ABSTRACT

The effect of partially thiolated polycytidylic acid (MPC) on the colony-forming ability of the progenitor cells (CFUC) of RF/Un leukemic mice was investigated using the plasma clot method in order to study the mode of action of the modified polynucleotide. The results showed that MPC inhibited the CFUC in a dose-dependent and time-dependent manner. Once a maximum level of inhibition of CFUC (~40%) was observed, no further inhibition occurred whether the concentration of MPC was increased or whether the duration of incubation was lengthened. High-specific-activity [3H]thymidine, an S-phase-specific agent, showed a similar inhibition profile on the CFUC as did MPC. When MPC and high-specific-activity [3H]thymidine were incubated together with the bone marrow cells, there was no additive or synergistic inhibitory effect on the CFUC. Thus, it appears that MPC is an S-phase-specific agent. When injected i.v. into the mice, MPC decreased the number of CFUC of both the bone marrow and the spleen significantly.

INTRODUCTION

Introduction of a mercapto group into the 5-position of some of the cytosine units of polycytidylic acid converts it into a biochemically active analog according to the antitemplate concept (1-3). Thus, MPC is a potent inhibitor of the RNA-dependent DNA polymerases of oncornaviruses (7, 8, 10, 22), the DNA polymerase α from Burkitt’s lymphoma cells (23) and regenerating rat liver (14), and the DNA-dependent RNA polymerases of mammalian (18) and bacterial (11, 16) origin. MPC forms a double-helical complex with polyinosinic acid which is readily taken up by tumor cells (12) and elicits interesting antiviral activity (19). Preliminary clinical results indicate that MPC is active against lymphocytic leukemia in humans (9). For the development of the antitemplates as a new class of chemotherapeutic agents, it is important to understand the mode of action of MPC in biological systems. In this communication, we report that MPC was active both in vitro and in vivo against the granulocytic progenitor cells of the myelogenous leukemic RF/Un mouse.
Effect of MPG on the clonogenicity of bone marrow cells from the myelogenic leukemic RF/Un mice

The assay was carried out as described in "Materials and Methods." Polynucleotide added (μg/ml incubation mixture) Colonies/10^5 cells % of control

<table>
<thead>
<tr>
<th>Polynucleotide added</th>
<th>Colonies/10^5 cells</th>
<th>% of control</th>
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<tbody>
<tr>
<td>MPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>652 ± 3.0^a</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>600 ± 9.85</td>
<td>95.0 ± 1.6</td>
</tr>
<tr>
<td>50</td>
<td>595 ± 10.4</td>
<td>91.1 ± 1.6</td>
</tr>
<tr>
<td>100</td>
<td>540 ± 2.87</td>
<td>83.5 ± 0.6</td>
</tr>
<tr>
<td>150</td>
<td>436 ± 9.6</td>
<td>67.2 ± 1.5</td>
</tr>
<tr>
<td>200</td>
<td>430 ± 4.67</td>
<td>66.8 ± 0.8</td>
</tr>
<tr>
<td>300</td>
<td>445 ± 2.87</td>
<td>66.4 ± 0.5</td>
</tr>
<tr>
<td>500</td>
<td>432 ± 1.45</td>
<td>66.3 ± 0.4</td>
</tr>
</tbody>
</table>

Polycytidylic acid

100 613 ± 24.0 94.8 ± 3.7
200 658 ± 29.6 101.1 ± 4.6

^a Exposure time of cells to polynucleotide, 1 hr.
^b Mean ± S.E.

RESULTS

The results of the effect of MPC on in vitro colony-forming units of the RF/Un leukemic mice assayed by the plasma clot cloning method were summarized in Table 1. Under the assay conditions, MPC inhibited the colony-forming ability of the murine bone marrow cells. The inhibition was dose dependent and reached a maximum at 34% at a concentration of 150 μg MPC per ml of assay mixture. Further increase of the concentration of MPC, up to 500 μg/ml, did not augment the inhibitory effect. By contrast, the unmodified polycytidylic acid at a concentration up to 200 μg/ml of incubation mixture had no observable effect on the colony-forming ability of the cells.

When bone marrow cells were exposed to MPC in vitro for different periods of time before they were plated in plasma clots, it was evident that the inhibition of the clonogenicity of the cells increased with time of exposure to the drug (Chart 1). The inhibition increased rapidly from 17 to 40% at 30 min and then leveled off to form a plateau through 4 hr of incubation of the cells with MPC.

In other experiments, bone marrow was incubated with HSA-[³H]dTThd to directly measure the progenitor of CFUC in S phase (thymidine suicide). The results are reported in Chart 2. These data show that labeled thymidine inhibited the colony-forming ability of the bone marrow cells in a time-dependent manner. The inhibition was 33% within 30 min and increased to 45% at 2 hr. Lengthening of the time of incubation for 6 additional hr did not increase the level of inhibition significantly. The results were similar to those with MPC. As shown in Table 2, there was no additive or synergistic effect on the clonogenicity of the murine bone marrow cells when MPC and HSA-[³H]dTThd were present together in the incubation mixture. Virtually similar inhibitory effect was observed when the cells were exposed to MPC alone, HSA-[³H]dTThd alone, or both agents together.

It was of interest to determine whether MPC would show a similar effect on the clonogenicity of the bone marrow and spleen cells in the mouse. Therefore, the in vivo study was initiated as described in "Materials and Methods." Assuming uniform distribution throughout the body, the dose of 50 mg MPC per kg of body weight would correspond to a concentration of approximately 100 μg per ml in the body fluid. The results in Table 3 indicate that MPC injected i.v. into the mouse decreased the clonogenicity of both the bone marrow and the spleen cells significantly. The potent inhibition of the bone marrow CFUC was particularly evident. The in vivo cytotoxic effect of MPC decreased with time as shown by the recovery of the number of clonogenic bone marrow and splenic cells.

DISCUSSION

The growing and quantitating of colonies of granulocytes...
cells. Once a maximum level of inhibition of CFUC (~40%) was produced, no further inhibition occurred whether the concentration of MPC was increased or the duration of incubation was extended. This observation suggests that the cytotoxic effect of MPC was cell cycle dependent, with MPC killing only those cells which were in S phase during the period of incubation with the modified polynucleotide. The absence of a progressive increase in toxicity with prolongation of the duration of incubation with MPC suggests that MPC may also arrest cells at the G1-S boundary (5) or that the MPC was degraded during prolonged incubations. If the MPC was an S-phase-specific drug, its addition to high-specific-activity thymidine should not produce more cytotoxic effect than that of high-specific-activity thymidine alone since all the cells in S phase would be killed by the radioactive thymidine. On the other hand, if MPC killed the cells in a nonspecific manner, additional cytotoxicity should be observed. As seen in Table 2, the cytotoxicity of MPC was not augmented by high-specific-activity thymidine nor was the converse true. Thus, the results indicate that MPC seems to be an S-phase-specific agent. This conclusion is in agreement with the finding of the biochemical studies that MPC is readily polymerizing enzymes.

The fact that MPC decreases the number of clonogenic cells in both the bone marrow and spleen of leukemic mice indicates that MPC was distributed to the marrow and the spleen and was cytotoxic to the clonogenic cells residing in those sites. The recovery of the progenitor cells at a later time, i.e., 25 hr after the administration of MPC into the mouse, implies that the toxic effects of a single dose of MPC were relatively short lived. More detailed pharmacological studies of the kinetics and distribution of MPC as well as the testing of various schedules and routes of administration are in progress in our laboratories.

Table 3

<table>
<thead>
<tr>
<th>Time (hr) after</th>
<th>% of control</th>
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<tbody>
<tr>
<td>i.v.</td>
<td>Marrow CFUC</td>
</tr>
<tr>
<td>19</td>
<td>8.1 ± 4.1*</td>
</tr>
<tr>
<td>25</td>
<td>21.6 ± 1.9</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

and/or macrophages in the semisolid system (6, 20) are believed to be a measure of the committed progenitor cells which will proliferate to form granulocytes and macrophages. Thus, the inhibition of the colony-forming units under the assay conditions can be ascribed to the killing of the committed progenitors. The inhibition by MPC of the marrow and splenic CFU-C represents the first example of such study, namely, the effect of a polynucleotide on the hematopoietic clonogenic

REFERENCES

7. Chandra, P., and Bardos, T. J. Inhibition of DNA polymerases from RNA tumor viruses by novel template analogues: partially thiolated polycytidylic acid.


Cancer Research

Effects of Partially Thiolated Polycytidylic Acid on the Clonogenicity of Murine Leukemic Stem Cells

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