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Urinary Polyamines in Cancer Patients

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SUMMARY

Patients with histologically diagnosed tumors and leukemias have an average 24-hr excretion value for polyamines that is severalfold greater than that detectable in "normals". In leukemias, the excretion level declines when the patients are in remission. After surgery for the removal of a portion of a solid tumor mass, polyamine levels in the urine decreased to near normal in all cases. It is possible, therefore, that polyamine excretion levels could serve as (*a*) a tool to monitor cancer and (*b*) a test that could lead to an early detection of cancer.

INTRODUCTION

Polyamines are small hydrocarbon-amine substances which occur ubiquitously in nature (18). Because of their polycationic nature, polyamines have a strong affinity for nucleic acids *in vitro*, and *in vivo* studies indicate a possible role for these compounds in the control of rRNA metabolism (2, 4, 8–11, 13). Studies of rapid-growth systems, both normal and neoplastic, indicate that polyamine synthesis and accumulation is markedly elevated early after tissue stimulation (1, 3, 5, 7, 10, 12–14, 16, 17).

The need for a test for cancer is apparent, both for initial detection and for quantification of residual tumor cells following therapy. It seemed probable that patients with active cancer might have elevated levels of these compounds in some body fluid. Examination of blood indicated no elevated polyamines in human or animal tumors (6). Preliminary examination of urine of a patient with a large metastatic ovarian teratoma indicated an astonishingly high level of all 3 polyamines (putrescine, spermidine, and spermine). Following surgical removal of the major portion of this solid tumor mass, the urine levels were markedly decreased. These findings suggested that a systematic screening of the urines of a large number of patients with varying types of diagnosed cancer would establish whether this finding was generalized enough to be of clinical significance.

MATERIALS AND METHODS

Selection of Patients for Urine Collections

Nontumor Volunteers. Twenty-four-hr urine samples were collected from a group of patients with no known cancer. These included 8 pregnant women and 35 hospitalized patients with a variety of medical illnesses not characterized by overt changes in body metabolism (myocardial infarction, cirrhosis, hypertension, diabetes, fractured mandible, and bacteremia). In addition, 50 normal volunteers submitted urines for analysis.

Cancer Patients. The Baltimore Cancer Research Center, a branch of the National Cancer Institute, admits patients with diagnosed leukemia, lymphoma, brain tumor, and metastatic solid tumors, for therapy with newer chemotherapeutic agents and/or intensive radiation therapy; surgery is performed when indicated. During the past 12 months, 24-hr urine samples, refrigerated under toluene, have been collected from a portion of the patients at the Baltimore Cancer Research Center. Collections were made on new patients before therapy and on some previously admitted patients who had received no recent drug or radiation therapy. Collections from some patients were repeated after surgical removal of tumor or following chemotherapy. All collections were from patients with normal renal function (normal blood urea nitrogen, urine volume, and serum and urine creatinines). Some patients had minor elevations in serum glutamic oxaloacetic transaminase, depressed albumin, or known tumor involvement of liver, but none had significant liver impairment as measured by serum bilirubin, serum glutamic oxaloacetic transaminase, lactic dehydrogenease, and alkaline phosphatase.

Determination of Polyamine Levels in 24-Hr Urine Samples

For quantification of the polyamines, previously described methodology was used with minor modifications (15). A 5-ml aliquot of a 24-hr urine sample was hydrolyzed in 6 N HCl for 12 to 16 hr at 110–120°. Presumably, the polyamines are excreted as conjugated compounds since they have different mobilities on electrophoresis prior to hydrolysis. After hydrolysis, the sample was adjusted to pH 9, and the amines were extracted into 1-butanol. The butanol was evaporated to dryness, and the residue was redissolved in 0.2 ml of 0.1 N HCl. A 10- to 50-μl aliquot of this solution was subjected to high-voltage electrophoresis at 80 V/cm for 2 hr, and the amine was quantified by staining with the following: 100 mg of cadmium acetate, 5 ml of glacial acetic acid, 10 ml of distilled H_2O, 100 ml of acetone, and 1 g of ninhydrin. Standards were run in concentrations of the same range found in the urines. ^{14}C-Labeled amines were used routinely to determine the recovery rates, and appropriate corrections were made.

RESULTS

The 50 normal volunteers (Table 1) all had polyamine levels that were less than 5 mg/24 hr for each amine. The hospitalized nontumor patients with presumed normal

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Table 1

Urinary polyamines in patients with cancer

Polyamine concentrations were determined by extraction of the amines into 1-butanol and separation by high-voltage electrophoresis as described in "Materials and Methods."

Disease	No. tested	Urinary polyamines (mg/24 hr[a]) Putrescine	Spermidine	Spermine
Normal volunteers	50	2.7 ± 0.53	3.1 ± 0.56	3.4 ± 0.67
Nontumor patients[b]	35	2.9 ± 0.51	4.7 ± 0.62	3.5 ± 0.69
Normal pregnancy	8	3.7 ± 0.58	7.7 ± 0.83	10.5 ± 1.80
Patients with cirrhosis	5	4.1 ± 0.65	5.1 ± 0.67	4.0 ± 0.60
Tumor patients[c]				
Acute myelocytic leukemia relapse	24	5.5 ± 1.10	26.4 ± 3.40	38.1 ± 4.20
Remission (complete or partial)	9	< 2.5	10.3 ± 1.30	17.7 ± 3.20
Acute lymphocytic leukemia relapse	5	7.0 ± 0.93	38.5 ± 5.50	65.9 ± 6.70
Chronic myelocytic leukemia	1	<2.5	3.0	5.6
Chronic lymphocytic leukemia	1	6.9	8.3	26.3
Multiple myeloma	2	<2.5	<2.5	7.8
Lymphoma				
Hodgkin's	16	7.9 ± 0.89	16.3 ± 2.4	27.2 ± 3.6
Lymphosarcoma	15	3.7 ± 0.40	15.4 ± 1.9	29.9 ± 3.8
Reticulum cell sarcoma	3	3.0 ± 0.29	13.6 ± 1.5	7.3 ± 0.88
Solid tumors[d]				
CNS	4	5.8 ± 0.76	21.1 ± 2.5	18.7 ± 2.1
Melanoma	3	<2.5	14.0 ± 2.3	19.8 ± 2.2
Rectal	7	5.7 ± 0.58	20.3 ± 2.8	31.1 ± 4.0
Testicular	4	7.6 ± 0.81	18.5 ± 2.1	43.0 ± 5.7
Ovarian	5	29.9 ± 5.7	43.5 ± 6.7	38.7 ± 6.1
Osteogenic sarcoma	1	3.0	30.9	18.8
Breast	1	10.7	7.4	18.4
Uterine leiomyosarcoma	1	<2.5	<2.5	70.4

[a] Each value is expressed as the mean ± S.E. Further, 2 separate 24-hr urine samples were analyzed for each patient.

[b] These patients suffered from various pathologies, *i.e.*, diabetes, cardiac infarction, hypertension, pneumonia, and bacteremia.

[c] All with diagnosed malignant tumors, initial urine collection postadmission, prechemotherapy, except in acute myelocytic leukemia patients, who were in relapse or in remission after chemotherapy.

[d] All patients with proven metastases except CNS malignant tumors.

metabolism all had normal polyamine excretion patterns. All but 1 of the diagnosed cancer patients who were tested (Table 1) showed an elevated polyamine pattern before therapy. These patients had an average urine polyamine excretion that was severalfold above that found in either normal volunteers or in nontumor patients. Urine of several women with normal pregnancies showed some elevation in polyamine excretion.

Certain patients with cancer were studied before and after therapy in an effort to ascertain the effects on polyamine excretion. Urine was collected from 4 patients (Table 2) with acute myelocytic leukemia during remission and during relapse. Each showed a marked change in polyamine levels with markedly higher values measurable during relapse. It will be of interest to determine whether polyamine excretion can be used as a guide to suggest timing of bone marrow evaluations.

Some patients with cancer were studied before and after surgery and/or chemotherapy. One patient (K. R., Table 3) had a marked drop in urinary polyamines following surgical removal of a large ovarian tumor mass and then a further drop following intensive chemotherapy. Another patient (M. R., Table 3) with acute myelocytic leukemia had elevated levels of urinary polyamines before chemotherapy, exhibited a severalfold increase in the level of polyamine excretion during daunomycin administration, and then entered a remission period in which the polyamines were excreted in normal amounts. Urinary polyamines fell to near normal after surgery

Table 2

Changes in urinary polyamines: acute myelocytic leukemia

Polyamine concentrations were determined by extraction of the amines into 1-butanol and separation by high-voltage electrophoresis as described in "Materials and Methods."

Patient	Disease status	Urinary polyamines Putrescine	Spermidine	Spermine
W. D.	Remission	<2.5	5.7	14.0
	Relapse	<2.5	78.0	<2.5
J. A.	Remission	<2.5	<2.5	<2.5
	Relapse	5.1	12.5	15.4
R. L.	Remission	<2.5	11.0	17.0
	Relapse	11.3	67.0	103.0
M. R.	Remission	<2.5	<2.5	<2.5
	Relapse	5.2	10.6	10.6

Table 3

Changes in urinary polyamines following surgery or drug therapy

Polyamine concentrations were determined by extraction of the amines into 1-butanol and separation by high-voltage electrophoresis as described in "Materials and Methods."

Patient	Disease status	Urinary polyamines		
		Putrescine	Spermidine	Spermine
K. R.[a] (ovarian teratoma)	Presurgery	72.0	84.0	64.0
	Postsurgery, prechemotherapy	10.0	28.0	<2.5
	Postchemotherapy	<2.5	5.6	<2.5
J. H. (brain tumor)	Presurgery	<2.5	8.5	14.7
	Postsurgery	<2.5	<2.5	<2.5
C. P. (brain tumor)	Presurgery	<2.5	20.3	<2.5
	Postsurgery	<2.5	13.7	3.8
C. D. (testicular tumor)	Presurgery	7.8	21.9	32.1
	After orchiectomy	5.4	6.1	5.0
M. R. (acute myelocytic leukemia)	Prechemotherapy (11/30/70)	5.2	10.6	10.6
	Prechemotherapy (12/1/70)	4.7	11.7	8.5
	During daunomycin (12/20/70)	<2.5	27.0	48.7
	During daunomycin (12/22/70)	<2.5	36.2	48.2
	Possible remission (1/7/71)	<2.5	<2.5	<2.5
	Remission (1/14/71)	<2.5	<2.5	<2 .5
A. V.[b] (Hodgkin's disease, Stage IV B)	Admission (11/7/70)	15.8	40.4	44.4
	During chemotherapy (11/16/70)	54.0	97.0	102.0
	During chemotherapy (11/25/70)	58.0	116.0	237.0
	Terminal (12/2/70)	31.0	101.0	171.0

[a] Removal of grapefruit-sized mass; peritoneal implants noted.
[b] Progressive worsening course to death.

in 3 patients, 2 with brain tumors and 1 with a testicular tumor (Table 3). A patient, A. V., had rising polyamine levels during the month before his death, a month marked by tumor progression despite intensive chemotherapy, i.v. hyperalimentation, and *Pseudomonas aeruginosa* and Group D streptococcal bacteremias.

DISCUSSION

These results indicate that patients with diagnosed cancer, with rare exception, excrete elevated levels of polyamines. The values for urinary polyamines in cancer patients were 5 to 10 times those of the control group. Further, surgical removal of all or a portion of the tumor mass always led to a decrease in the urinary polyamines. This is striking in the case of a patient whose large ovarian teratoma was removed and less striking in CNS[1] tumors. However, only a small portion of the CNS tumors could be removed, and patients with CNS tumors did not exhibit markedly elevated urinary polyamine excretion. This may be due to the slow growth rate usually attributed to CNS tumors.

[1] The abbreviation used is: CNS, central nervous system.

The cancer patients with solid tumors involved in these studies had proven metastases, except in the case of the CNS cancers. It is yet to be determined whether patients with localized cancers excrete elevated polyamines.

In several cases of acute myelocytic leukemia, chemotherapy culminated in a decreased level of polyamine excretion. However, urinary polyamine levels first increased during chemotherapy, in those cases studied, and then decreased after the patients entered into a partial or complete remission, as determined by bone marrow evaluations. In one case of acute myelocytic leukemia, the polyamine excretion level continued to increase in spite of chemotherapy, and the patient died (Table 3). In this case, the increasing polyamine load excreted indicated the lack of effectiveness of the chemotherapy.

Cancers are known to contain high concentrations of the polyamines (12, 14, 16), concentrations as high or higher than in other rapidly growing tissues (13, 14, 16). The data suggest that the polyamines excreted are from the tumor itself, as surgical removal of the tumor leads to decreased urinary excretion of polyamines. Also, urinary polyamines decrease in cases of effective chemotherapy, *i.e.*, effective chemotherapy in that the patient has decreased clinical signs of disease.

Further, those cancers with the most rapid growth and turnover rates are manifest in higher urinary excretion levels of the polyamines, another possible indication that the polyamines excreted arise from the cancer itself.

Since the original hypothesis was that polyamines would be elevated in rapidly growing and turning over tissues, the possibility does exist that other pathologies which cause metabolic hyperfunction may be reflected by elevated polyamine excretion. Nonetheless, the consistency of the findings would strongly suggest that polyamine excretion be considered as a means of cancer detection.

Further studies need to be conducted to establish: (*a*) whether elevated urinary polyamine levels are associated with all kinds of cancers. Limited kinds of cancers are treated at the Baltimore Cancer Research Center; (*b*) whether elevated excretion patterns of polyamines occur during the initiative stages of cancer; and (*c*) whether urinary polyamine excretion can be used to facilitate effective chemotherapy.

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