A Comparison of the Biological Properties of 6-Selenopurine, 6-Selenopurine Ribonucleoside, and 6-Mercaptopurine in Mice

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SUMMARY

6-Selenopurine per se, and not its riboside or the products of its decomposition, was found to be the active antitumor agent when tested in mice bearing lymphoma L1210. It is suggested that the extremely short biological half-life of 6-selenopurine riboside (about 1 hour at body temperature) may be an important factor in the lack of antitumor activity of this compound. 6-Selenopurine riboside formed a number of decomposition products when incubated in vitro at 37° C. but was not converted to 6-selenopurine under these conditions.

6-Selenopurine exhibited the same activity as 6-mercaptopurine against the growth of lymphoma L1210, and 6-mercaptopurine-resistant L1210 cells exhibited cross-resistance to 6-selenopurine.

A 6-mercaptopurine-insensitive tumor, L5178-Y, was more susceptible to combination therapy with azaserine and 6-mercaptopurine than was a 6-mercaptopurine-sensitive tumor, L1210. When azaserine and 6-selenopurine were administered to mice bearing L5178-Y or L1210 lymphomas in amounts equivalent on a molar basis to the most effective potentiating combination of azaserine and 6-mercaptopurine, only additive antitumor effects were noted.

It was reported previously (7) that 6-selenopurine (6-SeP), analogous in structure to 6-mercaptopurine (6-MP), is as potent as the latter in inhibiting the growth in mice of lymphoma L1210, but possesses weaker antitumor activity and greater host toxicity than does 6-MP when injected into mice bearing L5178-Y or Sarcoma 180. In contrast to 6-MP, 6-SeP is unstable at body temperatures, with a half-life of about 6 hours; accordingly, the magnitude of its antitumor activity implies that 6-SeP must be capable of exerting its effect upon the target cells rather swiftly. This possibility, plus the fact that 6-SeP is a more potent inhibitor than 6-MP in bacterial and cell culture systems (6) and was capable of inhibiting 6-MP-resistant microorganisms, provided the justification for further investigation of this compound. It was considered important to determine whether unaltered 6-SeP or a metabolic derivative of it was the active antitumor agent, whether 6-MP-resistant tumors selected by that agent would also be resistant to 6-SeP, and whether 6-SeP, known to be rather different from 6-MP in terms of electron distribution and degree of polarization, might behave differently than the latter in combination with other chemotherapeutic agents.

MATERIALS AND METHODS

Male and female (AKR X DBA/2) F1 hybrid mice, 6–8 weeks old and weighing 18–25 gm., were used in our study of L1210 and L5178-Y (tumors which were used in the form of lymphomas). The technic of tumor implantation has already been described.

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1 Obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

2 Donor mice carrying this lymphoma were provided by L. W. Law of the National Cancer Institute.

3 The Y-strain of leukemia L5178 was developed by G. A. Fischer and J. J. Jaffe from a single cell, isolated in vitro and grown initially in a medium which has been described by G. A. Fischer and A. D. Welch, Science, 126:1018–19, 1957. The original donor mice carrying L5178 were kindly provided by L. W. Law.
described (7). Each mouse received from 1 to $3 \times 10^6$ viable tumor cells; these were injected subcutaneously into the right flank. All animals were fed Purina Laboratory Chow, and drinking water was available ad libitum.

6-Selenopurine was prepared by a method reported elsewhere (5). A commercial sample of 6-mercaptopyrimidine was used. 4 One of the studies described herein called for the use of the ribonucleoside of 6-SeP (6-seleno-9-β-D-ribofuranosylpurine); a description of its synthesis with analytical data and the results of decomposition studies are presented in the following paragraphs.

**Synthesis of 6-selenopurine ribonucleoside.**—Into absolute methanol (8 ml.), sodium (0.9 gm., 0.0087 mole) was placed. Freshly generated hydrogen selenide was then bubbled through the solution. 6-Chloro-9-β-D-ribofuranosylpurine6 (1 gm., 0.0035 mole) was suspended in boiling absolute methanol (16 ml.). The solution of sodium hydroselenide was added, the mixture heated to reflux for 10 minutes, and the preparation chilled for 8 hours. At the end of that period, the product was filtered off and dissolved in sodium carbonate solution (2 per cent, 6 ml.). Colloidal selenium was filtered off. Acidification of the clear, yellow filtrate with glacial acetic acid produced glittering, bright yellow flakes melting at 212°–214°C. (yield: 0.55 gm.; 48.5 per cent of theory). The purification procedure was carried out at 5°C. to minimize decomposition.

**Analysis:** Calculated for C$_{10}$H$_{12}$N$_2$O$_4$Se: C, 36.26; H, 3.65; N, 16.92. Found: C, 36.19; H, 3.62; N, 16.80.

**Decomposition studies.**—Examination of a 1:100 solution of 6-selenopurine ribonucleoside (6-SePR) in phosphate-citrate buffer of pH 7 disclosed two UV-absorption peaks: 285 mμ (ε = 7,890) and 345 mμ (ε = 11,150), respectively. During incubation of the solution at 37°C, the absorption spectrum was redetermined at 30-minute intervals. Judging from the height of the 345 mμ peak, the half-life of 6-SePR under the above conditions was about 1 hour (as compared with 6 hours for 6-SeP); after 2½ hours, the 345-mμ peak had disappeared completely.

Because of the demonstrated instability of 6-SeP and 6-SePR, fresh solutions of these two compounds were made daily just before the time of injection. The stability of 6-MP permitted the preparation of sufficient amounts of the appropriate solution to last for the duration of each experiment. Solutions of azaserine6 (in physiological saline) were prepared at the outset to last for 6 days; they were kept in a refrigerator when not in use and were warmed slightly just prior to injection.

In studies of the responses of the lymphomas to drugs, treatment with any particular compound was begun 24 hours after subcutaneous inoculation of the tumor cells into individually weighed mice (7). The compounds were injected intraperitoneally once daily for 6 consecutive days, and no mouse received more than 1 ml. of solvent (saline or pH 8 buffer) daily. In each experiment, ten mice were used for study of the effect of each dose level of a drug or combination of drugs, while ten mice, which served as controls, were given injections once daily of a comparable amount of the appropriate vehicle.

On the day following the final dose, each mouse was weighed, sacrificed, and its tumor removed and weighed. The average tumor weight of each treated group was compared with that of the controls, and the result was recorded in terms of percentage inhibition of tumor growth.

**RESULTS**

Antitumor activity of decomposition products of 6-selenopurine.—It was shown previously that the half-life of 6-SeP in neutral aqueous solution at 37°C is approximately 6 hours. To determine whether the decomposition products of 6-SeP possess antitumor activity, 6-SeP was incubated for 24 hours at 37°C before being injected into mice bearing lymphoma L1210. The antitumor activity of a solution of this material was compared with that of 6-SeP, which was prepared fresh daily. The data summarized in Table 1 show that the antitumor activity of the decomposition products of 6-SeP is markedly lower than that of the original compound.

Antitumor activity of 6-selenopurine ribonucleoside (6-SePR).—It has been shown that a number of purine and pyrimidine analogs are metabolized to nucleosides and nucleotides, and there is evidence which suggests that ribose-containing derivatives of at least some of these bases are the active growth-inhibitory agents (1, 2, 11). On the assumption that 6-SeP also might be converted to a ribose-containing compound before it can exert antitumor activity and that the instability of the...
free base at body temperature limits its conversion to the active form, the ribonucleoside of 6-SeP was synthesized, and its antitumor activity was compared with that of 6-SeP and 6-MP. The data in Table 1 indicate the 6-SePR possessed little or no antitumor activity in vivo. The reason for this inactivity suggested itself when the stability of the ribose derivative was determined in vitro. The ribonucleoside is much less stable than is 6-SeP at body temperature, with a half-life of about 1 hour (cf. “Materials and Methods”), and forms upon decomposition a number of products, but not 6-SeP itself. While this observation does not eliminate the possibility that 6-SeP is active in vivo in the form of a ribose-containing derivative, it suggests that the administration of 6-SePR as an alternative to 6-SeP itself is contraindicated.

Effect of 6-selenopurine on the growth of a 6-mercaptopurine-resistant tumor.—Law et al. (4) have shown that, when a strain of L1210 is selected which is resistant to 6-MP, there is a concomitant development of resistance to a number of other purine analogs. The data in Table 1 show that this also occurs in the case of 6-SeP.

Comparison of effects of 6-SeP and 6-MP in combination with azaserine against a 6-MP-sensitive and a 6-MP-resistant tumor.—Skipper (10) and Tarnowski and Stock (12) have reported that azaserine potentiates the antitumor activity of 6-MP against a variety of experimental neoplasms. Since it was also found that azaserine potentiates the antitumor activity of another purine analog, 6-thioguanine (8), experiments were designed to compare the potency of combinations of (a) azaserine and 6-MP and (b) azaserine and 6-SeP against L1210, a 6-MP-sensitive tumor, and L5178-Y, previously shown to be relatively insensitive to 6-MP. The results of these experiments are summarized in Table 2. They show that, in the case of L1210, azaserine potentiated the antitumor activity of 6-MP, as was first reported by Skipper (10), but only in the following combination of doses: azaserine, 1.25 mg/kg, plus 6-MP, 6.25 mg/kg. Combinations of the two drugs at all

### Table 1

**Effects of Various Compounds on Growth of L1210 Lymphoma**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Compounds</th>
<th>Dosage levels* (mg/kg)</th>
<th>Av. change in body wt.† (gm.)</th>
<th>Mortality</th>
<th>Av. tumor wt.‡ (mg.)</th>
<th>Approximate percentage inhibition of tumor growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210</td>
<td>6-MP</td>
<td>20</td>
<td>+1.1</td>
<td>0/10</td>
<td>330 ± 26</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>6-SeP</td>
<td>25</td>
<td>0</td>
<td>0/10</td>
<td>330 ± 26</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Decomp. Products</td>
<td>25</td>
<td>-2.8</td>
<td>0/10</td>
<td>330 ± 26</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>6-SePR</td>
<td>50</td>
<td>-3.6</td>
<td>0/10</td>
<td>330 ± 26</td>
<td>10</td>
</tr>
<tr>
<td>L1210/6MP</td>
<td>6-MP</td>
<td>25</td>
<td>+0.9</td>
<td>0/10</td>
<td>384 ± 11</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>6-SeP</td>
<td>32</td>
<td>-1.6</td>
<td>3/10</td>
<td>384 ± 11</td>
<td>0</td>
</tr>
</tbody>
</table>

* The compounds were injected intraperitoneally once daily for 6 days, starting 24 hours after introduction of the tumor.
† In all cases, AKR × DBA/2 F1, hybrid mice, 6–8 weeks old, 18–25 gm., of either sex, were used. In general, the weight gain of untreated tumor-bearing mice, during the period of observation, averaged 2.5 gm. The size of each group is indicated by the denominator of the appropriate fraction in the columns headed “Mortality.”
‡ The average weight of the tumors, together with the standard error \( \sqrt{\frac{\sum d^2}{n(n-1)}} \) is given for each group of animals, in comparison with its control group; in each case, the latter figure is placed above that of the experimental group and is shown in italics.
TABLE 2

EFFECT OF 6-MERCAPTOPURINE, 6-SELENOPURINE, AND AZASERINE, ALONE AND IN COMBINATION, ON THE GROWTH OF LYMPHOMAS L1210 AND L5178-Y

<table>
<thead>
<tr>
<th>Dose Levels* (mo/kg)</th>
<th>L1210 Lymphoma</th>
<th>L5178-Y Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compounds</td>
<td>Av. change in body wt. (gm.)</td>
<td>Mortality</td>
</tr>
<tr>
<td>6-MP</td>
<td>-0.2</td>
<td>0/20</td>
</tr>
<tr>
<td>12.5</td>
<td>+1.0</td>
<td>0/20</td>
</tr>
<tr>
<td>6.2</td>
<td>+2.4</td>
<td>0/20</td>
</tr>
<tr>
<td>6-SeP</td>
<td>-1.0</td>
<td>2/10</td>
</tr>
<tr>
<td>16</td>
<td>-0.6</td>
<td>0/15</td>
</tr>
<tr>
<td>8</td>
<td>+0.7</td>
<td>0/15</td>
</tr>
<tr>
<td>Azaserine</td>
<td>-2.5</td>
<td>0/10</td>
</tr>
<tr>
<td>2.5</td>
<td>+0.4</td>
<td>0/15</td>
</tr>
<tr>
<td>1.2</td>
<td>+0.7</td>
<td>0/15</td>
</tr>
<tr>
<td>Azaserine + 6-MP</td>
<td>1.2 + 25</td>
<td>-1.2</td>
</tr>
<tr>
<td>1.2 + 12.5</td>
<td>-0.7</td>
<td>0/20</td>
</tr>
<tr>
<td>1.2 + 6.2</td>
<td>-0.8</td>
<td>0/20</td>
</tr>
<tr>
<td>2.5 + 6.2</td>
<td>-1.8</td>
<td>0/20</td>
</tr>
<tr>
<td>5 + 6.2</td>
<td>-2.3</td>
<td>0/20</td>
</tr>
<tr>
<td>Azaserine + 6-SeP</td>
<td>1.2 + 16</td>
<td>-0.8</td>
</tr>
<tr>
<td>1.2 + 8</td>
<td>0</td>
<td>0/10</td>
</tr>
<tr>
<td>5 + 8</td>
<td>-3.0</td>
<td>0/10</td>
</tr>
<tr>
<td>5 + 16</td>
<td>-2.3</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* The compounds were injected intraperitoneally once daily for 6 days, starting 24 hours after introduction of the tumor.

† In all cases, AKR X DBA/2 F1 hybrid mice, 6-8 weeks old, 18-25 gm., of either sex, were used. In general, the weight gain of untreated tumor-bearing mice, during the period of observation, averaged 2.5 gm. The size of each group is indicated by the denominator of the appropriate fraction in the columns headed “Mortality.”

‡ The average weight of the tumors, together with the standard error $\frac{2d^2}{n(n-1)}$ is given for each group of animals, in comparison with its control group; in each case, the latter figure is placed above that of the experimental group and is shown in italics.

$\frac{2d^2}{n(n-1)}$
other dosages tested gave only additive effects. At the same time, azaserine did not potentiate the antitumor activity of 6-SeP in any of the various combinations tested. In the case of L5178-Y, a tumor which is little affected by 6-MP alone, azaserine again markedly potentiated the antitumor activity of this purine analog. Here there were several combinations of doses with which potentiation was disclosed. The potentiating combinations had in common a small dose of 6-MP (6.25 mg/kg). As the dose of azaserine was increased (1.25, 2.5, and 5.0 mg/kg, respectively), the antitumor potency of the combination was increasingly enhanced, leading, in the case of the combination of azaserine (5.0 mg/kg) plus 6-MP (6.25 mg/kg), to almost complete suppression of growth, with no tumors being palpable in twenty of twenty mice. That the tumor cells were not destroyed by this treatment became apparent when, upon cessation of treatment after 1 week, the tumors in all animals commenced to grow and eventually caused the death of each host. One azaserine-6-SeP dosage combination (1.25 mg/kg plus 16 mg/kg, respectively) appeared to be potentiating against L5178-Y, while the same dosage combination (as well as all others tried) gave only additive antitumor activity against L1210. Whereas the most effective combination of azaserine and 6-MP against the growth of L5178-Y was in doses of 5.0 and 6.25 mg/kg, respectively, molar equivalent amounts of azaserine and 6-SeP gave only additive effects.

In experiments with both L1210 and L5178-Y, no indication of intolerable host toxicity, as determined by excessive weight loss (more than 20 per cent of the pretreatment level) or death, was found with the most effective combinations of azaserine and 6-MP.

**DISCUSSION**

The purine analog, 6-SeP, differs in structure from 6-MP only by the substitution of selenium for sulfur at C-6. The resulting change in electron distribution apparently is sufficient markedly to reduce the stability of the compound. A comparison of the biological activities of 6-MP and 6-SeP shows that the two compounds behave similarly. For example, both 6-MP and 6-SeP inhibited the utilization of formate-C\(^4\), but not that of adenine-8-C\(^4\), for the synthesis of nucleic acids by cells of the Ehrlich carcinoma (ascitic form) incubated in vitro. Also, tumor cells (L1210) resistant to 6-MP exhibited cross-resistance to 6-SeP. The evidence presently available suggests that 6-SeP is, on the whole, a less effective antitumor agent in vitro than is 6-MP, because it has a much lower biological half-life than does the latter. If, as has been suggested, the active forms of the purine analogs are ribose-containing derivatives, compounds such as 6-MP and 6-SeP, to be effective, require the presence of appropriate enzymic machinery within cells, in the liver or in the tumor itself, to assist in their conversion to the active form. In addition, these purines require the time and opportunity to reach the site or sites of metabolic conversion to the active form. All these considerations indicate that, compared with 6-MP, 6-SeP is at a disadvantage as an antitumor agent, since it has been shown that the selenium compound is unstable under in vivo conditions, and that the products of its decomposition, while toxic to the host, possess but little antitumor activity. In addition, the data suggest that the fraction of a given dose of 6-SeP which is converted to the active form(s) also has markedly short biological half-life. Certainly, this is true in the case of the ribonucleoside of 6-SeP. It is unfortunate that these are the characteristics of 6-SeP, for they greatly limit the potential usefulness of the compound.

An interesting development in these studies has been the finding, reported previously (9), that a 6-MP-insensitive tumor, L5178-Y, is more susceptible to combination therapy with azaserine and 6-MP than is a 6-MP-sensitive tumor, L1210. A similar effect has been observed by Sartorelli et al. (9), using the combination of azaserine and 6-thioguanine against 6-thioguanine-resistant Ehrlich ascites carcinoma. These findings add weight to the growing body of evidence indicating that, when populations of cells develop resistance to a particular chemotherapeutic agent, they may become much more dependent upon metabolic pathways the near-indispensability of which makes the cells unusually vulnerable to attack by agents hitherto ineffective (13). The agent to which the cellular population developed resistance originally may still be therapeutically useful in combination with another agent, perhaps by exerting its blocking action (to a lesser degree) on the residual portion of the incompletely abolished original metabolic pathway, or by permitting a sequential blockade of major proportions through acting together with another agent on a newly vulnerable biosynthetic path.

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A Comparison of the Biological Properties of 6-Selenopurine, 6-Selenopurine Ribonucleoside, and 6-Mercaptopurine in Mice

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