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Genotoxicity of low-dose Glyphosate by Sister Chromatid Exchange

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Purpose: Glyphosate (N-phosphonomethyl glycine) is widely used as an herbicide for weed control in rural areas. It is also readily available for suicide attempts. Glyphosate has high toxicity and negatively affects the human body. The aim of this investigation was to study the genotoxicity of a low-concentration of glyphosate through sister chromatid exchange (SCE) in human blood lymphocytes in vitro.

Methods: Primary lymphocyte cultures were obtained from blood samples of 11 males and seven females who had been exposed to glyphosate (0, 100, 200, and 300 ng/mL). The frequency of SCEs was examined and statistical analysis was performed.

Results: All doses of glyphosate induced a significant dose-dependent increase in SCE frequency compared with the control group (P<0.001). In particular, the SCE frequency for exposure to low-dose glyphosate was significantly higher in females than in males.

Conclusion: According to the result of this study, even a low-dose of glyphosate may damage DNA and females are more vulnerable to glyphosate.

Key Words: Glyphosate, Toxicology, Sister chromatid exchange

Introduction

Glyphosate (N-phosphonomethyl glycine) is widely used as a herbicide in rural area for weed control and is readily available for suicide attempts. The effects of glyphosate have been extensively investigated due to its potential to produce adverse health effects in humans since glyphosate was introduced in the market in the 1970s. Earlier studies on this herbicide suggested its minimal genotoxic activity[1-3]. A recent review on glyphosate also concluded that there is no strong evidence to support that glyphosate herbicide poses health risk to humans under present and expected conditions of novel use[4].

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However, there is a clear consensus among a number of studies that are recently published, that occupational exposure to pesticide is a risk factor of cancers[5-7]. Furthermore, they demonstrated that aerial spraying glyphosate could also damage DNA by comet assay[8] and by micronuclei test[7] in peripheral lymphocytes. In addition to its impact on genetic damages on human cells, Walsh et al.[9] showed that glyphosate interferes an enzyme involved in testosterone production in mouse cell culture. This difference originated from a wide variety of test systems and recent study pointed that some studies of glyphosate sponsored by companies were not included in the previous review[10]. Although glyphosate has been constantly re-evaluated by regulatory authorities supported by science, questions regarding its safety are periodically raised.

The SCE is a highly sensitive tool in the cytogenetic damage induced by various genotoxic agents or environments at very low concentrations[11]. The exact mechanisms of SCE formation have not been established, however, it has been suggested that these mechanisms may be associated with DNA replication. Some studies demonstrated that glyphosate significantly increased the frequency of SCE in human lymphocytes[1,12-14]. However, these results do not support the facts that their scoring experience was insufficient and they could not find statistically significant changes at any dose.

In this study, the genotoxicity of glyphosate by the SCE was evaluated in Korean population for the first time. Based on previous studies, low-dose glyphosate was not harmful. But, this study showed very low dose glyphosate could induce significant genotoxicity. The results of this study provide a new insight for the safe use of glyphosate.

Materials and Methods

1. Chemicals

Glyphosate (purity 95% powder; Sigma, St. Louis, MO, USA) was dissolved and diluted in sterilized DMSO. RPMI-1640, fetal bovine serum, phytohemagglutinin (PHA), 5-bromo-2-deoxyuridine (BrdU), and colcemid were purchased from Gibco (Uxbridge, UK).

2. Preparation of in vitro Experiments

Four milliliters of heparinized peripheral venous blood were obtained from the 18 healthy-volunteers (age range: 23 to 28 year-old, 11 males and 7 females), who were all non-smoker. None of the volunteers had undergone previous chemotherapy or radiotherapy, and they had no clinical history of chronic infection, drug intake or radiation exposure. The regional institutional review board (IRB 08-57) approved the research proposal, and informed consent was obtained from all the individuals involved in the study.

The peripheral blood of each person was divided into the control group and three exposure groups. The three exposure groups were classified at the final concentration of 100, 200, and 300 ng/mL. Glyphosate was diluted with culture medium to obtain the final concentration as described above. For control and glyphosate treatment groups, the same amount of absolute-ethanol was given as control stimulation. The exposure to glyphosate and control were performed simultaneously without changing the pH of the medium.

3. Sister Chromatid Exchange (SCE) Assay

Each blood sample (1.0 mL) was mixed with 9 mL of culture medium that consisteds of RPMI-1640 supplemented with 10% fetal bovine serum: 0.1 mL (1 g/mL) of PHA was supplemented as a mitogen and then this was incubated at 37° C for 72 hr. At 24 hr of culture, 0.1 mL (1 g/mL) of BrdU was added each culture. At 70 hr of incubation, 0.1 mL (10 μg/mL) of colcemid was added in order to arrest mitosis at metaphase. All the chromosome preparations were stained by using the BrdU-H?echst-Giemsa technique. The SCE of the lymphocytes was microscopically examined and counted based on the Cytovision Computer- Assisted Karyotyping System (Applied Imaging, Santa Clara, CA, USA). 20 well-spread sec-

ond division metaphases were scored by each subject. The results were used to determine the mean number of SCEs (SCEs/cell).

4. Statistical Analysis

In this experiment, the one-way ANOVA method was used to statistically compare the mean number of SCEs from each group. 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 present study was conducted to observe the frequency of SCEs in peripheral blood lymphocytes of 11 males and 7 females with and without the exposure of glyphosate. These subjects were divided into four groups. In addition to a control group, in which subjects did not receive any glyphosate, subjects assigned to each of the other three groups were treated with 100 ng/mL, 200 ng/mL, and 300 ng/mL glyphosate, respectively. The mean frequency of SCEs/cell was 6.55±2.25 (mean±SD) for the control group, 7.97±2.25 for the 100 ng/mL group, 9.13±2.4 for the 200 ng/mL group, and 10.37±2.54 for the 300 ng/mL group. This outcome demonstrates that glyphosate significantly increases the frequency of SCEs in a dose-dependent manner compared with that of control values (Fig. 1).

There was a statistically significant difference between males and females across all groups except the 300 ng/mL exposure. Females consistently showed a higher frequency of SCEs than males (Fig. 2). In the control group, the frequencies of SCEs/cell in male and female groups were 6.25±2.23 and 6.99 ±2.21, respectively. In the group treated with 100 ng/mL glyphosate, SCEs/cell in males and females were 7.60±2.33 and 8.5±2.29, respectively. In the group treated with 200 ng/mL glyphosate, SCEs/cell in males and females were 8.91±2.31 and 9.48±2.5,

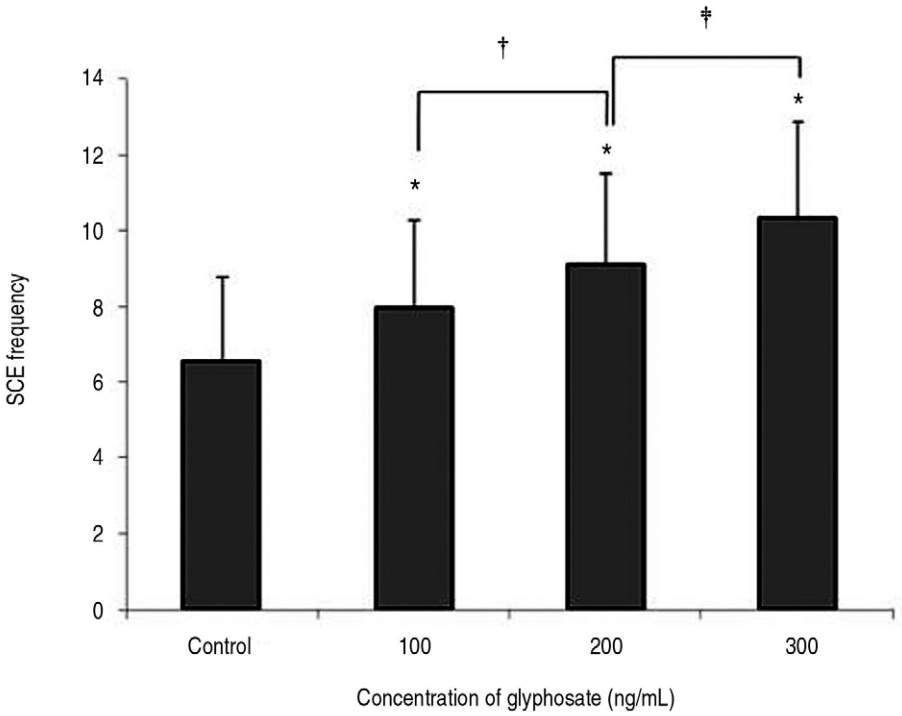

Fig. 1. Sister chromatid exchange (SCE) frequencies after the treatment of glyphosate.
* $p<0.001$ vs control
† $p<0.001$ vs 100 ng/mL of glyphosate
‡ $p<0.001$ vs 200 ng/mL of glyphosate.

respectively. In the group treated with 300 ng/mL glyphosate, SCEs/cell in males and females group were 10.34±2.55 and 10.42±2.53, respectively, showing no significant differences.

Discussion

A number of earlier studies demonstrated the risk of glyphosate to human health. However, their outcomes have been controversial. Li and Long[3] showed no genotoxic activity of glyphosate, using a variety of methods in vitro and in vivo assays including the Salmonella typhimurium and Escherichia coli WP-2 reversion assays, and recombination (rec-assay) with Bacillus subtilis. The review of glyphosate and glyphosate formulation (Roundup) also concluded that glyphosate causes no harm[4]. A prospective cohort study showed that most of the subtype cancers were not associated with glyphosate exposure, although multiple myeloma increased slightly[6]. Moreover, some studies showed that glyphosate did not induce genotoxicity by chromosomal aberrations and micronuclei analysis[15]. Glyphosate-induced oxidative stress did not show dose-dependent effect and did not pose significant health risk[16].

However, some studies demonstrated genotoxicity of purity glyphosate by significant increase of both structural chromosome aberrations and sister chromatid exchanges in vitro[14]. In addition, it changed cellular antioxidant status as a glutathione depletion, enzymatic disorder and increased lipid peroxidation[17]. Glyphosate induces cytotoxicity and genotoxicity on various cell types; human umbilical, embryonic, mammalian, and placental cells[18,19]. It was also investigated whether the exposure to glyphosate and Roundup induced cytogenic and genotoxic effects in buccal epithelial cell line given its frequent use for the suicide. Roundup turned out to be more genotoxic than glyphosate under various method assays[20].

On genotoxic studies of glyphosate, the products used in previous studies are various from pure glyphosate to pesticide Roundup. Roundup contains glyphosate as well as surfactant and other inert compounds. Interestingly, Roundup did not induce micronuclei in mice in vivo, but in fish erythrocytes[21]. Experiments with used sea urchin embryos introduced the synergic effect of glyphosate and formulation products which change the cell cycle regulation while purity glyphosate was ineffective[22]. Amer et al.

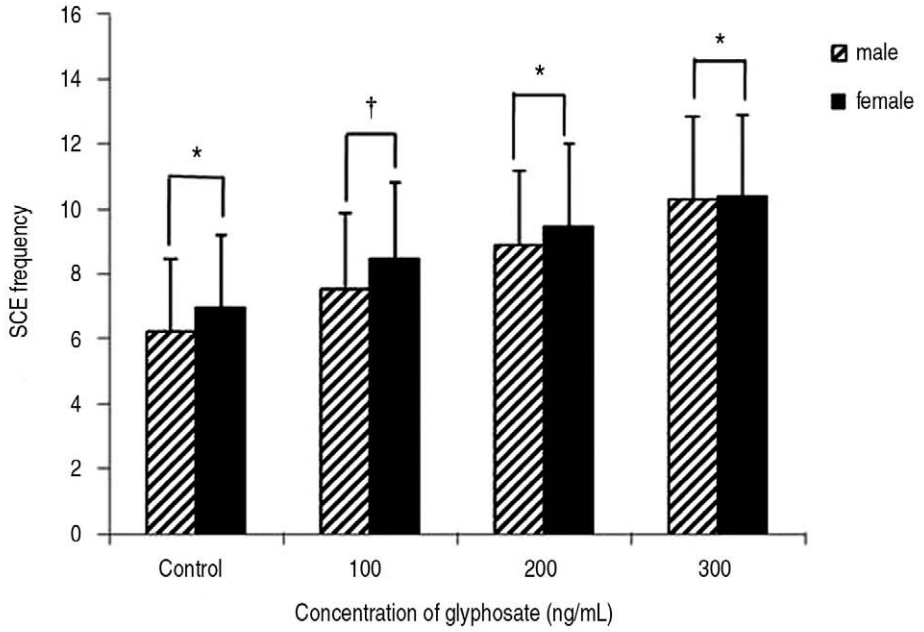

Fig. 2. Gender difference on sister chromatid exchange (SCE) frequency in the lymphocytes exposed to glyphosate.
* $p<0.05$
† $p<0.01$ between male and female.

reported the occurrence of chromosomal aberration at high glyphosate concentration (84% glyphosate and 16% solvent).

To further clarify the genetoxicity of glyphosate, the current study investigated the frequency of the SCEs in human lymphocytes using pure glyphosate. Preliminary data showed that pure glyphosate could induce SCEs under 500 ng/mL level. Therefore, this is the first study to have revealed that minimal doses of glyphosate could induce SCE. The results showed that all doses of glyphosate increased the SCE frequency significantly compared with the control group in a dose-dependent manner. This increase of SCEs frequency was weak but, extremely significant, suggesting that more considerate treatment of glyphosate is needed. However, previous studies using chromosomal aberration and micronuclei showed no cytotoxic potential of glyphosate, indicating that glyphosate could cause chromosomal instability specially related to SCE induction.

Interestingly, reported in the control and two other groups, in which subjects were treated with 100 and 200 ng/mL, respectively, the SCE frequency was significantly higher in females than in males. However, 300 ng/mL-group showed no significant difference between genders. Higher frequency of SCEs in females was well introduced although its correct mechanism was not identified[24]. These results demonstrated a synergistic effect of low-dose glyphosate and suggested that females were more vulnerable to the cytotoxicity of low-dose glyphosate.

This study has some limitations. Cytotoxicity should be examined not only in pure glyphosate but also in various concentrations of Roundup for safe use. Based on the results of the present study, various Roundup as a herbicide on sale will be identified. Furthermore, there is no additional molecular analysis that supports the result of this study. The SCE is merely one of the experimental methods of many genetic studies. This assay cannot explain the clinical symptoms and has different results by such various variations as smoking, age, sex, and others. This study was targeting at acute exposure to glyphosate only, not to chronic exposure in rural area. In addition, we do not know any exact toxicity dose in vivo because this study was performed only in vitro. Therefore, further research should be carried out.

Conclusion

The present study, clearly showed that minimal dose of glyphosate could induce SCE. As the SCE is one of the most sensitive markers to detect the severity of DNA damage in a cytogenetic level, this result may suggest the guideline for safe use of glyphosate using SCE analysis. Further molecular analysis with larger sample sizes is necessary to demonstrate the robustness of the outcome of this research on the cytogenetic damage by glyphosate exposure.

Conflict of interest statement

The authors declare that there are no conflicts of interest

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