**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 μm) concentrations of epigallocatechin gallate (EGCG) and glyphosate and high (100 μm) 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 activity¹, the lowering of plasma cholesterol and triglyceride levels² and anti-oxidant activity³. The 4 major catechins in GT are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin (EGC)⁴. In addition to commonly known effects of GT above, EGCG have anti-inflammatory and antiviral activities⁵ and prevent cardiovascular diseases⁶, neurological problems⁷, and...
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, non-cardiogenic pulmonary edema, mental change, metabolic acidosis and even to cardiac arrest and death\(^{14}\). Small amount of oral intake may be asymptomatic or cause nausea, vomiting, and diarrhoea, 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 tissues\(^ {17}\). 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) test\(^ {20-22}\). 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 μM of EGCG and Group 4 contains 300 ng/mL of glyphosate and 100 μM 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 μg/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.
by the same person. The results were used to determine the mean number of SCEs (SCEs/cell).

3. Statistical analysis

In this experiment, the statistical comparisons of the mean number of SCEs from each group were performed using the one-way ANOVA method. The TUKEY post-testing was utilized for multiple comparisons. All the statistical analyses were performed using SPSS software (version 18.0). A p value <0.05 was considered significant.

Results

The frequency of SCE was examined in the 10 healthy-male volunteers. The mean and standard deviation (SD) of each group were calculated and summarized in Table 1. Exposure group to glyphosate (Groups 2, 3 and 4) has a higher mean SCE frequency than Group 1, significantly. Compared to mean SCE frequency of Group 1 (6.38±2.28), Group 2 had extremely higher SCE (10.33±2.50, p<0.001). And Group 3 revealed decrease in mean SCE frequency (9.91±1.93) compared with that in group 2, however, this difference have no statistical value (p=0.219). The mean SCE frequency was lower in Group 4 (9.49±1.85) than group 2 with statistical significance (p=0.001). However, there was no significant difference between Group 3 and 4 (p=0.191), indicating no dose-dependent effect of EGCG in genotoxicity of glyphosate.

Average SCE frequency of each group in individual subject of this study was presented in Table 2. In all subjects, glyphosate increased SCE frequency significantly compared to control, respectively. EGCG treatment (Groups 3 and 4) reduced SCE frequency in the lymphocyte exposed to glyphosate, however statistical value was shown in only one subject (No. 5). Moreover, EGCG increased SCE frequency in two subjects (No. 7 and 8) though they did not have significance.

Discussion

The aim of this study was to know protective effect of EGCG in human blood lymphocyte exposed to genotoxicity of glyphosate by SCE method. GT is mainly comprised of EGCG, ECG, EGC and EC, therefore, their anti-genotoxic effects against glyphosate were analyzed in 3 subjects preliminarily. As a result, EGCG showed a lowest SCE frequency in the cytotoxicity by glyphosate compared to ECG, EGC and EC, though it did not have statistical significance. Of the catechins, polyphenolic components of GT, EGCG is the major constituent and also most active component with the highest antioxidant properties\(^2\). Therefore, our further main experiment was

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<th>Table 1. Mean SCE frequency of individual subject</th>
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GLY: glyphosate, SCE: sister chromatid exchange, EGCG: epigallocatechin gallate
* p<0.001 compared to Group 1
\(^*\) p<0.05 compared to Group 2
performed by EGCG and its anti-genotoxic effect against glyphosate was evaluated.

SCE is the exchange of genetic material between two identical sister chromatids. SCE method in the lymphocyte is well-known experiment to examine genotoxicity of agent or environment. After chromosomal double-strand breaks (DSBs), inter-strand cross-linking damage, and collapsed replication forks by DNA damage is occurred, the important pathway of genomic repair should be followed, named homologous recombination (HR). When demand of HR increased, available sequence from the sister chromatid is used and dysregulation of HR may occur. Therefore, SCE is originated from the result of above mechanism, so SCE frequency may correlated with the degree of DNA damage. Until now, it is widely accepted that SCE is closely related to genotoxicity.

Our result showed that glyphosate causes DNA damage and genotoxicity that related to mutagenicity and carcinogenicity, as previously described. Considering general concentration of GT by oral intake, the effective concentration (100 μM) in present study was relatively high. It is not clear whether the result of this study can be applied to glyphosate acute exposure patient. Accordingly, the experiments in the lymphocyte of its acute exposure patient and in vivo studies might figure out its cytotoxicity. Therefore, further study with clinically available dose should be performed, And the effect of other catechins in GT also should be studied in various subject based on various kinds of personal consequences and side effects of EGCG.

Conclusion

It was suggested that EGCG may be a potential supplement for the genotoxicity of glyphosate. The numerous health benefits of EGCG as a prophylactic, but also as a therapeutic, agent acting through different pathways are well identified though there were conflicting results about its effect. Therefore, EGCG is still most attractive naturally available products. For its safe and effective use for anti-genotoxicity, concerning personal bioavailability and potential side effect of EGCG remain to be addressed.

REFERENCES

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