The In Vitro Effect of β -Carotene and Mitomycin C on SCE Frequency in Down's Syndrome Lymphocyte Cultures

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— Down's syndrome (DS) has the highest incidence among chromosomal disorders and is a predisposing factor in acute leukemia pathogenesis. DS patients are sensitive to both physical and chemical inducers at the DNA level. Studies on β -carotene, an antioxidant, suggested that there is a relationship between high β -carotene diet and reduced tumor incidence in humans indicating that β -carotene is a chemopreventive agent against cancer. Sister chromatid exchange (SCE) is known as a sensitive parameter among the genotoxicity tests. In this study, we aimed to investigate the in vitro effect of β -carotene on SCE frequencies in 7 DS patients and 7 healthy controls aged between 0-16 years. A direct leukomogenic agent Mitomycin-C (MMC) was used as a powerful SCE inducer. Addition of MMC to the cultures alone resulted in a significant enhancement of SCE frequencies in both groups when compared to the spontaneous values. In the study, β -carotene seemed to decrease MMC induced mean SCE/cell values, but did not have an effect on unstimulated cells. As this is a limited study, it is hard to conclude that β -carotene is a chemopreventive agent in DS patients, although our results seem to support other investigators' reports. ——— Down's syndrome; sister chromatid exchange; mitomycin C; β -carotene © 1998 Tohoku University Medical Press

Down's syndrome (DS) is the most common chromosomal abnormality in man (Thompson et al. 1991). It is one of the commonest causes of mental retardation and its frequency is 1 in 700 live births (Shubber et al. 1991). Bone marrow cells of DS patients are highly sensitive to physical and chemical mutagens including UV or x-ray irradiation and viral infections (Major et al. 1985).

Clinical and experimental findings of the syndrome reported to date suggest

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that the chromosome disorder may be associated with defective DNA repair and predisposition to the development of leukemia in DS patients is well known (Aldenhoff et al. 1980; Heidemann et al. 1985).

Sister chromatid exchange (SCE), which occurs spontaneously in proliferating cells, is known among the mutagenicity tests and is a manifestation of damage to the genome (Latt et al. 1981). It has been shown to be a highly sensitive parameter for the monitoring of mutagenic and/or carcinogenic effects (Perry and Evans 1975). Carcinogen induced DNA damage, DNA repair and SCE are significant events during the initiation stage of carcinogenesis (Popescu et al. 1984).

Mitomycin C (MMC), a leukomogenic teratogen, acts as a cross-linking and bifunctional alkylating agent and is a powerful SCE inducer (Shubber et al. 1991). In this study for the induction of SCE, MMC was added to the cultures as the chemical inducer.

β -Carotene is an efficient antioxidant which quenches singlet oxygen and is accepted to be a preventive agent, particularly in chemical carcinogenesis (Manoharan and Banerjee 1985; Weitberg et al. 1985). In the present study, the in vitro effect of β -carotene on SCE frequencies induced with MMC as a chemical inducer was investigated.

MATERIALS AND METHODS

Patients

Peripheral blood samples obtained from 7 DS patients (3 boys and 4 girls) and 7 healthy control children (2 boys and 5 girls) were karyotyped. Both groups were aged between 0–16 years. Lymphocyte cultures were prepared both for cytogenetic analysis and SCE differentiation from the samples.

SCE differentiation

Sterile heparinized venous blood samples were obtained and 72 hours lymphocyte cultures were set up in 5 ml of culture medium consisting of RPMI 1640 (Seromed, Berlin, Germany) as the basal medium supplemented with 20% fetal calf serum (Seromed), 1.5% phytohemagglutinin (Seromed), 1% 200 mM L-glutamine (Seromed) and 100 U/ml penicillin, 100 μ g/ml streptomycin (Seromed). At the 24th hour, 5-bromo-2'-deoxyuridine (Sigma, St. Louis, MO, states USA) was added to the cultures at the final concentration of 10 mg/ml. The cultures were wrapped in aluminum foil and stored at 37°C in dark.

MMC (Sigma) and β -carotene (Sigma) were added to the cultures one at a time and together to the final concentrations of 10^{-7} M and 10^{-6} M, respectively, at the 48th hour of incubation. Five μ l of 99% ethanol was added to a separate culture tube as the solvent control (Ekmekçi et al. 1995).

The cultures were harvested at the 72nd hour after a 2 hour incubation period

with 0.1 mg/ml of colchicine (0.5 mg/ml, Seromed). Cells were treated with hypotonic potassium chloride (KCl) solution (0.075 M) at 37°C for 15 minutes, fixed in a fresh fixative of 3:1 methanol/glacial acetic acid and washed three times in fresh and cold fixative. Chromosome spreads were prepared on ice cold slides and differential SCE staining was performed by the slightly modified procedure of Wolff and Perry (Wolff and Perry 1974).

SCE's were counted by scoring at points of discontinuity in staining and reported as the mean number of exchanges per metaphase after at least 30 metaphases per data point were counted with at least 44 chromosomes. Metaphases were counted in a coded fashion without the scorer knowing the nature of the experimental incubation.

Cytogenetic studies

Conventional peripheral blood lymphocyte cultures were set up and Giemsa-trypsin (GTG) banding was performed in order to confirm DS diagnosis in patients and detect the chromosomal construction in the control group (Verma and Babu 1989a).

Statistical analysis

SPSS for Windows, release 6.0 (Microsoft, Redmond, WA, USA) was used for statistical evaluations. Mean values of independent groups were compared with student *t*-test.

RESULTS

According to the cytogenetic analysis, 2 of the 7 DS patients had 46, XX, t (21q, 21q) karyotypes, whereas 3 of them had 47, XY, +21 and 2 of them had 47, XX, +21 karyotypes. In the control group, consisting of 7 healthy children, 2 karyotypes were 46, XY and 5 were 46, XX.

Our results show that the mean SCE frequency was 6.36 ± 1.12 in DS patients ($n=7$) and 5.60 ± 0.65 in the control group ($n=7$). The difference between these two values was not significant ($p>0.05$) (Table 1). The ethanol, applied in experiments as a solvent, had no significant effect on the frequencies of spontaneous SCEs in all experiments ($p>0.05$) (Table 1).

The addition of MMC alone gave a significant enhancement of the frequencies of SCEs in DS and control groups ($p<0.001$) (Table 1). In the control group, when compared to spontaneous SCEs, when MMC was added to the cultures, mean SCE/cell significantly increased to 10.86 ± 2.49 from the spontaneous SCE value of 5.60 ± 0.65 ($p<0.001$) (Table 1). When the cell cultures were induced with MMC, the mean SCE/cell frequencies of DS patients were found to be significantly higher ($p<0.05$) than the MMC induced control group (Table 1). Addition of MMC and β -carotene together to the cultures decreased the mean SCE/cell values in both groups when compared with the MMC-induced SCE frequencies. When

TABLE 1. *Comparison of the mean SCE/cell of the DS patients and the normal control group in lymphocyte cultures*

Group and type of treatment	Mean SCE ($\pm$ s.d.) per cell		
	Down's syndrome	Normal	<i>p</i>
Spontaneous SCE	6.36 ± 1.12	5.60 ± 0.65	>0.05
Mitomycin-C	$17.36 \pm 5.61^{***}$	$10.86 \pm 2.49^{***}$	<0.05
Mitomycin-C + β -Carotene	$11.07 \pm 2.69^{**}$	$8.03 \pm 2.25^*$	<0.05
β -Carotene	5.86 ± 1.35	4.91 ± 0.89	>0.05
Ethanol	5.91 ± 1.042	5.74 ± 0.75	>0.05

The spontaneous mean SCE values with the values found after addition of various chemicals in normal controls and Down's syndrome patients are compared

Comparison of the spontaneous mean SCE/cell values with the values found after addition of chemicals for each group of patients indicated with '*' signs.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

the MMC and β -carotene added cultures were compared in the two groups, the mean SCE/cell was significantly higher ($p < 0.05$) in the DS patients (Table 1).

When β -carotene alone was added to the cultures, there was not a significant difference from the spontaneous SCE values in both groups ($p > 0.05$) (Table 1). Also, when the mean SCE/cell values were compared in β -carotene added cultures, we couldn't find a significant difference ($p > 0.05$) between the experimental and the control groups (Table 1).

DISCUSSION

As a mutagenicity test and a DNA damage indicator, SCE has a mean number of 5–8 in a normal proliferating cell (Verma and Babu 1989b). Although SCE frequencies were reported to be elevated in syndromes like Bloom's syndrome, acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), its frequencies in DS patients is still controversial (Shubber et al. 1991). Many conflicting results were reported in recent years (Heidemann et al. 1985; Shubber et al. 1991). In our study, regarding uninduced SCE frequencies of lymphocyte cultures of Down's patients and the control group, no significant difference was observed between the two groups ($p > 0.05$). These results are similar to those of Yu and Borgaonkar (1977).

MMC as a leukomogenic chemical inducer was added to the cultures to investigate the SCE response of DS and control leukocytes to DNA damaging agents. After the 24 hour exposure time to MMC, SCE frequencies were found to have a 2–3 fold increase in both groups. These findings were similar to the previously reported results of another study carried in our laboratories (Ekmekçi et al. 1995). An interesting finding was that mean SCE/cell had a significantly

higher increase in DS patients compared to the MMC induced controls ($p < 0.05$) which supports the hypothesis that DS patients have a sensitivity to chemical and physical inducers (Major et al. 1985).

In this study, β -carotene as an antioxidant is used in order to inhibit DNA damage, thereby to reduce SCE frequencies. β -Carotene quenches singlet oxygen and can function in the lipid phase as a chain breaking antioxidant by neutralizing peroxyradicals (Burton and Ingold 1984). Coincubation of eucaryotic cells with β -carotene prior to or during exposure to activated phagocytes or to a cell free, oxidant generating system prevented oxidant induced chromosomal damage, and a biologic role for this agent in cytoprotection against phagocyte-derived oxidants has been proposed (Weitberg et al. 1985).

According to our results, addition of MMC and β -carotene together to the cultures, decreased the mean SCE/cell values in DS patients when compared with the MMC induced SCE frequencies ($p < 0.05$). When only β -carotene was added to the cultures, mean SCE/cell values did not show a significant decrease when compared to the spontaneous SCE values ($p > 0.05$). This finding is consistent with the idea that β -carotene prevents chromosomal damage in the activated cells (Manoharan and Banerjee 1985). It did not have an effect on the unstimulated cells in this study.

The properties of β -carotene as an efficient antioxidant and its ability to trap organic free radical molecules are also likely to be conducive to its role as a preventive agent, particularly in chemical carcinogenesis (Manoharan and Banerjee 1985). β -Carotene also provides some additional advantages in that unlike the retinoids (vitamin A analogues), excess dietary β -carotene does not cause an adverse systemic effect, making it a more desirable dietary prophylactic agent (Manoharan and Banerjee 1985; Richards et al. 1990). It is proposed that β -carotene acts as a preventive agent at the initiation stage of carcinogenesis by its ability for trapping the organic free radicals and consequently reducing DNA alkylation caused by the electrophilic molecules. It can be regarded as a potential dietary chemopreventive agent (Manoharan and Banerjee 1985).

In our study β -carotene was found to be effective in vitro on reducing the chemically induced SCE frequencies in DS patients. DS patients are sensitive to both physical and chemical inducers at the DNA level, as we stated above. Although this is a limited study with a small group of patients and controls, our results seem to support the other investigators' reports.

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