

The Suppressing Effect of the Extract from *Cassia nomame* on Clastogenicity and Cytotoxicity of Mitomycin C in CHO Cells

Shin'ichi Kadowaki,* Kazufumi Naitou, Yoshinori Takahara, and Koji Yamamoto

Department of Bioengineering, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan

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Chinese hamster ovary (CHO) K1 cells were treated with 2.5 μM Mitomycin C (MMC) for one hour and incubated in Ham's F12 medium containing 10% fetal bovine serum or in the medium supplemented with the extract from *Cassia nomame* pods, leaves and stems. The frequency of cells with chromosome aberrations was significantly lowered by the extract. Proliferation of CHO cells was suppressed by a 1-hr MMC treatment, but the suppression was nullified by the presence of the extract. These findings suggest that the extract can prevent the physiological damage caused by MMC.

Key words — *Cassia nomame*, mitomycin C, suppressing effect, chromosome aberration;

INTRODUCTION

Recently the tea plant has been suggested be effective in suppressing carcinogenesis. An epidemiological study showed that the mortality from cancer in Sizuoka Pref., a major green tea producing region in Japan, was significantly lower than that in other prefectures.¹⁾ Sasaki *et al.* (1993) reported that the extract of Po-lei tea and Rooibos tea suppressed the frequency of chromosome aberrations induced by clastogens. Attention has been focused on the carcinogenesis-suppressing effect of catechins in tea plant extract. Epigallo-cathechin-3 gallate suppresses carcinogenesis by inhibiting the activity of urokinase which is needed for proliferation of can-

cer cells.²⁾

The aqueous extract from leaves, stems, and pods of *Cassia nomame* called "Hama-cha" is a conventional beverage in the San'in district of Japan. It is also used as a raw material for a diuretic or antidote in a folk remedy. Many species in the genus *Cassia* produce chemicals (second metabolites) which affect human physiology. Senna extracts, *i.e.*, extracts from Tinnevely senna (*Cassia angustifolia* Vahl) and Egyptian senna (*Cassia acutifolia* Delile), are used worldwide for the treatment of constipation. A recent study revealed no *in vitro* clastogenic activity of the senna extract in the mouse micronucleus assay, and there was no indication of a genotoxic risk for the therapeutic use of senna as a laxative.³⁾

In this paper, we report that the extract from *Cassia nomame* lowers the frequency of chromosome aberration in Chinese hamster ovary (CHO) K1 cells treated with 2.5 μM Mitomycin C (MMC), and restores the proliferation of CHO cells suppressed by MMC to the normal level.

MATERIALS AND METHODS

Preparation of the Extract from *Cassia nomame*

— Collected plants (*Cassia nomame*) dried in the shade and chopped into chips, were put into boiling water, 30 times in weight of the chips, and kept boiling for 1 hr. After cooling, the chips were strained out through gauze and the decoction was filtered through 50 μm nylon-mesh. The filtrate was centrifuged at approximately 60 $\times g$ for 4 min two or three times, and the supernatant was collected. The supernatant was centrifuged at about 560 $\times g$ for 10 min repeatedly until no sediment was seen. The supernatant was concentrated by vacuum distillation until it was evaporated to one fifteenth of its initial volume. The resulting extract was stored at 4°C. If sediment appeared in the extract, the supernatant was used.

Cell line and Culture — CHO cells used in this study were originally obtained from Dr. Sasaki (Hachinohe National College of Technology). The cells were derived from CHO K-1 (from American Type Cell Collection: ATCC) and screened by Sasaki as a population with a relatively short cell cycle. Cells were cultured in Ham's F12 medium (DAIGO, Wako Pure Chemical Industry, Japan) supplemented with 10% fetal bovine serum (GIBCO BRL Inc., U.S.A.) in a humidified atmosphere with 5% CO₂ at 37°C. The volume of the culture medium was 5 ml

*To whom correspondence should be addressed: Department of Bioengineering, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan. Tel.: +81-258-47-9434; Fax: +81-258-47-9400; E-mail: kadowaki@stn.nagaokaut.ac.jp.

per 60-mm petri dish.

Mitomycin C Treatment — As a mutagen, MMC was used. MMC (MITOMYCIN Kyowa S, azirizino[2',3':3,4]pyrrolo[1,2- α]indole-4,7-dione-6-amino-1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)8a-methoxy-5-methyl-carbamate) was purchased from Kyowa Hakko Kogyo Co., Ltd. in Japan. CHO Cells were inoculated at a density of 2.5×10^5 cells/60-mm dish. The cells were exposed to $2.5 \mu\text{M}$ -MMC for 1 hr after the incubation for 19 or 26 hr, when cell growth was in log phase.

Assay of Chromosome Aberrations — After MMC treatment, the cells were washed with Hank's balanced salt solution (Hank's BSS), and incubated in the medium mentioned above with or without 0.8v/v% extract. At 14, 17, 20, 23, 26 and 29 hr after the MMC treatment, colchicine solution was dropped into the dishes to make the final concentration $125 \mu\text{M}$, and incubated for one hour. Mitotic preparations were obtained by an air-drying method. Preparations were stained with 6% Wright Solution in 1/30 M phosphate buffer (pH 6.8). One hundred well-spread metaphases were observed for chromosome aberrations at each sampling time under a microscope. The frequency of aberrant cells was determined by counting metaphase cells with one or more chromosome aberrations. The experiments were repeated twice.

Measurement of Proliferation Rate of CHO Cells — CHO cells were inoculated at a density of 2.5×10^5 cells/60-mm dish. After a 19-hr incubation, half of the cells were treated with MMC, washed in Hank's BSS, and cultured on the medium with or without the extract added. The remaining half of the cells were washed with Hank's BSS without pretreatment with MMC at the 20th hour of incubation, and cultured in the medium with or without the extract added.

The medium was not exchanged in any group, except for that 20 hr after inoculation.

In all four groups, the number of cells was counted every 8 hr. To count the cells, we suspended the cells in 0.25% trypsin PBS(-)-solution (Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+}), measured the cell density with a counting chamber and calculated the number of cells per dish. The experiments were repeated twice.

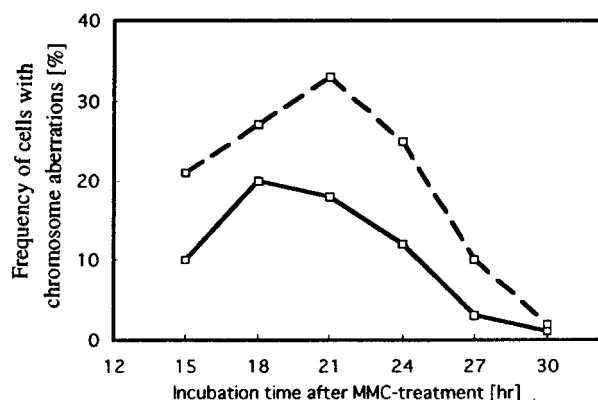


Fig. 1. Suppressing Effect of the Extract on Clastogenicity of MMC

The frequency of cells with chromosome aberrations is shown at each incubation time after a one-hour treatment with MMC. The solid and broken lines show the frequencies in the medium with and without the extract added, respectively.

Table 1. A 2×2 Ttable for χ^2 Test on Significance of the Differences in the Frequency of Cells with Chromosome Aberrations

Medium supplemented with	Normal cells	Aberrant cells	Total
None	482	118	600
The extract	536	64	600
Total	1018	182	1200

$\chi^2 = 18.9$

RESULTS

Suppressing Effect of the Extract on Clastogenicity of Mitomycin C

Figure 1 shows the frequency of cells with chromosome aberrations at various time points. The cells incubated in the medium with the extract showed chromosome aberrations at a lower frequency than those incubated in the medium without the extract. To test the significance of the difference in the frequencies, we conducted the χ^2 -test for 2×2 table (Table 1). The results indicated that the difference is significant at 99%. These findings suggest that chromosome lesions induced by a 1-hr MMC treatment would be partly restored by the extract.

Recovering from proliferation-inhibiting effect of MMC

Figure 2 shows the proliferation curves of the MMC-treated and non-treated cells in the medium with or without extract. The proliferation of the non-treated cells was not influenced by the addition of the extract.

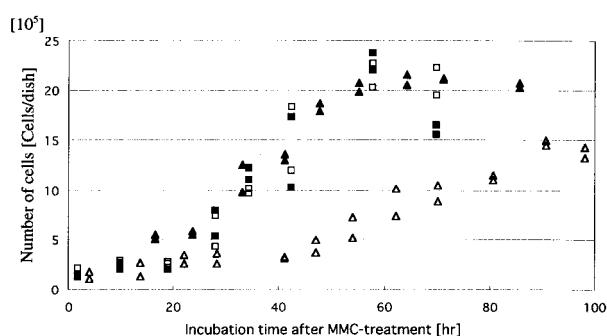


Fig. 2. Proliferation of MMC-Treated (,) and Non-Treated (,) Cells in the Medium with (,) and without (,) the Extract Added

On the other hand, the proliferation rate of the MMC-treated cells began to lower apparently at about 20 hr after the treatment, stopped increasing for the following 20 or more hours, and began to increase at about 40 hr after the treatment. When MMC-treated cells were incubated in the medium with the extract added, they proliferated normally as non-treated cells. The findings suggest that the suppression of cell proliferation by MMC is prevented by the addition of the extract to the medium after MMC treatment.

DISCUSSION

It is established that chromosome aberrations are produced by MMC when cells are treated during the G1 phase. According to Sasaki *et al.* (1989), the duration of G1, S, and G2 phases in CHO cells treated with 1.0 μM MMC for one hour was 9, 9 and 3 hr, respectively.⁴⁾ In the present study, the aberrations occurred at a maximum frequency at 21 hr after a 1-hr MMC treatment. This finding supports the view that MMC produces chromosome aberrations most effectively in G1 cells.

Addition of the extract after MMC treatment resulted in lowering the frequency of chromosome aberrations. This result suggests that chromosome lesions caused by MMC in G1 cells could be repaired during the progression from G1 to M phase at which chromosome aberrations are observed.

It is also known that MMC strongly inhibits division of human leukocyte *in vitro* when treated in the G1 or S phase, but not in the G2 phase.⁵⁾ In the

present study, suppression of cell proliferation was observed apparently about 40 hr after MMC treatment. These results suggest that division of cells which are in G1 or early S phase at the time of treatment is inhibited. Some of the cells could repair lesions induced by MMC and restart division after about 20 hr.

On the other hand, the results showed that MMC-treated cells proliferated normally when they were cultured in the medium containing the extract. The lesions induced by MMC in G1 cells may be restored before the cells reach the M phase. The extract from *Cassia nomame* may contain some chemicals which promote the repair of lesions induced by MMC. Molecular studies on these mechanisms are needed to obtain a definite conclusion.

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