

원 저

클라이포세이트 노출로 인한 DNA손상에 대한 녹차의 예방적 효과

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In vitro Effects of Epigallocatechin Gallate on Sister Chromatid Exchange in the Lymphocytes Exposed to Glyphosate

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Purpose: Green tea is known as a potent anti-oxidant, anti-carcinogen, and genetic protector. Glyphosate (N-phosphonomethyl glycine) is a widely used non-selective herbicide that causes DNA damage. The present study was conducted to investigate the protective effects of green tea in human blood lymphocytes exposed to glyphosate using the Sister Chromatid Exchange (SCE) frequency method.

Methods: Peripheral blood was obtained from 10 volunteers and cultured through four different conditions. Four groups were divided into control, glyphosate only (300 ng/mL), glyphosate and low (20 μ m) concentrations of epigallocatechin gallate (EGCG) and glyphosate and high (100 μ m) concentrations of EGCG.

Results: The glyphosate exposed groups had a higher mean SCE frequency (10.33 ± 2.50) than the control group (6.38 ± 2.28 , $p < 0.001$). The low concentrations of EGCG groups had a lower mean SCE frequency (9.91 ± 1.93) than the glyphosate-only group, although this difference was not significant ($p = 0.219$). However, the high concentration group (9.49 ± 1.85) had a significantly lower SCE frequency than the glyphosate-only group ($p = 0.001$).

Conclusion: EGCG has a gene protective effect in human lymphocytes exposed to the genotoxicity of glyphosate in the case of high concentrations.

Key Words: EGCG, Glyphosate, Sister chromatid exchange

Introduction

Green tea (GT), one of the most widely consumed beverage in the world, has been consumed by

Eastern Asian people as a medicinal beverage to promote health and stabilize body and soul. The commonly known effects of GT are anti-diabetic activity^{1,2}, the lowering of plasma cholesterol and triglyceride levels³ and anti-oxidant activity^{4,6}. The 4 major catechins in GT are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin (EGC)⁷. In addition to commonly known effects of GT above, EGCG have anti-inflammatory and antiviral activities⁸ and prevent cardiovascular diseases⁹, neurological problems^{10,11} and

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cancer as a potent genetic protector^{12,13}.

Glyphosate, chemically N-(phosphonomethyl) glycine, is widely used non-selective herbicide for both agricultural and non-agricultural purpose. Since then, constantly, glyphosate intoxicated patients frequently visited emergency room and their severity was dependent on intake concentration. Large amount of ingestion may cause gastrointestinal tract injury, such as erosion or ulcer or haemorrhage, and severe systemic effects. Severe systemic symptoms may occur from cardiotoxicity, hepatotoxicity, renal toxicity, non-cardiogenic pulmonary edema, mental change, metabolic acidosis and even to cardiac arrest and death¹⁴. Small amount of oral intake may be asymptomatic or cause nausea, vomiting, and diarrhea, however, we cannot guarantee its safety. Therefore, many previous studies about the safety of glyphosate formulation have been performed. These studies on this herbicide suggested its minimal genotoxic activity^{15,16} and a review on glyphosate also concluded that there is no strong evidence to pose a health risk to humans tissues¹⁷. However, latest studies showed a harmful effect of glyphosate variously as a potential endocrine disruptor and inducing reproductive disability on placental cells^{18,19}. And, occupational exposure to glyphosate is a risk factor of cancers by comet assay or Sister Chromatid Exchange (SCE) test²⁰⁻²². It is difficult to know the detail mechanisms of both 'genotoxic effect of glyphosate' and 'genetic protective effect of EGCG'. This study was done to clarify protective effect of EGCG in human blood lymphocyte exposed to genotoxicity of glyphosate by SCE frequency method.

Methods

1. Preparation of the *in vitro* experiments

Four milliliters of Peripheral blood of 10 healthy volunteers aged from 21 to 26 years was collected because aging can affects SCE frequency. To control the factors that can change SCE frequency, regular drug users, smokers and alcoholics were excluded. In addition anybody who had cancer, chronic infection,

history of chemotherapy, history of radiotherapy or radiation exposure history was also excluded. The regional institutional review board (IRB) approved the research proposal, and informed consent was obtained from all the individuals involved in the study.

Roundup UltraMax[®] (Monsanto, Roseville, CA, USA) was used as representative product of glyphosate herbicide. It contains 570 gram of active ingredient glyphosate in 1 liter and 2% ammonium sulphate as a surfactant. And EGCG made by Sigma (Saint Louis, MO, USA) was used.

All of blood samples were experimented together through four groups that divided by concentrations of glyphosate and EGCG. Group 1 is control group that contains no glyphosate and no EGCG. Group 2, 3 and 4 are experimental groups. Group 2 contains only 300 ng/mL of glyphosate and no EGCG and Group 3 contains 300 ng/mL of glyphosate and 20 μ M of EGCG and Group 4 contains 300 ng/mL of glyphosate and 100 μ M of EGCG.

2. Sister chromatid exchange (SCE) assay

Each sample of blood (1.0 ml) was mixed with 9 ml of culture medium that consists of RPMI-1640 (Gibco, UK) supplemented with 10% fetal bovine serum (FBS, Gibco, Uxbridge, UK): 0.1 mL (1 g/mL) of Phytohemagglutinin (PHA, Gibco, Uxbridge, UK) was supplemented as a mitogen and then this was incubated at 37°C for 72 hours. At 24 hours of culture, 0.1 ml (1 g/ml) of 5-bromo-2-deoxyuridine (BrdU) was added each culture. Different concentrations of glyphosate and EGCG according to groups were added after 48 hours of incubation. At 70 hours of incubation, 0.1 ml (10 μ g/mL) of colcemid (Gibco, Uxbridge, UK) was added to arrest mitosis at metaphase. All the chromosome preparations were stained using the BrdU-Hoechst-Giemsa technique. The SCE of the lymphocytes was microscopically examined and counted using the Cytovision Computer-Assisted Karyotyping System (Applied Imaging, Santa Clara, CA, USA). For each group in one subject, 20 of well-spread chromosome pairs in second division metaphase were included in results