Nutraceuticals in chemoprevention

Novi Sad, 14 December 2016
What is Cancer?

Cancer is a disease characterized by out-of-control cell growth leading to spread of abnormal cells to other body parts by local invasion and/or distant metastasis. It is one of the major and growing public health problems, currently accounting for over 12% deaths globally.
Cancer risk factors

Genetic factors contribute only around 5–10% of the cancer risk, while environmental factors account for 90–95% (10–15% from chemical and industrial carcinogens, 15–20% from infections, 25–30% from tobacco, and 30–35% from diet)


Lifestyle represents the major determinant of cancer risk, practical approaches in cancer prevention interventions include pursuing lifestyle or dietary changes.
Chemoprevention

“Chemoprevention” is defined as the use of natural dietary agents able to prevent or interfere with the development or progression of neoplastic processes that lead to the appearance of cancer.

- Primary Chemoprevention: aims to prevent the development of disease in the general population.

Chemoprevention

- **Secondary Chemoprevention**: this focuses on individuals who have been diagnosed with some type of premalignant lesions that may progress to invasive cancer. This strategy aims to limit the development and progression of malignant lesions.

- **Tertiary Chemoprevention**: this type of chemoprevention is directly aimed at preventing the recurrence of new secondary tumours in individuals who have developed a cancer.
Chemoprevention

Chemotherapy

Chemoprevention (primary)

Chemoprevention (population at high risk)

Chemoprevention (secondary)

Chemoprevention (tertiary)
# Chemopreventive agents

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Calcium, vitamin D, β-carotene, green tea polyphenols, resveratrol</td>
</tr>
<tr>
<td>Moderate</td>
<td>Aspirin, Ibuprofen</td>
</tr>
<tr>
<td>High</td>
<td>Retinoid acid</td>
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A good chemopreventive

Chemoprevention, through the use of synthetic or natural compounds, represents the possibility to inhibit, stop or reverse the process of carcinogenesis.

Blockage of carcinogen formation, the induction of detoxification enzymes,

Slowing of cell division, induction of apoptosis and induction of differentiation of neoplastic cells constitute important chemopreventive actions.

A promising chemopreventive agent must show selectivity towards cancer cells and low toxicity on non-transformed cells.
Large number of pure compounds and extracts from dietary sources has been evaluated in various experimental models for testing their chemopreventive efficacy.
Dietary cancer chemopreventive compounds

- **Resveratrol**
  - Grape and red wine
  - *Vinca vinifera*

- **Lycopene**
  - Tomato
  - *Lycopersicon esculentum*, Solanaceae

- **Quercetin**
  - Blueberry
  - *Vaccinium myrtillus*, Ericaceae

- **Daidzein**
  - Soya beans
  - *Glycine max*, Leguminosae

- **Curcumin**
  - Turmeric
  - *Curcuma domestica*, Zingiberaceae

- **Sulforaphane**
  - Broccoli, *Brassica oleracea*
  - Cruciferae

- **Folate**
  - *Glycine max*, Leguminosae

- **Silymarin**
  - Milk thistle,
  - *Silybum marianum*, Compositae

- **Sulforaphane**
  - Broccoli, *Brassica oleracea*
  - Cruciferae

- **Sinalbin**
  - White mustard
  - *Sinapis alba*, Cruciferae

- **Sinigrin**
  - Black mustard
  - *Brassica nigra*, Cruciferae

- **Glucobrassicin**
  - Horseradish
  - *Armoracia rusticana*, Cruciferae

- **Indole-3-carbinol**
  - Cruciferous vegetables
  - Cruciferae

- **Genistein**
  - Soya beans
  - *Glycine max*, Leguminosae

- **(-)Epigallocatechin-3-O-gallate (EGCG)**
  - Green tea
  - *Camellia sinensis*, Theaceae
Chemopreventive activity is generally investigated employing:

- cancer cell lines to investigate apoptosis, cell proliferation detoxification pathways and specific biochemical pathways.

- animal models with induced tumor to evaluate latency period and/or decrease in incidence.

Many dietary phytochemicals have been demonstrated to be effective reducing incidence and/or affecting latency period of carcinogen induced tumors at various organ sites in experimental rodent models.
Mechanisms of chemopreventive actions

Dietary phytochemicals

Anti-initiation
(Blocking agents)

- Inhibition of adduct formation/uptake
- Induction of phase II enzymes
- Inhibition of phase I enzymes
- Scavenging of ROS / carcinogens
- Induction of DNA repair
- Induction apoptosis of initiated cells

Anti-promotion
(Suppressing agents)

- Inhibition of clonal expansion (cell cycle arrest)
- Induction of terminal differentiation, senescence
- Modulation of signal transduction, angiogenesis, immunomodulation
Overall findings of experimental studies

1. Relatively long and repeated exposures to dietary phytochemicals have generally been needed for observing protective effects;

2. Most of the dietary phytochemicals have been demonstrated to be effective against several classes of environmental carcinogens at multiple organ sites;

3. Bioavailability of dietary phytochemicals and their metabolites have not been reported from most of the experimental studies that demonstrated their chemopreventive efficacy;

4. In most of these studies doses of chemopreventive agent(s) administered or effective doses appear to be much higher than normal dietary exposures in human.

Some examples

Isothiocyanates

Anthocyanins

Castanea sativa bark extract
## Isothiocyanates

![Isothiocyanate and Glucosinolate Reaction Scheme](image)

<table>
<thead>
<tr>
<th>Side chain (R)</th>
<th>Chemical name</th>
<th>Glucosinolate</th>
<th>Isothiocyanate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$S(O)CH$_2$CH$_2$CH$_2$CH$_2$-</td>
<td>4-methylsulfinylbutyl-</td>
<td>Glucoraphanin (GRA)</td>
<td>Sulforaphane (SFN)</td>
</tr>
<tr>
<td>CH$_3$SCH$_2$CH$_2$CH$_2$CH$_2$-</td>
<td>4-methylthiobutyl-</td>
<td>Glucoerucin (GER)</td>
<td>Erucin (ERN)</td>
</tr>
<tr>
<td>CH$_3$S(O)CH=CHCH$_2$CH$_2$-</td>
<td>4-methylsulfinyl-3-butenyl-</td>
<td>Glucoraphenin (GRE)</td>
<td>Sulforaphene (GRE-ITC)</td>
</tr>
<tr>
<td>CH$_3$SCH=CHCH$_2$CH$_2$-</td>
<td>4-methylthio-3-butenyl-</td>
<td>Glucoraphasatin (GRH)</td>
<td>Raphasatin (GRH-ITC)</td>
</tr>
</tbody>
</table>
Sulforaphane modulates carcinogens activation

Sulforaphane

Phase I enzymes (CYP450)

Precarcinogen

EXCRETION

Phase II enzymes (GST, UGT, NQO1)

Carcinogen

EXCRETION

Interazione col target

Growth and preparation for mitosis

DNA replication

Growth and normal metabolic roles

First growth phase

Second growth phase

Synthesis phase

Mitotic phase

Prophase

Metaphase

Anaphase

Telophase

Interphase

G1

G2

S
SF induces cell cycle arrest in Jurkat cells

SF induces apoptosis in Jurkat cells

8 h

Viable  Apoptotic  Necrotic

* *p<0.05, ** p<0.001 with respect to the control

24 h  48 h

Mean green fluorescence

*p<0.001 with respect to the control

Carcinogenesis 23: 581-6, 2002; Biochem Pharmacol 68: 1133-8, 2004
Molecular Targets of Sulforaphane

**Apoptosis**
- Caspases $\uparrow$
- Bcl-2 $\downarrow$
- Bax $\uparrow$
- Bax:Bcl-2 $\uparrow$
- p53 $\uparrow$
- PARP $\uparrow$
- IAP $\downarrow$
- Smac/Diablo $\uparrow$
- Calpain $\uparrow$
- p21$^{Cip1/Waf1}$ $\uparrow$
- Acetylated histones $\uparrow$

**Phase I enzymes**
- CYP1A1 $\downarrow$
- CYP2B1/2 $\downarrow$
- CYP3A4 $\downarrow$
- CYP2E1 $\downarrow$

**Phase II enzymes**
- GST1A1 $\uparrow$
- GSTA1/2 $\uparrow$
- GSTM1 $\uparrow$
- UGT1A1 $\uparrow$
- NQO1 $\uparrow$
- $\gamma$-glutamylcysteine synthetase $\uparrow$

**Cell cycle**
- Cyclin A $\uparrow$
- Cyclin B1 $\uparrow$
- Cyclin D1 $\downarrow$
- Cdk4 $\downarrow$
- p21$^{Cip1/Waf1}$ $\uparrow$
- Cdc25C $\downarrow$
- Kruppel-like factor 4 $\uparrow$
- GADD45b $\uparrow$
- PC3TIS21/BTG2 $\uparrow$
- SMAR1 $\uparrow$
- CKSHS2 $\uparrow$
- MCM4 $\downarrow$
- MCM7 $\downarrow$

**Angiogenesis**
- KDR $\downarrow$
- HIF-1 $\downarrow$
- VEGF $\downarrow$
- c-Myc $\downarrow$

**Metastasis**
- MMP-2 $\downarrow$
- Tissue inhibitor of metalloproteinase-2 $\downarrow$

**SULFORAPHANE**

SF is selective towards cancer cells

$IC_{50} = 50 \, \mu M$
Normal cells

$IC_{50} = 15 \, \mu M$
Jurkat cells
Anthocyanins in chemoprevention

Anthocyanidins & Anthocyanins

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
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<tbody>
<tr>
<td>Pelargonidin</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Peonidin</td>
<td>OMe</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Malvidin</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
</tr>
<tr>
<td>Pelargonidin-3-galactoside</td>
<td>H</td>
<td>H</td>
<td>galactose</td>
</tr>
<tr>
<td>Cyanidin-3-galactoside</td>
<td>OH</td>
<td>H</td>
<td>galactose</td>
</tr>
<tr>
<td>Cyanidin-3-rutinoside</td>
<td>OH</td>
<td>OH</td>
<td>rutinose</td>
</tr>
<tr>
<td>Cyanidin-3-glucosylrutinoside</td>
<td>OH</td>
<td>OH</td>
<td>glucose-rutinose</td>
</tr>
<tr>
<td>Delphinidin-3-galactoside</td>
<td>OH</td>
<td>OH</td>
<td>galactose</td>
</tr>
</tbody>
</table>
Malvidin and cyanidin inhibit cancer cells proliferation
Cyanidin 3-O-β-glucopyranoside induces apoptosis in Jurkat cells

Biochem Pharmacol 67: 2047-56, 2004
Overall findings of clinical trials
Overall findings of clinical trials

1. In most of the trials chemopreventive agents were administered to patients with cancer or high risk individuals, i.e., after occurrence of damage or disease;
2. Though there are several clinical trials conducted using dietary phytochemicals, results from only approximately 20% trials have been reported. Other trials have either not been completed or their results are not reported due to issues of toxicity, bioavailability and other unknown reasons under the conditions employed for the trials;
3. Bioavailability issues have been reported for curcumin, genistein, resveratrol, lycopene and green tea;
4. Toxicity issues have been reported in clinical trials conducted with tocopherols and retinoids;
5. Very few trials with curcumin and green tea have shown beneficial effects as judged by modulation of biomarkers and symptoms.
Clinical trials vs. experimental studies
Healthy Lifestyle

Eat More Fruits
Exercise
Drink More Water
Read Spiritual Books
Meditation
Walk More
Eat Well
Peace of Mind
Nome Cognome
Struttura
Contatti

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