Quantitative Analysis of Carotenoids and DNA Oxidation Products in Human Tissues Using LC-MS-MS

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Lycopene and Prostate Cancer Prevention

Lycopene

- Carotenoid responsible for red color of tomatoes
- Also found in watermelon and pink grapefruit
- Available as a dietary supplement
- Potent antioxidant
- Associated with reduced risk of prostate cancer
APCI vs Electrospray for LC-MS-MS of Carotenoids

\[ y = 0.0098x - 0.3288 \]

\[ R^2 = 0.9981 \]
Negative Ion APCI MS-MS of Lycopene

$[M - C_5H_9]^-$

$m/z$ 467, 536, 540

Relative Abundance

$m/z$ (m/z) 300, 340, 380, 420, 460, 500, 540, 580
Negative Ion Electrospray Product Ion MS-MS with CID of Lycopene and \(-\text{Carotene} \)
LC-MS and LC-MS-MS of the Tomato Dietary Supplement Lyc-O-Mato (LycoRed)

C$_{18}$ HPLC and Negative Ion APCI

lycopene

-carotene

-carotene

LC-MS SIM of m/z 536

LC-MS, extract spiked with - and -carotene

LC-MS-MS SRM of m/z 536→467 of spiked extract

C₃₀ HPLC Negative Ion APCI LC-MS-MS of Lycopene in Human Plasma

Lycopene standard
m/z 536  467

all trans-lycopene

Human plasma extract
m/z 536  467

all trans-lycopene

cis-lycopenes

5-cis- lycopene

Retention time (min)
### Accuracy and Inter-day Precision of LC-MS-MS Analysis of Lycopene (Using the TSQ Quantum)

<table>
<thead>
<tr>
<th>Concentration of standard solution (M)</th>
<th>Nominal amount injected on-column (pmol)</th>
<th>Measured amount (mean SD, pmol)</th>
<th>Accuracy (% Deviation)</th>
<th>Inter-day precision (RSD %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5</td>
<td>5.47 ± 0.37</td>
<td>9.36</td>
<td>6.80</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>9.22 ± 0.75</td>
<td>-7.8</td>
<td>8.09</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>50.73 ± 2.77</td>
<td>1.46</td>
<td>5.46</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>97.78 ± 3.81</td>
<td>-2.22</td>
<td>3.90</td>
</tr>
<tr>
<td>50</td>
<td>500</td>
<td>498.69 ± 3.06</td>
<td>-0.26</td>
<td>0.61</td>
</tr>
</tbody>
</table>

* n=5 on five different days
Hypotheses to be Tested

- Lycopene prevents DNA oxidation both *in vitro* and *in vivo*.
  - Does lycopene prevent formation of multiple DNA oxidation products or only certain products?
  - Is lycopene a *pro-oxidant*?
  - Can *in vitro* experiments predict *in vivo* effects of lycopene?
- Oral administration of lycopene results in increased concentration in the prostate
- Serum lycopene reflects prostate levels
Structures of Target Oxidized Deoxynucleosides

8-oxo-dG

8-oxo-dA

HMDU
LC-UV-MS-MS Measurement of Oxidized Deoxynucleosides

1. Isolate DNA from cells or tissue
2. Precipitate DNA with 70% cold ethanol
3. DNA Hydrolysis
   - Nuclease P1
   - Alkaline Phosphatase
4. Ultrafiltration (no SPE) to remove enzymes
5. UV quantitation of dG, dA, dC & T
6. MS-MS quantitation of 8-oxo-dG, 8-oxo-dA & HMDU
7. LC-UV-MS-MS Analysis
LC-UV-MS-MS of Hydrolysate of Fenton-oxidized Calf Thymus DNA

Retention time (min)

MS-MS relative response

Absorbance 260 nm

UV at 260 nm

TG 275>116
HMDU 257>141
8-oxo-dG 282>192
8-oxo-dA 267>176

UV at 260 nm
Results: Reduction of 8-oxo-dG Levels in Calf Thymus DNA by 1 µg Lycopene

Fenton-type reagent: 1 mM FeSO₄ / 1 mM H₂O₂
Clinical Study Design

Preliminary Dietary Intervention Study

- Tomato sauce (32 men) (equiv. to 30 mg lycopene/d)

Prostate Cancer (randomized, placebo-controlled, double blind)

- Placebo (30 men)
- Lycopene 30 mg/day (30 men)

BPH (randomized, placebo-controlled, double blind)

- Placebo (30 men)
- Lycopene 30 mg/day (30 men)
Human Study Design

- 60 men diagnosed with benign prostate hyperplasia and 60 with prostate cancer who will have prostatectomy or biopsy are being recruited (119/120 to date).
- Subjects are randomized into two groups who either take a pill containing lycopene (30 mg) or a placebo every day for 3 wk in a double-blind study.
- Blood samples are obtained at the beginning and end of the intervention period for the measurement of lycopene, the DNA oxidation products 8-oxo-dG and 8-oxo-dA, and prostate specific antigen (PSA).
- Prostate tissue samples are obtained at the end of the study either from resected tissue or biopsy for the measurement of lycopene and DNA oxidation products.
Men with elevated serum PSA were recruited into a tomato sauce whole-foods based study prior to prostate biopsy to confirm prostate cancer. Biopsy and blood samples were obtained for lycopene and DNA oxidation measurement.

32 men with stage T1 or T2 adenocarcinoma of the prostate completed the study.

Subjects consumed 200 g (3/4 cup)/d tomato sauce in pasta dishes for 21-days. (30 mg/d lycopene)

Total lycopene was measured in serum and prostate tissue obtained at the time of radical prostatectomy.

8-oxo-dG was measured in peripheral blood white blood cells and prostate tissue

PSA levels were measured in serum before and after intervention
Lycopene in Human Serum and Prostate Tissue After Tomato Sauce Intervention (30 mg lycopene/day)
Prostate Tissue Oxidative DNA Damage in Prostate Cancer Patients After 21-Day Tomato Sauce Whole Foods Intervention

**Leukocytes**

- Baseline: ~0.65
- Day 21: ~0.45

**Prostate**

- Reference group: ~1.15
- Intervention group: ~0.75
Leukocyte Oxidative DNA Damage (8-oxo-dG) vs. Serum Prostate Specific Antigen (PSA) in ng/mL.

Box plots showing changes in leukocyte 8-oxo-dG/10^5 dG and PSA levels before and after intervention.
Conclusions
Tomato whole-foods intervention

- Lycopene in serum increased 2-fold
- Lycopene levels in prostate tissue increased 3-fold
- DNA oxidation (measured as 8-oxo-dG) decreased 21% in leukocytes
- DNA oxidation decreased 28% in prostate tissue
- PSA in serum decreased 20%
- These results are consistent with epidemiological data showing an inverse correlation between tomato consumption and risk of prostate cancer

Conclusions, cont.

- TSQ Quantum provided >50-fold improvement in APCI performance for carotenoid analysis compared to the older model Quattro II it replaced.
- Quantum provided 10-fold enhancement of sensitivity for nucleoside analysis using electrospray.
- Phase IIb clinical investigations of lycopene (30 mg/d) are in progress in men with prostate cancer or benign prostate hyperplasia (119 out of 120 have completed the protocol).
Related Presentations at the 52nd ASMS Conference from this Laboratory

- **MPY 469**: Liu and van Breemen. Quantitative analysis of monoglutamyl and polyglutamyl folate by hydrophilic interaction LC-MS-MS.
- **WPK 182**: Zhu and van Breemen. LC-MS-MS determination of TBARs as a measurement of lipid peroxidation in human serum.
- **WPP 296**: Pajkovic et al. Ultrafiltration LC-MS screening of natural products for ligands to the androgen receptor.
- And 13 others
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