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Efficacious anti-cancer property of flavonoids from citrus peels

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Abstract

Cancer is one of the two leading human fatal diseases. Drug development for cancer intervention has progressed well in past decades yet existing drugs face many limitations in applications and effectiveness and are often associated with serious of side effects, which can further deteriorate the patients' quality of life. Recent development of natural product based and therapeutically sound anti-cancer agents have gained popularity in the field of functional foods, in which a few have demonstrated efficacy and minimal toxicity toward the prevention and treatment of carcinogenesis. With multiple active molecular components, citrus peels and derived extracts have demonstrated potent efficacious properties against various cancers due in large part to the rich content of flavonoids present in citrus peels. This review summarizes the results of currently available data regarding the *in vivo* anti-cancer activity of citrus peel flavonoids, and identifies opportunities for subsequent human clinical trials to assess preventive and therapeutic effects in the near future.

Keywords: Citrus peels; Flavonoids; Polymethoxyflavones; Efficacy; Cancer

1. Background

Medicinal use of citrus peels, such as aged tangerine peels in south east Asia, can be traced back to 10th century BC. However, the systematic in-depth exploration of the biological activity of citrus peels did not embark until the last decade, when advanced flavonoid profiles in citrus peels were established and isolation of majority of individual flavonoids became available. Since then, a plethora of biological properties important to health and diseases have been identified [1–3]. In addition to cancer prevention and intervention, other biological functions of compounds from citrus peels investigated include inflammation inhibition [4–7], hypolipidemia [8,9], regulation of metabolic syndrome [10–13], delayed onset of Alzheimer's disease [14,15] and more. Characterization of the phytochemical composition of citrus peels with modern analytical technology indicated that citrus peels are an abundant source of polyhydroxyl flavonoids (PHFs) such as hesperidin, neohesperidin and naringin; and almost the sole source of polymethoxyflavones (PMFs) with high content, which are mainly represented by nobiletin, tangeretin, sinesetin, 3,5,6,7,8,3′,4′-heptamethoxyflavone and 3,5,6,7,3′,4′-hexamethoxyflavone [3,16,17].

Research in anti-cancer activity of citrus flavonoids has been majorly focused on *in vitro* experiments to elucidate action mechanisms such as anti-proliferative effects, enzyme inhibition and cancer cell attenuation. PMFs have demonstrated the growth inhibition of human leukemic cell (HL-60) lines [18]. Of PMFs, tangeretin played important inhibitory roles in cancer-cell proliferation and metastasis stage by inhibiting cell adhesion and invasion [19]; showed cell cycle arrest in G1 phase by inhibiting cyclin-dependent kinases (Cdk) and enhancing Cdk inhibitor proteins [20]; inhibited extra cellular-signaling-regulated kinase 1/2 (ERK1/2) phosphorylation and growth of human mammary cancer cells and cytolysis by natural killer cells [21]; repressed induced and constitutively expressed cyclooxygenase-2 (COX-2) in human lung cancer cells [22]. In exploring the anticancer activity of citrus flavonoids, another major PMF, nobiletin was found to have effectively inhibited the proliferation and migration of human umbilical endothelial cells of human prostate,

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skin, breast and colon carcinoma cell lines [23]; reduced AOM-induced cell proliferation in colonic adenocarcinoma cells [24]; suppressed the proliferation, migration and tube formation on matrigel of human umbilical vein endothelial cells stimulated with endothelial cell growth supplement [25]; and attenuated the growth of prostate cancer cells and reduced azoxymethane (AOM)-induced large bowel carcinogenesis in rats [26], to name a few. Multiple biological pathways of anti-cancer mode of action by citrus flavonoids were also studied and summarized [27]. Furthermore, the study of structure activity relationship (SAR) of citrus flavonoids and cancer prevention was linked to the structural similarities between flavonoids and 17β-estradiol, suggesting interaction of flavonoids with estrogen receptors and also with estrogen metabolizing enzymes, such as cytochrome P450 enzymes CYP1A1 and CYP1B1, which are over-expressed in variety of tumor tissues [28].

Conclusions can be made from many *in vitro* studies including those mentioned above that citrus flavonoids exert strong anti-cancer activities. However, the beneficial effectiveness of a drug or a nutrient can only be attested by *in vivo* efficacy studies. In this review, we summarize the *in vivo* anti-cancer effect of bioactive compounds either as single molecules or as a mixture of molecules from citrus peels. The anti-cancer activity of citrus peel flavonoids has been evaluated on several animal models, including cancers of skin [17,29], colon [30–34], prostate [33,35], lung [36], and liver [37] among others.

2. *In vivo* anti-cancer efficacy of phytochemicals in citrus peels

2.1. Skin cancer

In the first reported *in vivo* experiment with citrus polymethoxylated flavonoids, nobiletin effectively inhibited the production of hydrogen peroxide (H_2O_2), attributed to reduced O_2^- generation because of the functional relationship between H_2O_2 formation and O_2^- dismutase or other nonenzymatic reaction [29]. Pretreatment with nobiletin remarkably reduced the weight of edema, thickness of epidermis, number of infiltrated leukocytes and H_2O_2 generation on TPA treated ICR mouse dorsal skin, indicating the efficacy of nobiletin in anti-inflammation and anti-carcinogenesis, because the TPA induced epidermal hyperplasia and edema formation are manifest of inflammatory processes leading to carcinogenesis [29].

Two-stage carcinogenesis skin model was used to characterize the inhibition of tumor promoting effects. The multistage skin carcinogenesis model has initiation, promotion, and progression three distinct stages [38], which serves as a major *in vivo* model for studying the sequential and stepwise evolution of the cancer process by chemical and physical carcinogens. The steps of this standard model include topical application of a single sub-carcinogenic dose of skin carcinogen, such as DMBA that causes irreversible DNA damage; then repeated application of promoters such as most commonly used TPA to induce cell proliferation and inflammation. This promotes the selective clonal expansion of initiated epidermal cells and leads to the formation of multiple squamous papillomas. Also in the same *in vivo* experiment, nobiletin at dosages of 0.16 and 0.32 μmol/L effectively inhibited the growth of mouse skin tumor initiated by DMBA and promoted by TPA in a dose-dependent manner [29]. The tumor incidence at 20 weeks was reduced by 33.5–43.3% in nobiletin treated groups *vs* in the control group. The average number of tumors per mouse was inhibited by 61.2% (0.80 ± 0.48) at dose of 0.16 μmol/L and 75.7% (0.50 ± 0.22) at dose of 0.32 μmol/L, respectively [29].

In the same skin cancer model, the topical application of a mixed citrus peel extract (CPE) also effectively inhibited molecular biomarkers of skin inflammation and attenuated TPA-induced skin tumor formation by reducing both the tumor incidence and tumor multiplicity of papillomas at 20 weeks, which demonstrated the efficacious anti-tumor effect of molecules from the citrus peel extract (CPE) and the capability of preventing inflammation-associated skin tumorigenesis [17]. The untreated positive control group of mice had 16 ± 3 papillomas per mouse and a 100% incidence of skin tumors at 20 weeks, whereas no tumors were observed in the negative control group following acetone application. However, when 100 μL of the CPE was applied to the shaven backs of mice 30 min prior to each TPA application, the average number of papillomas per mouse was 12 ± 4 (25% reduction compared to positive group). In addition, the tumor incidence was found to be 100% in positive group whereas extract-treated group showed significantly decreased at 81%. The number of papillomas (≥5 mm in diameter) per mouse was significantly decreased in the CPE treated group. In addition, in the positive control group in which mouse skin tumors were initiated by DMBA and promoted by TPA for 20 weeks, the protein expression of ornithine decarboxylase (ODC), COX-2, and VEGF (vascular endothelial growth factor) was apparently increased compared to healthy skin tissue. However, treatment with the CPE resulted in a strong reduction of ODC, COX-2, and VEGF protein levels in skin tumors. ODC is involved in cancer proliferation and both COX-2 and VEGF contribute to angiogenesis, hence we suggest that citrus flavonoids reduced tumor size by inhibiting the tumor growth and angiogenesis [17].

2.2. Colon cancer

Colorectal cancer (CRC) has high rates of mortality and morbidity and also has increasing prevalence even with current choices of diagnosis and medication [30]. In experimental models, development of CRC from normal colonic epithelium includes several distinct steps, *i.e.* colonic crypt hyperplasia, dysplasia, adenoma, adenocarcinoma and distant metastasis [30,31]. The formation of aberrant crypt foci (ACF) in early stage of the progression is widely considered a histological biomarker of colon tumorgenesis [31]. ACF also occurs very frequently in colon cancer patients, which proposed as a putative pre-neoplastic lesion [31]. Furthermore, increased number of incidence and multiplicity of ACFs are closely associated with CRC development [30,31]. The tumorigenesis of colon cancer is involved in various genetic and molecular changes in cell proliferation, inflammation, resistance to apoptosis and tumor angiogenesis.