Molecular Interactions of Turmeric with Cancer Chemotherapy

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What is Turmeric?

- Turmeric is a member of the *Curcuma* botanical group, which is part of the ginger family of herbs, the Zingiberaceae. The root and rhizome (underground stem) of the *Curcuma longa* L. plant is crushed and powdered into ground Turmeric. Ground Turmeric is used worldwide as a seasoning, to make curry, and for its therapeutic effects.
<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae (Plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta (Vascular plants)</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta (Seed plants)</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta (Flowering plants)</td>
</tr>
<tr>
<td>Class</td>
<td>Liliopsida (Monocotyledons)</td>
</tr>
<tr>
<td>Subclass</td>
<td>Zingiberidae</td>
</tr>
<tr>
<td>Order</td>
<td>Zingiberales</td>
</tr>
<tr>
<td>Family</td>
<td>Zingiberaceae (Ginger family)</td>
</tr>
<tr>
<td>Genus</td>
<td>Curcuma L. (Curcuma)</td>
</tr>
<tr>
<td>Species</td>
<td>Curcuma longa L. (Turmeric)</td>
</tr>
</tbody>
</table>
Rhizome or underground stem
A teaspoon of turmeric a day can keep cancers at bay, according to a senior nutrition expert. The good old grandmother's practice of putting a pinch of turmeric to spice and spruce up curries has now proved to be protecting the human body.
Dietary turmeric and Alzheimer’s disease

Diets rich in Curcumin, a compound found in the curry spice Turmeric, may help explain why rates of Alzheimer’s disease are much lower among the elderly in India compared with their Western peers.
"Curcumin reduces inflammation caused by a buildup of a protein known as beta-amyloid, a plaque-like substance that blocks brain cells from communicating with each other and eventually affects your ability to remember. Accumulations of beta-amyloid plaques are linked to Alzheimer's disease."
<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Incidence</th>
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</thead>
<tbody>
<tr>
<td>Melanoma of Skin</td>
<td>1%</td>
</tr>
<tr>
<td>Oral</td>
<td>5%</td>
</tr>
<tr>
<td>Lung</td>
<td>22%</td>
</tr>
<tr>
<td>Colon and Rectum</td>
<td>14%</td>
</tr>
<tr>
<td>Other Digestive</td>
<td>12%</td>
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<tr>
<td>Prostate</td>
<td>17%</td>
</tr>
<tr>
<td>Urinary</td>
<td>9%</td>
</tr>
<tr>
<td>Leukemia and Lymphomas</td>
<td>8%</td>
</tr>
<tr>
<td>All Other</td>
<td>12%</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Cancer Site</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Melanoma of Skin</td>
<td>1%</td>
</tr>
<tr>
<td>Oral</td>
<td>2%</td>
</tr>
<tr>
<td>Breast</td>
<td>27%</td>
</tr>
<tr>
<td>Lung</td>
<td>6%</td>
</tr>
<tr>
<td>Colon and Rectum</td>
<td>15%</td>
</tr>
<tr>
<td>Other Digestive</td>
<td>9%</td>
</tr>
<tr>
<td>Uterus</td>
<td>14%</td>
</tr>
<tr>
<td>Urinary</td>
<td>4%</td>
</tr>
<tr>
<td>Leukemia and Lymphomas</td>
<td>7%</td>
</tr>
<tr>
<td>All other</td>
<td>15%</td>
</tr>
</tbody>
</table>

*Excluding non-melanoma skin cancer and carcinoma in situ of uterine cervix.
Geography of colon cancer incidence
Geography of Breast cancer incidence

(h) Breast
Geography of Leukemia incidence

- Jews born in Europe and in America
- Canada
- Israel
- United States
- United States (Negro)
- Norway
- Jamaica
- England
- Colombia
- Japan
- South Africa (Bantu)
- Bombay

(e) Leukemia
Geography of lung cancer incidence

Figure 5-2 (Continued)
Geography of Prostate cancer incidence

- United States (Negro)
- Canada
- United States
- Norway
- Jamaica
- Colombia
- South Africa (Bantu)
- England
- Israel
- Bombay
- Japan

(g) Prostate
Geography of Skin cancer incidence

(c) Skin
Geography of Skin cancer incidence

(d) Skin (excluding melanoma)
Geography of Stomach cancer incidence

(a) Stomach
Results of Epidemiology

• Incidence of cancer varies nation to nation.
• High incidence reported in western countries.
• Indian (Bombay) results of low incidence for cancer are more convincing may be due to their large daily dietary intake of spice turmeric.
• Data on turmeric and chemotherapy are lacking from India.
Curcumin and Cancer

- "Therapeutic potential of Curcumin in human prostate cancer. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of prostate cancer cells."
Curcumin and colon cancer

- Curcumin therefore appears to exert its anti-carcinogenic properties by inhibiting proliferation and inducing apoptosis in certain gastric and colon cancer cells.
Curcumin and cardiovascular system

- Curcumin exhibits a protective role on the cardiovascular system which include lowering of cholesterol and triglyceride levels.
Turmeric extracts and colon cancer

"Turmeric extracts were responsible for dramatic improvements in some patients with oral cancer and significantly reduced urinary excretion of tobacco mutagens in smokers. Also, the American Health Foundation, biomedical research center in Valhalla, N.Y., has demonstrated inhibition of colon cancer and regression of colon tumors with Turmeric extracts."
Turmeric as an anti-oxidant

Turmeric and its active Curcuminoids or Curcuminins and the water soluble peptide turmerin, have antioxidant properties and effectively inhibit the free radical damage to biomolecules both in vitro and in vivo conditions. The fact that Curcuminoids act as antioxidants by prevention and intervention processes, makes them very unique natural antioxidants"
"Turmeric (especially the Curcumin component) has rich stores of antioxidants. In the body these important disease-fighting substances mop up unstable oxygen molecules called free radicals that can otherwise damage cells and cause diseases such as cancer."
Turmeric Side Effects and Warnings.

- Turmeric may increase the risk of bleeding or potentiate the effects of warfarin therapy. - Am J Health Syst Pharm 2000 Jul 1;57(13):1221-7; quiz 1228-30 -- Potential interactions between alternative therapies and warfarin. -- Heck AM, DeWitt BA, Lukes AL. -- School of Pharmacy and Pharmacal Sciences, Purdue University, Indianapolis, IN, USA.
Side effects of Turmeric

• Do not use as a supplement if you have gallstones or during pregnancy.

• Allergic contact dermatitis from turmeric.
Curcumin, isolated from turmeric, has beneficial properties as an anti-inflammatory and chemopreventive agent. It is present in the diet, and also used as a coloring and flavoring additive in many foods worldwide.

Chemical structure of curcumin
http://www.nirs.go.jp
Hypothesis

- Curcumin inhibits c-Jun-N-terminal kinase (JNK) activation\(^1,2\), and reactive oxygen species (ROS) generation\(^3,4,5\).
- Many cytotoxic chemotherapy agents activate JNK and ROS as they induce apoptosis
- Dietary curcumin may block chemotherapy-mediated JNK and ROS effects, thereby decreasing the effectiveness of cancer chemotherapy

\(^1\)Huang et al. PNAS 88: 5292, 1991 \\
\(^2\)Chen et al. Oncogene 17:173, 1998 \\
\(^3\)Kunchandy et al. Int.J.Pharmacol. 58:237, 1990 \\
Chemotherapy

- Drugs that kill cancer cells. There are different ways to kill the cancer cells.
- According to their mode of action chemotherapy has been classified as
  - alkylating agent, eg. mechlorethamine and cyclophosphamide.
  - anthracyclines, eg. adriamycin
  - topoisomerase inhibitor, eg. camptothecin
  - antimetabolites, eg. 5-flourouracil, methotrexate
RECENT TREND by Dr. Watson

Apoptosis

- Often apoptosis is called 'programmed cell death'.
- The apoptosis program can be activated by a wide diversity of triggers ranging from normal, physiological micro-environmental 'cues' to toxic stimuli, such as radiation-induced genomic damage.
- The classical hallmarks of apoptosis - the morphological changes and the degradation of DNA into the typical oligo-nucleosomal fragments - are dependent upon a family of protein-snipping enzymes known as caspases.
The Mechanisms of Apoptosis

- There are 3 different mechanisms by which a cell commits suicide by apoptosis.
  1. one generated by signals arising within the cell
  2. another triggered by death activators binding to receptors at the cell surface, eg, TNF-α, Lymphotoxin, Fas ligand (FasL)
  3. a third that may be triggered by dangerous reactive oxygen species.
1. Apoptosis triggered by internal signals: the intrinsic or mitochondrial pathway;

• In a healthy cell, the outer membranes of its mitochondria express the protein **Bcl-2** on their surface.
• Bcl-2 is bound to a molecule of the protein **Apaf-1**.
• Internal damage to the cell (e.g., from **reactive oxygen species**) causes Bcl-2 to release Apaf-1 that no longer keep **cytochrome c** from leaking out of the mitochondria.
1. Apoptosis triggered by internal signals:

- The released cytochrome c and Apaf-1 bind to molecules of **caspase 9**.
- The resulting complex of
  - cytochrome c
  - Apaf-1
  - caspase 9
  - (and **ATP**)
- is called the **apoptosome**.
- These aggregate in the cytosol.
1. Apoptosis triggered by internal signals:

- Caspase 9 is one of a family of over a dozen caspases. They are all proteases. They get their name because they cleave proteins - mostly each other - at aspartic acid (Asp) residues.
- Caspase 9 cleaves and, in so doing, activates other caspases.
- The sequential activation of one caspase by another creates an expanding cascade of proteolytic activity which leads to, a) digestion of structural proteins in the cytoplasm, b) degradation of chromosomal DNA and finally phagocytosis of the cell or cell death.
The intrinsic or mitochondrial pathway

- Release of Bcl-2, Apaf-1, and caspase-9
- Formation of apoptosome
2. Apoptosis triggered by external signals:

• the extrinsic or death receptor pathway: Fas and the TNF receptor are integral membrane proteins with their receptor domains exposed at the surface of the cell

• binding of the complementary death activator (FasL and TNF respectively) transmits a signal to the cytoplasm that leads to activation of caspase 8 which initiates a cascade of caspase activation leading to phagocytosis of the cell.
2. Apoptosis triggered by external signal

- When cytotoxic T cells recognize (bind to) their target, they produce more FasL at their surface.
- This binds with the Fas on the surface of the target cell leading to its death by apoptosis.
3. Apoptosis-Inducing Factor (AIF)

- Neurons, and perhaps other cells, have another way to self-destruct that - unlike the two paths described above - does not use caspases.
- Apoptosis-inducing factor (AIF) is a protein that is normally located in the intermembrane space of mitochondria. When the cell receives a signal telling it that it is time to die, AIF is released from the mitochondria (like the release of cytochrome c in the first pathway), migrates into the nucleus, binds to DNA, which triggers the destruction of the DNA and cell death.
Activation of Caspases

- In a given apoptosis pathway, activation of caspases may be initiated by death-receptor/death-factor interaction, or by the movement of cytochrome C molecules out of mitochondria, or both.
- c-Jun-N terminal kinases activate caspases for nucleosomal degradation.
In-vitro experiments to show the effect of chemotherapy on cancer

• Breast cancer cell lines: MCF-7, MDA-MB231, BT474

• Chemotherapies: Camptothecin, Adriamycin, Mechlorethamine, 5-Flurouracil and Methotrexate.
Tracing the programs…of cell death

- C-Jun-N kinase activation
- Caspase-3 activation
- Mitochondrial cytochrome C release
- DNA degradation
- Reactive oxygen species.
Camptothecin-Induced Apoptosis

- Camptothecin induces apoptosis-associated DNA fragmentation in MCF-7 breast cancer cells (lane 5)
- The presence of curcumin with camptothecin (lane 6) inhibits camptothecin-mediated apoptosis

DNA fragmentation analyzed by agarose gel electrophoresis
Quantitative Inhibition of Apoptosis

- Curcumin at 10 μM inhibits the ability of camptothecin to induce programmed cell death of MCF-7 cells by > 60%

DNA fragmentation analyzed by an ELISA
Curcumin Inhibits Caspase Activation

Other assays of apoptosis that are independent of DNA fragmentation confirm curcumin’s ability to block the action of camptothecin.

Apoptosis evaluated by a caspase activity assay
Concentration Dependence

- Curcumin blocks apoptosis of MCF-7 cells in a concentration-dependent manner

DNA fragmentation analyzed by an ELISA
• Even a brief, 3-hour exposure to curcumin can inhibit camptothecin-induced apoptosis of MCF-7 cells.

DNA fragmentation analyzed by an ELISA
Impact on Other Breast Cancer Models

- MDA-MB-231 and BT-474 cell lines were studied to see if curcumin had a similar impact on other breast cancer cells.
- Also, curcumin’s impact on alkylating agent- (mechlorethamine) and anthracycline (adriamycin)-induced apoptosis was studied, since these classes of drugs are used clinically in the care of patients with breast cancer.
Table 1. Inhibition of Chemotherapy-Induced Apoptosis

<table>
<thead>
<tr>
<th>Chemotherapeutic</th>
<th>Curcumin Concentration</th>
<th>MCF-7</th>
<th>MDA-MB-231</th>
<th>BT-474</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camptothecin</td>
<td>1 μM</td>
<td>9.0±4.7%</td>
<td>0.3±9.6%</td>
<td>27.5±16.7%</td>
</tr>
<tr>
<td></td>
<td>5 μM</td>
<td>43.9±0.9%</td>
<td>19.9±3.8%</td>
<td>50.5±2.5%</td>
</tr>
<tr>
<td></td>
<td>10 μM</td>
<td>66.3±1.6%</td>
<td>43.2±4.4%</td>
<td>71.2±10.5%</td>
</tr>
<tr>
<td>Methloretamine</td>
<td>1 μM</td>
<td>2.6±3.3%</td>
<td>5.3±6.0%</td>
<td>19.3±2.2%</td>
</tr>
<tr>
<td></td>
<td>5 μM</td>
<td>27.0±8.5%</td>
<td>20.5±5.2%</td>
<td>47.6±12.5%</td>
</tr>
<tr>
<td></td>
<td>10 μM</td>
<td>23.3±1.0%</td>
<td>22.7±5.4%</td>
<td>70.4±12.3%</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1 μM</td>
<td>47.2±6.9%</td>
<td>6.2±6.2%</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5 μM</td>
<td>55.5±4.4%</td>
<td>18.0±2.9%</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10 μM</td>
<td>65.3±6.7%</td>
<td>38.5±4.6%</td>
<td>ND</td>
</tr>
</tbody>
</table>

All results presented are the mean and standard error of the mean from four experiments; Abbreviations: ND Not done
Curcumin Blocks ROS Generation

- Curcumin blocks the ability of chemotherapy to induce ROS in a concentration-dependent manner.

Generation of reactive oxygen species assayed using dichlorodihydrofluorescein diacetate.
• Curcumin blocks the ability of chemotherapy to induce ROS in BT-474 breast cancer cells.
Curcumin Blocks JNK Activation

- Curcumin blocks the ability of camptothecin to activate JNK in MCF-7 breast cancer cells in a concentration-dependent manner

<table>
<thead>
<tr>
<th>0</th>
<th>1 μM</th>
<th>5 μM</th>
<th>10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Rabbit polyclonal α-phospho-c-jun antibody

JNK activity measured by an immunocomplex assay with GST-c-Jun substrate
Curcumin Blocks AP-1 Activation

- Curcumin blocks the activation of AP-1 by an alkylating agent and topoisomerase inhibitor.

AP-1 activity measured with an ELISA
AP-1 Activation II.

- Curcumin also blocks AP-1 activation by chemotherapy in BT-474 breast cancer cells

AP-1 activity measured with an ELISA
Curcumin & Cytochrome c Release

- JNK and ROS induce release of mitochondrial cytochrome c, which then activates apoptosis.
- Curcumin inhibits, in a dose-dependent manner, release of cytochrome c induced by mechlorethamine and camptothecin.

Western blot detection of cytochrome c in BT-474 cytosolic fractions after the indicated treatments.
Cytochrome c Release II.

• Mitochondrial release of cytochrome c is also inhibited by curcumin in MCF-7 breast carcinoma cells.

Cytochrome c in MCF-7 cells, with quantitation by densitometry.
In-Vivo Experiments

- Xenograft model in female nude mice (nu/nu)
- MCF-7 and BT-474 cells xenograft.
- Periodic tumor size measurements
- Dietary curcumin treatment
- Cyclophosphamide injection (i.p) followed by tumor measurements.
- Immunohistochemistry of tumor for apoptotic cells and c-Jun-N kinase activation.
Breast Cancer Models *in vivo*

- In a pilot experiment, tumor-bearing mice were randomized to either a standard diet or one supplemented with curcumin on day 0.
- On day 1 both groups were treated with a single dose of Cytoxan.
- The curcumin diet group has less tumor shrinkage.
- Similar results were noted in a BT-474 xenograft pilot.
Curcumin Blocks the Anti-Tumor Activity of Chemotherapy

• In a large trial, a diet containing curcumin inhibited the ability of Cytoxan to induce tumor growth delay in a murine model of human breast cancer (p<0.0001)

The diet supplemented with curcumin contains an intake representative of some human populations
Curcumin Blocks Apoptosis *in vivo*

- Curcumin reduced cyclophosphamide-induced apoptosis (in red; compare B and A) as well as JNK activation (also in red; compare D and C) *in vivo* compared with a standard diet.

Immunofluorescence analysis of tumor sections.
Curcumin Penetrates Into Tumors

• Curcumin is highly metabolized by the liver after oral intake
• A diet supplemented with curcumin at levels representative of some human populations, however, does result in detectable levels of curcumin reaching tumor tissue, where it may be available to inhibit the action of chemotherapy

Curcumin fluorescence in green
Conclusions

- Curcumin inhibits the induction of apoptosis by several chemotherapeutic agents in breast cancer cell lines
  - Inhibition is dose- and time-dependent
  - Curcumin also inhibits chemotherapy-induced ROS and JNK activation

- Mitochondrial release of cytochrome c is inhibited
- Dietary supplementation with curcumin inhibits the anti-tumor activity of cyclophosphamide in an in vivo model of human breast cancer
  - Inhibition of apoptosis and JNK is seen in vivo
Discussion

• Curcumin has an ability to inhibit cytochrome P450 enzymes in liver microsomes. This enzyme is necessary for the activation of cyclophospham ide prodrug to an active anticancer metabolite 4-hydroxy cyclophosphamide.

• Caution should be exercised for the use of antioxidant curcumin supplementation along with chemotherapy.
Implications

- Further studies of the anti-apoptotic activity of curcumin are needed
- Breast cancer patients undergoing cytotoxic chemotherapy may need to avoid dietary supplementation with curcumin
- Patients may need to limit their exposure to curcumin-containing foods while receiving chemotherapy
Acknowledgements

• Supported by a grant from the American Institute for Cancer Research

• Additional support was from the Department of Defense Breast Cancer Research Program