Altered Polyamine Metabolism in Cystic Fibrosis

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Summary

Children with cystic fibrosis excreted elevated urinary levels of all three polyamines—putrescine, spermidine, and spermine. Heterozygote parents excreted intermediate concentrations of the polyamines, but not levels significantly different from levels in normal controls. Patients with cystic fibrosis who were administered a tracer amount of [^{14}C]spermidine excreted 11–13% of the radiolabel within 72 hr whereas normal controls excreted 60–76% of the radiolabel within 72 hr. Spermine excretion was positively correlated with increased pathology as assessed by the National Institutes of Health (NIH) clinical score, whereas urinary putrescine and spermidine levels were negatively correlated with increased pathology.

Speculation

It seems that urinary polyamine levels may be used both as nonspecific biochemical markers of clinical disease and as prognostic indicators in cystic fibrosis patients.

Cystic fibrosis is an autosomal recessive genetic disease present mainly in Caucasian populations. The major manifestations, pancreatic dysfunction and chronic pulmonary disease, are associated with mechanical obstruction of organ ducts by abnormally viscous mucus secretions (1, 5, 6). DiSant'Agnese *et al.* (4) provided evidence that increased mucus secretion was a result of abnormalities in all exocrine glands. Numerous studies have uncovered a variety of biochemical abnormalities in patients with cystic fibrosis, but neither the basic biochemical defect nor the pathogenic mechanisms of the disease have been elucidated. Cystic fibrosis patients have been found to have an abnormally high NaCl content in their sweat. A test based on chloride sweat levels, however, is not predictive of disease severity (18). More recently, a ciliary inhibitory factor has been described in the serum of homozygous patients and some obligate heterozygotes. This cytotoxic substance, when added to the ciliary systems of oyster gill or rabbit trachea, results in an alteration or cessation of normal ciliary activity (2, 16). The cilia test has been useful as a developmental tool and has allowed for the partial purification of the ciliary inhibitory factor.

Several laboratories have reported altered polyamine levels in whole blood samples from patients with cystic fibrosis (3, 8, 10). In a recent study, Cohen *et al.* (3) analyzed various blood components to assess precisely which cells had altered polyamine concentrations. The spermidine concentration in erythrocytes of patients with cystic fibrosis was markedly elevated (150% of controls). Further, there were marked decreases in the spermine concentration in both lymphocytes and granulocytes. Thus, whole blood samples from cystic fibrosis patients had an elevated spermidine/spermine ratio. Although these observations are of interest, it is well documented that intracellular polyamine concentra-

tions can be altered in a variety of ways in both normal and pathologic tissues (13, 15). Rosenblum *et al.* (11) reported that patients with cystic fibrosis did not conjugate or excrete [^{14}C]spermidine as rapidly or extensively as normals or patients with cancer (12).

In this report, the authors studied the relation of extracellular polyamine metabolism to the expressed pathology of cystic fibrosis. It was found that children with cystic fibrosis excreted elevated urinary levels of all three polyamines—putrescine, spermidine, and spermine. Heterozygote parents excreted intermediate concentrations of the polyamines, but not levels significantly different from levels in normal controls. Patients with cystic fibrosis who were administered a tracer amount of [^{14}C]spermidine excreted 11–13% of the radiolabel within 72 hr whereas normal controls excreted 60–76% of the radiolabel within 72 hr. Spermine excretion was positively correlated with increased pathology as assessed by the NIH clinical score, whereas urinary putrescine and spermidine levels were negatively correlated with increased pathology.

MATERIALS AND METHODS

MATERIALS

[^{14}C]Spermidine (12.5 mCi/mm) was obtained from New England Nuclear, Boston, MA. Hank's solution, butterfly needles, and heparin were obtained from Abbott Laboratories, Chicago, IL.

METHODS

Urinary samples were collected from patients positively diagnosed as cystic fibrosis homozygotes (four males, three females, age range 5–11 yr, mean age 8), from their obligate heterozygote parents (five males, three females, age range 26–38 yr, mean age 30) (no evidence of disease), and from two groups of age-matched controls: heterozygote age-matched normal group (three males, three females, age range 20–27 yr, mean age 26) and cystic fibrosis age-matched normal group (four males, one female, age range 5–8 yr, mean age 7). Urine of age-matched children with asthma and lung disease also was analyzed. Urine was acidified with concentrated HCl and kept frozen until analysis. Polyamine concentrations in hydrolyzed urine samples were assayed on a Durum D-500 amino acid analyzer as previously described (17). Collections were 24-hr or 8-hr specimens. It was found that serial 8-hr or 24-hr urines from the same patient show remarkably consistent values for polyamine concentration when the excretion of the polyamine is expressed per mg of creatinine. When this normalizing technique is not used, values can be 2- to 3-fold different from day to day. It must be assumed that these fluctuations, when no reference is used, are due to improper collections or changes in kidney function, or both (7, 14).

[^{14}C]Spermidine (10 μCi) was added to 1 ml of sterile Hank's solution containing 1% human serum albumin and the pH adjusted to 7.2 with sterile NaOH. Butterfly needles were inserted

into both the left and right forearm veins. The iv lines were flushed with sterile 0.9% NaCl containing heparin (10%). One venous line was used exclusively for blood sampling after injection of the radioisotope through the opposite line. Venous blood samples (3 ml) were withdrawn at various times after injection (up to 60 min) and placed in 3-ml heparin Vacutainer tubes. These tubes were spun at 2000 rpm for 10 min and the plasma was decanted. Each plasma sample was assayed in duplicate for ^{14}C by counting a 100- μl sample in 10 ml Aquasol. The total urine output for 72 hr postinjection was collected in urine containers, acidified with concentrated HCl to prevent bacterial growth, and refrigerated. The total urine volumes were measured and a 100- μl aliquot of each sample was counted for ^{14}C activity in 10 ml Aquasol.

^{14}C Spermidine metabolism was studied in a group of six normal volunteers (four males, two females, age range 25–29 yr, mean age 26) and two cystic fibrosis patients (one male, age 23 and one female, age 26). All studies were approved by the Human Subjects Committee, University of Arizona, and informed, written consent was obtained before each study.

A group of patients with cystic fibrosis (five males, seven females, age range 4–23 yr, mean age 11) was examined and assigned a prognostic clinical score on the basis of several clinical tests and a physical examination (19). A urine specimen collected at the time of the evaluation was acidified and kept frozen until analysis. Polyamine content was analyzed as previously described. All of the patients with cystic fibrosis who were assigned an NIH clinical score as well as having urinary specimens evaluated for polyamine levels were receiving daily multivitamin therapy, sudafed (30 mg four times a day), Accelerase and cotazyme before

meals, and all received antibiotics based upon results of culture sensitivities. Most were receiving dicloxacillin (250 mg four times a day) or Bactrin (trimethoprim and sulfamethoxazole, 80 mg and 400 mg, respectively, four times a day).

RESULTS

URINARY EXCRETION OF POLYAMINES IN CYSTIC FIBROSIS PATIENTS, HETEROZYGOTE PARENTS, AND NORMALS

Urinary polyamine levels in patients with cystic fibrosis, their obligate heterozygote parents, and age-matched controls for both groups are shown in Table 1. In comparison to normals and heterozygotes, the cystic fibrosis patients had urinary levels of all three polyamines that were 2- to 10-fold higher. Elevations of putrescine, spermidine, and spermine in urine of children with cystic fibrosis were statistically significant ($P < 0.0001$) compared to both the heterozygote parents and to normal children. They were higher, also, than in the urine of children with other lung disorders (Table 1). In heterozygotes, no significant differences from controls were found in urine levels of putrescine ($P < 0.55$) or spermidine ($P < 0.25$); however, a slight elevation ($P < 0.011$) in spermine level in the urine of heterozygote parents was noted compared to age-matched controls.

^{14}C SPERMIDINE METABOLISM IN NORMALS AND CYSTIC FIBROSIS PATIENTS

Plasma decay curves for ^{14}C spermidine in normal and cystic fibrosis patients are plotted in Figure 1. At 1 min after ^{14}C -

Table 1. Urinary polyamines in patients with cystic fibrosis, in obligate heterozygotes, in patients with other lung diseases, and in age-matched normal volunteers

Mean values	Male/Female	Mean age	Urinary Polyamines ($\mu\text{g}/\text{mg}$ creatinine)		
			Putrescine	Spermidine	Spermine
Normals	3/3	25	2.73 ± 0.59	1.56 ± 0.42	0.06 ± 0.01
Heterozygotes	5/3	30	3.04 ± 0.90	1.80 ± 0.34	0.17 ± 0.09
Normals	4/1	7	2.09 ± 0.57	1.79 ± 0.49	0.14 ± 0.08
Asthmatics and severe lung disease	3/2	7	2.45 ± 0.65	2.07 ± 0.51	0.22 ± 0.15
Cystic fibrosis	4/3	8	9.80 ± 1.40^1	4.00 ± 0.45^1	1.81 ± 0.33^1

¹ Data differ from age-matched normals, heterozygotes, and patients with other lung disorders ($P < 0.0001$).

Fig. 1. Decline in plasma radioactivity at various times after ^{14}C spermidine injection (100 μCi , iv) in (A) normal volunteers ($N = 6$) and (B) in patients with cystic fibrosis ($N = 2$) was assessed in a 0.1 ml plasma aliquot by counting in 10 ml Aquasol scintillant.

spermidine injection, the patients with cystic fibrosis had about 50% the amount of radiolabel in plasma as compared to controls. This suggests a rapid sequestration of the radiolabel in patients with cystic fibrosis. However, in both groups there was a similar level of radioactivity within 10 min. Urinary excretion of the ^{14}C radiolabel was markedly reduced in cystic fibrosis patients compared to normals (Table 2). Cystic fibrosis patients excreted only 11–13% of the injected radiolabel within 72 hr as compared to 60–76% excreted by normal individuals within the same time period.

URINARY POLYAMINE LEVELS AND NIH CLINICAL SCORE

Correlations between the NIH clinical score and urinary polyamine levels in cystic fibrosis patients are shown in Figure 2. In all 12 patients studied, urinary levels of putrescine, spermidine, and spermine were elevated compared to normal values. However, the urinary level of spermine was positively correlated to extent of disease whereas putrescine and spermidine were negatively correlated to extent of disease. Those patients with advanced clinical disease (NIH score less than 70, $N = 4$) were found to have significantly lower urinary levels of putrescine and spermidine ($P < 0.05$) than those with less severe disease (NIH score greater than 70, $N = 8$). In contrast, spermine urinary levels were highest in patients with advanced disease (NIH score less than 70) and significantly lower ($P < 0.01$) in patients with less severe disease (NIH score greater than 70). The method of least squares was used to determine the best fit line and was plotted by a Wang computer.

DISCUSSION

Changes in polyamine levels in physiologic fluids have been correlated with alterations in cellular kinetics. In patients with metastatic cancer, the putrescine concentration correlates with the [^3H]thymidine labeling index of the tumor cells, and the spermidine concentration reflects the cell turnover or cell loss factor of the tumor (7, 14–16). In cancer patients, plasma or urine levels of spermidine accurately reflect tumor cell kill in response to chemotherapy (7, 16). Elevated extracellular polyamine levels also have been found in other pathologies characterized by abnormal cell growth fractions and cell death components (9, 15). There are not other well characterized extracellular markers of cell growth and cell death.

The elevated polyamine excretion observed in patients with cystic fibrosis may be related to destruction of excretory gland cells as the disease progresses (5, 6). Further, alterations in urinary levels of polyamines are related to the decreased ability of patients with cystic fibrosis to excrete polyamines. Patients with cystic fibrosis excreted only 11–13% of a [^{14}C]polyamine label in their urine within 72 hr whereas normals and patients with cancer excreted 70–76% (Table 2).

More surprisingly, patients with cystic fibrosis excreted very high concentrations of spermine compared to normals and patients with cancer (15). Spermine concentrations are higher in highly differentiated tissues such as exocrine glands, and this extracellular increase may be a reflection of the destruction of glandular cells (14, 15). Further, the spermine concentration in the urine is positively correlated with the extent of disease as established by the NIH clinical score. This is in marked contrast to the negative

Fig. 2. Urinary levels of (A) putrescine, (B) spermidine, and (C) spermine of 12 cystic fibrosis patients as a function of increased pathology as assessed by the NIH clinical score. (See *Materials and Methods*.)

correlation between the clinical score and the urinary levels of putrescine and spermidine. In our patient population, those cystic fibrosis patients with NIH scores greater than 70 ($N = 8$) (mild disease) excreted significantly higher levels of all three polyamines than age-matched normals (Table 1). Thus, urinary polyamine levels may be used both as nonspecific biochemical markers of clinical disease and as prognostic indicators in cystic fibrosis patients. Alterations in excretory patterns should precede major changes in extent of pathology and polyamine analysis would, therefore, be more useful and easier than the extensive clinical tests now utilized.

In summary, alterations in extracellular levels of polyamines related to pathologic growth appear quite general. However, urinary polyamine levels may be used as objective biochemical markers of clinical disease in patients with cystic fibrosis. In the case of cystic fibrosis, it will be important to establish the mechanism or mechanisms by which polyamine metabolism is altered as a function of increased pathology.

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Table 2. Excretion of [^{14}C]spermidine in normals and cystic fibrosis patients

Time after injection (hr)	(% of injected ^{14}C recovered in urine)		
	Normals ($N = 6$) ¹	CF patient 1	CF patient 2
4	22 ± 2	6.0	3.2
10	32 ± 3	7.0	4.0
24	47 ± 4	7.9	7.0
48	65 ± 7	10.7	9.7
72	68 ± 8	13.5	11.2

¹ Data are expressed as the mean ± SEM.

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