Effects of supplementing a low-carotenoid diet with a tomato extract for 2 weeks on endogenous levels of DNA single strand breaks and immune functions in healthy non-smokers and smokers

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Increased consumption of tomato products is associated with a decreased risk of cancer. The present study was performed to investigate whether consumption of a tomato oleoresin extract for 2 weeks can affect endogenous levels of DNA single strand breaks in peripheral blood lymphocytes in healthy non-smokers and smokers. We also assessed, the effect of the tomato oleoresin extract on various immunological functions of peripheral blood mononuclear cells.

A double-blinded, randomized, placebo-controlled study design was used. Over a period of 2 weeks 15 non-smokers and 12 smokers were given three tomato oleoresin extract capsules daily (each containing 4.88 mg lycopene, 0.48 mg phytoene, 0.44 mg phytofluene and 1.181 mg \(\alpha\)-tocopherol). The control group received placebos. The baseline level of endogenous DNA damage for non-smokers was slightly (13\%) and non-significantly \((P = 0.44)\) lower than that of smokers. Placebo supplementation of non-smokers and smokers for 2 weeks did not significantly affect lycopene plasma levels or DNA damage in either group. Intervention with tomato oleoresin extract resulted in significant increases in total plasma lycopene and resulted in decreased levels of DNA strand breaks of \(-32\) (non-smokers) and \(-39\%\) (smokers). However, this effect was not statistically significant in either group \((P = 0.09\) for non-smokers and \(P = 0.12\) for smokers). Analysis of the distribution pattern of DNA strand breaks showed a statistically significant \((P < 0.05)\) increase in the number of comets in class 0 (undamaged) and a decrease in classes 1 and 2 (damaged) after the tomato oleoresin extract intervention in non-smokers. The changes in the smoker group were not statistically significant. Treatment with the tomato extract had no effect on lymphocyte proliferation, NK cell activity, interleukin (IL)-2 production and tumor necrosis factor (TNF)\(\alpha\) production, but it significantly reduced IL-4 production in smokers \((P = 0.009)\).

Introduction

Epidemiological and experimental data suggest that an increased intake of tomato products can reduce the risk of cancers, especially prostate and colon cancer. Several lines of evidence suggest that tomato constituents, such as lycopene, affect biological mechanisms such as modulation of immune functions and of antioxidant status.

DNA damage is an important step in the initiation of cancer. Human intervention with tomato juice for 2 weeks significantly reduced endogenous levels of DNA strand breaks in peripheral blood lymphocytes (1) and tomato puree supplementation increased DNA protection against hydrogen peroxide (2,3). Research on the beneficial health effects of tomato products has focused mainly on lycopene, the major tomato carotenoid. However, supplementation with lycopene for 12 weeks had no effect on DNA damage in human lymphocytes (4). These observations indicate that a complex mixture of phytochemicals such as carotenoids, tocopherols, phenolic acids and flavonoids may be more effective than single isolated compounds. Indeed, mixtures of different carotenoids and mixtures of carotenoids with \(\alpha\)-tocopherol or with some polyphenols showed synergistic effects to inhibit lipid peroxidation, an important pathological process leading to the generation of free radicals, which are able to damage DNA (5,6).

Similarly, we have shown recently that under certain conditions supplementation of a low-carotenoid diet with tomato juice results in enhanced cellular immune functions (7,8). Such an immunomodulation may also contribute to the observed reduction in cancer risk.

In the same experimental setting, which resulted in reduced endogenous levels of DNA strand breaks in lymphocytes (1), we observed a stimulation of various cellular immune functions with tomato juice supplementation (7). These results were recently confirmed by a second study (8). Immune cell functions such as NK cell cytotoxicity play an important role in reducing the risk of cancer. Results from animal models as well as epidemiological data suggest that low NK cell cytotoxicity is associated with higher cancer rates (9,10). We, therefore, measured NK cell cytotoxicity and the production of several cytokines associated with the regulation of NK cell function.

Here, we investigated the effect of a tomato oleoresin extract that contains, in addition to lycopene, a number of lipid-soluble tomato compounds such as phytoene, phytofluene and \(\alpha\)-tocopherol. It is of interest to know whether or not such extracts are able to mimic the effects of tomato products, such as juice or puree, with respect to DNA damage and modulation of cellular immune functions.

There is considerable evidence that a diet high in carotenoids can improve health and no evidence of adverse effects has yet been found. However, supplementation with high-dose \(\beta\)-carotene in isolated form may increase the incidence of lung cancer in smokers or asbestos workers (11,12). Therefore, we investigated the effects of a tomato oleoresin extract, containing carotenoid lycopene with a high bioavailability, on DNA damage, immune and antioxidant status in non-smokers and smokers.

Abbreviations: IL, interleukin; TNF\(\alpha\), tumor necrosis factor \(\alpha\).
Materials and methods

Study design

A double-blind, randomized, placebo-controlled design was used. On the basis of a physical examination and medical history, 30 non-smoking and 25 smoking male volunteers were included in the study and randomly assigned into two groups. The extract group consisted of 15 non-smokers (aged 35.4 ± 11.8 years) and 12 smokers (aged 31.9 ± 12.1 years) and the placebo group was made up of 15 non-smokers (aged 31.2 ± 11.6 years) and 13 smokers (aged 38.6 ± 10.5 years). Each volunteer was placed on a restricted carrot and tomato product diet for 4 weeks. After a 2-week pre-treatment period the active treatment period began during which time each volunteer received three capsules containing either tomato oleoresin extract (Lyc-o-Mato) or placebo daily after dinner. All capsules were provided by LycoRed Natural Products Industries Ltd (Beer-Sheva, Israel). Each ‘Lyc-o-Mato’ capsule contained 4.88 mg lycopene, 0.48 mg phytomeno, 0.44 mg phytocarotene and 1.18 mg α-tocopherol. Blood samples were collected in the morning after an overnight fasting at the end of each treatment period.

Smoking is a more serious problem for women than for men. It has been reported that carotenoid bioavailability is influenced by the menstrual cycle and sex-dependent lipid metabolism. Although, only a 5–10% variation in plasma carotenoids during the menstrual cycle was observed, we decided to include only men in this study. The major reason for this decision was the high inter- and intra-subject variability found using the comet assay. To exclude the possible increase in variability, we decided to include only men. We are aware of this shortcoming and restrict our conclusions to men only. Further studies are needed to investigate such effects in women.

Chemicals

Unless otherwise stated, all chemicals were purchased from Merck (Darmstadt, Germany). Methanol, β-carotene and lutein were purchased from Carl Roth GmbH (Karlsruhe, Germany) and lycopene was a gift from BASF (Ludwigshafen, Germany). Tert-butyl methyl ether was from Riedel-de Haën (Seelze/Han, Germany).

Single cell microgel electrophoresis assay (comet assay)

DNA damage was measured by the single cell microgel electrophoresis assay (comet assay). Lymphocytes were isolated by density gradient centrifugation using Lymphoprep (Life Sciences, Eggenstein-Leopoldshafen, Germany). Twenty microliters of lymphocyte suspension (2 x 10⁶ cells) were mixed with 75 μl low-melting point agarose and were placed between two layers of agarose on a microscope slide. Three slides from each volunteer were placed in an electrophoresis chamber containing alkaline buffer (1 mM sodium carbonate, 10 mM Tris, 10% DMSO) for 1 h. Then, slides were placed in an electrophoresis chamber containing alkaline buffer (1 mM Na₂EDTA, 300 mM NaOH) for 40 min to allow DNA unwinding. Electrophoresis was carried out at 25 V, 300 mA for 20 min.

Fifty cells of each slide (150 cells/subject) were analyzed using the imaging software of Perceptive Instruments (Halstead, UK). The amount of damaged DNA was expressed as the percentage of DNA in the tail (tail intensity). To calculate the distribution of DNA damage within each sample, the images were grouped into classes with a different percentage of DNA in the tail [class 0 (0–6%), class 1 (6.1–17%), class 2 (17.1–55%), class 3 (55.1–60%), class 4 (60.1–100%)] in accordance with refs 15 and 16. Results are reported as means ± SD.

Plasma levels of fat-soluble compounds of the tomato oleoresin extract

Representative HPLC chromatograms of human plasma before and after the intervention are shown in Figure 1. Plasma levels of lycopene after 2 weeks of low-carotenoid diet were 249 ± 168 nM for non-smokers and 236 ± 123 nM for smokers. Intervention with the tomato oleoresin extract (three capsules daily) for 2 weeks resulted in significantly increased plasma levels of lycopene [from 275 ± 194 (week 0) to 785 ± 290 nM (week 2)] for non-smokers and [from 228 ± 101 (week 0) to 828 ± 204 nM (week 2)] for smokers. Intake of placebo between weeks 0 and 2 did not significantly change the lycopene plasma levels [from 223 ± 139 (week 0) to 145 ± 87 nM (week 2)] for non-smokers and [from 244 ± 144 (week 0) to 181 ± 162 nM (week 2)] for smokers.

Intervention with the tomato oleoresin extract-containing (in addition to lycopene) phytofluene and α-tocopherol led to a 1.8- (non-smokers) and 2.0-fold (smokers) increase in the plasma concentrations of phytofluene (data not shown). Placebo treatment caused a slight but not statistically significant decrease in the phytofluene plasma levels. The α-tocopherol baseline values of all subjects were within the normal range from 17 to 35 μM at baseline and were not affected by intervention [23 ± 6 (week 0) μM, 23 ± 7 μM (week 2) for non-smokers and 25 ± 9 μM (week 0), 26 ± 9 μM (week 2) for smokers]. Plasma concentrations of other carotenoids such as lutein, β-cryptoxanthin, α-carotene and β-carotene were not significantly different from baseline values neither after the ingestion period of the tomato oleoresin extract nor after the placebo treatment (data not shown).

DNA single strand breaks in lymphocytes

Non-smokers. There was no statistically significant difference in endogenous DNA strand breaks between the extract and placebo groups at week 0 (P = 0.31). Intervention with ‘placebo’ for 2 weeks did not affect DNA damage. The observed difference between the values at week 0 and at week 2 was
In contrast, intervention with tomato oleoresin extract for 2 weeks resulted in a decrease in DNA damage of ~32% (Figure 2). However, this effect was not statistically significant (P = 0.09). Additionally, we calculated each subject’s changes from baseline after 2 weeks in the placebo and tomato oleoresin extract groups. There was also no statistically significant difference (P = 0.27) between these groups.

Table IA shows the distribution of the degree of DNA damage expressed as the number of cells in the five comet classes, from 0 (undamaged) to IV (maximally damaged). More than 90% of cells were undamaged (class 0). Intervention resulted in a statistically significant (P < 0.05) increase in the percentage of undamaged cells and in a decrease in damaged cells in classes I and II. There was no statistically significant effect of intervention on the heterogeneity of DNA damage expressed as dispersion coefficient (Table IA).

Smokers. There was no statistically significant difference in DNA damage between the extract and placebo groups at week 0 (P = 0.39). Placebo supplementation showed a slight non-significant increase (16%) in DNA damage after 2 weeks. In contrast, intervention with tomato oleoresin extract for 2 weeks resulted in a decrease in DNA damage of ~39% (Figure 2B). However, this effect was not statistically significant (P = 0.12) either. The lack of significance may be explained by the high inter-individual variability of the low levels of DNA damage even in healthy smokers and the relatively small sample size (n = 12/13).

There was a tendency towards reduced DNA damage in the tomato oleoresin extract group analyzed as each subject’s changes from baseline within 2 weeks (P = 0.10). The changes in the intercellular distribution of the degree of DNA damage and the heterogeneity (H) of DNA damage were not statistically significant in the smoker group (Table IB).

**Immunological functions**

At week 0 non-smokers significantly differed from smokers in production of IL-2 (P = 0.022) and IL-4 (P = 0.026). Treatment with the tomato extract had no effect on lymphocyte proliferation, NK cell activity, IL-2 production and TNFα production (Table II) but significantly reduced IL-4 production in smokers (P = 0.009).
The 2-week intervention with tomato oleoresin extract (14.6 mg lycopene/day) resulted in a pronounced increase in plasma lycopene concentration (0.51 and 0.60 μM for non-smokers and smokers, respectively). The increase in plasma levels of lycopene in non-smokers was slightly and statistically significantly (P = 0.325) lower than that in smokers. The relation between smoking and lycopene plasma concentrations is not clearly understood. It has been reported that serum lycopene concentrations decreased by ~40% after subjects smoked three cigarettes in 30 min (19). In line with our data, there are a number of publications that have shown no statistically significant differences in plasma lycopene concentrations between non-smokers and smokers (19–22).

In this study, the increase in plasma levels of lycopene was slightly higher than that after the 2-week intervention with 330 ml tomato juice (40 mg lycopene) (increase in plasma concentration 0.41 μM or tomato puree (7 mg lycopene/day) (increase in plasma concentration 0.41 μM), which resulted in enhanced protection against DNA damage (1,2,23). We observed that tomato extract treatment showed a tendency to reduce endogenous DNA strand breaks for non-smokers (P = 0.09) and smokers (P = 0.12). Evaluation of distribution of DNA damage within cells showed a statistically significant increase in the frequency of cells with undamaged DNA (class 0) and concomitant decrease in the frequency of cells with damaged cells (classes 1 and 2).

There were no statistically significant differences in endogenous DNA damage in lymphocytes between smokers and non-smokers at baseline. Controversial data on the effect of smoking on DNA damage investigated by comet assay have been reported. There are at least two reports showing statistically significant differences in endogenous DNA damage and equally as many showing no differences (24–27). It appears, that other exogenous factors such as nutrition may be responsible for these differences. Supplementation with high dose β-carotene in isolated form and smoking has been shown to increase the risk of lung cancer (11,12). Also, adverse effects of other carotenoids have been discussed. Here we showed that a tomato oleoresin extract providing lycopene decreased the level of endogenous DNA damage in the lymphocytes of smokers.

Carotenoids are known to modulate immune function in humans (28). A number of immune cell structures (e.g. cell membrane receptors) or functions (e.g. NK cell cytotoxicity) are particularly sensitive to oxidative stress. Carotenoids with their antioxidative capacity may help to prevent such oxidative damage and thereby support appropriate immune functions (28). Therefore, we investigated the effects of the tomato extract on several biomarkers of immunocompetence.

**Table I.** Dispersion coefficient and frequency distribution of DNA strand breaks in human peripheral blood lymphocytes (A) for non-smokers (B) for smokers

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Time</th>
<th>H¹</th>
<th>Comet classes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>Placebo</td>
<td>Before</td>
<td>2.9 ± 0.5</td>
<td>93.8 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>2.6 ± 0.6</td>
<td>91.5 ± 4.5</td>
</tr>
<tr>
<td>Tomato extract</td>
<td>Before</td>
<td>2.9 ± 1.0</td>
<td>91.5 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>3.1 ± 0.9</td>
<td>95.8 ± 27*</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>Placebo</td>
<td>Before</td>
<td>3.4 ± 0.8</td>
<td>92.4 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>3.1 ± 0.9</td>
<td>92.4 ± 3.1</td>
</tr>
<tr>
<td>Tomato extract</td>
<td>Before</td>
<td>3.2 ± 0.8</td>
<td>92.5 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>3.2 ± 0.8</td>
<td>95.2 ± 4.2</td>
</tr>
</tbody>
</table>

*Coefficient of dispersion (H).

* Different from placebo, P < 0.05.

**Table II.** Immunological markers of subjects supplemented with a tomato oleoresin extract or placebo for a period of 2 weeks (n = 12–16, mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Extract</td>
</tr>
<tr>
<td><strong>Proliferation (O.D.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>1.67 ± 0.23</td>
<td>1.48 ± 0.20</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.81 ± 0.26</td>
<td>1.37 ± 0.17</td>
</tr>
<tr>
<td><strong>NK cell activity (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>54 ± 19</td>
<td>53 ± 16</td>
</tr>
<tr>
<td>Week 2</td>
<td>54 ± 20</td>
<td>54 ± 17</td>
</tr>
<tr>
<td><strong>IL-2 (µg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>0.85 ± 0.44</td>
<td>0.72 ± 0.27</td>
</tr>
<tr>
<td>Week 2</td>
<td>0.91 ± 0.37</td>
<td>0.78 ± 0.24</td>
</tr>
<tr>
<td><strong>IL-4 (pg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>22 ± 11</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>Week 2</td>
<td>20 ± 8</td>
<td>17 ± 7</td>
</tr>
<tr>
<td><strong>Tumor necrosis factor-α (µg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>4.7 ± 1.6</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>Week 2</td>
<td>4.6 ± 2.6</td>
<td>4.5 ± 1.5</td>
</tr>
</tbody>
</table>

*P = 0.009 compared with smokers at week 0.

**Discussion**

The 2-week intervention with tomato oleoresin extract (14.6 mg lycopene/day) resulted in a pronounced increase in plasma lycopene concentration (0.51 and 0.60 μM for non-smokers and smokers, respectively). The increase in plasma levels of lycopene in non-smokers was slightly and statistically significantly (P = 0.325) lower than that in smokers. The relation between smoking and lycopene plasma concentrations is not clearly understood. It has been reported that serum lycopene concentrations decreased by ~40% after subjects smoked three cigarettes in 30 min (19). In line with our data, there are a number of publications that have shown no statistically significant differences in plasma lycopene concentrations between non-smokers and smokers (19–22).

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Carotenoids are known to modulate immune function in humans (28). A number of immune cell structures (e.g. cell membrane receptors) or functions (e.g. NK cell cytotoxicity) are particularly sensitive to oxidative stress. Carotenoids with their antioxidative capacity may help to prevent such oxidative damage and thereby support appropriate immune functions (28). Therefore, we investigated the effects of the tomato extract on several biomarkers of immunocompetence. Lymphocyte proliferation, NK cell cytotoxic activity as well as the production of the cytokines IL-2 and TNFα were not affected by the tomato extract. This is in contrast to results from earlier studies with carrot and tomato juices that showed an enhancing effect on these immune parameters in males consuming a low-carotenoid diet (7,8). The main difference between these studies is that in the present study we used a tomato oleoresin extract. This
indicates that the oleoresin extract could not completely mimic the effect of tomato juice.

IL-4 production at baseline was significantly higher in smokers than in non-smokers, a fact that has also been reported by others (29). This high IL-4 production may contribute to the increased susceptibility of smokers to certain airway disease conditions such as viral or mycobacterial infections (30). Tomato extract intake in smokers resulted in significantly reduced IL-4 production similar to the level found in non-smokers. This suggests that the supplement was effective in normalizing the elevated IL-4 production in smokers. Since oxidative stress is known to enhance IL-4 gene expression (31), antioxidants in the tomato oleoresin extract may have mediated the reduction in IL-4 production. Whether the decreased IL-4 production in smokers taking a tomato oleoresin extract would reduce the risk of airway inflammation is currently not known. A recent study also reported that peripheral blood lymphocytes supplemented in vitro with vitamin E significantly reduced IL-4 production and down-regulated IL-4 mRNA expression (32). However, in the present study the slightly increased vitamin E intake (5.4 mg/day) with the tomato oleoresin extract did not affect vitamin E plasma levels suggesting that other tomato extract compounds, such as phytofluene, may be responsible for the suppressive effect on IL-4 production. In vitro studies with quercetin, another constituent of tomato with antioxidant activity, have shown inhibition of T-helper 2-lymphocyte-derived cytokine production such as IL-4 was observed (33). Overall, the data from the literature and the present data suggest that the antioxidative capacity of tomato oleoresin extract is responsible for the reduction in DNA damage and IL-4 production.

In conclusion, a tomato oleoresin extract showed a tendency to reduce endogenous DNA strand breaks in human peripheral lymphocytes in non-smokers and smokers. The damage distribution showed a statistically significant shift in the pattern—from more to less DNA damage in non-smokers. Minor effects on immune functions were observed with a significant reduction in IL-4 overproduction in smokers. However, other immune cell functions involved in tumor recognition and elimination, such as NK cell cytotoxicity, were not affected. The oleoresin tomato extract is less complex and contains a lower number of different compounds than tomato products. This extract was able, at least partly, to mimic the effects of tomatoes (effects on DNA damage and IL-4). This study should help to identify the compounds in tomatoes responsible for the beneficial health effects.

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References


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