EFFECTS OF ANTIOXIDANTS ON MITOMYCIN C- INDUCED SISTER CHROMATID EXCHANGES AND MICRONUCLEI

Antioksidanların, Mitomisin C'nin indüklediği kardeş kromatid değişimleri ve mikronükleuslar üzerine etkileri

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Abstract

Purpose: *This study was designed to investigate the effects of antioxidants on mitomycin C (MMC) induced sister chromatid exchanges (SCE) and micronuclei (MN).*

Material and Methods: *The action of vitamins C (10^{-4} M), E (10^{-6} M) and ß-carotene (10^{-6} M) on the genotoxic activity of mitomycin C (10^{-7} M) was evaluated in cultured human lymphocytes of five donors with the help of SCE and MN frequencies.*

Results: *It was found that SCE and MN levels were significantly increased by MMC. The presence of vitamins C, E and ß-carotene caused a significant reduction in the numbers of SCE and MN induced by MMC when used separately, but no antioxidant effect of ß-carotene was observed in MMC-induced MN frequency. On the other hand, in triple combinations of vitamins C, E and ß-carotene and MMC, MMC-induced SCE and MN frequencies were reduced significantly (22.02 % - 24.04 % for SCEs and 31.91 % - 57.55 % for MN).*

Conclusion: *These results indicated that the combinations of vitamins C, E and ß-carotene can modify the DNA damaging effect of the carcinogens and act as biological antioxidants under in vitro conditions.*

Key Words: *Ascorbic acid, Beta carotene, Micronuclei, Sister chromatid exchange, Vitamin E*

Özet

Amaç: *Bu çalışmada mitomisin C'nin (MMC) indüklediği kardeş kromatid değişimleri (KKD) ve mikronukleuslar (MN) üzerine antioksidantların rolü araştırılmıştır.*

Gereç ve Yöntem: *Beş kisinin kültüre edilmis insan lenfositlerinde KKD ve MN frekansları kullanılarak mitomisin C'nin (10^{-7} M) genotoksik aktivitesi üzerine vitamin C (10^{-4} M), E (10^{-6} M) ve ß-karotenin (10^{-6} M) etkileri değerlendirildi.*

Bulgular: *KKD ve MN degerlerinin MMC ile önemli derecede arttığı bulundu. Vitamin C, E ve ß-karoten tek başlarına kullanıldığında MMC ile artan KKD ve MN değerlerinde önemli oranda azalmaya sebep oldu. Ancak ß-karotenin antioksidant etkisi, MMC'nin artırdığı MN frekansında gözlenemedi. Ayrıca, vitamin C, E ve ß-karoten ve MMC'den oluşan üçlü kombinasyonlarda, MMC'nin artırdığı KKD ve MN frekansları önemli oranda azaldı (KKD için: % 22.02-% 24.04 ve MN için: % 31.91-% 57.55).*

Sonuç: *Vitamin C, E ve ß-karoten kombinasyonları, karsinojenlerin DNA hasarlayıcı etkilerini azaltabilir ve in vitro koşullarda biyolojik antioksidant olarak rol oynayabilir.*

Anahtar Kelimeler: *Askorbik asit, Beta-karoten, Kardes kromatid değişimi, Mikronukleus, Vitamin E*

The sister-chromatid exchange (SCE) and micronucleus (MN) assays on lymphocytes of mammalian species are sensitive cytological methods that is often used to evaluate exposure to mutagens and carcinogens (1,2). Antioxidant nutrients such as L-ascorbic acid (vitamin C), α-tocopherol (vitamin E) and ß-carotene are thought to give protection against the development of cancer at various sites (3,4).

Carcinogen induced DNA damage, DNA repair, SCE and MN are significant events during the initiation stage of carcinogenesis (5,6). Many studies suggest that antioxidant nutrients inhibit

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chromosomal damage, tumor promotion, cell transformation, and cancer induced chemical carcinogens or radiation (6-11). The research reported here was performed to determine the effects of ascorbic acid, ß-carotene and vitamin E on SCEs and MN induced by mitomycin C (MMC) in in vitro conditions in peripheral lymphocytes from healthy humans.

MATERIALS AND METHODS

Lymphocyte Culture

Heparinized blood samples were obtained and cultured from five healthy non-smoking individuals (3 females and 2 males) aged 25-30 yr, none of whom were known to be receiving drugs for any medical or other reasons. Whole blood, 0.4 ml was added to 5 ml of the culture medium F-10 HAM (Sigma) which was supplemented with 20 % fetal calf serum (Gibco), 1.5 % phytohemagglutinin (PHA) (Sigma) and 2 mM L-glutamine.

MMC (10^{-7} M, Sigma), ascorbic acid (10^{-4} M, Sigma), ß-carotene (10^{-6} M, Sigma) and α-tocopherol (10^{-6} M, Sigma) were each in turn added to cultures at 24 h of the incubation for SCE and at the beginning of the incubation for MN. MMC and α-tocopherol were dissolved in ethanol.

Sister-chromatid Exchange Assay

Frequency of SCEs in lymphocytes was assessed according to the method originally developed by Perry and Wolff (12). Bromodeoxyuridine (Brdu) (Sigma) was added to the cultures at the beginning of the culture (final concentration 10^{-4} M). Cells were harvested at the end of 72 h and 0.1 µg/ml colcemide was added 2 h before harvesting. The whole incubation was performed at 37 °C in a 5 % CO_2 atmosphere (in the dark). The cells were collected by centrifugation, treated for 20 min with 0.075 M potassium chloride and fixed with glacial acetic acid: ethanol (1:3). The cell suspensions were dropped on cold slides, dried at 37 °C for 24 h and stained by a modified fluorescence plus Giemsa method (12). The analysis of each sample was appropriately blinded. An average of 30 complete and well-spread metaphases were scored for each culture.

Micronucleus Test

Cytochalasin B (Cyt-B, Sigma) was made up as a stock solution in dimethyl sulfoxide (DMSO) at a concentration of 2 mg/ml and stored at -70 °C. 44 h after the start of culture, an aliquot of stock solution of cyt-B was first diluted in the culture medium and then added to the lymphocyte PHA stimulated cultures at a final concentration of 3 µg/ml, according to the method of Fenech and Morley (2). The cultures were stopped at 72 h, treated with hypotonic solution (0.1 mol KCL/L) by the method of Balasem and Ali (13) for 3 min and fixed in two changes of methanol-acetic acid (3:1). The cells were spread onto glass slides and stained with 5 % Giemsa for 6 min. The slides were coded and scored blind at 1000 x magnification. At least 500 binucleated cells with preserved cytoplasm were scored for each culture. For the identification of micronuclei published criteria were applied (14).

Statistical Analysis

Statistical comparisons on frequency of MN and SCE were made between exposed and control cultures using Student's t-test.

RESULTS

SCE Test

The mean SCE frequencies in the solvent, vitamin C, vitamin E and ß-carotene controls were not significantly different from the negative controls ($p>0.05$). MMC alone generated a significant increase in SCE values compared to controls ($p<0.001$). The presence of vitamins C, E and ß-carotene in the medium during the 48 hr treatment of the cell cultures with MMC caused a significant reduction of SCEs (32.19 %, 21.87 % and 23.38 %, respectively). At the same concentrations, triple combinations of MMC and vitamins C, E and A showed a reduction of SCEs, but MMC + vitamin C + vitamin E group failed to show significant reduction as compared to the MMC-treated group ($p> 0.05$) (Table I).

Micronucleus Test

Table II shows the effects of vitamins C, E and ß-carotene on the mitomycin C-induced micronuclei. The MN frequency was increased by the treatment of MMC from negative control group. This difference was statistically significant ($p<0.001$).

The vitamin C alone caused a small but statistically significant increase in the micronucleus frequency ($p<0.001$). However, both vitamin C and E significantly reduced the micronucleus frequency generated by MMC ($p<0.05$ and $p<0.01$, respectively). MMC induced micronucleus frequency was also reduced significantly (49.64 % and 31.91 %) in the presence of the ß-carotene, except MMC + ß-carotene group. Furthermore, in the triple combinations, the presence of vitamins C, E and ß-carotene in cultures treated with MMC caused a significant reduction of the MN frequency ($p<0.01$).

Table I. The in vitro effects of vitamins C, E and ß-carotene on mitomycin C-induced sister chromatid exchanges

Treatment	n	Mean SCE/cell ± SE	% Reduction of SCE[a]
1.Negative control	5	12.01 ± 0.11	-
2.Solvent control (10 µl)	5	11.87 ± 0.49	-
3.Vitamin C control (10^{-4} M)	5	12.13 ± 0.30	-
4.Vitamin E control (10^{-6} M)	5	11.81 ± 0.22	-
5.ß-carotene control (10^{-6} M)	5	12.21 ± 0.28	-
6.Positive control (MMC) (10^{-7} M)	5	72.85 ± 1.09[b]	-
7.MMC + Vitamin C	5	53.26 ± 0.68[b,c]	32.19
8.MMC + Vitamin E	5	59.54 ± 2.22[b,c]	21.87
9.MMC + ß-carotene	5	58.62 ± 1.38[b,c]	23.38
10.MMC + Vitamin C + ß-carotene	5	59.45 ± 2.40[b,c]	22.02
11.MMC + Vitamin C + Vitamin E	5	59.17 ± 5.68[b]	22.48
12.MMC + Vitamin E + ß-carotene	5	58.22 ± 3.61[b,c]	24.04

[a] $\%\ Reduction\ of\ SCE = \dfrac{(Mean\ SCE\ in\ MMC) - (Mean\ SCE\ in\ MMC + Vitamin\ C/E/\beta\text{-}carotene)}{(Mean\ SCE\ in\ MMC) - (Mean\ SCE\ in\ negative\ control)} \times 100$

[b] *$p<0.001$ when compared with controls*

[c] *$p<0.05$ when compared with MMC-treated group*

Table II. The in vitro effects of vitamins C, E and ß-carotene on mitomycin C-induced micronuclei

Treatment	n	Cells with MN (Mean % ± SE)	% Reduction of MN[a]
1.Negative control	5	1.27 ± 0.10	-
2.Solvent control (10 µl)	5	1.08 ± 0.10	-
3.Vitamin C control (10^{-4} M)	5	2.88 ± 0.24[b]	-
4.Vitamin E control (10^{-6} M)	5	1.57 ± 0.07	-
5.ß-carotene control (10^{-6} M)	5	0.97 ± 0.15	-
6.Positive control (MMC) (10^{-7} M)	5	12.27 ± 0.24[b]	-
7.MMC + Vitamin C	5	8.95 ± 0.98[b,d]	30.18
8.MMC + Vitamin E	5	5.11 ± 0.16[b,e]	65.09
9.MMC + ß-carotene	5	12.15 ± 0.24[b]	1.09
10.MMC + Vitamin C + ß-carotene	5	6.81 ± 0.66[b,e]	49.64
11.MMC + Vitamin C + Vitamin E	5	5.94 ± 0.79[e,e]	57.55
12.MMC + Vitamin E + ß-carotene	5	8.76 ± 0.15[b,e]	31.91

[a] $\%\ Reduction\ of\ MN = \frac{(Mean\ MN\ in\ MMC) - (Mean\ MN\ in\ MMC + Vitamin\ C/E/ß\text{-}carotene)}{(Mean\ MN\ in\ MMC) - (Mean\ MN\ in\ negative\ control)} \times 100$

[b] $p<0.001$ and [c] $p<0.01$ when compared with controls

[d] $p<0.05$ and [e] $p<0.01$ when compared with MMC-treated group

DISCUSSION

It has been shown previously that the antioxidant effects of ascorbic acid and a-tocopherol are responsible for the protection of the cultured rat embryos from the damaging effects of xanthine and xanthine oxidase (15). The results of the present study show that the SCE and MN frequencies can be induced in cultured human lymphocytes by MMC and confirm that the antioxidant vitamins C, E and ß-carotene can reduce such effects. Comparable anticlastogenic effects of vitamins C and E on the chromosome-damaging action of carcinogens were reported by Shamberger et al. (9) from human lymphocyte cultures.

Combinations of vitamins C, E and ß-carotene significantly reduced SCE and MN frequencies induced by MMC ($p<0.05$ and $p<0.01$, respectively), but in the combination of vitamins C and E the reduction in SCE frequencies (22.48 %) was not statistically significant ($p<0.05$). It has been shown that with different concentrations vitamin C can cause or prevent the SCE rates in cultured human lymphocytes exposed to Thiotepa or L-ethionine (16). In our study, although there was a significant increase in MN frequencies of vitamin C control compared to negative control ($p<0.001$), there was no statistical difference between the SCE frequencies of the negative control and vitamin C control. Similar results were obtained that vitamin C at the same concentration did not increase the SCE frequency in human lymphocyte cells (17). However, we observed that vitamin C significantly reduced both SCE and MN frequencies induced by the MMC.

Manoharan et al. (10) demonstrated that ß-carotene provides a marked protection to the mammary epithelial cells from the SCE inducing action of the carcinogens. It has been reported that cells preincubated with vitamin E and ß-carotene are protected from oxygen radical-induced SCEs (11). In this study, ß-carotene alone did not decrease the

micronucleus frequency induced by MMC, while ß-carotene significantly reduced the sister chromatid exchanges induced by MMC. However, a triple combination of vitamins C, E and ß-carotene and MMC produced a significant reduction of SCE and MN frequencies. Also, Deng et al. (18) demonstrated that combined application of α-tocopherol and ß-carotene inhibited SCE induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in cultured V79 cells, and no protective activity was observed when used separately.

In addition, because lymphocytes are important cells of the immune system, the presence of chromosomal damage in lymphocytes may disturb normal functions of the cells. The protective effects of these vitamins against chromosomal damage in lymphocytes may indicate the maintenance of normal function of lymphocytes.

Although both in vivo and in vitro studies on the anticlastogenic and antimutagenic action of vitamins C, E and ß-carotene have been contributed separately during the last years, no in vitro system on the combined action of these vitamins on both SCE and MN frequencies have been available. It, therefore, should be emphasized that the combinations of the vitamins tended to inhibit SCE and MN frequencies in human lymphocytes induced by carcinogens.

The present data clearly show that the antimutagenic effects of the combinations of these vitamins reduce the action of mitomycin C, and support that antioxidant nutrients inhibit carcinogenic activity of chemical mutagens.

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