Clinical Investigations of 6-Azathymine

A Thymine Analog*

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SUMMARY

Twenty-four patients received azathymine. All fourteen children and two of the adults had acute leukemia; the other adults had a variety of solid tumors. All of the children had previously responded to Methotrexate and had then become resistant to it. Azathymine did not produce any improvement in any patient.

The hepatotoxic effect of azathymine was striking in the adults and occurred in the absence of objective regression of the neoplastic disease. Liver damage also occurred in one child.

Azathymine exerts a specific renal effect, causing hyperuricemia associated with a decrease in the excretion of uric acid. This occurs early in the course of therapy and with doses that do not cause liver function changes.

In the search for agents which might prove useful in the chemotherapy of neoplastic disease, attention has been focused on the processes by which components of nucleic acids are synthesized. Attempts have been made both to inhibit the de novo biosyntheses of ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) and to interfere with the utilization for nucleic acid synthesis of preformed purine precursors. It is hoped that analogs of naturally occurring purines, pyrimidines, or precursors of either will preferentially inhibit the anabolic processes in the neoplastic cells.

Among the compounds studied in humans are several types of folic acid antagonists; i.e., the 4-amino-substituted derivatives of folic acid (aminopterin and Methotrexate) (17, 32), dianinodi chlorophenyl pyrimidines (30), and dihydrotriazines (28). These substances are thought to interfere with de novo synthesis of the purine bases of DNA and RNA and of the thymidine portion of DNA. The purine analogs tested clinically include 2,6-diaminopurine (6), 8-azaguanine (2, 9, 10, 20), 6-mercaptopurine (7), thioguanine (31), 6-chloropurine (15), and thioguanosine (25). These apparently inhibit incorporation into nucleic acid of preformed purine bases. Among the pyrimidine analogs studied in man are 5-bromouracil,1 azauracil (19), a group of fluorinated pyrimidines including 5-fluorouracil (12, 13, 40) and 5-fluorodeoxyuridine (1, 29), and azathymine (16). Of this group, azathymine is the only one that has not produced any antitumor or antileukemic effects in animals2 and has not affected the development of the chick embryo.4

6-Azathymine (6-methyl-asym-triazine-3,5-(2,4)-dione) (Chart 1) was shown by Prusoff, Holmes, and Welch (35) to inhibit competitively the growth of S. faecalis and L. casei when these organisms are grown in media supplemented by thymine or thymidine. The bacteriologic findings suggested a functional relationship of azathymine and the 4-amino analogs of folic acid as well as an effect of azathymine on pyrimidine metabolism. Therefore, a study of the effects of azathymine in neoplastic disease in man was undertaken (16).

1 J. H. Burchenal, M. L. Murphy, and C. T. Tan, unpublished observations.
2 J. H. Burchenal, unpublished observations.
3 D. A. Clarke, unpublished observations.
4 D. A. Karnofsky, unpublished observations.

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MATERIALS AND METHODS

In the initial trials, azathymine was administered to five adults and fourteen children. All of the children and two of the adults had acute leukemia resistant to other forms of therapy, including the folic acid antagonists. The other three adults had carcinoma of the ovary, carcinoma of the breast and rectum, and melanoma, all with metastases. The drug was given orally, and all tolerated it well initially. The three non-leukemic adults and seven of the children received the azathymine alone. The rest received it in combination with other antimetabolites such as 6-mercaptopurine, Methotrexate, Daraprim, and azaserine.

The amounts of azathymine received by the various groups of patients and the duration of the periods of therapy are shown in Table 1. The daily dose ranged from 2.5 to 5.0 gm. in the adults and 0.5 to 10.0 gm. in the children. The largest daily dose of 10 gm. was given to a child weighing 45 kg. He received a total of 3.3 gm/kg over a 27-day period. Another child had 6.7 gm/kg in 87 days while also receiving Methotrexate. All but one of the adults had 5 gm. daily.

Urine uric acid was measured by a modification of the method of Archibald (18) employing the enzyme, uricase. This method has been described in detail elsewhere (24). Serum uric acid was measured by the same method, except that only occasional specimens were subjected to incubation with uricase. Urine total nitrogen was measured by acid digestion and direct Nesslerization (23). Urine urea nitrogen was measured by incubation with urease and direct Nesslerization.

RESULTS

No clinical or hematologic improvement was noted in any of the patients treated.

TOXICITY

Symptomatic.—Subjectively, no ill effects occurred in any of the children, whereas two of the adults developed anorexia and nausea while receiving 5 gm. of azathymine daily. These symp-

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
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<tbody>
<tr>
<td><strong>THE DOSE OF AZATHYMINE AND THE DURATION OF TREATMENT</strong></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>CHILDREN</th>
<th>Treated with azathymine alone</th>
<th>Treated with azathymine and other antimetabolites</th>
<th>TOTAL NO. PATIENTS</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td>No. courses of therapy:</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Duration of therapy:</td>
<td>1-27</td>
<td>8-22</td>
<td>8-14</td>
<td></td>
</tr>
<tr>
<td>Range—days</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No. treated</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7-13 days</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
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<tr>
<td>&lt;7 days</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&lt;2 wks. or more</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total dose:</td>
<td>.08-.3</td>
<td>.07-.67</td>
<td>.10-.16</td>
<td></td>
</tr>
<tr>
<td>Range—gm/kg</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>No. received:</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>&gt;1 gm/kg</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>0.5-1 gm/kg</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>0.1-0.5 gm/kg</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>&lt;0.1 gm/kg</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Average daily dose</td>
<td>35-122</td>
<td>23-181</td>
<td>65-125</td>
<td></td>
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</table>

* One patient received two courses of azathymine, lasting 8 and 9 days, in a 37-day period.
toms slightly preceded laboratory evidence of liver damage. A third adult, a 58-year-old woman with papillary and solid carcinoma of the ovary with abdominal metastases, became stuporous on two occasions after receiving each time a total of 40 gm. of azathymine, 1.0 gm/kg in 8 days. There was no change in the status of her primary disease or in hepatic function studies on these occasions. The mental depression cleared within 2–3 days after the drug was stopped.

Hepatic.—Clinical as well as laboratory evidence of disturbed liver function occurred during treatment in five patients, four of the five adults but only one of the fourteen children. The fifth adult was the one noted above to have become stuporous, necessitating interruption of therapy twice. Only five of the eight children who received total doses of at least 0.5 gm/kg had adequate checks of liver functions throughout the period of treatment. A fifth child had normal liver function tests during the first course of therapy (6.7 gm/kg) but was not checked during the second course (1.4 gm/kg).

The earliest chemical change noted was elevation of the prothrombin time with progressive elevation continuing for as long as 5 days after the last dose of azathymine. Studies performed on one patient at a time when the prothrombin time of his undiluted plasma was 47.5 seconds (control, 38.6 seconds), a normal accelerator globulin, and a proconvertin level 35 per cent of normal. The "true" prothrombin concentration was 20 per cent of normal. The recalcification concentration was normal.

The prothrombin time reverted to normal within several days in all but one adult with acute leukemia who had a continued rise, to almost 2 minutes, despite the administration of vitamin K and blood. In two of the four cases the return of the prothrombin time to normal followed the administration of vitamin K. In four cases the prothrombin time elevation was followed by the development of jaundice, and other liver function tests became abnormal in three of the adults.

Charts 2, 3, and 4 show the course of three patients who developed impairment of liver function. The patient depicted in Chart 2 received a total of 60 gm., 1.4 gm/kg, of azathymine during a 5-week period. At the end of this time the prothrombin time, cephalin flocculation, thymol turbidity, and bromsulfalein retention became elevated, and there was a fall in the total cholesterol level with a marked rise in the percentage of free cholesterol.

Chart 3 shows the findings in a 4-year-old boy with acute leukemia who was treated with azathymine and Daraprim. This combination was
used because of the synergistic action noted by Elion and Hitchings (14) for these two drugs in *L. casei*. He had a fall in the leukocyte level from 7,000 to 700/cu mm after 3 weeks of this treatment. At the end of 6 weeks, after receiving 40 gm., 2.3 gm/kg, of azathymine, there was elevation of the prothrombin time to 26 seconds associated with icterus and cephalin flocculation abnormality. The prothrombin time returned to normal after he was given vitamin K intravenously for 2 days. The icterus cleared in several days.

A third patient (Chart 4), a 53-year-old man with melanoma who received a total dose of 90 gm., 1.6 gm/kg, of azathymine in a 19-day period, developed marked changes in all the liver function tests studied. These reverted to pretreatment levels within a month after the last dose of the drug.

**Hematopoietic.**—There was little hematologic effect in the leukemic patients studied. Two of the adults, however, showed marked decrease in hemoglobin with slight thrombocytopenia, 1–2 weeks after the last dose of azathymine. The platelet decrease was very brief in duration, but the hemoglobin required several months to return to normal.

In one of these two adults the marrow showed changes in the erythroid elements to a picture re-

### TABLE 2

**EFFECT OF AZATHYMINE ON URIC ACID EXCRETION**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>CONTROL PERIOD*</th>
<th>TREATMENT PERIOD</th>
<th>TREATMENT PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum uric acid, mg. % (mean)</td>
<td>Uric acid, mg/day (mean)</td>
<td>Blood urea nitrogen, mg. % (mean)</td>
</tr>
<tr>
<td>1</td>
<td>5.1</td>
<td>12.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>7.9</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>7.6</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>3.7</td>
<td>7.3</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>3.7</td>
<td>8.7</td>
<td>15.0†</td>
</tr>
<tr>
<td>6</td>
<td>3.7</td>
<td>7.5</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>3.7</td>
<td>6.5</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>3.7</td>
<td>5.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* These were periods identical in duration with and, in most cases, immediately preceding the treatment periods.
† Plus thymine, 5.0 gm/day.
‡ Thymine alone, 5.0 gm/day.
sembling megaloblasts. In the other, during treatment with azathymine, there was a marked decrease in the percentage of nucleated red blood cells in the marrow. After treatment there was a temporary increase of these cells above normal levels with no morphologic change.

Renal.—All of the five adults treated initially with azathymine were found to have elevated serum uric acid levels during the course of treatment. The data are difficult to evaluate in two of these patients (both of whom had acute leukemia), since one had no pretreatment determination of uric acid and the other had a rise in the blood urea nitrogen later to 68 mg. per cent. The serum uric acid was not determined regularly in most of the children. In one, there was a transient rise in the uric acid level from 1.4 to 7.1 mg. per cent (without blood urea nitrogen elevation) at the time of hepatotoxicity. Four others showed elevations of uric acid levels to 6.5–11.6 mg. per cent. The uric acid level reverted to normal in all patients within a week after the last dose of azathymine. Uric acid excretion was studied in five additional patients (Table 2) to determine the mechanism of the hyperuricemia. Urine uric acid was found to fall markedly in each instance while the serum uric acid level was rising. The change in urate excretion was noted as early as 24 hours after the first dose of azathymine. Chart 5 illustrates an example of the influence of azathymine on uric acid excretion. The inhibition of urate excretion occurred without significant change in blood urea nitrogen. Urine urea nitrogen and urine total nitrogen were measured in two patients, and no changes were observed. In one patient simultaneous determinations of urea and urate excretion and phenolsulfonphalein excretion were made during a control period and repeated during chronic azathymine administration. Of these parameters, urate excretion was the only one affected. The simultaneous administration of equal doses of azathymine and thymine to one patient resulted in inhibition of uric acid excretion similar to that which occurred when azathymine was given alone. (Hepatotoxicity was not noted in this group of patients, but all were treated for periods of less than a week and received less than 0.5 gm. of azathymine/kg.)

**Reversal Experiments**

In view of the competitive antagonism of thymine and azathymine in some bacteria, azathymine administration was repeated cautiously in one patient (Chart 4) with the simultaneous administration of an equal amount of thymine orally (5 gm. daily). The drugs were both discontinued as soon as there were signs of liver damage (when a total dose of 70 gm. of each had been given), so that the marked hepatotoxicity which was noted following 90 gm. of azathymine alone was not repeated. There was, however, a definite rise in the prothrombin time to 21 seconds, a decrease in the plasma albumin, a decrease in the total cholesterol, a rise in the cephalin flocculation, and a slight rise in bromsulfalein retention. The hemoglobin level, which had begun to rise, again fell, as did the percentage of nucleated red cells in the marrow. As noted in Table 2, elevation of the serum uric acid level again occurred. During the period of biochemical abnormalities, the patient experienced anorexia and nausea. When both compounds were discontinued, the patient was seen to improve subjectively first, and, following this, all chemical values reverted to pretreatment levels. During the period that the serum albumin was low the patient suddenly gained 17 pounds in weight and developed ankle edema and ascites. There was a rapid diuresis as soon as the plasma albumin rose.

No clinical or biochemical change followed subsequent administration of 90 gm. of thymine alone orally to this same patient over a 21-day period.

**DISCUSSION**

No improvement was noted in any patient treated with azathymine. The leukemic patients were all known to be resistant to Methotrexate, and the three patients with solid tumors would not have been expected to respond to folic acid antagonists. In order to check further on the activity of azathymine, it should be tested in patients expected to be sensitive to Methotrexate. However,
the hepatotoxicity observed in the adults as well as the rapid development of hyperuricemia precluded further clinical trials of azathymidine. The simultaneous administration of thymine did not prevent these toxic effects of azathymine. The mechanism of the development of liver damage is not known. The liver function test abnormalities suggest hepatocellular damage rather than intrahepatic obstruction by bile stasis. The rapid clearing of the abnormalities when drug administration was stopped indicates that this was not activation of a latent virus. There is no histologic material presently available to clarify this point.

The finding of hyperuricemia is due to a specific renal effect of azathymine on the excretion of uric acid. This was shown by the concomitant decrease in the total urinary excretion of uric acid in the absence of any change in the urinary volume or the blood urea nitrogen levels. Several compounds have been found to influence the urinary excretion of uric acid. Among these are probenecid (39), phenylbutazone (43), and large doses of salicylates (4), which augment urate excretion, and pyrazinamide (11), chlorothiazide (26), and small doses of salicylates (42), which inhibit excretion. Azathymine appears to belong to the latter group of compounds which selectively interfere with the excretion of uric acid.

Acute and chronic toxicity studies in mice and rats (27) indicated the lethal action of the drug to be associated entirely with the acute effects, i.e., production of paralysis and death. The dogs receiving 20 mg/kg/day for a total of ten doses. The dogs receiving this amount, however, became anorectic and very weak and developed hind-quarter weakness. These abnormalities disappeared when the drug was stopped. There were no hematopoietic effects in the dogs other than some reticulocytopenia. The only neurological abnormality noted in the patients was stupor, which developed twice in one woman.

Microbiologic studies with azathymine (35) indicated that this compound competitively antagonized the utilization of exogenous thymine or thymidine, thus inhibiting the growth of S. faecalis, L. casei, and L. leichmanii. The drug interrupted the utilization of the endogenously formed metabolites as well, as demonstrated by the inhibition of the growth of these organisms by azathymine when they are grown in media containing folic acid or vitamin B12, but no thymine or thymidine. The inhibitory effect of azathymine on these organisms seems to depend on the enzymatic formation first of azathymidine (33, 34, 37, 41). Azathymidine is a much more potent inhibitor of the growth of these microorganisms and, unlike azathymine, produces the same degree of inhibition with either thymine or thymidine present. The azathymidine is inhibitory not only when it is present in the medium initially but also when it is added during the rapid log phase of growth of the organism (37, 41). This inhibition can be prevented by simultaneous addition of excess thymidine. If the thymidine is added after the rapidly reproducing cells are exposed to azathymidine for 1–2 hours, however, the inhibition cannot be reversed unless the inhibited cells are removed from the azathymidine medium.

Although in S. faecalis azathymine-C14 is incorporated into DNA to the extent of 12–18 per cent of the cell complement of thymine (24), the inhibition is not considered to be due to such an irreversible reaction. It is thought that the inhibitory effect may be exerted on a reversible system; i.e., inhibition of formation of a precursor of DNA or of a hypothetical coenzyme which contains thymidine or which requires thymidine for its production.

Studies in two mammalian in vitro systems, rabbit bone marrow and mouse Ehrlich ascites tumor (21, 36), agreed with the microbiological findings in that azathymidine but not azathymine markedly depressed the uptake of C14 formate during in vitro incubation. (Under the conditions used, the uptake of formate-C14 is primarily into the methyl group of thymine, relatively little isotope appearing in the purines.)

Some of the findings with azathymine are analogous to those observed with other pyrimidine analogs. Evidence suggests that the active form of azathymine is the deoxyribotide, and similar evidence exists about the need for enzymatic transdeoxyribosidation of 5-bromouracil (3) and the 5-fluorinated pyrimidines (5). In addition, the incorporation of these various pyrimidine analogs into the cell nucleic acid in vitro does not seem to be the only mechanism by which some of these antimetabolites affect growth. Thus, azauridine or azauridylic acid acts by inhibiting the enzyme orotidylidic decarboxylase (22), thus inhibiting formation of uridine-5'-phosphate, and 5-fluoro-2'-deoxyuridine (after phosphorylation) prevents the methylation of deoxyuridine-5'-phosphate (5).

Although the active form of azathymine appears to be the deoxyribotide, the clinical trials were performed with azathymine. It is possible but does not seem likely that the renal and hepatotoxicity...
observed were related to the presence of the free base and would not occur with the deoxyriboside. These abnormalities were limiting factors in the administration of the drug and occurred without any evidence of therapeutic effect, suggesting that no further benefit would be obtained by the use of azathymidine alone. (It would also prove difficult to produce enough of this deoxyriboside to be clinically useful.) There is evidence, however, of synergism with other antimetabolites in microbiologic systems (14) and of potentiation of the effects of fluorinated pyrimidines in mouse leukemia (8), suggesting that such combination therapy is the next step to be tried clinically.

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