Published in final edited form as:

Nutr Cancer. 2001 ; 41(1-2): 98–103.

Inhibition by White Tea of 2-Amino-1-Methyl-6-Phenylimidazo[4,5-*b*]Pyridine-Induced Colonic Aberrant Crypts in the F344 Rat

Gilberto Santana-Rios, Gayle A. Orner, Meirong Xu, Maria Izquierdo-Pulido, and Roderick H. Dashwood

G. Santana-Rios, G. A. Orner, M. Xu, and R. H. Dashwood are affiliated with the Linus Pauling Institute, Oregon State University, Corvallis, OR 97331. M. Izquierdo-Pulido is affiliated with the Facultat de Farmacia, Universitat de Barcelona, Spain

Abstract

There is growing interest in the potential health benefits of tea, including the anticarcinogenic properties. We report here that white tea, the least processed form of tea, is a potent inhibitor of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced colonic aberrant crypts in the rat. Male Fischer 344 rats were treated for 8 wk with white tea (2% wt/vol) or drinking water alone, and on alternating days in experimental Weeks 3 and 4 the animals were given PhIP (150 mg/kg body wt po) or vehicle alone. At the end of the study there were 5.65 ± 0.81 and 1.31 ± 0.27 (SD) aberrant crypt foci per colon in groups given PhIP and PhIP + white tea, respectively ($n = 12$, $P < 0.05$). No changes were detected in *N*-acetyltransferase or arylsulfotransferase activities compared with controls, but there was marked induction of ethoxyresorufin *O*-deethylase, methoxyresorufin *O*-demethylase, and UDP-glucuronosyltransferase after treatment with white tea. Western blot revealed corresponding increases in cytochrome *P*-450 1A1 and 1A2 proteins. Enzyme assays and Western blot also revealed induction of glutathione *S*-transferase by white tea. There was less parent compound and 4'-hydroxy-PhIP but more PhIP-4'-*O*-glucuronide and PhIP-4'-*O*-sulfate in the urine from rats given PhIP + white tea than in urine from animals given carcinogen + drinking water. The results indicate that white tea inhibits PhIP-induced aberrant crypt foci by altering the expression of carcinogen-metabolizing enzymes, such that there is increased ring hydroxylation at the 4' position coupled with enhanced phase 2 conjugation.

Introduction

Tea is one of the most widely consumed beverages in the world. The popularity of tea has increased with reports of potential health benefits against such chronic diseases as cardiovascular disease and cancer (1–4), notwithstanding recent findings to the contrary for gastric cancer among the Japanese (5). The health benefits of tea have been attributed in large part to the high levels of catechins and related polyphenols, operating via one or more of the following mechanisms: 1) induction of various enzyme activities involved in drug metabolism and carcinogen activation/detoxification, 2) inhibition of the activated metabolites of carcinogens and mutagens, 3) scavenging of reactive oxygen species and nitric oxide, 4) modification of signal transduction pathways, and 5) alterations in cell cycle check points and apoptosis (reviewed in Refs. 1–4). Through these various mechanisms, tea has demonstrated excellent chemoprotective properties in animal models of skin, lung, esophageal, and gastrointestinal cancers (6–9). Included among these investigations of the inhibitory properties of tea are several reports that focused on protection against the mutagenic and carcinogenic effects of heterocyclic amines (9–12).

Address correspondence to R. H. Dashwood, Linus Pauling Institute, Oregon State University, Weniger Hall 503, Corvallis, OR 97331. E-mail: rod.dashwood@orst.edu.

Heterocyclic amines are potent mutagens created during the cooking of meat and fish (13). Some heterocyclic amines, such as 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), induce tumors of the colon in experimental animals, and for this reason, they have been used as model compounds for the study of events that occur during colon carcinogenesis (14). We reported in previous studies using the Fischer 344 (F344) rat (9) that green and black teas protected against IQ-induced aberrant crypt foci (ACF), which are putative preneoplastic lesions in the colon (15,16). Because green tea was more effective than black tea, it was suggested that the degree of protection against IQ-induced ACF might be related to the extent of tea processing.

Tea processing and manufacturing practices influence the types of tea commonly consumed. Green tea, which is most popular in Japan and China, is produced when the leaves of *Camellia sinensis* are subjected to withering and then are pan-fried/steamed, rolled, shaped, and dried. When tea is pan-fried or steamed, polyphenol oxidases in the leaf become inactive, which prevents compounds such as epigallocatechin-3-gallate (EGCG) from undergoing oligomerization (oxidation) to more complex polyphenols, such as theaflavins and thearubigins (17). However, if the leaves are deliberately crushed or broken to facilitate oxidation of EGCG and other polyphenols, a darker color is produced and unique flavor characteristics associated with black and oolong teas are generated. White tea is the least processed type of tea, in that, unlike other teas, it is simply steamed and dried without a prior withering stage.

Because catechins are converted to more complex polyphenols with extent of tea processing and green tea was more effective than black tea in inhibiting IQ-induced ACF (9), we hypothesized that white tea also might exhibit chemopreventive properties against heterocyclic amines. Indeed, in preliminary studies in vitro, four white tea varieties (Silver Needle, Flowery Pekoe, Mutan White, and Exotica China White) exhibited strong antimutagenic activity in the *Salmonella* assay, and the most effective of these teas, Exotica China White, was significantly more potent than Premium Green (“Dragonwell Special grade”) tea against several heterocyclic amines, including PhIP (18). Therefore, we have sought to determine whether white tea would protect against PhIP-induced ACF in the rat and, if so, to provide data on the possible inhibitory mechanism(s).

Materials and Methods

Chemicals

PhIP was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Tea standards used in the analytic studies were purchased from Sigma Chemical (St. Louis, MO), except caffeine and gallic acid, which were obtained from Acros (Fair Lawn, NJ). Unless stated otherwise, reagents and supplies used for enzyme assays and Western blotting were from sources described previously (9).

Tea Preparation and Analysis

Exotica China White tea (referred to hereafter as “white tea”) was a gift of Stash Tea (Portland, OR). As in previous studies (9), the concentration used during brewing was 2 g of tea leaves per 100 ml of hot water (2%, wt/vol), and the brewing time was 5 min. Tea, brewed fresh on alternating days throughout the study, was analyzed routinely by high-performance liquid chromatography (HPLC) using a Shimadzu VP series instrument and a mobile phase composed of methanol and water. Full details of the HPLC conditions and liquid chromatography-mass spectrometry analyses are described elsewhere (18). The composition of 2% (wt/vol) white tea beverage given to the rats was as follows (mg/ml): 0.041 gallic acid, 0.022 theobromine, 0.003 theophylline, 0.465 (–)-epigallocatechin, 0.109 (+)-catechin, 0.822 caffeine, 1.229 EGCG, 0.031 (–)-epicatechin, and 0.619 (–)-epicatechin gallate.

Animals and Treatments

Male F344 rats, 5–6 wk of age, were purchased from the National Cancer Institute and housed two to three animals per cage. AIN-93G diet (Dyets, Bethlehem, PA) was provided to all groups ad libitum. Animals were given PhIP, PhIP + white tea, or vehicle + white tea (Fig. 1). Throughout the 8-wk study, Group 1 was given drinking water, whereas Groups 2 and 3 were given 2% (wt/vol) white tea in place of the drinking water. At 2 wk after the start of the experiment, three rats were selected at random from Groups 1 and 2 and killed to study changes in hepatic carcinogen-metabolizing enzymes. Each of the rats remaining in Groups 1 and 2 was given PhIP by oral gavage, 150 mg/kg on alternating days, for a total of five treatments per rat. Animals in Group 3 were given the equivalent volume of vehicle alone. As part of this study, three rats in each group were treated as described above, but they were housed individually in metabolism cages; urine was collected for 0–48 h after the final dose of PhIP to study changes in carcinogen metabolite profiles. At the end of 8 wk, each of the seven to nine animals remaining in each group was killed, and ACF were scored as described previously (19).

Enzyme Assays

As described in detail elsewhere (20), hepatic microsomal and cytosolic fractions were prepared on the day of sacrifice and stored in aliquots at -80°C . Assays for methoxyresorufin *O*-demethylase, ethoxyresorufin *O*-deethylase, UDP-glucuronosyltransferase (UDPGT), glutathione *S*-transferase (GST), *N*-acetyltransferase, and arylsulfotransferase were performed as reported previously (20).

Western Blot

To distinguish between different isoforms of GST, proteins were separated on 4–12% Bis-Tris gels (Novex, San Diego, CA) and transferred to polyvinylidene difluoride membranes. Cytosolic protein was loaded in the amount of 5 μg (GST- α and - μ) or 20 μg (GST- π) per lane, and the corresponding purified rat GST proteins (Oxford) were used as standards (100 ng of GST- α and - μ or 20 ng of GST- π). Primary and secondary antibodies were as follows: for GST- π , 1:2,000 dilution of rabbit anti-human GST P1-1 (Calbiochem); for GST- α , 1:3,000 dilution of goat anti-rat GST Ya (Oxford); for GST- μ , 1:3,000 dilution of goat anti-rat GST Yb (Oxford); for rabbit anti-goat horseradish peroxidase, 1:80,000 dilution (Sigma Chemical); and for goat anti-rabbit horseradish peroxidase, 1:25,000 dilution (Bio-Rad, Hercules, CA). Detection was by chemiluminescence (NEN, Boston, MA) onto film (Amersham, Piscataway, NJ). Blots were quantified using NIH Image 1.58 or an Alpha Imager system (AlphaInnotech, San Francisco, CA). Western blot cytochrome *P*-450 (CYP) 1A proteins was undertaken exactly as reported elsewhere (20).

PhIP Urinary Metabolites

Aliquots of urine collected from each rat were treated with deconjugating enzymes (aryl sulfatase or β -glucuronidase), or they were left untreated, and the PhIP urinary metabolites were separated by HPLC, as described in detail in our previous studies (21). PhIP and the major metabolites PhIP-4'-*O*-glucuronide, PhIP-4'-*O*-sulfate, and 4-hydroxy-PhIP were quantified by integration of the peak areas and expressed as "percentage of identified urinary metabolites."

Statistics

Values are means $\pm$ SD, and comparisons were made between the group given PhIP + white tea and the positive control group given PhIP and drinking water (Student's *t*-test, using SigmaPlot 2001).

Results

There was no significant differences among the groups with respect to mean body weight, food, or tea/water consumption on the basis of daily records kept throughout the 8-wk study (data not presented). Figure 2 summarizes the key findings from the ACF studies. At the end of 8 wk, rats given PhIP alone had, on average, 5.65 ± 0.81 (SD) ACF/colon ($n = 12$), and this was reduced significantly to 1.31 ± 0.27 in the group given PhIP + white tea. None of the rats given vehicle and white tea had ACF. According to the criteria reported previously (19,20), white tea had no significant effects on the overall size of ACF on the basis of the mean number of aberrant crypts per focus or the distribution of ACF within each size category (data not presented).

Results from the enzyme assays are summarized in Fig. 3. Because the data are from rats killed before the first dose of PhIP (Fig. 1), changes in enzyme activity reflect the responses to white tea treatment. Compared with rats given drinking water alone, ethoxyresorufin *O*-deethylase activity (a marker for CYP 1A1) was induced >4.5-fold, methoxyresorufin *O*-demethylase activity (a marker for CYP 1A2) was induced 6.5-fold, and UDPGT activity was induced 3-fold in rats given white tea. A slight but statistically significant induction of GST activity also was observed in rats given white tea compared with controls. However, no significant changes were seen for *N*-acetyltransferase or arylsulfotransferase activities after treatment with white tea.

Western blot confirmed the strong induction of CYP 1A1 and 1A2 by white tea (Fig. 4). Densitometry measurements indicated very low levels of CYP 1A1 in controls, such that the relative induction of CYP 1A1 was >30-fold for rats given white tea vs. drinking water. Expression of CYP 1A2 was readily detected in controls, and the relative induction by white tea compared with drinking water was approximately threefold on the basis of scanning densitometry measurements (Fig. 4). It was noted, however, that the total expression of hepatic CYP 1A2 protein in groups given drinking water or white tea was markedly higher than the corresponding levels of CYP 1A1.

Western blot for GST isoforms revealed that white tea caused a slight induction of GST- π without corresponding changes in GST- α or - μ (Fig. 5). A significant induction of GST enzyme activity also was detected in rats given white tea compared with the drinking water controls (Fig. 3).

Urine collected 0–48 h after dosing was analyzed by HPLC for the presence of parent compound and major metabolites (Table 1). Major peaks were identified as PhIP-4'-*O*-glucuronide, PhIP-4'-*O*-sulfate, 4'-hydroxy-PhIP, and parent compound (Table 1). White tea reduced the proportion of PhIP and 4'-hydroxy-PhIP but increased the relative amounts of PhIP-4'-*O*-glucuronide and PhIP-4'-*O*-sulfate. The change in relative amount of PhIP-4'-*O*-glucuronide was highly significant, being ~20-fold higher in rats given white tea than in controls given drinking water alone. In addition to PhIP-4'-*O*-glucuronide, treatment of urine with β -glucuronidase suggested that other PhIP-derived glucuronides were increased in rats given white tea, but these (minor) metabolites were not identified in the present study. The overall profiles of PhIP metabolites in Table 1 were in accordance with our previous studies in the F344 rats showing chemopreventive effects of chlorophyllin and indole-3-carbinol (21).

Discussion

The present study has shown, for the first time, that white tea is a potent inhibitor of PhIP-induced ACF in the rat. The degree of protection by white tea in this investigation exceeded that by green tea and black tea in a previous study of IQ-induced ACF (9). However, the two

studies had certain elements in common, including induction of hepatic CYP 1A proteins and an increase in the relative levels of ring glucuronides and sulfates eliminated in the urine. The changes in metabolite profiles and carcinogen-metabolizing enzymes are consistent with a mechanism involving increased ring hydroxylation of PhIP at the 4' position followed by phase 2 detoxification in the form of augmented glucuronide and sulfate conjugation. Also noteworthy was the increase in GST activity by tea, since a recent report has shown that GST P1 protects against *N*-hydroxy-PhIP cytotoxicity and DNA adduct formation in human prostate (22). It is possible, therefore, that GST induction by white tea favors conversion of *N*-acetoxy-PhIP back to the parent compound, reducing overall DNA adduct formation.

White and green teas contain similar levels of EGCG but differ in the relative amounts of other polyphenols and caffeine (18). Caffeine has been demonstrated as an important protective constituent of tea in mice treated with ultraviolet-B light (23) and in rats given tobacco-specific nitrosamines (24). However, concurrent administration of caffeine and PhIP for 10 wk to male F344 rats resulted in an increase in ACF formation (25), and in female F344 rats caffeine inhibited PhIP-induced mammary tumors but increased the incidence of colon tumors (26). In the present investigation, there was significant protection, not enhancement, of PhIP-induced ACF formation by white tea, but the relative contributions of individual tea constituents require further examination. As a first step in this direction, we combined the nine major constituents of white tea (including EGCG and caffeine) in proportion to their levels in 2% tea (wt/vol); the "artificial" white tea was significantly less effective than complete tea at inhibiting several heterocyclic amines in the *Salmonella* assay (18). These findings suggest that minor constituents in white tea might be important, possibly acting synergistically with the major constituents. Synergistic effects of certain tea polyphenols have been reported in cultured human lung cancer cells (27).

In addition to studies with cells in culture, some recent reports have focused on in vivo effects of individual tea constituents and their possible mechanisms after initiation. In one investigation, (+)-catechin was shown to inhibit by >70% the number of intestinal tumors in *Min/+* mice (28). In a second study, also using *Min/+* mice (29), white tea suppressed intestinal polyp formation to a degree that was equivalent to that obtained with sulindac, a potent nonsteroidal anti-inflammatory agent. Tumor suppression was accompanied by marked reductions in β -catenin expression as well as decreased expression of downstream targets of β -catenin/Tcf signaling (cyclin D1 and c-Jun). These studies suggest that, in addition to modifying phase 1 and phase 2 enzymes, white tea downregulates the β -catenin/Apc pathway after initiation. Because Apc has been described as the gatekeeper of colorectal cancer (30), the results with white tea have potentially important implications for chemoprotection against one of the major causes of cancer-related death in humans.

In summary, the present investigation has shown that white tea is a potent inhibitor of PhIP-induced ACF in the rat, extending previous work on the antimutagenic effects of white tea in vitro. Mechanism studies suggested that the inhibition resulted, at least in part, from alterations in carcinogen-metabolizing enzymes (CYP 1A1 and 1A2, UDPGT, and GST), leading to augmented metabolism of the parent compound and enhanced elimination of detoxified metabolites in the urine. The data provide additional support for a possible chemopreventive role of tea against cancers of the large bowel.

Acknowledgements

The authors thank Carmen A. Blum for help with animal handling and dosing, Bill Amberg, Lou-Anne Amberg, and Ann Perez for care of the animals, and Joy Edlund (Stash Tea) for providing Exotica China White tea. This work was supported in part by National Cancer Institute Grants CA-65525 and CA-80176. G. Santana-Rios and G. A. Orner were supported by National Institute of Environmental Health Sciences Toxicology Training Grant T32 ES-0707060.

References

1. Tijburg LB, Mattern T, Folts JD, Wiesgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases: a review. *Crit Rev Food Sci Nutr* 1997;37:771–785. [PubMed: 9447275]
2. Dreosti IE, Wargovich MJ, Yang CS. Inhibition of carcinogenesis by tea: the evidence from experimental studies. *Crit Rev Food Sci Nutr* 1997;37:761–770. [PubMed: 9447274]
3. Mukhtar H, Ahmad N. Green tea in chemoprevention of cancer. *Toxicol Sci* 1999;52:111–117. [PubMed: 10630599]
4. Yang CS, Chung JY, Yang G, Chhabra SK, Lee MJ. Tea and tea polyphenols in cancer prevention. *J Nutr* 2000;130:472S–478S. [PubMed: 10721932]
5. Tsubono Y, Nishino Y, Komatsu S, Hsieh C-C, Kanemura S, et al. Green tea and the risk of gastric cancer in Japan. *N Engl J Med* 2001;344:632–636. [PubMed: 11228277]
6. Lou YR, Lu YP, Xie JG, Huang M-T, Conney AH. Effects of oral administration of tea, decaffeinated tea, and caffeine on the formation and growth of tumors in high-risk SKH-1 mice previously treated with ultraviolet B light. *Nutr Cancer* 1999;33:146–153. [PubMed: 10368809]
7. Zhang Z, Liu Q, Lantry LE, Wang Y, Kelloff GJ, et al. A germ-line *p53* mutation accelerates pulmonary tumorigenesis: *p53*-independent efficacy of chemopreventive agents green tea or dexamethasone/*myo*-inositol and chemotherapeutic agents taxol or adriamycin. *Cancer Res* 2000;60:901–907. [PubMed: 10706103]
8. Wang ZY, Wang LD, Lee MJ, Ho CT, Huang MT, et al. Inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. *Carcinogenesis* 1995;16:2143–2148. [PubMed: 7554067]
9. Xu M, Bailey AC, Hernaez JF, Taoka CR, Schut HAJ, et al. Protection by green tea, black tea, and indole-3-carbinol against 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced DNA adducts and colonic aberrant crypts in the F344 rat. *Carcinogenesis* 1996;17:1429–1434. [PubMed: 8706244]
10. Stavric B, Matula T, Klassen R, Downie RH. The effect of teas on the in vitro mutagenic potential of heterocyclic amines. *Food Chem Toxicol* 1996;34:515–523. [PubMed: 8690310]
11. McArdle NJ, Clifford MN, Ioannides C. Consumption of tea modulates the urinary excretion of mutagens in rats treated with IQ: role of caffeine. *Mutat Res* 1999;441:191–203. [PubMed: 10333533]
12. Hirose M, Hasegawa R, Kimura J, Akagi K, Yoshida Y, et al. Inhibitory effects of 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), green tea catechins and other antioxidants on 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazo (Glu-P-1)-induced rat hepatocarcinogenesis and dose-dependent inhibition by HTHQ of lesion induction by Glu-P-1 or 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx). *Carcinogenesis* 1995;16:3049–3055. [PubMed: 8603484]
13. Skog KI, Johansson MAE, Jägerstad MI. Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem Toxicol* 1998;36:879–896. [PubMed: 9737435]
14. Nagao M, Ushijima T, Toyota M, Inoue R, Sugimura T. Genetic changes induced by heterocyclic amines. *Mutat Res* 1997;376:161–167. [PubMed: 9202752]
15. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen—preliminary findings. *Cancer Lett* 1987;37:147–151. [PubMed: 3677050]
16. Pretlow TP, O'Riordan MA, Somich GA, Amini SB, Pretlow TG. Aberrant crypts correlate with tumor incidence in F344 rats treated with azoxymethane and phytate. *Carcinogenesis* 1992;13:1509–1512. [PubMed: 1394832]
17. Yang, CS.; Wang, Z. Chemistry of tea. <http://www.teatalk.com/science/chemistry.htm>
18. Santana-Rios G, Orner GA, Amantana A, Provost C, Wu S-Y, et al. Potent antimutagenic activity of white tea in comparison with green tea in the *Salmonella* assay. *Mutat Res* 2001;495:61–74. [PubMed: 11448643]
19. Tachino N, Hayashi R, Liew C, Bailey GS, Dashwood RH. Evidence for *ras* gene mutation in 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced colonic aberrant crypts in the rat. *Mol Carcinog* 1995;12:187–192. [PubMed: 7727039]

20. Xu M, Chen R, Dashwood RH. Effect of carcinogen dose fractionation, diet, and source of F344 rat on the induction of colonic aberrant crypts by 2-amino-3-methylimidazo[4,5-*f*]quinoline. *Carcinogenesis* 1999;20:2293–2298. [PubMed: 10590222]
21. Guo D, Schut HAJ, Davis CD, Snyderwine EG, Bailey GS, et al. Protection by chlorophyllin and indole-3-carbinol against 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced DNA adducts and colonic aberrant crypts. *Carcinogenesis* 1995;16:2931–2937. [PubMed: 8603466]
22. Nelson CP, La Creis RK, Sauvageot J, Isaacs WB, De Marzo AM, et al. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione *S*-transferase. *Cancer Res* 2001;61:103–109. [PubMed: 11196146]
23. Huang M-T, Xie J-G, Wang ZY, Ho C-T, Lou Y-R, et al. Effects of tea, decaffeinated tea, and caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice: demonstration of caffeine as a biologically important constituent of tea. *Cancer Res* 1997;57:2623–2629. [PubMed: 9205068]
24. Chung FL, Wang M, Rivenson A, Iatropoulos M, Reinhardt J, et al. Inhibition of lung carcinogenesis by black tea in rats treated with a tobacco-specific carcinogen: caffeine as an important constituent. *Cancer Res* 1998;58:4096–4101. [PubMed: 9751618]
25. Tsuda H, Sekine K, Uehara N, Takasuka N, Moore MA, et al. Heterocyclic amine mixture carcinogenesis and its enhancement by caffeine in F344 rats. *Cancer Lett* 1999;143:229–234. [PubMed: 10503909]
26. Hagiwara A, Boonyaphiphat P, Tanaka H, Kawabe M, Tamamo S, et al. Organ-dependent modifying effects of caffeine, and two naturally occurring antioxidants α -tocopherol and *n*-tritriacontane-16,18-dione, on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary and colonic carcinogenesis in female F344 rats. *Jpn J Cancer Res* 1999;90:399–405. [PubMed: 10363577]
27. Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, et al. Synergistic effects of (–)-epigallocatechin gallate with (–)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. *Cancer Res* 1999;59:44–47. [PubMed: 9892181]
28. Weyant MJ, Carothers AM, Dannenberg AJ, Bertagnolli MM. (+)-Catechin inhibits intestinal tumor formation and suppresses focal adhesion kinase activation in the *Min/+* mouse. *Cancer Res* 2001;61:118–125. [PubMed: 11196148]
29. Orner GA, Dashwood W-M, Blum CA, Darío Díaz G, Li Q, et al. Response of *Apc*^{min} and A33DN6-cat mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Mutat Res*. In press
30. Kinzler KW, Vogelstein B. Cancer-susceptibility genes: gatekeepers and caretakers. *Nature* 1997;386:761–763. [PubMed: 9126728]

Figure 1.

Protocol used to examine protective effect of white tea on 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP)-induced aberrant crypt foci (ACF) in Fischer 344 (F344) rat. Tea was brewed at a concentration of 2% (wt/vol) and given as sole source of drinking water throughout study (Groups 2 and 3). At 2 wk after starting experiment and before treatment with PhIP, 3 rats were selected at random and killed for study of changes in hepatic carcinogen-metabolizing enzymes (Groups 1 and 2). Urine was collected 0–48 h after last of 5 treatments with PhIP or vehicle (3 rats/group) and analyzed for changes in PhIP metabolite profiles. Animals remaining in each group were killed after 8 wk, and ACF were scored, as described in detail elsewhere (19,20).

Figure 2. Inhibition of PhIP-induced ACF by white tea. Values are means $\pm$ SD; $n = 7-9$ rats/group. *, $P < 0.05$ vs. PhIP + water controls (by Student's t -test). No significant differences were detected with respect to size of ACF on the basis of mean number of aberrant crypts per focus (data not shown).

Figure 3.

Effect of white tea on hepatic enzyme activities in rat. Enzyme assays were performed after 2 wk of treatment with white tea, and corresponding microsomal or cytosolic fractions were used to study ethoxyresorufin *O*-deethylase (EROD), methoxyresorufin *O*-demethylase (MROD), UDP-glucuronosyltransferase (UDPGT), glutathione *S*-transferase (GST), *N*-acetyltransferase (NAT), and arylsulfotransferase (AST). Values are means $\pm$ SD; $n = 3$. *, $P < 0.05$; **, $P < 0.01$ (by Student's *t*-test).

Figure 4.

Effect of white tea on hepatic cytochrome *P*-450 (CYP) 1A1 and 1A2 proteins. Western blot and immunodetection of proteins of interest were performed after rats had been given water (controls) or white tea (2% wt/vol) for 2 wk. Values are means $\pm$ SD from densitometry measurements of corresponding blot shown above. **, $P < 0.01$ vs. controls (by Student's *t*-test). Results are from a single blot in each case but are representative of results obtained from ≥ 2 separate determinations.

Figure 5.

Effect of white tea on hepatic glutathione *S*-transferase (GST) proteins. Western blot/immunodetection was performed after rats had been given water (control) or white tea (2% wt/vol) for 2 wk. Values are means $\pm$ SD from densitometry measurements for corresponding blots shown above. *, $P < 0.05$ vs. controls (by Student's *t*-test). Results are from a single blot in each case but are representative of results obtained from ≥ 2 independent experiments.

Table 1
Urinary Excretion Profiles 0–48 h After PhIP Dosing^{a,b}

Group	Percentage of Identified Urinary Metabolites			
	PhIP-4'-O-glucuronide	PhIP-4'-O-sulfate	4'-Hydroxy-PhIP	PhIP
PhIP + water	0.63 ± 0.12	45.2 ± 5.53	40.2 ± 1.19	17.5 ± 5.16
PhIP + white tea	11.3 ± 2.55 [†]	73.8 ± 2.37 [*]	7.9 ± 1.77 [*]	7.0 ± 1.02 [*]

^a Values are means ± SD; *n* = 3. Metabolites were identified by coelution with authentic standard, through use of deconjugating enzymes, and by comparison with reported profile of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) metabolites in Fischer 344 rat (see Fig. 4 in Ref. 21).

^b Statistical significance is as follows:

^{*} *P* < 0.05;

[†] *P* < 0.01 vs. PhIP + water.