Effect of Chemopreventive Agents on Intermediate Biomarkers during Different Stages of Azoxyymethane-induced Colon Carcinogenesis

Jagveer Singh, Gary Kelloff, and Bandaru S. Reddy

Abstract

Chemoprevention of colon cancer is emerging as an alternative to therapy with a broad potential for reducing cancer incidence in defined high-risk groups and the general population. Besides several chemopreventive agents in use and under investigation, D,L-α-difluoromethylornithine (DFMO) and piroxicam have been shown to effectively inhibit colon carcinogenesis in rodents. A variety of proliferation-related parameters have been suggested as potential intermediate markers of cancer risk that could be used to monitor the progress of chemoprevention in clinical trials. We have investigated the effect of chemopreventive agents, DFMO, and piroxicam on mucosal ornithine decarboxylase (ODC) and tyrosine-specific protein kinase (TPK) activities during different stages of azoxymethane (AOM)-induced colon carcinogenesis in male F344 rats in order to examine the plausibility of using these enzymes as intermediate biochemical markers of colon cancer. Groups of male F344 rats were fed modified AIN-76A diets containing 0 or 150 ppm piroxicam or 4000 ppm DFMO and given s.c. injections of AOM dissolved in normal saline at a dose of 15 mg/kg body weight/week, once weekly, for 4 weeks. Vehicle control groups received s.c. equal volumes of normal saline. Groups of animals were then sacrificed at 0, 4, 16, 24, and 32 weeks after AOM or saline treatment, and their colonic mucosa was analyzed for ODC and TPK activities. AOM treatment significantly increased mucosal ODC as well as TPK activities. AOM-induced ODC and TPK activities were significantly suppressed by dietary DFMO progressively at all stages of colon carcinogenesis. Dietary piroxicam increased AOM-induced mucosal ODC and TPK activities but significantly reduced tumor incidence as well as tumor multiplicity. DFMO exerted a more pronounced inhibitory effect on AOM-induced colon tumor development. These results emphasize the importance of development of agent-specific intermediate biomarkers to be used as effective predictors of colon carcinogenesis.

Introduction

Colorectal cancer is a major cause of morbidity and mortality among men and women in the United States, afflicting 167,000 people and causing about 65,000 deaths in 1991 (1). Despite several advances made in the treatment of colon cancer, the cure rate has remained largely unchanged in the past two decades (2). Chemoprevention, which is emerging as an alternative to therapy, relies on the concept of inhibition or reversal of cancer formation through chemical intervention (3, 4). Several chemopreventive agents in use and under investigation include retinoids, purified dietary constituents, vitamins, micronutrients, antimutagens, dithiolthiones, metabolic products, protease inhibitors, and polyamines and prostaglandin synthesis inhibitors. DFMO, a specific, enzyme-activated, irreversible inhibitor of ODC activity, and piroxicam, a nonsteroidal, antiinflammatory drug, have been shown to effectively inhibit carcinogen-induced colon tumor development in rodents (5, 6). Recent preclinical studies in laboratory animals and clinical investigations in high-risk patients of colon cancer have identified DFMO and piroxicam as ideal candidates for chemoprevention trials. However, the success of such clinical trials has been hampered by the lack of appropriate intermediate biomarkers or end points which could indicate the progress of chemoprevention and predict the subsequent reduction of cancer occurrence.

Intermediate end points may be defined as measurable markers of cellular or molecular events associated with specific stages of the multistep evolution and progression of carcinogenesis. This indicates that the risk of carcinogenic transformation must correlate with quantitative degree and pattern of biomarker expression. ODC, which catalyzes the conversion of ornithine to putrescine in a rate-limiting step of crucial polyamine biosynthesis, plays an important role in normal and neoplastic cell proliferation (7) and is associated with the tumor-promoting ability of a variety of agents (7–8). Increased ODC activity has been demonstrated in benign colonic adenomas (9–12) as well as normal appearing mucosa adjacent to adenomas (13–15), reflecting the underlying hyperproliferative state of colonic mucosa. Significantly

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2 To whom requests for reprints should be addressed, at American Health Foundation, Division of Nutritional Carcinogenesis, One Dana Road, Valhalla, NY 10595.

3 The abbreviations used are: DFMO, D,L-α-difluoromethylornithine; ODC, ornithine decarboxylase; TPK, tyrosine-specific protein kinase; AOM, azoxymethane; DTT, dithiothreitol.
high levels of ODC activity have been reported for sev-
eral rodent carcinogenesis models (16–17). TPks which catalyse phosphorylation of tyrosine res-
vides of protein substrates are important growth-pro-
moting signal-transducing enzymes involved in cellular
growth and differentiation (18–19). Tyrosine phos-
phorylation is now recognized as an important regulatory
mechanism in response to a number of processes, in-
cluding the action of several growth factors and onco-
genes. Enhanced TPK activity has been demonstrated in
neuroblastoma (20), rhabdomyosarcoma (21), and mam-
mary (22) and colon (23) carcinomas. A significant num-
ber of TPKs that are products of protooncogenes have
been implicated in the etiology of a number of human
cancers (24).

Do these intermediate biomarkers, ODC and TPK,
predict the known chemopreventive influence of DFMO
and piroxicam on experimental colon tumor develop-
ment? This study was designed to investigate the effect
of chemopreventive agents, DFMO and piroxicam, on
mucosal ODC and TPK activities during different stages
of AOM-induced colon carcinogenesis in male F344 rats
in order to examine the plausibility of using these en-
zymes as intermediate biochemical markers for colon
cancer. The major goal of this study was to determine
which intermediate biomarkers, if altered by these chem-
opreventive agents, could be used as effective predictors
of colon cancer.

Materials and Methods

Animals, Diets, and Carcinogen. Weaning male F344
rats were obtained from Charles River Breeding Labor-
yories (Kingston, NY). Piroxicam and DFMO were gen-
erous gifts from Pfizer Central Research (Groton, CT) and
Merrill-Dow Research Institute (Cincinnati, OH), respec-
tively. AOM (COS: 25843-45-2) was purchased from Ash-
Stevens (Detroit, MI). All ingredients of semipurified diet
were obtained from Dytts, Inc. (Bethlehem, PA) and
stored at 4 °C prior to preparation of the diets. Piroxicam
and DFMO were incorporated into the control diet at
dose levels of 150 ppm and 4000 ppm, respectively. All
control and experimental diets were prepared weekly in
our laboratory and stored in a cold room.

Male F344 rats received at weaning were quarantined
for 10 days. All animals were housed in plastic
 cages with filter tops under controlled environmental
conditions of 21 °C temperature, 50% humidity, and a
12-h light/dark cycle. At 5 weeks of age, all animals were
assigned to AOM-treated and vehicle-treated groups and
fed the control diet (Ref. 25; Table 1).

Experimental Procedure. Beginning at 6 weeks of age,
animals from AOM- and vehicle-treated groups were re-
allocated into different dietary subgroups and fed one of
the experimental diets containing 150 ppm piroxicam
or 4000 ppm DFMO or control diet (Table 1). The ration-
ale for selecting 150 ppm piroxicam and 4000 ppm
DFMO has been based on our previous results, which
indicated a colon tumor-inhibitory effect of these agents
at these levels (5). One week later, animals intended for
carcinogen treatment were administered s.c. AOM dis-
olved in normal saline at a dose rate of 15 mg/kg body
weight/week, once weekly, for 4 weeks. Animals in-
tended for vehicle treatment received s.c. an equal vol-
ume of normal saline. Animals were maintained on their
respective dietary regimens until termination of the ex-
periment. Body weights were recorded once weekly
during carcinogen treatment and then every 4 weeks.
Ten animals treated with AOM or vehicle from each
dietary subgroup were sacrificed by decapitation at 0, 4,
16, 24, and 32 weeks after the last AOM or saline
injection. Their colons were resected and opened longi-
tudinally, and the contents were flushed with saline.
Colon tumors, if any, were removed before scraping the
colon mucosa with a microscope slide. Mucosal scrapings
and tumors were quick-frozen in liquid nitrogen and
stored at −80 °C until use. No colon tumors were ob-
erved until 16 weeks after the AOM or vehicle
treatment.

Determination of ODC Activity. Mucosal scrapings
and tumor tissue were homogenized separately with polytron
homogenizer in 10 volumes of 50 mM sodium phosphate
buffer, pH 7.2 (containing 5 mM DTT, 0.2 mM pyridoxal
phosphate, 0.1 mM EDTA), and centrifuged at 40,000 ×
g for 30 min at 4 °C. Clear supernatant fraction was used
for ODC assay. Enzyme activity was determined by meas-
uturing 14CO2 liberated from L-[1-14C]ornithine as
described elsewhere (26). The standard assay mixture con-
tained, in a total volume of 0.25 ml, an aliquot of superna-
tant, 50 mM sodium phosphate buffer (pH 7.2), 0.2
mM pyridoxal phosphate, 5 mM DTT, 0.1 mM EDTA, 0.4
mM L-ornithine, and 0.50 μCi D,L-[1-14C]ornithine hyd-
chioride (56.6 mCi/mmol; Amersham Co., Arlington
Heights, IL). The reaction mixture was incubated at 37 °C
for 1 h in a 16 × 100 mm glass tube sealed with rubber
stopper supporting a center well (Kontes, Morton Grove,
IL). The released 14CO2 was trapped on a microglass-
fiber filter disc (934-AH, GFA; Whatman) soaked in satu-
rated solution of barium hydroxide. The reaction was
stopped by injecting 0.1 ml of 2 N sulfuric acid through
a rubber septum directly into the reaction mixture.
Incubation was continued for an additional 1 h to com-
pletely trap the released 14CO2. Filter paper along with
the center well was then transferred to a scintillation vial,
and radioactivity was counted in 10 ml of scintillation
cocktail (Scintisol; ISOLAB, Inc.) ODC activity is ex-
pressed as pmol 14CO2 released/mg protein/h.

Determination of Protein Tyrosine Kinase Activity. TPK
activity was measured in both cytosolic and membrane

<table>
<thead>
<tr>
<th>Table 1 Percentage composition of experimental semipurified diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet ingredients</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>α,β-Methionine</td>
</tr>
<tr>
<td>Cornstarch</td>
</tr>
<tr>
<td>Dextrose</td>
</tr>
<tr>
<td>Corn oil</td>
</tr>
<tr>
<td>Alphacel</td>
</tr>
<tr>
<td>Mineral mix, AIN-76A</td>
</tr>
<tr>
<td>Vitamin mix, AIN-76A</td>
</tr>
<tr>
<td>Choline bitartrate</td>
</tr>
<tr>
<td>Piroxicam</td>
</tr>
<tr>
<td>DFMO</td>
</tr>
</tbody>
</table>

* Adopted from American Institute of Nutrition Reference Diet (AIN-76A), with the modification of source of carbohydrate.
+ Piroxicam and DFMO were added to the diets at the expense of cornstarch.
fractions. Colonic mucosal or tumor samples were homogenized in 5 volumes of 10 mM Tris-HCl, pH 7.5, containing 0.25 M sucrose, 1 mM MgCl₂, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.5 mg/ml aprotinin. All debris and nuclei were removed by centrifuging at 800 X g for 10 min at 4°C. The supernatant was further centrifuged at 40,000 X g for 30 min at 4°C, and the resulting supernatant fraction was used as cytosol for assaying TPK activity. The 40,000 X g pellet (membrane fraction) was resuspended in 50 mM Tris-HCl, pH 7.5, containing 20 mM magnesium acetate, 5 mM NaF, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.5% Nonidet P-40; sonicated three times for 10 s each time; and centrifuged at 40,000 X g for 30 min at 4°C. The supernatant (solubilized membrane) was used to assay TPK activity. TPK activity was measured using a tyrosine kinase-specific synthetic polymer as substrate to assay TPK activity. TPK activity was measured using a tyrosine kinase-specific synthetic polymer as substrate to assay TPK activity.

Table 2: Body weights of male F344 rats treated with AOM or vehicle and fed control or experimental diets

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body weight (g) at weeks after carcinogen/vehicle treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>AOM-treated</td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>216 ± 4.2</td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>216 ± 2.2</td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>210 ± 1.8</td>
</tr>
<tr>
<td>Vehicle-treated</td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>239 ± 1.6</td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>238 ± 2.2</td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>235 ± 3.2</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM.

Table 3: Effect of DFMO and piroxicam on tumor incidence and multiplicity during different stages of AOM-induced colon carcinogenesis in male F344 rats

<table>
<thead>
<tr>
<th>Chemopreventive agents</th>
<th>0</th>
<th>4</th>
<th>16</th>
<th>24</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of</td>
<td>Total</td>
<td>No. of</td>
<td>Total</td>
<td>No. of</td>
</tr>
<tr>
<td></td>
<td>animals</td>
<td>tumors</td>
<td>animals</td>
<td>tumors</td>
<td>animals</td>
</tr>
<tr>
<td>Control diet</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate percentage of animals developing grossly visible tumors.

Statistical Analysis. Wherever applicable the data were analyzed using an unpaired t test and one-way analysis of variance.

Results

General Observations

The body weights were comparable in all the animals fed control and experimental diets (Table 2). However, the body weights of AOM-treated animals were slightly lower than those of vehicle-treated animals due to AOM carcinogenicity and consequent tumor burden.

Tumor Incidence

Table 3 presents gross observations of the effect of piroxicam and DFMO on incidence and multiplicity of colon tumors observed at different stages. AOM induced multiple tumors toward the end of experiment in about 100% of animals fed the control diet. Animals fed the piroxicam diet developed relatively small colon tumors. As reported earlier using different protocol (3), the colon (1:1) and 100% ether. Radioactivity was counted in 10-mI scintillation cocktails (Scintiverse; Fisher Scientific, Fair Lawn, NJ). TPK activity was corrected for endogenous phosphorylation by assaying in the absence of polymer substrate. The enzyme activity is expressed as pmol [32P] ATP incorporated/mg protein/min.

Protein Determination. Protein content in cytosol and membrane fractions was determined by the method of Bradford (29), using bovine serum albumin as the standard.

Table 1: Effect of DFMO and piroxicam on tumor incidence and multiplicity during different stages of AOM-induced colon carcinogenesis in male F344 rats

<table>
<thead>
<tr>
<th>Chemopreventive agents</th>
<th>0</th>
<th>4</th>
<th>16</th>
<th>24</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of</td>
<td>Total</td>
<td>No. of</td>
<td>Total</td>
<td>No. of</td>
</tr>
<tr>
<td></td>
<td>animals</td>
<td>tumors</td>
<td>animals</td>
<td>tumors</td>
<td>animals</td>
</tr>
<tr>
<td>Control diet</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate percentage of animals developing grossly visible tumors.

† Numbers in brackets are mean ± SEM.

‡ Significantly less than that in control group: 1, P < 0.01; 2, P < 0.001.
tumor multiplicity was significantly reduced in animals fed the piroxicam diet ($P < 0.01$). The incidence and multiplicity of AOM-induced colon tumors were significantly decreased in animals fed the experimental diet containing 4000 ppm DFMO ($P < 0.001$). The latency period was greatly extended by dietary DFMO and piroxicam.

**ODC Activity**

**Induction of Colonic Mucosal ODC Activity.** Table 4 summarizes the colonic mucosal ODC activity at different time periods after AOM or saline treatment. AOM administration induced persistently elevated levels of ODC activity throughout the duration of this experiment. Although AOM-stimulated ODC activity exhibited a distinctly biphasic elevation pattern as demonstrated earlier (16), the differences in levels of ODC activity between AOM-treated and vehicle-treated animals remained highly significant irrespective of dietary intake of chemopreventive agent ($P < 0.001$).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Weeks after carcinogen treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>AOM-treated</td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>0±1</td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>0±1</td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>0±1</td>
</tr>
<tr>
<td>Vehicle-treated</td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>0±1</td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>0±1</td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>0±1</td>
</tr>
</tbody>
</table>

*Table 4 Effect of DFMO and piroxicam on colon mucosal ODC activity during different stages of AOM-induced colon carcinogenesis in male F344 rats.*

* ODC activity is expressed as pmol CO$_2$ released/hr/mg protein; values are mean ± SEM (n = 10).

* Significantly different from corresponding vehicle-treated groups: 1, $P < 0.001$; 2, $P < 0.01$.

$^c$ Significantly different from their respective control groups: 1, $P < 0.001$; 2, $P < 0.01$.

ODC activity by piroxicam and DFMO followed somewhat similar patterns of ODC activity, except that the differences in levels of TPK activity were not significant in the beginning of the experiment. AOM treatment significantly increased membrane-bound as well as cytosolic TPK activity in animals on the control diet as compared to their vehicle-treated counterparts throughout the duration of the experiment ($P < 0.05-0.001$). In addition, AOM administration induced progressively increasing levels of membrane-bound as well as cytosolic TPK activity. Dietary piroxicam significantly raised the TPK activity in AOM-treated as well as saline-treated groups as compared to their corresponding AOM- and saline-treated animals fed the control diet ($P < 0.05-0.001$). In contrast, animals fed the DFMO diet exhibited progressively decreasing levels of TPK activity in AOM-treated as well as saline-treated animals. In these animals, the levels of TPK activity were significantly reduced as compared to their corresponding control dietary groups ($P < 0.05-0.001$).

**Colonic Tumor TPK Activity.** As summarized in Table 5, the TPK activity of colon tumors of animals fed the control diet was about 6 times higher than that of their uninjured colon mucosa. Interestingly, both cytosolic and membrane-bound TPK activities were significantly suppressed in colon tumors of animals fed piroxicam as compared to those fed the control diet ($P < 0.01$).

**Discussion**

The results of this experiment demonstrate that dietary DFMO and piroxicam significantly reduce colon tumor occurrence and multiplicity and greatly increase tumor latency in AOM-treated animals, corroborating earlier findings (5–6, 16–17, 30–32). Our data clearly demonstrate distinct peaks of ODC activity manifesting the multistep characteristics of AOM-induced colon carcinogenesis. The phasic increase in the colonic mucosal ODC activity of AOM-treated rats was 10–11 times higher than that of saline-treated rats. The levels of ODC activity in colon tumors of rats fed the control diet were 140-fold higher than the colonic mucosal ODC activity of vehicle-treated rats on the control diet. The results of present investigation and earlier studies (7–17) suggest that mucosal ODC activity can be used as a reliable and sensitive marker for colon carcinogenesis.

We expected that DFMO would inhibit the intermediate biomarkers, since it had inhibited colon tumor
development; this was the case. DFMO has been shown to strongly inhibit cell proliferation and tumor development in rodents presumably by blocking ODC-catalyzed decarboxylation of ornithine into putrescine, thereby depleting intracellular polyamines (5–6). These polyamines are known to play an important regulatory role in the control of normal growth and neoplastic transformation (33–34). In most of the animal studies on chemoprevention reported to date, ODC inhibitors are shown to be very effective antitumor agents when treatment was begun prior to, concomitant with, or shortly after tumor initiation (31–32). In this study, where DFMO treatment began 1 week before AOM administration, we observed more than 90% inhibition of AOM-induced increase in ODC activity in a time-dependent manner, as well as suppression of colon tumor development. Significant differences in the levels of colonic ODC activity, piroxicam significantly inhibited cell proliferation and tumor development. 

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Membrane-bound or cytosolic activity</th>
<th>Weeks after carcinogen treatment</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM-treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>Membrane 109 ± 5b1, 99 ± 3b1, 169 ± 6b1, 239 ± 9b1, 255 ± 6b1, 1513 ± 200</td>
<td>0, 4, 16, 24, 32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytosolic 46 ± 3b1, 48 ± 2b1, 76 ± 2b1, 61 ± 2b1, 61 ± 2b1, 498 ± 64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>Membrane 123 ± 5b1, 112 ± 4b1, 306 ± 10b1, 415 ± 16b1, 386 ± 11b1, 494 ± 58b1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytosolic 48 ± 2b1, 49 ± 2b1, 77 ± 2b1, 81 ± 3b1, 59 ± 2b1, 196 ± 57b1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFMO, 4000 ppm</td>
<td>Membrane 98 ± 4b1, 80 ± 3b1, 113 ± 5b1, 73 ± 5b1, 66 ± 2b1, 161 ± 4b1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytosolic 44 ± 3b1, 42 ± 2b1, 38 ± 2b1, 24 ± 1b1, 24 ± 1b1, 65 ± 2b1</td>
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</tr>
<tr>
<td>Vehicle-treated</td>
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</tr>
<tr>
<td>Control diet</td>
<td>Membrane 85 ± 4, 77 ± 4, 91 ± 4, 86 ± 3, 80 ± 2</td>
<td>0, 4, 16, 24, 32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytosolic 37 ± 2, 31 ± 2, 41 ± 2, 39 ± 2, 42 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piroxicam, 150 ppm</td>
<td>Membrane 89 ± 3, 83 ± 4, 129 ± 5b1, 163 ± 7b1, 198 ± 6b1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytosolic 40 ± 2, 41 ± 3b2, 50 ± 2b2, 61 ± 3b1, 40 ± 3</td>
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<tr>
<td>DFMO, 4000 ppm</td>
<td>Membrane 80 ± 5, 66 ± 2b1, 60 ± 2b1, 50 ± 2b1, 53 ± 3b1</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Cytosolic 36 ± 2, 36 ± 2, 28 ± 2b1, 18 ± 1b1, 12 ± 1b1</td>
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</tr>
</tbody>
</table>

a TPK activity is expressed as [32P]ATP phosphorylated/mg protein/min; values are mean ± SEM (n = 10).

b Significantly different from corresponding vehicle-treated groups; 1, P < 0.001; 2, P < 0.01; 3, P < 0.05.

c Significantly different from their respective control groups; 1, P < 0.001; 2, P < 0.01; 3, P < 0.05.

To our knowledge, there are no data pertaining to the modulation of ODC activity by piroxicam. Indomethacin is the only compound of this category so widely studied in the context of its chemopreventive action. DeRubertis et al. (38), in a related study, demonstrated that treatment of rats with indomethacin rapidly increased colonic mucosal ODC activity, [3H]thymidine incorporation into mucosal DNA, and enhancement of proliferative activity of colonic epithelium. These authors also showed that concurrent administration of prostaglandin analogue in rats suppressed an indomethacin-induced increase in colonic mucosal ODC activity, DNA synthesis, and epithelial proliferative activity. Recently, Noguchi et al. (39) demonstrated that indomethacin decreased mammary tumor incidence and tumor multiplicity and increased tumor latency but significantly promoted proliferation of DMBA-induced tumors in both high- and low-fat diet groups as indicated by increased tumor size, increased bromodeoxyuridine labeling index, and decreased tumor doubling time. Furthermore, since prostaglandins have a major role in the maintenance of normal gastrointestinal physiology, it is not surprising that drugs which inhibit the formation of prostaglandins interfere with normal gastrointestinal function. The nonsteroidal antiinflammatory drugs have been shown to cause gastric mucosal cell loss due to gastric erosion and peptic ulcer formation and perforation (40), which may initiate several rounds of compensatory hyperproliferation in gastrointestinal mucosa. In several other studies, aspirin and indomethacin have been shown to increase gastric and duodenal epithelial proliferation (41–42).

TPK activity has only recently been implicated in cellular proliferation, differentiation, and neoplastic transformation. The biochemical actions of most of the oncogenes and certain growth factors are thought to be mediated through the effects of specific protein kinases that are involved in selective phosphorylation of cellular proteins (18–20). Hennipman et al. (22) observed progressively increasing levels of TPK activity in benign to malignant breast tumors as compared to normal breast tissue. Elevated levels of TPK activity have been demonstrated in human colon cancer and colon cancer cell...
lines. Arlow et al. (43) recently reported that an AOM-induced increase in colonic ODC activity in rats was associated with increased TPK activity. In our study, colonic mucosal TPK activity has exhibited a pattern similar to that of ODC activity. There was a consistent and significant increase in AOM-induced TPK activity in both cytosolic and membrane fractions of rats fed the control diet as compared with their saline-treated counterparts. A similar pattern of difference in AOM-induced TPK activity was observed in animals fed the piroxicam or DFMO diet. Although piroxicam appears to moderately enhance TPK activity both in AOM-treated as well as saline-treated animals, the dietary DFMO significantly suppressed the TPK activity in a systematic time-dependent manner (Table 5). Furthermore, we observed drastically reduced levels of membrane-bound TPK activity in relation to cytosolic TPK activity in AOM-treated animals fed the piroxicam diet both in colonic mucosa and tumors as compared to those in AOM-treated animals fed the control diet. Sakanoue et al. (44) have reported reduced levels of cytosolic tyrosine kinase activity and increased levels of membrane TPK activity in human colon carcinomas as compared with their adjacent normal mucosa. It is possible that the progressively increasing translocation of TPK from cytosol to membrane may be occurring with the advancing sequence of events in AOM-induced tumor development as in the case of phorbol ester-induced translocation of protein kinase C (45). It may also be possible that the cytosolic (nonreceptor) tyrosine kinase could be an antioncogene product itself. Recently, multiple genetic alterations have been detected that appear to contribute to the malignant transformation of colonic mucosa. Allelic deletions of chromosomes 5, 17, and 18 sequences have been identified in human colorectal carcinoma and in adenomas, suggesting that tumor suppressor genes may be important regulators of cell growth (46).

In conclusion, the results of this study demonstrate the usefulness of mucosal ODC and TPK in analyzing the state of proliferating mucosa and events leading to tumor development. But these results also point to the fact that some chemopreventive agents, such as piroxicam, can inhibit cancer development without reducing proliferation. This of course raises interesting questions about the diverse mechanisms by which different agents exert their chemopreventive effects and the general usefulness of proliferating markers such as ODC as an intermediate end point. We suggest that it may be necessary to use "agent-specific intermediate biochemical markers" for evaluating the effectiveness of specific agent(s) in chemopreventive studies.

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Effect of chemopreventive agents on intermediate biomarkers during different stages of azoxymethane-induced colon carcinogenesis.

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