We determine the superoxide formed in the self-degradation of mutagens activated by cytochrome enzymes and evaluated the scavenging effect of various tea extracts. Benz[a]pyrene (B[a]P) and 2-amino-6-methylpyridido(1,2-a:3',2'-d)imidazole (Glur-P-1) each produced a large amount of superoxide after activation by cytochrome enzymes. However, 2-amino-3-methyl-imidazo(4,5-f)quinoline (IQ), 3-amino-1,4-dimethyl-5H-pyridol(4,3-b)indole (Trp-P-1) and aflatoxin B1 (AFB1) failed to generate a significant amount of superoxide. The addition of a tea extract to the reaction system markedly inhibited the activation of superoxide from Glur-P-1. However, the tea extracts showed weaker inhibition of the B[a]P-mediated formation of superoxide. Among the four teas tested, the oolong tea extract tended to exhibit the strongest inhibitory effect. Our results suggest that the chemopreventive efficacy of a tea extract is partly associated with its antioxidant activity.

Key words: tea extract; superoxide; scavenging effect; mutagen

Accumulating evidence shows that free radicals and reactive oxygen species are involved in the pathogenesis of certain human diseases such as inflammation, cancer, aging and atherosclerosis. The production of oxyradicals in the activation process of chemical carcinogens by cytochrome P450 is a key step in tumor promotion and progression. Oxyladicals can also induce DNA damage, resulting in the specific activation of a human proto-oncogene. Certain scavengers of active oxygen can decrease the mutation induced by certain mutagens; therefore, the chemopreventive efficacy of antioxidants is becoming increasingly recognized.

Tea is one of the most widely consumed beverages in the world. Numerous biological activities of tea have been reported including its antimutagenic, antioxidative, antitumoral and cancer-preventive effects. In our previous study, we showed that certain tea extracts exhibited strong antimutagenic and antioxidative activity. The scavenging effect of a tea extract toward superoxide, hydrogen peroxide, and the hydroxyl radical was observed. Moreover, the antimutagenicity of the tea extract was related to its antioxidative activity. We reasoned, therefore, that a tea extract may prevent damage induced by active oxygen derived from the metabolism of mutagens. We address here evidence to back this reasoning.

Leaves of green tea, puchong tea, oolong tea, and black tea were purchased at a local market in Taichung, Taiwan. The tea leaves (20 g) were extracted with boiling water (400 ml) for 5 min, and the filtrate was freeze-dried. Each sample was prepared in triplicate, and the results were averaged.

Measurement of the superoxide derived from mutagens including IQ, Glur-P-1, Trp-P-1, B[a]P and AFB1 was assayed according to the method of Wataya et al. A mutagen (1 ml, in dimethylsulfoxide) was added to an S9 mix solution (9 ml, containing 4% S9 mix, 4 mM nicotine adenine dinucleotide, 5 mM glucose-6-phosphate, 80 mM MgCl2 and 130 mM KCl in a 0.2 M sodium phosphate buffer pH 7.4), while maintaining the final concentration of the mutagen to 3 mM. The S9 mix (37.6 mg of protein/ml; Organ Teknika Co., Switzerland) was prepared from Sprague-Dawley male rats treated with Aroclor 1254. The reaction mixture was incubated at 37°C for 30 min and then centrifuged at 4000 rpm for 10 min. The resulting supernatant was incubated with an equal volume of nitroblue tetrazolium (NBT, 60 μM) and a tea extract or phosphate buffer (control) at 37°C, and the absorbance at 490 nm was determined at intervals during the incubation.

The formation of superoxide was markedly catalyzed by B[a]P and by Glur-P-1 when activated by cytochrome P450 enzymes. The amount of superoxide increased with increasing incubation time, especially in the case of B[a]P (Fig. 1). Flowers et al. have demonstrated that rat hepatocytes treated with B[a]P-7,8-dione, a genotoxic metabolite of B[a]P, produced a large quantity of superoxide. They also indicated that B[a]P-7,8-dione caused damage to DNA via the generation of reactive oxygen species. In addition to superoxide, hydrogen peroxide was also generated when B[a]P was incubated with rat microsomes. Wataya et al. have indicated that the superoxide was derived from the N-hydroxy metabolites of 3-amino-1-methyl-5H-pyridol[4,3-bj]indole (Trp-P-2) during its autooxidation process. Sato et al. have also reported the generation of a measurable amount of superoxide in the activation of various heterocyclic amine mutagens mediated by NADPH/cytochrome P450 reductase. In all tests, the IQ type of mutagen such as 2-amino-3,5-dimethylimidazo[4,5-f] quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline (MeIQx), and IQ produced more superoxide than the other heterocyclic amines. The pro-
duction of superoxide from the heterocyclic amine mutagens was correlated well ($r=0.88$) with their mutagenicity in *Salmonella typhimurium* by the Ames test. In this study, however, no significant promotion of superoxide generation by IQ was apparent.

With a realization of the role of oxyradicals in mutagenesis and carcinogenesis, the chemopreventive efficacy of antioxidants and radical scavengers has been intensively investigated. A number of antioxidants and related compounds such as butylated hydroxyanisole, butylated hydroxytoluene, tocopherols and carotenoids have been tested for their effects on mutagenesis or carcinogenesis. Many researchers have recently focused on the chemopreventive ability of polyphenols, and especially of tea catechins.

The effects of various tea extracts on the formation of superoxide from B[a]P and Glu-P-1 are summarized in Table 1. The addition of a tea extract to the reaction system resulted in marked inhibition of the generation of superoxide from Glu-P-1. All the tea extracts tested, except black tea, exhibited over 95% inhibition at a dosage of 1 mg. However, these extracts showed weak inhibition of the B[a]P-mediated formation of superoxide. Except for oolong tea, the other three kinds of tea inhibited the generation of superoxide by less than 50% at a 5 mg dosage. In our previous study, we reported that an oolong tea extract expressed higher antimutagenic activity, antioxidative activity, and scavenging effect on superoxide than the other kinds of tea. The data also indicated that the antimutagenicity of each tea extract toward B[a]P was significantly correlated with its scavenging effect on superoxide. Based on the foregoing results, we consider that the antimutagenicity of the tea extracts, especially toward B[a]P, was due to their scavenging effect on active oxygens.

The antioxidative activity of catechins has been suggested to play an important role in their antimutagenic and anticarcinogenic effects. The tea component, epigallocatechin gallate (EGCG), can reduce the DNA damage induced by the N-OH-Glu-P-1 mutagen in a dose-dependent manner. The N-hydroxyl derivative of Trp-P-2 was degraded in aqueous media to form active oxygen species such as hydroxyl radicals, resulting in single-strand DNA breaks. Our result also shows that Glu-P-1 produced superoxide via microsomal enzyme activation. Hence, the preventive action of the tea extracts may be inhibiting the oxidative degradation of heterocyclic amine derivatives or by scavenging the active oxygens produced in the process of oxidative degradation. Hochstein and Atallah have suggested that the antimutagenic and anticarcinogenic effects of antioxidants may be due to their scavenging effects on free radicals or their ability for inducing antioxidative enzymes. Tea polyphenols exhibited a strong free-radical-scavenging effect.

The importance of the antioxidant action of each tea extract and its antimutagenic effect was further recognized by studying the role of 8-hydroxy-2′-deoxyguanosine (8-OH-2′-dG) in tumor progression. EGCG could inhibit the TPA-induced formation of 8-OH-2′-dG in SENCAR mice. Inagake et al. reported a significant increase in 8-OH-2′-dG in DNA of the rat colon and liver after a 1,2-dimethylyhydrazine injection. However, drinking green tea could inhibit this formation of 8-OH-2′-dG in the colon and provide protection from oxidative mutagenesis.

In conclusion, our results show that activated mutagens, especially B[a]P, produced large amounts of superoxide, and that this could be inhibited by a tea extract. Among the four types of tea tested, the oolong tea extract exhibited the strongest inhibition of the formation of superoxide by activated mutagens. This finding supports the hypothesis that oxyradicals play an important role in chemical mutagenesis. Our results also show that the chemopreventive efficacy of a tea extract was partly associated with its antioxidative activity. However, the precise action of the tea extracts on chemical mutagenesis in vivo needs to be examined in future.

Table 1. Inhibition of Various Tea Extracts on the Formation of Superoxide in the Activation Process of Mutagens by S9 Mix

<table>
<thead>
<tr>
<th>Tea extract (mg)</th>
<th>Green tea</th>
<th>Pouchong tea</th>
<th>Oolong tea</th>
<th>Black tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu-P-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>64.2</td>
<td>63.1</td>
<td>64.5</td>
<td>60.0</td>
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<td>95.6</td>
<td>95.1</td>
<td>95.9</td>
<td>88.3</td>
</tr>
<tr>
<td>B[a]P</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>18.9</td>
<td>21.2</td>
<td>18.6</td>
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</tr>
<tr>
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<td>31.3</td>
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<td>46.6</td>
<td>49.7</td>
<td>53.3</td>
<td>42.9</td>
</tr>
</tbody>
</table>

* A mutagen was preincubated with S9 mix at 37°C for 30 min, and then centrifuged at 4000 rpm for 10 min. The supernatant was mixed with a tea extract and NBT, and then incubated at 37°C for 120 min. The final concentration of the mutagen in the reaction mixture was 1 mM.
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References