Research Paper

SCREENING FOR ANTICANCER ACTIVITY OF LYCOPENE IN COMBINATION WITH VITAMIN E AGAINST MCF-7 AND HEP-2 CANCER CELL LINES

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Abstract
Numerous epidemiological studies have demonstrated that consumption of vegetables and fruits reduces the risk of many types of human cancers. Recently, there has been a growing interest in the role of the tomato carotenoid, lycopene, in cancer prevention and treatment. The hallmark of the cancer cell is the deregulation of molecular mechanisms responsible for control of cell growth. Therefore, possible mechanisms underlying the anticancer activity of lycopene has been investigated paying special attention to anticancer activity of lycopene in combination with vitamin E against MCF-7 and Hep-2 cancer cell lines. Cell viability was also analyzed by MTT assay. The combination of low concentrations of lycopene with vitamins exhibited a synergistic effect on cell proliferation and differentiation and an additive effect on cell cycle progression. Further research need to be explored to make use of lycopene as an effective anti-cancer drug.

Key words: Lycopene, Vitamin E, MCF-7, Hep-2, MTT.

INTRODUCTION
Cancer is a major public health problem, with significant associated death and disability and it is the second leading cause of death in developed countries. Globally, cancer is a major public health burden, accounting for one in eight deaths overall more than AIDS, tuberculosis and malaria combined. Despite advances in treatment, deaths from cancer are projected to rise, with an estimated 17 million deaths in 2030 [Boyle et al., 2008]. Globally, breast cancer affects more women than any other type of cancer and is the leading cause of cancer-related deaths among women. Lung cancer kills more people every year than breast, colon, and prostate cancer combined. The World Health Organization (WHO) reports that over 1.1 million people die of lung cancer each year [Hortobagyi et al., 2008].

Considerable interest and research efforts have been expended in an effort to uncover the potential roles of carotenoids in human health and disease. Recently, there has been a growing interest in non-provitamin A carotenoids in the tomato, lycopene, as a cancer preventive agent. Lycopene unique antioxidant capacity, to remove free radicals, protecting cells from damage to DNA and gene that can prevent the cancer process. Dietary intake of tomatoes and tomato products has been found to be associated with a lower risk of a variety of cancers in several epidemiological studies. Lycopene is the most abundant carotenoid in tomatoes (Lycopersicon esculentum L.) with concentrations ranging from 0.9–4.2 mg/100 g depending upon the variety. Other edible sources of lycopene include rosehips, watermelon, papaya, pink grapefruit, and...
guava [Bohm et al., 2003]. Epidemiological studies have suggested that lycopene decreases the risk of several types of human malignancies, such as breast [Zhang et al., 1997] and lung cancer [Michaud et al., 2000]. Several reports have demonstrated potential beneficial effects of lycopene, especially antioxidant function, enhanced cellular gap junction communication, anti-angiogenesis, inhibition of cell proliferation and induction of apoptosis [Adams et al., 2007]. Carotenoids like lycopene are unstable and highly reactive towards oxygen and free radicals. This reactivity of lycopene is the basis for its anti-oxidant activity in biological systems that might contribute to its efficacy as a chemoprevention agent. Moreover, it has been suggested that lycopene can exert modulatory action on cancer by interacting with a wide spectrum of molecular targets central to the cell signaling machinery [Rao et al., 2000]. Several tissue culture studies have also shown the protective effect of lycopene against the growth of different cancer cell lines. Animal studies have also shown the protective effect of dietary lycopene against colon, lung and breast cancers [Heber, 2000]. Evidence in recent years has shown that vitamin E has the anticancer potential and in addition to its anti-cancer property, it also facilitates the mechanism of action of lycopene when supplemented along with them [Duffield et al., 2002]. The findings point out the anticancer activity of lycopene in combination with vitamin E against breast cancer cell line MCF-7 and lung cancer cell line Hep2.

MATERIALS AND METHODS

Cell lines
MCF-7 (breast cancer) and Hep-2 (lung cancer) cell lines were kindly provided by the Department of Endocrinology, Dr ALM PG Institute of Basic Medical Science, Chennai. Cells were cultured in DMEM medium and supplemented with 10% of fetal bovine serum (FBS) then the culture flasks were incubated for 3-4 days at 37°C in 5% CO₂ incubator.

Lycopene preparation
The compound lycopene was mixed with 75mg of vitamin E and dissolved in DMSO.

Analysis of cell viability by MTT assay
Cell viability was measured quantitatively by using MTT, showed the activity of living cells [Plumb et al., 1989]. MCF-7 and Hep2 cells were seeded into 24 well plates and treated with 100µl/ml, 150µl/ml, 200µl/ml, 250µl/ml and 300µl/ml of lycopene mixture dissolved in DMSO. The treated mixture was then incubated at 37°C with 5% CO₂ for 24 hours. After incubation, 2µl/ml of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] labeling reagent was added to each well followed by incubation for 3hours at 37°C with 5% CO₂ and then the medium was discarded and formazan crystals were dissolved in 1.0 ml of 0.04N HCl. The absorbance of cells was measured at 570 nm with an ELISA reader. MTT assay was performed in the Department of Endocrinology, Dr ALM PG Institute of Basic Medical Science, Chennai.

STATISTICAL ANALYSIS
Each data point was obtained by making at least 3 independent measurements. All data are expressed as mean ± S.D. Data were analyzed by an analysis of variance (p<0.05) and the means separated by one way ANOVA.

RESULTS
Lycopene in combination with vitamin E significantly reduced the growth of MCF-7 cell line at the concentrations of 100µl/ml and 150µl/ml by 55.83% and 48.3% respectively. The growth inhibition at 200µl/ml, 250µl/ml and 300µl/ml of lycopene mixture was observed to be 93.3%, 70% and 93% respectively [Figure 1]. Figure 2 represents the evaluation of MTT reduction of Hep2 cell lines treated with lycopene mixture at 570nm in which the cell growth was significantly reduced at the concentrations of 100µl and 300µl by 48.3% and 40.6% respectively and the growth reduction rate was observed to be 82%, 75.8% and 71.91% for 150µl, 200µl and 250µl respectively.
Fig 1. Evaluation of MTT reduction of MCF-7 cell line treated with lycopene mixture at 570nm using ELISA reader

Fig 2. Evaluation of MTT reduction of Hep2 cell line treated with lycopene mixture at 570nm using ELISA reader
Dietary chemoprevention has emerged as a cost effective approach to control most prevalent chronic diseases including cancer and so natural products with anticancer properties could be valuable substances in cancer treatment. The anticancer activity of lycopene has been demonstrated both in in vitro and in vivo tumour models. In addition to its anti-carcinogenic activities, lycopene shows an array of biological effects including cardioprotective, anti-inflammatory, antimutagenic and antioxidant properties [Krinsky and Johnson, 2005]. Among naturally occurring carotenoids, lycopene has shown the strongest ability to scavenge free radicals and chemically quench singlet oxygen, being 2-fold and 10-fold more effective at quenching singlet oxygen than β-carotene and α-tocopherol, respectively [Conn et al., 1991].

The combination of low concentrations of lycopene with vitamins exhibited a synergistic effect on cell proliferation and differentiation and an additive effect on cell cycle progression. Such synergistic antiproliferative and differentiating effects of lycopene and other compounds found in the diet and in plasma suggest that phytochemicals such as vitamin E and lycopene,

**DISCUSSION**

Table: 1 One way ANOVA for evaluation of MTT reduction of MCF-7 cell lines treated with lycopene mixture at 570nm

<table>
<thead>
<tr>
<th>Source of variation for MCF-7 cell line</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.041917</td>
<td>5</td>
<td>0.008383</td>
<td>9.550633</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.010533</td>
<td>12</td>
<td>0.000878</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.05245</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table: 2 One way ANOVA for the evaluation of MTT reduction of Hep2 cell line treated with lycopene mixture at 570nm using ELISA reader

<table>
<thead>
<tr>
<th>Source of variation for Hep2 cell line</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.03096</td>
<td>5</td>
<td>0.006192</td>
<td>162.8863</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.000684</td>
<td>18</td>
<td>0.000038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.031644</td>
<td>23</td>
<td></td>
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with separate modes of action, may unexpectedly combine to promote anticancer effects not seen with either agent alone. Various mechanisms have been proposed to explain the inhibitory effects of lycopene, including cell cycle arrest and/or apoptosis induction via a modulation of redox status, a regulation of growth factor signaling, changes in cell growth-related enzymes, an enhancement of gap junction communication, and a prevention of smoke-induced inflammation [Richard and Breemen, 2008].

Lycopene induced phosphorylation of PDGF receptor beta and attenuated several kinases (extracellular signal-regulated kinase 1/2, p38 and c-Jun N-terminal kinase). Lycopene directly trapped the platelet-derived growth factor [Chiang et al., 2007]. It may act as anti-cancer agent by inhibiting stromal cells, tumour cells and their interactions [Wu et al., 2007]. A number of studies showed that lycopene inhibited the growth of human cancer cells grown in cultures. The growth-inhibitory effects of lycopene were observed not only in lung cancer cells, but also in other cell types, including prostate, breast, hepatoma, stomach, colon and oral cancer cells. In particular, lycopene has been reported to decrease cyclin D1 and to increase p53, p21Waf1/Cip1 protein levels in cancer cells. The study demonstrates the induction of cell death in MCF-7 and Hep2 cell lines upon treatment with lycopene mixture at various concentrations. MTT assay is the most sensitive cytotoxicity assay that is mainly based on the enzymatic conversion of MTT with in mitochondria by succinate dehydrogenase. Apoptotic cell death is accompanied by a series of complex biochemical events and definite morphological changes. Despite the complexity of the mechanisms involved, mitochondria appear to release apoptosis inducing factors that may trigger DNA fragmentation in nuclei [Susin et al., 1999].

Oxidative stress is recognized as one of the major contributors of increased risk of cancer, and in chemical assays, lycopene is the most potent antioxidant among various common carotenoids [Mascio et al., 1989]. Lycopene has unique antioxidant capacity, to remove free radicals, protecting cells from damage to DNA and gene that can prevent the cancer process. Further research need to be exploited for the successful application of lycopene as an effective chemotherapeutic drug to combat cancer metastasis.

Levy et al., (1995) who showed that lycopene is a stronger cell growth inhibitor than β-carotene. Cell proliferation is controlled by a series of cell cycle regulators, including cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors, which regulate cell cycle progression. The growth inhibition of lycopene onMCF-7 breast cancer cells was associated with decreased G1-S cell cycle progression, decreased cyclin D1 expression, and stabilization of p27 in the cyclin E-CDK complex. In addition to cell proliferation inhibition. The growth inhibitory effect of lycopene may also be attributed to induction of apoptosis [Nahum et al., 2006].

**Conclusion**

To gain a better understanding of the beneficial biological activities of lycopene upon cancer prevention, a greater knowledge of the metabolism of lycopene is needed. More research is clearly needed to identify and characterize additional lycopene metabolites and their biological activities, which will potentially provide invaluable insights into the mechanisms underlying the beneficial effects of lycopene to humans, especially in terms of cancer prevention. If such studies succeed in identifying an active lycopene derivative, it could be used as a parent compound for the development of potent anticancer drugs.

**ACKNOWLEDGEMENTS**

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**REFERENCES**