Inhibition of Intestinal Tumorigenesis in A\textit{pc}\textsuperscript{Min/+} Mice by Green Tea Polyphenols (Polyphenon E) and Individual Catechins

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\textbf{Abstract:} In this work, we compared the cancer preventive activities of Polyphenon E (PPE), a standardized green tea polyphenol preparation given in diet versus drinking fluid as well as the activities of PPE versus individual catechins. We treated A\textit{pc}\textsuperscript{Min/+} mice for 9 wk with 0.08\% (-)-epigallocatechin-3-gallate (EGCG), 0.08\% (-)-epicatechin-3-gallate, or 0.12\% PPE in drinking fluid or diet. Only 0.12\% dietary PPE and 0.08\% EGCG in drinking fluid significantly decreased tumor multiplicity (70\% and 51\%, respectively). Compared to PPE in drinking fluid, dietary PPE delivered twofold more EGCG to the small intestine. Immunohistochemistry showed that adenomas in groups treated with PPE and EGCG had decreased cell proliferation, \(\beta\)-catenin nuclear expression, and phospho-Akt levels; higher cleaved caspase-3 levels, and partially restored retinoid X receptor \(\alpha\) expression. The results suggest that these molecular events contribute to the cancer prevention activity of EGCG and PPE. Furthermore, diet appears to be a better route of administration for PPE than drinking fluid.

\textbf{Introduction}

Green tea, made from the dried leaves of the plant \textit{Camellia sinensis}, has been studied extensively for possible cancer preventive effects. The most abundant and biologically active tea polyphenol is (-)-epigallocatechin-3-gallate (EGCG). Other catechins such as (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epicatechin (EC) may also contribute to the biological activities of green tea.

The possible inhibitory effects of green tea extracts and related polyphenols have been studied in different rat models using colon tumor or aberrant crypt foci (ACF) formation as endpoints, but the results are not conclusive; inhibitory effects (1,2), marginal inhibitory effects (3,4), and lack of an inhibitory effect (5–8) have been reported. On the other hand, the inhibitory activities of green tea and tea polyphenols have been consistently demonstrated in mouse models [reviewed in (9,10)]. Green tea extracts, alone and in combination with sulindac, have been shown to decrease intestinal tumor formation in the A\textit{pc}\textsuperscript{Min/+} mouse model (11,12). We have systematically investigated the effect of EGCG on intestinal tumorigenesis in A\textit{pc}\textsuperscript{Min/+} mice and found that oral administration of EGCG (0.08–0.32\% in drinking fluid) inhibited tumorigenesis (13). Oral administration of green tea extracts or EGCG has also been shown to inhibit the formation of azoxymethane (AOM)-induced ACF and colon tumor formation in mice [(14) and unpublished results].

In theory, the intestine is a promising site for chemoprevention with polyphenols that have low systemic bioavailability. EGCG, the major polyphenol in green tea, has only limited systemic bioavailability after oral ingestion; even the absorbed EGCG is excreted mostly into the intestine through the bile (9). Therefore, the intestine may actually be exposed to high levels of EGCG after ingestion. The reasons for the inconsistency among the different studies are complex and may be related to several factors: 1) the animal species used, 2) the diet used, 3) the protocol of tumor initiation and tumor yield, and 4) the type and dose of tea polyphenols or extracts used, when they were given, and whether they were administered through drinking fluid or through the diet.

Polyphenon E (PPE) is a standardized green tea polyphenol preparation containing about 65\% EGCG and 25\% other catechins. It has high potential for use in human cancer prevention trial; extensive studies on toxicology and pharmacokinetics have been conducted (14,15). This work was initiated to compare the inhibitory activities of PPE when given in the drinking fluid versus in the diet as well as the activities of PPE versus individual catechins, EGCG and ECG, in the A\textit{pc}\textsuperscript{Min/+} mice. ECG was included in the study because of the proposal that ECG may be a more active preventive agent than EGCG due to its higher activity in inducing nonsteroidal anti-inflammatory drug (NSAID) activated gene (NAG-1) (16).
Chemicals and Diets

EGCG, ECG, and PPE were provided by Dr. Yukihiko Hara (Mitsui Norin Co. Ltd., Tokyo, Japan). The PPE preparation contained 65.6% EGCG, 7.4% ECG, 3.0% EGC, 9.3% EC, 3.1% (-)-gallocatechin-3-gallate (GCG), and 0.7% caffeine. The EGCG (0.08%), ECG (0.08%), and PPE (0.12%) solutions were prepared in a 0.5% citric acid solution in deionized H2O and used for drinking fluid. The citric acid was added to the drinking fluid to stabilize the catechins and to mask their bitter taste. PPE diet consisted of 0.12% PPE in the American Institute of Nutrition (AIN)-93G diet (Research Diets, Inc., New Brunswick, NJ).

Breeding and Genotyping of ApcMin/+ Mice

Male C57BL/6J-ApcMin/+ and female wild-type littermate mice were initially purchased from The Jackson Laboratory (Bar Harbor, ME) as founders, and a breeding colony was established in the animal facility of the Susan Lehman Cullman Laboratory for Cancer Research (Rutgers, The State University of New Jersey, Piscataway, NJ). Pups were produced from the colony and weaned at 3 wk of age. Genotyping was done by routine polymerase chain reaction assays (13).

Diet Treatment and Tissue Harvesting

Male and female C57BL/6J-ApcMin/+ mice (5–6 wk old) on a basic AIN-93G diet were administered the following treatments: 0.08% EGCG (n = 24), 0.08% ECG (n = 24), 0.12% PPE as sole source of drinking fluid (n = 30), or 0.12% PPE (n = 26) in the diet. Body weight, food consumption, and fluid consumption were measured weekly. After 9 wk of treatment, mice were sacrificed by CO2 asphyxiation, and blood was withdrawn via cardiac puncture. The entire intestinal tract was harvested, flushed thoroughly with cold 0.9% saline, cut open longitudinally, and flattened on filter paper to expose tumors in the lumen. The flattened tissues on filter paper were placed on dry ice briefly to aid in scoring the visible tumors. A 150 mm segment of small intestine (from the jejunum) from 3 males and 3 females in each group was fixed in 10% buffered formalin for 24 h and then Swiss rolled for routinely pathological processing. Intestinal tissues were collected and frozen for bioavailability studies.

Immunohistochemistry

Embedded tissue blocks were cut serially for at least 30 slides and labeled numerically. Slides 1, 10, 20, and 30 were stained for haematoxylin and eosin for histopathological evaluation, and the remaining were used for immunohistochemistry. A standard avidin-biotin complex (ABC) method was used for immunohistochemistry as previously described (17). In brief, tissue sections were deparaffinized in xylene and rehydrated to distilled water, and the endogenous peroxidase was quenched in 0.3% hydrogen peroxide in methanol for 30 min. Subsequently, sections were subjected to antigen retrieval by heating the slides in sodium citrate buffer (0.01 M, pH 6.0) in a pressure cooker for 3 min after reaching full pressure. Nonspecific staining was blocked with either 10% normal horse or goat serum. Antibodies diluted to appropriate concentrations were applied to tissue sections, and the slides were incubated in a humidified chamber overnight at room temperature. Following rinsing in phosphate-buffered solution, the sections were incubated with the appropriate biotinylated antibody and then stained using the Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) for 30 min. 3-3′ Diaminobenzidine (Vector Laboratories) was used as the chromogen.

Proliferative cells were identified by staining with antibodies against Ki-67 (Dako North America, Inc., Carpinteria, CA). Quantification of the number of total cells and Ki-67 positive cells in adenomas was performed by using the Image-Pro Plus system (Silver Spring, MD). The color image containing tumor cells was converted into black and white. The area of tumor cells was selected manually and circled with a green line on a black and white image. The selected cells were highlighted with red and adjusted according to cell intensity to insure that the highlighted cells were matched well with that in the color image. The number of Ki-67 positive cells and the total number of tumor cells were counted automatically. Proliferation index was expressed as the percentage of the number of Ki-67 positive cells in the total number of tumor cells. Apoptotic cells were stained with an antibody against cleaved-caspase 3 (Cell Signaling Technology, Inc., Danvers, MA) and were quantified similarly. Positivity of nuclear staining for β-catenin (antibody purchased from Cell Signaling Technology) phospho-Akt (antibody purchased from BD Biosciences, San Jose, CA) and retinoid X receptor α (RXRα, antibody purchased from Santa Cruz Biotechnology, Santa Cruz, CA) was counted manually and expressed as the percentage of positive-staining cells in the total number of tumor cells. All of the tumor cells in the tissue sections were counted.

Quantification of EGCG in Biological Samples

Plasma samples were analyzed by previously described methods (18). In brief, plasma (100 µl) was extracted with methylene chloride and ethyl acetate, and the ethyl acetate fraction was dried in vacuo. The sample was then resuspended in 10% acetonitrile and analyzed by high-performance liquid chromatography (HPLC) with electrochemical detection (HPLC-ECD). The results represented unconjugated levels of EGCG in the plasma. To determine the levels of total EGCG (i.e., conjugated plus unconjugated EGCG), plasma samples were hydrolyzed with β-glucuronidase (250 U) and sulfatase (1 U) before the solvent extraction procedure.
Table 1. Effect of Treatment With Tea Polyphenols on Tumorogenesis of ApcMin/+ Micea

<table>
<thead>
<tr>
<th>Diet (n)</th>
<th>Region</th>
<th>Size</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Middle</td>
<td>Distal</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (11)</td>
<td>7.45 ± 1.61*</td>
<td>15.27 ± 3.26*</td>
<td>22.18 ± 4.28*</td>
</tr>
<tr>
<td>0.08% EGCG (12)</td>
<td>5.42 ± 1.06*</td>
<td>7.75 ± 1.21*</td>
<td>8.83 ± 1.79†</td>
</tr>
<tr>
<td>0.08% ECG (12)</td>
<td>5.33 ± 1.13*</td>
<td>9.17 ± 2.40*</td>
<td>11.67 ± 3.29†</td>
</tr>
<tr>
<td>0.12% PPE fluid (14)</td>
<td>4.43 ± 0.89*</td>
<td>10.14 ± 2.07*</td>
<td>19.36 ± 3.55†</td>
</tr>
<tr>
<td>0.12% PPE diet (14)</td>
<td>3.00 ± 0.67†</td>
<td>4.50 ± 1.33†</td>
<td>5.71 ± 1.19†</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (12)</td>
<td>7.08 ± 1.28</td>
<td>11.83 ± 1.96</td>
<td>14.83 ± 2.50</td>
</tr>
<tr>
<td>0.08% EGCG (11)</td>
<td>5.00 ± 0.93</td>
<td>6.27 ± 1.99</td>
<td>9.00 ± 1.38</td>
</tr>
<tr>
<td>0.08% ECG (11)</td>
<td>6.55 ± 1.18</td>
<td>9.45 ± 1.30</td>
<td>10.18 ± 1.75</td>
</tr>
<tr>
<td>0.12% PPE fluid (16)</td>
<td>5.63 ± 1.01</td>
<td>11.56 ± 2.17</td>
<td>11.81 ± 2.22</td>
</tr>
<tr>
<td>0.12% PPE diet (9)</td>
<td>4.56 ± 1.09</td>
<td>6.11 ± 2.65</td>
<td>12.44 ± 3.73</td>
</tr>
</tbody>
</table>

a: Abbreviations are as follows: AIN, American Institute of Nutrition; EGCG, (-)-epigallocatechin-3-gallate; ECG, (-)-epicatechin-3-gallate; PPE, polyphenon E. The 5-wk-old male and female ApcMin/+ mice on AIN93G diet were randomized into 5 groups: control (no treatment), 0.08% EGCG or ECG (as sole source of drinking fluid), 0.12% PPE (as sole source of drink), or 0.12% PPE in the diet. This treatment was administered for 9 wk, and mice were sacrificed at 14 wk of age. Each value represents mean ± SE. Values with different symbols (*, †, ‡) are different with statistical significance at P < 0.05 (1-way analysis of variance combined with Tukey’s posttest).

Small intestine and colon samples were prepared by using the method of Chu et al. (19) with modifications. In brief, tissue samples were homogenized in 5 volumes of methanol:ethyl acetate:dithionite (2:1.3, vol/vol/vol) with 14 passes of a mechanical dounce homogenizer. Aliquots were centrifuged in vacuo. The resulting residue was resuspended with water (200 µl) and then hydrolyzed and extracted in a manner analogous to plasma.

EGCG levels were analyzed using an HPLC system consisting of two ESA model 580 dual-piston pumps (Chelmsford, MA), a Waters Model 717plus refrigerated autosampler (Milford, MA), and an ESA 5500 coulochem electrode array system (CEAS). The potentials of the CEAS were set at −100, 100, 300, and 500 mV. Separation was achieved using previously described methods (18).

Statistics

One-way analysis of variance (ANOVA) with either Tukey’s honestly significant difference post hoc tests was used for statistical analysis of tumor multiplicity and immunohistochemistry results, respectively. Two-way ANOVA was used to combine tumor data from males and females. Student’s t-test was used for analysis of plasma and tissue levels of tea catechins.

Results

Effect of EGCG, ECG, and PPE on Intestinal Tumorogenesis in ApcMin/+ Mice

EGCG in the drinking fluid and PPE in the diet significantly reduced the total number of small intestinal tumors in female ApcMin/+ mice by 51% and 70.6%, respectively. Table 1. Tumors in the small intestine were mainly less than 1 mm in diameter and located in the distal region. The effect of EGCG on total tumor multiplicity in the female mice was mainly due to inhibition in the distal region of the small intestine. With the dietary PPE treatment, tumors of all size groups and in the different regions were significantly decreased. ECG or PPE in the drinking fluid also appeared to have inhibited total tumor multiplicity, but the effects were not statistically significant. A similar inhibitory trend was observed in the male mice; however, the effects were not significant. The two data sets of male and female mice were analyzed by 2-way ANOVA, and results indicated that there was no effect of either gender or the interaction between gender and treatment on tumor number. The male and female data sets were therefore combined and reanalyzed together. Both EGCG in the drinking fluid and PPE in the diet significantly reduced total tumor multiplicity by about 50% compared to the control group (data not shown). This was mainly due to the significant inhibitory effect observed in the distal small intestine (51.2% inhibition from the control group). EGCG significantly inhibited tumors less than 2 mm (38.7%), whereas PPE diet treatment demonstrated an inhibitory effect on tumors of all size groups (38.7–57.4%). No significant difference in body weight, food consumption, and fluid intake among the different treatment groups were found (data not shown).

Effects of Treatment on Cell Proliferation and Apoptosis

Antibodies against Ki-67 and cleaved caspase-3 were used in immunohistochemistry to determine the treatment effect on cell proliferation and apoptosis. Both antibodies showed
staining in the nucleus (Fig. 1). Strong Ki-67 expression was found in the lower third but not the upper two-thirds of normal crypts (results not shown). In the EGCG and PPE treated group, the Ki-67 staining was decreased in tumors but not in the normal crypts (Fig. 1, A1–A3). The proliferation index in adenomas in the treatment groups with 0.08% EGCG, 0.12% PPE in the diet, and 0.12% PPE in the drinking fluid were significantly lower as compared with the proliferation index in adenomas from the control group (Table 2). Caspase-3 positive (apoptotic) cells were observed in tumors but rarely in the normal mucosa. The number of apoptotic cells, expressed as apoptotic index, was markedly increased in the tumors of the EGCG and PPE treated mice (Fig. 1, B1–B3; Table 2). No appreciable change was observed in the normal epithelia.

**Effects of Treatment on β-Catenin Expression and Levels of Phosphorylated Akt**

Normal mucosa in the upper two-thirds of the crypts showed a distinct membranous staining of β-catenin, whereas some cells at the base of crypts displayed

| Table 2. Effect of EGCG and PPE on Cell Proliferation, Apoptosis, and Nuclear Staining Positivity of β-Catenin, Phospho-Akt, and RXRα in Tumors |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment       | Adenomas Analyzed | Proliferation Index | Apoptotic Index | Nuclear Positivity (%) |
| Control         | 18               | 59.0 ± 9.9*       | 2.2 ± 0.6*      | 31.7 ± 7.6*       |
| 0.08% EGCG      | 26               | 24.0 ± 7.7†       | 4.1 ± 1.3†      | 17.8 ± 6.2†       |
| 0.12% PPE in fluid | 19             | 31.0 ± 6.9†       | 4.2 ± 1.5†      | 16.0 ± 5.1†       |
| 0.12% PPE in diet | 16             | 22.0 ± 8.5†       | 6.9 ± 1.7†      | 9.0 ± 7.5†        |

a: Abbreviations are as follows: EGCG, (−)-epigallocatechin-3-gallate; PPE, polyphenon E; RXRα, retinoid X receptor α. Mice were treated with 0.08% EGCG in drinking fluid, 0.12% PPE in the diet, and 0.12% PPE in drinking fluid. Proliferation index was determined by immunohistochemistry using antibody against Ki-67, and apoptotic index was determined by immunohistochemistry using antibody against cleaved caspase-3 (Asp175). Nuclear staining positivity is shown as the number of positively stained cells expressed as a percentage in the total number of tumor cells counted. Values with different symbols (*, †, ‡) are different with statistical significance at P < 0.05 (1-way analysis of variance combined with honestly significant difference post hoc test).
cytoplasmic and sporadic nuclear staining. All adenomas from the control group had enhanced nuclear and cytoplasmic but reduced membranous \(\beta\)-catenin staining (Fig. 2, A1–A3). Significantly reduced nuclear staining of \(\beta\)-catenin, as expressed in nuclear staining positivity, was observed in the adenomas from mice treated with EGCG and PPE (Table 2). In adenomas from the treated groups, the intensity of cytoplasmic staining was reduced to moderate expression levels, whereas membranous staining was increased or even restored totally (Fig. 2, A1–A3).

In the adenomas from untreated \(Apc^{Min/+}\) mice, phospho-Akt staining was observed in both the nucleus and cytoplasm (Fig. 2, B1–B3), whereas in the normal mucosa, low level of staining in the basal cells in lower third and no staining in the differentiated cells in the upper two-thirds of the crypt were observed (results not shown). The treatment with EGCG and PPE lowered the nuclear staining of phospho Akt in the adenomas, both in terms of staining intensity and number of positive-staining cells. The percent of phospho-Akt nuclear positive-staining cells in the adenomas was significantly decreased by these treatments (Table 2). The cytoplasmic phospho-Akt staining was also lower in the adenomas from the groups that were treated with EGCG and PPE.

Effects on RXR\(\alpha\) Expression

In both the untreated and treated \(Apc^{Min/+}\) mice, uniformly strong nuclear staining of RXR\(\alpha\) was found in all normal epithelial cells, and the staining intensity and number of positive-staining cells were lower in adenomas (Fig. 3). Nevertheless, the RXR\(\alpha\) positive cells in adenomas from mice treated with EGCG and PPE were significantly higher than in tumor tissues from the control group (Table 2), showing a partial restoration of RXR\(\alpha\) expression.

Intestinal and Plasma Levels of Catechins After PPE Administration: Diet Versus Drinking Fluid

Administration of PPE through the diet resulted in higher levels of total EGCG in the small intestine (2.2-fold) and colon (1.9-fold) as compared to mice treated with PPE through the drinking fluid (Table 3). The small intestinal and colon levels of EC, ECG, and EGC in mice that received PPE from the diet were also about twice the levels of those in mice that received PPE in the drinking fluid; however, the differences were not statistically significant, possibly because of the larger standard deviations. The plasma levels of catechins were not different between these two groups.

Discussion

This study confirms the inhibitory activity of EGCG in the \(Apc^{Min/+}\) mouse intestinal tumorigenesis model as we...
reported previously (13). We further demonstrated the inhibitory activity of PPE, especially when it was administered in the diet. The PPE preparation contained 65.6% EGCG; therefore, 0.12% PPE and 0.08% EGCG were similar in EGCG content. Because of the large standard deviations in tumor multiplicity, our data do not allow a precise comparison between the effects of 0.12% PPE and 0.08% EGCG when both were administered in the drinking fluid. Nevertheless, the results do not suggest that the ECG, EGC, GCG, and EC (which accounted for 23% by weight) in the PPE preparation contributed significantly to the inhibitory effects. When administered at 0.08% in drinking fluid, ECG appeared less effective than EGCG; even though according to statistical analysis, we can only conclude that the ECG was not more effective than EGCG. EGC differs from EGCG in having one less phenolic group on the B-ring and has generally been shown to have similar and lower biological activities in previous studies (20,21) except a report showing higher activity in inducing NAG-1 (16). The Apc<sup>Min/+</sup> mice provide a genetically relevant model for intestinal tumorigenesis. Nevertheless, there is an inherent problem in the large variability in the tumor multiplicity among individual mice and the large differences between batches of mice. In this study, the treatment effects were more pronounced in female mice than in male mice. When the results from the female and male mice were combined and analyzed together, the preceding conclusions can still be drawn.

One interesting observation that we found is that PPE was more effective when administered in the diet than in the drinking fluid. This is likely to be due to the higher EGCG levels delivered to the intestinal tissues by the dietary route (Table 3). We estimated that the amounts of PPE consumed through the diet and fluid were approximately the same. The differences in the intestinal catechin levels are likely to be due to the longer transient time for solids compared to liquids (22,23). When PPE is administered in the diet, it is expected to have a longer transient time through the gastrointestinal tract, which allows catechins to be more extensively absorbed by enterocytes in comparison to administration through the drinking fluid. This observation suggests that diet is a more

**Table 3.** Plasma and Tissue Levels of Catechins in Apc<sub>Min/+</sub> Mice Treated With PPE in the Diet or Drinking Fluid<sup>a</sup>

<table>
<thead>
<tr>
<th>Catechin Levels (ng/g or ng/ml)</th>
<th>EGCG</th>
<th>ECG</th>
<th>EGC</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPE diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>28.0 ± 4.7</td>
<td>N/D</td>
<td>1.9 ± 0.43</td>
<td>16.8 ± 3.7</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1234.8 ± 197.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107.4 ± 21.0</td>
<td>169.0 ± 30.6</td>
<td>241.2 ± 53.0</td>
</tr>
<tr>
<td>Colon</td>
<td>230.6 ± 74.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N/D</td>
<td>8.4 ± 1.8</td>
<td>10.3 ± 2.0</td>
</tr>
<tr>
<td><strong>PPE fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>24.3 ± 3.8</td>
<td>N/D</td>
<td>4.5 ± 1.9</td>
<td>14.4 ± 5.2</td>
</tr>
<tr>
<td>Small intestine</td>
<td>550.0 ± 169.0</td>
<td>48.4 ± 14.6</td>
<td>90.1 ± 26.5</td>
<td>97.9 ± 54.6</td>
</tr>
<tr>
<td>Colon</td>
<td>117.3 ± 32.5</td>
<td>N/D</td>
<td>5.3 ± 1.3</td>
<td>8.0 ± 1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations are as follows: PPE, polyphenon E; EGCG, (-)-epigallocatechin-3-gallate; ECG, (-)-epicatechin-3-gallate; EGC, (-)-epigallocatechin; EC, (-)-epicatechin. Plasma and tissue samples were collected at time of sacrifice. Values represent the mean ± standard error of measurement of 7–13 samples.

<sup>b</sup> PPE diet versus PPE fluid <i>P = 0.06</i> by Student’s <i>t</i>-test.

<sup>c</sup> PPE diet versus PPE fluid <i>P < 0.05</i> by Student’s <i>t</i>-test.
effective route for administering catechins to animals for cancer prevention studies, at least for intestinal cancers. Another advantage of administering catechins through the diet is that it does not affect the amount of diet consumed. In contrast, administration of high concentrations of catechins through the drinking fluid may cause the reduction of fluid intake by certain strains of mice, such as the NCR nu/nu mice, due to the bitterness of the catechin solution. Although tea is consumed as a beverage by humans, the dietary administration may resemble the situations of a clinical trial when PPE is given in capsules with food or when consumers taking tea extracts capsules or tablets as supplements.

Our observed suppression of cell proliferation and enhancement of apoptosis in the intestinal adenomas by treatment with EGCG and PPE are consistent with similar observations in the 4(methyl nitrosamino)-1-(3-pyridyl)-1-butaneone-induced lung adenomas in A/J mice, which we proposed to be key events in the inhibition of tumorigenesis (24,25). It is worth noting that such effects only occurred in the tumors and not in normal epithelia (Fig. 1). In the Apc\(^{Min/+}\) mice, tumorigenesis is driven by the defective Apc gene, which causes aberrant nuclear localization of β-catenin (26). The nuclear β-catenin interacts with transcription factor TCF4 and activates many growth promoting and oncogenic genes. In this work, nuclear staining of β-catenin was observed in tumor cells in contrast to the membrane staining observed in normal epithelial cells. Treatment with EGCG or PPE decreased the levels of nuclear β-catenin staining and increased the levels of membrane staining. Thus, it partially restored the β-catenin staining pattern to that in normal epithelial cells. These observations are consistent with our previous Western blotting results that have shown that EGCG treatment of Apc\(^{Min/+}\) mice decreased β-catenin in the nuclear preparations from intestinal tumors (13). If the decreased nuclear β-catenin levels, as well as suppressed cell proliferation and enhanced apoptosis, are events closely related to the inhibition of tumorigenesis, we expect to observe stronger effects by PPE administered in the diet than in the drinking fluid. A hint of such a difference was observed, but the difference between the 2 groups was not statistically significant.

The observed decrease in phospho-Akt levels is likely to be related to changes in nuclear β-catenin levels because both phosphorylation of Akt and nuclear translocation β-catenin can be induced by the same upstream signaling including activated insulin-like growth factor-1 receptor (27,28). RXR\(\alpha\) is a nuclear receptor that acts as a transcription factor on the binding of the ligand and retinoids and plays a significant role in regulating cell growth and differentiation as well as suppressing tumorigenesis (29,30). RXR\(\alpha\) also mediates the degradation of β-catenin in an Apc-independent pathway (31). It was reported by Issa et al. (32) that RXR\(\alpha\) expression was lost in the adenomas in Apc\(^{Min/+}\) mice. Our results confirmed this observation. We further demonstrated the RXR\(\alpha\) expression was partially restored in the EGCG and PPE treated mice.

In summary, this work demonstrates the inhibitory effects of green tea polyphenols in the Apc\(^{Min/+}\) mouse model, and the effect is more pronounced when PPE is administrated in the diet than the drinking fluid. The inhibitory activity is likely to be due to the suppression of cell proliferation, enhancement of apoptosis, decrease of nuclear β-catenin levels, inhibition of Akt phosphorylation, and restoration of RXR\(\alpha\) expression.

Acknowledgments and Notes
This work was supported by NIH grant CA88961. We thank Dr. Y. Hara (Mitsui Norin Co. Ltd., Tokyo, Japan) for providing tea polyphenols for the study. X. Hao and M. Bose contributed equally to this study. Address correspondence to Dr. Chung S. Yang, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8209. Phone: 732-445-3400, ext. 248. FAX: 732-445-0687. E-mail: csyang@rci.rutgers.edu.

Submitted 17 November 2006; accepted in final form 26 February 2007.

References


