Ornithine Decarboxylase and Polyamines in Colorectal Neoplasia and Mucosa

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Abstract

Ornithine decarboxylase (ODC) and polyamines are intimately involved in normal cellular proliferation and are likely to play a role in carcinogenesis. ODC activity and polyamine content were measured in tissue samples obtained during colonoscopy from 48 benign neoplastic polyps (20 tubular adenomas; 28 villous adenomas), 18 cancers (including 5 malignant polyps), and adjacent mucosa. ODC activity in polyp and cancer tissue specimens was higher than in adjacent mucosa in 75 and 83% of pairs, respectively. Similarly, putrescine, spermidine, and spermine contents were higher in the majority of polyps and cancers compared to adjacent mucosa. ODC activity and polyamine content in colonic mucosa from 10 patients without a history of colorectal neoplasia were not different from adjacent mucosal values in the patients with neoplasia. In conclusion, ODC and polyamines are elevated in the majority of colorectal neoplasms, but amounts in normal mucosa do not differentiate between patients with cancer, benign neoplastic polyps, and normal subjects.

Introduction

A variety of intracellular processes requisite for cellular proliferation and differentiation including transcription and translation regulation require polyamines (1). Intracellular concentrations of spermine, spermidine, and their precursor putrescine have in turn been shown to be highly regulated (1-5). In mammalian cells, putrescine is synthesized solely from the amino acid ornithine, although it can also be formed catabolically from spermidine in some circumstances (2). The first and generally rate limiting step in polyamine biosynthesis is catalyzed by ODC2 (6-8). ODC activity is low in quiescent cells, but can be decreased up to 30-fold within 4 hours by a variety of stimuli, such as feeding, after exposure to various growth factors, hormones, and drugs and after tissue injury during regenerative growth (9-11).

Several lines of evidence suggest that ODC plays an important role in carcinogenesis. Studies in tumor promotion models have shown a strong relationship between the tumor-promoting ability of carcinogens and induction of ODC activity (12-15), and inhibition of ODC activity can inhibit carcinogenesis (16, 17). A variety of tumors demonstrate increased ODC activity and polyamine content relative to normal tissue of the same organ system, including colorectal cancer compared to uninvolved normal appearing mucosa (18-31). Multiple investigators have also assessed ODC activity in benign colorectal neoplastic polyps, the putative transition lesion between normal mucosa and cancer, and have reported results varying from higher, to similar, to lower relative activity in polyp tissue versus adjacent mucosa (20-22, 27, 31-35). A variety of methodological factors may have contributed to these discrepant results, since ODC is known to be a labile enzyme and the ODC assay is very sensitive to seemingly minor technical factors (36). We report our experience using a standardized tissue procurement method (biopsy at the time of endoscopy) to assess both ODC activity and polyamine contents in colorectal neoplasia and flat mucosa.

Materials and Methods

Patients

Patients scheduled to undergo colonoscopy at the Tucson Veterans Affairs Medical Center and University of Arizona Medical Center for clinical indications gave consent for biopsy of neoplastic appearing lesions and flat mucosa from the opposite wall at the level of the lesion or from normal mucosa at the rectosigmoid junction if the patient had no history or current evidence of colorectal neoplasia. Patients with inflammatory bowel disease or a familial polyposis syndrome were excluded. The protocol was approved by the University of Arizona Institutional Review Board.

Colonoscopy was performed between 8 and 12 am and patients were fasting. Bowel preparation was performed with polyethylene glycol lavage. Colonoscopy was performed with an Olympus CF-1T10L colonoscope (Olympus Corp., Lake Success, NY) and biopsies were obtained with an Olympus FB-13U "jumbo" forceps. Biopsies from neoplastic lesions ≥1 cm in size were obtained either during colonoscopy or after a polyp had been excised with electrocautery and retrieved from the patient. Biopsies were taken from the surface of the polyp away from any apparent cautery injury. Tissue was promptly snap-frozen at the bedside and stored in liquid nitrogen until assayed within the following 4 months. All

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2 The abbreviations used are: ODC, ornithine decarboxylase; DFMO, difluoromethyl ornithine.
biopsies from an individual case were stored for identical time periods. Histological interpretation was performed by staff pathologists.

Methods

ODC Assay. Biopsy specimens were each homogenized in 600 µl ice-cold buffer (25 mM Tris-HCl, pH 7.5-0.1 mM EDTA-2.25 mM dithiothreitol) and centrifuged for 5 min at 10,000 g using a chilled Beckman microfuge. The supernatant was assayed within 5 min by addition of 100 µl supernatant to 150 µl buffer so that the final concentration of the reaction mixture was 15 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 2.25 mM dithiothreitol, 0.01 mg/ml pyridoxal-5-phosphate, and 500 µM L-ornithine (specific activity, 1.2 mCi/mmol). After a 30-min incubation at 37°C, the reaction was stopped by addition of 0.5 ml 5 N HCl. The 14CO2 released was trapped on a filter paper containing NCS tissue solubilizer and counted after a 1-h incubation at room temperature. A DFMO-corrected blank was used for all calculations and was produced by incubating tissue supernatant with 10 mM DFMO for 10 min before assaying as above.

Protein Determination. Protein content was measured with bovine serum albumin as the standard and using the Read and Northcote modification of the Bradford protein assay (37). The sample homogenates were stored refrigerated until the protein assay could be performed, which was within 24 h.

Polyamine Assay. Polyamines were analyzed by reverse-phase, ion-paired high-performance liquid chromatography, using the method of Seiler and Knodgen (38) as applied to colorectal tissue by us (39). Briefly, tissue samples were dissected and disrupted in ODC assay buffer (see previous section on ODC analysis) and clarified at 2000 x g. The soluble fraction was made 0.2 N HClO4 and acid-soluble and -insoluble fractions were obtained by centrifugation at 12,000 x g. After addition of dianinoheptane as an internal standard, up to 200 µl of the acid soluble fraction was analyzed by high-performance liquid chromatography for polyamine contents. Using this method, the limit of detection was ~0.01 nmol for the amines putrescine, spermidine, spermine, and their acetyl derivatives. For the studies conducted here, in which tissue samples generally were prepared at a concentration which corresponded to ~1 mg/200 µl, the limit of polyamine detection was ~0.01 nmol polyamine/mg soluble protein. Protein contents were determined after solubilization in 0.5 NaOH, using the Bradford method (37).

Histology. Benign polyps with any component of villous architecture were categorized as villous adenomas secondary to the possibility that random sampling with endoscopic biopsies obtained a predominance of villous tissue. All other benign neoplastic polyps were designated as tubular adenomas.

Results

Sixty-six paired biopsies were obtained from a neoplastic lesion and adjacent mucosa in 55 patients (46 males; 9 females) ranging in age from 54 to 94 years (mean age, 69 years). Eighteen patients had either a cancer (n = 13) or cancerous polyp (n = 5). Of 48 benign neoplastic polyps, 20 were tubular adenomas and 28 villous adenomas. The distribution of lesions is given in Table 1. Eighty-seven % of neoplasms were located distal to the splenic flexure. Polyp sizes ranged from 1 to 5 cm.

Putrescine, spermidine, and spermine were the only amines detectable in neoplastic and normal mucosa. Although spermine and spermidine were measurable in all samples, putrescine was undetectable in 30% of polyps and 15% of normal mucosa specimens.

Values of ODC activity and polyamine content in neoplastic tissue relative to adjacent flat mucosa within individuals are illustrated in Fig. 1. Tubular and villous adenomas were analyzed separately to determine if ODC or polyamines could differentiate tissues of different malignant potential. Fig. 1 presents the difference between the ODC or polyamine determinations in neoplastic minus normal mucosa. A positive number indicates that activity or content was higher in the neoplasm than in the normal mucosa. ODC and polyamine levels in neoplastic tissues were greater than from the mucosa in the majority of polyp-mucosa (both tubular and villous adenomas) and cancer-mucosa pairs, except for a reversed trend with putrescine content in villous adenomas relative to mucosa.

Despite a reversal in some lesion-mucosa pairs, cancer and polyp tissue overall demonstrate significantly higher ODC activity and spermidine content than adjacent mucosa when analyzed by paired t test (Table 2). Although spermine content in benign polyps was also significantly greater than adjacent mucosa, the difference between cancer tissue and mucosa did not reach significance. Conversely, putrescine concentrations were significantly higher in cancers than mucosa, but no difference was found in polyp-mucosa pairs. When analyzed collectively, mean cancer tissue ODC activity is significantly greater than in benign polyp tissue (Table 2: P = 0.01 by t test). However, mean spermidine, spermine, and putrescine content in cancers and polyps were not different. Additionally, ODC activity and polyamine content within tubular adenomas and villous adenomas were not significantly different.

Spermine and spermidine contents were highly correlated both in neoplasia (r = 0.86) and flat mucosa (r = 0.85). Modest correlations were evident between putrescine and spermine in neoplasia (r = 0.21) and mucosa (r = 0.35) and between putrescine and spermidine in neoplasia (r = 0.35) and mucosa (r = 0.33). Conversely, ODC activity was not significantly related to polyamine amounts in either tissue type (r ranging from -0.24 to 0.10) except for putrescine content in neoplasia (r = 0.64).

Table 1 Distribution of colorectal neoplasia in 55 patients

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Cecum</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>3</td>
<td>4.5</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Descending colon</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>18</td>
<td>31.8</td>
</tr>
<tr>
<td>Rectum</td>
<td>14</td>
<td>25.4</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>100</td>
</tr>
</tbody>
</table>
Mucosal biopsies from the rectosigmoid junction (20 cm from the anus) were obtained in 10 patients (8 males; 2 females) ranging in age from 48 to 79 years (mean age, 67 years) without a history of colorectal neoplasia who underwent colonoscopy for various clinical indications. These examinations were normal including no evidence of mucosal inflammation. This group was well matched by age, sex, and site of mucosal biopsy (80% of mucosal biopsies taken from patients with neoplasia were from the sigmoid colon or rectum) with the subgroups harbor-
ODC and Polyamines in Colorectal Neoplasia and Mucosa

ing neoplasia. Flat mucosa ODC activity and polyamine content in these "normals" are compared with the mucosal values from patients with neoplasia in Table 3. Although the mean ODC activity in flat mucosa from normals is lower than in patients with polyps or cancer, there is no statistical difference between groups.

Discussion

ODC activity is highly inducible and increased activity with comparatively high levels of polyamines accompanies normal cellular proliferation and differentiation (1-11). Enhanced ODC activity is also evident during the initiation and promotion phases of experimental carcinogenesis (12-15). A variety of neoplasms exhibit increased ODC activity relative to normal tissue of the same organ. We evaluated this relationship in colorectal tissues and also measured polyamine content, supposing these parameters would reflect ODC activity in vivo. The results are in accordance with previous studies reporting elevated ODC activity in most colorectal carcinomas and benign neoplastic polyps (19-31, 35) and also demonstrate increased relative amounts of spermine and spermidine in polyps in addition to cancers. Although putrescine was elevated in cancer tissue, this was not found in polyps.

Although we found colonic neoplastic tissue in general to demonstrate high relative ODC activity and polyamine content, these parameters did not discriminate between neoplastic tissue types with different malignant potential (e.g., tubular adenoma; villous adenoma; carcinoma), except that ODC was higher in cancers compared to polyps. In addition, a minority of neoplasms had ODC activity and polyamine content lower than a paired sample of adjacent normal-appearing mucosa. This reversal in ODC activity ratio in a subset of lesions might not be representative of the general proliferative activity. Hietala (23) has described 2- to 4-fold differences in ODC activity between multiple samples from different areas within individual tumors. We also found differences in ODC activity within biopsy samples obtained on different days from the same tumor (data not shown). Although less likely, the concept of biological heterogeneity related to ODC activity might also apply to normal-appearing colorectal mucosa, similar to focal islands of dysplasia of varying severity in the setting of ulcerative colitis defined by flow cytometry (45). Levine (46) has also described fields of hyperproliferation defined by flow cytometry in flat mucosa adjacent to some colonic neoplasms.

Several technical factors may account for some of the divergence of findings between studies. Porter (20) reported substantially lower ODC activity in both tumor and normal mucosa specimens obtained from surgical resections compared with preoperative endoscopic biopsies of the same areas. The duration between tissue resection or biopsy and tissue freezing may have been longer with the surgical specimens, an obvious confounding issue with the extremely short half-life of ODC. We have examined the effect of different methodological factors on calculated ODC activity and found significant variation dependent upon the type of buffer, the protein content of the reaction mixture, and the nature of the blank used (36). Data has been reported that ODC activity within biopsy samples significantly diminishes with prolonged storage at -20°C (47).

Collectively, the multitude of potential biological and technical sources of variation in ODC activity likely account for discrepancies reported by different investigators, including the very large incongruity between ranges of ODC activity (values ranging from a few pmol to nmol CO2/mg protein/h). Different reports by the same investigators have described ODC activity ranges varying to this extent (35, 48-50).

ODC activity in flat colorectal mucosa has been reported as highly discriminatory in defining risk for familial polyposis coli (35). These investigators and others

<table>
<thead>
<tr>
<th>Table 3</th>
<th>ODC activity and polyamine content (mean ± SD) in normal colorectal mucosa*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplasia status of subjects</td>
<td>n</td>
</tr>
<tr>
<td>Cancer</td>
<td>18</td>
</tr>
<tr>
<td>Benign neoplastic poly</td>
<td>37</td>
</tr>
<tr>
<td>Adenoma</td>
<td>12</td>
</tr>
<tr>
<td>Villous</td>
<td>25</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
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</tbody>
</table>

* Means do not differ significantly between categories of patients by ANOVA analysis of variance.

$^{ac}$ Subjects categorized by most advanced lesion.

$^{ad}$ One unit = 1 nmol CO2/60 min/mg protein.

$^{ac}$ Non-detectable levels of putrescine were assigned a value of 0.01 nmol (the lower limit of detection); thus the actual mean values are ≤ to those shown.
have extended this observation to patients with sporadic colorectal neoplasia, in that ODC activity within normal mucosa was found to be significantly higher in individuals with neoplasia compared to those without these lesions (although a large overlap in ODC activity between patient groups was noted) (19, 21, 25, 32, 48, 50, 51). Mucosal polyamine content has also been reported to be significantly increased in this context (21, 30, 51). The primary implication from these studies is that elevated ODC activity reflects a presumed generalized mucosal hyperproliferation (field defect hypothesis) and might therefore serve as a marker for the presence of neoplasia elsewhere in the colon or rectum. In this context, rectal mucosal ODC activity and/or polyamine content obtained at the time of screening sigmoidoscopy could potentially be utilized to determine which patients should undergo screening colonoscopy. Although no longitudinal data has been reported to support the notion that mucosal ODC activity predicts future risk for neoplasia development (as opposed to concurrent, synchronous lesions as referred to above), a marker with this ability could be utilized to tailor future surveillance strategy for individuals (i.e., how frequently to examine the colon) and to identify appropriate candidates for intervention therapy with dietary alterations or pharmacological agents. We, however, did not find a significant difference in ODC activity or polyamine content in the flat mucosa from subjects without neoplasia compared to those harboring neoplastic polypos or cancer. Our small sample size raises the issue of a type II error; however, other investigators with dietary alterations or pharmacological agents. We, however, did not find a significant difference in ODC activity or polyamine content in the flat mucosa from subjects without neoplasia compared to those harboring neoplastic polypos or cancer. Our small sample size raises the issue of a type II error; however, other investigators have also found ODC not to be discriminatory for neoplasia risk (31–34, 49, 52, 53). These divergent reports again emphasize that although most studies find ODC and polyamine content activity to be generally elevated in colorectal neoplastic tissue, substantial variability and overlap between tissues and patients exist. Thus far colon neoplasia risk stratification utilizing tissue bioanalysis has generally concentrated on a single marker, such as mucosal crypt labeling index or ODC. As discussed above for ODC, these assays when used alone do not appear to be highly discriminatory for neoplasia risk assessment. Consequently, future efforts should attempt to use a combination of markers, perhaps together with other parameters such as diet or family history, to determine a risk profile capable of predicting the presence of neoplasia in an individual subject.

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References


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