Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

ROLE OF ASCORBIC ACID AS AN ANTI OXIDANT IN GASTRIC CANCER PATIENTS IN SOUTH INDIAN POPULATION

MADHURI K [1], VANI.K [1], RABBANI SYED[*2], ALI A. ALSHATWI [3]

Toxicology Lab, Osmania University College for Women, Central Research Institute of Unani Medicine, Hyderabad, Dept. of Food Science and Nutrition, College of Food and Agriculture Sciences King Saud University. Email: rabbanisyd@gmail.com

Received: 23 June 2011, Revised and Accepted: 8 Aug 2011

ABSTRACT

Dietary chemo preventive substances are regarded as being generally safe and some of them may even have efficacy by preventing or reversing premalignant lesions. Vitamin supplementations have marked potentiality against toxic effects of diversified chemicals. Blood samples were collected from 80 human subjects with gastric cancer patients receiving the chemotherapy and , blood samples from 100 healthy individuals were collected as controls for the purpose of comparison Cytogenetic studies were carried out in peripheral blood lymphocytes of study population before and after Vitamin C by adopting standard cytogenetic protocols such as (a) Chromosomal aberrations (CA) (b) Sister chromatid exchanges (SCE). Vitamin C prophylaxis was studied in a group of 80 gastric cancer patients receiving the adjuvant chemotherapy. An increase frequency of Total chromosomal aberrations was observed in the cancer patients receiving chemotherapy as compared to the control group ($p < 0.001$, $t=11.11$) and the mean SCE rate per cell in the cancer patients with out Vitamin C prophylaxis is higher than the cancer patients in comparison to control group ($p<0.001$,$t=32.22$). A statistically significant decrease in total chromosomal aberration and mean SCE was reported among the cancer patients receiving chemotherapy, supplemented with Vitamin C for 3 months when compared to before Vitamin C prophylaxis ($p<0.001$, $t=9.39$) and there is no significant difference found with in the controls after Vitamin C Prophylaxis.

Keywords: Ascorbic acid, Gastric Cancer.

INTRODUCTION

.Gastric cancer, commonly referred to as stomach cancer, can develop in any part of the stomach and may spread throughout the stomach and to other organs; particularly the esophagus, lungs, lymph nodes, and the live (1). Gastric cancer (GC) treatment and prognosis vary in different regions of the world; incidence of the disease, approach to early diagnosis and treatment varies greatly between western and the eastern hemispheres. It is the second leading cause of cancer-related death worldwide (2).

Supplementation of the chemo preventive compounds has been known to be a strategy to protect against oxidation damage and to slow the process of cancer development by intervening in the process of carcinogenesis. These antioxidants protect, prevent or reduce the extent of oxidative destruction of cellular tissues. Elevated levels of lipid peroxidation products and the simultaneous decline of antioxidant defense mechanism has been suggested to be harmful through disruption of membrane lipid and damage of cellular organelles resulting in oxidative stress (3). In recent years, there has been increasing interest in the potential cancer chemo protective properties of diet derive other botanical agents. Several epidemiological evidences suggest that intake of fruits, vegetables and whole grains may reduce the cancer risk and this has been attributed to the foods having rich in bioactive compounds (4). Dietary chemo preventive substances are regarded as being generally safe and some of them may even have efficacy by preventing or reversing premalignant lesions. It has been reported that the common use of anticarcinogens and antimutagens in every days life will be the most effective against the genetic and other related diseases (5). Vitamin supplementations have marked potentiality against toxic effects of diversified chemicals.

Ascorbic acid (Vitamin C) is known to act as an antimutagen and anticarcinogen in various test systems (6). Under in Vitaminro conditions, it decreasescarcinogen-induced gene mutations (7,8), SCEs (9), and chromosomal breakages (10,11). Vitamin C (ascorbic acid) is an antioxidant that can scavenge free radicals and protect macromolecules, including DNA, from oxidative damage induced by different agents Vitamin C may play a role in the pathogenesis prevention and therapy of cancer, although its role has been the subject of controversy. In humans, ascorbic acid prophylaxis has been reported to decrease chromosomal damage in peripheral lymphocytes. Ferguson (1994) (12) suggested that the use of antimutagens and anticarcinogens in everyday life would be the most effective procedure for preventing human cancer and to some extent genetic diseases

The frequency of chromosome instability in peripheral blood lymphocytes (PBL) is generally indicative of increased cancer risk for those exposed to DNA damaging agent (13) . The aim of the present study was to investigate chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes of gastric cancer patients. The role Vitamin C as antimutagenic agents modulating the frequency of these chromosomal aberrations and sister chromatid exchange in gastric cancer patients receiving chemotherapy regime .

MATERIALS AND METHODS

Selection of subjects

Blood samples were collected from 80 human subjects with gastric cancer patients receiving the chemotherapy in a combination of ECF (Epirubicin, cisplatin and 5-fluorouracil) from recognized cancer hospitals under the supervision of oncologist. The gastric cancer patients were receiving an adjuvant therapy in combination of the drugs in different cycles the dosage information is obtained from the cancer hospital and also from the medical oncologist). The mean age group of the patients was in the range of 46 – 50 yrs and belongs to the same socio-economic status. blood samples from 100 healthy individuals were collected as controls for the purpose of comparison.

All the participants were given informed about the objective of the study and a written consent was obtained from each subject .The blood samples were collected and further manipulated in accordance with the recommendations of the bio-medical ethical guidelines. This study has been approved by the Institutional ethics committee. Cytogenetic studies were carried out in peripheral blood lymphocytes of study population before and after Vitamin C by adopting standard cytogenetic protocols such as (a) Chromosomal aberrations (CA) (b) Sister chromatid exchanges (SCE)

Vitamin C prophylaxis was studied in a group of 80 gastric cancer patients receiving the adjuvant chemotherapy with ECF (Epirubicin, Cisplatin & 5-Fluorouracil). A daily dose of 1g for five consecutive days in a week was given systematically to the patients under clinical oncologists supervision in tablet form with the brand name Limcee (Glaxo) from February 2008 to May 2008. The effectiveness of Vitamin- C prophylaxis was evaluated before and after 3 months treatment with Vitamin-C.

Chromosomal aberration assay

All chemical reagents were purchased from Sigma Chemicals, except colcemid (Gibco Laboratory). Leukocyte cultures were set up following standard procedures in our laboratory. 0.5 ml blood was added to 4.5 ml RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 1% streptomycin-penicillin, 0.2 ml reagent grade phytohemagglutinin, and was incubated at 37ºC. After 50 h, cultures were treated with 0.1 mg/ml colcemid to block cells in mitosis. Lymphocytes were harvested after 52 h by centrifuging cells to remove culture medium (800–1000 rpm), addition of hypotonic solution (KCl 0.075 M) at 37ºC for 20 min to swell the cells, and treated twice with Carnoy's fixative (3:1 ratio of methanol: acetic acid). Slides were carefully dried on a hot plate (56ºC, 2 min). Three days later, slides were stained using the Trypsin-giemsa technique. For the CA analysis, 100 complete metaphase cells in first cell cycle were evaluated per subject under a Leica microscope (100x)

Statistical Method

The student t- test (14) was employed to analyze the statistical significance in the frequency of mean chromosomal aberrations (CA) and Sister chromatid exchanges (SCE) per cell between the control and the cancer patients. The use of student t –test for sister chromatid exchange is based upon the assumptions that the number of sister chromatid exchange per cell is very small and it is following the poison distribution

RESULTS

In the present investigation, (table 1) analysis of chromosomal aberrations in the lymphocytes of patients was studied using standard metaphase analysis method. The difference in the incidence of chromosomal aberrations between the control, Cancer patients and patients receiving chemotherapy were subjected for statistical analysis and the values were found to be statistically significant at the level $p < 0.05$.

An increase frequency of Total chromosomal aberrations was observed in the cancer patients receiving chemotherapy as compared to the control group ($p < 0.001$, $t=11.11$). The % of Total aberrations in cancer patients receiving chemotherapy before Vitamin C administration was 10.13 ± 5.54 as against to 3.65 ± 1.63 in control group. A statistically significant decreased in Total aberrations was reported among the cancer patients receiving chemotherapy, supplemented with Vitamin C for 3 months when compared to total aberration before Vitamin C prophylaxis ($p<0.001$, $t=9.39$).Frequencies of chromosomal aberrations in cancer patients receive chemotherapy was 6.10 ± 2.72 as compared to that of 10.13 ± 5.54 in cancer patients before Vitamin C and there is no significant difference found with in the controls after Vitamin C Prophylaxis.

In the present study, (table 2) the mean SCE rate per cell in the cancer patients with out Vitamin C prophylaxis is higher than the cancer patients in comparison to control group ($p<0.001$,$t=32.22$). The percentage of SCE rate per cell in cancer patients before Vitamin C prophylaxis 9.27± 0.14 followed by 3.30±0.04 in control group. A statistically significant decrease in SCE was reported among the cancer patients receiving chemotherapy, supplemented with Vitamin C for 3 months when compared to SCE before Vitamin C prophylaxis ($p<0.001$, $t=9.39$) and there is no significant difference found with in the controls after Vitamin C Prophylaxis.

DISCUSSION

Evidence is accumulating in support of a role for ROS in the etiology of cancer. Inflammatory cells, such as neutrophils, macrophages, and eosinophils, are an important endogenous source of oxygen radicals. Stimulation of these cells by tumor promoters or by foreign bodies causes the release of ROS. Chromosomal instability is considered to be a primary vent in neoplastic transformation and also as a marker of cancer progression (15,16). Further Antunes *et al.,* (1999) (17) reported a significant reduction in chromosomal aberrations and number of abnormal metaphases induced by doxorubicin in human peripheral lymphocytes by Vitamin C.

In the present Investigation we intend to see the antimutagetic activity of Vitamin c and its role in increasing the antioxidative metabolism which in turn decrease DNA damage in cancer patients receiving chemotherapy regime. A significant decrease in Total chromosomal aberrations (CA) ($p<0.001$) was observed after administration of Vitamin C for 3 months as the frequency of total CA was higher in gastric cancer patients in comparison to control group. The observations made by Giri *et al.*, (1998) (18) in cisplatin and cyclophosphamide treated patients against Vitamin C prophylaxis, supports the present investigation. Ascorbic acid is an antioxidant, possessing substantially nucleophilic character. Alkylation of ascorbic acid should effectively compete with the alkylation of other nucleophilic sites. The protective effects of ascorbic acid may be due to the antagonistic effect of this Vitamin at all levels of genotoxicity initiation. It may also be due to the self-alkylation of ascorbic acid, which prevents or reduces the cellular alkylation of macromolecules. Vitamin C may also quench reactive oxygen metabolites in stomach or deodenum and prevent the formation of N nitroso compounds that are mutagenic. Nitrosamines have been linked to gastric cancer. Formation of nitrosamines in the gastro intestinal track can be decreased by administration of Vitamin C (19). High dietary Vitamin C intake correlates with reduced gastric cancer risk (20), it is not certain what confers protection: Vitamin C itself are the other components of foods , particularly fruits and vegetables, that also happen to contain Vitamin

In this present study an significant decrease in the frequency of SEC of the cancer patients after Vitamin C administration, as the frequency of SCE was increased in comparison to control group ($p < 0.001$) . Edenharder *et al.,* (1998) (21) stated that Vitamins and related compounds were having the ability to modulate the sister-chromatid exchanges (SCEs) induced by Trp-P-2 or cyclophosphamide (CP) in human peripheral lymphocyte cultures in the presence of an exogenous metabolizing system.Modulatory effect of Vitamin C on the mutagenic effect of the antineoplastic drug Cyclophosphamide (CP) was assessed in the invivo mice by using micronucleus test. Simultaneous oral administration of Vitamin C with i.p. administration of CP was found to decrease the frequency of micro-nucleated polychromatic erythrocytes elevated by CP. Vitamin C exhibited a significant antimutagenic effect over a wide dose range(1.56-200mg/kg) (22) . Further, Anderson 1994 (23) reported the effect of the antioxidant nutrient such as Vitamin C and E has not been offered any protection in the bacterial mutation assay against bleomycin treatment, whereas Vitamin C offered protection in HPL and both Vitamins showed a marked reducing response in micro nucleated cells of mice.

Vitamin C (ascorbic acid) is an antioxidant that can scavenge free radicals and protect macromolecules, including DNA, from oxidative damage induced by different agents. The protective effect of Vitamin C on cisplatin induced chromosome aberrations has been determined in the human peripheral lymphocyte aberration test *inVitro*. The results of treatments with Vitamin C indicated that it statistically significantly decreases the number of chromosome aberrations and number of metaphases with aberrations induced with cisplatin.

Conflict of interest

No competing interests.

Table 1: Effect of 3 months Vitamin – C prophylaxis on frequency of chromosomal aberrations in Study group

Group	Sample	Vit -C	No. of patients	No. of metaphases	% of the metaphase	No. of aberrant cells	% of aberrant cell
Control	Feb' 2008	-	100	1927	96.35	73	3.65 ±1.63
	May' 2008	+	100	1914	95.70	86	4.30 ±1.92
Cancer patients with chemo-therapy	Feb' 2008	-	80	2696	89.86	304	10.13 ±5.546.1 **
	May' 2008	+	80	1878	93.90	122	6.10 ±2.72 **

* 100 Metaphases were scored for each sample; Values are in mean ± SE; * *p < 0.001

Table 2: Effect of 3 months Vitamin -C prophylaxis on SCE's in Study group

Group	Sample	Vit-C	No. of patients	No. of metaphases	No. of SCE's	SCE's/ cell ± SE	PRI
Control	Feb' 2008	-	100	600	1985	3.30 ± 0.04	1.43
	May'2008	+	100	600	2098	3.49 ± 0.06	1.48
Cancer patient+	Feb'2008	-	80	900	9250	9.27 ± 0.14 **	1.85
chemotherapy	May' 2008	+	80	600	2180	3.63 ± 0.10 **	1.64

* 30 Metaphases were scored for each sample; Values are in mean ± SE; * *p < 0.001

REFERENCES

1. World Health Organization ,Cancer (Fact sheet N°297)". http://www.who.int/mediacentre/factsheets/fs297/en/. Retrieved 2009-05-11
2. Sugano K. Gastric cancer: pathogenesis, screening and treatment. Gastrointest. Endocopy Clin N Am 2008; 18:513-22.
3. Ifeoma N, Obioma C and Chinyere C. Effect of vitamin on malondialdehyde and glutathione levels in type 2 diabetic Nigerians. The Internet Journal of Pharmacology (2009); 7.1.
4. Surh, Y.-J. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory actiVitaminies: a short review, Food Chem. Toxicol. 2002: 40, 1091-1097.
5. Chatterjee and Rahaman MJ .Protective effect of cysteine against X-ray And bleomycin-induced chromosomal aberrations and cell cycle delay . Mutat Res 1993; 290(2):231-8.
6. Shamberger, R. J. Genetic toxicoloaj@of ascorbic acid. Mutat. Rca, 1984 : 133: 135â€"159,.
7. Guttenplan, J. B. Inhibition by L-ascOrbate of bacterial mutagenesis induced by two N-nitroso compounds. Nature (Lond.),1977:268: 368â€"370.
8. Raina, V., and Gurtoo, H. L. Effect ofvitamins A, C, and E on aflatoxin B,- induced mutagenesis in Salnwnella typhimurium TA-98 and TA-l00. Tera togen.Carcinogen.Mutagen. 1,985: 5: 29â€"40.
9. Galloway, S. M., and Painter, R. B. Vitamin C is positive in the DNA synthesis inhibition and sister-chromatid exchange tests. Mutat. Res., 1979 :60: 321â€"327,.
10. Gebhart, E., Wagner, H., Grziwok, K., and Behnsen, H. The action of anticlastogens in human lymphocyte cultures and their modification by rat liver S9. II. Studies with vitamins C and E. Mutat. Res., 1985:149: 83â€"94,.
11. Shamberger, R. J., Baughman, F. F., Kalchert, S. L, Willis, C. E., and Hoffman, G. C. Carcinogen-induced chromosomal breakage decreased by antioxidants. Proc. Nati. Acad. Sci. USA ,1973:, 70: 1461â€"1463,.
12. Ferguson LR., Antimutagens as cancer chemopreventive agents in the diet. Mutat Res. 1994: 1;307(1):395-410.
13. Bonassi S, Abbondandolo A, Camurri L, Dal Pra A, De Ferrari M, Degrassi F, Forni A, Lamberti L, Lando L, Padovani M, Sbrana I, Vecchio D, Puntoni R .Are chromosome aberrations in circulating lymphocytes predicitve of future cancer on set in humans Prelimiary results of an Italian cohort study. Genet. Cytogenet. 1995 :79:133-335.
14. Goldstein, A Biostatistics : An introductory test. The Mac Millian.Co. New York collies. 1965.
15. Hawkins NJ, Gorman P, Tomlinson IP, Bullpitt P, Ward RL .Colorectal carcinomas arising in the hyperplastic polyposis syndrome progress through the chromosomal instability pathway. Am. J. PathoI. 2000:1(57):385 - 392.
16. Burt EC, James LA, Greaves MJ, Birch JM, Boyle LM, Varley JM . Genomic alterations associated with loss of heterozygosity for TP53 in Li-Fraumeni syndrome fibroblasts. Br. J. Cancer. 2000 :(83):467- 472.
17. Antunes, F.; Barclay, L.R.C.; Ingold, K.U.; King, M., Norris, J.Q.; Scaiano, J. C.; Xi, F.
18. On the Antioxidant ActiVitaminy of Melatonin. Free Radic. Biol. Med.1999: 26, 117-128
19. Giri A., Khynriam D, Prasad SB . Vitamin C mediated protection on cisplatin induced Mutagenicity in mice. Mutat Res. Nov1998 ;421(2):139-48.
20. Helser MA, Hotchkiss JH, Roe DA: Influence of fruit and vegetable juices on the endogenous Formation of N-nitrosoproline and N-nitrosothiazolidine-4-carboxylic acid in humans on controlled DIets. Carcinogenesis .1992 :13:2277–2280.
21. Byers T, Guerrero N: Epidemiologic evidence for Vitamin C and Vitamin E in cancer prevention. American Journal of Clinical Nutrition .1995 ;62:1385S–1392S.
22. Edenharder R., Kerkhoff G, Dunkelberg H . Effects of beta-carotene, retinal, riboflavin, Alpha-tocopherol and Vitamins C and K1 on sister-chromatid exchanges induced by 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) and cyclophosphamide in human lymphocyte cultures. Food Chem Toxicol. Nov 1998 ;36(11):897-906.
23. Ghaskadbi S., Rajmachikar S, Agate C, Kapadi AH. Vaidya VG .Modulation of Cyclophosphamide mutagenicity by Vitamin C in the in vivo rodent micronucleus assay. Teratog Carcinog Mutagen;1992 :12(1):11-7.
24. Anderson D, Yu TW, Phillips BJ, Schemezer P The effect of various antioxidants and Other modifying agents on oxygen-radical generated DNA damage in human lymphocytes in the COMET assay. Mutation Res.1994: 307:261–271