Letter to the Editor

Enhanced Urinary Excretion of N'-Acetylspermidine and the Presence of Tumors

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ABSTRACT

We have studied the urinary excretion of free and acetylated polyamines in hepatoma-bearing Buffalo rats during the period of linear growth of the tumor mass. The excretion of nonconjugated polyamines was unchanged. N'-Acetylspermidine excretion did not parallel the linear increase in tumor mass but increased exponentially. Enhancement of N^2-acetylspermidine excretion above control levels was observed only at a time when the average tumor mass was 35 ± 9 (S.D.) g, shortly before the period when necrosis is usually observed. These data taken together with the analysis of urinary acetylpolyamines in rats bearing mammary tumors and in two melanoma patients show that the determination of the N'/acetylspermidine/N^2-acetylspermidine ratio in urine may be of only limited value as an indicator for the presence of tumors.

Approximately 80% of the spermidine found in the urine of healthy volunteers occurs as the 2 monoacetyl derivatives, N'/acetylspermidine and N^2-acetylspermidine (2, 11), and only a small portion of this amine is excreted in the nonconjugated form. Abdel-Monem et al. (2-4) have reported that cancer patients may show an enhancement of N'/acetylspermidine excretion and suggest the ratio of N'/acetylspermidine to N^2-acetylspermidine as an indicator for the presence of tumors. Since suitable methods are now available (1, 11, 12), the N'/acetylspermidine/N^2-acetylspermidine ratio can be routinely and precisely determined, and it can be performed even on incomplete urine collections. If an increased ratio reliably indicated tumors, this approach could replace the more tedious and less well reproducible determination of total polyamines in urine (7).

As a highly reproducible animal model of a rapidly growing solid tumor, we chose a rat hepatoma. A suspension of cultured rat Morris 7288C hepatoma cells (2 x 10^6 cells in 0.2 ml 0.9% NaCl solution) was injected i.m. into the hind leg of 4 Buffalo rats. Four rats served as controls. [Initial weight of the animals was 235 ± 16 (S.D.) g.] The animals were individually housed in stainless steel metabolic cages. They were kept under their usual 12-hr light-12-hr dark cycle and had access to food and water ad libitum. As soon as palpable tumors appeared, urine collections were started, using polyethylene bottles containing 1 ml of 4 N HCl. In parallel, the size of the tumors was measured by estimation of 2 diameters. (The values of the thickness of skin and fur of control animals were subtracted.) The cross-section was calculated and taken as a measure of tumor size. At the end of the experimental period, the tumors weighed 35 ± 9 g. The individual weights correlated well with the cross-sections. Free and acetylated polyamines were determined in the 24-hr urine samples using a reverse-phase, high-pressure liquid chromatographic method (12). As is shown in Chart 1, the tumor size increased linearly with time from 14 to 49 days after inoculation of the hepatoma cells.

Neither in the control nor in the tumor-bearing rats did free polyamine excretion change significantly (not shown). N'-Acetylspermidine excretion was above control levels from Day 35 on. Its excretion increased thereafter more rapidly than did the tumor size. N^2-Acetylspermidine was greater than control only at Day 49. As a consequence, the N'/acetylspermidine/N^2-acetylspermidine ratio was significantly higher than the control value (2.3 ± 0.2) from Day 35 on.

It appears from these results that the presence of a hepatoma of more than 10 to 15 g is necessary in order to create a significant elevation of the N'/acetylspermidine/N^2-acetylspermidine ratio in rat urine. The tumor mass in our previous study on 7,12-dimethylbenzanthracene-induced mammary tumors (13) never reached this size. This may explain why we were unable to observe significant changes in the urinary acetylpolyamine pattern, although the direct comparison of these 2 tumor lines may not be justified.

N'-Acetylspermidine not only is an excretory product but also appears to be the natural substrate of a cellular polyamine oxidase (5, 8) which converts spermine into spermidine and spermidine into putrescine (6). This enzyme is present in excess in all tissues studied (10). One must assume, therefore, that a major proportion of N'-acetylspermidine formed within a given tissue or organ will be degraded to putrescine.

It is likely that the disproportionate increase of N'-acetylspermidine excretion by animals with large tumors is due to a less efficient supply of the tumor with oxygen and, consequently, a decrease in the rate of N'-acetylspermidine degradation by polyamine oxidase.

Whether tumor cells have an increased capacity for N'-acetylspermidine formation cannot at present be decided. It will be necessary to separate the acetylating enzyme from polyamine oxidase or to specifically inhibit the latter enzyme before a reliable assay of the acetyltransferase is feasible.

Treatment of rats with thioacetamide induced enhanced formation not only of putrescine and spermidine but also of N'-acetylspermidine (9, 13). This finding argues in favor of the notion that the enhancement of the N'-acetylspermidine/N^2-acetylspermidine ratio in urine is not necessarily an indication for the presence of a tumor. It rather indicates a metabolic situation which is characterized by an increased rate of polyamine degradation along the acetylation pathway. It may signal the presence of a tumor as well as the presence or the formation of amounts of polyamines which exceed the physiological...
The study of the urinary polyamine pattern in 2 melanoma patients gave inconsistent results with respect to the $N^1$-acetylspermidine/$N^3$-acetylspermidine ratio (13). However, it may be too early to decide whether the findings in hepatoma-bearing rats are directly applicable to humans. Rats and mice excrete a considerable portion of free putrescine and spermidine (13), and the normal $N^1$-acetylspermidine/$N^3$-acetylspermidine-ratio is significantly higher in rodents (13) than in humans (2, 3, 11). These differences not only are indicating species differences but also can be considered an argument that the $N^1$-acetylspermidine/$N^3$-acetylspermidine ratio may be a more sensitive indicator for changes in polyamine metabolism in humans than in rodents.

REFERENCES

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