Effects of black tea, green tea and wine extracts on intestinal carcinogenesis induced by azoxymethane in F344 rats

Giovanna Caderni, Carlotta De Filippo, Cristina Luceri, Maddalena Salvadori, Augusto Giannini, Annibale Biggeri, Sophie Remy, Veronique Cheynier and Piero Dolora

Department of Pharmacology, University of Florence, 6 Viale G.Pieraccini, 50134 Florence, 1USL 10H, Antella, Florence, 2Department of Statistics, University of Florence, 59 Viale Morgagni, 50134 Florence, Italy and 3Research Unit Biopolymers and Aromas INRA-ISVV, 2 Place Viala, 34060 Montpellier cedex, France

To whom correspondence should be addressed
E-mail: gioca@pharm.unifi.it

We investigated whether polyphenolic extracts from black tea, green tea or red wine affect azoxymethane (AOM)-induced intestinal carcinogenesis. Male F344 rats were treated 10 times (1 week apart) with AOM (7.4 mg/kg, s.c.) and then allocated into groups receiving black tea, green tea or red wine extracts mixed in the diet at a dose of 50 mg/kg body weight for 16 weeks. In the rats treated with black tea or wine extracts, there were significantly fewer colorectal tumours than in controls (the mean ± SE number of tumours/rat was 2.54 ± 1.6 in controls, 1.54 ± 1.4 in the black tea group, 3.2 ± 1.9 in the green tea group and 1.63 ± 1.6 in the wine extract group). Significantly fewer rats in the black tea and wine extract groups had adenomas than in controls (86%, 59%, 90% and 50% of rats in the control, black tea, green tea and wine extract groups, respectively, had adenomas). The tumours from the black tea group and, to a lesser extent, those from the wine group, had a significantly greater apoptotic index than tumours in controls (mean ± SE apoptotic index: 2.92 ± 0.25, 4.13 ± 0.46, 2.88 ± 0.30 and 3.72 ± 0.46 in controls, black tea, green tea or wine extract groups, respectively). In contrast, the apoptotic index of the normal mucosa did not vary among groups. These data indicate that black tea and wine extracts, but not green tea extracts, can protect against AOM-induced colon carcinogenesis by a mechanism probably involving increased apoptosis in tumours.

Introduction

Although genetic background plays a role in the aetiology of human colon cancer, epidemiological and experimental studies indicate that environmental factors and diet in particular are probably important determinants of this neoplastic disease (1). Many foods or food components affect the risk of developing colon cancer, either increasing or decreasing it (1–5). In recent years, growing attention has been paid to food components with a potential cancer-inhibiting effect, with the hope of identifying effective chemopreventive diets or dietary supplements for human use.

Among potentially chemopreventive food components, polyphenols, a heterogeneous group of chemicals characterized by hydroxylated aromatic rings, have been considered with interest. They decrease experimental carcinogenesis in various models (6–9) and are abundant in fruits and vegetables, the consumption of which has been consistently associated in humans with a lower risk of different types of cancer, including colon cancer (1).

Polyphenolic powders obtained from red wine delay tumour onset in transgenic mice spontaneously developing neurofibroma-like tumours (9). Polyphenolic extracts from green or black tea have also been reported to decrease experimental carcinogenesis in various rodent organs such as skin, lung and oesophagus (6–8,10), whereas the effect of these compounds on the colon is less clear. In rats, polyphenolic extracts from green tea have been demonstrated to decrease colon cancer induced by carcinogens, but some authors report conflicting evidence (2,11–13). Similarly, contradictory results have been reported on the effect of polyphenol extracts from black tea on experimental colon carcinogenesis (5,14).

Given these results and the fact that, in developed countries, where colon cancer is one of the most common neoplastic diseases, black tea and other beverages, such as red wine, may be significant sources of polyphenols, we thought it important to ascertain whether polyphenol extracts from black tea, green tea and red wine affect azoxymethane (AOM)-induced intestinal carcinogenesis in rodents. Since it has been suggested that chemopreventive factors may act by increasing apoptosis in tumours and in the colon mucosa (15–17), we also determined whether this parameter was affected in the colon mucosa and in tumours in rats treated with polyphenols.

Materials and methods

Materials

AOM was purchased from Sigma (Milan, Italy) and dietary components from Piccioni (Gessate, Milan, Italy).

Wine polyphenolic extracts (WE) were prepared from a 2-year-old red wine, 1994 vintage, made from Cabernet Sauvignon grapes by standard red wine making procedures at the Arzens Cooperative winery (Arzens, Aude, France), as follows: alcohol was eliminated by distillation of 40 l batches and the remaining solution was deposited on a vinyl-divinyl benzene column. After rinsing with water to remove sugars and organic acids, the phenolic pool of chemicals present in wine was eluted with 90% ethanol in water, concentrated by vacuum, evaporation and atomized. The WE, analysed by High Performance Liquid Chromatography-Array Detector (HPLC-DAD) as described earlier (18), contained 4.4% (w/w) anthocyanins, 0.8% flavonols, 2.0% phenolic acids, 1.4% catechin, 1.0% epicatechin and 28.0% proanthocyanidin units, consisting of 18.0% epigallocatechin, 13.2% catechin, 65.0% epicatechin and 3.8% epicatechin gallate, with a mean degree of polymerization of 6.8. Together, these compounds accounted for 45% of the WE in weight. Approximately half of the WE consisted phenolic species derived from genuine grape constituents in the course of wine making and ageing and of various derived phenolic species, including dimeric flavanol–anthocyanin adducts (19). The phenolic composition of the WE powder was similar to that of the initial wine. WE also contained about 5.0% polysaccharides and 2.8% proteins.

Green tea (GT) and black tea (BT) extracts were provided by Unilever Research (Colworth Laboratory, Sharnbrook, Bedford, UK). The extracts were ‘decaffeinated’ total tea solids and had the following composition (green tea): 1.91% gallic acid, 0.39% theobromine, 5.76% epigallocatechin, 1.44%...
catechin, 1.07% caffeine, 4.03% epicatechin, 13.11% epigallocatechin gallate, 0.87% gallocatechin gallate, 7.96% epicatechin gallate. These compounds account for 36% of the total green tea solids present in a water extract, a value similar to that reported by Wang et al. (7). The composition of the black tea extract was 2.93% gallic acid, 0.45% theobromine, 1.47% epigallocatechin, 0.61% catechin, 0.23% caffeine, 1.76% epicatechin, 2.44% epigallocatechin gallate, 0.16% gallocatechin gallate, 2.55% epicatechin gallate, 0.32% theaflavin, 0.83% theaflavin 3-methylethyl gallate, 0.36% theaflavin-3'-methylethyl gallate and 1.01% theaflavin digallate.

Animals

We used 4–5 week old male F344 rats (Nossan, Correzzana, Milan, Italy). The animals were housed in plastic cages with wire tops and bottoms and maintained at a temperature of 22°C, with a 12 h light–12 h dark cycle, according to the European Union Regulations on the Care and Use of Laboratory Animals (20). The experimental protocol was approved by the Commission for Animal Experimentation of the Ministry of Health, Rome, Italy. After their arrival from the supplier, the animals (n = 99) were quarantined for 1 week, during which time they were fed a standard lab chow. They were then shifted to a high-fat diet, the composition of which was based on the AIN76 diet, modified to contain a high level of fat (230 g of corn oil per kilogram), a low level of cellulose (20 g/kg w/w) and a low level of calcium (1.3 g/kg), to mimic the diet typical of western human populations at high risk of colon cancer (the caloric content of this diet was 4814 kcal/kg) (22). Rats were then treated s.c. with 10 weekly injections (1 week apart) of AOM (7.4 mg/kg; total dose 74 mg/kg) following the protocol of Yamane et al. (2). One week after the last AOM injection, rats were randomly allocated to receive a high-fat diet alone (control group), or supplemented with BT, GT or WE. Each polyphenolic extract was administered mixed in the diet as described for red wine polyphenolic extracts delaying tumorigenesis in transgenic mice (9) and for different compounds tested in chemoprevention in the control, BT, GT and WE groups, respectively. Some studies of colon carcinogenesis (3,15,16). rats died during the experiment, seven during the treatments to de...
Table I. Number of tumours (adenomas and cancers)/rat and their location in AOM-induced rats treated with different polyphenol extracts

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Tumours/rat</th>
<th>Colon–rectum</th>
<th>Small intestine</th>
<th>Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (22)</td>
<td>2.54 ± 1.6</td>
<td>0.18 ± 0.39</td>
<td>0.27 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>BT (22)</td>
<td>1.54 ± 1.4*</td>
<td>0.27 ± 0.55</td>
<td>0.36 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>GT (20)</td>
<td>3.2 ± 1.9</td>
<td>0.25 ± 0.44</td>
<td>0.3 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>WE (22)</td>
<td>1.63 ± 1.6*</td>
<td>0.23 ± 0.43</td>
<td>0.27 ± 0.45</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se; numbers in parentheses represent the number of rats in each group.

*pSignificantly different (P < 0.05) from the control group by Poisson regression.

Table II. Number of adenomas or cancers in the colon/rectum of AOM-induced rats treated with different polyphenolic extracts

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Tumours in the colon/rectum/rats</th>
<th>Adenomas</th>
<th>Cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (22)</td>
<td>1.72 ± 1.31</td>
<td>0.82 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>BT (22)</td>
<td>1.00 ± 1.15*</td>
<td>0.54 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>GT (20)</td>
<td>2.55 ± 1.50</td>
<td>0.70 ± 1.03</td>
<td></td>
</tr>
<tr>
<td>WE (22)</td>
<td>1.09 ± 1.30</td>
<td>0.54 ± 0.74</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se; numbers in parentheses represent the number of rats in each group.

*pSignificantly different (P < 0.05) from controls by Poisson regression.

Table III. Incidence of tumours in different locations

<table>
<thead>
<tr>
<th>Group</th>
<th>Colorectum/small intestine</th>
<th>Adenomas</th>
<th>Cancers</th>
<th>Ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>19/22 (86%)</td>
<td>10/22 (45%)</td>
<td>6/22 (27%)</td>
<td></td>
</tr>
<tr>
<td>BT</td>
<td>13/22 (59%)*</td>
<td>10/22 (45%)</td>
<td>8/22 (36%)</td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>18/20 (90%)*</td>
<td>12/20 (60%)</td>
<td>5/20 (25%)</td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>11/22 (50%)**</td>
<td>13/22% (59%)</td>
<td>6/22 (27%)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent number of rats with tumours in the different locations/group; the numbers in parentheses are values expressed as percentage.

*p, **Significantly different from controls (P < 0.05 and P < 0.01, respectively) by Poisson regression.

The results of this study indicate that chronic treatment of F344 rats with a diet supplemented with polyphenols from black tea or red wine inhibited the process of AOM-induced intestinal carcinogenesis. Green tea polyphenols were not effective in our experiments.

Weisburger and co-workers (14) gave black tea to rats in drinking water and studied the effects on colon carcinogenesis; they reported a reduction in AOM-induced preneoplastic lesions in the colon. In a later long-term carcinogenesis experiment (5), the same authors did not contain a protective effect; in fact, they found that black tea promoted colon tumours in the post-initiation phase. In this study, rats were killed after 50 weeks of treatment—a long period, after which the tumour rate was very high. In contrast, we decided to kill the animals 26 weeks after the first AOM treatment. Weisburger et al. (5) found only intestinal cancers in the treated animals, whereas we found a mixture of adenomas and cancers. In our study black tea and wine polyphenols had a greater effect on adenoma than on cancer formation.

A protective effect of black tea on colon carcinogenesis had been documented previously in a study of aberrant crypt foci, induced by AOM or by food carcinogens such as 2-amino-3-methyl-imidazo (4,5-f)quinoline (14,26). Therefore, it is possible that the inhibitory effect of black tea polyphenols is more evident in the early stages of colorectal carcinogenesis (the formation of aberrant crypt foci and adenomas), while advanced lesions are refractory to inhibition by polyphenols.

In the present study we also found that green tea polyphenols did not affect colon carcinogenesis. Yamane et al. (2) reported that green tea was protective against colon carcinogenesis induced by AOM; they administered tea extracts in drinking water at a dosage roughly equivalent to 10–100 mg polyphenols/kg body weight (2,12). A similar protective effect of a green tea extract was found in N-methyl-N-nitrosourea-induced colon carcinogenesis by Narisawa et al. (11), who administered tea in drinking water to rats at different doses (6–150 mg tea extract/kg body weight). At variance with previous studies in which tea extract was administered as a drink (2,5,11,12,14), we administered a dried polyphenol powder with the food, a protocol that has been adopted in several chemoprevention studies of colon carcinogenesis (3,15,16) and, most importantly,
in previous experiments showing a protective effect against tumour induction by red wine total extracts (9). Although it is possible that this decision could have affected the results, green tea extracts, administered in drinking water, have been reported to increase AOM-induced colon carcinogenesis (13).

The discrepancies among all these studies, including ours, could be due to the variable composition of the tea preparations used in different experiments. Catechins, which are thought to be anticarcinogenic, were present at high concentrations (75%) in the work of Yamane et al. (2), but at lower concentrations (33.2%) in our green tea extract. Yet, the concentration of catechin in our extract (~26%) was similar to that used in other studies in which a protective effect on chemical carcinogenesis was documented (7,8,12). It should also be noted that most of the published studies on green and black tea did not use decaffeinated extracts (2,5,13). Decaffeination could alter the effectiveness of polyphenols, as documented in skin carcinogenesis (8). Given the complexity of tea composition, it is obvious that more studies are needed to identify individually active components of these mixtures and to determine the possibility of antagonistic or synergistic effects.

In the present paper we also reported that wine polyphenolic extracts partially inhibit the induction of colorectal carcinogenesis by AOM. The alcohol-free polyphenolic extract from red wine used in the present experiments contained monomeric polyphenols such as anthocyanins, monomeric flavanols, flavonoids and phenolic acids, along with complex phenols and tannins with an average degree of polymerization of 6. It has been reported that wine solids with a similar phenolic composition but containing relatively large amounts of non-phenolic compounds (tartaric acid and sugars) delay spontaneous tumour onset in transgenic mice (9). The effect of wine extracts on colon carcinogenesis had not been tested before. Recently, we reported that extracts of red wine rich in complex polyphenols and tannins, but free of low-mass phenols such as anthocyanins or catechins, did not modify carcinogen-induced preneoplastic lesions in the colon of rats (26). The fact that in the present study we found a protective effect of wine extract suggests that such low-mass compounds may be responsible for any protective effect observed. Accordingly, we also recently demonstrated that resveratrol, a low-mass polyphenol present in grapes and in red wine, depresses the growth of preneoplastic lesions in the colon of rats treated with AOM (28). As discussed above in regard to tea, given the complexity of composition of wine extracts, further studies are needed to elucidate which particular compounds are responsible for the observed effect.

It should also be noted that rats in the WE group, as well as those in the GT group, weighed less than controls at the end of the experiment. Caloric restriction, resulting in a body weight reduction of 60–77%, has been reported to decrease AOM-induced colon carcinogenesis in rats (29). However, the difference in rat weight in some experimental groups was statistically significant but not very marked (94% of the body weight of controls) and was found only at the end of the experiment. Moreover, both GT- and WE-treated rats weighed less than controls, but an inhibitory effect on colon carcinogenesis was observed only in the WE group.

In this study we also observed an increased AI in the tumours but not in the normal mucosa of the animals treated with black tea and red wine polyphenols, an effect that could explain the inhibition of tumour growth. The induction of apoptosis in neoplastic cells is considered a possible mechanism for eliminating cells with a high level of DNA damage (15). With an approach similar to that used in the present study it was demonstrated that chemopreventive substances such as sulindac, curcumin, phenethyl-3-methylcaffeate and 6-phenylhexyl isothiocyanate increase apoptosis in experimentally induced colonic tumours (15), suggesting increased apoptosis as a mechanism of chemoprevention (16).

The results reported here indicate that polyphenol-rich extracts from black tea and red wine inhibit colon carcinogenesis induced by AOM in rodents. Whether such an effect is relevant for humans is not known. A recent prospective cohort study (30) has shown that regular consumption of black tea significantly reduces the risk of colon cancer, although previous epidemiological studies did not show strong associations between the consumption of black tea or wine and colorectal cancer (1).

In conclusion, we demonstrated that polyphenols of widely consumed beverages could inhibit colon carcinogenesis and we suggested that such compounds might be effective in inhibiting carcinogenesis in humans. It will now be important to obtain information on the biological activity of individual components in the complex mixture of polyphenols present in wine and tea.

Acknowledgements

This work was financially supported by grants from the European Community FAIR programme (grant no. CT95/0653), QLRT 1999-00505 and QLKJ-1999-00346, by the Ministero della Università e della Ricerca Scientifica e Tecnologica and by the University of Florence, Italy.

References

Polyphenolic extracts and intestinal carcinogenesis


