Protective Effects of (–)Epigallocatechin Gallate and (+)Catechin on Nitrogen Oxide-Induced Sister Chromatid Exchange

Reiko Tanaka*

Department of Nursing, Akita Keijo Junior College, 2–3–4 Shimizu, Odate-shi, Akita 017–0046, Japan
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The protective effects of green tea polyphenols on nitrogen oxide (NOx)-induced sister chromatid exchange (SCE) in cultured cells were studied. (–)Epigallocatechin gallate (EGCG) and (+)catechin (CT), the major polyphenol constituents of green tea, were found to reduce the frequency of SCE induced by NOx. NO releaser (NOR4)-released NO and $NaNO_2$ were used as NOx. Significantly fewer SCEs were induced by 30 μM NOR4 and $NaNO_2$ after the treatment of cells with 0.1 μM EGCG or 0.5 μM CT. NOx (NO, NO_2^-) is a potent environmental pollutant owing to its carcinogenic properties. Therefore these experimental results indicate the protective effects of green tea against NOx-type carcinogens.

Key words —— green tea, nitrogen oxide, sister chromatid exchange, (–)epigallocatechin gallate, (+)catechin

INTRODUCTION

The protective effects of (–)epigallocatechin gallate (EGCG) and (+)catechin (CT) on paraquat-induced sister chromatid exchanges (SCEs) have been reported previously.[1] These polyphenols are major constituents of green tea. Tea is one of the most common beverages consumed worldwide, and its possible beneficial health effects have received a great deal of attention, particularly in recent years. The effectiveness of green tea and its extracts as possible cancer-preventing agents has been evaluated in clinical trials and *via* animal models.[2–6]

Nitrogen oxide (NOx) is a potent environmental pollutant owing to its carcinogenetic properties. Isomura *et al*.[7] demonstrated the induction of mutation and chromosome aberrations in rat lung cells following *in vivo* exposure to NO and NO_2^-. In another study,[8] NOx induced chromosomal aberrations in cultured chinese hamster lung (CHL) cells. In that study, NO induced the most aberrations, while NO_3^- induced the least aberrations, while NO releaser (NOR4)-released NO and $NaNO_2$ have been found to increase the frequency of SCE.[9]

These results indicate the preventive effects of green tea polyphenols on NOx-induced SCE in cultured cells. Therefore, in the present study, we examined the effects of EGCG and CT on NOx-induced SCE.

MATERIALS AND METHODS

CHL cells (CHL/IU) were obtained from Dainippon Pharmaceutical Co. (Osaka, Japan). Cells were seeded at a density of 10^5 cells/ml in rectangular bottles, and were then incubated for about 20 hr at 37°C in Eagle's minimum essential medium (MEM; Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% calf serum (Dainippon Pharmaceutical Co.).

After washing cells with phosphate-buffered saline (PBS, 0.01 M, pH 7.4) and adding fresh medium which does not contain phenol red or serum, cells were exposed to EGCG (Kurita Industry Company, Tokyo, Japan), CT (Kurita Industry Company), NOx and 5-bromo 2′-deoxyuriden (BrdU; final concentration, 10 μM; Sigma Co., Louis, MO, U.S.A.) in the dark at 37°C. EGCG and CT were added 30 min prior to NOx and BrdU addition. After 5 hr of exposure to NOx, cells were again washed with PBS and incubated in MEM containing phenol red and serum for 20 hr at 37°C. Four hours before the end of incubation, colcemid (final concentration, 0.2 μg/ml; GIBCO Laboratories, New York, U.S.A.) was added to each bottle. After the incubation period, cells were harvested using trypsin (Merck, Darmstadt, Germany), and were then centrifuged ($112 \times g$, 5 min). Following treatment with 0.04 M KCl for 15 min at 37°C, chromosomes were obtained by centrifugation ($174 \times g$, 5 min) and were fixed with methanol-acetic acid (3 : 1, v/v). These procedures were performed in a darkened laboratory because BrdU is sensitive to light. The chromosomal preparations were stained with 2% Giemsa solution (Merck) in 0.3 M Na_2HPO_4 at pH 10.4 for 30 min.[10]

*To whom correspondence should be addressed: Department of Nursing, Akita Keijo Junior College, 2–3–4 Shimizu, Odate-shi, Akita 017–0046, Japan. Tel.: +81-186-45-1749; Fax: +81-186-43-6713; E-mail: r-tanaka@kjo.ac.jp

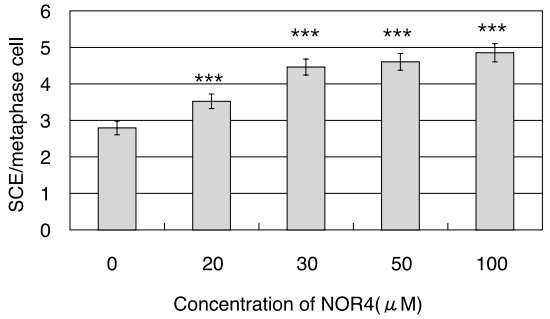

Fig. 1. Frequency of SCE Induced by NOR4

CHL cells were exposed to NOR4 for 5 hr. Values are the mean ± S.E. Number of recorded metaphase cells was 50–60. ***$p < 0.001$ *vs*. 0 μM NOR4.

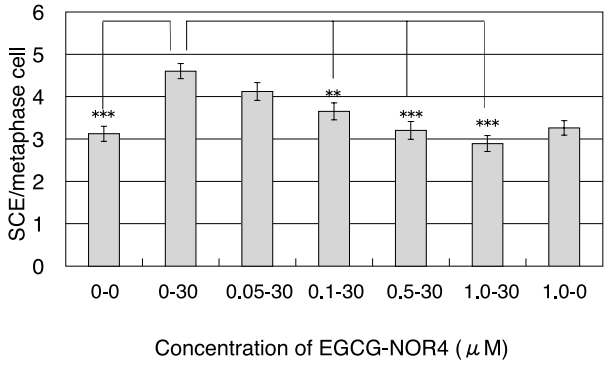

Fig. 2. Effects of EGCG on the NOR4-Induced SCE

Cells were exposed to EGCG and NOR4 for 5 hr. Values are the mean ± S.E. Number of recorded metaphase cells was 50–60. ***$p < 0.001$, **$p < 0.01$ *vs*. 30 μM NOR4.

This technique darkly stains chromatids that have incorporated BrdU. The results were recorded as the frequency of SCE/metaphase cells for chromatids that had undergone two replication cycles.[11] As NOx, NOR4, (±)-N-[(E)-ethyl-2-[(Z)-hydroxyimino]-5-nitro-3-hexene-1-yl]3-pyridine carboxamide (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) and $NaNO_2$ (Wako Pure Chemical Industries Co. Ltd.) were used. In a previous study,[9] $NaNO_3$ was found to have no effect on the frequency of SCE. NOR4 releases NO, which is oxidized to NO_2^- with little or no formation of NO_3^- in aqueous solutions.[12] At 37°C, 100 μM NOR4 was found to release 20 μM NO_2^- for 5 hr in MEM.

Statistics ——— All data are expressed as the mean ± S.E. Statistical significance was determined using Student's *t*-test.[13]

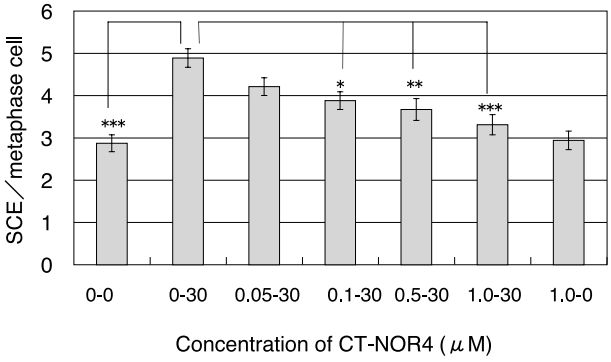

Fig. 3. Effects of (+)CT on NOR4-Induced SCE

Cells were exposed to CT and NOR for 5 hr. Values are the mean ± S.E. Number of recorded metaphase cells was 50–60. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs*. 30 μM NOR4.

RESULTS

SCE analysis was used as an index of genotoxic activity. Cells were exposed to NOR4 or $NaNO_2$ for 5 hr. Experiments were repeated 5 times.

Figure 1 shows NOR4-induced SCE in CHL/IU cells. The frequency of SCE was dose dependent. Based on these results, 30 μM NOR4 was used to determine the effects of EGCG and CT in this report.

The effects of EGCG and CT on NOR4-induced SCE are shown in Figs. 2 and 3. EGCG and CT exhibited similar effects, and were effective in significantly decreasing the frequency of NOR4-induced SCE at more than 0.1 μM.

Figure 4 shows the $NaNO_2$-induced SCE in CHL/IU cells after 5 hr of treatment. Frequencies

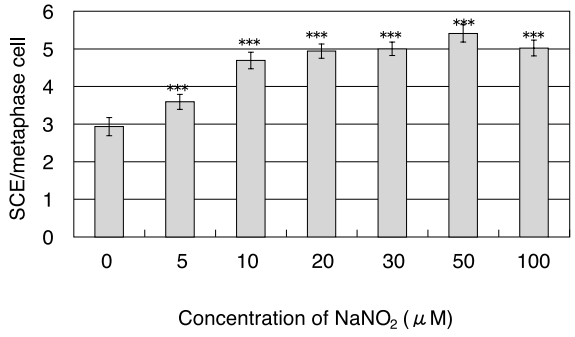

Fig. 4. Frequencies of SCE Induced by $NaNO_2$

CHL cells were exposed to $NaNO_2$ for 5 hr. Values are the mean ± S.E. Number of recorded metaphase cells was 50–60. ***$p < 0.001$ *vs*. 0 μM $NaNO_2$.

of SCE increased at concentrations of 5–50 μM of $NaNO_2$. Based on these results, 30 μM $NaNO_2$ was used to determine the effects of EGCG and CT. The

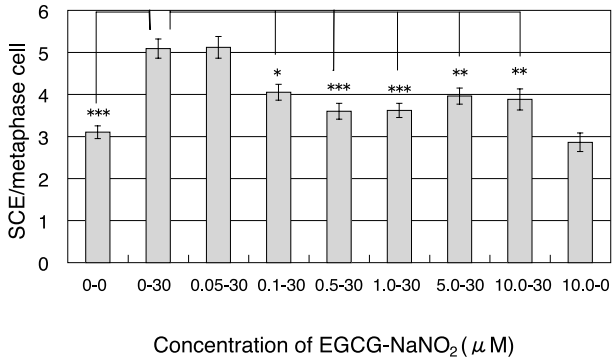

Fig. 5. Effects of EGCG on $NaNO_2$-Induced SCE

Cells were exposed to EGCG and $NaNO_2$ for 5 hr. Values are the mean ± S.E. Number of recorded metaphase cells was 50–60. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs.* 30 μM $NaNO_2$.

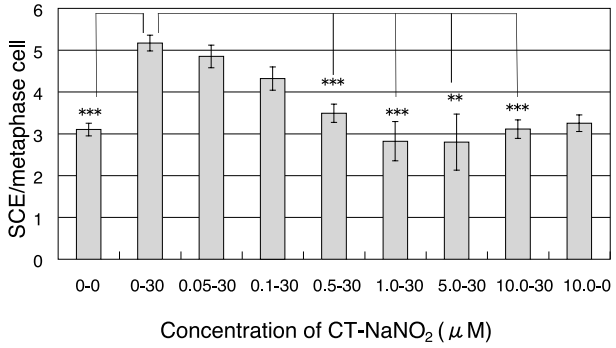

Fig. 6. Effects of (+)CT on $NaNO_2$-Induced SCE

Cells were exposed to CT and $NaNO_2$ for 5 hr. Values are the mean ± S.E. Number of recorded metaphase cells was 50–60. ***$p < 0.001$, **$p < 0.01$ *vs.* 30 μM $NaNO_2$.

effects of EGCG and CT on $NaNO_2$-induced SCE are shown in Figs. 5 and 6. EGCG was significantly effective at more than 0.1 μM, and CT was significantly effective at more than 0.5 μM.

As shown in Figs. 2, 3 ,5 and 6, the effects of EGCG (1.0, 10.0 μM) and CT (1.0, 10.0 μM) on SCE frequency were not obtained in the absence of NOR4 nor $NaNO_2$.

These results indicate significant protective effects of EGCG and CT on NOx-induced SCE.

DISCUSSION

Previous studies[1] have demonstrated the protective effects of EGCG and CT, which are catechins found in green tea, on paraquat (superoxide anion generator)-induced SCE. In this study, EGCG and CT were found to protect against NOR4 and $NaNO_2$-induced SCE (Figs. 2, 3, 5 and 6). Based on these findings, EGCG and CT were confirmed to be effective in protecting against NOx-induced genotoxicity.

The catechins and polyphenols are known to be scavengers of reactive oxygen species *in vitro* and to act as antioxidants through their effects on transcription factors and enzyme activities.[14] The ability of catechins to scavenge reactive oxygen species and nitrogen species has been confirmed in many *in vitro* systems.[14]

The finding that catechins are rapidly and extensively metabolized by salivary and intestinal microflora, as well as in the liver,[14] led to *in vivo* and epidemiologic studies. However, evaluation of their effectiveness as anitioxidants *in vivo* is complex. Therefore, studies using catechin metabolites may be necessary for *in vivo* systems.

Nanjo *et al.*[15] reported stronger radical scavenging activities for EGCG than for CT. In the present study, the protective activity of EGCG against $NaNO_2$- and NOR4-induced genotoxicity was approximately equal to that of CT. Therefore, EGCG- and CT-containing foods may be beneficial to human health due to their ability to reduce reactive oxygen species-induced cancer. However, further investigation of the effectiveness of these green tea metabolites on human health is necessary.

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