High Ornithine Decarboxylase Activity and Polyamine Levels in Human Colorectal Neoplasia

GLENN M. LAMURAGLIA, M.D. FRANÇOIS LACAINE, M.D. RONALD A. MALT, M.D.

Polyamines are required for cell proliferation, and ornithine decarboxylase (ODC) is the first and probably rate-limiting enzyme in their synthesis. Tissue containing colonic or rectal adenocarcinomas (N = 34) or polyps (N = 6) and noninvolved paired colonic mucosa were obtained from fresh surgical specimens. ODC activity was elevated (mean: 320%) in both the cancer and polyps. In noninvolved colonic mucosa of tumor-bearing specimens, ODC activity was 165% that of colonic mucosa of nonneoplastic disease. Concentrations of polyamines in neoplasms were 121–214% increased, as compared with normal mucosa; those of spermidine and spermine varied inversely with the histological grade of the tumor. High levels of ODC activity and of polyamines were features of neoplasia, but not of malignancy alone. These characteristics of colonic neoplasia suggest its susceptibility to control by inhibition of ODC.

From the Surgical Services, Massachusetts General Hospital and Shriners Burns Institute, and the Department of Surgery, Harvard Medical School, Boston, Massachusetts

THE ALIPHATIC POLYAMINES putrescine, spermidine, and spermine are ubiquitous intracellular bases required for normal and neoplastic growth.[1] Among their other functions, they facilitate transcription, translation, and initiation of protein synthesis. Tropic stimuli can greatly increase polyamine concentrations, largely by increasing the activity of ornithine decarboxylase (ODC), which catalyzes the first and probably rate-limiting step in their synthesis: the conversion of ornithine to putrescine.[2]

Sustained levels of ODC have been implicated as an essential component of tumor development.[3–5] Elevated levels of ODC are found in proliferating normal cells[6,7] and during neoplastic transformation.[2,8–11] Promoters of colonic tumors such as sodium deoxycholate and high-fat diets increase ODC activity in rats.[12,13] During dimethylhydrazine (DMH)-induced carcinogenesis, rat colonic mucosa has increased ODC activity,[14,15] and treatment with α-difluoromethylornithine (DFMO, an irreversible inhibitor of ODC) reduces the incidence of DMH-induced colorectal tumors in mice by 94%.[16]

We examined spontaneous human colorectal adenocarcinomas, polyps, and normal-appearing mucosa from operative specimens to determine both the relative activities of ODC and the polyamine profile of these human tissues. A preliminary account of this work has been published.[17]

Materials and Methods

Operative specimens of human colon were obtained from nonobstructed patients (N = 47) and processed within 10 minutes of colectomy. No patients had received chemotherapy or radiotherapy. After the bowel was slit lengthwise and washed in ice-cold water, samples of nonnecrotic tumor, of nearby normal-appearing mucosa, of polyps, and of small bowel, when applicable, were placed in iced buffer (50 mM sodium phosphate, pH 7.2, 1 mM EDTA, and 2 mM dithiothreitol).

Assays were performed on tissue suspensions homogenized on ice for 6 seconds using a Polytron (Brinkman Instruments Inc., Westbury, NY). Samples for enzymic activity were centrifuged at 35,000 × g for 20 minutes at 4 C. The supernatant was immediately frozen at −85 C for subsequent analysis. ODC activity on an ornithine substrate was determined in duplicate using a modification of the method of Lapointe and Cohen to assay $^{14}CO_2$ generated.[18] For a final volume of 300 μl, the incubation wells contained 160 μl of supernatant, 1 mM pyridoxal phosphate, 0.2 mM L-ornithine, and 0.25 μCi of DL-[1-^{14}C]ornithine (specific activity, 47.2 mCi/mmol, New England Nuclear Corp., Boston, MA). The mixture was incubated at 37 C for the collection of $^{14}CO_2$ in barium

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Reprint requests: R. A. Malt, M.D., Massachusetts General Hospital, Boston, MA 02114.

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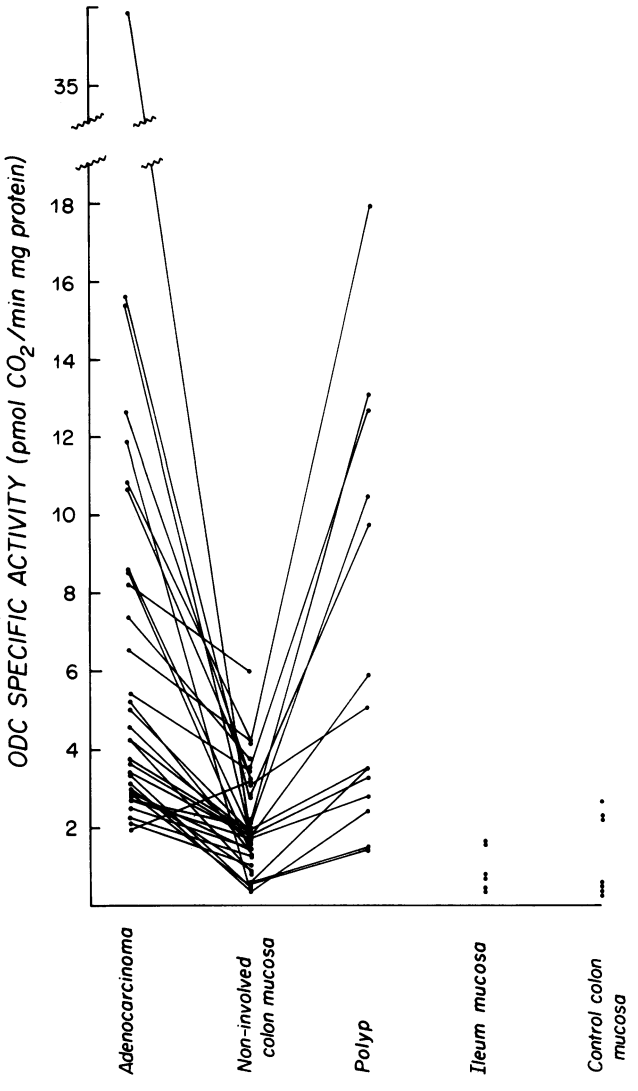

FIG. 1. Ornithine decarboxylase activity of specimens. Lines connect samples taken from the same patients.

hydroxide-saturated discs. After the reaction was stopped at 1 hour by adding 50 μl of 2 N sulfuric acid, $^{14}CO_2$ was collected for another 30 minutes. $Ba^{14}CO_3$ was counted in toluene:Triton X-100 media at 78% efficiency by internal standard. Controls were heat-inactivated supernatants.

Kinetic studies were performed on tissue harvested in 0.9% NaCl and fragmented with a scalpel. Fragments from the same specimen were incubated in 0.5 ml 0.9% NaCl aliquots at 37 C. The suspensions of tissues were then combined with 1.5 ml iced buffer, kept at 4 C, and processed as regular samples. Half-life tissue measurements were determined from ODC activity, using the formula $t_{1/2} = {}^{0.693}/K$ (ref. 19).

Protein content was analyzed using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA).

Quantitation of putrescine, spermidine and spermine[20] was kindly carried out by Dr. Peter P. McCann and Mr. Keith A. Diekema at Merrell Dow Research Center, Cincinnati, Ohio. Homogenates were brought to 0.4 M perchloric acid with 5 M perchloric acid. After 12–24 hours at 4 C, the specimens were centrifuged at 15,000 × g for 15 minutes and were filtered through a 0.45 μm filter.

Official reports of the specimens by the Department of Pathology were accepted for histopathologic classification.

Statistical analysis was performed using Student's t-test for paired data and the Wilcoxon T-test, using normal colonic mucosa as a reference to minimize individual variations caused by surgical technique, nutritional state of the patient, and circadian rhythm of ODC.[21-23]

Results

Pathology

Specimens were classified into three groups: (1) no neoplastic tissue present, (2) only benign neoplastic tissue present, and (3) malignant neoplastic tissue present.

Group 1 (N = 7) was composed of one suspected angiodysplasia, one nonobstructing stricture, and five diverticulosis specimens. Group 2 (N = 6) contained all villous adenomas. Group 3 (N = 34) contained adenocarcinomas (right colon 11, transverse colon 2, left colon 5, rectosigmoid 16), 26 of which were moderately differentiated. Of the colons containing adenocarcinomas, 29 also contained polyps elsewhere in the specimen: ten adenomatous, nine villous, and one both adenomatous and villous. Of these synchronous polyps, eight were processed for biochemical analysis.

ODC Activity

In all except one adenocarcinoma specimen, ODC activity was higher in the neoplastic tissue than in the corresponding normal-appearing colonic mucosa (Fig. 1). The observed increases in enzymic activity of adenocarcinoma (314%) and in polyps (324%) were similar.

No differences were identified in comparing the ODC activity of the noninvolved colonic mucosa bearing benign or malignant neoplasms (groups 2 and 3). However, their average ODC activity was 165% that of the colonic mucosa of nonneoplastic bearing specimens (group 1). The enzymic activity of the terminal ileum mucosa was 70% that of the colon mucosa of nonneoplastic bearing specimens.

Histologic grade analyzed by paired differences showed that well-differentiated adenocarcinomas had the highest ODC activity as compared with polyps, moderately differentiated adenocarcinoma, and poorly differentiated adenocarcinomas (Fig. 2).

The half-lives of ODC activity were similar in the adenocarcinomas, polyps, and colon mucosa (Table 1).

Polyamines

Putrescine levels were equivalent in all specimens of colonic tumor and mucosa. The concentration of putrescine in ileal mucosa was approximately 50% that of the colonic samples (Table 1). Paired differences comparisons of putrescine levels with histologic grades of tumors showed a direct correlation of ODC activity with the degree of differentiation (Fig. 2).

Spermidine and spermine concentrations in human colonic neoplasms were 121–214% those of noninvolved colon mucosa (Table 1). Greater spermidine and spermine levels were found in the polyps. Paired differences of spermidine and spermine levels in colonic tumors also showed a direct correlation with the degree of differentiation (Fig. 2).

No significant differences were noted between the polyamine profile from colonic mucosa of specimens bearing benign or malignant neoplasms. Colonic mucosa from specimens not bearing neoplasms, however, had lower levels of spermidine ($p < 0.02$) and spermine ($p < 0.0001$), as compared with the colonic mucosa of tumor-bearing specimens.

Discussion

These data indicate markedly elevated ODC activity in human colorectal neoplasms just as in human renal carcinomas,[34] cutaneous epithelioma,[5] and brain.[25] However, the enzymic activity in colonic neoplasms is not a distinguishing feature between benign and malignant disease. These results coincide with previous observations in rat and human colonic neoplasms.[12]

During carcinogenesis of the colon, there is a generalized shift of the major site of DNA synthesis from the lowest third of the colonic crypts to the middle and upper third.[26] This alteration throughout the colon mucosa, seen in very high risk patients and DMH-treated mice, occasionally results in synchronous neoplasia.[27] In human beings, the synchronous and metachronous potentiality of colon adenocarcinoma[28,29] indicates that the disease is generalized throughout the colon mucosa. Our data comparing tumor-bearing mucosa to nontumor-bearing mucosa support this argument. The higher ODC activity and the increase of spermidine and spermine concentration in tumor-bearing colonic mucosa approach the levels found in neoplastic tissue. The increased ODC activity of

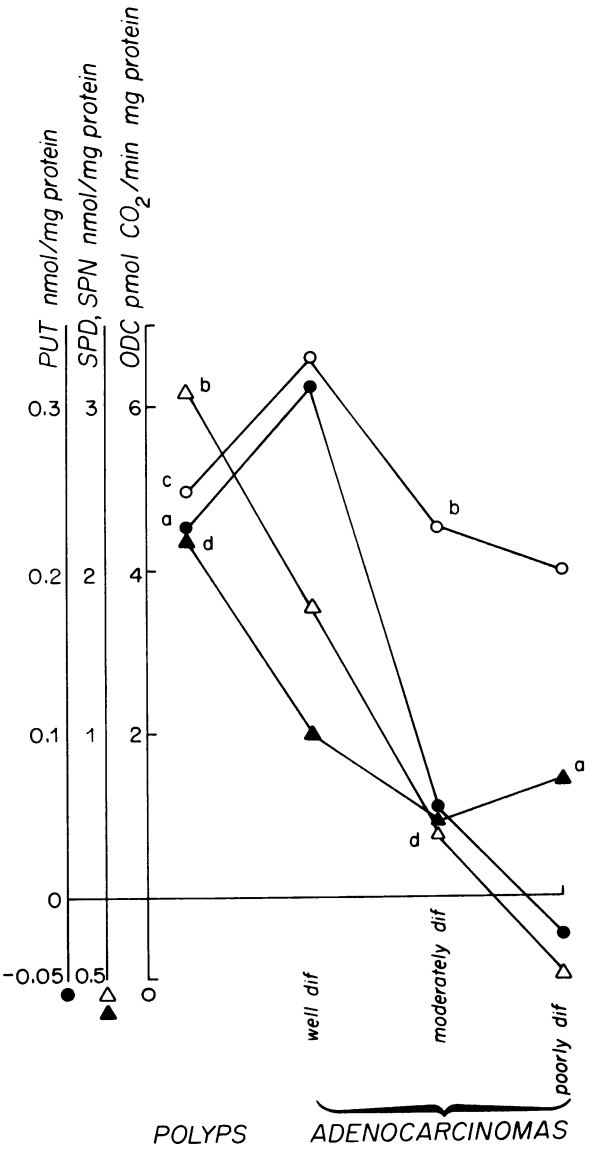

FIG. 2. Paired differences between tumors and adjacent non-involved colon mucosa in putrescine (PUT, ●), spermidine (SPD, ▲), spermine (SPN, △) and ODC activity (○) according to histological grade. Significance: a: $p < 0.05$; b: $p < 0.02$; c: $p < 0.01$; d: $p < 0.001$.

TABLE 1. *Ornithine Decarboxylase Activity and Polyamine Concentrations*

	N	$T_{1/2}$ Min	ODC Activity*	Polyamines†		
				Putrescine	Spermidine	Spermine
Adenocarcinoma	34	169 ± 46	6.71 ± 1.10	0.52 ± 0.09	2.47 ± 0.21	3.63 ± 0.31
Polyp	14	128 ± 27	6.95 ± 1.48	0.54 ± 0.09	4.24 ± 0.54	6.78 ± 0.92
Non-involved colon mucosa	40	133 ± 26	2.14 ± 0.22	0.43 ± 0.07	1.98 ± 0.14	3.42 ± 0.23
Control colonic mucosa	7		1.30 ± 0.40	0.58 ± 0.12	1.14 ± 0.13	1.24 ± 0.08
Ileal mucosa	6		0.91 ± 0.20	0.23 ± 0.06	1.57 ± 0.42	2.94 ± 0.73

* pmol $CO_2 \cdot min^{-1} \cdot mg$ protein.

† $nmol \cdot mg \cdot protein^{-1}$.

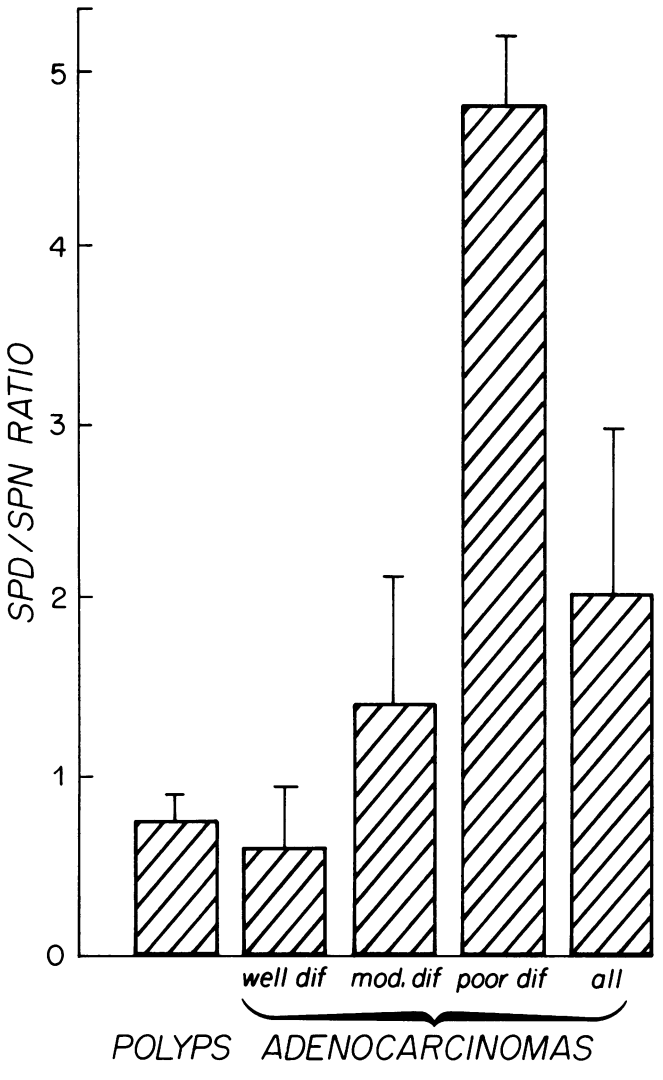

FIG. 3. Ratio of spermidine (SPD) to spermine (SPN) by histological grade of neoplasms (±S.E.).

the colon mucosa in familial colonic polyposis further substantiates this argument.[30]

Similar half-lives in both tumors and adjacent noninvolved colon mucosa verify that the differences in ODC activity assayed in tumors were not merely differences of enzyme inactivation inherent in the experimental protocol. The longer half-life observed in the noninvolved mucosa of tumor-bearing specimens compared with the half-life of nontransformed cells[31] may be the result of aberrations in the enzyme inactivating mechanisms, which occur in preneoplastic and neoplastic tissue.[32]

The polyamine content of human colonic neoplasms increases along with the ODC activity. The concomitant increase of polyamines and ODC activity implies a breakdown in the feedback inhibition in neoplasia.[1,11] The fact that the highest polyamine content is present in the nonmalignant polyps, but at a lower level than is found in the adenocarcinomas, may be a result of decreased synthesis of polyamines and increased polyamine degradation through the formation of the N′-acetyl derivatives.[33]

Because the polyamine levels have an indirect correlation with histologic grade, the spermidine:spermine ratio, described as an indicator of malignancy in some tumors,[11] increases with the cellular atypia of the specimens (Fig. 3).

Specific irreversible nontoxic inhibitors of polyamine synthesis such as DFMO have become available. DFMO inhibits growth of mouse colon cancer *in vitro* and *in vivo*,[34] potentiates the effect of 5-fluorouracil in cultured human colon adenocarcinoma cells,[35] and reduces the incidence of DMH-induced colon tumors by 94% in mice.[16] Inhibition of ODC and polyamine synthesis might be used as chemoprevention[36] in autosomal dominant members at risk for colonic polyposis[30] or as one arm of a multidrug protocol for treatment of colon cancer.

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