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Green Tea Polyphenols Inhibit Colorectal Tumorigenesis in Azoxymethane-Treated F344 Rats

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In studying the cancer-preventive activities of green tea polyphenols, we previously demonstrated that dietary administration of polyphenon E (PPE) inhibited the formation of aberrant crypt foci (ACF) in the colon of azoxymethane (AOM)-treated F344 rats. Herein, we reported cancer-preventive activity of PPE using colorectal cancer as an end point. F344 rats were given two weekly injections of AOM, and then maintained on a 20% high-fat diet with or without 0.24% PPE for 34 wk. In the control group, 83% of rats developed colorectal tumors. Dietary PPE treatment significantly increased the plasma and colonic levels of tea polyphenols, and decreased tumor multiplicity and tumor size. Histological analysis indicated that PPE significantly decreased the incidence of adenocarcinoma, and the multiplicity of adenocarcinoma as well as the multiplicity of adenoma. PPE treatment significantly decreased plasma levels of proinflammatory eicosanoids, prostaglandin E2, and leukotriene B4. It also decreased β-catenin nuclear expression, induced apoptosis, and increased expression levels of RXRα, β, and γ in adenocarcinomas. In conclusion, our results convincingly demonstrated the inhibitory effects of orally administered PPE on colon carcinogenesis in AOM-treated rats and suggested possible biomarkers for the biological effects of green tea polyphenols.

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Oral administration of green tea or tea preparations were found to inhibit tumorigenesis in different animal models, such as ovarian, pancreatic, gastric, esophageal, prostate, breast, and skin cancers (4,5). The inhibitory effects of tea and tea polyphenols on intestinal tumorigenesis were reported in both 1,2-dimethylhydrazine-induced colon carcinogenesis and ApcMin/+ mouse models (6–9). Similar effects of tea preparations on azoxymethane (AOM)-induced rat colon tumorigenesis were also demonstrated (10). Green tea extract (2% in drinking fluid) significantly inhibited aberrant crypt foci (ACF) formation (an early premalignant lesion in colon carcinogenesis) in 2-amino-3-methylimidazole [4,5-f] quinoline-treated rats (11). However, two other studies showed that green tea extract (1 or 2% in drinking fluid) only had a marginal inhibitory effect on ACF formation in AOM-treated rats (12,13). Some studies also failed to show the inhibitory effect of tea polyphenol preparations...
in either 1,2-dimethylhydrazine- or AOM-induced rat colon tumorigenesis models (14–17). This may be due to the different diets used, the different protocols of tumor initiation, and the different types and doses of tea preparations used in the studies.

In our previous studies, we found that dietary PPE treatment significantly decreased the total number of ACF and the percentage of ACF with high-grade dysplasia in AOM-treated rats. The high-grade dysplastic ACF from rats treated with 0.24% PPE in the diet had decreased nuclear expression levels of β-catenin and cyclin D1, but increased apoptosis and retinoid X receptor α (RXRα) expression, in comparison to the control rats (18). In the present study, we further demonstrated the inhibitory effect of PPE on colorectal carcinogenesis in the same AOM-treated rat model, in a longer-term experiment, using visible colorectal tumors as an end point and characterized the associated molecular changes.

Materials and Methods

Animal Treatment

All animal experiments were conducted in accordance to the protocol (#02–027) approved by the Institutional Animal Care and Use Committee of Rutgers, the State University of New Jersey. Male F344 rats at 6 wk of age were purchased from Taconic Farms (Germantown, NY). All animals were housed in a plastic cage with a filter top (3 rats per cage). The animal room was controlled at 20 ± 2°C, 50 ± 10% humidity, and a 12-h light/12-h dark cycle. Animals had free access to food and water at all times. Food cups were replenished with fresh diet twice weekly. After 1 wk of acclimation, animals were randomly distributed into control and experimental groups.

At 7 wk of age, all rats were subjected to two weekly subcutaneous injections of AOM (Midwest Research Institute, Kansas City, MO) at a dose of 15 mg/kg each (18). One day after the second injection, rats were fed a modified 20% high-fat AIN-76A diet (19) containing 0 or 0.24% PPE (30 rats per group). PPE, a gift from Dr. Yukihiko Hara of the Mitsui Norin Co., Ltd. (Tokyo, Japan), is a standardized green tea polyphenol preparation containing 65% EGCG, 7% ECG, 3% EGC, 9% EC, 3% galloycatechin gallate, and 0.6% caffeine. Dose selection for PPE was based on our previous studies, which showed that PPE at 0.12% or 0.24% in the diet significantly inhibited the formation of ACF in AOM-treated rats (18). In order to minimize possible degradation of tea polyphenols, fresh stocks of animal diets were made every two and half months, and all stock diets were stored at 4°C for the entire period of the experiment. Body weight was monitored once a week until the termination of the experiment at 34 wk after the second AOM injection.

Tissue Harvesting

At termination of the experiment, all animals were sacrificed by CO2 asphyxiation. After laparotomy, the liver, spleen, and entire large intestine were harvested. Liver and spleen were washed with ice-cold saline, blotted, dried, and then weighed. The large intestine from cecum to anus was longitudinally opened and flushed with ice-cold saline. Tumor number was recorded. Tumor size was measured by length, width, and height using a digital caliper. Tumors with length ≥ 0.5 cm were dissected and then halved; one half was snap-frozen in liquid nitrogen for biochemical analyses, and the other half was fixed in 10% buffered formalin for 24 h. For blood sample collection, five rats from each of the control and PPE-treated groups were sacrificed during early morning (8:00–10:00 AM), and blood was collected by heart puncture. This was designed to obtain the relatively higher blood levels of green tea polyphenols than at later time points, because rats are nocturnal and feed during night.

Histopathological and Apoptosis Analyses

Tumor tissues were processed and appropriately oriented in paraffin blocks for serial sectioning at 4-μm thickness. Sections 1, 10, and 20 were stained with hematoxylin and eosin (HE) for histological evaluation, which allowed tumor visualization at different sectioning levels. The sections adjacent to HE sections that contained histologically confirmed tumors were used for immunohistochemistry (IHC). Adenomas and adenocarcinomas were identified as described previously (20). For apoptosis analysis, cells showing morphological changes including blebbing, shrinkages, and chromatin condensation were regarded as apoptotic bodies. At least 1000 cells were analyzed for each tumor, and apoptotic index was defined as the percentage of apoptotic cells in all tumor cells analyzed on each tissue section (21).

Immunohistochemistry

A standard avidin-biotin peroxidase complex method was employed as previously described (20). In brief, after dewaxing and rehydration, the slides were heated in sodium citrate buffer (0.01 M, pH 6.0) in a pressure cooker for 3 min after reaching full pressure. Endogenous peroxidase was quenched using 3% hydrogen peroxide in methanol. Sections were then blocked for 1 h at
room temperature in phosphate-buffered solution (PBS) containing 3% normal horse or goat serum depending on the origin of the primary antibody. The sections were then immunostained with anti-β-catenin (1:2000, BD Biosciences, Franklin Lakes, NJ) or RXRα, β, or γ (1:1000 for RXRα and 1:2000 for both RXRβ and γ, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at room temperature. The antibodies were diluted in either 10% goat or horse serum. The sections were rinsed in PBS and incubated with a biotinylated secondary antibody and subsequently incubated in VECTASTAIN ELITE ABC reagent for 30 min, using 3,3’-diaminobenzidine (Vector laboratories, Burlingame, CA) as the chromogen. Sections were then counterstained for 2–3 min with hematoxylin (Sigma, St. Louis, MO) and mounted with Permount. Both positive- and negative-stained cells in tumor regions were counted for the nuclear expression of β-catenin as well as RXRα, β, and γ. The results were expressed as the percentage of positive cells in total tumor cells (at least 1000 cells were analyzed).

### Enzyme Immunoassay for Prostaglandin E2 (PGE2) and Leukotriene B4 (LTB4)

Procedures for PGE2 and LTB4 enzyme immunoassay (EIA) were the same as previously described (6). In brief, plasma was centrifuged for 10 min at 12,000 × g (Sorvall RT 6000B) to remove any possible blood cells. The supernatants were used for immunoassay. For the determination of PGE2 and LTB4 levels, the plasma samples were mixed with ethyl acetate, vortexed for 30 min, and then centrifuged at 10,000 × g for 20 min. The organic layer was collected and dried using a Speed Vacuum Evaporator (VWR International, Inc., West Chester, PA). The dried samples were then reconstituted in EIA buffer (Cayman Chemical), and the levels of PGE2 and LTB4 were determined using EIA kits (Cayman Chemical).

### Statistical Analysis

Tumor incidence was analyzed by Chi-square test. Tumor multiplicity, apoptotic index, and positivity of staining were analyzed by Mann-Whitney U test. The data on tissue levels of polyphenols, and the levels of PGE2 and LTB4 were analyzed using Student’s t-test.

### Results

#### General Observations

All animals showed a steady body weight gain during the experimental period, and the administration of 0.24% PPE did not affect the growth of the rats during the first 17 wk of treatment. Starting from Week 18, rats treated with PPE showed slightly lower body weight than the control (data not shown), and by the end of the experiment, the body weights of PPE-treated rats were about 5% lower than those of the control rats ($P < 0.05$, see Table 1). During the entire experiment, there were no signs of toxicity or conditions suggesting adverse effects caused by dietary administration of PPE. At the time of sacrifice, there were no differences in average liver or spleen weights between the control and PPE treatment groups (Table 1).

### Dietary PPE Treatment Inhibits Colon Tumorigenesis

After 34 wk of dietary treatment with PPE, the rats were sacrificed, and the entire colon was harvested. On gross colonic examination, 83% of control rats and 68% of PPE-treated rats developed visible tumors. The tumors were located mainly in the distal colon and rectum. All the tumors were of exophytic growth. Control rats had an average of 3.64 tumors per rat, while PPE-treated rats only had 1.64 tumors per rat (reduced by 55%, $P < 0.01$) (Table 1). PPE treatment also reduced the tumor size by 45% (31.78 vs. 57.97 mm³, $P < 0.05$). Based on histopathological characteristics, tumors were categorized into adenomas and adenocarcinomas (Fig. 1). Interestingly, almost all of the adenomas from both control and PPE-treated groups were tubular adenoma with high-grade dysplasia (Fig. 1A and B). All adenocarcinomas from both groups were found to be derived from adenomas, and were well differentiated with glandular formation and at an early stage, without invasion beyond muscular layer and without lymph

### Table 1. Effects of dietary administration of PPE on body, liver, and spleen weights as well as on tumor multiplicity and tumor size of AOM-treated F344 rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Spleen weight (g)</th>
<th>Tumor multiplicity(tumor/rat)</th>
<th>Tumor size (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (23)</td>
<td>508.4 ± 4.2</td>
<td>15.7 ± 0.3</td>
<td>0.95 ± 0.01</td>
<td>3.64 ± 0.31</td>
<td>57.97 ± 7.85</td>
</tr>
<tr>
<td>PPE (22)</td>
<td>482.5 ± 7.21</td>
<td>16.6 ± 0.3</td>
<td>0.96 ± 0.02</td>
<td>1.64 ± 0.21</td>
<td>31.78 ± 5.37</td>
</tr>
</tbody>
</table>

Male F344 rats were subject to two weekly injections of AOM (each at 15 mg/kg, s.c.), and then fed modified high fat (20%) diet with or without 0.24% PPE for 34 weeks. Data represent mean ± SD. The tumor size is the mean of the sum of tumors in each animal. The numbers of rats per group are shown in parentheses. Mann-Whitney U test was used for statistical analysis of tumor multiplicity and size ($P < 0.05$, and $P < 0.01$).
node metastases (Fig. 2C and D). The tumor multiplicity of PPE group was significantly lower than that of control group \((P < 0.05)\). The incidence of adenocarcinoma in PPE group was significantly reduced compared with control \((Table 2, 23\% \text{ vs. } 57\%, P = 0.02)\), and the multiplicity of adenocarcinoma per rat in PPE group was also significantly decreased compared to control group \((by 80\%, 0.24 \text{ vs. } 1.17 \text{ per rat, } P < 0.03, Table 2)\). The incidence and multiplicity of adenoma per rat in PPE group was lower than those in control group; however, this trend did not reach statistical significance \((P = 0.26, P = 0.08, \text{ respectively})\), which may be due to the inhibition of adenoma progression to adenocarcinoma by PPE.

**PPE Treatment Increases Apoptosis and Decreases \(\beta\)-Catenin Nuclear Accumulation**

The effect of PPE treatment on apoptosis was analyzed under a microscope by visualizing apoptotic bodies characterized by blebbing, shrinkages, and chromatin condensation \((Fig. 1A–D)\). PPE treatment significantly increased cellular apoptosis in both adenomas and adenocarcinomas, in comparison with the control; the apoptotic index increased approximately 40\% \((P < 0.05)\) in adenomas and about 100\% \((P = 0.01)\) in adenocarcinomas \((Table 3)\). There was no appreciable change in the level of cellular apoptosis in normal mucosa of rats treated with PPE. \(\beta\)-catenin is an important component in the Wnt signaling pathway; we and others have demonstrated that aberrant \(\beta\)-catenin expression is a common event in both human and rat colorectal cancer \((20,22,23)\). In the present work, we compared \(\beta\)-catenin nuclear expression in adenocarcinomas from the control and PPE-treated rats, and found that PPE treatment decreased nuclear levels of \(\beta\)-catenin expression by 55.0\% \((P < 0.01, Fig. 1E–F; Table 3)\).

**PPE Treatment Increases the Expression of RXR\(\alpha\), \(\beta\), and \(\gamma\)**

We previously demonstrated that PPE treatment enhanced RXR\(\alpha\) expression in dysplastic ACF of AOM-treated rats \((18)\). In the present study, we analyzed the expression levels of RXR\(\alpha\) and its two family members, namely RXR\(\beta\) and \(\gamma\) in adenocarcinomas from both the control and PPE-treated rats \((Fig. 2)\). PPE treatment significantly increased the nuclear expression level of RXR\(\alpha\) \((by 50\%, P = 0.01)\), RXR\(\beta\) \((by 38\%, P < 0.02)\), and RXR\(\gamma\) \((by 75\%, P = 0.05)\) \((Table 3)\). Due to the heterogeneous expression patterns of RXR\(\alpha\), \(\beta\), and \(\gamma\) in adenomas from both the control and PPE treatment groups, their changes of expression were not evaluated in adenomas.

**PPE Treatment Decreases PGE2 and LTB4 Levels**

Since inflammation has been associated with colon carcinogenesis, we measured levels of proinflammatory eicosanoids, PGE2 and LTB4, in the plasma of rats \((Fig. 3)\). In the control group, plasma levels of PGE2 and LTB4 were \(328 \pm 214 \text{ ng/mL and } 374 \pm 52 \text{ ng/mL}\), respectively. In the PPE-treated group, plasma levels of PGE2 and LTB4 were \(132 \pm 96 \text{ ng/mL and } 263 \pm 35 \text{ ng/mL}\), representing a 60\% and 30\% reduction in PGE2 \((P = 0.05)\) and LTB4 \((P < 0.01)\) levels, respectively.

**Levels of Catechins in Plasma and Colorectal Mucosa**

The levels of major tea polyphenols in the plasma and colonic mucosa of PPE-fed rats were quantified with a previous method using high performance liquid chromatography \((HPLC)\) \((24)\). Four major tea polyphenols, namely EGCG, EGC, ECG, and EC, were measured...
In plasma, EC showed the highest level (92 ng/ml), followed by EGC, EGCG, and ECG (28, 18, and 3.2 ng/ml, respectively). Higher levels of tea polyphenols were detected in the colonic mucosa of PPE-fed rats with higher EGCG (570 ng/g), followed by ECG, EGC, and EC (98, 45, and 25 ng/g, respectively). The low levels of EGCG detected in the colon samples of the control group could be due to the cross-contamination between samples.

### Table 2. Effects of dietary administration of PPE on histopathological lesions in AOM-treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence (%)</th>
<th>Multiplicity (tumor/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenoma</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Control</td>
<td>83 (19/23)</td>
<td>57 (13/23)</td>
</tr>
<tr>
<td>PPE</td>
<td>68 (15/22)</td>
<td>23 (5/22)</td>
</tr>
</tbody>
</table>

Rats were treated as described in Table 1. Colon tumors were dissected and processed for histopathological analysis. Adenoma and adenocarcinoma were identified as described in Figure 2. Differences on lesion incidence were analyzed with Chi-square test. The numbers of rats in each group is shown in parentheses. Tumor multiplicity data are shown in mean ± SD and analyzed by Mann-Whitney U test (\(^1\)P = 0.02, \(^2\)P < 0.08 and \(^3\)P < 0.03).

In our previous study, dietary PPE treatment led to a decreased number of AOM-induced colonic ACF in F344 rats, especially the percentage of those with high-grade dysplasia (18). This effect was associated with increased cellular apoptosis, decreased nuclear expression levels of both \(\beta\)-catenin and cyclin D1, and increased expression level of nuclear RXR\(\alpha\) in dysplastic ACF. These results demonstrate that dietary

**Discussion**

In our previous study, dietary PPE treatment led to a decreased number of AOM-induced colonic ACF in F344 rats, especially the percentage of those with high-grade dysplasia (18). This effect was associated with increased cellular apoptosis, decreased nuclear expression levels of both \(\beta\)-catenin and cyclin D1, and increased expression level of nuclear RXR\(\alpha\) in dysplastic ACF. These results demonstrate that dietary...
administration of PPE inhibited the carcinogenic events during the early stage of colon carcinogenesis in rats. In the present study with the same rat model, we demonstrated that long-term (34 wk) dietary PPE treatment significantly decreased the incidence and multiplicity of colonic adenocarcinomas in AOM-treated rats. The effect was associated with decreased $\beta$-catenin nuclear expression levels, increased apoptosis, and increased nuclear expression of RXR$\alpha$, $\beta$, and $\gamma$ in colorectal tumor tissues as well as decreased levels of proinflammatory eicosanoids (PGE2 and LTB4) in plasma.

The anti-inflammatory activity of tea catechins has been discussed extensively (5). In the present study, we observed that plasma levels of PGE2 and LTB4 in the AOM-treated rats were significantly reduced by PPE treatment. PGE2 and LTB4 are the major metabolites of arachidonic acid via the cyclooxygenases (COX1 and COX2) pathway and lipoxygenase pathway, respectively. COX-1 is constitutively expressed, while COX-2 is upregulated in colorectal carcinogenesis in both humans and animal models (25,26). EGCG was demonstrated to significantly inhibit constitutive COX-2 mRNA and protein overexpression in multiple human colorectal cancer cell lines (27,28). The observed decreased plasma levels of PGE2 in PPE-treated rats might be due to the inhibition of COX expression or activities by PPE. The reduced plasma level of LTB4 could be due to the reduced inflammation state (by the anti-inflammatory activity of PPE) or a direct inhibition of lipoxygenase activity by PPE.

Many mechanisms have been proposed for the cancer-preventive actions of tea polyphenols, and the inhibition of aberrant Wnt signaling pathway has been proposed for colon cancer prevention (5). $\beta$-catenin plays a pivotal role in the Wnt signaling pathway. After its translocation into the nucleus, it transcriptionally activates a variety of genes including CyclinD1 and c-Myc, which promote tumorigenesis. Aberrant expression of $\beta$-catenin has been associated with both human and AOM-induced rat colorectal carcinogenesis (20,22,26). We previously found that PPE treatment decreased $\beta$-catenin nuclear expression in AOM-induced ACF in F344 rats. Herein, we showed that $\beta$-catenin nuclear accumulation was also lowered by PPE in the AOM-induced colon adenocarcinomas in F344 rats. These results were in line with the effects of PPE or EGCG on $\beta$-catenin in adenomas formed in colorectal cancer cell lines and APC$^\text{Min+}$ mouse model as we previously reported (7,8). The above-mentioned findings supported the notion that $\beta$-catenin-mediated oncogenic signaling is

### Table 3. Effects of PPE treatment on apoptotic index and nuclear expression of $\beta$-catenin and RXR$\alpha$, $\beta$, and $\gamma$ in AOM-induced colon lesions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Adenoma Apoptotic index</th>
<th>Adenocarcinoma Apoptotic index</th>
<th>$\beta$-catenin</th>
<th>RXR$\alpha$</th>
<th>RXR$\beta$</th>
<th>RXR$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.62 ± 2.40% (32)</td>
<td>3.11 ± 1.20% (43)</td>
<td>28.85 ± 0.11 (43)</td>
<td>1.7 ± 0.58 (43)</td>
<td>1.44 ± 0.55 (43)</td>
<td>1.2 ± 0.42 (43)</td>
</tr>
<tr>
<td>PPE</td>
<td>5.34 ± 2.68% (20)</td>
<td>6.33 ± 1.19% (10)</td>
<td>13.20 ± 0.08 (10)</td>
<td>2.6 ± 0.70 (10)</td>
<td>2.0 ± 0.47 (10)</td>
<td>2.1 ± 0.32 (10)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The apoptotic bodies and $\beta$-catenin nuclear staining were manually counted and the expression of RXR$\alpha$, $\beta$, and $\gamma$ was semi-quantified as described in materials and methods. The data were shown in mean ± SD, and Mann-Whitney U test was used for statistical analysis.

![Figure 3](image-url). Effects of dietary PPE administration on plasma levels of PGE2 and LTB4 in AOM-treated rats. At week-34 after the last AOM injection, blood was collected. Levels of PGE2 and LTB4 were analyzed by EIA as described in Methods. Data represent mean ± SD (n = 10 for the control group, and n = 9 for the PPE-treated group). Statistically significant difference was tested by Mann-Whitney U test.
a major target of PPE, and its inhibition by PPE treatment might be critical for the overall inhibition of colorectal tumorigenesis.

The family of retinoid X receptors (RXRs), including RXRα, β, and γ, is involved in regulating cell proliferation, differentiation, apoptosis, and development. Targeted loss of the RXRα gene led to embryonic lethality (29,30), and conditional disruption of the RXRα allele in mouse prostate epithelium resulted in the development of prostate intraepithelial neoplasia (31). Several studies showed that reduced expression of RXRα, β and γ was correlated with the development of skin (32), gastric (33), prostate (34), breast (35), pancreatic (36), and thyroid (37) cancers. We found that reduced expression of RXRα, β, and γ was associated with both AOM-induced rat and human colorectal carcinogenesis (unpublished data). We previously observed that PPE treatment of rats increased the expression of RXRα in AOM-induced colonic ACF (18). In this study, we observed that PPE treatment led to significantly increased nuclear expression of not only RXRα but also RXRβ and γ in AOM-induced colorectal adenocarcinomas. These data suggest that RXRα, β, and γ are potential targets of PPE treatment.

Bioavailability is an important factor that influences the biological functions of orally administered reagents. Our results showed that PPE feeding resulted in the different levels of tea polyphenols in the plasma and colonic mucosa of the rats. Even though PPE contained a much higher level of EGCG (65%) than other polyphenols such as EGC (3%), EC (9%), the plasma levels of EC and EGC were higher than those of EGCG. This is due to the lower systemic bioavailability of EGCG than the nongallated tea polyphenols, namely EGC and EC. The unabsorbed EGCG would go to the colon and be absorbed into the colonic mucosa, which led to the high level of colonic EGCG. We believe most of the colonic mucosal tea polyphenols quantified by HPLC method were intracellular, because the colonic tissues were extensively washed. However, the possibility that some of the polyphenol molecules were adsorbed to the colonic mucosa cannot be completely eliminated. EGCG is the most biologically active polyphenol in tea. The rather high concentration of EGCG in the colon is apparently responsible for the inhibitory activity observed herein. Based on the allometric principle of dose translation from animals to humans (38), a diet containing 0.24% PPE is equivalent to the consumption of 1,200 mg PPE for a person that requires 2,500 kcal per day. According to the USDA database, a cup of tea contains approximately 300 mg of catechins (the actual levels of catechins consumed may be lower than this value). Therefore, the presently used dose corresponds to the consumption of 4–5 cups of tea a day for humans. This will be a situation of heavy tea drinkers.

Results on the inhibitory effects of tea polyphenols against colon carcinogenesis in rat models have not been consistent. The present study provides strong evidence for the effectiveness of tea polyphenols in inhibiting colorectal carcinogenesis. Our results also demonstrated the inhibitory effects of tea polyphenols on the molecular events during colorectal carcinogenesis, such as enhanced cellular apoptosis, reduced aberrant nuclear β-catenin expression, and increased nuclear expression of RXRα, β, and γ. These events may be used as biomarkers to monitor the effects of tea polyphenols on humans.

### Funding

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### References

34. Mao GE, Reuter VE, Cordon-Cardo C, Dalbagni G, Scher HI, et al.: Decreased retinoid X receptor-alpha protein expression in basal cells occurs in the early stage of human


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