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

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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 NaNO2
were used as NOx. Significantly fewer SCEs were in-
duced by 30 μM NOR4 and NaNO2 after the treatment
of cells with 0.1 μM EGCG or 0.5 μM CT. NOx (NO,
NO2−) is a potent environmental pollutant owing to its
carcinogenic properties. Therefore these experimen-
tal results indicate the protective effects of green tea
against NOx-type carcinogens.

Key words — green tea, nitrogen oxide, sister chro-
matid exchange, (−)epigallocatechin gallate, (+)catechin

INTRODUCTION

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

Nitrogen oxide (NOx) is a potent environmen-
tal pollutant owing to its carcinogenic properties.
Isomura et al.7) demonstrated the induction of muta-
tion and chromosome aberrations in rat lung cells
following in vivo exposure to NO and NO2−. In an-
other study,8) NOx induced chromosomal aberrations
in cultured chinese hamster lung (CHL) cells. In that
study, NO induced the most aberrations, while NO2−
induced the least aberrations, while NO releaser
(NOR4)-released NO and NaNO2 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 cul-
tured cells. Therefore, in the present study, we ex-
amined the effects of EGCG and CT on NOx-in-
duced SCE.

MATERIALS AND METHODS

CHL cells (CHL/IU) were obtained from Dainippon Pharmaceutical Co. (Osaka, Japan). Cells
were seeded at a density of 105 cells/ml in rectangu-
lar 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 Phar-
maceutical Co.).

After washing cells with phosphate-buffered
saline (PBS, 0.01 M, pH 7.4) and adding fresh me-
dium which does not contain phenol red or serum,
cells were exposed to EGCG (Kurita Industry Com-
pany, Tokyo, Japan), CT (Kurita Industry Company),
NOx and 5-bromo 2′-deoxyuriden (BrdU; final con-
centration, 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 × g, 5 min). Following treatment with 0.04 M
KCl for 15 min at 37°C, chromosomes were obtained
by centrifugation (174 × g, 5 min) and were fixed
with methanol-acetic acid (3 : 1, v/v). These proce-
dures were performed in a darkened laboratory be-
cause BrdU is sensitive to light. The chromosomal
preparations were stained with 2% Giemsa solution
(Merck) in 0.3 M Na2HPO4 at pH 10.4 for 30 min.10)
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. As NOx, NOR4, (±)-N-[2-(E)-ethyl-2-[(Z)-hydroxyimino]-5-nitro-3-hexene-1-yl]-3-pyridine carboxamide (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) and NaNO2 (Wako Pure Chemical Industries Co. Ltd.) were used. In a previous study, NaNO3 was found to have no effect on the frequency of SCE. NOR4 releases NO, which is oxidized to NO2⁻ with little or no formation of NO3⁻ in aqueous solutions. At 37°C, 100 µM NOR4 was found to release 20 µM NO2⁻ for 5 hr in MEM.

Statistics —— All data are expressed as the mean ± S.E. Statistical significance was determined using Student’s t-test.

RESULTS

SCE analysis was used as an index of genotoxic activity. Cells were exposed to NOR4 or NaNO2 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 NaNO2-induced SCE in CHL/IU cells after 5 hr of treatment. Frequencies of SCE increased at concentrations of 5–50 µM of NaNO2. Based on these results, 30 µM NaNO2 was used to determine the effects of EGCG and CT.
effects of EGCG and CT on NaNO₂-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 NaNO₂.

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

**DISCUSSION**

Previous studies¹ 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 NaNO₂-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 antioxidants 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 NaNO₂- 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.

**REFERENCES**


