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Review

On the Potential Use of Flavonoids in the Treatment and Prevention of Pancreatic Cancer

ALEXANDRA B. ROGINSKY, MICHAEL B. UJIKI, XIAN-ZHONG DING and THOMAS E. ADRIAN

Northwestern University Feinberg School of Medicine, Department of Surgery, 303 East Chicago Avenue, Tarry 4-711, Chicago, IL 60611, U.S.A.

Abstract. *Pancreatic cancer is a disease carrying a dismal prognosis, with overall 5-year survival at around 4%. Recent clinical trials of adjuvant therapies have not found a dramatic increase in median survival. In the current review, we examine the available literature on flavonoids, a group of naturally occurring substances, for their effects on cancer cells and potential for therapy of pancreatic cancer in the future. With the available in vitro and in vivo data, it is likely that flavonoids will move into the clinical arena as therapeutic or preventive tools for cancer.*

Pancreatic cancer is a disease with a dismal prognosis. The American Cancer Society estimates that 30,700 Americans will have been diagnosed in 2003 and that there will have been 30,000 deaths from pancreatic cancer in 2003. The five-year survival of pancreatic cancer is 4%, with no difference reported among the races or both sexes, and no trend of decline since the 1930s (1). There have been numerous clinical trials in the past few years regarding various drugs and combinations on pancreatic cancer patients, and, disappointingly, none have found dramatic changes in median survival (2). There is a clear need for advancements in the therapy of pancreatic cancer. Naturally occurring compounds are clearly a major source for new anti-cancer drugs and, among these, flavonoids hold considerable promise.

Background

In recent years there has been a dramatic increase in the investigation of naturally occurring products and their potential role for therapy of various cancers. The increasing market for research on natural products and resources led to the Convention on Biological Diversity, establishing national sovereignty over natural resources, commitment to conservation, development and sharing of benefits. Natural resources can no longer be simply taken from source countries without regard to applicable treaties and laws (3). One group of naturally occurring substances with considerable promise for cancer therapy is the flavonoids. The term "flavonoid" refers to over 4,000 naturally occurring substances from dietary plants. All flavonoids, by definition, have a common phenylbenzopyrone C6-C3-C6 structure(4), with one or more hydroxyl group substitutions, illustrated by the letter "R" below (see Figure 1).

There are six large groups of flavonoids, classified by the functional state of substitutions on the carbon skeleton, connection between the B and C ring, the functional groups on the C ring and their degree of oxidation (4).

Flavonoids have probably existed in the plant kingdom for over one billion years. Historically, herbs containing flavonoids have been used in folk medicine throughout the world (5). There are multiple epidemeological reports in the literature that diets rich in flavonoids reduce the incidence of various cancers, probably by playing a role in the *prevention* of cancer (6). There have also been studies addressing dietary flavonoid intake and heart disease. An inverse relationship between flavonoid intake and heart disease has been documented in several multi-national studies (7-9). Animal studies have demonstrated that flavonoids have anti-oxidant and cardio-protective properties *in vivo* (10, 11). The American Heart Association has added to its recommendations consumption of a diet with soy proteins including the intrinsic isoflavones for cardiac health and longevity (12). Similarly, daily intake of moderate amounts of alcohol is associated with a reduction in cardiovascular disease. The flavonoid resveratrol present in red wine is believed to favorably influence biochemical reactions such as decreasing platelet aggregation, endothelial adhesion and increasing nitric oxide production (13).

Correspondence to: Prof. Thomas E. Adrian, Northwestern University Feinberg School of Medicine, Department of Surgery, 303 East Chicago Avenue, Tarry 4-711, Chicago, IL 60611, U.S.A. Tel: (312) 503-3489, Fax: (312) 503-3491, e-mail: tadrian@northwestern.edu

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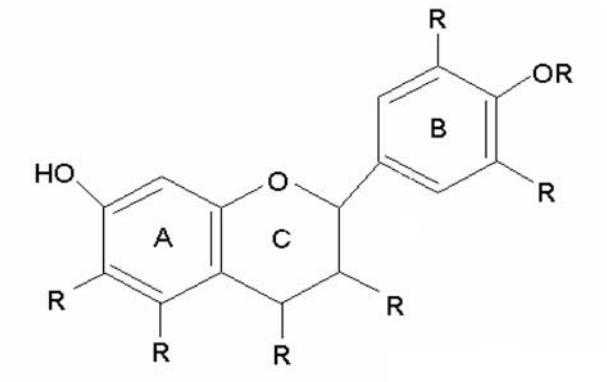

Figrure 1. *Basic flavonoid structure, with three six-carbon rings (A, B, and C).*

The focus of our laboratory is the study of pancreatic cancer. Therefore, in the current review we assess the available data on flavonoids and their effects on pancreatic cancer *in vitro* and *in vivo*.

Flavonoids and pancreatic cancer

Genistein. Genistein is a soy isoflavonoid that has been widely studied and cited in the literature as having a host of potentially beneficial clinical properties. Furthermore, of all the flavonoids, genistein is the most investigated in the literature regarding cancer. Genistein causes growth arrest of human melanoma cells *in vitro* (14). Genistein inhibits skin carcinogenesis in hairless mice following ultraviolet B exposure, in a dose-dependent fashion, with topical application being more effective than oral dosing (15) . Genistein has also been shown to inhibit growth and induce apoptosis in HepG2 cells (16) and inhibit growth of human breast carcinoma cells (17).

Similar to its effects in other types of cancer, genistein inhibits pancreatic cancer cell growth. One of genistein's main mechanisms of action involves the inhibition of tyrosine kinases (18). The interest in tyrosine kinase and pancreatic cancer stems from the evidence that pancreatic cancers have increased tyrosine kinase activity (19). In one study, growth of MIA PaCa-2 and PANC-1 cells was inhibited at a concentration of 1 μM genistein (20), a concentration previously shown to inhibit tyrosine kinase activity (21). In MIA PaCa-2 and PANC-1 cells, maximal stimulation of growth receptor tyrosine kinase by exogenous factors including epidermal growth factor resulted in maximal growth. Genistein blocked tyrosine kinase activation and cell growth on both cell lines (22). Genistein not only inhibited basal growth of MIA PaCa-2 and PANC-1 cells, but also the growth induced by stimulatory effects of epidermal growth factor and bombesin (20).

Another group investigated genistein as well as two other tyrosine kinase inhibitors, lavendustin and herbimycin A. Genistein significantly inhibited growth of the MIA PaCa-2, PANC-1 and BxP-3 cells lines at concentrations of 188, 260 and 220 μM, respectively (18).

Ding *et al.* studied genistein as part of an investigation of lipoxygenase and its products on pancreatic cancer cell growth. The enzyme 12-lipoxygenase is overexpressed in pancreatic cancer cells and its blockade inhibits proliferation and induces apoptosis (23). The same group investigated the mechanism by which the 12-lipoxygenase metabolite, 12(S) hydroxyl eicosatetraenoic acid (12-HETE) stimulates pancreatic cancer cell growth, and found it to be through a tyrosine kinase-dependent mechanism. Genistein (100 μM) abolished the effect of 12(S) HETE on proliferation (24).

In his famous paper *The Metabolism of Tumors*, Otto Warburg elegantly outlined that cancer cells have a tremendously high affinity for glucose compared with normal tissues (25). Genistein has also been studied in its effect on various pathways in glucose metabolism of pancreatic cancer cells. Genistein treatment of MIA PaCa-2 cells affects cell metabolism in multiple ways. It causes a decrease in tricarboxylic acid cycle activity, and reduces glucose oxidation as well as ribose synthesis through the pentose cycle. The non-oxidative pentose cycle is critical for nucleic acid synthesis and acts as a salvage pathway for both purines and pyrimidines. Blocking this pathway appears to be a major mechanism of growth inhibition by genistein (26).

Genistein has also been studied as an agent to enhance the effectiveness of chemotherapeutic agents. One hypothesis for the current failure of chemotherapy in pancreatic cancer is that chemotherapeutic agents induce nuclear factor kappa B (NF-Î B) (27-29). There is evidence that NF-Î B is constitutively up-regulated in cancer cells (30, 31). NF-Î B is a molecule that plays a major role in survival of cells when exposed to stress. Li *et al.* showed that pretreatment of cells with genistein, an NF-Î B inhibitor, followed by treatment with docetaxel or cisplatin, significantly inhibited growth of Bx-PC3 cells and induced apoptosis (32).

Epidermal growth factor receptor and tyrosine kinase were also investigated in MIA PaCa-2 cells with the flavonoids, quercetin and luteolin. Exposure of MIA PaCa-2 cells to quercetin and luteolin decreased growth and cellular protein phosphorylation in a time-dependent fashion. Exposure of the cells to quercetin and luteolin also led to apoptosis in this study, evidenced by cell shrinkage, DNA fragmentation and poly (ADP-ribose) polymerase (PARP) cleavage (33). Another group reported that genistein inhibits cell growth through its effect on TGF-beta (TGFb) pathways (34).

Mouria *et al.*, studied the ability of several flavonoids to induce apoptosis. They investigated quercetin, rutin, transresveratrol and genistein *in vitro* and quercetin *in vivo* in a nude mouse model. Quercetin, transresveratrol and genistein caused apoptosis *in vitro* through the mitochondrial pathway, as evidenced by cyctochrome c release and caspase 3 activation in MIA PaCa-2 and BSp73AS (a rat pancreatic cell line) (35). In contrast to other reports in the literature (32), genistein did not inhibit NF-kB, although quercetin and transresveratrol did. The *in vivo* experiments involved injection of MIA PaCa-2 cells subcutaneously, and treating the experimental mice with quercetin. Treated mice had statistically significant increased survival, decreased growth of primary tumor and prevention of metastasis (35).

Pancreatic cancer cells live in a relatively hypoxic environment. There is evidence that the PO_2 in pancreatic lesions is 14 mm mercury (36). There are reports that the ability to survive in a hypoxic environment has a profound effect on the survival of cancer cells and treatment failure in multiple other systems (37, 38). Hypoxia inducible factor 1 (HIF-1) is a newly described transcription factor for vascular endothelial growth factor (VEGF). There is an increase in HIF-1 protein and mRNA in Capan-1, Capan-2, PANC-1 and MIA PaCa-2 cells in response to hypoxia (48). Hypoxic cancer cells treated with genistein showed no detectable HIF-1 DNA binding activity, in contrast with control hypoxic cells. Genistein also decreased the expression of VEGF *in vitro* (36).

The same group further studied the effect of genistein *in vitro* and on an *in vivo* orthotopic murine model of pancreatic cancer. In all five cell lines tested, AsPC-1, Capan-1, Capan-2, MIA PaCa-2 and PANC-1, genistein significantly inhibited VEGF secretion at a concentration of 50 μM. In the *in vivo* model, experimental animals received 1.3mg of genistein intraperitoneally. Tumor growth was slower than in control animals, but a statistically significant difference in tumor size after euthanasia was seen only in the mice injected with Capan-1 cells (39). Another publication by the same authors also described an orthotopic model of the disease, wherein genistein significantly increased survival in treated animals and almost completely inhibited the formation of metastatic tumors; only one out of eight genistein-treated animals developed metastases (40).

Lian *et al.* studied the effects of signal transducer and activator of transcription 3 (STAT 3), a transcription factor required for cellular proliferation and differentiation. This group demonstrated that STAT3 is constitutively activated in MIA PaCa-2 and PANC-1 cells, and that 10μM genistein could inhibit the activation (41).

Because genistein and other isoflavones are phytoestrogens, they exhibit estrogenic properties (42). There have been studies regarding differences in their effects on cells, based on the estrogen receptor status. Su.86.86 cells, from a female patient, were inhibited by equol and coumestrol, while HPAF-II cells, from a male patient, were stimulated by these agents. Genistein also stimulated the HPAF-II cells with no significant effect on the Su 86.86 cells (43). Because most human pancreatic cancers have a k-ras mutation (44), the authors assessed k-ras expression in response to treatment. Equol and coumestrol decreased k-ras expression in the cells obtained from a female. Genistein, however, independently of its growth-inhibitory effects, caused a marked three-fold increase in the expression of k-ras in cells both from male and female sources. Genistein also increased the expression of the multi-drug resistance (*Mdr-1*) gene in male pancreatic cancer cells. This study has profound implications on potentially treating male pancreatic cancer patients with genistein (43). There are also similar surprising findings with regard to quercetin. There is evidence that quercetin can also potentiate carcinogenesis *in vivo*. In one study, rats were injected once with nitrosomethylurea (NMU), and fed either the standard or quercetin-supplemented diet. The rats given quercetin in addition to NMU had a statistically higher incidence of dysplastic foci than NMU-treated rats alone (45).

Daidzein. Daidzein is an isoflavone found in soy products and legumes (4). Daidzein has been shown to inhibit growth of human cervical cancer cells (HeLa cells) *in vitro* and to cause cell cycle arrest (46). The same group demonstrated LoVo colon cancer cells to undergo cell cycle arrest and apoptosis upon treatment with daidzein (47). The investigations on daidzein and pancreatic cancer are just starting.

Daidzein, another phytoestrogen isoflavonoid, was tested on estrogen receptor-positive and-negative pancreatic cancer cells *in vitro*. MIA PaCa-2 (estrogen receptor-positive) and PANC-1 cells (estrogen receptor-negative) were treated with daidzein. Daidzein inhibited the growth of both cell lines. There also appeared to be a saturation phenomenon in the estrogen receptor-positive cells, with a lack of increase in inhibition when the daidzein concentration was increased above the IC_{50} (48).

Resveratrol. Resveratrol is an anti-oxidant found in grapes, raspberries, blueberries, peanuts and some pine trees (49). A few years ago, Jang *et al.* showed that resveratrol is effective in preventing initiation, promotion and progression, all three steps of carcinogenesis (50). There is published data that resveratrol inhibits NF-Î B (51), a molecule known to be up-regulated in cancer cells (30, 31). Resveratrol has also been shown in other reports to inhibit the growth of breast, colon and prostate cancer cells *in vitro* (52-54).

Ding *et al.* investigated the effects of resveratrol, a polyphenol compound found in the skin of red grapes. Resveratrol inhibited growth of the AsPC-1 and PANC-1 cell lines, and induced apoptosis as evidenced by propidium iodide staining, morphological appearance and TUNEL assay (55). A group in Austria reported a decrease in the proliferative response of pancreatic PANC-1 and BxPC3 cells in conditioned media (56). Fulda and Debatin outlined how resveratrol induces apoptosis in MIA PaCa-2 and other cancer cell lines by sentitizing the cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (57).

Baicalein. Baicalein is a flavonoid extracted from *Scutellaria baicalensis* Georgi (58) and used in Japan and China as a component of the herbal medicine Sho-saiko-to to treat chronic hepatitis (41).

A study from Japan evaluated nine components of Sho-saiko-to and found that baicalein inhibited growth of human hepatoma cells (Hep G2) as well as the pancreatic cancer cell line BxPC-3 (59). Another group also documented that baicalein was a powerful antioxidant (58).

Baicalein is a selective inhibitor of 12-lipoxygenase. As previously mentioned, the 12-lipoxygenase metabolite, 12(S) HETE, is a stimulatory factor for pancreatic cancer cell growth (23). This phenomenon is also well documented in the urologic literature, where there is a known increase in expression of 5-lipoxygenase and 12-lipoxygenase in bladder cancer (60, 61). Inhibition of 12-lipoxygenase by the flavonoid baicalein causes a dose-dependent inhibition of growth and apoptosis induction in bladder (62) and renal cancer cells *in vitro* (63).

Treatment with baicalein induced apoptosis in the pancreatic cancer cells PANC-1, MIA PaCa-2, Capan-2 and HPAF, as evidenced by DNA propidium iodide staining, TUNEL assay and DNA fragmentation. Treatment with baicalein also caused marked morphological changes (64, 65). The same group demonstrated that baicalein inhibited the growth of subcutaneously transplanted HPAC and AsPC-1 tumors in nude mice with no apparent toxicity (64).

Flavopiridol. Flavopiridol is a new isoflavone that is an inhibitor of cyclin-dependent kinases (66) that has been studied in some clinical trials for both solid tumors and hematological malignancies (67, 68). In an *in vitro* study involving pancreatic, colon and gastric cancer cell lines, flavopiridol enhanced apoptosis induced by gemcitabine in all three. In the Capan-2 human pancreatic cancer cell line, the greatest induction of apoptosis occurred when gemcitabine treatment was followed by flavopiridol. Flavopiridol may serve as a tool to help overcome gemcitabine resistance in pancreatic cancer cells (69).

Apigenin. Apigenin (4',5,7,-Trihydroxyflavone) is one of the most common naturally occurring flavonoids and is widely distributed in fruits and vegetables (70). Apigenin has been shown to possess anti-inflammatory effects, free radical scavenging properties, and anti-carcinogenic effects (71). It has been shown to possess growth inhibitory properties in several cancer lines, including breast, colon, skin, thyroid and leukemia cells (72-76).

Recent data from our laboratory indicate that apigenin inhibits growth of human pancreatic cancer cell lines *in vitro* as well. Apigenin caused both time- and concentration-dependent inhibition of DNA synthesis and cell proliferation in AsPC-1, CD18, MIA PaCa2 and S2-013 cell lines. The inhibition of cell growth was through a G2/M-phase cell-cycle arrest through reduced levels of cyclin A, cyclin B, cdc25A and cdc25C (77). In addition to cell cycle arrest, apigenin also appears to cause apoptosis and even enhance gemcitabine-induced pancreatic cancer cell apoptosis when used in combination (78).

Catechins. Tea from the plant *Camellia sinensis* has a tremendous amount of flavonoids, which comprise approximately 20% of the dry weight of tea. The most prominent are the flavanols, including epigallocatechin, epicatechin, gallocatechin and catechin (79).

Epigallocatechin-3-gallate (EGCG), the most abundant green tea polyphenol (80), has been studied regarding its effect on pancreatic cancer cell proliferation, using *in vitro* techniques to assess proliferation and invasion ability. EGCG significantly inhibited growth of PANC-1, MIA PaCa-2 and BxPC-3 cells at 25 μM. Incubation with EGCG also caused a decrease in the invasive ability of all three cell lines through Matrigel (81). EGCG, epicatechin-3-gallate, green and black tea polyphenols also inhibited growth of the human pancreatic cancer cell line HPAC, decreased expression of k-ras, and, somewhat surprisingly, also increased expression of the multi- drug resistance gene (82). Hong *et al.* studied flavonoids and other tea polyphenols. All green tea polyphenols inhibited lipogenase and cyclooxygenase arachidonic acid metabolism (83). Lipoxygenase and cyclooxygenase are known to be critical in the progression of pancreatic cancer (84).

Conclusion

There is an ever-increasing interest in the role of natural products to treat cancer. There have been many studies in various systems on the flavonoids, with more literature recently appearing regarding flavonoids and pancreatic cancer. It is certain, however, that research will continue and more potential agents will be identified in this very large and ubiquitous group of compounds in the plant world. Perhaps future recommendations by agencies such

as the American Cancer Society will include recommendations for a diet high in flavonoids to prevent pancreatic cancer, and that these agents, in higher concentrations, will be used to treat cancer when it does occur. Furthermore, based on existing experimental evidence, it is likely that cancer preventive effects of dietary flavonoids will be identified. Perhaps a daily glass of wine will be found to be beneficial in contributing to cancer prevention as well as for reducing the risk of cardiovascular diseases.

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