Changes in Serum Putrescine and Spermidine Levels following Local Radiation to Hepatoma 3924A of the Rat


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SUMMARY

Local irradiation of tumors of rats bearing 3924A hepatomas resulted in more than a doubling of the putrescine level and nearly a doubling of spermidine concentration in the serum within 12 hr. Within 24 hr, the putrescine concentration had increased four-fold in the serum, along with a continued increase in the spermidine concentration. The decrease in the spermidine concentration of the tumor paralleled increased levels of spermidine in the serum, whereas the concentrations of polyamines in the liver were unchanged. These changes are similar to previously reported changes in the spermidine concentration in sera and in tumors following the administration of a single dose of 5-fluorouracil to rats with 3924A hepatomas. Since local irradiation was confined to the tumor, we conclude that the increases in putrescine and spermidine detected in the serum are derived from the tumor tissue with no involvement of the host tissues.

INTRODUCTION

It has been previously hypothesized that an elevation of spermidine in the serum of cancer patients reflects tumor cell loss (1, 9, 13, 14). This hypothesis is based on the study of intracellular and extracellular polyamine concentrations in 2 animal model systems: (a) a hormone-dependent mammary tumor (MTW9) which spontaneously regresses after ablation of all sources of hormone (10, 11); and (b) a rapidly growing rat hepatoma (3924A) and its response to a single injection of 5-fluorouracil (13, 14). We have reported rapid increases in the level of spermidine in the serum in response to either spontaneous regression, as in the case of the mammary tumor, or to chemotherapy.

After chemotherapy, it was not possible to determine what portion of the elevation in polyamines in serum was due to release of polyamines from tumor tissue and what portion might be released from host tissues. However, the extracellular fluid of the MTW9 mammary tumor during regression did contain higher concentrations of spermidine than were present in the extracellular fluid prior to the initiation of regression (11). To assess the contribution of tumor tissue compared with host tissues, we have studied the levels of putrescine, spermidine, and spermine in serum of rats with 3924A hepatomas that have been locally irradiated with 3750R.

MATERIALS AND METHODS

Putrescine, spermidine, and spermine hydrochloride standards were obtained from Calbiochem (San Diego, Calif.) and recrystallized 3 times with ethanol before use. 14C-labeled polyamines were used to determine the recovery rates of the polyamines and were obtained from New England Nuclear (Boston, Mass.). Nin-Sol, a ready-to-use ninhydrin solution, and thiodiglycol were purchased from Pierce Chemical Co., Rockford, Ill.

Serum samples (2 to 4 ml) from normal and tumor-bearing rats were collected by cardiac puncture and analyzed for polyamines as previously described (4, 5, 6, 15). Separate rats were used for time studies since only one cardiac puncture is feasible. Tumor and liver samples were homogenized in 4 volumes of cold 5% trichloroacetic acid with a Polytron homogenizer. The homogenates were centrifuged at 1000 x g for 15 min. An aliquot of a supernatant was analyzed for individual polyamines after separation by a Model D-500 amino acid analyzer (Durrum Instrument Corp., Palo Alto, Calif.). This machine was equipped with a 5-mm path-length flow cell. A PDP8/M computer, made by Digital Equipment Corp. (Maynard, Mass.) but standard on the Durrum D-500, controls the entire assay procedure, including sample injection, time of buffer changes, and calculation of the peak areas of the polyamines. A 7.5-cm column (1.75 mm, inside diameter) packed with Durrum DC-4A sulfonated polystyrene cation-exchange resin was used for all separations (bead diameter, 8.0 ± 1 μm; 12% cross-linkage). The column temperature used was 65°C.

Buffers. Preparation of the 3 buffers used is described elsewhere (4). They are: Buffer A, 0.2 mole of Na+ per liter, pH 6.16, the loading buffer for serum and tissue samples; Buffer B, 2.4 moles of Na+ per liter, pH 4.68, the 2nd buffer for serum and tissue samples; and Buffer C, 3.05 moles of Na+ per liter, pH 4.68, the 3rd buffer used for serum samples.

Tumor Growth. Female ACI rats (125 to 150 g) were inoculated s.c. in the back with 3924A hepatoma cells. The rats

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were maintained under a 12-hr lighting schedule, the dark period beginning at 8:00 p.m. Commercial laboratory rat chow (Charles River Laboratories, Wilmington, Mass.) and water were supplied ad libitum. Tumor volumes (cu mm) were calculated \((\frac{1}{2}L \times W \times H)\) from measurements of length (L), width (W), and height (H), made sequentially during the experiment. Regression analysis of a number of methods for volume measurements has indicated that \(\frac{1}{2}LWH\) best describes the relationship between size and time. Furthermore, variability of growth rates in individual tumors determined by this method decreases considerably after individual tumors have reached a minimum of 200 cu mm (2). For this reason, experiments were scheduled when animals could be grouped uniformly with a mean tumor volume of 200 ± 50 cu mm. A complete treatise of methods and radiation-induced changes in growth characteristics of this tumor has recently been published (3). Tumors were weighed (wet weight) immediately upon sacrifice and removal from the animal.

**Tumor Radiation.** Radiations were carried out with a 250-kV, 29 ma General Electric Maxitron 250 using filters of 0.25 mm copper and 1.0 mm aluminum. Prior to radiation, the animals were anesthetized with ether and placed in a lead-shielded box through which the tumor protruded. Controls were anesthetized with ether for the same period of time and subjected to the same conditions, with the exception of the radiation treatment. The midpoint of the tumor was approximately 6 cm from the X-ray tube target and received 3750 R, while the animal body received 0.5% of the dose delivered to the radiated tumor. Radiation dosage was determined by measurement in air. Radiation and subsequent sacrifice were performed between 9 and 11 a.m. to avoid possible diurnal variations in tissue or extracellular polyamines.

In the experiments reported herein, the effects of a single local radiation of the tumor with 3750 R were assessed on the levels of polyamines in serum, tumor, and liver. The effects were monitored for 168 hr with daily samples being taken after the 1st day in which the effects were assayed 3 times. Thereafter, intermittent samples were taken up to 33 days after the initial irradiation.

**RESULTS**

**Effect of Local Radiation on the Level of Putrescine and Spermidine in Serum.** Within 12 hr of local radiation of the hepatoma, spermidine concentration in the serum more than doubled (Chart 1). Within 24 hr, the concentration was nearly 3-fold that detected prior to irradiation. Thereafter, the serum concentration of spermidine dropped and was near control level within 96 hr of initial irradiation. The spermidine concentration followed a similar pattern to that of spermine; i.e., rapid increase with maximal concentrations in the serum detectable within 24 hr of radiation of the tumor (Chart 2). Putrescine concentration had returned to control value within 72 hr.

**Polyamine Concentrations in Tumor Tissue after Local Irradiation.** Similar changes were seen after local irradiation of the tumor as had been demonstrated for tumor tissue after 5-fluorouracil administration (13, 14). That is, within 48 hr, the level of spermidine in the tumor dropped from about 1200 nmoles/g to about 800 nmoles/g (Chart 3). This constituted a loss of approximately one-third of the spermidine pool. This loss in spermidine concentration in the tumor paralleled the increase in spermidine in the serum. There are only slight changes in putrescine and spermine concentration in the tumor after local radiation.

**Polyamine Concentrations of the Liver of Tumor-bearing Rats after Radiation.** Local radiation of the tumors of rats with 3924A hepatomas had no effect on the level of polyamines in the livers. Liver concentrations (mean ± S.E.) for putrescine were 18 ± 5, for spermidine were 1020 ± 130 and for spermine were 790 ± 62. The levels were not different in tumor-bearing rats that did not receive radiation, and also were not significantly different from the levels of polyamines in the livers of normal controls. After a single injection of 5-fluorouracil (150 mg/kg), we found a slight
regenerative process which was evident within 72 hr of the initial chemotherapy (13). Therefore, we could not rule out the possibility that some of the changes in extracellular polyamines seen after giving 5-fluorouracil might be due to changes in liver metabolism of polyamines. However, this study would indicate that host tissues are minimally involved in this process.

DISCUSSION

Chart 4 illustrates a model that we propose to explain the relationship between polyamine levels in tumor tissue and polyamines in extracellular fluids. Those tumors with high cell-loss factors would be associated with the highest levels of spermidine in serum and urine prior to chemotherapy and/or radiation therapy. These assumptions are corroborated by the animal model studies previously published (11, 13) and also by the data presented herein, as well as being compatible with studies of human cancer patients (1–9, 12, 15). It is interesting that the putrescine concentration also increases in the serum in response to local radiation of these tumors. Also note that these tumors have significant concentrations of putrescine and have considerable ornithine decarboxylase activity, as previously reported for this hepatoma and the liver of ACI rats (16). Furthermore, the level of putrescine in the liver is almost nondetectable.

In general, that is true of unstimulated liver of adult rats. We have found in studies of human cancer patients in response to chemotherapy that an increase in putrescine in the serum and urine best correlates with the labeling index. Therefore, it is likely in this system that there is a fairly high labeling index and that putrescine is being actively synthesized and excreted by the tumors as a response to radiation.

It appears at this time, both from studies of animal model systems and of body fluids of human cancer patients in response to either chemotherapy or radiation therapy, that both putrescine and spermidine will be useful markers of the efficacy of the therapeutic regime.

REFERENCES


Chart 3. Concentrations of putrescine, spermidine, and spermine in 3924A rat hepatomas at various times after local tumor radiation of 3750 R (see "Materials and Methods" for details). Each point represents the mean ± S.E. for 8 separate tumors assayed individually.
Serum Polyamines after Tumor Radiation

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