Levels of Colorectal Ornithine Decarboxylase Activity in Patients with Colon Cancer, a Family History of Nonpolyposis Hereditary Colorectal Cancer, and Adenomas

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Abstract

Ornithine decarboxylase (ODC), a key enzyme in mammalian polyamine biosynthesis, has been proposed to be a marker of colonic epithelial cell proliferation and risk for colorectal cancer. We investigated the basal levels of ODC activity in sigmoid and rectal mucosae, and basal and tumor promoter 12-O-tetradecanoylphorbol-13-acetate-induced levels of skin ODC activity in individuals with a personal history of colon cancer (n = 9 colon; n = 58 skin), a family history of nonpolyposis hereditary colorectal cancer (n = 49; n = 42), adenomas (n = 16; n = 40), and healthy, family history-negative control subjects (n = 40; n = 79). Using a fresh tissue assay and samples obtained after a standard colon lavage preparation, colon mucosal ODC levels ranged from 0 to 192 pmol/mg/h (sigmoid, 0–163 pmol/mg/h; mean, 36 ± 32 pmol/mg/h; rectum, 0–192 pmol/mg/h; mean, 35 ± 32 pmol/mg/h). No differences among the four groups of subjects were found for either colon or skin ODC levels, and there were no sex differences overall or in any group. These results are not compatible with the suggestion that ODC levels are a useful marker of risk for colorectal cancer.

Introduction

Colorectal cancer is a major cause of morbidity and mortality in western societies. While some modest improvements in survival may be evident in certain studies in the United States, primarily consequent to greater frequency of diagnosis of earlier stage disease, there are major needs to define reversible or removable etiological factors for this disease and to be able to identify individuals at risk. Recent studies of colon mucosa have suggested some specific epithelial proliferative changes which may be useful indicators of early stages of colon carcinogenesis in individuals. Lipkin et al. (1, 2) have described an assay which quantitates DNA synthesis and may allow characterization of high-risk colon epithelium. A possibly easily measurable "marker," ODC3 has also been described in reports by Luk et al. (3, 4). ODC is a particularly interesting enzyme because of its role in the promotion of stage of carcinogenesis (5). Studies of ODC in colonic tissues have been small and have been conducted under a variety of methodological and clinical conditions (3, 4, 6–10). Because ODC is present in many human tissues and is more accessible in the skin, we initiated studies of this enzyme in punch skin biopsies as a means of monitoring potential chemopreventive agent effects (11). We report here our investigation of this enzyme marker in colon and skin biopsies in 226 individuals with a personal history, at risk for, and without risk factors for colorectal cancer. Our results, using a fresh tissue assay, are at variance with other reports in several respects and suggest that many variables other than risk status for colorectal cancer influence the measurable tissue ODC activity.

Materials and Methods

Subjects. Individuals were recruited and managed under a protocol approved by the University of Wisconsin Committee for the Protection of Human Subjects (86-840-264). Two hundred and twenty-six individuals were recruited over a 3-year period through different mechanisms which related to their risk status. Individuals with colorectal cancer (n = 58) (mean age, 63.5 years; range, 31–86 years) or adenomas (n = 40) (mean age, 60.9 years; range, 35–84 years) and some healthy, risk factor-free subjects (n = 39) were recruited at the time of endoscopic screening procedures in a gastrointestinal endoscopy clinic. The medical records of these subjects were reviewed and confirmed their respective risk status. Individuals with family history of nonpolyposis hereditary colorectal cancer (n = 49) (mean age, 38 years; range, 18–64 years) were recruited from 17 separate families registered in the University of Wisconsin Clinical Cancer Center Prevention Clinic registry (12). These individuals also had confirmation of their risk status through extensive medical record documentation of their family histories (13) and had to be first-degree relatives of affected individuals, such that they appeared to be at 50% risk for cancer. The remaining healthy, risk factor-free subjects (n = 40) were recruited under a separate protocol
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Procedures. All colon mucosal biopsy specimens were obtained using cold 2-mm forceps through either a colonoscope or a flexible sigmoidoscope as the instrument was being withdrawn. Pairs of colon biopsies were obtained at each of two sites: at about 10 cm and at 30 cm from the anal verge. All colon mucosal biopsies were obtained after lavage preparation. Subjects were given a 1-gallon “Colyte” lavage to take 12 h before the endoscopic procedure, along with one 10-mg tablet of metachlopramide. After this they ate nothing until the procedure was completed; this was usually in the morning. Biopsies were immediately transferred to ice-cold homogenization buffer in coded vials and transported to the laboratory, where measurements were usually initiated within 30 min.

Pairs of skin biopsies were obtained using a 2-mm biopsy punch after brief cleansing of the inner aspect of the upper arm with an alcohol swab. Local anesthesia of the biopsy sites was achieved by creating a stretching of the skin with an intradermal injection of normal saline (11).

ODC Induction in Skin Biopsies by TPA. A single skin-punch biopsy sample was taken and transferred to a 15-ml Corex tube containing 1 ml serum-free minimal essential medium at 37°C. The medium was then gassed with 95% O₂ and 5% CO₂ for 1 min. TPA (2.5 × 10⁻⁵ M) was then added in ethanol (final concentration in the medium, 0.5%), and the tubes were sealed and incubated in a shaking water bath at 37°C for 6 h. Skin biopsy samples were removed to determine ODC activity (14).

ODC Assay. Mucosal biopsies were homogenized in 500 μl of the homogenization buffer (50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.1 mM pyridoxal phosphate) and then centrifuged. ODC activity in the soluble extract was determined by measuring the release of CO₂ from D,L-[¹⁴C]ornithine hydrochloride. The assay mixture contained 20 mM Tris-HCl (pH 7.5), 0.32 mM pyridoxal phosphate, 4 mM dithiothreitol, 0.4 mM EDTA, 12 μM L-ornithine hydrochloride, 0.02% Brij 35 containing 0.25 μCi D,L-[¹⁴C]ornithine hydrochloride, and enzyme in a total volume of 0.25 ml. After incubating at 37°C for 60 min in 15-ml Corex centrifuge tubes equipped with rubber stoppers and center well assemblies, the reaction was stopped by adding 0.5 ml of 2 M citric acid. The incubation was continued for at least another hour to ensure complete absorption of ¹⁴CO₂ by the ethanola-mine:methoxyl ethanol (0.2 ml; 2:1 v/v) contained in the center well. Finally, the center well containing the ethanala-mine:methoxyl ethanol was transferred to a vial containing 5 ml toluene-based scintillation fluid and 1 ml ethanol, and the associated radioactivity was determined in a liquid scintillation counter. The ODC assay was linear with time up to 60 min at both 2.5 and 50 nmol (200 μM) L-ornithine and with extract volume up to an equivalent of 300 μg protein. The ODC assay mixture contained less than 200 μg protein from soluble mucosal extract.

The specific assay procedures for colon and skin biopsies including TPA-induced skin ODC activity have been described in further detail previously (9, 10). In preliminary investigations to define how to detect reproducibly and with reasonable sensitivity the level of ODC activity in a small colon mucosal sample, we characterized ODC activity in a surgical colon specimen. In this investigation, ODC activity in the whole homogenate was compared with the soluble (30,000 × g) and particulate fractions.

Additionally, we determined (a) the effect of substrate (L-ornithine) concentration, (b) sensitivity to α-difluoromethylornithine; and (c) the effect of freezing the mucosal extract on ODC activity. The results of these studies (see below) led to the standard procedure, of which three methodological features should be emphasized. First, we measured supernatant fractions (after 30,000 × g centrifugation) ODC activity after homogenization of the colon mucosal biopsies. Unfractionated whole homogenate ODC activity has been measured in other studies (3). Second, the concentration of L-ornithine used was 12 μM, which is one-eighth of the concentration used in other studies (3). Finally, fresh tissue was assayed in the study reported here, while measures in other reports have been made after freezing specimens to −70°C (3, 6, 8).

Statistical Analyses. The distributions of values for levels of colon ODC activity were skewed to the left. When data were logarithmically transformed (log value + 1) the distributions became more symmetric. Comparisons of ODC activity levels by group were done with one-way analysis of variance.

Results

The results of the preliminary investigations are shown in Tables 1–3. Clearly, ODC is exclusively localized in the soluble fraction (Table 1). These results are in accord with previous findings, in which ODC has been shown to be an entirely soluble enzyme (5). Furthermore, substrate saturation is the result of enzyme concentration and the amount of colon mucosal extract (less than 450

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total ODC activity (pmol/60 min)</th>
<th>Specific activity (pmol/60 min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>Whole homogenate</td>
<td>569</td>
<td>27.45</td>
</tr>
<tr>
<td>Supernatant</td>
<td>614</td>
<td>44.7</td>
</tr>
<tr>
<td>Pellet</td>
<td>96</td>
<td>64.1</td>
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Table 1 Characterization of ODC activity in human colon mucosal specimen

A human colon specimen obtained during surgery was immediately taken to the laboratory in homogenization buffer. Mucosa was dissected from the colon specimen, homogenized in 1 ml buffer (whole homogenate), and centrifuged at 30,000 × g for 30 min (supernatant). The pellet was washed once with the homogenate and reconstituted in 1 ml homogenization buffer. The whole homogenate, supernatant, and the pellet were used for assaying ODC activity, using either 2.5 or 50 nmol L-ornithine in substrate concentrations.
ODC activity was determined 4 weeks later. The numbers apply to sigmoid and rectum results, respectively. There are no significant differences among the groups for sigmoid or rectal values. Basal skin ODC levels ranged from 0 to 192 pmol/mg/h (SD 35). There were no significant differences among groups of patients for either skin ODC measure, and there were no significant differences in the measured levels in any group when results were subgrouped by sex. There were no significant correlations for any group between the sigmoid and rectum levels and the basal skin ODC levels. There was no significant correlation between basal skin ODC levels and age. There was a small negative correlation between TPA-induced ODC levels and age for all groups combined (r = −0.21; P < 0.01; n = 154).

Discussion

Studies of biological markers of disease risk are remarkably complex. Complete control of the precise patient populations and the circumstances of obtaining and assaying the specific marker is difficult. The reported study was conducted over 3 years, during which controls and other subjects were not studied at evenly distributed time points. The laboratory procedures for the ODC assay differ from those used by other investigators in what may be important respects: fresh instead of flash-frozen tissue is assayed; the concentration of L-ornithine is lower (which increases test sensitivity); and the supernatant fraction of ODC is what we have measured, although our comparative study (Table 1) suggests a minimal contribution of this last procedure to differences in results.

There are, however, significant strengths to the reported work. We have studied significantly larger numbers of subjects than others have reported upon (3, 4, 6–8). The longer period of study allows peculiar variables (seasonal effects, or the particular technique of an individual laboratory technician) to become less important. We initially studied some patients whose specimens were not assayed for over 2 h after procurement; ODC levels in these were markedly lower. The vast majority of subjects had medical record confirmation of their risk assessment for differences based on sex also showed no differences for either sigmoid or rectal values (Table 5). The sigmoid and rectal measures for each group are positively correlated (Spearman correlation coefficients 0.41–0.54; P = 0.16–0.0009). There were no significant associations between sigmoid and rectal ODC levels and age for the four groups together or within each group.

Nonpolyposis hereditary colorectal cancer.
status, and colon samples were all obtained at standard conditions, an important consideration since we have found that endoscopy preparation significantly influences measured ODC activity levels (9).

Our results, all of which suggest no helpful role for the measurement of ODC levels in colon or skin specimens in the assessment of risk status of individuals, differ from the results of several small published studies (3, 4, 6–8). The higher levels of ODC activity in colon mucosae as compared to basal skin samples, obtained with the known rapid inducibility of this enzyme and the large SD, suggest that in the colon local inducers are often active. Further evidence for this is provided in another study in which we found an absence of correlation between ODC levels in colon mucosae measured at two time points separated by 8 weeks (10). Studies of large populations, under very carefully specified conditions, with the development of replicable and strongly positive findings will be necessary to further support the case that ODC tissue activity levels in individuals can be measured with any expectation that colorectal cancer risk status is being assessed.

References
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