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Age-related Increase of Sister Chromatid Exchange Frequency in Bone Marrow Cells of Senescence Accelerated Mouse and Its Inhibition by Chronic Treatment of Ginseng

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***ABSTRACT** : Age-related change in the frequency of spontaneous sister chromatid exchange (SCE) and chromosomal aberrations were investigated in bone marrow cells of accelerated senescence-resistant mice (SAM R1) and senescence accelerated ones (SAM P1). And the effect of chronic treatment of ginseng extract (Panax ginseng C.A. Meyer) on these chromosomal abnormalities was tested in SAM P1. SCE frequency in the cells was progressively increased with age in both mice, but it was consistently higher in SAM P1 than in SAM R1 at all corresponding age. Chromosomal aberrations were, however, not significantly changed with age except that it was slightly increased in only aged SAM P1. Interestingly, the rate of these genetic instabilities in SAM P1 was remarkably retarded by long-term administration of ginseng water extract (0.05% in drinking water). These results suggest that frequency of spontaneous SCE in bone marrow cells increase in parallel with senescence of the mice, and SAM P1 is in the condition of being more exposed than SAM R1 to DNA damaging factors. These also indicate that long-term treatment of ginseng may reduce the genetic damage.*

***Key Words** : Aging, Senescence accelerated mouse, Bone marrow cells, SCE, Ginseng*

I. INTRODUCTION

It is generally accepted that DNA damage such as single strand breaks or chromosomal lesions is increased with advancing age and the accumulation of altered DNA leads to genetic disorders such as chromosome aberrations (Schneider *et al.*, 1982; Ghosh *et al.*, 1989; Park *et al.*, 1992). In normal condition, most of the damaged DNA are repaired by various repair mechanisms, but a part of unrepaired or misrepaired DNA causes to induce sister chromatid exchange (SCE). SCE is the result that two chromatid arms of a chromosome are interchanged, and oxygen free radicals seem to be involved in arising this abnormality (Tayama and Nakagawa, 1994). Although biological significance of the exchange is still poorly understood, SCE has been accepted as a sensitive indicator of cellular response to DNA damage induced by a wide variety of mutagens and carcinogens, and the change in the frequency seems to be linked to the incidence of cytogenetic diseases (Popp *et al.*, 1994; Sardas *et al.*, 1994). Most cross sectional studies have showed that spontaneous chromosomal abnormalities in human lymphocytes are increased with age, on the contrary, their repairing capability is declined (Ames, 1989). However, age-related alteration in the frequency of SCE and chromosomal aberrations was inconsistent according to cells observed (Schneider *et al.*, 1982; Ghosh *et al.*, 1989; Ghosh *et al.*, 1991; Park *et al.*, 1992; Lazutka *et al.*, 1994).

The senescence accelerated mouse (SAM) is a unique animal model for studying aging, which was established by Takeda *et al.* (1981). The SAM consists of two strains, one is accelerated senescence-resistant (SAM R) which is the control strain that undergoes the normal process of aging. The other is senescence accelerated mouse (SAM P) which has a mean life span of about 12 months

and shows various accelerated changes including cataracts (Hosokawa *et al.*, 1988), amyloidosis (Takeshita *et al.*, 1982), and spontaneous neoplasia (Takeda *et al.*, 1981). Cytogenetic deficit in this senescence prone strain might be a factor responsible for the accelerated aging, however, little is known about it.

Recently, many peoples in oriental countries have been popularly used herbal medicines for the purpose of prevention or cure of degenerative diseases. Korean ginseng (*Panax ginseng* C.A. Meyer) is a representative one which has been known for thousands of years as a mysterious folk medicine. It is also utilizing as an indispensable raw materials for various prescriptions. Ginseng revealed to have various pharmacological efficacies through clinical and animal studies (Lee, 1992). Especially, it enhances synthesis of DNA and mitosis of lymphocytes, and delays growth of tumor cells (Fulder, 1977; Odashima *et al.*, 1985; Oura, 1988; Riklis *et al.*, 1988; Kim and Choi, 1992; Saita *et al.*, 1994). The mechanism of such beneficial actions of ginseng on cytogenetic activity was not clearly elucidated. However, its antioxidant activity which was demonstrated in various models for oxidative stress (Lee *et al.*, 1993; Saita *et al.*, 1994; Lee *et al.*, 1995 a,b) would be a candidator of the effect. Because the incidence of most degenerative diseases including cancer is closely related to free radical reaction (Adelman *et al.*, 1988; Ames, 1989).

In this study, we investigated the change in SCE frequency and chromosomal aberrations in bone marrow cells from SAM mice with age to manifest its age-related alteration and the effect of long term administration of ginseng extract on the rate of these cytogenetic abnormalities in the senescence prone strain.

II. MATERIALS AND METHODS

1. Materials

L-Glutamate, sodium bicarbonate, colcemide, heparin and Hoechest 33258 were purchased from Sigma Chemicals Co., and fetal bovine serum (FBS), RPMI 1640 medium, penicillin, streptomycin and 5-bromo-2-deoxyuridine (Brdu) were purchased from Gibco Laboratories, NY. Other chemicals were of the first grade.

2. Preparation of ginseng extract

Korean red ginseng (roots of 6 years old) produced by Korea Tobacco and Ginseng Corporation was used. The extract was made from ginseng powder (30-40 mesh) by soaking it for 4 hrs in 5 volume of hot water. Temperature of water was maintained at 70°C to prevent saponins and other phenolic compounds from being destroyed by heat. This procedure was repeated more than twice and the extract was pooled. The extract concentrated by using a vacuum evaporator was characterized by HPLC, which revealed to contain 36% moisture and 7% saponins. The major saponins present in the extract were composed of ginsenoside-Ro (0.09%), -Ra (0.06), -Rb_1 (0.32%), -Rb_2 (0.01%), -Rc (0.18%), -Rd (0.22%), -Re (0.08%), -Rf (0.05%), -Rg_1 (0.15%), and -Rg_2 (0.82%).

3. Animals

Accelerated-senescence resistant mouse (SAM R1) and senescence accelerated one (SAM P1) were kindly provided by professor Toshio Takeda of Kyoto University in Japan and bred in the animal facility of Korea Ginseng and Tobacco Research Institute. Only male mice were used for this study and reared in a clean conventional system under condition of a 12 hr light and dark cycle at 200-300 lux, at 20±2°C in temperature, and 40-60% in humidity.

Our experiments were designed for two aims. One is to compare the rate of SCE frequency and chromosome aberrations between SAM R1 and SAM P1 with age and the other is to find the effect of ginseng on such DNA damage. All mice were received chow and water *ad libitum*. To investigate the effect of ginseng, we used 16 animals of SAM P-1 which had been freely accessed water extract of ginseng (0.05%) with drinking water from 6 weeks of age until all experiments has been completed. Control mice were given only drinking water. The ginseng solution and water were replaced everyday.

4. Harvest and Culture of Bone Marrow Cells

Bone marrow was removed from femurs of mice at the indicated ages in a clean bench by washing with RPMI 1640 (5% FBS) culture medium. Cells were collected by centrifugation at 600×g for 5 mins. Following removal of the supernatant, cells were resuspended in a RPMI 1640 medium (10% FBS) and pelleted again by centrifugation. The harvested bone marrow cells were cultured in RPMI 1640 medium containing 10% heat-inactivated FBS, penicillin (100 U/ml), streptomycin (100 μg/mg), L-glutamine (0.3 mg/ml). Cells (2.1×10^6 cells per flask) were incubated at 37°C for 72 hrs.

5. Staining and Scoring

Differential staining was achieved by a slight modification of previously described (Julian *et al.*, 1987). Briefly, after 48 hrs of incubation at 37°C, 5-Brdu (8 μg/ml) was added for an additional 24 hrs incubation in the dark in a 5% CO_2 atmosphere. Cells were harvested by adding colcemide (0.1 μg/ml) for the final 2 hrs of incubation, and washed three times with Hank's balanced salt solution (HBSS). The cells were exposed to a hypotonic solution (75 mM KCl) for 15 mins and then fixed in freshly prepared methanol: glacial acetic acid (3 : 1, v/v) at ambient temperature. Air-dried slides were prepared and aged for 3 days. And then slides were finally stained in Hoechest 33258 solution and 4% Gimsa solution for the detection of chromosomal abnormalities. The frequency of SCE and chromosomal aberrations per metaphase was scored.

III. RESULTS AND DISCUSSION

1. Age-related Change of SCE Frequency in Bone Marrow Cells

The first aim of this study was to elucidate the age-related change in SCE frequency and chromosomal aberrations in bone marrow cells. Data in Table 1 compare the rate of SCE frequency in SAM R1 and SAM P1 in relation to age. The rate of spontaneous SCE frequency in SAM R1 was gradually increased with the increasing age, and in both groups, old mice (over 12 monthsold) showed higher SCE frequencies than young ones (3 month old) ($P < 0.05$). SAM P1 also showed a similar age trend in the frequency. But they have maintained consistently a higher value than SAM R1 at all corresponding ages, although there was no significant difference. The increase in the frequency of SAM P1, was more rapid than that of SAM R1. The data indicate that SCE frequency in bone marrow cells of SAM mice is clearly increased with the senescence.

Table 1. Age-related increase of SCE frequency in bone marrow cells from SAM R1 and SAM P1

Age (months)	SAM R1	SAM P1
3	3.80±0.28	3.91±0.29
6	4.42±0.34	5.96±0.43
12	5.22±0.48	6.53±0.43
15	5.30±0.28	7.58±0.48*
18	6.15±0.52*	
24	5.87±0.37*	

Data were expressed as frequency per metaphase. One hundred clear metaphases spreaded were scored to determine the mean SCE frequency (Data: Mean±SEM). * Significantly different from 3 months old mice in the same group ($p < 0.05$).

The age-related alteration in SCE in lymphocytes has been intensively studied by Ghosh *et al.* (1989, 1991) and others (Park *et al.*, 1992; Lazutka *et al.*, 1994). The rate of spontaneous SCE in peripheral lymphocytes of human was increased with age (Dearce, 1981; Franceshi *et al.*, 1992); Park *et al.*, 1992). In this study we demonstrated that the incidence of SCE frequency in a dividing cell also appears in parallel with the aging process as in a cell completed differentiation.

Chromosomal aberrations are also considered as an index of genomic instability, which have been described in a numerous heritable human diseases such as Bloom's syndrome that are characterized by a putative DNA repair defect and a predisposition to the development of neoplasia (Schneider, 1982; Hecht and Hecht, 1988; Illeni *et al.*, 1991). Chromosomal aberrations are classified into five types including breaks, gap, and ring so on. An age related increase in this parameter has been reported even if contradictory data exist in the literature (Nakanishi *et al.*, 1979; Martin *et al.*, 1985; Ghosh *et al.*, 1991). In this study, no significant change was found between strains or with age (Table 2). Dicentric and acentric a-

Table 2. Age-related change in chromosomal aberration in bone marrow cells from SAM R1 and SAM P1 and its modulation by chronic ginseng treatment

Age (months)	Frequency of chromosomal aberration/cell		
	SAM R1	SAM P1	SAM P1-GIN*
3	0.86±0.77	0.85±0.77	0.45±0.55
6	0.81±0.72	0.78±0.69	-
12	0.80±0.63	1.13±0.60	0.63±0.63
15	0.85±0.73	1.05±0.80	0.78±0.78
18	0.90±0.68	-	-
24	0.72±0.67	-	-

*: Ginseng treatment. No significant differences between age or groups.

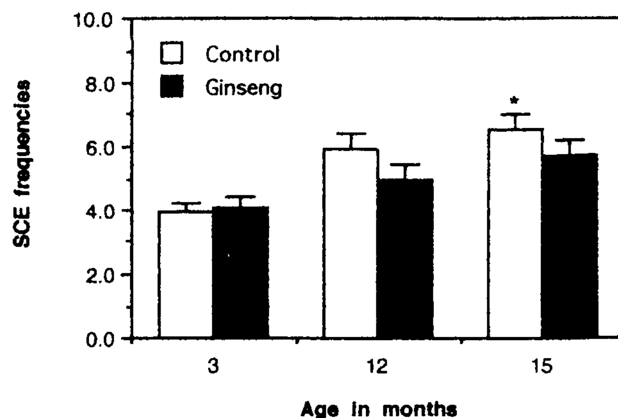

Fig. 1. The effect of long-term administration of ginseng extract on the rate of SCE in bone marrow cells from SAM P1. Values were the number of SCE frequencies± SEM counted in one hundred metaphases from mice of each group. *Significantly different from 3 months old mice in the control group ($p < 0.05$).

berrations were also not observed. However, the number of chromatid breaks was few and that of chromatid gap was prevalent in both SAM P1 and SAM R1. Although the rate of chromosomal aberrations per cell was not changed with age, it was somewhat high in animals over 12 months of age in SAM P1 when compared to that in young or age matched SAM R1. The observation in bone marrow cells shows that genetic instability was clearly related to aging process. Furthermore, it may be an evidence showing that elders have more probability to get genetic disorders.

Most physiological functions such as detoxification, antioxidant defense and DNA repairing capability are declined with the senescence (Chetsanga *et al.*, 1995; Lee, *et al.*, 1992). There are many evidences that reactive oxygen free radicals causes to induce various types of DNA damage (Ames, 1987; Halliwell, 1993; Tayama and Nakagawa, 1994). This cytotoxic event might be caused from the decline of repairing ability or antioxidant capacity of organisms. Senescence prone strains have relatively less antioxidant defense capability when compared to accelerated-senescence resistant ones (Nomura *et al.*, 1989; Liu and Mori, 1993). Therefore, the increased frequency in SCE and chromosomal aberrations in especially aged SAM P1 might be related to the increased status of oxidative stress *in vivo*.

2. Effect of Ginseng Extract on the Frequency of SCE

As mentioned previously, because of ginseng has been known to have anticancer, antitumor and antioxidant actions (Yamamoto *et al.*, 1990; Park *et al.*, 1992; Lee *et al.*, 1993; Lee *et al.*, 1995a) and such effects were more prevalent when animals were treated for long term (Lee *et al.*, 1993; Lee, *et al.*, 1995a, b), we investigated whether long-term administration of ginseng suppresses the spontaneously raised SCE and chromosomal aberrations in SAM P1 strain.

Interestingly, the rate of SCE frequency was much lower in the ginseng-treated mice than in the untreated ones, although it showed a similar value in mice of 3 months old (Fig. 1). Frequency of chromosomal aberrations also showed less increase in mice treated with ginseng extract chronically (Table 2). Although there was no significance, these data show clearly that long term ginseng treatment can retard the DNA damage occurring naturally with advancing age. It is not clear how ginseng can protect chromosomes from the damage raised spontaneously. Yamamoto *et al.* (1990) demonstrated that panaxytriol, a ginseng saponin, significantly delays the development of tumor mass in mice. Saita *et al.* (1994) observed the same effect in cell culture system using MK 1 cell line. In their studies, polyacetylene revealed to protect cell membrane from oxidative damage. These results suggest that such a protective effect of ginseng seems to be resulted from its antioxidant ac-

tion.

In conclusion, our result suggest that SCE frequency in bone marrow cells is increased age dependently, and SAM P1 is in the condition of being more exposed than SAM R1 to DNA damaging factors, and that long-term administration of ginseng may be beneficial for the reduction of genetic instability.

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