Preclinical and Clinical Evaluation of Sulforaphane for Chemoprevention in the Breast

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**Lifetime Probability of Developing Cancer by Site, Women, US, 1998-2000**

<table>
<thead>
<tr>
<th>Site</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites</td>
<td>1 in 3</td>
</tr>
<tr>
<td>Bladder</td>
<td>1 in 7</td>
</tr>
<tr>
<td>Colon &amp; Rectum</td>
<td>1 in 7</td>
</tr>
<tr>
<td>Breast</td>
<td>1 in 7</td>
</tr>
<tr>
<td>Endometrium</td>
<td>1 in 6</td>
</tr>
<tr>
<td>Heart</td>
<td>1 in 23</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>1 in 17</td>
</tr>
<tr>
<td>Larynx</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Leukemia</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Nervous system</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Prostate</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Skin</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Uterus</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Uterine corpus</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>1 in 30</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>1 in 30</td>
</tr>
</tbody>
</table>

**Women's Cancers in the U.S. (Mortality Top 10)**

- Lung
- Breast
- Colon & Rectum
- Ovary
- Pancreas
- Leukaemia
- Non-Hodgkin Lymphoma
- Uterus
- Multiple Myeloma
- Brain & Other Nervous System

**DNA to Enzymes...**

- DNA
- mRNA
- Transcription
- "Chromosomes"
- "Gene Expression"
- "Gene Transcription"
Some Good News…

Vegetable Consumption and Breast Cancer Risk

- Epidemiological studies (10 case-control and 7 prospective cohort) provide evidence for a modest inverse relationship between quantities of fruit and vegetables consumed and the risk of developing breast cancer.
- In the majority of the studies, only limited information on cruciferous intake was available.

Cruciferae (aka Brassicaceae, mustard family)

- Cruciferous vegetables:
  - Broccoli
  - Brussels sprouts
  - Cauliflower
  - Chinese cabbage
  - Horseradish
  - Mustard
  - Chinese radish
  - Wasabi
  - Watercress
  - White mustard

Cruciferous Vegetables and Chemoprevention

- In cruciferous vegetables, substances known as isothiocyanates exist as stable sugar conjugates called glucosinolates.
- Glucosinolates, the predominant glucosinolate in broccoli sprouts, is converted to sulforaphane by myrosinase, a plant enzyme, during chewing of plants or hydrolysis by gut microflora.

Sulforaphane’s Mechanism of Action Inside the Cell
A major mechanism for protection against carcinogenesis involves induction of cytoprotective enzymes.

**Phase 1 Enzymes**
- Cytochrome P450s
- GSTs, UGTs, QR, γ-GCS, AFAR

**Phase 2 Enzymes**
- HO-1, ferritins, MnSOD, catalase, LTB4DH
- GSTs, NQO1, SULT1A1

**Cruciferous Vegetables and Chemoprevention**

*Cruciferous Vegetables and Chemoprevention* (T Kensler, 2006 Breast Spore Update)

**Market Stage Broccoli** (150 grams)
**3-Day Sprouts** (3 grams)
**Freeze-Dried Sprout Extract** (150 mg)

All preparations contain the same quantity of enzyme inducer activity.

**Anthracenes and Cancer**

www.cardmine.co.uk
Prevention of DMBA-induced Mammary Tumors

- At age 47, 48, 49, 50, and 51 days each female Sprague-Dawley rat received by gavage either vehicle, 75 or 150 μmol sulforaphane.
- On day 50, all rats received DMBA (8.0 mg).
- Overall, the incidence, multiplicity, and weight of mammary tumors were significantly reduced, and their development was delayed.

Broccoli sprouts, which contain abundant quantities of glucoraphanin, the sulforaphane precursor, are safe, effective, inexpensive and a practical means to achieve chemoprevention against breast cancer.

Hypothesis

Preclinical Assay: Specific Aims

Determine the pharmacokinetic and pharmacodynamic action of sulforaphane in the mammary epithelium of rodents and develop and validate biomarkers to assess chemopreventive efficacy.

1. What is the distribution pattern of a single dose of sulforaphane in the mammary gland over time?
2. How does a single dose of sulforaphane affect cytoprotective gene transcript levels over time (NQO1, HO-1)?
3. How does a single dose of sulforaphane affect cytoprotective enzyme activity over time (NQO1)?
4. In which cells of the mammary gland do we see cytoprotective protein induction (HO-1, SULT1A1)?

Preclinical Assay Design

- 150 μmol sulforaphane delivered in 400 μl corn oil after overnight fasting.

Pharmacokinetics of Sulforaphane
Pharmacokinetics of Sulforaphane

HPLC & Photodiode Array Detector

(Data ± SEM, n = 3 rats/group)

Effect of Sulforaphane on NQO1 and HO-1 Transcripts

RNA Bioanalyzer & RT Detection System

(Data ± SEM, n = 3 rats/group)

Effect of Sulforaphane on NQO1 Enzymatic Activity

Micro Plate Reader

(Data ± SEM, n = 3 rats/group)
Effect of Sulforaphane on NQO1 Enzymatic Activity

(distance ± SEM, n = 3 rats/group)

Localization of HO-1 and SULT1A1 Induction by Sulforaphane

Localization of HO-1 Induction by Sulforaphane

Original magnification X 100; Scale bar = 50 μm

Localization of SULT1A1 Induction by Sulforaphane

CONTROL

TREATED (48 Hours)
Preclinical Assay: Specific Aims

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Preclinical Assay Major Aim

Determine the pharmacokinetic and pharmacodynamic action of sulforaphane in the mammary epithelium of rodents and develop and validate biomarkers to assess chemopreventive efficacy and translate these findings to a human population at elevated risk for breast cancer.

Clinical Pilot Study

Primary aim: Determine whether sulforaphane and/or its metabolites directly reach human breast tissue.

Eligibility criteria:
- Non-smokers
- No prior history of cancer
- Not on aspirin, NSAIDs, antibiotics (for at least 1 week before surgery)
- Scheduled to undergo a reduction mammoplasty
- Be 18 yrs or older
- Have signed informed consent

Clinical Pilot Study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N= 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yrs)</td>
<td>30 (21-50)</td>
</tr>
<tr>
<td>Race Caucasians</td>
<td>1</td>
</tr>
<tr>
<td>African Americans</td>
<td>9</td>
</tr>
<tr>
<td>Pre-op Weight (kg)</td>
<td>99 (68.3 -158.8)</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>9</td>
</tr>
<tr>
<td>Current ETOH drinkers</td>
<td>6</td>
</tr>
</tbody>
</table>
**Clinical Pilot Study**

- Ten women were recruited from April 05 to March 06
- BSP dose (200 µmol ITC) was given on average 53 min (range 16 – 82) before surgery
- Side effects: 2 individuals complained of a bitter taste in mouth
  - 2 burning sensation in throat
  - 1 burning in stomach

**Clinical Pilot Study**

- Average time between BSP and removal was similar between right and left breast tissue:
  - left: 117 min ± 36
  - right: 103 min ± 26
- Mean weight of breast tissue removed:
  - left: 846 grams ± 412
  - right: 916 grams ± 433

**Clinical Pilot Study (Pharmacokinetics)**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pre-dose</th>
<th>Mean ± SE</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Breast Tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(pmol/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post dose (~110 minutes)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
</tr>
<tr>
<td>Breast Tissue</td>
<td>N/A</td>
<td>2.00 ± 1.95</td>
</tr>
<tr>
<td>Urine (µM)</td>
<td>4.1 ± 5.2</td>
<td>158.9 ± 93.9</td>
</tr>
<tr>
<td>Plasma (µM)</td>
<td>0.01 ± 0.02</td>
<td>0.9 ± 0.7</td>
</tr>
</tbody>
</table>

**Clinical Pilot Study (Pharmacodynamics)**

- Due to short interval between ingestion of broccoli sprouts preparation and breast resection unable to evaluate pharmacodynamic effect
- Did detect and measure NQO1 and HO-1 transcripts and NQO1 enzymatic activity in the collected mammary tissue
- Developing immunostaining assays to enable future localization of cytoprotective enzyme induction

**Randomized Case-Control Clinical Trial**

Diagram of Study Plan:

- Reduction
- Pre-treatment
- Post-treatment
- Administration
- Surgery

Diagram showing steps:

1. Reduction
2. Pre-treatment
3. Post-treatment
4. Administration
5. Surgery
Note: Data from participating states and the District of Columbia were aggregated to represent the United States.

Trends in Consumption of Five or More Recommended Vegetable and Fruit Servings for Cancer Prevention, Adults 18 and Older, US, 1994-2002

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