지 역 사 회 영 양 학 회 지 2(5) : 735~744, 1997
Korean J Community Nutrition 2(5) : 735~744, 1997

☐ **Original Article** ☐

Effects of Green Tea Infusion on the Preneoplastic Lesions and Peroxidation in Rat Hepatocarcinogenesis

Hee Seon Kim,*† Hyung-Sook Kim, Haymie Choi

Department of Food Science and Nutrition, College of Natural Sciences, Soonchunhyang University, Asan, Korea*
Department of Foods and Nutrition, College of Human Ecology, Seoul National University, Seoul, Korea

ABSTRACT

The effect of green tea drinking on the hepatocellular chemical carcinogenesis have been studied. Placental glutathione S-transferase(GST-P) positive foci area in a liver tissue, contents of thiobarbituric acid reactive substances(TBARS), total cytochrome P450 and glucose 6-phosphatase(G6P) activity in hepatic microsomes were investigated.

Weaning Sprague-Dawley male rats were fed AIN-76A diet with deionized water or green tea infusion. Rats of CTR and CTR+ groups were provided deionized water while GTI and GTI+ groups were provided green tea instead of deionized water for the entire experimental period of 13 weeks. Rats of GTP and GTP+ groups had deionized water for the first 6 weeks and switched to green tea for the last 7 weeks of the experimental period. CTR+, GTI+, and GTP+ groups were carcinogen treated groups. Diethylnitrosamine(DEN) was injected as a single dose of 200mg/kg body weight intraperitoneally after 4 weeks of feeding. 2-Acetylaminofluorene(AAF) was used as a carcinogen proliferater and supplied in the diets of carcinogen treated rats as 0.02% content for the last 6 weeks starting from 2 weeks after DEN injection. Rats were sacrificed after 13 weeks of feeding.

The area and number of GST-P positive foci detected in carcinogen treated rats were decreased by green tea ingestion but when timing and duration of green tea ingestion was delayed after promotion period as in GTP+ group, GST-P positive foci were not decreased as much as in GTI+ group. TBARS contents of carcinogen treated rats decreased by 13 weeks of green tea ingestion but GTP groups did not show statistically significant differences. G6P activities tended to decrease by carcinogen treatment but changes were not statistically significant by green tea ingestion. Total cytochrome P450 contents were increased by carcinogen treatment. Thirteen weeks of green tea ingestion(GTI) also increased total cytochrome P450 contents while 7 weeks of green tea ingestion(GTP) did not show any effects.

These results suggest that green tea has suppressive effects on hepatocellular chemical carcinogenesis probably through the activities of antioxidant compounds. (*Korean J Community Nutrition* 2(5) : 735~744, 1997)

KEY WORDS : green tea · placental glutathione S-transferase positive foci · hepatocellular chemical carcinogenesis · rats.

†**Corresponding author** : Hee Seon Kim, P.O.Box 97, Asan-si, Choongchungnam-do, Korea, 336-600
Tel) 0418) 530-1263, Fax) 0418) 530-1264

Introduction

Free radicals, such as oxygen radical, organic hydroperoxides or other types are produced by certain chemical carcinogens and appear to play a role in the carcinogenic process(Cerutti 1985 ; Kensler & Trush 1984). In cultured cells, extracellular superoxide radical($O_2 \cdot$) production by xanthine oxidase can promote the transformation of initiated mouse fibroblasts(Zimmerman & Cerutti 1984). Thus, many tumor promoters can induce a pro-oxidant state in their target tissue and this action may be related to their carcinogenic activity(Cerutti 1985). Antioxidants, therefore, should possess some anticarcinogenic activity.

Antioxidants have been reported to modify carcinogen activation, detoxification and mutagenicity aside from their ability to scavenge reactive carcinogen metabolites and free radicals(Kahl 1986 ; Yang & Wang 1993). Natural and synthetic antioxidants such as vitamins C and E, beta-carotene, butylated hydroxyanisole and butylated hydroxytoluene have been shown to inhibit chemically induced carcinogenesis although the mechanism of these agents to modify chemical carcinogenesis is unclear(Kahl 1986).

Prevention of carcinogenesis is one of the major strategies for cancer control. The inhibitory effects of several preventive agents in experimental carcinogenesis have been reported. However, some of these agents have harmful effects and are usually expensive. We considered that a natural product in the diet, having an anticarcinogenic effect, is safer and can be obtained easily at a low cost. Green tea has been found to influence the development of cancer in both epidemiological(Kono et al. 1988 ; Wu & Wang 1991) and experimental studies(Kada et al. 1985 ; Okuda et al. 1985). Green tea contains a large amount of polyphenols(up to 30% of the dry leaf weight), most of which are flavan-3-ols, commonly known as catechins. The major flavan-3-ols are (−)-epicatechin derivatives including (−)-epicatechin(EC), (−)-epicatechin gallate(ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate(EGCG)(Shi et al. 1994). Catechins are known to have antioxidant activity(Hatano et al. 1984 ; Kimura et al. 1984 ; Matsuzaki & Hara 1985 ; Okuda et al. 1985 ; Ruch et al. 1989 ; Zhao et al. 1989). An antioxidant fraction of green tea containing several catechins was previously shown to have antimutagenic and anticarcinogenic activities(Fujiki et al. 1992 ; Tang et al. 1987 ; Wang et al. 1987 ; Wu et al. 1987). Other studies have also documented that tea catechins possess antihepatotoxic (Hikino et al. 1985) and antihypercholesterolemic (Muramatsu et al. 1986) activities. However, most studies were conducted with concentrated solution of purified green tea fractions in animal experiment or in the cell culture with high concentration of green tea. Therefore, the effects of green tea could be overestimated. A long term feeding model of green tea infusion was needed to verify the anticarcinogenic effects of green tea when ingested with meals.

In the present study, possible modifying effects of green tea on diethylnitrosamine(DEN)-induced hepatocarcinogenesis were examined in rats. The choice of this experimental design to induce hepatocarcinogenesis was based on its established application to detect modifying effects of many chemicals on liver carcinogens(Osigo et al. 1985). The frequency and area of glutathione S-transferase placental form(GST-P)-positive foci were analyzed. Results of these quantitative analysis of altered liver cell foci have been proved to agree with the data on frequency of liver neoplasm. These liver cell foci are generally recognized as preneoplastic lesions in the line age of hepatocellular carcinoma development and are considered to reflect the carcinogenic potential due to the consistent manner in which liver cell foci appear during the post-initiation stage of hepatocarcinogenesis(Tanaka et al. 1990). We also evaluated the influence of green tea on thiobarbituric acid reactive substances(TBARS) and glucose 6-phosphatase(G6P) in order to test the hypothesis that the antitumor effect of green tea involves a protective action against oxidative damage. Total cytochrome P450 activity was investigated to examine green tea effects on the drug metabolizing enzyme systems when a chemical carcinogen was introduced.

Materials and Methods

1. Preparation of Green Tea infusion

Tea was brewed by adding 25g green tea(Sullok Green Tea, Pacific Corporation, Seoul, Korea) in 1L of 80℃ boiled deionized water and allowed to steep at room temperature for 5 minutes. The brewed tea was filtered using several layers of cheese cloth. The composition of brewed green tea was analyzed by HPLC. Amino acids, vitamin C, caffeine, and several phenolic acids were analyzed(Ikegaya et al. 1990) using C18 column(C18 Shiseido Cap Cell Pack, 10μm particle size, 250×4.6mm, Shiseido Co. Inc., Japan) and UV detector. The mobile phase contained 250 mL of 25% THF(1,2,3,4-tetrahydro-9-fluorenone) and 750mL of 1% phosphoric acid(H_3PO_4). The column was maintained at room temperature and the flow rate of mobile phase was 0.8ml/min. Table 1 shows the composition of green tea infusion used in the experiment.

2. Animals and diet

Male Sprague-Dawley rats(80－90g) were supplied from Seoul National University Animal Facility. The rats were housed two per suspended stainless steel cage with wire mesh bottoms and they were kept in a humidity and temperature controlled room with a 12h light : dark cycle. The rats had free access to deionized water and a commercial diet(Rat chow, Sam Yang Animal Chow, Co., Korea) for a week prior to the start of the study. Diet(Table 2) used for the experiment was formulated to meet recommended nutrient levels for rats(AIN-76A). After rats were accustomed to the experimental environment, they were fed standard AIN-76A diet and either deionized water or green tea infusion. Rats were allocated to 6 groups of 6 rats each so that each group had similar mean body weight(Table 3). Control groups (CTR and CTR+) were fed standard diet and deionized water ad libitium for the entire experimental period of 13 weeks. One of the green tea groups (GTI and GTI+) had free access to the standard diet and tea infusion instead of deionized water throughout the entire experimental period(13 weeks). The other green tea groups(GTP and GTP+) were fed standard diet and deionized water for the first 6 weeks of the experimental period. After that, deionized water was exchanged to green tea infusion for the rest of the experimental period(7 weeks) for which the carcinogen treated rats were fed carcinogen promoter. Groups with+sign(CTR+ GTI+ and GTP+) were treated by chemical carcinogen 4 weeks after initiation of the experiment. Hepatocell-

Table 1. Conposition of green tea infusion

Components		Tea infusion (mg/100ml)
Amino acid		20.79
Vit. C		22.32
Caffeine		66.71
Polyphenols	EGC	11.68
	EC	5.36
	EGCG	33.89
	ECG	6.00
	Total	56.93

EGC : epigallocatechin
EC : epicatechin
EGCG : epigallocatechin gallate
ECG : epicatechin gallate

Table 2. Compositions of experimental diets

Component	g/100g diet
Casein	20
DL-Methionine	0.3
Corn starch	65
α-Cellulose	5
Corn oil	5
Choline bitartrate	0.2
AIN Mineral Mix[1]	3.5
AIN Vitamin Mix[2]	1
Total	100%

1) AIN Mineral Mixture(g/kg) : Calcium phosphate dibasic 500g, Sodium chloride 74g, Potassium citrate monohydrate 220g, Potassium sulfate 52g, Magnesium oxide 24g, Manganous carbonate(43－48% Mn) 3.5g, Ferric citrate(16－17% Fe) 6g, Zinc carbonate (70% ZnO) 1.6g, Cupric carbonate(53－55% Cu) 0.3g, Potassium iodate 0.01g, Sodium selenite 0.01g, Chromium potassium sulfate 0.55g, Sucrose finely powdered 118g.

2) AIN Vitamin Mixture(g/kg) : Thiamin hydrochloride 600mg, Riboflavin 600mg, pyridoxine hydrochloride 700mg, nicotinic acid 3g, D-calcium pantothenate 1.6g, Folic acid 200mg, D-biotin 20mg, Cyanocobalamin 1mg Retinyl palmitate pre-mix(250,000 IU/g) 1.6g, DL-alpha-tocopherol acetate(250IU/g) 20g, Cholecalciferol(400,000IU/g) 250mg, Menaquinone 5mg Sucrose, finely powdered 972.9g.

Table 3. Effects of gree tea drinking on body weight gain of rats

Group	Number of animals	Week 0	Week 13
CTR	6	84.17±2.54[a]	548.17±66.23[a]
CTR+	6	87.50±1.87[a]	432.83±61.47[bc]
GTI	5	89.25±2.09[a]	529.60±40.41[a]
GTI+	5	85.75±1.93[a]	403.40±55.01[c]
GTP	6	85.25±1.90[a]	488.67±47.48[ab]
GTP+	6	86.00±2.05[a]	421.50±37.19[bc]

CTR : Control
CTR+ : Carcinogen treatment
GTI : Green tea infusion from initiation
GTI+ : Carcinogen treatment with green tea infusion from initiation
GTP : Green tea infusion from promotion
GTP+ : Carcinogen treatment with green tea infusion from promotion
Values are mean±SD.
Means with the same alphabet are not significantly different at $p<0.05$ by Duncan's multiple range test

ular chemical carcinogenesis was induced using Solt and Farber protocol(1976) with a slight modification where the carcinogen treated animals recei-ved a single intraperitoneal injection of diethylnitrosamine (DEN)(200mg/kg body wt) dissolved in saline. CTR, GTI and GTP groups were the counter groups for each treatment and given a single intraperitoneal injection of saline solution. After DEN injection, the carcinogen treated groups were fed standard diet for 2 weeks. After that, the carcinogen treated groups were fed 0.02% of 2-AAF containing diet for 6 weeks. After 6 weeks of 2-AAF feeding, carcinogen treated groups were switched to the standard diet. Rats were killed at week 13. GTP+ and GTI+ groups were compared to investigate the effect of duration and timing of green tea ingestion on the process of chemical carcinogenesis. The experimental design is on Fig. 1.

3. Placental glutathione S-transferase(GST-P) positive foci

Upon killing of the animals by decapitation after 12 hours of fasting, the livers were immediately excised and rinsed with saline solution. Blot dried livers were weighed and cut into 2 3mm thick sections with a blade. These liver slices were fixed in ice cold acetone for immunohistochemical examination of GST-P positive foci. The avidin-biotin peroxidase complex method was used to demonstrate GST-P positive liver foci, a putative preneoplastic lesion(Sato 1988). Immunohistochemical analysis was carried out with sequential treatment of rabbit anti-rat placental glutathione S-transferase(BMI Inc., Tokyo, Japan) as a primary antibody, swine anti-rabbit IgG antibody as a secondary antibody(BMI Inc., Tokyo, Japan) and peroxidase-antiperoxidase complex(Vectastain Elite ABC kit, Vecta Laboratories, InC, USA). Final visualization of GST-P positive foci was enzymatically activated by 3,3-diaminobenzidine and H_2O_2 as substrates(Sigma Chemical Co. St. Louis, USA). The area of the GST-P positive foci bigger than 0.2mm in diameter were counted and measured, and the total areas of liver sections examined were measured using an image analyzer. Data were calculated as BMI plus image analyzer program produced in Bummi Universe(Ansan-si, Kyunggi-do, Korea).

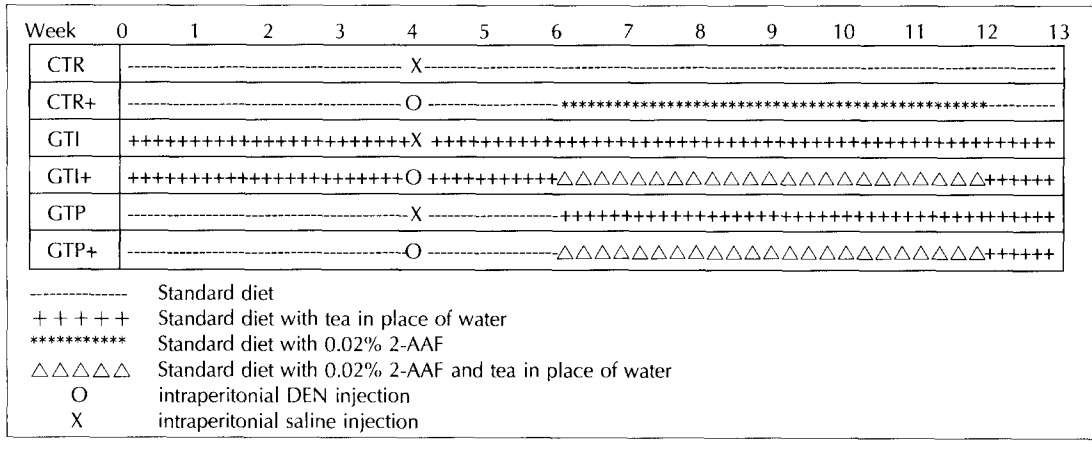

Fig. 1. Experimental design.

4. Preparation of microsomal and cytosolic fractions

Microsomal and cytosolic fractions were prepared as in modified methods of Sohn et al.(1994). A portion of the liver was finely minced in 3 weight volume of ice cold 50 mM Tris-HCl buffer, pH 7.4 containing 1 mM EDTA, and then homogenized. Homogenates were centrifuged at 12,000g for 20 minutes and microsomes were obtained by centrifuging the resulting supernatant at 105,000g for 1 hour. After collecting cytosolic fractions, the microsomes were resuspended in 3 weight volume of 50 mM Tris-HCl buffer, pH 7.4. Cytosols and microsomal suspensions were stored in small aliquots at −70℃ until assayed.

5. Thiobarbituric acid reactive substances assay

Lipid peroxides of hepatic microsomes were determined by measuring the formation of thiobarbituric acid reactive substances(TBARS)(John, Steven 1978). Malondialdehyde as the product of lipid peroxidation reacted with thiobarbituric acid and the absorbance of the resulting chromophore was measured at 535 nm.

6. Glucose 6-phosphatase assay

Glucose 6-phosphatase(G6P) activity was determined by measurement of the inorganic phosphate liberated from glucose 6-phosphatase by the method of Baginski et al.(1983). The absorbance was determined at 700 nm and the amount of phosphate liberated by enzyme from glucose 6-phosphatase was calculated by calibration with the standard(1.15μmole Pi/volume of the assay mixture).

7. Determination of total cytochrome P450 content

Total cytochrome P450 contents were determined by the method of Omura and Sato(1964). Sodium dithionate was added to the fresh liver microsomes and the reduced hemoprotein was combined with carbon monooxide by bubbling CO through the solution. The characteristic absorbance at 450 nm was determined by dual beam spectroscopy.

8. Protein Assay

Protein amounts of hepatic microsomes were determined by Lowry's method(1951).

9. Statistical analysis

All statistical analyses were carried out by Duncan's multiple range test using SPSS program. A p-value of less than 0.05 was selected as a limit of statistical significance.

Results and Discussion

Table 1 shows that green tea infusion supplied to the rats contains (−)-EC, (−)-ECG, (−)-EGC, (−)-EGCG, ascorbic acid, caffeine and a few amino acids. In this green tea, EGCG was the major constituent and represent approximately 5% of the dry weight of green tea leaves or 33.9mg/100ml green tea infusion. Among the catechins in green tea, EGCG has been proved to reduce different types of spontaneous or chemically induced tumors of liver, stomach, skin, lung, and esophagus in laboratory animal studies(Huang et al. 1992).

The body weight gain over a 13-week period was similar within three carcinogen treated groups and within three corresponding counter groups(Table 3). Rats consumed less diet after DEN injection while counter groups showed no differences in diet consumption after saline injection. After DEN injection, rats in carcinogen treated groups gradually consumed more diet, and finally consumed about the same amount as their counter groups, during 2 weeks of standard diet feeding. However, diet consumption was remarkably reduced in carcinogen treated groups when a 0.02% 2-AAF containing diet started to be provided. After all, the food intake of carcinogen treated groups was less than that of their counter groups for the rest of the experimental period. Therefore the final weight gain of carcinogen treated groups were depressed compared to their counter groups. The rats consuming green tea weighed somewhat less than the controls on water although the differences were not statistically significant.

Placental glutathione S-transferase is an enzyme

that shows a remarkable increase in the liver of rats treated with hepatocarcinogens and hepatocellular carcinoma in humans(Grame et al. 1993 ; Sato et al. 1981). Several types of GST forms are known to be elevated, but GST-P positive foci is the most effective marker for DEN-initiated lesions(Tatematsu et al. 1985). Osigo et al.(1985) have proved that the degree of induction of GST-P positive foci and nodules in this bioassay protocol for liver carcinogen directly corresponds with the incidence of hepatocellular carcinomas revealed in long-term in vivo systems. Therefore, GST-P positive foci can be considered to reflect the carcinogenic potential due to the consistent manner in which liver cell foci appear during the post-initiation stage of hepatocellular carcinogenesis. Thirteen weeks of green tea ingestion(GTI+) inhibited GST-P positive foci in the rat hepatocarcinogenesis (Fig. 2). When green tea was offered for the last 6 weeks of the experimental period(GTP+), GST-P positive foci was not decreased compared to the control group(CTR+). Green tea inhibited DEN-induced hepatocarcinogenesis in rats only when given before DEN was introduced. Green tea only when ingested before carcinogen injection showed positive effects on hepatocarcinogenesis. The-refore, the inhibitory effect of green tea was apparent from the initiation of the chemical carcino-genesis or even before. Results of the quantitative area analysis of liver

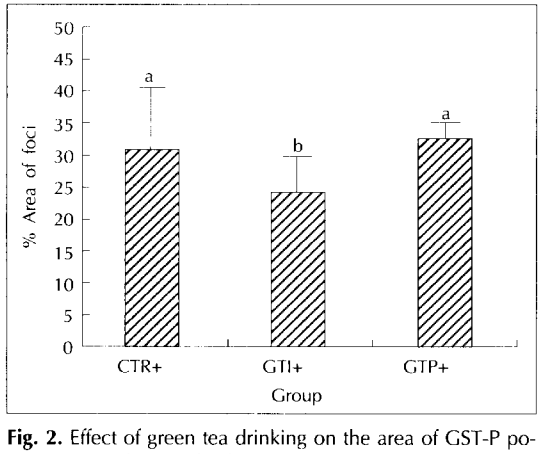

Fig. 2. Effect of green tea drinking on the area of GST-P positive foci in the hepatocarcinogenesis. CTR+ : Carcinogen treatment, GTI+ : Carcinogen treatment with green tea infusion from initiation, GTP+ : Carcinogen treatment with green tea infusion from promotion

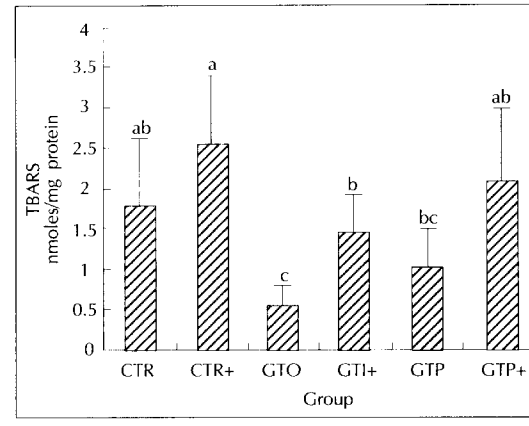

Fig. 3. Effect of green tea drinking on the hepatic microsomal thiobarbituric reactive substances. CTR : control, CTR+ : carcinogen treatment, GTI : green tea infusion from initiation, GTI + : carcinogen treatment wit tea infusion from initiation, GTP : green tea infusion from promotion, GTP+ : carcinogen teatment with green tea infusion from promotion

cell foci were in agreement with the data on the number of foci(Table 4).

Lipid peroxidation and toxicity associated with oxygen radicals have been suggested as major causes of cancer. Therefore, the lipid peroxidation was reported to be increased in rats treated with various carcinogens and xenobiotics(Kagawa et al. 1986). Lipid peroxidation decreased the microsomal membrane integrity and influenced the G6P activities(Poli & Gravela 1982), and was associated with the promotion of carcinogenesis(Slaga et al. 1981). Hepatic microsomal lipid peroxidation determination by TBARS of green tea groups decreased compared to the control

Table 4. Effect of green tea drinking on the area and number of GST-P positive foci in rat hepatocarcinogenesis

Group	Number of rats	% Area of foci	Number of foci
CTR+	6	31.25 ± 9.43^{a}	67.00 ± 5.29^{a}
GTI+	5	23.89 ± 5.47^{b}	37.67 ± 11.02^{b}
GTP+	6	32.36 ± 2.73^{a}	55.33 ± 16.04^{a}

CTR+ : Carcinogen treatment
GTI+ : Carcinogen treatment with green tea infusion from initiation
GTP+ : Carcinogen treatment with green tea infusion from promotion
Values are mean±SD.
Means with the same alphabet are not significantly different at $p<0.05$ by Duncan's multiple range test

groups, but the difference was significant only for the green tea groups(GTI, GTI+) with 13 weeks of ingestion(Table 5). Green tea given during the promotion stage did not show statistically significant differences compared with the control groups as observed in GST-P positive foci. The TBARS were moderately correlated with the number of GST-P positive foci($r=0.3998$, $p<0.05$). If there were more animals(more than 20 animals) in each group, the correlation between TBARS and GST-P positive foci could be more significantly showed. All of carcinogen treated groups showed higher TBARS than their corresponding counter groups while CTR+ groups showed the highest TBARS(Fig. 3). These results showed effective antioxidant function of green tea by decreasing the lipid peroxides formed from DEN-induced hepatocarcinogenesis.

Hepatic microsomal glucose 6-phosphatase(G6P) has been found to reflect the stability of the microsomal membrane(Kim & Choi 1994 ; Poli & Gravela 1982). In the carcinogen-treated green tea groups (GTI+ and GTP+), the activities of G6Pase decreased steadily. The activity was the highest in the 13 weeks of green tea group(GTI) although the difference between it and the control group(CTR) was not statistically significant. These results could not prove that green tea, rich in various polyphenolic compounds, has a positive effect on the stability of hepatic microsomal membranes. Therefore, membrane stability could not be a direct influence on the decrease of GST-P positive foci from green tea. Alternative mechanisms could be speculated to involve other antioxidant effects such as reducing pro-oxidant agents which directly influence DNA, intercellular communication, hormone or growth factor responsiveness. Further experiments should be conducted to prove whether green tea shows these antioxidant effects.

The green tea effect in decreasing GST-P positive foci proved to be related to the antioxidant effect in decreasing lipid peroxidation but that effect was not enough to keep the microsomal membrane stability. A previous study showed green tea effects of antioxidation as dose dependent manner. If green tea was ingested longer than 13 weeks, or a higher concentration of tea was provided, microsomal membrane stability may have been shown. However, further investigation is required to draw a conclusion.

Total cytochrome P450 contents did not show statistically significant differences except in the GTP group(Table 5). Green tea significantly induced total cytochrome P450 contents in the GTI group(0.95 ± 0.336nmol/mg protein), while the GTP group showed low cytochrome P450 contents(0.47 ± 0.401 nmol/mg protein). Cytochrome P450 enzymes became sensitive by the duration of green tea ingestion, so the induction of the enzymes by the GTI group was higher than by the GTP group. Total cytoch-rome P

Table 5. Effect of green tea drinking on the hepatic microsomal thiobarbituric acid reactive substances, G6P activitites and total cytochrome P450 in rat hepatocarcinogenesis

Group	Number of rats	TBARS (nmoles/mg protein)	G6P(nmoles Pi liberated/ min/mg protein)	Total cytochrome P450 (nmol cytochrome P450/mg protien)
CTR	6	1.78 ± 0.796^{ab}	13.37 ± 3.983^{a}	0.61 ± 0.115^{ab}
CTR+	6	2.61 ± 0.817^{a}	11.69 ± 3.578^{ab}	0.90 ± 0.195^{b}
GTI	5	0.59 ± 0.187^{c}	14.93 ± 5.691^{a}	0.95 ± 0.336^{b}
GTI+	5	1.46 ± 0.463^{b}	7.24 ± 2.009^{b}	0.94 ± 0.217^{b}
GTP	6	0.90 ± 0.479^{bc}	11.69 ± 2.577^{ab}	0.47 ± 0.401^{a}
GTP+	6	2.13 ± 0.806^{ab}	7.14 ± 1.809^{b}	0.95 ± 0.144^{b}

CTR : Control
CTR+ : Carcinogen treatment
GTI : Green tea infusion from initiation
GTI+ : Carcinogen treatment with green tea infusion from initiation
GTP : Green tea infusion from promotion
GTP+ : Carcinogen treatment with green tea infusion from promotion
Values are mean±SD.
Means with the same alphabet are not significantly different at $p<0.05$ by Duncan's multiple range test

450 contents showed moderate correlation with the number of GST-P positive foci($r=0.3943$, $p<0.05$).

Farber(1984) reported that the nodules showed large decreases(75 - 89%) in total microsomal cytochrome P450 content and in several mixed-function oxygenase(phase I enzymes) activities. Tsuda et al. (1987) also demonstrated that the more advanced lesions expressed fewer P450 enzymes, suggesting that decreased expression of P450 enzymes may be important in the growth and development of these lesions. However, in our experiment, the activities of cytochrome P450 enzymes of carcinogen treated groups were somewhat higher than corresponding counter groups although the difference was significant only in GTP groups. Upon subchronic intake of green tea, most likely through their active polyphenol fractions, a number of cytochrome P450 xenobiotic metabolizing enzyme systems in rats increased (Sohn et al. 1994). It seems logical to propose that green tea would decrease the carcinogenic effect of specific carcinogens that undergo biochemical detoxification by the cytochrome P450 enzymes. However, the question of whether the induction cytochrome P 450 enzymes is beneficial must be further investigated since the various isozymes of cytochrome P450 enzymes showed different effects in chemical hepatocarcinogenesis(Degawa et al. 1991 ; Park & Choi 1997 ; Sohn et al. 1994).

Summary and Conclusion

The subchronic(13 weeks) ingestion of green tea with meals was effective in inhibiting GST-P positive foci, lowering the lipid peroxidation and modulating cytochrome P450 enzymes in rat hepatocarcinogenesis treated with DEN and 2-AAF. However, the stability of microsomal membrane was not influ-enced by green tea ingestion. Therefore, the antioxidative effects and the induction of cytochrome P450 enzyme activities could be considered to be the mechanism of chemoprevention against chemical carcinogenesis although further study is need to clearly explain the specific mechanisms. Future research planning is in progress to determine green tea effects on certain isozymes of cytochrome P450 enzymes and other phase I and phase II enzymes which are known to have suppressive or progressive effects in rat hepatocarcinogenesis.

References

Baginski ES, Foaa PP, Zak B(1983) : Glucose 6-Phosphatase. In : Bergmeyer ED, ed. Methods of enzymatic analysis, Vol. 2, pp876-880, CRC.

Cerutti PA(1985) : Prooxidant states and tumor promotion. *Science* 227 : 375-381

Degawa M, Mura S, Hashimoto, Y(1991) : Expression and induction of cytochrome P450 isozymes in hyperplastic nodules of rat liver. *Carcinogenesis* 14 : 2151-2156

Farber E(1984) : The multistep nature of cancer development. *Cancer Res* 44 : 4217-4223

Fujiki H, Yoshizawa S, Horiuchi T, Suganama M, Yatsunami J, Nishiwaki S, Okabe S, Nishiwaki-Matsushima R, Okuda T, Sugimura T(1992) : Anticarcinogenic effects of ( )-epigallocatechin gallate. *Preventive Medicine* 21 : 503-509

Grame IM, Richard JW, William TM(1993) : The expression of cytochrome P450 epoxide hydroxylase and glutathione S-transferase in hepatocellular carcinoma. *Cancer* 671 : 36-43

Hatano T, Yoshida T, Fujita Y, Okada T, Kimura Y, Okada H, Arichi S(1984) : Effect of tannins of wakan-yaku on lipid peroxidation and fat cell. *Wakan-Yaku Gakkaishi* 1 : 40-41

Hikino H, Kiso Y, Hatano T, Yoshida T, Okuda T(1985) : Antihepatotoxic actions of tannins. *J Ethanopharmacol* 14 : 19-29

Huang MT, Ho CT, Lee CY(1992) : Phenolic coumpounds in food and their effects on health II. In : American Chemical Society Symposium Series 507. ACS, Washington, DC.

Ikegaya K, Takayanagi H, Anan T(1990) : Quantitative analysis of tea constituents. *Tchia Renbo* 71 : 43-74

John AB, Steven DA(1978) : Microsomal lipid peroxidation. Method in enzymology vol 52 pp302-310, Academic press, New York.

Kada T, Kaneko K, Matsuzaki S, Matsuzaki T, Hara Y (1985) : Detection and identification of natural bio-antimutagens. A case of the green tea factor. *Mutat Res* 150 : 127-132

Kagawa K, Matsutaka H, Yamaguchi Y, Fukuhama C(1986) : Garlic extract inhibits the enhanced peroxidation

and production of lipid in carbon tetrachloride-induced liver injury. *Jpn J Pharmacol* 42 : 19-26

Kahl R(1986) : The dual role of antioxidants in the modification of chemical carcinogenesis. *J Environ Sci Hlth C4* : 47-92

Kensler T, Trush M(1984) : Role of oxgen radical in tumor promotion. *Environ Mutagen* 6 : 593-619

Kim SH, Choi H(1994) : Effects of N-6, N-3 fatty acids and vitamin E supplement on the induction of preneoplastic lesion in murine hepatocarcinogenesis model. *Korean Biochem J* 27 : 125-131

Kimura Y, Okuda H, Mori K, Okuda T, Arichi S(1984) : Effects of extracts of various kinds on lipid metabolic injury in rats fed peroxidized oil. *J Jpn Soc Nutr Food Sci* 37 : 223-232

Kono S, Ikeda M, Tokudome S, Kiratsune M(1988) : A case-control study of gastric cancer and diet in northern Kyushu, Japan. *Jpn J Cancer Res* 79 : 1067-1974

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ(1951) : Protein measurement with the folin phenol reagent. *J Biol Chem* 193 : 265-275

Matsuzaki T, Hara Y(1985) : Antioxidative activity of tea leaf catechins. *J Agric Chem Soc Jpn* 59 : 129-134

Muramatsu K, Jukuyo M, Hara Y(1986) : Effect of green tea cathechins on plasma cholesterol level in cholesterol-fed rats. *J Nutr Sci Vitaminol* 32 : 613-622

Okuda T, Mori K, Hayatsu H(1985) : Inhibitory effects of tannins on mutagen. *Wakan-Yaku Gakkaishi* 2 : 168-169

Omura T, Sato R(1964) : The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* 239 : 2370-2378

Osigo T, Tatematsu M, Tamano S, Tsuda H, Ito N(1985) : Comparative effects of carcinogenesis on the induction of placental glutathione S-transferase positive liver nodules in a short-term assay and of hepatocellular carcinogenesis in a long-term assay. *Toxicol Pathol* 13 : 257-265

Park KA and Choi H(1997) : Modification of hepatic microsomal cytochrome P4502E1 enzyme by garlic powder in rat hepatocarcinogenesis. *J Biochem Mol Biol* 30(1) : 73-79

Poli G and Gravela E(1982) : Lipid peroxidation in isolated hepatocytes. In : Mcbrien DCH and Slater TF eds. Free radicals, lipid peroxidation and cancer, pp 215-242, Academic Press

Ruch RJ, Chen SJ, Klaunig JE(1989) : Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea. *Carcinogenesis* 10 : 1003-1008

Sato K(1988) : Glutathione S-transferase and hepatocarcinogenesis. *Jpn J Cancer Res* 79 : 556-572

Sato K, Hatayama I, Hoshino K, Imai F, Tsrchida S, Sato T, Nishimura K, Tatematsu M, Ito N(1981) : Enzyme derivation patterns in primary rat hepatomas induced by sequential administration of two chemically different carcinogens. *Cancer Res* 41 : 4147-4153

Shi ST, Wang ZY, Smith TJ, Hong JY, Chen WF, Ho CT, Yang CS(1994) : Effects of green tea and black tea on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation, DNA methylation, and lung tumorigenesis in A/J mice. *Caner Res* 54 : 4641-4647

Slaga TJ, Klein-Szanto AJP, Triplett LL, Yotti LP, Troko JE (1981) : Skin tumor-promoting activity of benzoyl peroxide, a widely used free radical-generating compound. *Science* 213 : 1023-1025

Sohn OS, Surace A, Fiala ES, Richie Jr JP, Colosimo S, Zang E, Weisburger JH(1994) : Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. *Xenobiotica* 24 : 119-127

Solt D, Farber E(1976) : New principles for the analysis of chemical carcinogenesis. *Nature* 263 : 701-703

Tanaka T, Mori Y, Morishita Y, Hara A, Ohno T, Kojima T, Mori H(1990) : Inhibitory effect of sinigrin and indole-3-carbinol on diethylnitrosamine-induced hepatocarcinogenesis in male ACI/N rats. *Carcinogenesis* 11 : 1403-1406

Tang GZ, Li Y, Tang LL, Duan XX, Yan RQ(1987) : Effect of green tea on the induction of liver cancer in rat by AFB_1. *Abstracts of International Conference on Tea Quality-Human Health*. Hangzou, China, p45.

Tatematsu M, Mera Y, Ito N, Stoh K, Sto K(1985) : Relative merits of immunohistochemical demonstration of placental A, B, and C forms of glutathione S-transferase as markers of altered foci during liver carcinogenesis in rats. *Carcinogenesis* 6 : 1621

Tsuda H, Moore MA, Asamoto M, Inoue T, Fukushima S, Ito N, Satoh K, Amelizad Z, Oesch F(1987) : Immunohistochemically demonstrated altered expression of cytochrome P450 molecular forms and epoxide hydrolase in N-ethyl-N-hycroxylnitrosamine induced rat kidney and liver lesions. *Carcinogenesis* 8 : 711-717

Wang ZY, Khan WA, Bicker DR, Mukhtar H(1987) : Protection against experimental skin chemical carcinogenesis in mice by green tea polyphenol. *Abstracts of International Conference on Tea Quality-Human Health*. Hangzou, China, p44

Wu RR, Lin IP, Cheng HW(1987) : Effects of Fujian Oolong tea, Jasmine tea, green tea and tea standing overnight on urethan-induced lung neoplasia in mice. *Abstracts of International Conference on Tea Quality-Human*

Health. Hangzou, China, p42

Wu Y, Wang H(1991) : The effects of tea polyphenol on endogenous nitrosation in humans. *J Hyg Res* 20(1), 26-29

Yang CS, Wang ZY(1993) : Tea and cancer. *J Natl Cancer Inst* 85 : 1038-1049

Zhao B, Li XJ, He RG, Cheng SJ, Xin WJ(1989) : Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys* 14 : 175-186

Zimmerman R, Cerutti P(1984) : Active oxygen acts as a promoter of transformation in mouse embryo C3H/10T1/2/c18 fibroblasts. *Proc Natl Acad Sci USA* 81 : 2085-2087