ABSTRACT

In the present study protective effects of lycopene on cultured human lymphocytes against Bleomycin-induced cytogenetic alteration in the form of chromosomal aberrations (CAs) has been observed. Four different cultures were set up. First culture vial was kept untreated, second was treated with lycopene (8μg/ml), third was treated with bleomycin (15μg/ml) and fourth received combined treatment of lycopene (8μg/ml) and bleomycin (15μg/ml). There was significant reduction in the chromosomal aberration frequency, particularly chromosome gaps, chromosome breaks and dicentric chromosomes, in those cultures treated with both lycopene as well as bleomycin when compared to the culture treated alone with bleomycin.

KEYWORDS: Lycopene, Bleomycin, Chromosomal aberrations, Human Lymphocytes.

INTRODUCTION

Lycopene is a carotenoid responsible for the red colour of tomato and some other fruits. It was first discovered in tomato.[1] Epidemiological and small experimental studies indicate that lycopene – rich diets may have a protective effect against chronic diseases, including cancer and heart disease.[2] These carotenoids are suggested to play a role in protection against light dependent damage.[3] Pre-treatment with lycopene offered protection against γ-
radiation induced cellular damage and could be developed as an effective radio protector during radiotherapy.\[4\]

Bleomycin sulphate is a mixture of glycopeptides\[5\], antibiotic containing approximately primarily Bleomycin A\(_2\) (70%) and B\(_2\) (30%). It is isolated from *Streptomyces verticillus*. The drug binds to DNA, inhibits DNA synthesis and causes single strand scission of DNA *in vivo* and *in vitro* at specific base sequences. Exposure of cells to ionizing radiation during the G\(_0\) or G\(_1\) phases of the cell cycle causes chromosomal aberrations (CAs) as breaks, gaps, dicentrics, fragments, rings and translocations. CAs are used as biomarkers of radiosensitivity after medical, accidental and occupational exposure. Bleomycin also induces similar chromosomal aberrations in cultured cells and has often been used as radiomimetic drug. The studies on the biological effects of ionising radiation are carried out in mammalian tissues such as testes of rat, eggs of ascaris, testes of grasshoppers, larval cells of amphibians, somatic and meiotic cells of plants.\[6\] Nevertheless, human lymphocytes are predominant for cytogenetic analysis because they circulate in the blood and so receive equal radiation dose.\[7\]

Because radiation-induced cellular damage is attributed primarily to the harmful effects of free radicals, molecules with radical scavenging properties are particularly promising as radio protectors.\[8, 9\] Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against free radicals.\[10, 11\] In the present study, attempt has been made to observe possible radio-protective property of lycopene against the cytogenetic damage in cultured human lymphocytes caused by radiomimetic drug –Bleomycin.

**MATERIALS AND METHODS**

The present study was carried out on blood samples collected from 10 healthy, non smoking, and randomly selected individuals. The age of the donors ranged from 20-25 years. Four separate culture vials were set up from each of the ten blood samples collected. First culture vial was kept untreated so as to act as control. After 24 hours of initiation, second culture vial was treated with lycopene at the concentration of 8µg/ml. The third culture vial was treated with 15µg/ml of bleomycin and fourth vial received combined dose of lycopene and bleomycin at the concentration of 8µg/ml and 15µg/ml respectively.

About 5ml of RPMI 1640 medium was taken by a sterile syringe into sterile vial. To this 50µl of heparin, 0.1ml of phytohaemagglutinin, 0.1ml of streptomycin and 1ml of foetal calf
serum were added. About 0.6ml of whole blood (collected in sodium heparinised tubes) was added to above mixture and culture vials were incubated at 37ºC for 72hrs. After 69th hour of incubation the cultures were treated with 100 µL colchicine and left for 2 hour so as to arrest mitotic division. Then standard cytogenetics procedures as hypotonic treatment, fixation (1: acetic acid/ 3: methanol), slide preparation, staining (5% Giemsa solution) and air dried preparations were performed.[12] The metaphase preparations were made by dropping 4-5 drops of cell suspension from a convenient height on clean pre-chilled slide. The slides were immediately blind coded and routinely stained with 2% Giemsa before scoring. From control and all treatment groups, 100 metaphases were scored under compound light microscope for CAs such as breaks, gaps, dicentrics, tricentrics, acentric fragments, ring and prematurely separated centromeres.

RESULTS AND DISCUSSION

Table I: Chromosomal aberrations induced in *in vitro* cultured human lymphocytes after addition of lycopene alone as well as with bleomycin. (Figures indicate total aberrations per 1000 cells)

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Control</th>
<th>Lycopene (8µg/ml)</th>
<th>Bleomycin (15µg/ml)</th>
<th>Lycopene+Bleomycin (8µg/ml+15µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatid Gap</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>4*</td>
</tr>
<tr>
<td>Chromatid Break</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2**</td>
</tr>
<tr>
<td>Chromosome Gap</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Chromosome Break</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Dicentric</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1***</td>
</tr>
<tr>
<td>Ring</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Acentric Fragment</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prematurely Separated Centromeres</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Telomeric Association</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Satellite Association</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total Aberrations</strong></td>
<td><strong>10</strong></td>
<td><strong>15</strong></td>
<td><strong>40</strong></td>
<td><strong>20</strong>*</td>
</tr>
</tbody>
</table>

* Significantly less than alone bleomycin treatment at (P < 0.5), ** Significantly less than alone bleomycin treatment at (P < 0.1)

*** Significantly less than alone bleomycin treatment at (P < 0.01)

The data obtained from the study (Table I) showed that the frequency of CAs induced by bleomycin in human lymphocytes is significantly affected by the presence of lycopene. Addition of lycopene (8µg/ml) along with bleomycin (15µg/ml) showed significantly less (P<0.01) number of total CAs when compared with alone treatment of bleomycin in the same concentration. There was significant reduction in chromosome gaps (P<0.5), chromosome
breaks (P<0.1) and dicentric chromosomes (P<0.01), from those cultures treated with both lycopene as well as bleomycin when compared to the cultures treated alone with bleomycin. **Fig 1** indicates normal Giemsa stained human metaphase chromosomes. **Fig 2** indicates Dicentric chromosome, while chromatid gap and break are shown in **Fig 3**.

Ionizing radiations are one of the predominant exogenous factors that have deleterious consequences to human life. With respect to radiation damage to humans, it is important to protect them from harmful effects of ionizing radiation. Research in the development of radio protectors worldwide has focused on screening a variety of chemical and biological compounds. The first report\(^{[13]}\) on *in vivo* radioprotection was reported by Patt *et al.* where pre-treatment with a naturally occurring amino acid, cysteine was shown to increase survival of lethally irradiated mice and rats. The Waller Reed Army Research Institute synthesized and tested over 4,000 compounds and found the most effective compound to be WR-2721 (Amifostine).\(^{[14]}\) It has limited use due to its cumulative toxicity on daily administration with radiotherapy, which is manifested as nausea, vomiting, hypotension, allergic reactions, etc.\(^{[15]}\) Thus, there is still need and want to identify novel, nontoxic, effective and convenient compounds to protect humans against radiation.

To this problem, natural sources especially edible medicinal plants/herbs provide ideal solution as these are regarded as non-toxic even at higher concentrations. In the view of low cost, easy accessibility and less toxic effects, there is a growing interest on ethno medicines even among the common people.\(^{[16]}\) Randomized clinical trials on antioxidant vitamins to prevent acute adverse effects of radiation, suggest that use of high doses of antioxidants as adjuvant therapy might compromise radiation treatment efficacy.\(^{[17]}\)

The present study was carried out on *in vitro* cultured human lymphocytes. The cultures were treated either alone with lycopene as well as in combination with radiomimetic drug bleomycin. Positive implications about radio protective effects by lycopene on bleomycin induced cytogenetic alteration in the form of chromosomal aberrations in *in vitro* human lymphocyte cultures were recorded. The ameliorative effects of lycopene may be attributed to its property of scavenging the free radicals, which cause damage to the cells and tissues.

Dusinska *et al.*\(^{[18]}\) suggested that nutritional supplementation with antioxidants decreases chromosomal damage in humans. It was also found that lycopene can prevent carcinogenesis by protecting vital molecules including the nutritious property of lycopene. In a rat model,
pre-incubation of spermatozoa with lycopene offered protection against oxidative DNA damage \textit{in vitro}.\textsuperscript{[19-21]}

In the present study, it was observed that lycopene has a protective role against chromosomal damages induced by bleomycin in human lymphocytes. Therefore lycopene can be explored as radio protective agent.

Fig 1: Giemsa stained human metaphase chromosomes

Fig 2: Dicentric chromosomes

Fig 3: Chromatid gap and breaks
REFERENCES


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