Terminal Differentiation

<table>
<thead>
<tr>
<th>Addition</th>
<th>Benzidine Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>DMSO 1.8%</td>
<td>65</td>
</tr>
<tr>
<td>Tiazofurin 7 μmol/L</td>
<td>3</td>
</tr>
</tbody>
</table>

MEL cells were treated with drug for 48 hours and plated in 1.8% methylcellulose as described in the Materials and Methods Section. The percentage of benzidine-positive colonies was determined after three days.

MEL cell differentiation induced by DMSO and HMBA is also associated with a biphasic decrease in the level of c-myc and c-myb transcripts. Our results indicate that tiazofurin fails to induce changes in c-myc and c-myb expression, despite the inhibitory effects of this agent on MEL cell growth. In this regard, we previously demonstrated that arrest of MEL cell proliferation by ornithine decarboxylase inhibition is not associated with detectable changes in c-myc expression. Furthermore, in other cell lines, reduced myb and myc expression may not be obligatory for phorbol ester-induced growth arrest to occur. Our results also demonstrate that tiazofurin has no detectable effect on commitment to terminal MEL cell differentiation. This finding and the absence of changes in c-myc expression are in concert with recent studies on the effects of constitutively expressed c-myc genes transfected into MEL cells that suggest that a decrease in c-myc expression is necessary for commitment to terminal differentiation. Together, these results suggest that a decrease in c-myc and/or c-myb may be necessary for commitment; however, such decreases are not obligatory for induction of hemoglobin production in MEL cells.

Finally, recent studies have demonstrated that c-myc protein levels are maintained in the presence of declines in c-myc transcripts during erythroid differentiation of MEL cells by DMSO and hypoxanthine. These results question the significance of the biphasic decline in c-myc RNA levels in the induction of MEL cell differentiation by DMSO and certain other agents. Furthermore, these studies question whether declines in c-myc protein are necessary for commitment to terminal differentiation. The identification of tiazofurin as a compound which dissociates hemoglobin accumulation from changes in c-myc expression and commitment should provide an opportunity to examine further the molecular mechanisms responsible for induction of the differentiated MEL phenotype.

ACKNOWLEDGMENT

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Tiazofurin induction of mouse erythroleukemia cell hemoglobin production in the absence of commitment or changes in protooncogene expression

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