Polyamine Depletion of Tumor Tissue and Subsequent Elevation of Spermidine in the Sera of Rats with 3924A Hepatomas after 5-Fluorouracil Administration


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SUMMARY

The level of spermidine in the sera of rats with 3924A hepatomas more than doubled within 36 hr of the administration of 5-fluorouracil. During the same time period, the concentration of spermidine in the tumor dropped to 67% of that detectable prior to treatment. The level of spermidine in the liver of tumor-bearing rats decreased slightly within the first 24 hr and then increased within 72 hr to a level higher than that present initially. These data suggest that the elevated level of spermidine in the sera of tumor-bearing rats after 5-fluorouracil administration is mainly a result of spermidine release from the tumor tissue.

INTRODUCTION

One of the primary objectives of cancer chemotherapy is to maximize the effects on the tumor and minimize toxicity to normal cells. The antitumor agent, 5-FU, has been the drug of choice for many human tumors including breast, colon-rectal, pancreas, and stomach tumors (1-3). Because of its widespread clinical efficacy, 5-FU has been used in order to quantitate the changes in tumor cellularity of the 3924A rat hepatoma in response to chemotherapy. Rapidly growing tumors are known to respond favorably to chemotherapy, and the 3924A hepatoma has been extensively studied as a model of tumor growth.

Prior to any therapy, the 3924A hepatoma is composed of approximately 51% hepatoma cells, 26% connective tissue, 18% necrotic tissue, and 5% blood. Within 48 hr of treatment with 5-FU, these percentages have changed markedly; e.g., the tumor is then composed of about 27% hepatoma cells, 40% connective tissue, and 30% necrotic tissue. Within 24 hr of treatment with 5-FU, there is the largest decrease in the percentage of hepatoma cells (from 51% to 30%).

In Paper 1 in this series (11), we have reported that the level of spermidine in the sera of rats with regressing mammary tumors is markedly elevated. Further, there are elevations of spermidine in tumor interstitial fluids of regressing tumors and corresponding decreases in spermidine in the tumor tissue itself. These data indicated that intracellular spermidine levels, which increase during tumor growth, are lowered by excretion and that spermidine levels in the serum reflect the tumor cell loss. Results of the above study suggested that fluctuations in total tumor burden as a result of chemotherapy or radiation therapy might also be assessed as a function of serum polyamine levels.

In order to elevate this hypothesis further in an animal model system, we have studied polyamine levels in the sera, livers, and tumors of rats with rapidly growing 3924A hepatomas, at various times after a single injection of 5-FU.

MATERIALS AND METHODS

Putrescine, spermidine and spermine hydrochloride standards were obtained from Calbiochem (Los Angeles, Calif.) and recrystallized 3 times with ethanol before use. 14C-Labeled polyamines were used to determine recovery rates of the polyamines and were obtained from New England Nuclear (Boston, Mass.). Buffers for the amino acid analyzer were prepared from Beckman buffer concentrates with appropriate amounts of sodium chloride added to adjust to the proper molarity. Ninhydrin solutions were made from prepackaged ninhydrin kits from BioRad Laboratories (Richmond, Calif.).

Sera samples (2 to 4 ml) from normal and tumor-bearing rats were collected by cardiac puncture and analyzed as previously described (5). There were no detectable differences in the concentrations of polyamines present in plasma samples as compared to serum samples. Therefore, the data reported are for serum samples. Tumor and liver tissues were homogenized in 4 volumes of cold 0.1 N HCl in a Dwell tissue
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grinder. Dry sulfosalicylic acid was added to a final concentration of 4%. The homogenates were centrifuged at 1000 \( \times g \) for 15 min. An aliquot of a supernatant was analyzed for individual polyamines after separation by a Beckman Model 121 automatic amino acid analyzer as previously described (5).

Tumor Transplantation. Female ACI rats were inoculated s.c. in the back with 3924A hepatoma cells by Dr. Harold Morris in Washington, D. C., and shipped to the University of Virginia School of Medicine. The rats were maintained under standard laboratory conditions including commercial laboratory rat chow (Charles River Laboratories, Wilmington, Mass.) supplied \textit{ad libitum}, and on a 12-hr photoperiod, 8:00 a.m. to 8:00 p.m.

Tumor volumes (cu mm) were calculated (\( \frac{1}{2}L \times W \times H \)) from measurement of length, width, and height, made 3 times weekly. Variability of growth rates in individual tumors determined by this method have been shown to decrease considerably after individual tumors have reached a minimum of 200 cu mm (4). For this reason, experiments were scheduled when animals could be grouped uniformly with a mean tumor volume of 200 ± 50 cu mm.

5-FU (Roche Laboratories, Hoffman-La Roche, Inc., Nutley, N. J.) prepared in sterile 0.9% NaCl solution was injected i.p. between 8:00 a.m. and 9:00 a.m.; control animals were given injections of NaCl solution.

In the experiments reported herein, the effects of a single administration of 5-FU on tumor, liver, and serum levels of polyamines were monitored for 168 hr.

RESULTS

Effect of S-FU on the Level of Spermidine in Serum. A single administration of 5-FU results in a rapid increase in the serum level of spermidine (Chart 1). Within 36 hr the level has more than doubled and then gradually declines. This time course of increase of the spermidine level in serum correlates well with the relative decrease in tumor tissue and the relative increase in necrotic tissue. The mean size of the treated tumors is less than the control tumors within 48 hr of 5-FU administration (5). Administration of 5-FU to control rats causes a much smaller increment of serum spermidine within 24 hr (Chart 1).

Content of Putrescine, Spermine, and Spermidine in Tumor Tissue. Within the 1st 48 hr after 5-FU administration, the level of spermidine in the tumor drops from about 1200 nmoles/g to about 800 nmoles/g (Chart 2). This constitutes a loss of approximately one-third of the spermidine pool. Again, this time course parallels the increase in spermidine in the serum, and therefore it appears likely that a major percentage of the serum spermidine is derived from tumor tissue. There is little change in the spermine concentration at any time, and there is only a slight increase in putrescine concentration immediately after drug administration.

Changes in Polyamine Levels of the Liver in Tumor-bearing Rats and in Normal Controls. The concentration of spermidine in the liver of rats with 3924A hepatomas declines slightly within 24 hr of 5-FU administration (Chart 3). Thereafter, it increases to a level above that present before drug administration. This initial decline constitutes approximately 10% of the total spermidine pool. The spermine concentration drops slightly within the 1st 24 hr and then appears stationary for a period up to 168 hr of drug administration. Putrescine concentrations were almost nondetectable in the liver at all times. The liver of normal rats given injections of 5-FU exhibit changes similar to those of the tumor-bearing rats (Chart 3). That is, there is an initial early small fluctuation in the spermidine concentration followed by what appears to be a regenerative process. The ratio of spermidine to spermine changes from approximately 1 in the controls to 2 within 168 hr after 5-FU. This increase in the ratio of spermidine to spermine is due not only to an increase in the spermidine concentration but also to a slight decrease in the spermine concentration.

![Chart 1](https://example.com/chart1.png)

**Chart 1.** Changes in spermidine levels in the sera of rats with 3924A hepatomas and in normal rats after a single injection of 5-FU (150 mg/kg i.p.). Mean ± S.E. of data from 4 different rats assayed at each time and at Time 0. The study was reproduced 3 times with similar results.

![Chart 2](https://example.com/chart2.png)

**Chart 2.** Concentrations of putrescine, spermidine, and spermine in 3924A rat hepatomas at various times after a single injection of 5-FU (150 mg/kg i.p.). Mean ± S.E. of samples from 4 different rats. The study was reproduced 3 times with similar results.
The importance of the cell loss factor to the elevations of extracellular polyamines (15, 16). That is, tumor growth rate is best described not by the mitotic rate, but by the cell loss factor (15). Certain tumors have cell loss factors as high as 70 to 80%. From animal model systems we have seen that the elevation of spermidine in the sera is associated with tumor cell death. Therefore, we would predict that those tumors with a high cell loss factor would be associated with the highest levels of spermidine in sera and urines. Further predictions that can be made on the basis of this model are: (a) after chemotherapy or radiation therapy, there should be increases in the level of polyamines in sera and urines. The extent of the elevation should be related to the efficacy of the therapy; e.g., effective therapy regimens that result in high cell kill also would result in the largest manifestation of polyamines in sera and urines; (b) surgical ablation of large portions of a tumor would result in an immediate reduction in the level of polyamines excreted without any intervening fluctuations; (c) false positives would be expected in patients with other pathological conditions that are expressed through a high cell loss factor. Preliminary studies of serum and urine levels of polyamines conducted on patients having cancer support the model that polyamines are markers of tumor cell death. Studies are in progress to further test this model.

REFERENCES


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