Inhibition of Hydroxymethylglutaryl Coenzyme A Reductase Activity Induces a Paradoxical Increase in DNA Synthesis in Myeloid Leukemia Cells

By Raymond J. Hohl, Richard A. Larson, Valsa Mannickarottu, and Stanley Yachnin

The effects of competitive inhibition of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase by compactin on the in vitro proliferation of peripheral blood myeloid leukemia cells were studied using the cells from 45 patients with acute myeloid leukemia or chronic myelogenous leukemia in blast phase. The cells from 58% of these patients showed a dose-related inhibition of DNA synthesis when incubated with compactin. Unexpectedly, cells from 18% of the patients were resistant to the inhibitory effects of compactin on DNA synthesis and responded to the HMG CoA reductase inhibition with an actual increase in the incorporation of 3H-labeled thymidine into DNA. Another 18% of the patients studied displayed both inhibition and stimulation of DNA synthesis in a biphasic response depending on the particular concentration of compactin used. The maximum enhanced rates of cellular DNA synthesis were observed with lower compactin concentrations (5 × 10⁻⁷ mol/L) than were required for maximum inhibition of DNA synthesis (10⁻⁶ mol/L). Leukemia cells displaying a stimulated response to compactin had a significantly lower baseline DNA synthetic rate than did cells that showed an inhibitory response of DNA synthesis to compactin. There was no correlation between these cells’ varying DNA synthetic response to compactin and measures of baseline HMG CoA reductase activity or acetate conversion to cholesterol. Whereas the observation of cellular DNA synthesis stimulation by HMG CoA reductase inhibition has not been observed in other mammalian cells and seems paradoxical, explanations may emerge in light of our growing knowledge concerning the importance of isoprenylation for the function of certain cell regulatory proteins.

C CHOLESTEROL SYNTHESIS has long been recognized as playing an important role in the growth and replication of mammalian cells. Cells that proliferate rapidly have an increased requirement for cholesterol necessary for the synthesis of new membrane components. The increased requirement for sterols that accompanies cell growth can be met by either enhanced cellular uptake of exogenous cholesterol or by increased intracellular cholesterol biosynthesis. The key regulatory enzyme in cholesterol biosynthesis is hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, which catalyzes the conversion of HMG CoA to mevalonic acid. Agents that inhibit HMG CoA reductase therefore inhibit cholesterol biosynthesis. Compactin (ML236B) was the first competitive inhibitor of HMG CoA reductase to be used in laboratory investigations deciphering the interrelations between the availability of mevalonic acid and its metabolites and cell growth.

Studies with such inhibitors of HMG CoA reductase have shown that the requirement of mevalonic acid for cell growth is only partly a consequence of the requirement of proliferating cells for the incorporation of this precursor into additional cholesterol. Cells incubated with high concentrations of these enzyme inhibitors fail to proliferate when supplemented with adequate extracellular cholesterol unless critical amounts of mevalonic acid are also supplied. These observations form the basis for the hypothesis that one or more of the nonsterol products of mevalonic acid are required for normal cell growth.

HMG CoA reductase inhibitors have been shown to inhibit the growth of mouse L cells, human fibroblasts, baby hamster kidney cells, monkey arterial smooth muscle cells, and Swiss 3T3 cells. There are reports of cells displaying spontaneous resistance to the growth inhibitory effects of high concentrations of these compounds. In addition, resistant cells can be produced in the laboratory by serial incubation with increasing inhibitor concentrations. Resistant cells produced in this way have markedly increased HMG CoA reductase activity, correlated with evidence of HMG CoA reductase gene amplification.

Our laboratory has previously demonstrated that normal mitogen-stimulated lymphocytes and spontaneously growing neoplastic lymphocytes are susceptible to DNA synthesis inhibition when incubated with compactin. The addition of mevalonic acid to such compactin inhibited lymphocytes restored DNA synthesis. We also observed that mevalonic acid was a mitogen for normal peripheral blood lymphocytes, as well as for the lymphocytes of some patients with chronic lymphocytic leukemia.

The present studies examine the effects of compactin on in vitro proliferation of human myeloid leukemia cells. We have determined the degree to which leukemia cell DNA synthesis is reduced in response to incubations with various concentrations of compactin. Whereas cells from a majority of the myeloid leukemia patients studied showed the expected inhibition of DNA synthesis by exposure to compactin, we noted a paradoxical dose-related stimulation of DNA synthesis in the cells of a substantial minority of these leukemic patients.

MATERIALS AND METHODS

R(-)-S(+)-mevalonic acid lactone was purchased from Sigma Chemical Co (St Louis, MO) and was dissolved in RPMI 1640 (GIBCO, Grand Island, NY) titrated to pH 7.5 with 10N NaOH, sterilized by Millipore filtration (Millipore, Bedford, MA), and stored at −20°C. The salt form of compactin (ML-236B), a gift of Dr Akiri Endo (Tokyo Noko University, Tokyo, Japan), was

From the Section of Hematology/Oncology, Department of Medicine, The University of Chicago Medical Center, Chicago, IL. Submitted June 18, 1990; accepted October 25, 1990. Supported in part by Grant No. 90-04, American Cancer Society, Illinois Division, Inc., and National Institutes of Health Contract 2 T32 DK 07134.

Address reprint requests to Raymond J. Hohl, MD, Department of Medicine, Box 420, University of Chicago, 5841 S Maryland Ave, Chicago, IL 60637.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.


1064
dissolved in sterile phosphate-buffered saline.\textsuperscript{19} Plasmagel was purchased from Roger Bellon (Neuilly, France) and Ficoll-Paque from Pharmacia LKB Biotechnology Inc (Piscataway, NJ). [2\textsuperscript{14}C]-thymidine (56 mCi/m mole), DL-3-hydroxy-3-methyl[3\textsuperscript{14}C]-glutaryl coenzyme A (56 mCi/m mole), [4\textsuperscript{14}C]-cholesterol, and ACS scintillation counting fluid were obtained from Amersham Corp (Arlington Heights, IL). [1\textsuperscript{4}C]-sodium acetate (57 mCi/m mole), and RS-[5\textsuperscript{3}H(N)]-mevalonolactone were from New England Corp (Boston, MA).

Isolation of peripheral blood mononuclear cells (PBMCs). Before the initiation of any chemotherapy, anticoagulated (heparin) peripheral blood was obtained from patients with various myeloid leukemias by venipuncture after their informed consent. The blood was immediately subjected to gravity sedimentation for 40 minutes after the addition of 20\% (vol/vol) Plasmagel; mononuclear cells, including myeloid cells less differentiated than the metamyelocyte, were isolated from the supernatant plasma by the Ficoll-Hypaque technique.\textsuperscript{20}

The diagnosis of myeloid leukemia was confirmed in all patients using morphology and cytochemical results of bone marrow specimens, and the patients were classified using the French-American-British (FAB) criteria.\textsuperscript{21,22} The leukemia cell fraction of the leukocyte count was calculated to include blasts, promyelocytes in acute promyelocytic leukemia (FAB M3 disease), and immature monocytes in monocytic leukemias. The percentage of leukemia cells in the Ficoll-Hypaque preparations were similarly calculated after excluding cells more differentiated than the myelocyte.

DNA synthesis measurements. The Ficoll-Hypaque leukemia cell isolates were diluted to 1.5 \times 10\textsuperscript{6} cells/mL in RPMI 1640 supplemented with antibiotics, glutamine, and 12.5\% human AB serum (previously heated to 56\textdegree C for 30 minutes) to a final volume of 1.0 mL and incubated in triplicate at 37\textdegree C in a humidified 5\% CO\textsubscript{2}; air incubator. Some cultures were preincubated with compactin and/or mevalonate, usually for 72 hours, before the addition of 0.2 \mu g [2\textsuperscript{14}C]-thymidine. Sixteen hours later the cultures were harvested onto glass fiber filters and the DNA isolated by precipitation with trichloroacetic acid. Radiolabeled DNA was measured as counts per minute (cpm) by scintillation spectrophotometry (model 3375; Packard Instrument Company, Downers Grove, IL).\textsuperscript{23,24}

The activity index was calculated as the ratio of thymidine incorporation into DNA by cells with exposure to compactin. Unexpectedly, cells from 3 of these patients (6\%) did not respond to the HMG CoA reductase inhibitor with a statistically significant increase in HMG CoA reductase activity. Another 18\% of the patients studied displayed both inhibition and stimulation of DNA synthesis in a biphasic response depending on the particular concentration of compactin used. The cells from three patients (6\%) did not

### Table 1. Effects of Compactin on Myeloid Leukemia Cell DNA Synthesis

<table>
<thead>
<tr>
<th>Nature of the Change in DNA Synthesis as Compared With Control Unstimulated Leukemia Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only Inhibition</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>No. of patients (n = 45)</td>
</tr>
<tr>
<td>Proportion of patients (%)</td>
</tr>
<tr>
<td>Degree of effect at varying compactin concentrations:†</td>
</tr>
<tr>
<td>5 \times 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>10\textsuperscript{-4}</td>
</tr>
<tr>
<td>5 \times 10\textsuperscript{-7}</td>
</tr>
<tr>
<td>2 \times 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>5 \times 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>10\textsuperscript{-4}</td>
</tr>
</tbody>
</table>

*Cells were incubated with compactin for 72 hours before labeling for 16 hours with [\textsuperscript{14}C]-thymidine and were compared with the same patient's control cells (preincubated for 72 hours without compactin).

†Compactin concentrations are in moles per liter. The degree of effect is expressed as the activity index for DNA synthesis; values are the mean activity index (see text) ± 1 SD.
the cells from three individual patients, each of whom displayed a different response as described in Table 1. Figure 1A illustrates results obtained when leukemia cells demonstrated only inhibition of DNA synthesis with increasing concentrations of compactin. Maximum inhibition is achieved at a compactin concentration of $10^{-3}$ mol/L (activity index of 0.09). Cells displaying only stimulation of DNA synthesis in response to compactin over the concentration range tested are shown in Fig 1B; maximum stimulation is seen at $5 \times 10^{-7}$ mol/L compactin (activity index of 3.1). The results for leukemia cells showing both stimulation and inhibition of DNA synthesis are presented in Fig 1C. Maximum stimulation was at $5 \times 10^{-7}$ mol/L compactin and maximum inhibition was at $10^{-3}$ mol/L compactin; the corresponding activity indices were 1.7 and 0.7, respectively.

As shown in Table 2, cells showing only inhibition of DNA synthesis by compactin have a significantly ($P < .05$) lower activity index ($0.36 \pm 0.18$) at the maximum inhibitory concentration of compactin than do cells that exhibit both inhibition and stimulation of DNA synthesis (activity index $= 0.52 \pm 0.24$). Cells that show only stimulation of DNA synthesis in response to compactin have a significantly ($P < .005$) higher maximum activity index ($2.19 \pm 0.60$) than the maximum achieved by cells that show both responses (activity index $= 1.38 \pm 0.31$). The compactin concentrations that resulted in the maximum inhibition and/or stimulation of DNA synthesis for each group are also listed in Table 2. Maximum inhibition was seen at the highest concentrations of compactin used (usually $10^{-3}$ mol/L). In contrast, stimulation occurred at intermediate concentrations of compactin with the greatest effect seen at a modal value of $5 \times 10^{-7}$ mol/L compactin. In both respects no difference was observed between these two groups and cells responding in a biphasic manner with both inhibition and stimulation.

The stimulatory effect of compactin on DNA synthesis in leukemia cells was inversely related to the time in culture (Fig 2). The greatest relative increase in DNA synthesis was observed after a 24-hour preincubation with compactin. Increasing the duration of compactin exposure to 48 and 72 hours diminished the maximum relative increase in DNA synthesis observed. Similar experiments performed on the cells from 10 other patients showed the same temporal sequence of stimulatory growth in response to compactin. In contrast, cells subject to inhibition of DNA synthesis by exposure to compactin were maximally inhibited after the longest preincubation periods.

Table 3 shows the patients' initial white blood cell (WBC) count and proportion of circulating leukemia cells according to the cells' DNA synthetic response to compactin. No differences in initial WBC count were observed among the three groups. The percentage of leukemia cells, as well as the proportion of leukemia cells enriched in the Ficoll-Hypaque population actually cultured, was significantly higher in those instances where only compactin-induced stimulation of DNA synthesis was observed in comparison with cells showing only inhibition. Furthermore, leukemia cells displaying stimulated DNA synthesis

show any significant change of DNA synthesis when exposed to compactin.

Figure 1 shows examples of the changes in DNA synthesis seen in response to various compactin concentrations in
Table 2. Maximal Changes in DNA Synthesis and the Distribution of Compactin Concentrations Inducing These Changes in Leukemia Cells

<table>
<thead>
<tr>
<th>Nature of the Change in DNA Synthesis as Compared With Control Unstimulated Leukemia Cells*</th>
<th>Only Inhibition</th>
<th>Only Stimulation</th>
<th>Both Inhibition and Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal inhibition:</td>
<td>0.36 ± 0.18 (0.02-0.77)</td>
<td></td>
<td>0.52 ± 0.24 (0.10-0.84)</td>
</tr>
<tr>
<td>Maximal stimulation:</td>
<td></td>
<td>2.19 ± 0.60 (1.19-3.11)</td>
<td>1.38 ± 0.31 (1.12-2.02)</td>
</tr>
</tbody>
</table>

Compactin concentrations inducing these maximal changes in DNA synthesis:

- Inhibition: 10^{-4} mol/L (n = 25)
- 5 x 10^{-4} mol/L (n = 1)
- 5 x 10^{-5} mol/L (n = 4)
- 10^{-5} mol/L (n = 1)
- 10^{-6} mol/L (n = 1)

- Stimulation: 10^{-3} mol/L (n = 1)
- 2 x 10^{-3} mol/L (n = 1)
- 5 x 10^{-3} mol/L (n = 3)
- 10^{-3} mol/L (n = 1)
- 5 x 10^{-4} mol/L (n = 1)
- 10^{-4} mol/L (n = 1)

*Cells were incubated with compactin for 72 hours before labeling for 16 hours with ^14C-thymidine and were compared with the same patient's control cells (preincubated for 72 hours without compactin).

†Expressed as the activity index for DNA synthesis; values are mean activity index ± 1 SD. Ranges for the means are listed in parentheses.

Fig 2. Mean DNA synthesis of leukemia cells from a patient with myeloblastic (FAB M1) leukemia when incubated with various compactin concentrations. The brackets represent 1 SD. Control cpm (without compactin) expressed as mean ± 1 SD, are 4,678 ± 1,034 at 24 hours; 10,401 ± 168 at 48 hours; and 6,846 ± 144 at 72 hours of preincubation.

In response to compactin, there was a significantly lower baseline (unstimulated) DNA synthetic rate than did cells in which DNA synthesis in response to HMG CoA reductase inhibition was decreased. No correlation existed between the FAB subtypes of the leukemias studied and the discernable patterns of alterations in DNA synthesis. Of interest, all of the possible patterns of response to compactin seen in the cells from patients with CML in blast phase.*

The results of studies of baseline HMG CoA reductase activity, acetate conversion to cholesterol, and the concentration of compactin required to inhibit by 50% acetate conversion to cholesterol (ID_{50}) are shown in Table 4. There was no statistically significant correlation between the cells' DNA synthetic response to compactin and any of these measures of cholesterol biosynthesis, except for the comparison of the compactin ID_{50} for acetate conversion to cholesterol between cells that were only inhibited and cells that were both inhibited and stimulated (P < .001). None of the cells showed an enhanced conversion of acetate to cholesterol in the presence of any compactin concentration.

Finally, the ability of mevalonic acid to overcome the inhibition of DNA synthesis by compactin was evaluated. In parallel experiments, when 10^{-2} mol/L RS-mevalonic acid was added to the leukemia cell cultures in 17 cases that

*The distribution of myeloid leukemia FAB subtypes according to the differing DNA synthetic responses to compactin is as follows: only inhibited: M0 (2), M1 (2), M2 (6), M3 (2), M4 (7), M5 (2), CML blast phase (4), and myeloid leukemia from a preexisting myelodysplastic syndrome (1); only stimulated: M1 (1), M2 (2), M4 (2), M5 (1), and CML blast phase (2); both inhibited and stimulated: M1 (1), M2 (2), M4 (3), M5 (1), and CML blast phase (1); no effect: M2 (1), M5 (1), and CML blast phase (1).
been previously described. We, as well as other investiga-
tors, have concluded that the ability of HMG CoA reduc-
tase inhibitors to block cell growth is critically related to a
requirement for a nonsterol product of mevalonic acid.15-19 None of the cells from the AML/CML patients in this study
displayed a mitogenic response to the sole addition of 10-2
mol/L mevalonic acid.

DISCUSSION

Our investigations show that the malignant peripheral
blood cells from approximately 40% of patients with various
AMLs exhibit a seemingly paradoxical increase in DNA
synthesis in the presence of compactin. While competitive
HMG CoA reductase inhibitors have been previously
shown to inhibit the proliferation and DNA synthetic ability
of many cell types and to prevent cells from entering the
S-phase of the cell cycle in a concentration-dependent fashion,3,19 their ability to stimulate DNA synthesis has not
been previously described. We, as well as other investiga-
tors, have concluded that the ability of HMG CoA reduc-
tase inhibitors to block cell growth is critically related to a
requirement for a nonsterol product of mevalonic acid.15-20
The great majority of myeloid leukemia cells studied in this
report (75%) behave in a similar manner because at high
compactin concentrations, even in the presence of low-
density lipoprotein cholesterol, their DNA synthetic ability
can both be inhibited by compactin and partially or com-
pletely restored by the addition of exogenous mevalonic acid.

Human peripheral blood monocytes are capable of greater
rates of cholesterol biosynthesis than lymphocytes or granu-
locytes.27-29 As peripheral blood monocytes differentiate
along the adherent macrophage pathway, they display an
additional fourfold to sixfold increase in HMG CoA reduc-
tase activity and cholesterol biosynthesis.30 Early changes
along the pathway of monocyte differentiation (FAB M4
and M5 leukemia cells) result in acquisition by the leukem-
ial cell population of the enhanced sterol synthetic capac-
ity characteristic of the mature normal human monocyte.31
These earlier studies invite the speculation that inhibition
of cholesterol biosynthesis in undifferentiated malignant
monocyte precursor cells might retard cellular progression
through partial differentiation along the monocyte-macro-
phage pathway. Such an inhibition of cholesterol biosynthe-
sis might result in the preservation of the undifferentiated
state in these cells and be observed as an enhancement of
their ability to synthesize DNA in tissue culture. This
speculation might suffice to explain the compactin-en-

Table 3. Initial WBC Count, Proportion of Leukemia Cells, and Baseline DNA Synthetic Rates According to the Differing Leukemia Cell DNA Synthetic Responses to Compactin

<table>
<thead>
<tr>
<th>Effects of Compactin on DNA Synthesis</th>
<th>Median Initial WBC (cells/μL × 10-9)</th>
<th>% of Initial WBC</th>
<th>% of Ficoll-Hypaque Cells</th>
<th>Baseline 3H-thymidine Incorporated into DNA (cpm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only inhibited</td>
<td>28 (7-352)†</td>
<td>57 ± 20</td>
<td>70 ± 19</td>
<td>33,014 ± 24,546</td>
</tr>
<tr>
<td>Only stimulated</td>
<td>44 (4-224)</td>
<td>85 ± 9.0</td>
<td>90 ± 14</td>
<td>9,656 ± 5,160</td>
</tr>
<tr>
<td>Both inhibited and stimulated</td>
<td>16 (8-76)</td>
<td>58 ± 30</td>
<td>71 ± 25</td>
<td>22,959 ± 7,118</td>
</tr>
</tbody>
</table>

*Mean values ± 1 SD.
†Range.
‡Only significantly different comparisons are indicated with respective P values.

Table 4. Baseline HMG CoA Reductase Activities and Rates of Acetate Conversion to Cholesterol in Leukemia Cells With Differing DNA Synthetic Responses to Compactin

<table>
<thead>
<tr>
<th>Effects of Compactin on DNA Synthesis</th>
<th>Mean HMG CoA Reductase Activity*</th>
<th>Mean Acetate Incorporation into Cholesterol†</th>
<th>Geometric Mean Compactin ID50‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only inhibited</td>
<td>13.1 ± 10.05 n = 10</td>
<td>61.1 ± 60.25 n = 16</td>
<td>1.18 × 10^-1 (4.21 × 10^-8-3.31 × 10^-7) n = 15</td>
</tr>
<tr>
<td>Only stimulated</td>
<td>26.8 ± 20.22 n = 6</td>
<td>20.0 ± 16.2 n = 5</td>
<td>6.49 × 10^-4 (8.40 × 10^-5-5.01 × 10^-3) n = 3</td>
</tr>
<tr>
<td>Both inhibited and stimulated</td>
<td>11.7 ± 12.0 n = 8</td>
<td>48.8 ± 16.7 n = 5</td>
<td>4.10 × 10^-1 (3.39 × 10^-1-4.95 × 10^-2) n = 5</td>
</tr>
</tbody>
</table>

*Enzyme activities expressed as picomoles mevalonolactone formed per minute per milligram of protein.
†Expressed as picomoles per 5 × 10^7 cells per 4 hours.
‡The molar concentration of compactin required to inhibit by 50% the cellular incorporation of acetate into cholesterol; ranges indicating ± 1
geometric SD for the ID50 values are listed in parentheses.
§Mean ± 1 SD; n = the number of determinations in each group.
hanced DNA synthesis observed in M4 and M5 leukemia cells; however, it would not obviously apply to those leukemia cells from patients with other forms of AML whose DNA synthesis was also stimulated by HMG CoA reductase inhibition.

While the observation of cellular DNA synthesis stimulation by HMG CoA reductase inhibition seems paradoxical, explanations may emerge in light of our growing knowledge concerning the importance of isoprenylation in cell regulatory protein function. Recent evidence has clarified the nature of the nonsterol products of mevalonic acid that are important for the regulation of cellular growth and differentiation. It is now clear that a variety of cell proteins, including the nuclear lamins \(^{35}\) and the protein products of the RAS oncogene, \(^{34,36}\) as well as other members of the regulatory G-protein family, \(^{32,38}\) are posttranslationally modified by isoprenoids such as farnesyl \(^{34-38}\) and geranylgeranyl. \(^{39}\) Such posttranslational modifications augment the function of isoprenylated proteins by enhancing their capacity for membrane localization required for biologic activity. Furthermore, the discovery that the RAS protein, recognized to be the product of a growth-promoting gene, \(^{40}\) depends on farnesylation \(^{41,42}\) to achieve its membrane-localized regulatory functions \(^{41,42}\) has offered a possible explanation for the ability of HMG CoA reductase inhibitors to impair DNA synthesis and cell growth. However, in the context of the observations presented in this report, it should also be noted that an isoprenylated growth-suppressing protein KREV1 (RAP1A) has recently been described. \(^35\) The KREV1 gene encodes a protein that will reverse the malignant growth characteristics of NIH 3T3 cells that have been transfected and transformed by activated RAS. \(^41\) KREV1 contains the C-terminal amino acid sequence required for isoprenylation, \(^41\) is isoprenylated, \(^44\) and competes with the RAS protein for the same effector protein (guanosine triphosphatase-activating protein). \(^45\)

An alternative hypothesis to account for the stimulation of DNA synthesis by inhibition of HMG CoA reductase depends on the existence of such isoprenylated growth-suppressing proteins. Interference with the isoprenylation of such a putative protein as a consequence of compactin exposure \(^47\) could result in the enhancement of DNA synthesis and cell division, and might account for some of our observations. In this context, it is important to note that leukemia cells which display only stimulated DNA synthesis as a result of compactin exposure have a significantly lower baseline DNA synthetic rate than is observed in the two other groups of leukemia cells (see Table 4). Furthermore, the possibility of the simultaneous existence of isoprenylated growth-promoting and growth-suppressing proteins, and the relative affinity and cellular localization of the enzyme(s) \(^49\) responsible for their modification with respect to the isoprenoid precursor, could be invoked as an explanation for the biphasic response that some of our leukemia cell populations displayed. Competitive interaction between sterol and nonsterol pathways for an HMG CoA reductase-derived concentration-limiting precursor has been previously described in the case of sterol versus dolichol cellular biosynthesis, \(^46\) and has also been implicated as governing competition for a common substrate between the sterol and ubiquinone biosynthetic pathways. \(^50\)

The inhibition of cell growth by HMG CoA reductase inhibitors and possibly by inhibitors of enzymes downstream in the polyisoprenoid-sterol synthetic pathway, together with the presence of mutationally activated RAS proteins in certain known malignant tumors, has suggested that the protein isoprenylation pathways might be rational targets for the chemotherapy of human malignancies. Our observation that the proliferation of some malignant cell populations can be enhanced by HMG CoA reductase inhibition suggests that caution is required in determining the suitability of such an approach for any specific malignant phenotype.

ACKNOWLEDGMENT

We thank R. Mick, MS, for assisting with the statistical analysis, and M. Diaz, MD, for helpful discussion.

REFERENCES

12. Habenicht AJR, Glomset JA, Ross R: Relation of cholesterol and mevalonic acid to the cell cycle in smooth muscle and...


26. Larson RA, Chung J, Scaru AM, Yachnin S: Neutrophils are required for the DNA synthetic response of human lymphocytes to mevalonic acid: Evidence suggesting that a nonsterol product of mevalonate is involved. Proc Natl Acad Sci USA 79:3028, 1982


34. Hancock JR, Magee AJ, Chilis JE, Marshall CJ: All ras proteins are polyisoprenylated but only some are palmitoylated. Cell 57:1167, 1989


Inhibition of hydroxymethylglutaryl coenzyme A reductase activity induces a paradoxical increase in DNA synthesis in myeloid leukemia cells

RJ Hohl, RA Larson, V Mannickarottu and S Yachnin