Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay

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Abstract

There is growing interest in the potential health benefits of tea, including the antimutagenic properties. Four varieties of white tea, which represent the least processed form of tea, were shown to have marked antimutagenic activity in the Salmonella assay, particularly in the presence of S9. The most active of these teas, Exotica China white tea, was significantly more effective than Premium green tea (Dragonwell special grade) against 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and four other heterocyclic amine mutagens, namely 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2). Mechanism studies were performed using rat liver S9 in assays for methoxyresorufin O-demethylase (MROD), a marker for the enzyme cytochrome P4501A2 that activates heterocyclic amines, as well as Salmonella assays with the direct-acting mutagen 2-hydroxyamino-3-methylimidazo[4,5-f]quinoline (N-hydroxy-IQ). White tea at low concentrations in the assay inhibited MROD activity, and attenuated the mutagenic activity of N-hydroxy-IQ in the absence of S9. Nine of the major constituents found in green tea also were detected in white tea, including high levels of epigallocatechin-3-gallate (EGCG) and several other polyphenols. When these major constituents were mixed to produce ‘artificial’ teas, according to their relative levels in white and green teas, the complete tea exhibited higher antimutagenic potency compared with the corresponding artificial tea. The results suggest that the greater inhibitory potency of white versus green tea in the Salmonella assay might be related to the relative levels of the nine major constituents, perhaps acting synergistically with other (minor) constituents, to inhibit mutagen activation as well as ‘scavenging’ the reactive intermediate(s). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Salmonella assay; Tea polyphenols; Heterocyclic amines; Catechins; Caffeine; EGCG; IQ; PhIP

1. Introduction

Tea is the second most widely consumed beverage in the world. Reasons for its great popularity range from cultural traditions to purported health benefits. Results from epidemiological studies as well as laboratory experiments suggest that consumption of tea
confers protection against the development of chronic diseases, such as cardiovascular disease and cancer [1–4].

The health benefits of tea have been mainly attributed to the relatively high levels of flavonoids, including catechins and other polyphenols. Precisely how tea polyphenols might provide health benefits remains unclear, but the following mechanisms have been proposed: scavenging of reactive oxygen species (ROS) [5], modification of signal transduction pathways, cell cycle checkpoints, and apoptosis [6–9], and the induction of various enzyme activities involved with drug metabolism and carcinogen activation/detoxification [10–12]. Through these various mechanisms, tea has demonstrated excellent chemoprotective effects in animal models of skin, lung, esophageal, and gastrointestinal cancers [13–18]. Included among the latter studies are investigations in vitro and in vivo of heterocyclic amine-induced mutagenesis and carcinogenesis [16–18].

Heterocyclic amines are procarcinogens created during the cooking of foods that contain sugars, amino acids and creatinine, namely meats and fish [19–21]. Certain heterocyclic amines induce tumors of the small intestine and 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 [22–26]. In our previous work, tea was demonstrated to protect against the heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), using the colonic aberrant crypt focus as an intermediate biomarker [18]. The degree of protection by tea appeared to be related to the extent of processing, since green tea was generally more effective than black tea in vitro and in vivo [16–18]. This suggested the possibility that higher antimutagenic or

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Fig. 1. Tea manufacturing processes, showing oxidation steps that increase as tea is converted from white → green → oolong → black. The minimal processing of white tea, plus the higher overall proportion of buds to leaf, gives a pale beverage with a slightly sweet, subtle flavor compared with other teas.
anticarcinogenic activity might be expected from teas that have undergone the least amount of processing.

Green tea, consumed mainly in Japan, China and Korea, is produced when freshly harvested leaves of *Camellia sinensis* are subjected to withering, and then they are panfried/steamed prior to rolling/shaping and drying (Fig. 1). Black tea, which represents >90% of the total consumption worldwide, follows some of the processing steps used for green tea, but with the critical difference that the leaves are bruised, crushed, or broken, thus allowing polyphenol oxidases in the leaf to generate theaflavins, thearubigins and other complex polyphenols from the endogenous catechins. Oolong tea, popular in China and Japan, goes through an intermediate process involving withering, bruising, brief oxidation, and firing/drying (Fig. 1). White tea, which has received little if any attention for its health benefits, represents the least processed of teas in that it goes through steaming and drying without a prior withering stage. Because the catechins are converted to theaflavins, thearubigins, and more complex polyphenols as green tea is processed into oolong and black teas, and the catechins generally are assumed to be more active based on their antioxidant and other protective properties [5–12], we hypothesized that white tea might prove to be more inhibitory than green tea in the *Salmonella* assay.

Therefore, we examined the antimutagenic activity of several white tea varieties in the *Salmonella* assay against IQ and other heterocyclic amines, including studies of the possible inhibitory mechanisms, and compared the results with those for green tea. Subsequently, several of the major constituents in white tea and green tea were reconstituted to form ‘artificial’ teas that were tested for antimutagenic activity. Finally, because white tea contains both leaves and buds, these were tested separately and in combination for their inhibitory activity in the *Salmonella* assay.

## 2. Materials and methods

### 2.1. Chemicals

The heterocyclic amines IQ, 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx), 2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), and 3-amino-1-methyl-5H-pyrido [4,3-b]indole (Trp-P-2) were from Toronto Research Chemicals (Ont., Canada), whereas 2-hydroxyamino-3-methylimidazo[4,5-f]quinoline (N-hydroxy-IQ) was from SRI International (Menlo Park, CA). Tea standards (+)-catechin (CAT), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), epigallocatechin-3-gallate (EGCG), theobromine (TB), theophylline (TP), and monochloroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Caffeine (CAF) and gallic acid (GA) were from Acros (Fair Lawn, NJ). For chemical structures of tea constituents, see [17]. Methanol (HPLC grade) was from Fisher Scientific (Fair Lawn, NJ). Materials used for the mutagenicity assays were from sources previously described [16,17].

### 2.2. Tea preparation

Three white teas (Exotica China white, Flowery Pekoe, and Mutant white) and the green tea Premium green (“Dragonwell special grade”) were generous gifts of The Stash Tea Co. (Portland, OR). A fourth white tea, Silver Needle, was obtained from Tea and Company (San Francisco, CA). Teas were brewed fresh immediately before each experiment using double distilled-deionized water. The concentration during brewing was 2 g tea leaves per 100 ml (2%, w/v) and the brewing time was 5 min, unless indicated otherwise. Teas were sterilized by passage through a 20 μm filter before testing for inhibitory activity in the *Salmonella* mutagenicity assay.

### 2.3. Analysis of teas

High performance liquid chromatography (HPLC) analysis of teas was performed on a Shimadzu VP series instrument equipped with a 25 cm × 4.6 mm, 5 μm particle size, reverse-phase column (Supelcosil™ LC-18). The mobile phase was composed of methanol (buffer A) and water (buffer B). Chloroacetic acid was added to both solvents at a final concentration of 0.3% (w/v) and the pH was adjusted to 4.5 using 1N NaOH. The gradient program used for separation of the tea components was as follows: 10% buffer A at 0 min, increased linearly to 40% buffer A at 50 min and returned to 10% buffer A at 60 min. The flow rate was 1 ml/min and detection was at 273 nm. The major
fractions were identified by co-elution with authentic standards, and subsequently confirmed by LC-MS using a Perkin-Elmer Sciex API III + triple-quadruple mass spectrometer with an atmospheric pressure chemical ionization (APCI) source.

2.4. Mutagenicity assays

The antimutagenic activities of white tea and green tea were studied against IQ and other heterocyclic amines using Salmonella typhimurium strain TA98 in the presence of aroclor-induced rat liver S9, using the method of Hernaez et al. [16,17]. Some experiments used the direct-acting mutagen N-hydroxy-IQ in the absence of an exogenous activation system. All tests were performed under subdued lighting. Unless stated otherwise, assays were conducted at 37°C for 30 min by preincubation of the inhibitor (0.2 ml, adjusted to 1 ml with water) with 10% S9 activation system (0.2 ml, or the equivalent amount of buffer alone), mutagen (0.01 ml, in DMSO), and bacteria (0.2 ml), followed by the addition of 2 ml soft agar and pouring onto minimal glucose plates. Each tea was brewed at a concentration of 2% (w/v), but the results are given for final concentrations in the preincubation assay, expressed as “% equivalent” after taking into account the dilution factor. All experiments were conducted at least twice, and more often three times, using triplicate plates for each dose tested. Appropriate solvent and vehicle controls were used in each assay; these included experiments in which the tea volume was varied from 0.05 to 1 ml and the volume was made up to 1 ml in each test tube with water, for both positive and negative (no mutagen, vehicle) controls. His+ revertant colonies were counted after 48 h of incubation at 37°C in the dark. Under the conditions reported here none of the tests gave evidence for toxicity, as determined in three ways. First, background lawns and spontaneous revertants counts were carefully monitored in each experiment. Doses of inhibitor that reduced the spontaneous count or produced thinning of the background lawn when inspected under the microscope were regarded as toxic, and were avoided in subsequent experiments. Second, smaller ‘suspect’ colonies, if detected, were picked from the plate and confirmed to be His+ revertants by streaking on agar plates in the presence and absence of added histidine. Third, to ensure against an effect on cell survival, the highest dose of antimutagen was tested as reported before [27], by serial dilution of the preincubation mixtures and pouring onto plates containing histidine and biotin. In addition to experiments with complete tea, purified tea components were mixed to produce ‘artificial’ teas and tested with the appropriate solvent and vehicle controls. None of the assays using tea or purified tea constituents in the absence of mutagen gave evidence for toxicity under the conditions reported here. Mutagen doses were selected from the linear region of the dose-response curve, as determined in preliminary experiments (not presented).

2.5. MROD assays (inhibition of CYP1A2)

To test for inhibitory effects of tea on the isozyme primarily responsible for the activation of heterocyclic amines, namely cytochrome P4501A2 (CYP1A2), we conducted methoxyresorufin O-demethylase (MROD) assays, as described before [18]. Reactions contained rat liver S9 plus co-factors, and they were started by the addition of NADPH. To parallel the mutagenicity assays, tea was added in increasing concentrations, and the final incubation was brought up to 1 ml with water. Resorufin product was determined by its fluorescence (540 nm excitation, 580 nm emission filters); to test for possible quenching, in some experiments the reaction was allowed to proceed in the absence of tea, and the appropriate concentration of tea was added after stopping the reaction.

2.6. Statistics

Group comparisons were made using one-way analysis of variance (SAS version 8 or StatView 5.01).

3. Results

3.1. Antimutagenic activities of white and green teas against heterocyclic amines

Initial assays compared the inhibitory activities of four different white tea varieties brewed for various times up to 5 min (Fig. 2). All four teas exhibited good antimutagenic activity against IQ in the presence of S9, with Mutant white and Exotica China white varieties being more potent than Silver Needle and
Fig. 2. Antimutagenic activities of four white teas in Salmonella typhimurium strain TA98. Each tea was brewed for 5 min at 2% (w/v) and 0.2 ml was added to the preincubation assay containing IQ + S9. See Section 2 for further details. Data points and bars indicate mean ± S.D. from triplicate plates and they are actual plates counts. In this experiment, spontaneous counts were in the range of 37–45 revertants per plate in the absence of mutagen (with DMSO alone) following each tea treatment, with normal growth of the background lawn. Dashed line indicates spontaneous counts (mean) for DMSO in the absence of tea. The results are from a single assay, but are representative of data obtained in separate tests with each tea individually.

Flowery Pekoe teas. In most cases, a brew time of only 1 min appeared to be sufficient to release most of the antimutagens, although Exotica China white tea (referred to hereafter as “Exotica”) showed evidence for increasing inhibition with brew time. Thus, Exotica tea was selected for further study, including direct comparison with green tea.

Exotica white tea and Premium green tea, each brewed for up to 10 min at 2% (w/v), were tested against IQ in the presence of S9. Premium green tea is a high-end variety with a large rolled leaf that opens completely during brewing, allowing for the rapid release of antimutagens, typically within 2–3 min of brewing (Fig. 3a). Exotica white tea showed consistently greater inhibitory activity than green tea under the same assay conditions (Fig. 3a, open symbols). The antimutagenic activity of both teas increased with concentration (Fig. 3b), but Exotica white tea was consistently more potent than Premium green tea at all concentrations tested. At the maximum concentration in the assay, neither tea gave evidence of overt toxicity based on normal growth of the background lawn and spontaneous counts in the normal range. The lack of toxicity for each tea was confirmed both in the presence and absence of mutagen, using DMSO as the vehicle control.
In subsequent experiments, each tea was tested against four other heterocyclic amines, namely, MelIQx, Trp-P-2, 4,8-DiMeIQx, and PhIP (Fig. 4). In all cases, white tea was more effective than green tea under the conditions of the assay. Occasionally, a slight enhancement was detected at the 0.1% green tea concentration (e.g. Fig. 4b and c); we do not know the significance, if any, of these increased responses. However, this is certainly not a rare phenomenon, since previous studies have shown slight enhancement of mutagenesis, for example, with low but not high doses of chlorophyllin against benzo[a]pyrene, copper chlorin against Glu-P-2, retinoic acid against aflatoxin B1, hemin against the tobacco-specific nitrosamine 4-(N-methyl-N-nitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK), and chlorophyll, Ge-protoporphyrin, and the food coloring agent Monascus red against 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole (N-hydroxy-Trp-P-2) [27–33].

Because some inhibitors, such as chlorophyllin [17], rely on complex formation for their antimutagenic activity, and this mechanism depends on a large molar excess of inhibitor to mutagen, we included higher mutagen doses in some experiments. For example, in assays using 1 mg PhIP per plate (Fig. 4d), white tea and green tea both exhibited potent antimutagenic activity, without toxicity, implying that mechanism(s) other than complex formation might be important.

One alternative mechanism, namely direct inhibition of the ultimate carcinogen, was tested using N-hydroxy-IQ in the absence of an exogenous activation system. In contrast to the results from experiments in which S9 was included in the assay (Figs. 3 and 4), green tea and white tea exhibited more or less equivalent inhibitory activity against N-hydroxy-IQ, even at high concentrations (Fig. 5a). This suggested that inhibition of the enzymes in S9 might be a factor
Fig. 4. Antimutagenic activities of Exotica white tea and Premium green tea against several heterocyclic amines. Assays were performed with Salmonella strain TA98 in the presence of S9 under the conditions used for IQ (Fig. 3b). Results are given as mean ± S.D. of actual plate counts from triplicate plates, and they are representative of the data obtained from two or more separate experiments for each mutagen/tea combination. Concurrent tests using the same amounts of tea in the absence of mutagen (DMSO alone) showed no evidence of toxicity, based on normal growth of the background lawn and spontaneous counts similar to those in the absence of tea. The dashed line in each figure represents the mean value for spontaneous counts, using DMSO (no mutagen) plus the maximum dose of tea. Each mutagen dose was selected from the approximate linear portion of the dose-response curve, based on preliminary assays conducted in the absence of tea (not shown).

in explaining the more potent antimutagenic activity of white tea versus green tea.

White tea at a concentration of 0.0125% inhibited MROD activity by 50%, and 0.05% white tea inhibited enzyme activity completely (Fig. 5b). At these concentrations of tea, there was minimal interference via direct quenching of fluorescence (closed symbols, Fig. 5b). When the data were corrected for quenching and converted to ‘percent inhibition’, EROD activity was attenuated at concentrations similar to those required for inhibition of IQ mutagenicity in the Salmonella assay, but at concentrations below those needed to inhibit the mutagenicity of N-hydroxy-IQ (Fig. 5c).

One of the major polyphenols in tea, namely EGCG, was tested in the MROD assay, together with a known inhibitor of CYP1A2 (3,3′-diindolylmethane, I33′). Concentrations of EGCG required to give a similar profile of MROD inhibition as seen for white tea were in the range 10–100 μM, the highest concentration being more effective than an equivalent level of I33′ (Fig. 5d).
Fig. 5. Mechanism studies with white tea and green tea. (a) Inhibition of N-hydroxy-IQ in the Salmonella assay in the absence of S9. Assays were conducted with Salmonella strain TA98, using triplicate plates per dose; data are given as mean ± S.D. and are representative of two separate experiments. Dotted line, spontaneous counts for vehicle (DMSO) plus the highest concentration of tea; no toxicity was observed under the conditions of the assay, using the criteria stated in Section 2. (b) Inhibition of MROD activity, a marker for CYP1A2. Assays were conducted with rat liver S9 and co-factors in the presence or absence of tea, including incubations with addition of tea after stopping the reaction (test for quenching). Data are given as mean ± S.D. from triplicate test tubes, and are representative of results from three such assays. (c) In order to directly compare results for white tea in three separate test systems, namely IQ-induced mutagenicity (+S9), N-hydroxy-IQ-induced mutagenicity (−S9), and CYP1A2 (MROD) enzyme assays, background counts or quenching results were subtracted and the data were converted to ‘percent inhibition’ using the following formulae: 1 − [induced TA98 revertants + tea]/[induced TA98 revertants − tea] × 100, or 1 − [MROD activity + tea]/[MROD activity − tea] × 100. (d) Inhibition of MROD activity by EGCG, a major polyphenol in tea, in comparison with a positive control inhibitor, 133’. Data are given as mean ± S.D., n = 3.

3.2. Tea composition

EGCG is a major polyphenol in green tea, but the levels of this compound and other catechins were not known in white tea. Therefore, quantitative and qualitative analyses of white tea and green tea were undertaken. Fig. 6 shows a typical HPLC chromatogram for Exotica white tea, with several peaks detected at 273 nm. The identities of nine of the major constituents were confirmed by UV spectral
Fig. 6. Analysis of the major constituents in Exotica white tea. (a) White tea was brewed for 5 min at 2% (w/v) and 20 ml were subjected to separation by reverse-phase HPLC, using conditions described in Section 2. Identification was based on (i) UV–VIS spectra for each of the fractions collected; (ii) co-elution with authentic standards; (iii) mass spectral analysis (data not shown). (b) HPLC analysis of Exotica white tea after different brew times. Bars indicate mean ± S.D. from three separate determinations. For the abbreviations used, see Section 2.

analyses, co-elution with authentic standards, and CI mass spectrometry (not shown). When green tea was separated using the same HPLC conditions (not shown), there were higher levels of CAT and EC, but lower concentrations GA, TB, ECG, and caffeine compared with white tea (Table 1). Interestingly, EGCG was present at equally high levels in both teas. One peak with Rt 6.1 min (Fig. 6a) was detected at higher levels in white tea than green tea, plus several minor peaks, but the identity of these constituents could not be confirmed in the present study.

The nine major tea components identified in Table 1 were detected after a brew time of only 0.5 min, and each continued to increase in concentration with brew times up to 5 min (Fig. 6b). These results suggested that the overall ratios of the nine major constituents were constant, and that the changes in tea composition with brew time were quantitative rather than qualitative.
Table 1
Concentrations of major constituents in white tea (WT) and green tea (GT)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Rt</th>
<th>WT (µg/ml)</th>
<th>WT (%)</th>
<th>GT (µg/ml)</th>
<th>GT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>4.5</td>
<td>0.041</td>
<td>0.203</td>
<td>0.005</td>
<td>0.027</td>
</tr>
<tr>
<td>TB</td>
<td>12.9</td>
<td>0.022</td>
<td>0.113</td>
<td>0.009</td>
<td>0.045</td>
</tr>
<tr>
<td>TP</td>
<td>15.1</td>
<td>0.003</td>
<td>0.015</td>
<td>0.005</td>
<td>0.027</td>
</tr>
<tr>
<td>EGC</td>
<td>19.7</td>
<td>0.465</td>
<td>2.324</td>
<td>0.044</td>
<td>0.219</td>
</tr>
<tr>
<td>CAT</td>
<td>20.8</td>
<td>0.109</td>
<td>0.543</td>
<td>0.405</td>
<td>2.024</td>
</tr>
<tr>
<td>CAF</td>
<td>26.1</td>
<td>0.822</td>
<td>4.109</td>
<td>0.403</td>
<td>2.014</td>
</tr>
<tr>
<td>EGCG</td>
<td>28.6</td>
<td>1.229</td>
<td>6.146</td>
<td>1.222</td>
<td>6.110</td>
</tr>
<tr>
<td>EC</td>
<td>31.8</td>
<td>0.031</td>
<td>0.153</td>
<td>0.190</td>
<td>0.949</td>
</tr>
<tr>
<td>ECG</td>
<td>40.1</td>
<td>0.619</td>
<td>3.095</td>
<td>0.403</td>
<td>2.014</td>
</tr>
</tbody>
</table>

\(^a\) Teas were brewed at 2% (w/v) for 5 min and separated using the HPLC conditions shown in Fig. 6a. Percent values in the table (mg/100 mg dry wt.) were calculated by peak area integration, with reference to the standard curve for the corresponding compound (not shown). Rt: retention time (minutes); for other abbreviations see Section 2.

3.3. Antimutagenic activities of artificial teas, leaves and buds

Due to the different composition of white and green teas (Table 1), the nine major constituents were mixed in concentrations identical to those in each tea, and these ‘artificial’ teas were tested for inhibitory activity in the MROD and *Salmonella* assays versus complete tea. In the MROD assay (Fig. 7a), the artificial white and green teas proved to be more effective than the corresponding complete tea, whereas the reverse was true in the *Salmonella* assay using IQ + S9 (Fig. 7b). In both assays, however, white tea or artificial white tea inhibited more effectively than the corresponding green tea or artificial green tea.

Finally, because Exotica tea is a ‘cut’ version of a loose leaf variety (Mutant white), we obtained the uncut tea and carefully separated the leaves and buds. The antimutagenic activity of cut leaf tea was greater than that of cut buds alone, or the mixture of cut leaves and buds (Fig. 7c). The uncut tea was significantly less effective than the cut variety against IQ (compare the final two bars in Fig. 7c). Testing of cut and uncut Premium green tea showed only slightly higher antimutagenic activity of the former (data not shown).

4. Discussion

In this report, we describe for the first time the antimutagenic activities of four white tea varieties in the *Salmonella* assay, and include results from studies of the inhibitory mechanisms and characterization of the major constituents. The most effective of the white teas, namely Exotica China white, showed better antimutagenic activity than Premium green tea against IQ and against four other heterocyclic amines in the presence of S9. Interestingly, both teas were equally effective against the direct-acting mutagen \(N\)-hydroxy-IQ (−S9), but the maximum inhibition was \(\sim 60\%\) versus \(>95\%\) in assays containing the promutagen in the presence of S9. In MROD assays containing S9, inhibition by white tea occurred at concentrations similar to those used in *Salmonella* assays with IQ (+S9), suggesting that constituent(s) in white tea might prevent the metabolic activation of heterocyclic amines via interference with CYP1A2. A concentration of 0.05% white tea inhibited MROD activity by approximately the same degree as 50–100 \(\mu\)M EGCG.

EGCG is a major polyphenol in green tea, but little was known about the content of EGCG or other constituents in white tea. One hypothesis for the different antimutagenic activities of white tea and green tea was that the more limited processing of the former tea (Fig. 1) might allow for higher levels of EGCG and related catechins to be recovered after brewing. Previous work indicated that green tea was more effective than black tea in inhibiting IQ-induced colonic aberrant crypt foci in vivo [18], possibly due to the conversion of catechins to theaflavins and higher molecular weight polyphenols during tea processing. A comparison of Exotica white and Premium green teas by HPLC revealed similar overall profiles for the major UV absorbing peaks, suggesting that the greater antimutagenic activity of the former tea was not due to a ‘unique’ constituent, present solely in the
Fig. 7. Inhibitory activity of ‘artificial tea’ (a mixture of nine major constituents) in comparison with complete tea in the MROD assay and the *Salmonella* assay, and antimutagenic activity of white tea leaves and buds. Based on the data in Table 1, each of the nine major tea constituents was mixed in the proportions and concentrations equivalent to those in green or white teas, and then tested (a) in the MROD assay and (b) in the *Salmonella* assay according to the protocols used previously. Results are given as mean ± S.D., n = 3. Controls for background counts and quenching were included as described before (Figs. 3b and 5b, respectively). (c) Leaves and buds from white tea were separated by hand and tested separately for antimutagenic activity; the tea was cut to a consistency used in commercial teabags, or in some cases left uncut. Results are given as mean ± S.D., n = 3; statistical analysis using ANOVA. Bars with different superscripts were statistically different, P < 0.05.

White tea. However, certain quantitative differences were detected, including higher levels of gallic acid, theobromine, EGC, caffeine, and ECG in white tea (Table 1).

When the nine major constituents were mixed to produce ‘artificial’ teas, according to their concentrations in white and green teas (Table 1), inhibitory activities in the MROD assay were generally higher than for the corresponding complete tea (Fig. 7a). However, complete tea was more effective than the corresponding artificial tea in the *Salmonella* assay. One interpretation of these results is that
inhibition of IQ-induced mutagenicity involves all of the constituents in tea, including minor constituents not present in the artificial tea. The major constituents might contribute primarily to inhibition of carcinogen activation via effects on CYP1A2, but minor constituents (not identified) could act synergistically to inhibit the activated carcinogen(s) (Fig. 5a). The results are in general accordance with previous work showing inhibition of the mutagenicity of N-hydroxy-IQ by EGCG and EGC concentrations in the range 10–50 μM [17]. These concentrations of tea polyphenols appear to be achievable in vivo, and relevant to human exposures. Thus, in rats given decaffeinated green tea (25 mg/kg body weight, i.v.), EGCG reached a peak concentration of ∼55 μM in the intestine, and maintained steady state levels of 15–20 μM for 4 h [34]. In humans consuming green tea, the circulating plasma levels of total catechins were in the order of 1–10 μM, but higher concentrations might be achieved in the tissues of the GI tract after oral ingestion of tea [35].

In contrast to other teas, white tea has a relatively high content of buds, which are covered with fine silvery hair and impart a white/gray appearance to the dried tea. It was possible that the more potent antimutagenic activity of white tea might be due to higher levels of antimutagens in the buds. However, when the leaves and buds were carefully separated and tested for antimutagenic activity, leaves alone showed better inhibition than buds alone, or a mixture of buds and leaves (Fig. 7c). When white tea was cut to a consistency found in commercially available tea bags, this increased the antimutagenic activity, and HPLC experiments revealed improved overall extraction of the major UV absorbing constituents (not shown). Although the content of chlorophyll-related compounds was not examined, these were most likely present only in the beverage obtained from green tea leaves, judging by the hues of the brewed beverage (Fig. 1). Chlorophylls and chlorophyllins are potent antimutagens against heterocyclic amines in the Salmonella assay [17,29–32,36], and chlorophyll-related compounds isolated from the non-polyphenolic fraction of green tea exhibit strong antioxidant activities in vitro [37]. However, it is evident from the results of the present investigation that chlorophyll-related compounds did not contribute to a higher degree of antimutagenic activity of green versus white teas. Additional studies with white tea are needed, including testing for possible synergistic effects of the individual constituents, as reportedly recently for EGCG in the presence of (−)-epicatechin using a human lung cancer cell line [38]. It also will be important to compare the inhibitory activities of white tea and green tea in vivo and cell culture assays, focusing on mechanisms such as induction of detoxification enzymes, scavenging of reactive species (free radicals and electrophilic intermediates of carcinogens), alterations in signal transduction pathways, and changes in cell cycle regulation and apoptosis [5–12].

In summary, results from the present study demonstrate for the first time that white teas exhibited potent antimutagenic activity against a number of heterocyclic amines in the Salmonella assay. Exotica China white tea was more effective than Premium green tea in these assays, and mechanism studies suggested inhibition of carcinogen activation via interference with the enzymes in S9, plus inhibition of the direct-acting mutagen(s). EGCG was identified as the major polyphenol in both white and green teas, but caffeine, gallic acid, theobromine, EGC, and ECG were present at higher concentrations in white tea. Thus, the greater inhibitory activity of white tea versus green tea appears to be related to higher concentrations of several of the major constituents, perhaps acting synergistically with minor constituents to prevent mutagenicity via multiple mechanisms.

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References


