Enhancement of DNA Repair Enzyme O^6-Alkylguanine-DNA Alkyltransferase by (-)-Epigallocatehin Gallate from Green Tea

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ABSTRACT

O^6-Alkylguanine-DNA alkyltransferase (AGT) is an important DNA repair enzyme that protects cells from killing and mutagenesis by alkylating agents. This protein can correct DNA damage on O-6 position of guanine DNA. The purpose of this study was to determine effects of (-)-epigallocatechin gallate (EGCG) from green tea on increasing of AGT level in hepatocyte primary rat liver cell culture for 2 days. AGT activity was identified by radioactivity method and counted by Liquid Scintillation Counter. The results show that 48 hours after EGCG exposure by 8.3 ppm, 16.7 ppm, 33.3 ppm and 66.7 ppm, AGT activity was found 16.1, 23.5, 33.7 and 41.2 fmol/ug DNA, respectively ($p<0.01$). It has the meaning that alkyltransferase level increase 1.4, 2.1, 3.0 and 3.7 fold, respectively. These finding suggest that EGCG plays important role as chemopreventive agent for cancer prevention and possibly using for regulate cancer incidence.

Keywords: O^6-Alkylguanine-DNA alkyltransferase, (-)-Epigallocatechin Gallate, Liquid Scintillation Counter.

INTRODUCTION

Cancer is a major health problem world-wide. An alternative cancer control strategy such as chemoprevention needs to be developed to reduce cancer incident. In this case, the consumption of fruits and vegetables apparently plays a dominant role. One potential considerable mechanism is the strong antioxidant effect of tea polyphenols. Consumption of tea (*Camellia sinensis*) has been suggested to prevent cancer and has shown as non-toxic in both acute dosages and high long-term dosage**.** Studies with human cancer cell lines have demonstrated that epigallocatechin-3-gallate (EGCG), a major constituent of green tea, inhibits mitogen-activated protein kinases, cyclin-dependent kinases, growth factor-related cell signaling, activation of activator protein 1 (AP-1) and nuclear factor κB (NFκB), topoisomerase I and matrix metalloproteinases and other potential targets (Lambert and Yan, 2003). In order to be used as cancer chemoprevention, the effect of EGCG to increase O^6-Alkylguanine-DNA alkyltransferase (AGT) level is very important because of this enzyme can restore alkylated DNA damage.

AGT is known as a protein involved in DNA repair system. This protein has mechanism of action to remove alkyl groups created by alkylating agent from DNA. AGT is 22 kDa protein that plays an important role in cell maintenance. In normal tissues, AGT mitigates alkylation DNA damage caused either endogenously or by environmental toxins. It affects this by a suicidal transfer of the alkyl group from the O6-position of alkylated guanine residues on DNA to a cysteine moiety at position

145 on the binding site of the protein, thus regenerating native DNA (Shankar et al., 2005).

Cellular levels of AGT are restored only by de novo synthesis and modulation of AGT levels has emerged as a strategy for inhibiting or enhancing this repair process (Abril et al., 1999). Inhibition of the repair process may be used to deplete cellular AGT levels prior to alkylating agent chemotherapy in order to enhance their therapeutic efficacy, but enhancing it can be used to prevent alkylating agent or carcinogens causes DNA damage. These two ways of AGT role are important to decrease cancer incident.

The mechanism of the cancer chemo-prevention effect of green tea is being explored. In the present study, we determine effects of EGCG on AGT activity by using *N*-[^{3}H]methyl-*N*-nitrosourea (MNU) as an alkylating agent. Radioactivity of MNU binding AGT was evaluated by Liquid Scintillation Counter.

MATERIAL AND METHOD

EGCG and calf thymus DNA was purchased from Sigma Chemical and *N*-[^{3}H]methyl-*N*-nitrosourea from Amersham Co. Ltd. (*sp.act.* 18,6 Ci/mmol). Wistar rat was obtained from Animal Laboratory, Faculty of Pharmacy, Airlangga University. We use instrument of High Performance Liquid Chromatography (HPLC) (Shimadzu), Partisil SCX (10 x 45) column, and Liquid Scintillation Counter (RACKBETA. 12009-006).

Determination of DNA Concentration

Four milliliter of hepatocyte suspension was washed by PBS and lysed by lysis bufer. Protein was aglutinated by phenol solution and water fraction was taken 3.5 ml. On the water fraction was added chloroform-isoamylalcohol, mix and takes 3.0 ml water fraction. Soluble DNA in the water fraction was extracted by Na-acetate and absolutly ethanol, dryed and then solved in 2.0 ml TE. DNA in TE solution was read its absorbance on UV-VIS spectrophotometer at 258,4 nm and DNA concentration was determinated based from linier standard curve.

Determination of AGT Activity

AGT activity was measured by determining the transfer of [^{3}H]methyl groups to AGT from *O*6-[^{3}H]methylguanine in [^{3}H]methylated calf thymus DNA [8]. Purified AGT preparations were incubated with15 µg of [^{3}H]methylated DNA substrate in 1.0 ml 50 mM Tris-HCl pH 7.6 and 0.1 mM EDTA for 30 min at room temperature. The AGT reaction was terminated by addition of 1 ml of denaturation buffer (20 mM Tris-HCl (pH 7.6), 0.6 M NaCl and 8 M urea) to the samples and further incubation at room temperature for 60 min. The samples were then passed through 0.45 µm (2.5 cm) nitrocellulose filters on a Millipore holder unit. The reaction tubes were washed with 5 ml of denaturation buffer, and the wash was applied onto the filters. The filters were further washed with fresh denaturation buffer (2 x 5.0 ml) followed by 2 washes with 10% ethanol in water (each 5 ml). The filters were dried and the radioactivity was counted by scintillation counter in 3 ml of scintillation fluid (Amersham).

RESULT AND DISCUSSION

There were 80 plate of primary liver rat culture (10^6 cell/ml) used in the study Sixty four of cell culture (n = 4) received EGCG at 4 dose levels: 8.6 ppm, 16.6 ppm, 33.3 ppm and 66,7 ppm while controls (n = 4) were treated with solvent vehicle only. The AGT level were determined at 12, 24, 36 and 48 hours after EGCG exposure The results show that EGCG from green tea can increase the AGT activity in primary rat liver cell cultur, to repair DNA damage by removes alkyl group from DNA (Figure 1). In 48 hours, giving of EGCG 66.7 ppm can increase the AGT activity 3.7 fold compared with control (Table 1).

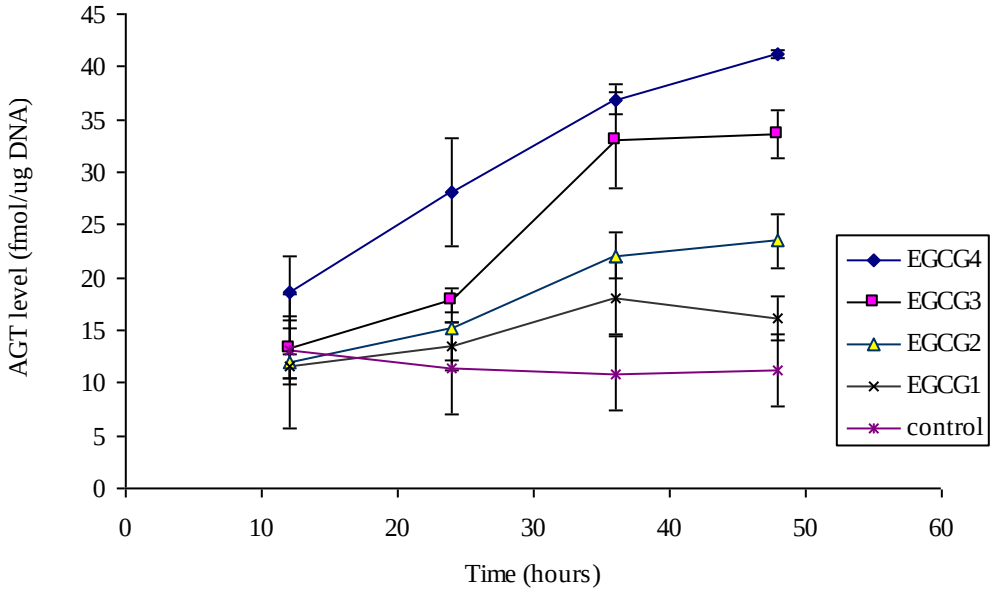

Figure 1. EGCG from green tea increase the AGT level in primary rat liver cell cultur. EGCG1 =8.6 ppm; EGCG2 =16.7 ppm; EGCG3 = 33.3 ppm; EGCG4 = 66.7 ppm.

Table 1. Increasing of AGT activity induced by EGCG for 48 hours (n = 4).

	EGCG concentration			
	8.6 ppm	16.7 ppm	33.3 ppm	66.7 ppm
Increasing of AGT activity	1.4 fold	2.1 fold	3.0 fold	3.7
p	-	-	<0.05	<0.01

In recent year, cancer chemoprevention is a new approach in cancer prevention. Natural products, such as grape seed, green tea, and certain herbs have demonstrated anti-cancer effects (Zoul et al., 2005). The effect of natural product on anti mutagenic and anti cancer activity is very important to develop. AGT, as DNA repair enzyme was intensively reduce mutagenic effect of some substances. In

bacteria that expressing wild-type AGT observes mutation rate reduction more 50 folds than bacteria with AGT-deficient (Palman et al., 1997). Also in transgenic mice, overexpressing AGT can reduce methylnitrosourea induced *O*6-methylguanine formation (Dumenco et al., 1993). A retrospective study founds that loss of AGT mRNA expression was observed in 20% and 57% of non-tumorous esophagus epithelial and esophageal squamous cell carcinoma (ESCC) samples, respectively (Fang et al., 2005). These findings prove that AGT plays important role in cancer prevention. The ability of EGCG increases AGT activity in this research, supports the potential use of green tea extract as cancer chemopreventive agents.

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