Selective Elevation of the N\(^1\)-Acetylspermidine Level in Human Colorectal Adenocarcinomas\(^1\)

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ABSTRACT

The association of N\(^1\)-acetylspermidine with human colorectal adenocarcinomas has been evaluated in this study. Free polyamines and their monoacetylated forms in adenocarcinomas, adenomas, and apparently healthy mucosae were determined using high-performance ion-exchange chromatography. The N\(^1\)-acetylspermidine levels in well- and moderately differentiated adenocarcinomas were 27.30 ± 3.13 (S.E.) (n = 99) and 22.86 ± 3.60 (n = 22) nmol/g, wet weight, respectively. These values were significantly higher than those of benign adenomas (5.38 ± 0.85 nmol/g, n = 31) and of control mucosa. The N\(^1\)-acetylspermidine levels in control mucosa on the oral and anal side of adenocarcinomas were 5.84 ± 1.44 (n = 57) and 7.92 ± 2.89 (n = 50) nmol/g, respectively; no significant difference was observed between control mucosae and adenomas.

The mean levels of three polyamines, putrescine, spermidine, and spermine in both adenomas and adenocarcinomas were about twice as high as those of control mucosae. The molar ratios of spermidine to spermine were significantly greater in both adenomas and adenocarcinomas than in control tissues. There was no obvious correlation between the free polyamine concentrations and the degree of malignancy of the colorectal tumors. These results suggest that the metabolism of N\(^1\)-acetylspermidine in colorectal adenocarcinomas is quite different from that in adenomas and in nonneoplastic mucosae and that N\(^1\)-acetylspermidine can be a promising biochemical marker of cancer in the human large intestine.

INTRODUCTION

Measurements of polyamines including putrescine, spermidine, and spermine in erythrocytes, urine, cerebrospinal fluid, and pathological tissue specimens have been shown to be useful in the diagnosis of cancers and in assessing the effects of anticancer treatment (9, 19). However, the rise in tissue polyamine levels is not specific for malignant tumors, but common to rapidly growing tissues, for example, embryonic and hormonally stimulated tissues (3). Because of this lack of specificity, one may suspect that polyamines cannot be reliable markers of cancers.

Recently, it has been shown that the rate of acetylation of spermine and spermidine is augmented under some stimulated conditions (10, 13, 17). Matsui and Pegg (11) showed that spermidine N\(^1\)-acetyltransferase in rat liver is activated by the treatment with dialkylnitrosamines. Intracellular conversion of [\(^{14}\)C]putrescine into N\(^1\)-acetylspermidine and N-acetylputrescine is increased, while that into N\(^6\)-acetylspermidine remains unchanged in Friend erythroleukemia cells when erythroidifferentiation of the cells is induced by dimethyl sulfoxide and hexamethylen bisacetamide (8). Accumulation of N\(^1\)-acetylspermidine is, however, minimal, even in rapidly growing tissues, except in hamster epididymis (12). In contrast, urinary excretion of acetylated polyamines has been repeatedly shown to be increased in leukemic patients (6, 21, 22) and in other cancers (1, 2, 21). The molar ratio of urinary N\(^1\)-acetylspermidine to N\(^6\)-acetylspermidine was reported to be increased in patients with cancer (1, 2).

These data suggest that there is a close relationship between N\(^1\)-acetylation of polyamines and the malignant state. These findings prompted us to analyze acetylated polyamines in human colorectal adenomas and adenocarcinomas. Care was taken to detect minute amounts of acetylated polyamines in cancerous tissues by means of highly sensitive high-performance liquid chromatography. We now report that N\(^1\)-acetylspermidine is a good indicator of cancer in human colorectal tumors.

MATERIALS AND METHODS

Chemicals. N\(^1\)- and N\(^6\)-acetylspermidine synthesized according to the published method (7, 21) were kindly supplied by Dr. T. Nakajima of Osaka University, Japan. N\(^1\)-Acetyspermine and N-acetylputrescine were synthesized in our laboratories according to the method of Dubin and Rosenthal (7). 1-Methylhistamine was purchased from Calbiochem-Behring Corp. (La Jolla, CA).

Specimens. Specimens of the colon and rectum were obtained at surgery from patients with colorectal adenocarcinoma and with polyp and then frozen and stored at −70°C. For the histological studies, preservation and processing of tissues were done routinely: tissues were fixed in 10% buffered formalin solution and embedded in paraffin. Multiple 5-μm sections were prepared and stained with hematoxylin and eosin. Colonic mucosa was taken at 3 cm distant from the margins of adenocarcinomas. Apparently normal mucosa on the oral side of tumors are referred to as “OS,” and those on the anal side are referred to as “AS.” The mucosa has histologically no signs of cancer. Cancerous tissues were taken at 2 opposite protruding portions near the periphery of the tumors; necrotic tissues were excluded. Tissues were washed free of feces with ice-cold 0.9% NaCl solution prior to homogenization. 4 ml of 10% buffered formalin solution was added to 25-ml aliquots of the supernatants on the column after neutralization with the same volume of a citrate buffer (pH 5.55).

N\(^1\)-Acetyspermidine Assay. Acetylated polyamines were assayed by a slightly modified method of Prussak and Russel (18). Briefly, all 3 buffers contained potassium in place of sodium; Buffer A (pH 4.25) was a citrate buffer containing 0.2 equivalent potassium; Buffer B (pH 10.1) contained boric acid and 0.2 equivalent potassium; and Buffer C (pH 17.14) contained glycerol and 0.2 equivalent potassium.
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10.1) contained boric acid and 0.44 equivalent potassium. The buffer flow rate was 0.4 ml/min. A short column (4.8 × 80 mm) of cation-exchange resin (62210F; Kyowa Seimitsu Co., Ltd.) was used for chromatographic separation. Column effluent and o-phthalaldehyde solution were mixed, and fluorescence was measured on a JASCO Model EP-110 spectrofluorometer. The recorder was set at 1 and 10 mV for 100% relative fluorescence. All other details of the microdetermination method were described previously (16). With this method, not only acetylated polyamines but also histamine and 1-methylhistamine could be measured simultaneously. The lower limit of detection was approximately 0.5 nmol/g wet tissue for each compound. Identification of monoacetylated polyamines was assisted by acid hydrolysis and thin-layer chromatography as described previously (12).

Polyamine Assay. Polyamines in HClO₄ extracts were measured as described previously (12, 16). With this method, N₁- and N₈-acetylspermidine were not resolved, while other naturally occurring polyamines were almost completely resolved.

RESULTS

Identification of N₁-Acetylspermidine in Human Colorectal Tissues. The modified method of Prussak and Russell (18) applied in our study separated N₁-acetylspermidine eluting at 45.3 min from N₈-acetylspermidine which eluted at 36.6 min. The peak which corresponded to N₁-acetylspermidine disappeared completely upon acid hydrolysis of colorectal specimens, showing that the peak contained only conjugate(s). When analyzed by the polyamine assay method, the spermidine peak increased in height after acid hydrolysis. Furthermore, isolated "N₁-acetylspermidine" peak fractions stoichiometrically yielded spermidine upon acid hydrolysis. The identity of the isolated fractions as N₁-acetylspermidine was also demonstrated by thin-layer chromatography (12). These results show the identity of the peak eluting at 45.3 min as N₁-acetylspermidine.

N₁-Acetylspermidine Contents in Adenocarcinomas and Adenomas. N₁-Acetylspermidine was detected in almost all specimens examined, while no appreciable amounts of N-acetylputrescine, N₈-acetylspermidine, and N₈-acetylspermine were detected. Chart 1 shows the N₁-acetylspermidine contents of well- and moderately differentiated adenocarcinomas, adenomas, and apparently "normal" mucosae (OS and AS). Adenomas included tubular, villous, and tubulovillous types. The mean N₁-acetylspermidine levels were several times higher in adenocarcinomas than in adenomas and control mucosa (Chart 1). In 2 cases of poorly differentiated adenocarcinoma, the N₁-acetylspermidine level was similarly elevated. Some solitary polyps showed elevated levels of N₁-acetylspermidine (more than 20 nmol/g wet tissue); the histological examination revealed cancers in all these cases. When the value of 15 nmol N₁-acetylspermidine/g, wet weight, was used as our cutoff for the normal value, the false-positive rate was 6.4 and 7.0% in adenomas and control mucosae, respectively. About two-thirds of adenocarcinomas had N₁-acetylspermidine in concentrations greater than 15 nmol/g. Although the other one-third showed lower levels than this cutoff, they showed higher levels than their respective control mucosae.

Histamine eluting at 31.9 min on the chromatograms was detected in all the specimens, while 1-methylhistamine eluting at 35.6 min was detected only in a limited number of specimens. Histamine levels were reduced in some adenocarcinomas and extremely elevated in others (data not shown).

Polyamine Levels in Adenomas and Adenocarcinomas. Putrescine, spermidine, and spermine were all detected in the intestinal mucosa, muscularis, and serosa, and polyamine levels were highest in the mucosa among the colonic layers examined. The levels of putrescine, spermidine, and spermine in both adenomas and adenocarcinomas were approximately twice as high as those of control mucosae (Table 1). The molar ratio of spermidine to spermine increased slightly but significantly in both adenomas and adenocarcinomas. The spermine level was significantly higher in moderately differentiated adenocarcinoma than in well-differentiated adenocarcinoma, leading to a lower ratio of spermidine to spermine in the former group.

DISCUSSION

Since levels of acetylated polyamines are usually very low in normal cells and even in neoplastic cells, it has been rather difficult to detect changes in these compounds. Our microdeter-
The assay of this acetylated compound method, however, enabled us to detect N1-acetyl spermidine in not only neoplastic tissues but also apparently normal colorectal mucosa. The high sensitivity was achieved by using a fluorometric technique and a highly sensitive spectrophotometer and by adopting carefully selected resins with a narrow range of bead diameter below 10 μm (16). The detection limit was about 1 pmol for each free and conjugated polyamine.

The present study showed that there is a great accumulation of N1-acetyl spermidine in colorectal adenocarcinomas but that there is no difference in N1-acetyl spermidine concentration between normal mucosae and benign adenomas. This compound may originate from adenocarcinoma cells but not from intestinal flora, for it was accumulated selectively in adenocarcinomas but not in adjacent control mucosae. Moreover, there is evidence to show that malignant cells produce much more N1-acetyl spermidine than do normal cells (8, 11). Spermidine N1-acetyltransferase activity is induced by dialkylnitrosamines in the rat liver (11). One cannot exclude, however, the possibilities that colorectal adenocarcinomas preferentially take up N1-acetyl spermidine of bacterial origin and that degradation of N1-acetyl spermidine by polyamine oxidase is suppressed in the adenocarcinomas. In any case, the results suggest that this acetyl polyamine can be used as an additional diagnostic marker to discriminate adenocarcinomas from benign adenomas in human large intestine. It remains to be established, however, whether or not the elevation of N1-acetyl spermidine is specific for adenocarcinomas in the human colon. Our preliminary study has shown that no elevation of the N1-acetyl spermidine level is observed in patients with juvenile poly, lymphoid poly, or ulcerative colitis. Furthermore, it remains unknown whether or not elevated levels of this compound are characteristic of all the carcinomas. Studies are under way to investigate these possibilities.

The elevation of polyamine levels and that of the ratio of spermidine to spermine are characteristic of rapidly growing tissues, including neoplastic tissues (3, 9, 19). The activity of polyamine-synthesizing decarboxylases is increased in rat colonic epithelium by carcinogens, such as dimethylhydrazine (4) and N-methyl-N′-nitro-N-nitrosoguanidine (20). It is not known yet whether or not these carcinogens induce spermidine N1-acetyltransferase activity in the colonic epithelium. Usually, the accumulation of N1-acetyl spermidine is coupled with the increase in ornithine decarboxylase activity (10, 13, 17) except in the rat pancreas (5). In a high percentage of patients with colorectal carcinomas, elevated serum concentrations of one or more polyamines have been reported (14, 15). However, elevated tissue levels of polyamines were observed in not only adenocarcinomas but also adenomas in our present study, showing that elevated polyamine levels alone are not typical for cancer in the large intestine. Since N1-acetyl spermidine is accumulated selectively in colorectal adenocarcinomas, it would be a better indicator of cancer than are free polyamines. The assay of this acetylated compound may be useful in distinguishing carcinomas from benign tumors, especially in solitary polyps. A single polyp weighing over 5 mg is enough for the determination of N1-acetyl spermidine and polyamines. The presence of cancer in polyps removed by endoscopic snare can be diagnosed by N1-acetyl spermidine assay as well as by microscopic examination.

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