Mechanisms of Action of 6-Thioguaine, 6-Mercaptotpurine, and 8-Azaguanine

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SUMMARY

The effects of 6-thioguaine on purine biosynthesis and cell viability have been examined in H.Ep. 2 cells grown in culture. Toxicity is not reversed by aminoimidazolecarboxamide, suggesting that inhibition of purine biosynthesis de novo is not the sole mechanism of toxicity. Also, 6-(methylmercaptopurine)purine ribonucleoside, a potent inhibitor of purine biosynthesis de novo, produces more marked reductions in cellular pools of purines than does 6-thioguaine without killing cells. There is no apparent inhibition by 6-thioguanosine 5'-monophosphate of other enzymes leading to the synthesis of guanosine 5'-triphosphate as determined in whole cells by measurements of radioactive hypoxanthine or guanine incorporation. Inhibition of DNA synthesis by 1 mm thymidine protects cells from 6-mercaptopurine or 6-thioguanine but fails to protect cells from 8-azaguanine toxicity. On the other hand, inhibition of RNA synthesis by 6-azaauridine plus deoxyctydine protects cells against 8-azaguanine but does not protect against 6-thioguanine or 6-mercaptopurine toxicity. In agreement with the in vitro data, arabinosylcytosine (a potent inhibitor of DNA synthesis) fails to protect mice against 8-azaguanine but has previously been shown to protect mice from 6-mercaptopurine or 6-thioguanine toxicity. The results support the hypotheses of others that incorporation into DNA (as 6-thioguanine nucleotide) is a mechanism of toxicity for these thiopurines, whereas 8-azaguanine is toxic due to its incorporation into RNA.

INTRODUCTION

The antitumor agent, 6-TG, is metabolized to the corresponding ribonucleoside mono-, di-, and triphosphate derivatives and is incorporated into the DNA and RNA of treated cells (Chart 1). Metabolism to the nucleotide level occurs via HGPRTase and is necessary for cytotoxicity (37). The ribonucleoside monophosphate, 6-thioguanosine 5'-monophosphate, presumable as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38). The radioactivity in nucleic acids of tumor cells treated with radioactive 6-MP appears to be due to the conversion to and subsequent utilization of 6-thioguanosine 5'-monophosphate, presumably as a result of the actions of IMP dehydrogenase and GMP synthetase, since this conversion is blocked by mycophenolic acid (40), an inhibitor of these enzymes (38).
Gradient used was from 5 mM KH₂PO₄, pH 3.35, to 250 mM K₂HPO₄. A Waters Associates ALC202 high-pressure liquid chromatograph equipped with a Reeve Angel pellicular column (Pellionex SAX, 1 mm inside diameter x 300 cm) was used to measure nucleotide pools. The linear gradient was 5 mM KH₂PO₄, pH 3.35, to 250 mM KH₂PO₄ plus 250 mM KCl, pH 4.4, in 35 min. The gradient was formed by electronically varying the output of 2 pumps using the Waters Associates Model 600 gradient programmer. The flow rate was 0.8 ml/min, and the column temperature was 80°C [heating of the column was accomplished by a circulating water bath and a rubber tubing jacket (29)]. Samples (25 to 100 μl) were introduced by means of the Model U6K LC injector system (Waters Associates, Milford, Mass.). Detection of eluting materials was by UV absorption at 254, 280, or 350 nm using 8-μl flow cells of the UV monitor supplied in the ALC202 instrument or an LDC Model 1205 monitor. A Hewlett-Packard Model 3380A digital electronic integrator was used to quantitate the UV signals. Endogenous purine and pyrimidine nucleotides were quantitated in terms of the respective ribonucleoside monophosphates at 254 and 280 nm, except for CTP which was quantitated at 280 nm only. An authentic sample of MMPR-5'-P was used to quantitate this metabolite of MMPR at 280 nm. Ribonucleoside mono- d-, and triphosphates of 6-TG were quantitated at 350 nm with respect to 6-thioguanosine, since purified samples of the nucleotides were not readily available. The metabolites of 6-TG in extracts of H.Ep. 2 cells followed the pattern previously observed by this methodology in Sarcoma 180 cells (28).

Radioactive Precursor Experiments. Incorporation of radioactive [8-14C]hypoxanthine into adenine and guanine nucleotides was measured in cell extracts by collecting fractions of the eluant at 1- to 2-min intervals from the high-pressure liquid chromatograph described above. A Gilson Model TDCE fraction collector was used, and the samples were collected directly into liquid scintillation vials (Gilson Model B100 base). Radioactivity was determined by liquid scintillation spectrometry after the addition of 10 ml of Aquasol (New England Nuclear, Boston, Mass.). Incorporation of [8-14C]hypoxanthine into the RNA and DNA of H.Ep. 2 cells was determined as described in detail elsewhere (5).

Reversal of Toxicity. In order to determine the importance of RNA or DNA synthesis to the toxicity of purine analogs, procedures were established to inhibit these processes selectively. Thymidine at a concentration of 1 mM is known to inhibit DNA synthesis without marked effect on RNA synthesis (25). Reversal of such inhibition was accomplished by washing the cells free of thymidine. We have used 6-azauridine plus deoxycytidine to inhibit RNA synthesis selectively. The dose (5 μg/ml) of 6-azauridine used produces a profound lowering of CTP and UTP pools (unpublished observation) which is consistent with its recognized site of action, i.e., inhibition of orotidine 5'-monophosphate decarboxylation by 6-azaaridine 5'-monophosphate (9). Deoxycytidine is included since it will supply the pyrimidine deoxyribonucleotides needed to maintain DNA synthesis, namely, dCMP (deamination of dCMP yields dUMP from which thymidylate acid is formed). Reversal of the 6-azauridine block of RNA synthesis is accomplished by washing the cells and adding uridine (to restore UTP and CTP pools).

The role of DNA synthesis in the toxicity of 8-aza-G was...
also determined in vivo by observing the toxicity to female C57BL × DBA/2 F₁ (hereafter called BD2F₁) mice when this analog was administered in combination with ara-C, a potent inhibitor of DNA synthesis. The protocol and treatment of data for combination toxicity studies of this nature have been described (36).

Materials. [8-¹⁴C]Hypoxanthine (49.4 μCi/μmole) was obtained from Schwarz/Mann, Orangeburg, N. Y. MMPR-5'-P was synthesized by the Organic Chemistry Department of this Institute and was provided by Dr. John A. Montgomery. [6-³²P]TG was prepared by Dr. S. H. Chu of Brown University as previously described (28). Other samples of purines, pyrimidines, or analogs and derivatives thereof were commercially supplied by either P-L Biochemicals, Milwaukee, Wis., or Sigma Chemical Co., St. Louis, Mo.

RESULTS

Role of Inhibition of Purine Biosynthesis in the Mechanism of Cytotoxicity due to 6-TG or 6-MP. If inhibition of purine biosynthesis de novo is critical for the action of 6-TG, protection against 6-TG toxicity should be afforded by AIC, which will “by-pass” the blockade (Chart 1). AIC only slightly protects Adenocarcinoma 755 or H.Ep. 2/S cells from 6-TG toxicity (Table 1). AIC provides better protection against 6-MP than against 6-TG in these cell lines. AIC produces a dramatic reversal of the toxicity due to MMPR (Ref. 2; Table 1). MMPR is the immediate precursor to PRPP amidotransferase (10, 11, 13, 21). Treatment of H.Ep. 2 cells with 0.1 μg of MMPR per ml resulted in accumulation of MMPR-5'-P to high levels (~1 μmole/10⁹ cells) and reduction of ATP and GTP pools to as low as 20 to 40% of control values in 24 hr (Chart 2). Exposure of the cells to this dose of MMPR for these time periods was without effect on cell viability (Chart 2, upper numbers). This suggests that depletion of purine nucleotide pools (or inhibition of PRPP amidotransferase) must be greater than that observed in order to produce cytotoxicity. Treatment of cells with 0.167 μg of 6-TG per ml produced effects on ATP, GTP, CTP, and UTP levels which were qualitatively similar to but quantitatively less than that of MMPR (Chart 3). However, the viability of the cells was dramatically reduced during the time course examined, with particular toxicity being manifest 24 hr after the drug was added to the medium. The level of 6-TG used in this acute experiment (Chart 3, 24 hr) exceeds by 33-fold the level required to kill cells after chronic exposure (Table 1, 0.005 μg/ml), whereas the level of MMPR used in acute experiments (Chart 2) is approximately the same as that required to kill cells after chronic exposure (Table 1). Nonetheless, a dissociation between effects of 6-TG on purine nucleotide pools and cytotoxicity is apparent. Levels of 6-TG nucleotides (about 60 to 70% was 6-thioGMP at all times shown) reached about 0.2 μmole/10⁹ cells, and by 24 hr these metabolites were virtually not detectable. Correspondingly, ATP and GTP levels were returning toward control values during the time interval in which 6-TG nucleotides were reduced, i.e., between 4 and 24 hr. The disappearance of 6-TG nucleotides from the cells probably reflects, at least in part, utilization of the drug from the medium for nucleic acid synthesis. Addition of 6-TG (0.167 μg/ml) to the medium of the cells treated with 6-TG for 24 hr resulted in subsequent incorporation of the drug into 6-TG nucleotides in 1 hr to levels

<table>
<thead>
<tr>
<th>Effect of AIC on the inhibition of tumor cell growth by purine analogs</th>
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</thead>
<tbody>
<tr>
<td>Growth or viability (% of control)</td>
</tr>
<tr>
<td>Without AIC</td>
</tr>
<tr>
<td>Adenocarcinoma 755a*</td>
</tr>
<tr>
<td>6-MP (5 μg/ml)</td>
</tr>
<tr>
<td>6-TG (0.2 μg/ml)</td>
</tr>
<tr>
<td>H.Ep. 2 cellsb</td>
</tr>
<tr>
<td>6-MP (0.05 μg/ml)</td>
</tr>
<tr>
<td>6-TG (0.002 μg/ml)</td>
</tr>
<tr>
<td>6-TG (0.005 μg/ml)</td>
</tr>
<tr>
<td>MMPR (0.05 μg/ml)</td>
</tr>
<tr>
<td>MMPR (0.2 μg/ml)</td>
</tr>
</tbody>
</table>

* Growth during 48 hr, treated versus control cells.
* Results are means ± range for 2 to 3 determinations.
* Cloning efficiency of treated versus control cells. Cells were exposed to the drugs for 7 to 14 days.

Chart 2. Effects of MMPR on nucleotide pools and viability of H.Ep. 2/S cells grown in tissue culture. Flasks containing approximately 250,000 tumor cells/ml were treated with MMPR, and aliquots of cells were removed 1, 2, 4, and 24 hr later for measurement of nucleotide pools by liquid chromatography and cell viability by cloning (see “Materials and Methods”). % viable cells, cloning efficiency compared to simultaneous control. Control nucleotide levels in μmoles/10⁹ cells were: ATP, 7.51; GTP, 2.06; UTP, 4.08; CTP, 0.53.

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similar to that seen 1 hr after the 1st treatment (data not shown).

Possible effects of 6-thioGMP on IMP dehydrogenase, HGPRTase, and ATP:GMP phosphotransferase activities in intact H.Ep. 2 cells were evaluated by treating cells for 1 hr with 6-TG, washing the cells free of nonincorporated drug, and adding [8-14C]hypoxanthine. Although high levels of 6-thioGMP are maintained during the uptake of radioactive hypoxanthine, there is no apparent inhibition of these enzymes by 6-thioGMP in intact cells (Table 2). If such inhibition had occurred, one would expect a marked lowering of radioactivity associated with GTP as was observed with mycophenolic acid, a known inhibitor of IMP dehydrogenase (38). In agreement with its effect on precursor labeling, mycophenolic acid produced a decrease in GTP pools to 15 and 39% of control levels in these experiments. Apparent lack of inhibition of HGPRTase or of ATP:GMP phosphotransferase by high levels of 6-thioGMP has been noted previously in intact Sarcoma 180 or L5178Y cells (6) by a similar technique using radioactive [8-14C]guanine. In experiments not shown, no effect of 6-thioGMP on [8-14C]guanine incorporation into GMP, GDP, or GTP was observed in H.Ep. 2 cells in which 6-thioGMP levels were as high as 200 nmoles/10⁹ cells.

Role of RNA and DNA Incorporation in the Toxicity of Purine Analogs. The importance of RNA or DNA incorporation to the toxicities of 6-TG, 6-MP, or 8-aza-G was evaluated by inhibiting RNA or DNA synthesis during exposure to the drugs. Thymidine at 1 mM produced a rather selective inhibition of DNA synthesis, whereas the combination of 6-azauridine plus deoxycytidine inhibited RNA synthesis more than DNA synthesis (Table 3). When 1 mM thymidine was used in combination with the purine analogs, the toxicities of 6-TG and 6-MP were dramatically reduced (Table 4). On the other hand, inhibition of RNA synthesis was without marked effect on 6-TG or 6-MP toxicity but afforded protection against 8-aza-G toxicity (Table 5).

Simultaneous administration of ara-C with 6-MP or 6-TG protects mice against the toxicities of the thiopurines (17, 33, 34, 36), and the ara-C plus 6-TG combination is less than additive against H.Ep. 2 cells in culture (43). ara-C failed to protect mice from the toxicity of 8-aza-G (Chart 4), i.e., the combination was additive in toxicity. The combination toxicity index of 1.1 for ara-C plus 8-aza-G is markedly different from the ara-C plus 6-MP or ara-C plus 6-TG value, i.e., ~2.0 (36).

Rates of 6-ThioGMP and 6-ThioGTP Formation in Intact Sarcoma 180 Cells. In an effort to understand the lack of effect of 6-thioGMP on enzymes required for GTP synthesis (Ref. 6; Table 2), an approximation of HGPRTase activity in whole cells was made (Table 6). The apparent rate of 6-thioGMP formation from 6-TG in Sarcoma 180 cells is about 0.006 μmole/min/g. Pretreatment with MPPR increases the rate of 6-thioGMP formation, probably due to the elevation of PRPP pools (28). The rate of 6-thioGTP formation is appreciable, being about 0.004 μmole/min/g at high levels of 6-thioGMP [MMPR-pretreated cells (Table 6)]. This rate should not reflect that for the GMP to GDP conversion since the Vmax values of GMP and 6-thioGMP for the ATP:GMP phosphotransferase are very different (23). Conversion of 6-thioGMP to 6-thioGDP by this enzyme appears to be limiting for 6-thioGTP formation since pools of 6-thioGDP are almost not measurable (28). The numbers of cells taken from the 2 groups of mice were probably about the same since the rates of 6-thiouric acid formation were identical (Table 6).

DISCUSSION

Effects of 6-TG on purine biosynthesis do not appear to be causally related to cell toxicity since: (a) effects on
Inhibition of RNA or DNA synthesis in H.Ep. 2/S cells grown in tissue culture

Flasks containing 50,000 tumor cells/ml were treated with 6-azauridine (5 μg/ml) plus deoxycytidine (20 μg/ml) or with 1 mM thymidine. [8-14C]Hypoxanthine (0.5 μCi/ml) was added at the same time and cumulative uptake of radioactivity was determined at 1, 2, 4, and 6 hr as previously described (5). The uptake of radioactivity into RNA and DNA was essentially linear in the controls during the 6-hr incubation period, averaging 6.2 nCi/hr in RNA and 1.1 nCi/hr in DNA for each sample of 100,000 cells assayed.

<table>
<thead>
<tr>
<th>[8-14C]Hypoxanthine incorporation (% of control)</th>
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<tbody>
<tr>
<td>Azauridine + deoxycytidine</td>
</tr>
<tr>
<td>Time (hr)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>6</td>
</tr>
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* Results are average values ± range for 2 separate experiments.

Effect of inhibition of DNA synthesis on the toxicities of purine analogs

Approximately 100 H.Ep. 2/S cells were attached to glass by overnight incubation. The cells were then exposed for 1 hr to the drugs shown in the presence or absence of 1 mM thymidine. Thymidine, where indicated, was included for an additional 5 hr, at which time all cells were again washed. Viability refers to clones formed 7 to 14 days later.

<table>
<thead>
<tr>
<th>Cell viability (% of control)</th>
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<tbody>
<tr>
<td>Without thymidine</td>
</tr>
<tr>
<td>Drug</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>6-TG (0.1 μg/ml)</td>
</tr>
<tr>
<td>6-MP (2 μg/ml)</td>
</tr>
<tr>
<td>8-aza-G (2 μg/ml)</td>
</tr>
</tbody>
</table>

* Results shown are mean values ± S.E. n = 3 to 5.

Hand, protection against 6-TG toxicity by inhibition of DNA synthesis in vivo with ara-C (17, 33, 34, 36) or in vitro with 1 mM thymidine (Table 4) suggests that incorporation into DNA is a mechanism of action. This result is strengthened by the observation that inhibition of RNA synthesis fails to protect cells against 6-TG (Table 5).

Although an effect of 6-TG on purine biosynthesis de novo is apparent in H.Ep. 2 cells (Chart 3), the toxicity of 6-TG appears unrelated to this effect since major loss in cell viability occurred at a time (24 hr) when purine nucleotide pools were recovering and the level of 6-TG nucleotides was very low. Also, MMPR produced more marked effects than 6-TG on ribonucleotide pools without killing cells (Charts 2 and 3).

Table 5

Effect of inhibition of RNA synthesis on the toxicities of purine analogs

The experiments were performed exactly as described in Table 4 except that 6-azauridine (5 μg/ml) plus deoxycytidine (20 μg/ml) was used instead of thymidine. Also, after the cells were washed at 6 hr, uridine (20 μg/ml) was added to all samples.

<table>
<thead>
<tr>
<th>Cell viability (% of control)</th>
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<tbody>
<tr>
<td>Without deoxycytidine</td>
</tr>
<tr>
<td>Drug</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>6-TG (0.1 μg/ml)</td>
</tr>
<tr>
<td>6-MP (2 μg/ml)</td>
</tr>
<tr>
<td>8-aza-G (2 μg/ml)</td>
</tr>
</tbody>
</table>

* Results shown are mean values ± S.E. n = 3 to 4.

Significantly different from 0, p < 0.05.
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and 3). Failure of AIC to reverse 6-TG toxicity (Table 1) also suggests that inhibition of PRPP amidotransferase is not a major site of toxicity of 6-TG. The effects of 6-TG to increase pyrimidine and to decrease purine ribonucleotide pools (Chart 3) are similar to those of MMPR (Chart 2; Refs. 28 and 41). The increase in pyrimidine ribonucleotides is thought to be due to increased availability of PRPP for pyrimidine biosynthesis resulting from inhibition of PRPP amidotransferase (28, 41).

A probable explanation for the lack of effect of 6-thioguanine on guanine utilization in whole cells (Ref. 6; Table 2) is that the enzymes involved are not sufficiently inhibited to become "rate-limiting." In the case of ATP:GMP phosphotransferase, an approximation of the rate can be made for Sarcoma 180 cells. In this cell line, as with the H.Ep. 2 cells, 6-thioguanine fails to interfere with [8-14C]guanine conversion to GTP (6). The ATP:GMP phosphotransferase activity is about 0.5 to 1.0 μm unit/g cells (Ref. 23; unpublished measurements by Y-C. Cheng) in the Sarcoma 180 tumor. The in vivo rate of HGPRTase is approximately 0.006 μm unit/g (Table 6) with 6-TG as substrate; however, this rate should approximate that for GMP formation from guanine since the Kₘ and Vₘₐₓ values are identical (30). Nucleoside diphosphate kinase is extremely active, being about 128 μm units/g (unpublished data of Y-C. Cheng). Therefore, if the in vitro ATP:GMP phosphotransferase level approximates the in vivo rate, this enzyme must be inhibited greater than 95% before it would become rate limiting. The problem is analogous to that described in the paper of Maren (19). Due to excess carbonic anhydrase in the mammalian kidney, about 99% inhibition of the enzyme is required before bicarbonate reabsorption becomes impaired (19). If we assume the GMP level to be equal to its Kₘ for ATP:GMP phosphotransferase, 10 μm, and the level of 6-thioguanine to be 100 μm, an approximation of the relative velocity in whole cells can be made from the relationship for a competitive, reversible inhibitor:

\[
\frac{V_i}{V_o} = \frac{1 + \frac{K_m}{S}}{1 + \frac{K_m}{S}(1 + I/K_I)} = \frac{1 + 1}{1 + 1 + 1.7} = 0.54
\]

where \(V_i/V_o\) is inhibited velocity (\(V_i\)) relative to uninhibited velocity (\(V_o\)); \(I\) is 6-thioguanine level of 100 μm; \(S\) is GMP level of 10 μm; \(K_I\) is 60 μm (23); and \(K_m\) is 10 μm (23). Although enzyme concentration is ignored, this equation may be valid since \(I\) and \(S\) are relatively large. The 46% inhibition is below that necessary to make this step rate limiting. Furthermore, the GMP level measured in Sarcoma 180 cells is about 50 μm (unpublished data), which would reduce the inhibition further. To obtain 95% inhibition would require 6-thioguanine levels in excess of 1 mm, a situation that does not occur at usual pharmacological doses. The apparent failure (Table 2) of 6-thioguanine to act as a product inhibitor of HGPRTase (12) in whole cells is not so readily explained.

The protection of H.Ep. 2 cells from toxicity of 6-MP by 1 mm thymidine (Table 4) supports the hypothesis that 6-MP is toxic due to its conversion to 6-TG nucleotides and subsequent incorporation into cellular DNA (40). The protection of cells against 8-aza-G toxicity by inhibition of RNA synthesis (Table 5) but not by inhibition of DNA synthesis (Table 4) is in agreement with the hypothesis that 8-aza-G acts due to its incorporation into RNA (18, 20).

Although these findings emphasize the importance of incorporation of these purine analogs into nucleic acids as mechanisms of action against H.Ep. 2 cells in culture, it would seem conceivable that in some cells the toxicity may be found to be due to effects on purine-biosynthetic pathways. This is especially true of 6-MP since AIC clearly protects against 6-MP toxicity (Ref. 7; Table 1), and 6-MP is known to reduce purine pools in Sarcoma 180 cells (35).

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