Polyphenolic Phytochemicals as Colorectal Cancer Chemopreventive Agents: An Intelligent Alternative to NSAIDs?

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**Abstract:** To review the evidence for using of polyphenolic phytochemicals as cancer chemopreventive agents. A literature search was undertaken from 1963 to the present day, using Medline and Pubmed. Epidemiological data suggests that diets rich in polyphenols confer significant protection against the risk of colorectal cancer. These observations are supported by in vitro and in vivo data. Polyphenols are polymeric in the anti-cancer action, but a common mechanism to all is their anti-oxidant property. Poor bioavailability, however, is potentially a limiting factor to their further development as clinical agents. Data regarding the chemopreventive efficacy of polyphenolic compounds is growing. Clinical trial data, although limited, suggests that such compounds show great potential for use as chemopreventive agents. The poor bioavailability of polyphenols could be advantageous in achieving a localised therapeutic effect in the gastrointestinal tract, thus minimising the risk of unwanted effects in organs distant from the locus of absorption.

**Key words:** Polyphenols, colorectal cancer, chemoprevention

**INTRODUCTION**

Colorectal cancer is the second most common cause of cancer death in the western world. Efforts to reduce mortality from this disease are currently focussed on early detection of precursor lesions and polyps and early diagnosis of established cancers. Other health strategies include chemoprevention, using either synthetic drugs or naturally occurring agents, that interfere with the multi-step pathway of carcinogenesis. Chemoprevention can be applied in three different scenarios. Primary chemoprevention entails administering a chemopreventive agent to the general population regardless of individual risk. Secondary chemoprevention involves a more focussed intervention where populations with an inherited or familial risk are targeted and tertiary chemoprevention selects populations following resection of a colorectal cancer, in an attempt to reduce the risk of local recurrence or metastatic spread\[^{[1]}\].

Recent randomised clinical trials have shown that Non-steroidal Anti-inflammatory Drugs (NSAIDs) such as the relatively non-selective Cyclooxygenase (COX) inhibitors, piroxicam and sulindac and the relatively selective COX-2 inhibitor, celecoxib, are able to cause regression of adenomas in patients with familial adenomatous polyposis, by up to 100%\[^{[2]}\]. The administration of aspirin to patients with recurrent colonic adenomas, or previous colorectal cancer, has also been shown to reduce new polyp formation by up to 35%\[^{[3]}\]. The structures of common NSAIDs can be seen in Fig. 1. Such studies suggest that chemoprevention could become a clinical reality, although to be effective patients would be required to take these drugs for many years. It is well documented that long term administration of NSAIDs such as aspirin is associated with side-effects

![Chemical Structures](image)

Fig. 1: Structures of common NSAIDs

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such as gastric mucosa inflammation, bleeding, dyscrasias, gastric ulceration and renal impairment. The inevitable morbidity associated with such side-effects may obfuscate any benefit gained from reduction of colorectal cancer risk. The recent worldwide withdrawal of the COX-2 inhibitor, Rofecoxib, further highlights the potential problems of long-term NSAID use. In this short report, evidence is reviewed that supports the notion of polyphenolic phytochemicals being considered an efficacious and safe alternative to NSAIDs.

Why polyphenolic phytochemicals?: Polyphenolic phytochemicals make up a large proportion of the constituents of the human diet, the main sources being fruits, chocolate, vegetables, cereals, legumes and beverages such as tea, coffee and wine. The exact classification and definition of polyphenolic compounds is open to debate and not the focus of this review. This report will centre mainly on three compounds namely curcumin, the major yellow pigment in the spice turmeric, resveratrol, a component of grapes and red wine and tea polyphenols with particular emphasis on epigallocatechin gallate (EGCG) (Fig. 2).

The confection that polyphenolic phytochemicals possess colorectal cancer chemopreventive properties is based on epidemiological findings that suggest the intake of polyphenol rich foods may delay the onset of cancer via antioxidant activity, induction of phase I and II detoxifying systems or by preventing the formation of carcinogenic precursors, such as heterocyclic amines. More recently there is evidence to suggest that certain polyphenols have a direct inhibitory effect on cancer cell growth by inhibiting proliferation, promoting apoptosis and inhibiting angiogenesis. Observational and case-control studies indicate that intake of fruit and vegetables is associated with a lower risk of colorectal cancer. The protective effect of high fruit intake is also evident amongst cohorts of individuals who are at high risk of colorectal cancer, including those with previous cancer history, colonic polyps and ulcerative colitis. Several retrospective epidemiological studies have shown that regular ingestion of green tea can be chemopreventive. One large Japanese study followed 8552 patients over a nine-year period and observed an average delay of cancer onset of 4 years (p=0.01) in individuals who consumed 10 or more cups of green tea daily, when compared to those consuming less than 3 cups daily.

Mechanisms of NSAID-and Polyphenolic Phytochemical-Mediated Cancer Chemopreventive Activity: A wide range of polyphenolic agents have been identified and are currently under evaluation using in vitro and in vivo models of carcinogenesis. To fully understand the potential of these polyphenolic phytochemicals, we must understand how they exert chemopreventive activity. Some of the known mechanisms of NSAID-and polyphenol-mediated chemopreventive activity are outlined in Table 1 and 2, respectively. These data show that both groups of compounds act via multiple mechanistic pathways, some of which overlap, for example antioxidant. The exact mechanistic pathways associated with cancer chemopreventive effects are, as yet, unclear. Some of the potential chemopreventive mechanisms associated with curcumin, green tea and resveratrol will now be considered.

Curcumin is a bright yellow pigment derived from the rhizome Curcuma Longa (Fig. 2 for chemical structure). It is found in the spice turmeric, which is widely used in Indian cuisine as a colouring and flavouring. Like many polyphenols curcumin has been shown to inhibit COX-2 expression in both human colorectal tumour cell lines in vitro and to decrease PGE-2 expression in humans in vivo. Curcumin has been shown to inhibit oxidative DNA adduct formation as measured by levels of the
Table 1: Mechanistic targets of NSAIDs potential related to cancer chemoprevention

<table>
<thead>
<tr>
<th>Therapeutic target</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-1 and/or COX 2 inhibition</td>
<td>Flurbiprofen</td>
</tr>
<tr>
<td></td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td>Sulindac</td>
</tr>
<tr>
<td></td>
<td>Celecoxib (COX-2 only)</td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td>Flurbiprofen</td>
</tr>
<tr>
<td></td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td>Sulindac</td>
</tr>
<tr>
<td></td>
<td>Celecoxib (COX-2 only)</td>
</tr>
<tr>
<td>Modulation of LOX</td>
<td>Flurbiprofen</td>
</tr>
<tr>
<td></td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td>Sulindac</td>
</tr>
<tr>
<td></td>
<td>Celecoxib (COX-2 only)</td>
</tr>
<tr>
<td>Suppression of prostaglandin synthesis</td>
<td>Flurbiprofen</td>
</tr>
<tr>
<td></td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td>Sulindac</td>
</tr>
<tr>
<td></td>
<td>Celecoxib (COX-2 only)</td>
</tr>
<tr>
<td>Induction of cell cycle arrest</td>
<td>Celecoxib (COX-2 only)</td>
</tr>
<tr>
<td></td>
<td>Sulindac</td>
</tr>
<tr>
<td>Inhibition of angiogenesis</td>
<td>Aspirin</td>
</tr>
<tr>
<td></td>
<td>Celecoxib (COX-2 only)</td>
</tr>
</tbody>
</table>

Table 2: Mechanistic targets of polyphenolic phytochemicals potentially related to cancer chemoprevention

<table>
<thead>
<tr>
<th>Therapeutic target</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2 inhibition</td>
<td>Curcumin</td>
</tr>
<tr>
<td></td>
<td>Black tea</td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td>Curcumin</td>
</tr>
<tr>
<td></td>
<td>Epigallocatechin gallate</td>
</tr>
<tr>
<td>Immune system modulation</td>
<td>Curcumin</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
</tr>
<tr>
<td>Inhibition of cell signalling pathway via cyclin D1</td>
<td>Resveratrol</td>
</tr>
<tr>
<td>Induction of GST Phase II detoxifying enzymes</td>
<td>Curcumin</td>
</tr>
<tr>
<td>Inhibition of nitric oxide synthase</td>
<td>Black tea extract</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
</tr>
<tr>
<td>Inhibition of oxidative DNA adduct formation</td>
<td>Black tea extract</td>
</tr>
<tr>
<td>Anti-oxidant mechanism</td>
<td>Curcumin</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
</tr>
<tr>
<td></td>
<td>Green tea extract</td>
</tr>
<tr>
<td></td>
<td>Black tea extract</td>
</tr>
</tbody>
</table>

pyrimidopurine DNA-adduct (M,G) decrease the expression of the oncoprotein beta-catenin, induce apoptosis in Colo 320 colon cancer cells and ACC-induced colon tumor. and induce the glutathione-S-transferase (GST) de-toxification enzyme system. Curcumin can also modulate immune system-mediated tumour cell killing by increasing the numbers of intestinal CD4+ T cells and B cells.

Resveratrol is a polyphenolic compound found in grapes, peanuts, berries and red wine (Fig. 2 for chemical structure). Experiments using cancer cell lines in vitro have shown resveratrol has an anti-proliferative effect and in CaCo2 human colon cancer cells, resveratrol induced the accumulation of cells in the S/G2 phase of the cell cycle, reflected by a 70% inhibition of growth.

Similarly in HT29 colon adenocarcinoma cells, resveratrol induced cell cycle arrest at the G2 phase via inhibition of CDK7 kinase activity. Resveratrol can also preferentially alter the levels of proteins involved in apoptotic pathways. For example, in CaCo2 colon cancer cells, high concentrations of resveratrol activated the proapoptotic protein caspase-3. In HCT116 cells resveratrol has been shown to activate a p53-independent apoptotic pathway that is potentially linked to cell differentiation and to induce both Bax-mediated and Bax-independent mitochondrial apoptosis.

Green tea contains a number of polyphenolic phytochemicals known as catechins. The epigallocatechin gallate (EGCG) has been shown to induce apoptosis and interfere with cell cycle progression in a number of colorectal tumour cell lines in vitro. This cytostatic effect was specific to tumour cells alone. Other effects include inhibition of DNA adduct formation, preservation of the colonic microflora and electrophile scavenging.

Epigallocatechin Gallate (EGCG) has been shown to inhibit DNA topoisomerase I, an enzyme involved in cell survival and DNA metabolism and structure, in numerous human colon carcinoma cell lines. Tea polyphenols have also been shown to induce enzymes involved in Phase II detoxification of dietary carcinogens.

With regard to chemoprevention mediated by NSAIDs, it is thought that inhibition of COX-2 activity is an important mechanism of action in colorectal cancer, however, other mechanisms are documented (Table 1) and include modulation of apoptosis, induction of cell-cycle arrest and inhibition of angiogenesis. Furthermore, sulindac has been shown to modulate the β-catenin/Tcf4 pathway via induction of p21 expression and celecoxib can decrease phorbol-ester-induced COX-2 expression and AP-1 DNA binding and can inhibit NF-κB activation.

Chemopreventive efficacy of polyphenolic phytochemicals and NSAIDs in vivo: The data demonstrate that there is a degree of overlap between the mechanisms of chemopreventive activity of polyphenols and NSAIDs and support the notion that polyphenols are effective chemopreventive agents. But an important consideration is the relative efficacy of polyphenols as chemopreventive agents when compared to NSAIDs in vivo.

Studies using genetic models of cancer and carcinogen-induced aberrant crypt foci models suggest that under certain conditions the chemopreventive efficacy of polyphenolic phytochemicals and NSAIDs can be similar (Fig. 3). Indeed, polyphenols such as green tea and soy appear to be more effective than NSAIDs at inhibiting carcinogen-induced ACF in vivo. The chemopreventive activity of NSAIDs and polyphenols against carcinogen-induced tumour models in vivo are compared in (Fig. 4). These data demonstrate that under...
Fig. 3: Comparison of efficacy of NSAIDs and polyphenols in genetic animal models and carcinogen-induced cancer models. Bubble size relates to the number of studies evaluating that particular agent.

Table 3: Chemopreventive activity of NSAIDs and polyphenolic phytochemicals in the ApcMin\(^+\) mouse. Treatment efficacy has been determined by inhibition of adenoma development.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (ppm)</th>
<th>Duration (days)</th>
<th>Adenoma development inhibition (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piroxicam</td>
<td>25-220</td>
<td>7-180</td>
<td>34-95</td>
<td>[53-56, 60]</td>
</tr>
<tr>
<td>Sulindac</td>
<td>30-300</td>
<td>7-80</td>
<td>32-99</td>
<td>[53, 57-62, 94]</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>150-1500</td>
<td>25-55</td>
<td>27-71</td>
<td>[55]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>1000-2000</td>
<td>70-75</td>
<td>6-64</td>
<td>[28, 73, 95]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>100 (in water)</td>
<td>49</td>
<td>70</td>
<td>[52]</td>
</tr>
<tr>
<td>Tea extract</td>
<td>1000</td>
<td>70</td>
<td>22</td>
<td>[63]</td>
</tr>
</tbody>
</table>

Table 4: Inhibition of aberrant crypt formation following dietary intervention with NSAIDs and polyphenolic phytochemicals in the AOM-induced colon carcinogenesis rodent model.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (ppm)</th>
<th>ACF development inhibition (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piroxicam</td>
<td>75-400</td>
<td>38-70</td>
<td>[96-99]</td>
</tr>
<tr>
<td>Sulindac</td>
<td>100-320</td>
<td>36-53</td>
<td>[98, 100-103]</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1500</td>
<td>41</td>
<td>[101]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>2000</td>
<td>42-57</td>
<td>[64, 67, 104]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>200</td>
<td>38</td>
<td>[105]</td>
</tr>
<tr>
<td>Tea extract</td>
<td>200-1200</td>
<td>35-57</td>
<td>[65, 66]</td>
</tr>
</tbody>
</table>

Table 5: Decrease of tumour incidence following dietary intervention with NSAIDs and polyphenolic phytochemicals in the AOM-induced colon carcinogenesis rodent model.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (ppm)</th>
<th>Decrease of tumour incidence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piroxicam</td>
<td>200-400</td>
<td>64-85</td>
<td>[106-108]</td>
</tr>
<tr>
<td>Sulindac</td>
<td>320</td>
<td>55</td>
<td>[109]</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1500</td>
<td>78-93</td>
<td>[110, 111]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>600-4000</td>
<td>25-42</td>
<td>[112-114]</td>
</tr>
<tr>
<td>Tea extract</td>
<td>1000</td>
<td>51</td>
<td>[115]</td>
</tr>
</tbody>
</table>

Fig. 4: Comparison of NSAIDs and polyphenols, ranked on potency to inhibit carcinogen-induced tumours. Bubble sizes relate to number of studies evaluating that particular agent.

Using the AOM-induced adenocarcinoma rat model, curcumin and green tea extracts showed similar efficacy to NSAIDs at inhibiting aberrant crypt foci formation, although NSAIDs were superior at reducing tumour incidence\(^{51-53}\).

Combined with the apparent lack of unwanted side effects following long term administration, these data suggest that certain polyphenolic phytochemicals may prove a sensible alternative to NSAIDs for use in colorectal cancer chemoprevention.

**Bioavailability of polyphenolic phytochemicals:** It is known that NSAIDs show a relatively high bioavailability. For example, aspirin is a weak acid that remains largely unionised in the acidic environment of the stomach thereby facilitating its absorption\(^{69}\). Current preclinical data from *in vivo* models suggests that polyphenols are poorly absorbed and avidly metabolised. Small polyphenols such as caffeic acid are most bioavailable following oral administration showing up to 27% recovery from urine, whereas tea polyphenols are poorly bioavailable showing around 0.00006% recovery from urine\(^{70}\). Curcumin is poorly bioavailable and is subject to a rapid first pass metabolism\(^{71}\) with only trace amounts detectable in the
peripheral circulation following oral administration\(^{72,73}\). Similarly other polyphenols such as resveratrol display poor bioavailability\(^{74-76}\). A further reduction in polyphenol bioavailability can also occur via bacteria-mediated degradation in the large bowel\(^{77,78}\).

Polyphenols can conjugate with glucuronide moieties and such conjugation may assist in their absorption from the small intestine\(^{39}\). In an attempt to increase absorption and consequently bioavailability, polyphenols have been co-administered with compounds such as lipids and emulsifiers\(^{79}\). Such a protocol significantly enhanced the absorption of the polyphenol quercetin and might therefore prove beneficial to other polyphenolic agents. The co-administration of curcumin with a pepper constituent has also been shown to increase curcumin absorption by a factor of 2\(^{80}\).

Certain polyphenols, such as epigallocatechin gallate from green tea, are excreted in bile\(^{81}\). Enterohpatic recirculation of bile excreted metabolites, a process that has been postulated following administration of resveratrol to rats\(^{79}\), might act to sustain therapeutic drug concentrations in the gut following oral administration and may therefore potentiate chemopreventive activity. It should be noted that the major metabolites of polyphenols and their intrinsic chemopreventive efficacy are still largely uncharacterised. It is possible that the beneficial effects of polyphenols are not reliant on their absorption through the gut barrier and that their efficacy may result from a direct anti-oxidative effect on mucosal cells\(^{82}\), beneficial effects on gastrointestinal micro-flora\(^{83}\), localised absorption and distribution to gastric epithelial cells, or may be attributable to their metabolites.

The differences in bioavailability of certain NSAIDs and polyphenols do not appear to affect their chemopreventive efficacy in the gastrointestinal tract, for example the bioavailability of curcumin is poor, however, in the Apc\(^{min}\) mouse has shown considerable efficacy (Table 3). Although poor absorption of polyphenols is likely to hinder their chemopreventive activity in cells distant from the gastrointestinal tract, such limitations may result in localised accumulation in the gastrointestinal tract thereby dramatically decreasing the risk of untoward side-effects in distant organs.

**Clinical studies of polyphenolic phytochemicals:** As yet few polyphenolic phytochemicals have been investigated in clinical trials. Curcumin has thus far been the most investigated. Serum levels of curcumin have been shown to be low following oral administration to patient volunteers, with measured levels of <0.03 \(\mu\text{M}\) following doses of up to 2 g\(^{80}\) and 1.75 \(\mu\text{M}\) after 8 g oral administration\(^{84}\). In one study, no detectable levels of curcumin were found in urine or blood following oral administration to human volunteers at doses from 36-180 mg\(^{92}\). However, curcumin sulphate was detected in the faeces of one patient at the 180 mg dose level, thus supporting previous work showing polyphenol conjugation can occur in the gastrointestinal tract\(^{93}\).

Clinical data suggest that curcumin is non-toxic and does not accumulate within the body. Daily oral doses as high as 8 g have been administered to patients for 3 months with no adverse effects\(^{94}\) and other studies involving curcumin administered at doses of 180 mg to 200 mg daily failed to demonstrate any toxicity\(^{92}\). In a recent clinical trial\(^{95}\) a daily 3.6 g dose of curcumin for 7 days resulted in curcumin accumulation in colorectal tissue to concentrations equivalent to those (5-5 \(\mu\text{M}\)) required for pharmacological activity in cells in vitro\(^{96-98}\). This same study\(^{99}\) also found that patients receiving 3.6 g of curcumin had a significant reduction in tumour levels of oxidative DNA damage (\(p<0.05\), student t-test) and trace levels of curcumin were only detected in the peripheral circulation one hour after administration of the highest dose. These data show that despite its poor absorption and rapid elimination from the body, pharmacologically active levels of curcumin can be achieved in the colorectal mucosa when administered at high enough doses.

Clinical studies using green tea are relatively limited. The administration of standardised green tea solids (0.6-1.8 g), dissolved in warm water, to human volunteers has been shown to result in a rapid decrease of rectal mucosa PGE\(_2\) levels within 8 h of consumption\(^{101}\). Unfortunately, the clinical use of such a regimen may be marred by reports of side-effects including bloating, nausea, vomiting, agitation, dizziness and restlessness\(^{102}\). Such side-effects are probably due to the caffeine content within green tea, often up to 79\(^{83}\). Decaffeination is therefore an option, however, caffeine has been reported to enhance the chemopreventive efficacy of green tea\(^{93}\) and removing it may reduce efficacy.

Recent data suggests that polyphenolic phytochemicals possess colorectal chemopreventive properties in both in vitro and in vivo models of colorectal carcinogenesis. Indeed, under certain conditions polyphenolic phytochemicals have been shown to be as effective as NSAIDs. The major confounding factor in the development of polyphenols as chemopreventive agents is their poor bioavailability. Although pharmacologically active concentrations are achievable in the intestinal mucosa, the dose required to achieve this may prove unpalatable to patients needing to be maintained on the medication for many years. The poor bioavailability of polyphenolic phytochemicals may,
however, prove to be a great asset. Although, limiting their use solely to the chemoprevention of colorectal cancer, the restriction of polyphenols to the gastrointestinal tract is likely to decrease the risk of untoward side-effects in organs distant to the locus of absorption. It remains to be seen if randomised clinical trials show polyphenols to be less, similarly, or more effective than NSAIDs in human colorectal cancer. The vast number of available polyphenols makes their development into clinical drugs a daunting but exciting project.

REFERENCES


