EFFECTS OF AQUEOUS GREEN TEA EXTRACT ON ACTIVITIES OF DNA TURN-OVER ENZYMES IN CANCEROUS AND NON-CANCEROUS HUMAN GASTRIC AND COLON TISSUES

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Aim • The purpose of this study was to investigate possible effects of green tea extract on the activities of DNA turn-over enzymes, namely adenosine deaminase (ADA) and xanthine oxidase (XO) in gastric and colon tissues from patients with stomach and colon cancer.

Materials and Methods • Six cancerous and 6 non-cancerous adjacent human gastric tissues, and 7 cancerous and 7 non-cancerous adjacent colon tissues obtained surgically were treated with aqueous green tea extract at 3 different concentrations for 1 hour, and then ADA and XO activities were measured.

Results • In all of the tissues, XO activities were found to elevate after treatment with green tea extract. Additionally, ADA activity was found to be inhibited in the cancerous gastric tissues by the green tea extract. Elevated XO and reduced ADA activities due to treatment with green tea extract may lower salvage pathway activity and lead to inhibition in carcinogenesis.

Conclusion • Our data suggest that green tea may support the medical treatment of stomach and colon cancer. (*Altern Ther Health Med*. 2008;14(3):30-33.)

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Tea is the second most consumed drink (after water). It is generally used as green tea and black tea. Green tea (*Camellia sinensis*) is a non-fermented form and includes more antioxidant molecules and has more antioxidant potential than black tea.[1] Approximately 30% of its dry weight is polyphenols, most of which are called catechins. The main catechins in green tea are epigallocatechin-3 gallate (EGCG), epicatechin-3 gallate, epigallocatechin, and epicatechin.[2] It is shown that a cup of green tea contains 50 mg to 100 mg of polyphenols, and this amount is enough to provide adequate daily antioxidant effect.[2,3] EGCG is a stronger antioxidant agent than vitamins E and C.[4,5] Various studies have reported that cancer chemopreventive mechanisms of tea polyphenols consist of enhancement of detoxification enzymes; inhibition of cytochrome P450, mutagenicity, genotoxicity, and urokinase activity; induction of apoptosis and cell cycle arrest; activation of mitogen-activated protein kinases; suppression of extracellular signals and cell proliferation; scavenging of free radicals; and inhibition of reactive oxygen species.[4,6-12] Adenosine deaminase (ADA) and xanthine oxidase (XO) are enzymes that play a role in the metabolism of the adenine nucleotides. ADA is a regulatory enzyme in this pathway. It irreversibly converts deoxyadenosine to deoxyinosine and ammonia and also converts adenosine to inosine and ammonia via the hydrolytic deamination.[13] XO is the last enzyme functioning in the purine metabolism, which catalyzes oxidation of xanthine and hypoxanthine, and it can produce superoxide radicals.[14] However, as far as we know, purine metabolism–related effects of green tea have not yet been investigated. The present study aims to establish whether green tea has anti-cancerous potential with regard to purine-metabolizing enzyme activities in the human stomach and colon cancers.

MATERIALS AND METHODS

Patients were randomly chosen from among subjects who had surgery for stomach and colon cancer in the Ankara University Faculty of Medicine, Department of General Surgery, Turkey. Six cancerous and 6 non-cancerous adjacent human gastric tissues, and 7 cancerous and 7 non-cancerous adjacent colon tissues obtained surgically were treated with aqueous green tea extract at 3 different final concentrations (0.05%, 0.5%, and 1.25%) for 1 hour. Activity assays were also performed in the same samples without green tea extract. Protein levels of the tissues were studied using Lowry's method[15] and adjusted to equal concentrations.

Before and after the incubation period, XO and ADA

enzyme activities were measured by spectrophotometric methods. Xanthine oxidase activity was determined by measuring uric acid formation from xanthine at 293 nm as described.[16] Adenosine deaminase activity was studied as described.[17] The activity results were expressed as μIU/mg-protein and mIU/mg-protein, respectively.

Percentage changes for the 3 different concentrations of green tea extract vs no green tea extract were calculated. The Wilcoxon signed-rank test was used for statistical evaluation of the percentage changes. Values of $P<.05$ were evaluated as significant.

RESULTS

Percentage changes are shown in Tables 1-4. In both cancerous and non-cancerous tissues, XO activities were found to elevate in correlation with increased extract concentrations in both cancer types. Additionally, ADA activity was found to decrease in the cancerous part of stomach tissue and to increase in the non-cancerous part.

DISCUSSION

Since ancient times, people have drunk green tea. Recent human studies report that green tea reduces risk factors for cardiovascular disease and cancer formation.[18,19] Additionally, it has been found to have antihypertensive, antibacterial, antiviral, antifibrotic, and neuroprotective effects in addition to body weight control, protection from ultraviolet radiation, and fortification of bone mineral density.[1] Recently, it has also been shown that the consumption of green tea has led to increased total serum antioxidant potential in normal and dyslipidemic subjects.[2]

Flavonoids have anticarcinogenic effects through inducing apoptosis. It has been suggested that the cancer cell line is more sensitive to polyphenols than normal cells.[20] Immunotherapy and chemotherapy are usually effective in treatment of small tumors in animals, but these remedies are generally ineffective in the treatment of large tumors. It has been suggested that DNA vaccination and EGCG as a chemotherapeutic agent are effective in inhibiting the growth of large tumors. Additionally, it has

TABLE 1 Percentage Change of Adenosine Deaminase Activities in Colon Tissues*

	Non-cancerous Colon Tissue		Cancerous Colon Tissue	
Green tea concentrations (%)	Without incubation	With incubation	Without incubation	With incubation
0.05	-2.02 (A)	28.04 (D)	17.37 (G)	-18.24 (J)
0.5	12.89 (B)	0.60 (E)	31.00 (H)	13.02 (K)
1.25	-11.35 (C)	15.10 (F)	5.05 (I)	4.33 (L)

*$P<.05$ for Wilcoxon test: B vs C, E vs K.

TABLE 2 Percentage Change of Xanthine Oxidase Activities in Colon Tissues*

	Non-cancerous Colon Tissue		Cancerous Colon Tissue	
Green tea concentrations (%)	Without incubation	With incubation	Without incubation	With incubation
0.05	43.87 (A)	26.42 (D)	64.33 (G)	340.63 (J)
0.5	71.88 (B)	63.02 (E)	125.39 (H)	646.82 (K)
1.25	212.77 (C)	190.11 (F)	336.18 (I)	934.99 (L)

*$P<.05$ for Wilcoxon test: A vs C, B vs C, D vs F, E vs F, G vs I, H vs I, J vs L, K vs L.

TABLE 3 Percentage Change of Adenosine Deaminase Activities in Stomach Tissues*

	Non-cancerous Colon Tissue		Cancerous Colon Tissue	
Green tea concentrations (%)	Without incubation	With incubation	Without incubation	With incubation
0.05	-3.75 (A)	13.43 (D)	-14.84 (G)	-31.09 (J)
0.5	-0.28 (B)	12.59 (E)	-3.79 (H)	-12.33 (K)
1.25	0.93 (C)	8.23 (F)	-9.96 (I)	-17.58 (L)

*$P<.05$ for Wilcoxon test: D vs J, E vs K, F vs L.

TABLE 4 Percentage Change of Xanthine Oxidase Activities in Stomach Tissues*

	Non-cancerous Colon Tissue		Cancerous Colon Tissue	
Green tea concentrations (%)	Without incubation	With incubation	Without incubation	With incubation
0.05	-19.90 (A)	6.08 (D)	7.86 (G)	13.39 (J)
0.5	20.91 (B)	55.48 (E)	54.29 (H)	33.79 (K)
1.25	76.02 (C)	116.12 (F)	133.89 (I)	366.42 (L)

*$P<.05$ for Wilcoxon test: A vs B, A vs C, B vs C, D vs E, D vs F, E vs F, G vs H, G vs I, H vs I.

been found that EGCG induces apoptosis in a dose-dependent manner. The treatment combination causes increased tumor-specific T-cell (CD8+ T cell) immune response, enhanced antitumor effects in mice with cancer, and long-term antitumor protection in cured mice.[21] It has been suggested that regular consumption of green tea (>3 cups per day) may decrease the development of lung cancer in smokers.[22] Irinotecan (IT) is a highly effective chemotherapeutic agent, but its use is limited due to severe intestinal toxicity. Green tea administration has reduced IT-induced side effects in mice.[23]

In a study performed on women, an inverse association was reported between green tea consumption and distal gastric cancer.[24,25] Likewise, in another human study, the protective effect of green tea consumption against stomach cancer formation was observed.[26] In cultured cells derived from human gastric carcinoma, it has been shown that EGCG inhibits cell growth and induces apoptosis.[27] Among both men and women, colorectal cancers are a significant cause of cancer-related deaths.[28] It has been suggested that green tea has anti-cancer properties via inhibition of cyclooxigenase (COX-2) in colon cancer.[29] EGCG has been identified as the most potent chemopreventive agent that can induce apoptosis, suppress the formation and growth of cancer cells, and inhibit mutagenesis in human colon cancers.[30] The apoptotic effect of EGCG via AMP-activated protein kinase has been demonstrated in HT-29 colon cancer cells.[31] tNOX is a cancer-specific cell surface protein, and recent studies have demonstrated that green tea catechins are its potent inhibitor.[32] It has been suggested that density-enhanced phosphatase (DEP-1) transmembrane protein-tyrosine phosphatase, a tumor suppressor protein, inhibits proliferation and migration of colon carcinoma cells in the colon epithelium by upregulation with protective nutrients such as green tea and apple polyphenols. These nutrients have the capacity to elevate transcription of DEP-1 m RNA.[33] It has been reported that EGCG is an effective therapy in colon tumors bearing *Ras* mutations[34] and shown that EGCG directly binds to DNA and RNA by using surface plasmon resonance assay and cold spray ionization-mass spectrometry. This binding prevents dsDNA oligomers from constituting single-strand DNA and accounts for cancer prevention. The direct binding may trigger oxidative stress and induces apoptosis in cells.[35]

ADA has an important role in maturation and activation of lymphocytes and monocytes. Its elevated levels in various biological fluids have been used as a rapid diagnostic test in tuberculosis. It has been reported that the enzyme activity progressively increases in human immunodeficiency virus infections. The physiopathological mechanism has not been fully understood yet; however, CD4+ lymphocytes and macrophages are shown to be responsible for elevated ADA activity. Therefore, ADA has been used as a marker of the cellular immune response. Congenital lack of the ADA enzyme has been reported in severe combined immunodeficiency.[13]

The inhibition of ADA activity leads to accumulation of adenosine nucleotides. These nucleotides are converted rapidly into their deoxy forms. Deoxyadenosine triphosphate (dATP) is one of the nucleotides generated in this way. This compound prevents DNA replication because it inhibits the synthesis of deoxyribonucleotides from ribonucleotides by interfering with ribonucleotide reductase.[36]

It has been reported that a decreased level of XO is a marker for gastric cancer with poor prognosis, as was previously suggested for breast cancer.[37] Additionally, reactive oxygen species (ROS) generated by XO are toxic to malignant B lymphoma cells.[38] In the present study, XO activities have been found to elevate in correlation with increased concentration of green tea extract in the cancerous and non-cancerous tissues of both types. On the other hand, in the present study, ADA activity was found to be inhibited in the cancerous gastric tissues by the green tea extract. It has been reported that ADA inhibitors inhibit growth of various types of cancer cells by increasing adenosine level.[39] Additionally, 2-chlorodeoxy-adenosine, which has been known as purine analog, has been found to be effective in hematological malignancies such as leukemia and lymphoma.[40,41] Another study suggested that human melanoma cells are highly sensitive to deoxyadenosine and deoxyinosine.[42]

In light of these findings, our results suggest that on one hand, increased XO activity may deplete the xanthine pool necessary for the hypoxanthine-guanine phosphoribosyl transferase enzyme to support salvage pathway activity. On the other hand, our results also suggest that decreased ADA activity due to green tea treatment may lead to inhibition of carcinogenesis through formation of deoxyinucleotide burden in the cancerous tissue. This is an in vitro study, and further in vivo studies should be conducted about the effects of green tea in colon and gastric cancers.

In conclusion, our study suggests that green tea consumption may promote cancer-preventive effects in people at risk for cancer, in addition to supporting the medical treatment of some kinds of cancers.

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