CORRESPONDENCE

A Combination of Retinoic Acid and Proteasome Inhibitors for the Treatment of Leukemias Is Potentially Dangerous

To the Editor:

Retinoic acid (RA) is used in the treatment of leukemias; however, the phenomenon of RA resistance is often the cause of relapse in some patients. In a recent report, Fanelli et al showed, using an acute promyelocytic leukemia–derived cell line NB4, that the treatment of RA-resistant cells with proteasome inhibitors restores RA sensitivity.

Proteasome inhibitors are already being tested in phase I of clinical trials as anticancer agents and are proposed to be useful in the treatment of chemoresistant and radioresistant leukemias. One might speculate that combination therapy of RA and proteasome inhibitors can be advantageous and may potentially overcome RA-resistant cases. A selective inhibitor of the proteasome [PSI; N-benzyloxycarbonyl-Ile-Glu-(O-t-butyI)-Ala-leucinal] induces apoptosis of murine lymphocytic leukemia L1210 cells, while RA treatment inhibits their proliferation, abolishes clonogenicity, and causes G1 arrest. L1210 is sensitive to RA, regardless of the fact that it does not express PML/RARα binding proteins.

We pretreated L1210 cells for 72 hours with either a control solvent (dimethyl sulfoxide [DMSO]) or 5 µmol/L and 50 µmol/L RA. Cells were then further treated for 24 hours with a combination of those agents and 500 nmol/L PSI or DMSO alone as control. Treated cells were fixed in 70% ethanol, stained with acridine orange, and analyzed using a FACScalibur flow cytometer (Becton Dickinson, San Diego, CA).

We have found that treatment with 5 µmol/L RA, which by itself is not cytotoxic, has a cytotoxic effect when combined with 500 nmol/L PSI (Table 1). However, a combination of 50 µmol/L RA, which is by itself cytotoxic, with 500 nmol/L PSI surprisingly diminishes the cytotoxic effects on L1210 cells, allowing a survival of 21.4% of the cells compared with 12.2% for PSI alone (χ² P < .0001). Moreover, while RA or PSI alone caused a G0/G1 block, this effect was partially abolished by a combination of both drugs (Table 2). PSI or 5 µmol/L RA treatment did not increase granulosity of the cells, as judged by the flow cytometric SSC-H parameter, while 50 µmol/L RA increased mean granulosity by 62%. The combination of 50 µmol/L RA and PSI increased mean granulosity by 50%.

Although 5 µmol/L RA does not induce differentiation—it causes only a moderate G1 phase block or retardation—50 µmol/L RA treatment most probably induces differentiation of a fraction of L1210 cells, as judged by increased granularity, and these cells became resistant to proteasome inhibition. Although proteasome inhibitors cause apoptosis of proliferating cells, they prevent apoptosis of some differentiated cells, such as neurons or thymocytes. Our results suggest that any attempt to treat leukemia with a combination of RA and proteasome inhibitors should be made with great caution. It potentially allows the survival of cells, which after the withdrawal of the drugs can cause a fast relapse of the leukemic disease, regardless of the presence of the PML/RMRα fusion protein.

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Table 1. Percentages of Apoptotic, Necrotic, and Living L1210 Cells, After 72 Hours’ Pretreatment With Either a Control Solvent or RA at the Indicated Concentrations, Followed by a 24-Hour PSI or Control Treatment

<table>
<thead>
<tr>
<th>Cells</th>
<th>Apoptotic</th>
<th>Necrotic</th>
<th>Living</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.0</td>
<td>4.5</td>
<td>83.5</td>
</tr>
<tr>
<td>RA 5 µmol/L</td>
<td>4.2</td>
<td>9.0</td>
<td>86.8</td>
</tr>
<tr>
<td>RA 50 µmol/L</td>
<td>23.6</td>
<td>11.9</td>
<td>64.5</td>
</tr>
<tr>
<td>PSI 500 nmol/L</td>
<td>27.2</td>
<td>60.6</td>
<td>12.2</td>
</tr>
<tr>
<td>PSI 500 nmol/L RA 5 µmol/L</td>
<td>22.0</td>
<td>75.2</td>
<td>2.8</td>
</tr>
<tr>
<td>PSI 500 nmol/L RA 50 µmol/L</td>
<td>20.6</td>
<td>58.0</td>
<td>21.4</td>
</tr>
</tbody>
</table>

Data were obtained in each group for 10,000 cells separated from other events based on light scatter characteristics. Apoptotic, necrotic, and living cells were discriminated with the use of CELLQuest v.1.2 software system.

Table 2. Percentages of L1210 Cells in Different Phases of the Cell Cycle, After 72 Hours’ Pretreatment With Either a Control Solvent or RA at the Indicated Concentrations, Followed by a 24-Hour PSI or Control Treatment

<table>
<thead>
<tr>
<th>Cells</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.8</td>
<td>11.1</td>
<td>55.1</td>
</tr>
<tr>
<td>RA 5 µmol/L</td>
<td>43.9</td>
<td>11.0</td>
<td>45.1</td>
</tr>
<tr>
<td>RA 50 µmol/L</td>
<td>51.5</td>
<td>7.2</td>
<td>48.3</td>
</tr>
<tr>
<td>PSI 500 nmol/L</td>
<td>49.8</td>
<td>4.5</td>
<td>45.7</td>
</tr>
<tr>
<td>PSI 500 nmol/L RA 5 µmol/L</td>
<td>55.7</td>
<td>2.9</td>
<td>41.4</td>
</tr>
<tr>
<td>PSI 500 nmol/L RA 50 µmol/L</td>
<td>44.9</td>
<td>7.0</td>
<td>48.1</td>
</tr>
</tbody>
</table>

Data were obtained for the living cells fraction of the same groups as in Table 1. Cell-cycle percentages were calculated depending on the DNA content with the use of CELLQuest v.1.2 software system.
Double-Homozygosity for Factor V Leiden and the Prothrombin Gene G20210A Variant in a Young Patient With Idiopathic Venous Thrombosis

To the Editor:

There is growing evidence that environmental and genetic risk factors often interact to induce clinically manifest venous thromboembolism (VTE). The role of gene-gene interactions, although much rarer, is supported by cosegregation of genetic defects observed in patients with familial thrombophilia. In this context, factor V (FV) Leiden and the prothrombin G20210A gene (FII) mutation are of particular interest because of their high prevalence in the normal population; about 5% of individuals harbor a prothrombin G20210A gene (FII) mutation.1,2 The FII mutation has been reported in about 10% of FV Leiden carriers with VTE,3 while FV Leiden is present in 30% to 40% of symptomatic carriers of the FII mutation.2,4 Homozygosity for these mutations is less common, with a prevalence of 0.02% for the FII mutation and 0.014% for the FII mutation.1,2 We present here a case of double-homozygosity for these defects.

A 34-year-old man presented with a first episode of idiopathic deep-vein thrombosis. He had never been exposed to environmental risk factors for thrombosis. Treatment consisted of subcutaneous low-molecular-weight heparin for 1 week followed by acenocoumarol for 3 months. At 18 months follow-up, he had experienced no recurrence.

Laboratory studies for thrombophilic disorders showed double-homozygosity for FV Leiden and the FII mutation, detected by polymerase chain reaction. None of his 15 relatives had a history of VTE. Four were double-heterozygous carriers, 4 were single carriers of the FII mutation, and 3 were noncarriers (Fig 1). Four relatives, 2 of whom had died, were not tested but they were obligate carriers of at least 1 (N = 1) or both (N = 3) mutations. The patient’s father, who had a history of smoking and hypercholesterolemia, died of myocardial infarction at 57 years of age and a son died from cribdeath. His mother experienced a myocardial infarction when she was 61 years old; she had a history of hypertension and diabetes mellitus. Fetal loss had occurred only in 1 noncarrier. Table 1 summarizes characteristics of this family.

Double-homozygosity for FV Leiden and the FII mutation is extremely rare. Theoretically, it is expected in 3 per 100 million whites. The thrombotic risk of this combined abnormality is unknown. Homozygosity for FV Leiden increases the risk of VTE approximately 80-fold compared with noncarriers.5 The risk in homozygous carriers of the FII mutation has not yet been estimated; only case reports have been published.

Remarkably, only our double-homozygous patient has developed...
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