Mechanism of Polymeric Black Tea Polyphenols in Chemoprevention

Prepared By:
Khushboo A. Gandhi,
Anand Pharmacy College
Cancer - Uncontrolled proliferation of cell with potential to spread to other organs

Carcinogenesis - Complex, multistep and multifactorial process
Consist of initiation, promotion and progression

Schematic representation of multistep-carcinogenesis

(Adopted from Surh et al., 1999)
CHEMOPREVENTION

Use of natural or synthetic compounds to prevent, suppress or delay the process of carcinogenesis.
List of Herbal anti-oxidants Studied for chemoprevention

- Aegle Marmelos (Singh et al., 2000)
- Garlic (Yang et al., 2001)
- Neem (Dasgupta et al., 2004)
- Onion (Belman, 1983)
- **Black Tea**/ Green Tea (Lambert & Yang, 2003)
- Turmeric (Aziune et al., 1992)
- Amla (Jose et al., 2001)
- Clove (Zheng et al., 1992)
- Capsicium (Surh, 2002)
- Grapes (Aziz et al., 2003)
Polymeric Black Tea Polyphenols

Polyphenols - Most significant group of components in tea

Green tea (20%)

Tea leaves -> Dried

PPO inactivated

Catechins 90%

Black tea (78%)

Tea leaves -> Dried

PPO mediated oxidation

Thearubigins / PBPs 47%

Catechins 30%

Theaflavins 13%

Polyphenol Content of Green and Black tea

<table>
<thead>
<tr>
<th></th>
<th>g % of dry solid extracted *</th>
<th>g % of dry total polyphenols content</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLACK TEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechins (Monomers)</td>
<td>3-10</td>
<td>30</td>
</tr>
<tr>
<td>Theaflavins (Oligomers)</td>
<td>3-6</td>
<td>13</td>
</tr>
<tr>
<td>Thearubigins/PBPs (Polymers)</td>
<td>12-18</td>
<td>47</td>
</tr>
<tr>
<td>GREEN TEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechins (Monomers)</td>
<td>30-42</td>
<td>90</td>
</tr>
<tr>
<td>Theaflavins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thearubigins/PBPs</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* % of solid extracted from black tea = 25-35%

(Adopted from Kumar et al., 2010, MRMC, 10: 492-505)

- PBPs content of an average cup of tea is 65mg/100ml (150mg per 235ml cup)
Previous studies shown that PBPs possess anti-initiating and anti-promoting activity \textit{in vivo} in different animal models

(Patel et al., 2008; Kumar et al., 2012)
OBJECTIVE

Evaluation of Chemopreventive Efficacy and Mechanisms of anti-initiating activities of Polymeric Black Tea Polyphenols (PBPs) / Thearubigins (TRs) in B(a)P-induced skin epidermis
Extraction of Black Tea Polyphenols (PBPs)
(By Soxhlet Based Solid Liquid Extraction Method)

(Krishnan et al., 2006, Food chemistry; 94: 331-340)
450 gm Black tea powder

Extracted with CHCl₃ in Soxhlet continuous extractor

- **CHCl₃ extract**
  - Dried tea powder
    - Extracted with ethyl acetate in Soxhlet continuous extractor
      - **Ethyl acetate extract**
        - Vacuum Dried
        - Dissolve in acetone
        - ppt. with diethyl ether (10 Vol.)(3 times)
        - PBP-1

- **Dried tea powder**
  - Extracted with butanol in Soxhlet continuous extractor
    - **Butanol extract**
      - Vacuum Dried
      - Dissolve in methanol
      - ppt. with diethyl ether (10 Vol.)(3 times)
      - Acidified with sulfuric acid & extracted with n-butanol
      - n-butanol extract II
      - Process same as for PBP-2 & 3
      - PBP-4 and PBP-5

(Krishnan et al., 2006, Food chemistry; 94: 331-340)
Evaluation of contamination of catechins and TFs in PBPs

EC = Epicatechin, ECG = Epicatechin gallate, EGC = Epigallocatechin, EGCG = Epigallocatechingallate, GCG = Gallocatechin gallate, C = Catechin, TF = Theaflavin, CF = Caffeine, TR = Thearubigin (PBPs)

Demonstrates the absence of known biologically active black tea-derived contaminants (Caffeine, C, EC, ECG, EGC, EGCG and TFs)

Yield of different PBPs

<table>
<thead>
<tr>
<th>PBPs</th>
<th>Wt.(gm)/450 gm of dry tea</th>
<th>% of dry tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBP-1</td>
<td>12.06</td>
<td>2.68</td>
</tr>
<tr>
<td>PBP-2</td>
<td>17</td>
<td>3.79</td>
</tr>
<tr>
<td>PBP-3</td>
<td>6.03</td>
<td>1.34</td>
</tr>
<tr>
<td>PBP-4</td>
<td>9.9</td>
<td>2.2</td>
</tr>
<tr>
<td>PBP-5</td>
<td>1.44</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Different fractions of PBPs obtained from Black tea

PBP-1 PBP-2 PBP-3 PBP-4 PBP-5
# Physicochemical Properties of Polymeric Black Tea Polyphenol Fractions

<table>
<thead>
<tr>
<th>property</th>
<th>PBP-1</th>
<th>PBP-2</th>
<th>PBP-3</th>
<th>PBP-4</th>
<th>PBP-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Brown</td>
<td>Light Brown</td>
<td>Light Yellow</td>
<td>Dark Brown</td>
<td>Brownish Black</td>
</tr>
<tr>
<td>pH of 1% aq. Solution</td>
<td>5.53</td>
<td>5.55</td>
<td>5.40</td>
<td>4.26</td>
<td>4.09</td>
</tr>
<tr>
<td>λ max1 (nm)</td>
<td>211</td>
<td>219</td>
<td>217</td>
<td>211</td>
<td>210</td>
</tr>
<tr>
<td>λ max2 (nm)</td>
<td>272</td>
<td>272</td>
<td>268</td>
<td>263</td>
<td>270</td>
</tr>
<tr>
<td>λ max1/λ max2</td>
<td>2.78</td>
<td>1.68</td>
<td>1.78</td>
<td>3.21</td>
<td>6.28</td>
</tr>
<tr>
<td>FeCl3 reactivity</td>
<td>Weakly Positive</td>
<td>Weakly Positive</td>
<td>Weakly Positive</td>
<td>Weakly Positive</td>
<td>Weakly Positive</td>
</tr>
</tbody>
</table>
### Animal Study

All animal studies were conducted after approval from the Institutional Animal Ethics Committee (ACTREC, Mumbai) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>Pre-treatment</th>
<th>Treatment after 20 mins of pre-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>Acetone</td>
<td>Acetone</td>
</tr>
<tr>
<td>2</td>
<td>PBP-5 control</td>
<td>PBP-5</td>
<td>Acetone</td>
</tr>
<tr>
<td>3</td>
<td>PBP-3 control</td>
<td>PBP-3</td>
<td>Acetone</td>
</tr>
<tr>
<td>4</td>
<td>PBP-mix control</td>
<td>PBP-mix</td>
<td>Acetone</td>
</tr>
<tr>
<td>5</td>
<td>B(a)P control</td>
<td>Acetone</td>
<td>B(a)P</td>
</tr>
<tr>
<td>6</td>
<td>PBP-5 + B(a)P</td>
<td>PBP-5</td>
<td>B(a)P</td>
</tr>
<tr>
<td>7</td>
<td>PBP-3 + B(a)P</td>
<td>PBP-3</td>
<td>B(a)P</td>
</tr>
<tr>
<td>8</td>
<td>PBP-mix + B(a)P</td>
<td>PBP-mix</td>
<td>B(a)P</td>
</tr>
</tbody>
</table>

Animal Model: Swiss bare mice (6-8 weeks)
For 3 days

Acetone/ 200 µg PBP5/
PBP3/ PBPmixture in 0.1 ml acetone

20 mins

On 3rd day

Acetone/ 1 mg B(a)P in 0.1 ml acetone

24 hrs
Effect of PBPs on CYP1A1 and CYP1A2 activity

Data represent mean ± standard error of four observations (three pooled epidermis for one sample). Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, p 0.05. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective P5+BP.
Effect of PBPs on CYP1A1 and CYP1A2 expression

Data represent mean ± standard error of five observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, p ≤ 0.05. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective B+P5.
Effect of PBPs on mRNA levels of CYP1A1 and CYP1A2
Effect of PBPs on Basal levels of AhR
Effect of PBPs on AhR levels

- Total AhR
- β-actin
- Cytosolic AhR
- Tubulin
- Nuclear AhR
- Histone H1

Comparison of relative optical density with different PBPs and controls.
Effect of PBPs on AhR related Proteins (WB)

- PBPs did not alter levels of Hsp90 and XAP-2
**Effect of PBPs on Hsp90 ATPase activity**

Data represent mean ± standard error of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, \( p \leq 0.05 \). ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective P5+BP.
Effect of PBP on binding of ligand: AhR complex to Arnt

Data represent mean ± standard error of three observations. Band density of IP Arnt was normalized with band density of Arnt. Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, p ≤ 0.05. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘£’ significant when compared with respective P5+BP.
Effect of PBPs on phosphorylation of AhR
Effect of PBPs on AhR: DNA binding (EMSA)
Effect of PBPs on DNA adduct formation
Effect of PBPs on DNA adduct formation

Results are presented as representative photomicrographs at X400 magnification. Quantitative analysis was done by digital image analysis in minimum 10 photomicrographs with at least three mice per group. Whereas semi-quantitative analysis was done by counting percentage of nuclei with BPDE-DNA adducts in only epidermis of 10 randomly selected images with at least three mice per group. Data represent mean ± SE of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, p ≤ 0.05. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective P5+BP.
Effect of PBP5s on levels of COX-2

Data represent mean ± standard error of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, p ≤ 0.05. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective B+P5.
Effect of PBPs on levels of PGE2

Data represent mean ± standard error of three observations. Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, $p \leq 0.05$. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective B+P5.
Effect of PBPs on B(a)P induced Hyperplasia
Data represent mean ± standard error of three pooled sample (3 animals per sample). Differences among groups were determined by one-way ANOVA followed by Bonferroni’s test, $p \leq 0.05$. ‘*’ significant when compared with B(a)P; ‘#’ significant when compared with Acetone; ‘¥’ significant when compared with respective controls; ‘₤’ significant when compared with respective P5+BP.
Reference


Pongratz I. et al. Inhibition of the specific DNA binding activity of the dioxin receptor by phosphatase treatment. J. Biol. Chem. 1991; 266, 16813–17
Thank You