The Effect of 4-Amino-5-imidazolecarboxamide on the Toxicity of 8-Azaguanine*

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The guanine analog, 8-azaguanine, has been shown to possess carcinostatic properties against transplantable tumors in experimental animals (7, 8). It was believed that the compound inhibited the proliferation of tumor cells by acting as an antimetabolite in the biosynthesis of nucleic acid purines of the neoplastic tissue. To elucidate the mechanism of action of the drug, the possibility of an interference with the incorporation of nucleic acid purine precursors was examined. The direct incorporation of guanine-C\(^14\) and radiocarbon from 4-amino-5-imidazolecarboxamide-C\(^14\) into nucleic acids isolated from liver and tumor tissues of mice bearing Sarcoma 37 was investigated, and the effect of the administration of 8-azaguanine simultaneously with either of the precursors was studied (1). During this study it was observed that relatively large doses of 8-azaguanine could be administered with guanine to tumor-bearing mice without apparent toxic effects. However, serious toxic symptoms were observed when 8-azaguanine and 4-amino-5-imidazolecarboxamide were administered simultaneously in doses which separately produced no toxic effects. At the same time the growth of the tumor was noticeably retarded when sublethal combinations of 8-azaguanine and 4-amino-5-imidazolecarboxamide were injected. This observation was considered to be of practical value if the carcinostatic action of 8-azaguanine had been enhanced by the use of such a drug combination.

To examine further the potentiating effect of the drug combination, a study of the subacute toxicity of the drugs in normal CAF\(_1\) mice was made. The LD\(_{50}\)'s of 8-azaguanine and of 4-amino-5-imidazolecarboxamide were estimated individually, and the toxicity of various combinations of doses of the two compounds was investigated.

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It had been shown earlier that in the mouse 8-azaguanine was hydrolytically deaminated to 8-azaxanthine (9). Hirschberg et al. (6) had found an inverse relationship between the carcinostatic action of 8-azaguanine and the concentration of azaguanine deaminase in most of the tumors examined. Since 8-azaxanthine was noncarcinostatic and relatively nontoxic, an inhibition of the biochemical processes responsible for the metabolism of 8-azaguanine might then potentiate the toxic and carcinostatic effects of the latter drug. The possible effect of 4-amino-5-imidazolecarboxamide on the deamination of 8-azaguanine by an 8-azaguanine deaminase preparation was therefore studied. Preliminary results of these investigations have been presented (2).

METHODS

TOXICITY STUDY

Materials.—4-Amino-5-imidazolecarboxamide hydrochloride was prepared as reported previously (3). An aqueous solution of this compound neutralized with sodium carbonate was used for administration.

8-Azaguanine was synthesized as described earlier (9) and was dissolved in 0.5 per cent sodium carbonate solution prior to injection.

Administration.—Each drug was injected intraperitoneally into CAF\(_1\) mice at 12-hour intervals for a total of five injections. This protocol was similar to that used in previous experiments with 8-azaguanine (1, 10). A rough estimate of the dose range to be used was determined, and the experiment was then run on groups of seven mice for each dose selected.

For drug combinations, the dosage of one of the drugs was kept constant, while that of the other was changed. The dosages, expressed in mg/kg of body weight/injection are shown in Table 1.

ENZYME INHIBITION STUDY

The extent of deamination of 8-azaguanine was determined by measuring the amount of ammonia produced in the presence of liver homogenate.

Preparation of enzyme systems.—An 8-azagua-
nine deaminase preparation similar to that described by Hirschberg et al. (6) was used. A rat was anesthetized with chloroform, and the liver was removed and immediately was cut into small pieces. The tissue was rinsed with ice cold isotonic saline, and was weighed. It was then homogenized in ice cold 0.05 M phosphate buffer at pH 7.3 for 4 minutes in a micro-Waring Blendor kept at -15°C. The final preparation contained 15 per cent of liver tissue (wet weight).

### Table 1

**Mortality Produced in Mice after a Series of Five Injections at Varying Dosages of 8-Azaguanine and 4-Amino-5-Imidazolecarboxamide**

<table>
<thead>
<tr>
<th>8-Azaguanine (mg/kg/injection)</th>
<th>4-Amino-5-imidazolecarboxamide (mg/kg/injection)</th>
<th>Deaths, total</th>
<th>Per cent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0</td>
<td>0/7</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>2/7</td>
<td>29</td>
</tr>
<tr>
<td>145</td>
<td>0</td>
<td>4/7</td>
<td>57</td>
</tr>
<tr>
<td>220</td>
<td>0</td>
<td>7/7</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>2</td>
<td>2/7</td>
<td>29</td>
</tr>
<tr>
<td>&quot;</td>
<td>4</td>
<td>4/7</td>
<td>57</td>
</tr>
<tr>
<td>&quot;</td>
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<td>7/7</td>
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<td>4/7</td>
<td>57</td>
</tr>
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<td>7/7</td>
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</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0/7</td>
<td>0</td>
</tr>
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<td>14</td>
</tr>
<tr>
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<td>515</td>
<td>5/7</td>
<td>71</td>
</tr>
<tr>
<td>0</td>
<td>700</td>
<td>7/7</td>
<td>100</td>
</tr>
</tbody>
</table>

Procedure for the determination of 8-azaguanine deaminase activity.—One ml. of liver homogenate was introduced into each of a series of side arm test tubes. Into one tube 1 ml. of water was then added to serve as blank to measure the ammonia normally produced by the homogenate. Into all other tubes 0.5 ml. of an 8-azaguanine solution (10 mg/ml in 0.05 M sodium carbonate) was added to serve as substrate. One of these tubes containing the substrate received 0.5 ml. of water to serve as control. To all the remaining tubes varying amounts of a 4-amino-5-imidazolecarboxamide solution (2 mg/ml) were added, and the final volume of the preparation was made up to 2 ml. with distilled water. The tubes then were incubated in a water bath at 37°C. for 30 minutes, when the reaction was stopped by the addition of 3 drops of 8 M H2SO4. Another experiment was carried out similarly with a 60-minute incubation period. One drop of n-octyl alcohol was added to each tube to prevent excessive foaming in the subsequent treatment. The ammonia produced was determined by the micro-volumetric method of Sobel et al. (13), by titrating the amount of ammonia liberated against dilute sulfuric acid. The results were expressed in micrograms of ammonia evolved. The experiments were run in duplicate, and averages from simultaneous runs are presented in Chart 1.

**RESULTS**

Toxicity study.—It is evident from Table 1 that a pronounced potentiation in toxicity was observed when 8-azaguanine and 4-amino-5-imidazolecarboxamide were administered simultaneously in doses which separately were nontoxic. When injected individually as five doses at 12-hour intervals, the LD50 of 8-azaguanine was of the order of 120 mg/kg of body weight/injection; that of 4-amino-5-imidazolecarboxamide was 450 mg/kg. When administered simultaneously, however, doses of 75 mg/kg of 8-azaguanine with as little as 8 mg/kg of 4-amino-5-imidazolecarboxamide produced 100 per cent mortality.

The toxic symptoms observed after the administration of the combination of the two drugs were similar to those observed after the administration of large doses (250 mg/kg) of 8-azaguanine alone. These symptoms consisted first of tremors, anorexia, and a marked drop in body temperature, followed later by complete muscular relaxation, prostration, and death.
In a corresponding experiment on mice of DBA strain a similar potentiation of the toxicity of 8-azaguanine in the presence of 4-amino-5-imidazolecarboxamide was observed.

Enzyme inhibition study.—In the in vitro study consisting of a liver homogenate system, it has been shown clearly that the deamination of 8-azaguanine was inhibited by 4-amino-5-imidazolecarboxamide. This inhibition was produced by concentrations of 4-amino-5-imidazolecarboxamide as low as 0.05 mg. in 2 ml. of the reaction mixture. The homogenate alone in the absence of 8-azaguanine produced 4 and 8 μg. of ammonia in the 30- and 60-minute incubation experiments, respectively. All values therefore were corrected for the corresponding blanks. The results are given in Chart 1.

DISCUSSION

An examination of the values reported in Table 1 indicated that the potentiation of the toxic effects was far more pronounced when small doses of 4-amino-5-imidazolecarboxamide were given with large doses of 8-azaguanine than when the relative doses were reversed. The small dosage of 4-amino-5-imidazolecarboxamide compared to that of 8-azaguanine which was capable of producing a decisive potentiation of toxicity and the similarity of the toxic symptoms observed after the administration of high doses of 8-azaguanine alone suggested that the observed toxicity was probably due to 8-azaguanine. The 4-amino-5-imidazolecarboxamide only modified the toxicity in a quantitative manner.

The inhibition produced by small concentrations of 4-amino-5-imidazolecarboxamide in the in vitro 8-azaguanine deaminase system was additional evidence to support this hypothesis. It was therefore concluded that the toxicity potentiation produced by the drug combination was probably due to an inhibition of the system responsible in vivo for the deamination of the relatively toxic 8-azaguanine to the nontoxic 8-aza-xanthine.

Experiments are now in progress to determine whether a correlation exists between the potentiation of the carcinostatic effect as well as the toxicity during the simultaneous administration of 4-amino-5-imidazolecarboxamide and 8-azaguanine. Preliminary evidence on these experiments (11) has indicated that the survival time of tumor-bearing mice is increased.

The in vitro inhibition of guanase by xanthopterin, 6-hydroxymethylpteridine, and 6-formylpteridine has been reported by Dietrich and Shapiro (4). The potentiation produced by 6-formylpteridine of the effect of 8-azaguanine (12) probably acted by the same mechanism as that reported in the present investigations with 4-amino-5-imidazolecarboxamide. The potentiation of the effects of 8-azaguanine produced by the prior administration of 6-chloro-9-(1'-p-sorbityl)-isalloxazine (flavotin) has been postulated to be due to inhibition of tumor xanthine oxidase (3). The resulting inhibition of guanase caused by the accumulation of xanthine would then give rise to the potentiating effect.

SUMMARY

1. The simultaneous administration to mice of small doses of 4-amino-5-imidazolecarboxamide and 8-azaguanine produced a pronounced potentiation of toxicity.

2. The symptoms of toxicity resembled those produced by large doses of 8-azaguanine.

3. In in vitro experiments using a rat liver homogenate, the deamination of 8-azaguanine to the relatively nontoxic 8-aza-xanthine was inhibited in the presence of small amounts of 4-amino-5-imidazolecarboxamide.

4. It appeared probable that 4-amino-5-imidazolecarboxamide interfered with the in vivo detoxication of 8-azaguanine by deaminase, thus giving rise to a potentiation in toxicity.

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