Observations on the Action of Azaserine in Mammalian Tissues*

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Azaserine has been shown to be an inhibitor of de novo purine synthesis (4, 8). In studies with mice bearing ascites cell tumors, inhibition of purine synthesis was shown to correlate with increased survival time of the hosts (7). It was further demonstrated that the significant effect of the drug on the tumor cells involved a reaction between azaserine and a constituent of the cells (3, 7). This reaction was irreversible in that the drug could not be washed away and that a single dose inhibited de novo purine synthesis for approximately 12 hours. Reaction between the drug and the cells took only 8-10 minutes of direct contact. This type of study has now been extended to experiments with solid tumors growing subcutaneously.

This drug is unable to completely inhibit tumor growth because it blocks only one of two pathways of purine nucleotide synthesis, both of which are available to all experimental neoplasms that we have tested (6). The incorporation of preformed adenine into nucleic acids was not inhibited by azaserine (2). Treated tumor cells can, therefore, continue to synthesize nucleic acids, which are essential for their further growth and multiplication. Azaserine also produces deleterious side effects in humans, which complicate its use as a chemotherapeutic agent (1). In view of the nature of action of this drug, which appears to be a "titration" of an enzyme in the tumor cells, the side effects might be due to a similar titration of a constituent of normal cells. With a drug having this mode of action, it would be important that it be present for only sufficient time to titrate the desired tissue. Its continued presence could be expected to result in toxicity to other tissues. In consideration of these points, it seemed to be of interest to determine how long azaserine remains in the blood after a single intravenous dose. We were unable to find any published analyses of blood for this drug. The in vitro ascites cell system in which de novo purine synthesis is measured by determining the extent of glycine-2-C^14 incorporation (3, 5) appeared to lend itself to the assay of azaserine in blood sera. This system is sensitive to as little as 0.01 µg. of azaserine.

METHODS AND RESULTS

Azaserine was assayed by measuring the inhibition of glycine-2-C^14 incorporation into ascites cell purines. Female Swiss mice were each given an intraperitoneal injection of 5 x 10^6 Ehrlich carcinoma ascites cells and were used as a source of tumor cells at 6-7 days. The cells were withdrawn under light ether anesthesia with a #18 needle and hypodermic syringe, or by pipette after laparotomy. These cells were washed four times by centrifugation and resuspension in isotonic saline (6-7 vols.) to remove endogenous glycine. The assays were conducted in Warburg respirometer vessels of 60-ml. capacity with 30 mg. dry weight of cells in Robinson’s medium plus glucose in a total volume of 12 ml., to which had been added 150 µg. of glycine-2-C^14. A standard curve was established with the use of known quantities of azaserine in the system, and it is illustrated by data given in Table 1. In each assay

<table>
<thead>
<tr>
<th>µg. Azaserine</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>97</td>
</tr>
<tr>
<td>0.1</td>
<td>84</td>
</tr>
<tr>
<td>0.05</td>
<td>71</td>
</tr>
<tr>
<td>0.02</td>
<td>55</td>
</tr>
</tbody>
</table>

Various amounts of azaserine were added to 0.1 ml. normal serum, and its effect upon the incorporation of glycine-2-C^14 in Ehrlich ascites cells was determined in vitro.

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standards containing known amounts of azaserine in control sera were included. From such standards the azaserine content of the test sera could be calculated. Assays were repeated whenever standards and unknowns differed widely from the standard curve. Control flasks also contained serum from untreated mice, as the presence of serum had a definite effect on the system. This is shown in Table 2. Incubation was for 60 minutes. The cells were extracted with 0.2 M perchloric acid (PCA) to obtain the acid-soluble fraction. Acid-soluble purine nucleotides were hydrolyzed at 120°C in an autoclave for 45 minutes with 0.2 M PCA. The purines were chromatographed on Dowex-50 columns, then on paper, and the radiocarbon was counted directly with discs cut from the paper chromatograms. The paper discs were then eluted and their purine content determined spectrophotometrically. Further details of these methods have been described elsewhere (5).

The presence of azaserine in the blood of mice and of humans after injection was studied. Two relatively high doses were used in mice, 20 mg/kg and 6 mg/kg. The drug was injected intravenously (by tail vein) into normal female Swiss mice of 25–28 gm. weight. Groups of three mice were exsanguinated at 5, 10, 20, 30, 60, and 120 minutes after injection, their bloods pooled, and the serum separated. Azaserine assays were conducted as described earlier.

Blood was also obtained from two human patients, who were females diagnosed as having cancer but without liver dysfunction or other organic disorder. One received a dose of 1.25 mg/kg, the other 0.4 mg/kg, and blood samples were taken by venipuncture at 10, 20, 30, 60, and 120 minutes. The following day the dose schedule was reversed and blood samples taken as before from the same patients. Azaserine assays were conducted as above on the sera collected. The results of one such assay are given in Table 3.

The results of these experiments are shown in Chart 1. It can be seen that in mice the drug was removed from the blood very rapidly. On the 20 mg/kg dose, only 1 per cent of the injected drug remained after 5 minutes, and it was not detectable after 120 minutes. On the lower dose (6 mg/kg) the results are similar, but no azaserine could be detected in the blood after only 60 minutes.

The results in humans were quite different. At a dose of 1.25 mg/kg, appreciable amounts of azaserine remained in the blood, even at 120 minutes. Although considerable variation in response to this dose existed between the two patients, it is apparent that the drug was removed from the blood much more slowly than in mice. At the 0.4 mg/kg dose, the removal was more rapid, but a small amount remained at 120 minutes. It is interesting to note that, at each dose level, the curve representing a less rapid removal of azaserine from the blood was from the patient who had received the drug 24 hours previously. However, since only two patients were studied, no conclusions can be drawn from this observation.

Previous work in this laboratory has shown that an intraperitoneal injection of 0.2 mg/kg of azaserine causes 90 per cent inhibition of glycine-2-C\textsuperscript{14} incorporation into Ehrlich ascites cells in vivo and has also shown that the inhibition remains at about this level for 12 hours, declining thereafter (7). The present work reports similar studies on solid Ehrlich carcinomas. Intraperitoneal injections into ascites tumors are essentially intratumoral and largely eliminate circulatory and excretory features from consideration. Therefore it is to be expected that larger doses will be required to affect solid tumors. The above work with blood levels shows the very rapid elimination of azaserine from the circulation.

Swiss mice were injected subcutaneously, each at two sites, with 5 × 10\textsuperscript{6} Ehrlich carcinoma...
ascites cells. In 6 days, when the two tumors on each mouse weighed a total of 200–500 mg., these animals were injected with various doses of azaserine and 15 minutes later with 250 µg. of glycine-2-C\textsuperscript{14} per mouse. One hour following the administration of the glycine they were killed by decapitation, the tumors removed and broken up in 10 volumes of 0.2 M PCA in a homogenizer. Acid-soluble and nucleic acid purines were isolated and treated as described earlier (5). The results are shown in Table 4. In order to get inhibition approaching 90–100 per cent, it was necessary to use a dose of 6 mg/kg.

**Table 4**

| INHIBITION OF GLYCYNE-2-C\textsuperscript{14} INCORPORATION INTO SOLID EHRICH CARCINOMAS IN VIVO BY AZASERINE |
|---|---|---|
| **Dose** (mg/kg) | **Nucleic acid** | **Adenine** (per cent) | **Guanine** (per cent) |
| 0.4 | 69 | 55 |
| 2.0 | 76 | 82 |
| 4.0 | 80 | 92 |
| 6.0 | 92 | 94 |

To investigate the duration of action in solid tumors, groups of mice with 6–7-day old subcutaneous tumors were injected with azaserine at 6 mg/kg body weight, the amount shown above to cause 90 per cent inhibition of glycine-2-C\textsuperscript{14} incorporation. At 6, 10, 12, 16, and 24 hours, a 250 µg. dose of glycine-2-C\textsuperscript{14} was injected intraperitoneally into each mouse. One hour later the animals were decapitated, the tumors removed, broken up in 0.2 M PCA, and the specific activities of the acid-soluble and nucleic acid purines determined. The results are shown in Chart 2. This demonstrates that inhibition remains at a high level for 10–12 hours, although the drug was present in the circulation for only a small fraction of this time.

To determine whether the above biochemical effect of the drug on solid tumors correlated with growth of solid tumors, tumors were implanted as above and on the 2d day were divided into two groups of six mice each. One group was injected with azaserine at 6 mg/kg at 12-hour intervals; the other group received saline. The drug was administered for 7 days, and on the 8th the mice were decapitated, the tumors removed as quantitatively as possible, and weighed. Both the Ehrlich carcinoma and Sarcoma 180 were used in this manner. The results for two such experiments are given in Table 5. This clearly demonstrates that inhibition of solid tumor growth correlates with the biochemical effect of inhibition of glycine incorporation.

**DISCUSSION**

The present study supports the concept that the biological effect of inhibition of solid tumor growth by azaserine is correlated with the biochemical inhibition of de novo purine biosynthesis by this drug. Evidence for this correlation has been obtained previously for ascites cell tumors (2, 3).

While the present data do not prove the point conclusively, it can at least be inferred that the side effects observed in humans treated with azaserine are due to the slow rate of its elimination from the blood. This is to be contrasted with the very rapid removal of the drug from the blood of mice, in which such side effects are not seen.
SUMMARY

Correlation between the biological effect of azaserine in inhibiting growth of solid tumor implants and its biochemical inhibition of de novo purine synthesis has been obtained.

CHART 2.—Duration of the inhibition of de novo purine synthesis in solid Ehrlich carcinoma implants after a single 6 mg/kg dose of azaserine.

TABLE 5
INHIBITION OF SOLID TUMOR GROWTH BY AZASERINE
(6 mg/kg every 12 hours for 7 days)

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich</td>
<td>994</td>
<td>100-710</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>554</td>
<td>149-407</td>
</tr>
</tbody>
</table>

* Six animals/group.

Assays of both human and mouse blood after a single dose of azaserine have been performed to determine the rate of removal of this drug from the blood stream. This process is very rapid in mice but considerably slower in humans.

It has been suggested that side effects seen in humans treated with azaserine are due to this slow rate of removal of the drug from the blood. In mice, in which such side effects are not observed, azaserine is eliminated from the blood very rapidly.

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