Metabolism of \([^{14}\text{C}]\)Spermidine and \([^{14}\text{C}]\)Putrescine in Normal Volunteers and in Cancer Patients

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ABSTRACT

The administration of \([^{14}\text{C}]\)putrescine or \([^{14}\text{C}]\)spermidine (i.v., 100 µCi) to normal volunteers or patients with advanced cancer resulted in \(\alpha\)-phase half-lives of 40 and 30 sec and \(\beta\)-phase half-lives of 30 and 60 min, respectively. No significant differences were found between the plasma decay curves of normals and those of cancer patients. Urinary excretion was similar with both groups excreting approximately 45% of \([^{14}\text{C}]\)putrescine within 24 hr and 60 to 76% of \([^{14}\text{C}]\)spermidine within 48 hr. Dowex chromatography indicated that >90% of the radiolabel in the urine was in a conjugated form. Plasma conjugation studies of \([^{14}\text{C}]\)putrescine and \([^{14}\text{C}]\)spermidine in both groups indicated near-total conjugation of the radiolabel within 4 to 5 min of i.v. injection. Since putrescine and spermidine are markers of disease activity, characterization of the conjugates will be important for the development of rapid, specific tests of altered disease activity in response to multimodality therapy.

INTRODUCTION

Russell (12) reported in 1971 that cancer patients had elevated levels of polyamines in their urine. Since that time many additional studies have shown that polyamine levels in urine or serum are elevated in patients with solid and hematological neoplasms (1-5, 7, 8, 14-16, 18, 20), in cerebrospinal fluid of patients with brain tumors (6), and in body fluids of patients with other pathological growth states such as psoriasis (13, 17) and cystic fibrosis (9).

Russell et al. (14) and Durie et al. (3) showed that changes in polyamine levels in the plasma or urine of diagnosed cancer patients were associated with alterations in tumor growth kinetics. A direct correlation was found between increases in the \([^{3}\text{H}]\)thymidine labeling index and an increase in the urinary putrescine level. Conversely, an increased rate of tumor cell death after chemotherapy or radiation therapy resulted in a markedly increased level of spermidine in plasma and urine.

The diamine, putrescine, and the polyamine, spermidine, therefore are useful tumor kinetic markers since changes in tumor growth and tumor cell death parameters closely parallel changes in the levels of putrescine and spermidine in the plasma and urine of cancer patients. Monitoring polyamine levels in cancer patients may ultimately provide clinically useful markers of tumor disease activity and could assist in designing more effective antineoplastic therapy.

Although many studies have shown that extracellular levels of polyamines are elevated in metastatic cancer, little is known about the extracellular metabolism of polyamines in humans. We therefore studied extracellular polyamine metabolism in urine and plasma of both normal volunteers and cancer patients by using \([^{14}\text{C}]\)putrescine and \([^{14}\text{C}]\)spermidine. Plasma clearance of both labels was extremely rapid. Both \([^{14}\text{C}]\)putrescine and \([^{14}\text{C}]\)spermidine were almost completely conjugated within 4 to 5 min of injection. Urinary excretion of these amines was almost exclusively in the conjugated form. No significant tissue uptake of the label was found; 40% of the putrescine label was excreted within 24 hr and >60% of the spermidine label was excreted within 48 hr.

MATERIALS AND METHODS

Materials. Aquasol, \([^{14}\text{C}]\)spermidine (12.5 mCi/mmole), and \([^{14}\text{C}]\)putrescine (12.5 mCi/mmole) were obtained from New England Nuclear (Boston, Mass.). Butterfly infusion sets and Panheprin were obtained from Abbott Laboratories (Chicago, Ill.). Dowex 50W-X8 (20 to 400 mesh) cation-exchange resin was obtained from Bio-Rad Laboratories (Richmond, Calif.).

\([^{14}\text{C}]\)Putrescine and \([^{14}\text{C}]\)Spermidine Plasma Decay Curves. \([^{14}\text{C}]\)Spermidine or \([^{14}\text{C}]\)putrescine (100 µCi) was added to 1 ml of sterile Hanks' solution with 1% human serum albumin (Cutter Laboratories, Berkeley, Calif.). The pH was adjusted to 7.2 with sterile NaOH. Normal volunteers were selected from the staff. Cancer patients were selected on the basis of demonstrated advanced disease as measured by several clinical parameters and by elevated urine and plasma polyamine levels. Only patients with normal renal and hepatic function were selected. None of the cancer patients studied had recently received chemotherapy or diuretics. All studies were approved by the Human Subjects Committee, University of Arizona, and informed, written consent was obtained prior to each study. Butterfly needles were inserted into the left and right forearm veins. The i.v. lines were flushed with sterile 0.9% NaCl solution containing heparin (10%). One venous line was used exclusively for blood sampling following injection of the radioisotope through the opposite line. Venous blood samples (3 ml) were drawn at various times after injection (up to 60 min) and placed in 3-ml Vacutainer tubes. The tubes were centrifuged (2000 rpm) for 10 min (Sorval GLC-2 centrifuge) and the plasma was decanted. A 0.1-ml plasma sample was assayed in duplicate for \([^{14}\text{C}]\) label in 10 ml Aquasol scintillant on a Beckman Model LS-250 liquid scintillation counter.

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Urinary Excretion of [14C]Putrescine and [14C]Spermidine. The total urinary output was collected from the subjects at 2, 4, 6, 8, 16, 24, 48, and 72 hr postinjection, acidified with concentrated HCl to prevent bacterial growth, and kept refrigerated. Total urinary volume was measured, and 0.1-ml aliquot of each sample was counted for 14C radiolabel.

Conjugation of [14C]Spermidine and [14C]Putrescine in Normals and Cancer Patients. Plasma samples were chromatographed on a Dowex resin 50W-X8 cation-exchange column (1.2 x 20 cm), and the free polyamines and conjugates were eluted with a 2 to 5 M linear NaCl gradient as described previously (10). Duplicate columns were run for 4 normal volunteers and for 6 cancer patients.

RESULTS

Plasma Decay of [14C]Putrescine and [14C]Spermidine. The plasma decay curve of [14C]putrescine in normals and cancer patients is shown in Chart 1. No significant differences in rate of decay were found between normals (N = 4) and cancer patients with advanced disease (N = 6). [14C]Putrescine declined rapidly in plasma with a calculated $\alpha$-phase half-life of approximately 40 sec (Chart 1A). The $\beta$-phase half-life for [14C] was approximately 30 min.

The plasma decay curves of [14C]spermidine in normals (N = 4) and in cancer patients (N = 6) are shown in Chart 1B. Again, no significant differences were found between normals and cancer patients with advanced disease. The calculated $\alpha$-phase half-life for spermidine was approximately 30 sec, whereas the $\beta$-phase half-life was approximately 60 min.

Urinary Excretion of [14C]Putrescine and [14C]Spermidine. Urinary excretion of [14C]putrescine in normal volunteers and in cancer patients is shown in Chart 2. No significant differences were found in the excretion patterns of normals and cancer patients with advanced disease. Chart 2A shows that approximately 45% of the [14C]putrescine was excreted within 24 hr. Urinary excretion of [14C]spermidine is shown in Chart 2B. In contrast to putrescine excretion 60 to 76% of the radiolabel was excreted within 48 hr by both normals and cancer patients. Dowex chromatography of urinary [14C]putrescine and [14C]spermidine showed that >90% of the radiolabel of both normals and cancer patients was in the conjugated form.

Plasma Conjugation of [14C]Putrescine and [14C]Spermidine. Plasma conjugation of [14C]putrescine and [14C]spermidine in normals and cancer patients is shown in Table 1. Within 4 min, approximately 90% of the radiolabeled putrescine was in a conjugated form. Cancer patients apparently conjugated the putrescine radiolabel initially faster than did normals (at 1 min after injection). On the other hand normals and cancer patients totally conjugated the [14C]spermidine label within 5 min after injection.

DISCUSSION

This study clearly shows that plasma clearance of [14C]putrescine and [14C]spermidine is extremely rapid in both normal volunteers and patients with metastatic cancer.

The almost complete clearance of both putrescine and spermidine label within 3 min is in marked contrast to plasma decay curves of anesthetized rats (10). However, the necessity to anesthetize rats to obtain plasma samples may account for a slower rate of conjugation and excretion.

The total amount of urinary excretion of [14C] label in humans was markedly above that detected in rats. Rats excreted only 5 to 7% of the administered spermidine or putrescine in their urine within 24 hr (11). After [14C]spermidine injection, 20% of the label was excreted within 48 hr in the urine (19). The fact that normal volunteers and patients with metastatic cancer excreted 70% of the administered [14C]spermidine in their urine within 48 hr suggests that, rather than degradation of the label or tissue uptake, the label is rapidly conjugated and excreted. This conservation of polyamines in extracellular fluids may explain the increased levels of urinary polyamines in patients with metastatic cancer. The rapid and extensive conjugation of putrescine and spermidine in both normals and can-
Polyamine Metabolism in Cancer Patients

Table 1
Conjugation of 14C-labeled polyamines in normals and cancer patients after i.v. injection

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Normals (N = 4)</th>
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