

Effect of Catechins on Mutagenesis of *Salmonella Typhimurium* TA 102 Elicited by *tert*-Butyl Hydroperoxide (*t*-BuOOH)

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ABSTRACT. Green tea, one of the most popular beverages consumed in Asian countries, has been reported to possess anticarcinogenic and antimutagenic properties. The aim in this study is to test the radical scavenging effect of catechins and caffeine, which were major components of green tea, and if they really prevent oxygen radical-induced mutagenesis. We used TA102 strain of *Salmonella typhimurium* which is sensitive to hydroxyl radical in the Ames mutation assay. We found that caffeine did not show any effects on mutagenesis in this system, but catechin significantly reduced mutagenesis or genotoxicity caused by hydroxyl radical. This radical-scavenging action of catechins may indeed contribute to the anticarcinogenic activity of green tea as has been proposed.

KEY WORDS: ames test, catechin, green tea.

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Green tea, one of the most popular beverages consumed in Asian countries, has been reported to possess antimutagenic properties against polycyclic aromatic hydrocarbons (PAH) and oxygen radicals [5]. Green tea contains catechins, caffeine, vitamins and amino acid. Because of their strong antioxidant activities, catechins have been believed to be the most important factor in prevention activity in mutagenicity by green tea. It has been reported that catechins directly inhibited the formation of lipid peroxide [8]. This is considered to be due to the radical scavenging effect of catechins.

Nevertheless, other workers reported that the inhibition of cytochrome P450 (CYP) mediated activation of carcinogens is a more important effect of catechins in the prevention of carcinogenesis [4]. In fact catechins inhibit CYP dependent monooxygenase reactions and, at the same time, metabolic activations of some carcinogens *in vitro* [12]. The antioxidant nature of catechins has been demonstrated [8] whether if they really prevent oxygen radical-induced genotoxicity has not been demonstrated. Although Grey and Adlercreutz reported that green tea inhibited the revertant induction of TA102 strain of *Salmonella typhimurium* which is sensitive to hydroxyl radical in the Ames mutation assay, they also showed that catechin did not have any effects on OH• mutagenicity of H₂O₂ or *tert*-butyl hydroperoxide (*t*-BuOOH) [2].

Green tea consists of numerous structures of catechins and we have suggested that crude catechin extracts of green tea might possess antimutagenic effects of oxygen radicals. In this study, we used TA102 to test the radical scavenging effect of a catechin mixture and if they really prevent oxy-

gen radical-induced mutagenesis. To determine the direct scavenging effect of catechins, *t*-BuOOH was chosen as the mutagen. *t*-BuOOH produces the hydroxyl radical by reacting with Fe (II), and this is not enzymatic reaction [3]. In addition to catechin, we tested the other constituent of green tea, caffeine, in this assay. Caffeine has been also reported to be a scavenger of oxygen radicals [6, 11]. The aim of the present study is to test the radical scavenging effect of catechins and caffeine, which were major components of green tea, and if they really prevent oxygen radical-induced mutagenesis.

Caffeine monohydrate was purchased from Wako Pure Chemical Co. (Tokyo, Japan). Glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADPH were purchased from Oriental Yeast Co. (Tokyo, Japan). *t*-BuOOH was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). A catechin mixture (catechins) was provided by Food Research Laboratories, Mitsui Norin Co. (Shizuoka, Japan). In our previous study, we have already analyzed the concentrations of catechins in the mixture [9]. The other reagents were of analytical grade.

Male Wistar rats (7 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan) and used for experiments after 1 week of acclimatization. All experiments on the animals were performed under the supervision and with the permission of the University's Institutional Animal Care and Use Committee. Hepatic S-9 was prepared as described by Omura and Sato [10]. The protein concentration of hepatic samples was determined as described by Lowry *et al.* [7].

The ames-assay was performed as described by Ames *et al.* [1] with some minor modifications, with *S. typhimurium* TA102. Preincubation mixtures (0.7 ml) consist of overnight culture of *S. typhimurium* TA102, hepatic S-9 and S-9 cofactor mix (Oriental East Co., Tokyo) containing a complete NADPH generating system and NADH. The S-9 mix-

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ture was used for production of Fe(II) and acceleration of peroxidation. The substrate (2 μ mol *t*-BuOOH /plate) as chemical oxidant was added to preincubation mixtures containing various concentration of catechins or caffeine. Immediately after 20 min preincubation, 2 ml of soft agar containing of 0.5 mM L-histidine and 0.5 mM biotin was added. The mixtures were poured onto a glucose agar plate. The numbers of revertant colonies were counted after 48 hr of incubation at 37°C. Statistical significance was determined by means of Dunnet test.

In Ames-assay with TA102 strain of bacteria, the numbers of revertant colonies elicited by *t*-BuOOH were dose-dependently decreased by the addition of catechins to preincubation mixtures containing hepatic S-9 and *t*-BuOOH as a mutagen (Fig. 1A). Caffeine on the other hand did not show significant effect on mutagenesis (Fig. 1B).

In conclusion, we were able to demonstrate the reductive effect of catechins on mutagenesis or genotoxicity caused by the hydroxyl radical. This radical-scavenging action of catechins may indeed contribute to the anticarcinogenic activity of green tea as has been proposed. Although previous studies reported caffeine was one of the radical scavengers [6, 11], caffeine did not show a significant effect on the mutagenesis of oxygen radicals in this system. Our previous study [9] showed that caffeine induces CYP1A2 and UDP-glucuronyltransferase simultaneously, and enhances the elimination of PAH carcinogens. These results suggest a difference between the anti-mutagenic effects of catechin and caffeine: green tea may reduce the chemical carcinogenesis due to OH• by scavenging OH• with catechins, but not with caffeine.

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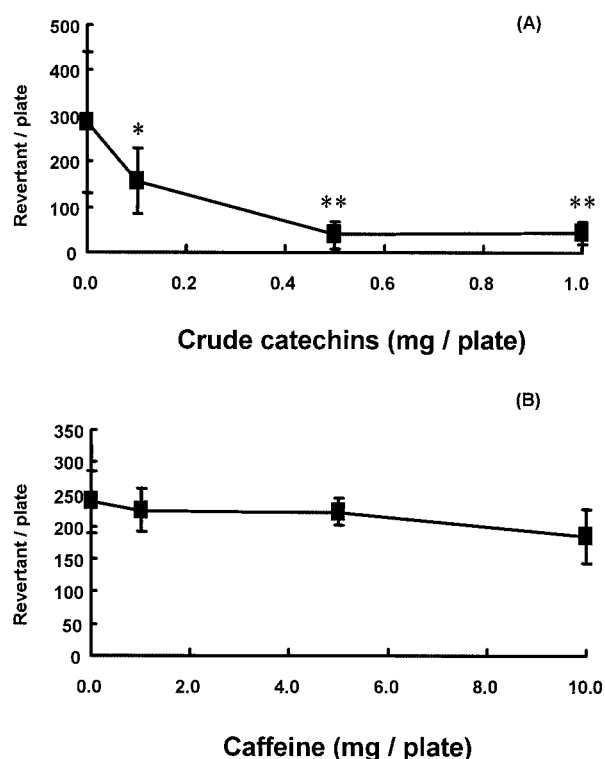


Fig. 1. Effect of the addition of (A) crude catechins and (B) caffeine on mutagenesis produced by *t*-BuOOH with *S. typhimurium* TA102. The preincubation in Ames assay was performed with hepatic S-9 from untreated rats. Each value is expressed as the Mean \pm SD from 4 rats. The spontaneous revertants are subtracted. * $p < 0.05$, ** $p < 0.01$, Significantly different compared to catechin or caffeine non-treatment.

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