Berry Polyphenols in the Prevention of Primary and Recurrent Breast Cancer

Harini S Aiyer, Ph.D.
Lombardi Comprehensive Cancer Center
Georgetown University

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Overview

• Breast cancer – incidence and risk factors
• Selection of berries for breast cancer prevention
• Berries in mammary tumor prevention
• Mechanisms by which berries prevent breast cancer
• Future directions
Breast Cancer - Causes

Sporadic breast cancer: 90%

Familial breast cancer: 10%
# Breast cancer - risk factors

## Non-Modifiable Risk Factors
- Age ↑
- Gender
- Genetics
- Family history ↑
- Previous breast disease ↑

## Modifiable Risk Factors
- Diet
- Alcohol ↑
- Smoking ↑
- Body weight ↑
- Exercise ↓
- Radiation

## Hormonal Risk Factors
- Cumulative exposure to estrogen ↑
- Age at menarche and menopause
- Parity
- Lactation ↓
- Hormone replacement therapy ↑
Berry polyphenols

- Excellent antioxidant properties
- Interaction with Estrogen receptor
- Cause cell-cycle arrest
- Interfere with cell-signaling pathways
- Interact with multiple pathways involved in carcinogenesis
Effect of ellagic acid on 4-hydroxy etsradiol-induced oxidative DNA damage

Aiyer et al., Int J Mol Sci. 2008 Mar;9(3):327-41
Next step-in vivo study (Short-Term)

- Received Mice
- Day 1
- Day 5
- Day 25

Control Diet

Supplemented Diet
- 5% (w/w) berries based on EA content
  - Blueberry (low <100 ppm)
  - Strawberry (moderate ≈ 600 ppm)
  - Red raspberry (high ≈ 1500 ppm)
- Ellagic acid (400 ppm)

DNA damage
(\(^{32}\)P-postlabeling)

Genomics
(microarray)

Euthanized

1- Daniel et al., 1993
These diets also significantly reduced baseline hepatic-DNA damage in a similar fashion.

Aiyer et al., *Int J Mol Sci.* 2008 Mar;9(3):327-41
Next step- tumorigenesis study

- 3 - 2 0 24 Weeks

Control diet

Experimental diet

Termination

Sham or 17ß-Estradiol Implants

<table>
<thead>
<tr>
<th>DIET</th>
<th>DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (AIN 93M)</td>
<td>NA</td>
</tr>
<tr>
<td>Blueberry</td>
<td>2.5% (w/w)</td>
</tr>
<tr>
<td>Black raspberry</td>
<td>2.5% (w/w)</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>400 ppm</td>
</tr>
</tbody>
</table>

Receive Animals
### Rationale for berry types used

<table>
<thead>
<tr>
<th>DIET</th>
<th>DOSE</th>
<th>Ellagic acid content</th>
<th>Anthocyanin content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry</td>
<td>2.5% (w/w)</td>
<td>Low (&lt;100 ppm)</td>
<td>Moderate (≈ 4000 ppm)</td>
</tr>
<tr>
<td>Black raspberry</td>
<td>2.5% (w/w)</td>
<td>High (≥1500 ppm)</td>
<td>High (≈ 7000 ppm)</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>400 ppm</td>
<td>Pure compound</td>
<td></td>
</tr>
</tbody>
</table>

**Blueberry**
- Cyanidin: 4.3 mg/g dry wt
- Petunidin: 10.7 mg/g dry wt
- Delphinidin: 17.2 mg/g dry wt
- Malvidin: 7.7 mg/g dry wt
- Pelargonidin: 31.2 mg/g dry wt
- Peonidin: 97.4 mg/g dry wt

**Black Raspberry**
- Cyanidin: 12 mg/g dry wt
- Petunidin: 20 mg/g dry wt
- Delphinidin: 33.8 mg/g dry wt
- Malvidin: 3.8 mg/g dry wt
- Pelargonidin: 18.6 mg/g dry wt
- Peonidin: 2.5 mg/g dry wt

*Aiyer et al., Berries and Cancer Prevention, Eds Stoner GD and Seeram NP, Springer, 2011*
Berry diets increase mammary tumor latency

Estrogen treatment associated morbidity in ACI rats
Berry diets reduce treatment-associated morbidity in ACI rats
Berry diets diminish treatment-associated mortality

Morbidity score – 1 to 5
(1-best; 5-worst.)

- Weight loss > 1 g per day
- Hair loss ≥ 3
- Crouch ≥ 3
- Eye deposits ≥ 3
- Loss of balance ≥ 3
- Tumor size ≥ 1.3 cm (not included in mortality index)

Berry diets decrease tumor volume and multiplicity

Aiyer et al., Nutr Cancer. 2008;60(2):227-34.
BERRY DIETS REDUCE TUMOR INCIDENCE

BERry diets modulate phase I enzymes

BERRY diets also modulate phase II enzyme

Berry diets inhibit E2-induced cell proliferation

PCNA positive cells (%)

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Diet</th>
<th>Control</th>
<th>Control</th>
<th>Blueberry</th>
<th>Black raspberry</th>
<th>Ellagic acid</th>
</tr>
</thead>
</table>
Berry phytochemicals act as antiestrogens

Adapted from Aiyer et al., Berries and Cancer Prevention, Eds Stoner GD and Seeram NP, Springer, 2011
Berry phytochemicals act as antiestrogens

Adapted from Aiyer et al., Berries and Cancer Prevention, Eds Stoner GD and Seeram NP, Springer, 2011
Overall mechanism of primary cancer prevention by berries

Aiyer et al., Berries and Cancer Prevention, Eds Stoner GD and Seeram NP, Springer, 2011
So how much berries do you need to eat?

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Blueberry diet</th>
<th>Black raspberry diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Units</strong></td>
<td></td>
<td>1%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Berries consumed by rats based on caloric intake (1g feed = 4 kcal)</td>
<td>mg/kcal</td>
<td>-</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allometric scaling to human consumption (2,000 kcal/day)</td>
<td>g dried berry</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>powder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common conversion for dried berries</td>
<td>Tbsp/d</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>Common conversion for fresh berries</td>
<td>Cups/d</td>
<td>-</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Source:** Aiyer et al., *Berries and Cancer Prevention*, Eds Stoner GD and Seeram NP, Springer, 2011

1 cup a day
Future- questions & directions

• Mechanistic
  – What is the effect of berry diets on E2 metabolism and clearance in the liver?
  – What is the effect of berry diets on pituitary-ovarian axis?
  – Do berry diets affect other cell-signaling pathways (Erbb2, MAPK etc) involved in E2-induced tumorigenesis?

• Translational
  – What is the effect of berry-intervention on women who have been diagnosed with breast cancer?
  – What are the possible interactions between berry constituents and current chemotherapy regimens in breast cancer?
Proposed mechanisms of AntiEstrogen action

- **Stromal Cells**: Paracrine effects
- **Cell membrane**: Free radicals, NFkB mediated signaling
- **Cytoplasm**: ER, CoA, CoR, E2
  - **Transcriptional effects**
- **Nucleus**: ER-independent signaling, Kinase signaling

**Liver**: Metabolism of and to estrogens

**Immune function**: Autocrine effects

Adapted from Clarke et al., 2001
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  - Gupta Lab

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