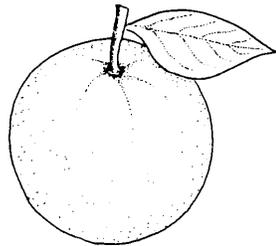


The Potential of Citrus Limonoids as Anticancer Agents

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Nutrition research on the health benefits of substances in plant foods (phytochemicals) has recently advanced to a new stage. The research frontier has moved from study of classical vitamin deficiency diseases, to study of the thousands of phytochemicals that may have important physiologic effects. The value of vitamin C for preventing scurvy during long ocean voyages was recognized by the New World explorers centuries ago, however the value of vitamin C as an antioxidant which may help prevent chronic diseases has been revealed only during the past several decades. Population studies show that cancer risk is about twice as great for individuals who consume few fruits and vegetables compared to those who consume a large amount. Increased intake of vitamin C, an important antioxidant found in fruits and vegetables, is strongly linked to reduced risk of many types of cancers (Block 1991). However, the health benefits of plant foods go beyond vitamin C and antioxidant protection. During the past decade, information on the health benefits of many classes of phytochemicals has appeared, such as for carotenoids, flavonoids, and folate. Along with vitamin C, these phytonutrients



are abundant in citrus fruit, and all show physiologic actions that may contribute to cancer prevention.

Vitamin C and flavonoids are antioxidants, substances that neutralize reactive oxygen species which can damage body cells and contribute to chronic diseases including cancer. Carotenoids, colored pigments in fruits and vegetables such as beta-carotene, lycopene, and lutein, also provide some antioxidant protection, but have other beneficial actions involving cell growth and vision. Folate is a B vitamin that is needed for the synthesis of DNA, and therefore is important for the integrity of genetic material in cells and the healthy growth of tissues. Recent information indicates that mild folate deficiency alters the structure of DNA in a way that may decrease the expression of tumor suppressor proteins. A survey of food folate sources showed that orange juice is the largest contributor to the food folate intake in the U.S. population. This is not only because of the high folate content of orange juice, but also because of the high frequency of orange juice consumption. Recent research suggests that U.S. consumers may be getting another health benefit from orange juice and other citrus products – phytochemicals called limonoids which appear to possess substantial anticancer activity.

Evidence for Anticancer Actions of Citrus Limonoids

Limonoids are unique highly oxygenated triterpenoid compounds long recognized as significant biologically active natural compounds. Citrus limonoids appear in large amounts in citrus juice and citrus tissues as water soluble limonoid glucosides or in seeds as water insoluble limonoid aglycones (Ozaki et al 1995). Limonoid glucoside concentrations can reach levels of 350-400 ppm in orange juice. The limonoid aglycones are responsible for the development of delayed bitterness in citrus (Maier et al 1977) and are converted to the non-bitter limonoid glucosides during fruit maturation (Hasegawa et al 1991).

Several citrus limonoids have recently been subjected to anticancer screen procedures utilizing laboratory animals and human breast cancer cells in culture. In mice, it was found that five limonoid aglycones (limonin, nomilin, obacunone, iso-obacunoic acid, ichangin) induced significant amounts of glutathione-S-transferase (GST) in the liver and intestinal mucosa (Lam et al 1994). GST is a major detoxifying enzyme system which catalyzes the conjugation of glutathione with many potentially carcinogenic compounds which are highly electrophilic in nature. A study of the inhibitory effects of two limonoid aglycones (limonin and nomilin) on the formation of benzo[a]pyrene induced neoplasia in the forestomach of ICR/Ha mice showed that incidence of tumors could be reduced by more than 50% at 10mg/dose (Lam and Hasegawa, 1989). In hamster, limonin was found to be a potent inhibitor of 7,12-dimethylbenz[a]anthracene induced oral carcinogenesis (Miller et al 1989). Topical application showed a 60% reduction in tumor burden. Nomilin was less effective. Topical application of the limonin glucoside to the same oral tumors in hamsters showed a 55% reduction in tumor burden (Miller et al 1992). This observation has added significance considering the predominance of limonin glucoside among the large amount of limonoid glucosides present in fruit tissues and juice. Most recently several limonoid aglycones and a mixture of limonoid glucosides were administered in vitro to estrogen dependent and estrogen independent human breast cancer cell lines (Table 1) (Guthrie et al 2000). The results showed that the limonoids were equally potent as tamoxifen

Table 1. Activity of citrus limonoids against proliferation of breast cancer cells

Limonoid Tested	Estrogen	Estrogen
	Dependent ^a MCG-7 (+)	Independent ^a MDA-MB-435 (-)
Limoinin	2.0	12.5
Limoinin glucoside	35.0	75.0
Nomilin	0.05	0.04
Limonoid glucoside mixture	0.05	0.08
Tamoxifen	0.04	90.0

^aIC₅₀ in mg/ml (concentration of test compound that inhibits growth by 50%).

for inhibiting the proliferation of estrogen-dependent breast cancer cells, and more potent than tamoxifen for activity against estrogen-independent cancer cells. Limonin and obacunone fed to rats have also been shown to increase GST and quinone reductase activities in liver and colon mucosa and was correlated with the prevention of colon carcinogenesis in rats (Tanaka et al 1999).

Future Research on Citrus Limonoids

The experimental results described above indicate that citrus limonoids may provide substantial anticancer actions. The compounds have been shown to be free of toxic effects in animal models so potential exists for use of limonoids against human cancer in either the natural fruit, in citrus fortified with limonoids, or in purified forms of specific limonoids. Although the initial studies are very promising, they have been conducted primarily with in vitro cell culture and animal models. Thus, research is needed to determine whether the limonoids may be useful in preventing or treating cancer in humans. The first step is to assess the bioavailability of the compounds for humans – are they absorbed after ingestion, do they appear in the blood and tissues, and for how long. If limonoid compounds are found to be bioavailable, further human studies will be needed to assess the effects of limonoid ingestion on biomarkers related to cancer. Experimental studies include measures of effects on enzyme systems which detoxify carcinogens, oxidative damage to DNA, immunocompetence, and fecal mutagenicity. Other needed studies include those which further establish the link between consumption of citrus and reduced cancer risk in

large populations, and long-term clinical trials to test the efficacy of limonoid ingestion for preventing cancer in healthy adults. As to whether isolated limonoids or a natural citrus product might be tested, it should be noted that the whole fruit contains other phytonutrients that have been identified as probable cancer preventatives, including vitamin C, flavonoids, carotenoids, and folate.

Establishment of the bioavailability and anticancer actions of limonoid compounds in humans will enhance citrus marketability and the demand for citrus and citrus products. It will further endorse citrus as an important functional food which may prevent cancer in humans. In addition, it will increase prospects for revenue generation from the reclamation of limonoids from citrus production by-products currently marketed as cattle feed.

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