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Preventive Effect of *Ecklonia stolonifera* on the Frequency of Benzo(a)pyrene-Induced Chromosomal Aberrations

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Abstract

Chromosomal aberration tests *in vitro* using Chinese hamster lung(CHL) cells were carried out to evaluate the possible role of the MeOH extract of *Ecklonia stolonifera* in modulating the chromosomal damage induced by Mitomycin C(MMC) and Benzo(a)pyrene(B(a)P), respectively. The MeOH extract of *Ecklonia stolonifera* (250μg/ml) reduced significantly the incidence of chromosomal aberration induced by treatment with B(a)P by 80%. The suppressive effect was much stronger than that of β-carotene, which is a well known antimutagen. However, there was no marked decrease in the chromosomal aberration induced by MMC. In the tests involving chromosomal aberration induced by the treatment of the MeOH extract of *Ecklonia stolonifera* alone, there was no significant increase in comparison with the negative control. The results would seem to indicate that, at least under the conditions examined, the MeOH extract of *Ecklonia stolonifera* decreased the chromosomal aberrations induced by B(a)P in the CHL cells, but had little effect on the chromosomal aberration induced by MMC.

Key words: *Ecklonia stolonifera*, marine algae, anti-chromosomal aberration, benzo(a)pyrene

INTRODUCTION

In recent years, it has become more important to evaluate the factors which modify the mutagenic or carcinogenic activities of environmental contaminants. Many naturally occurring compounds of plants and seaweed are known to inhibit chemical mutagenesis and carcinogenesis in a variety of *in vitro* and *in vivo* test systems(1-5). We have been interested in different kinds of seaweed, not only because many marine algae such as green and brown algae are commonly used as foods, but also because they have been used as a form of folk medicine in the curing of curare, gout, eczema and gallstones.

Marine organisms have been shown to have active antitumor compounds which contain polysaccharides (4,6,7). We have previously tested the MeOH extract of different kinds of seaweed as to their antioxidizing activity by measuring lipid peroxides produced when a mouse liver homogenate is exposed to the air at 37°C, using 2-thiobarbituric acid(TBA) and the radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The MeOH extract of *Ecklonia stolonifera* and the phloroglucinol isolated were shown to have a strong antioxidizing activity(8,9).

There are a variety of compounds in nature which work as antioxidants, and it is believed that these inhibit mutagenesis(10). Therefore, in this study, we examined the antimutagenic activity of the MeOH extract of *Ecklonia stolonifera* by analysing their effect on the frequency of mutagen-induced chromosomal aberration. *Ecklonia stolonifera* is an edible brown algae which belongs to the *Laminaria* species. It has been reported that phloroglucinol and ecklonialactones isolated from *Ecklonia stolonifera* have a feeding-deterrent effect on the sea urchin and abalone(11-14). The protein-polysaccaride fraction extracted from *Ecklonia stolonifera* also has an antitumor effect on Sarcoma-180 cells(15). But, no study of *Ecklonia stolonifera* has yet examined the frequency of mutagen-induced chromosomal aberration. Hence the present study was carried out with the objective of evaluating the possible influence of the brown algae, *Ecklonia stolonifera*, on *in vitro* chromosomal aberration induced by MMC and B(a)P.

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MATERIALS AND METHODS

Algae material

All the seaweed of *Ecklonia stolonifera* used was collected at Tae Jong Dae, Pusan in July, 1990. The algae were identified by the botanist Prof. H. G. Kim, and a voucher specimen is now deposited in the auther's laboratory(J. S. Choi). All the seaweed was washed with fresh water and air-dried in the shade. The dried material(2.9kg) was extracted three times with methanol at room temperature, and the solvent was removed under reduced pressure to a dark blue semisolid(500g).

Cell culture

A clonal sub-line from a Chinese hamster lung fibroblast cell line(CHL) was obtained from the National Institute of Safety Research, Seoul, Korea. The karyotype of these CHL cells consisted of 25 chromosomes. The cells had been maintained by 3~4 day passages and grown in a monolayer with Eagles' minimum essential medium(Gibco, 410-1100EA) supplemented with 10% fetal bovine serum(Gibco, 26140-020). These cells were maintained at 37°C in 5% CO_2 atmosphere.

Reagents

The trypsin-EDTA and colcemid were products of GIBCO BRL Life Tech. Inc.(Gaithersberg, U.S.A.). The mitomycin C(MMC) and benzo(a)pyrene(B(a)P) were purchased from Sigma Chemical Co.(St. Louis, MO, USA). The test compounds were dissolved in dimethylsulfoxide(DMSO) or saline. The preparation of a rat liver S-9 fraction for metabolic activation system has been previously reported(16). The S-9 fraction was prepared and stored immediately at -80°C before use.

Determination of the 50% growth inhibition concentration(Cytotoxicity)

Growth inhibition tests(17) were carried out on each sample before the chromosome aberration tests were started. The CHL cells were seeded at densities of 2.0 $\times 10^4$ cells/2ml in 24 well plates. Twenty-four hours after seeding, several different doses of each sample were separately added and incubated for a further 24 hr. The cells in the monolayer were then washed, treated with methanol, and stained with a 5% Giemsa solution (pH 6.8. Sigma. G4507) for 5min. Finally the 50% inhibition concentration(IC_{50}) values were calculated.

Chromosomal aberration test

Five different doses were prepared, and separately added to 3-day-old cultures(about 5×10^5cells/60mm dish). Where there was no metabolic activation, the chromosome preparations were made 24hr after treatment. However, it was necessary to change to a fresh medium after 6hr during the 24hr exposure in cases where an addition of S-9 mix for metabolic activation. The cells were treated with colcemid(0.2μg/ml) for 2hr, then harvested by centrifugation after trypsinization. The cell pellets were incubated in 0.075M KCl hypotonic solution for 20min at 37°C. After being spun down, the cell pellets were treated with ice-cold fixative(methanol : acetic acid=3 : 1(v/v)) twice. After centrifugation, the fixative was removed, and the cell pellet solutions were prepared by pipetting gently. A few drops of the cell pellet suspension were then placed on some precleaned dry slides, and then the chromosome was spread out on the slides by slanting them. After being allowed to dry, the slides were stained with 5% Giemsa's buffered solution(pH 6.8) for 30min. The number of cells with chromosomal aberrations was then recorded on 100 well-spread metaphases at a magnification of 1,000 with a microscope. The cells treated only with solvents served as a control(16).

Evaluation

CHL cells usually have less than 3.0% cell chromosomal aberration. Therefore, the data from the count up of the well-spread 100 metaphase chromosomes were expressed as a percentage.

RESULTS AND DISCUSSION

Cytotoxicity of the MeOH extract of *Ecklonia stolonifera*

Before we examined the effect of the MeOH extract on chromosomal aberration, the cytotoxicity of the MeOH extract was tested using the Chinese hamster lung cells. The MeOH extract showed no cytotoxicity even at the highest concentration of 250μg/ml in CHL cells treated for 24hr.

Effects of the MeOH extract and β-carotene on MMC-induced chromosome aberration

The incidences of chromosomal aberration by MMC in CHL cells after *in vitro* treatment with β-carotene and the methanol extract are given in Table 1 and 2. MMC at a dose of 0.1μg/ml produced a significant increase in chromosomal aberrations. The addition of β-carotene(250~15.625μg/ml) and the MeOH extract of *E. stolonifera*(250~15.625μg/ml) suppressed slightly the frequency of chromosomal aberrations by the MMC, but the effect was not significant. β-Carotene alone at the highest dose(250μg/ml) did not produce chromosomal aberrations, nor did the MeOH extract at the highest dose(250μg/ml). The results showed that beyond this dose range, no effective protection by the β-carotene and the MeOH extract of *E. stolonifera* could be observed.

Effects of the MeOH extract and β-carotene on B(a)P-induced chromosome aberration

The effects of the MeOH extract of *E. stolonifera* and β-carotene on B(a)P-induced chromosomal aberrations are given in Table 3 and 4. B(a)P(20μg/ml) with a metabolic activation system has induced about 15% chromosomal aberrations in CHL cells. However, the addition of β-carotene(250~15.625μg/ml) highly suppressed the B(a)P-induced chromosomal aberrations by 60~87%. There was also no dose dependence. The addition of the MeOH extract of *E. stolonifera*(250~15.625μg/ml) also suppressed significantly the B(a)P-induced chromosomal aberrations by 67~80%. Neither

Table 1. Effect of *β*-carotene on the frequence of cells with chromosome aberration induced by MMC in the absence of S9

Treatment MMC+β-carotene (μg/ml)		No. of cells analyzed	Types of aberration							Suppression rate(%)
			gap	ctb	cte	csb	cse	pol	nor	
−	−	100	2		1				97	
0.1	−	100	6	1	17		1		75	
0.1	250	100	3		11		2		84	36
0.1	125	100	3	1	11		3		82	28
0.1	62.5	100	4		13		5		78	12
0.1	31.25	100	2		15				83	32
0.1	15.625	100	2		16		1		81	24
−	250	100	2						98	

gap: chromatid and isochromatid gap, ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange, MMC: mitomycin C

$$\text{Suppression rate}: 100 - \frac{\text{aberration cells of sample}}{\text{aberration cells of positive control}} \times 100$$

Table 2. Effect of MeOH extract of *E. stolonifera* on the frequence of cells with chromosome aberration induced by MMC in the absence of S9

Treatment MMC+MeOH extract (μg/ml)		No of cells analyzed	Types of aberration							Suppression rate(%)
			gap	ctb	cte	csb	cse	pol	nor	
−	−	100	2		1				97	
0.1	−	100	6	1	17		1		75	
0.1	250	100	4		14		2		80	20
0.1	125	100			23		3		74	
0.1	62.5	100	1		20		1		78	12
0.1	31.25	100	2		19		3		76	4
0.1	15.625	100	4	1	19		3		73	
−	250	100	1						99	

gap: chromatid and isochromatid gap, ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange, MMC: mitomycin C

$$\text{Suppression rate}: 100 - \frac{\text{aberration cells of sample}}{\text{aberration cells of positive control}} \times 100$$

Table 3. Effect of β-carotene on the frequence of cells with chromosome aberration induced by B(a)P in the presence of S9

Treatment B(a)P+β-carotene (μg/ml)		No. of cells analyzed	Types of aberration							Suppression rate(%)
			gap	ctb	cte	csb	cse	pol	nor	
−	−	100	3						97	
20.0	−	100	12		3				85	
20.0	250	100	5		1				94	60
20.0	125	100	1		1				98	87
20.0	62.5	100	3						97	80
20.0	31.25	100	3						97	80
20.0	15.625	100	3		1				96	73
−	250	100	3						97	

gap: chromatid and isochromatid gap, ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange, B(a)P: benzo(a)pyrene

$$\text{Suppression rate} = 100 - \frac{\text{aberration cells of sample}}{\text{aberration cells of positive control}} \times 100$$

Table 4. Effect of MeOH extract of *E. stolonifera* on the frequence of cells with chromosome aberration induced by B(a)P in the presence of S9

Treatment B(a)P+MeOH extract (μg/ml)		No. of cells analyzed	Types of aberration							Suppression rate(%)
			gap	ctb	cte	csb	cse	pol	nor	
−	−	100	3						97	
20.0	−	100	12		3				85	
20.0	250	100	2		1				97	80
20.0	125	100	2		2				96	73
20.0	62.5	100	3						97	80
20.0	31.25	100	5						95	67
20.0	15.625	100	3		2				95	67
−	250	100	1						99	

gap: chromatid and isochromatid gap, ctb: chromatid breakage, cte: chromatid exchange, csb: chromosome breakage, cse: chromosome exchange, B(a)P: benzo(a)pyrene

$$\text{Suppression rate} = 100 - \frac{\text{aberration cells of sample}}{\text{aberration cells of positive control}} \times 100$$

β-carotene alone nor the MeOH extract of *E. stolonifera* produced chromosomal aberrations. These experiments showed that the MeOH extract of *E. stolonifera*, as well as β-carotene had a particularly strong suppressive effect on B(a)P-induced chromosomal aberrations, but not on the MMC-induced chromosomal aberration. This suggests that the MeOH extract of *E. stolonifera* may prevent the metabolic activation of indirect mutagens. B(a)P, a carcinogenic polycyclic aromatic hydrocarbon, is metabolized by mammalian enzymes to ultimate mutagenic and carcinogenic metabolites (18,19). Recent studies have shown that rivoflavin 5'-phosphate, ellagic acid, and several hydroxylated flavonoids, commonly found in plants, are potent inhibitors of the mutagenic activity of B(a)P(20,21). In this study, the MeOH extract of *E. stolonifera* seemed to be particularly effective against BP. This preferential activity appears to be due to its ability to interfere with the metabolic activation of B(a)P and to block the formation of DNA adducts. The present study also concluded that *E. stolonifera* be proposed as a strong candidate for a potential application as anticlastogenic material for in the case of indirect mutagens.

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REFERENCES

1. Cho, K. J., Lee. Y. S. and Ryu. B. H. : Antitumor effect

and immunology activity of seaweeds toward Sarcoma-180. *Bull. Koresn Fish. Soc.*, **23**, 345(1990)
2. Hah, J. C., Choe, E. S., Rhew, T H., Young, H. S. and Park, K. Y. · Antitumor effects of selected medicinal plant components to implanted Sarcoma 180 in the mouse. *J. Korean Cancer Association*, **23**, 197(1991)
3. Hiroyuki, N., Hideomi, A., Koichi. A., Shinziro, H. and Kazutosi, N. : Studies on the antitumor activity of marine algae. *Nippon Suisan Gakkaishi*, **55**, 1259(1989)
4. Hiroyuki, N., Hideomi. A., Koichi, A.. Shinziro, H. and Kazutosi. N. · Antitumor activity of polysaccharides and lipids from marine algae. *Nippon Suisan Gakkaishi*, **55**, 1265(1989)
5. Yamamoto, I., Nagumo, T., Yagi, K., Tominaga, H. and Aoki, M. · Antitumor effect of extracts from Sargassum and Laminaria. *Japan J. Exp. Med*, **44**, 543(1974)
6. Michio, F., Noriko, I., Yamamoto. I. and Nagumo, T. : Purification and chemical and physical characterization of an antitumor polysaccharide from the brown seaweed *Sargassum fulvellum*. *Carbohydate Research*, **125**, 97(1984)
7. Terukazu, N., Noriko, I., Michio, F.. Junko, H., Kanki, K. and Iwao, U. · Separation of sulfated, fucose-containing polysaccharides from the brown seaweed *Sargassum kjellmanianum* and their heterogeneity and antitumor activity. *Kitasato Arch. Exp. Med.*, **61**, 59(1988)
8. Choi, J. S., Lee. J. H., Kim. H. G., Young, H. S. and Mun, S. I. · Screening for antioxidant activity of plants and marine algae and its active principles from *Prunus davidiana*. *Kor. J. Pharmacogn.*, **24**, 299(1993)
9. Lee. J. H., Park, J. C. and Choi, J. S. : The antioxidant activity of *Ecklonia stolonifera*. *Arch. Pharm. Res.*, **19**, 223(1996)
10. Shiraki, M.. Hara, Y., Osawa, T., Kumon, H., Nakayama, T. and Kawakishi, S. : Antioxidative and antimutagenic effects of theaflavins from blacktea. *Mut. Res.*, **323**, 29(1994)
11. Kurata, K., Taniguchi. K., Shiraishi, K.. Hayama, N., Tanaka, I. and Suzuki, M. : Ecklonialactone-A and -B, two unusual metabolites from the brown algae *Ecklonia stolonifera* Okamura. *Chemistry Letters*, p.267(1989)
12. Kurata, K., Taniguchi, K., Shiraishi, K. and Suzuki, M. : Ecklonialactones-C-F from the brown alga *Ecklonia stolonifera*. *Phytochemistry*, **33**, 155(1993)
13. Taniguchi, K., Kurata, K. and Suzuki, M. : Feeding-deterrent effect of phlorotannins from the brown alga *Ecklonia stolonifera* against the abalone *Haliotis discus* hannai. *Nippon Suisan Gakkaishi*, **57**, 2065(1991)
14. Taniguchi, K.. Kurata, K. and Suzuki, M. : Feeding-deterrent activity of some Laminariaceous brown algae against the Ezo-abalone. *Nippon Suisan Gakkaishi*, **58**, 577(1992)
15. Lee. Y S., Kim, D. S., Ryu, B. H. and Lee, S. H. : Antitumor and immunomodulating effects of seaweeds toward Sarcoma-180 cell. *J. Korean Soc. Food Nutr.*, **21**, 544(1992)
16. Ishidate, M. and Sofuni, T. : The *in vitro* chromosomal aberration test using Chinese hamster lung(CHL) cells in culture. *Progerss in Mutation Research*, **5**, 427(1985)
17. Ryu, J. C., Lee, S. Y., Kim, K. R. and Park, J. S. · Evaluaton of the genetic toxicity of synthetic chemicals (I). Chromosome aberration test on Chinese hamster lung cells *in vitro*. *Environmental Mutagens & Carcinogens*, **14**, 138(1994)
18. Yang, S. K., McCourt, D. W., Roller, P. P. and Gelboin, H. V. : Enzymatic conversion of benzo(a)pyrene leading predominantly to the diol epoxide through a single enantiomer. *Proc. Natl. Acad. Sci.*, **73**, 2594(1976)
19 Sims, P., Grover. P. P., Swaisland, A., Pal, K. and Hewer, A. : Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide. *Nature*, **252**. 326(1974)
20. Wood, A. W., Sayer. J. M., Newmark, H. L., Yagi. H., Michaud, D. P., Jerina, D. M. and Conney, A. H. : Mechanism of the inhibition of mutagenicity of a benzo(a) pyrene 7,8-diol-9,10-epoxide by riboflavin 5'-phosphate. *Proc. Natl. Acad. Sci.*, **79**, 5122(1982)
21. Huang, M. T., Wood, A. W., Newmark, H. L., Sayer, J. M., Yagi, H., Jerina, D. M. and Conney, A. H. : Inhibition of the mutagenicity of bay region diol-epoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids. *Carcinogenesis*, **4**, 1631(1983)

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