

Control of the Aggressive Capacity of Prostate Cancer by Nutritional Inhibitors of Urokinase and Lipoxxygenase

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ABSTRACT Phytochemicals of food and herbs are very potent antioxidants and free radical scavengers. It is accepted that these chemicals minimize DNA damage by reacting with free radicals and in this way they could prevent cancer. However, in some studies antioxidants increase incident of cancers instead of lowering it. These trials have caused a rethinking of the use of natural compounds as chemoprevention agents utilizing antioxidant concept. It was hypothesized instead that elements of food could react with enzymes crucial for cancer formation and growth. We choose two of these as an illustration of a concept. Urokinase and lipoxxygenases are enzymes of a profound impact on cancer cells growth, metastasis and invasiveness. This article provides review of natural compounds, mostly antioxidants that are inhibitors of lipoxxygenase and urokinase. Inhibition of these enzymes by food elements could prevent or reduce cancer growth in this way rather than utilizing antioxidant pathway.

INTRODUCTION

Epidemiological studies are showing that cancer incidents are differing depending on geographical locations. Furthermore, when person moves from low to high cancer incident geographical location, his/her risk of developing cancer increases to the level observed in a population in which he/she is going to live. It is generally believed that this phenomenon is related to chemopreventing agents of food. Most widely circulating hypothesis is that ingredients of food with anti-oxidant properties minimize DNA damage by reacting with free radicals and consequently prevent cancer. Unfortunately, in the USA 18,000 high-risk smokers found that diet rich in a combination of β -carotene and retinyl palmitate resulted in a 28% increase in the incidence of lung cancer instead preventing it. A similar study conducted in Finland utilizing α -tocopherol and β -carotene, had similar findings for the group taking β -carotene (Goodman 2000).

In the different studies flavonols and flavones were investigated to determine chemoprevention activity against cancer. The study cohort consisted of 27,110 male smokers, aged 50-69 years, without history of cancer. They were participants of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study in Finland. During an average 6.1-year follow-up, 791 lung cancers, 226 prostate cancers, 156 urothelial cancers, 133 colorectal cancers, 111 stomach cancers, and 92 renal cell cancers were diagnosed. Intake of flavonols and flavones was inversely associated with the risk of lung cancer but no association was found between flavonol and flavone intake and the risk of other cancers (Hirvonen et al. 2001).

These two trials have caused a rethinking of the use of natural compounds as chemoprevention agents utilizing antioxidant concept. It is therefore likely that these chemicals (antioxidants) are acting in different way than expected. One of the possibilities is that they are disrupting specific pathways or inhibit enzymes that are important in cancerogenesis and cancer formation. Naturally found antioxidants are reacting with large number of enzymes from which we chose two as an illustration of this concept.

UROKINASE PLASMINOGEN ACTIVATOR

Proteolytic activity driven by urokinase plasminogen activator (uPA) or other proteolytic enzymes, such as metalloproteases, is commonly recognized as an important factor in metastasis. The urokinase activates plasminogen (nonactive form) turning it into its active form called plasmin. Plasmin is a strong proteolytic enzyme and hydrolyzes proteins of connective tissue and basement membranes. It activates other latent proteolytic enzymes, broadening the spectrum of proteins attacked. Pro-collagenase is activated

to collagenase in this way. Plasmin is a key enzyme in the mechanism responsible for tissue remodeling, tumor invasion, angiogenesis and development of distant metastasis.

An increased amount or activity of uPA, or urokinase plasminogen activator receptor (uPAR) per cell, has been found in human cancer cells lines with metastatic behavior (Conese and Blasi 1995). Moreover, animals injected with PC3 prostatic cancer cells expressing higher amounts of uPA and/or uPAR develop metastatic lesions including skeletal metastasis earlier and more frequently than animals injected with the same cell expressing lower amounts of uPA/uPAR (McMahon et al. 2001). Additionally, it has been reported that uPA activity is increased in metastatic tumors compared with primary tumors in experimental animals (Achbarou et al. 1994; Festuccia et al. 1995). The other plasminogen activator - tissue type plasminogen activator (tPA) is rarely overexpressed in malignant tumors and does not seem to be relevant in the metastatic process (Jankun et al. 1993; Wilson and Sinha 1993).

The ability of human carcinoma cells (expressing uPA) to invade the choriallantoic membrane and metastasize from it to the embryo, while treated with the antibody against the active site of uPA, was dramatically reduced in comparison to non-treated cells in the chicken embryo model (Ossowski 1988). Cells transfected with a plasmid, causing an overexpression of uPA in prostate cancer cells, showed a marked increase in metastasis, in comparison with the parental cell phenotype in the rat model. From the same phenotype, the cells underexpressing uPA were selected and these cells displayed drastically decreased metastasis. In this model prostatic PC3 cancer were used and decreased number of metastasis include skeletal metastasis (Kwaan et al. 1991). Reduction of metastatic potential by uPA inhibitors was shown on many *in vitro* and *in vivo* models also (Jarrard et al. 1994; Conese and Blasi 1995; Duffy 1998; McGowen et al. 2000).

It has been shown recently that inhibitors of uPA could reduce tumor size. Billstrom et al. showed that p-aminobenzamidine a competitive inhibitor of uPA, caused dose-dependent inhibition of uPA activity and decreased tumor-growth in DU-145 (human prostate cancer cells) inoculated SCID mice, when compared with non-treated animals (Billstrom et al. 1995). Amiloride,

another uPA inhibitor, reduces tumor growth and decreases the proliferation of the tumor cells in the hepatomas and intestinal carcinomas and LnCAP prostatic cancer xenografts grown in SCID mice (Kellen et al. 1988; Jankun et al. 1997, 1999; Evans and Sloan; Stakleff et al. 2000; Magdolen et al. 2000). In animals treated with a different uPA inhibitor, B623, a reduction in tumor size was observed. Unfortunately all these uPA inhibitors are toxic to humans in inhibitory activities or they are weak inhibitors (Kellen et al. 1988; Magdolen et al. 2000; Swiercz et al. 2001).

It has been reported that reduction of cancer growth by uPA inhibitors is related to anti-angiogenic activity (Bajou et al. 2001; Swiercz et al. 2001). The tip of neovascular advancing capillary vessels surrounding tumors has been reported to contain high amounts of uPA and its receptor. Binding of proteolytically inactive ligand to uPA receptor, reduces amount of uPA on the surface of capillary endothelial cells and reduces tumor growth. Also, our studies have shown that uPA inhibitors reduce angiogenesis in chick embryo model, and reduce length and number of sprouts of human umbilical vascular endothelial cells (HUVEC). In both cases inhibition of uPA activity on tip of capillary vessel or sprout prohibits cell migration and reduces their growth. Goodson et al. has shown that binding of proteolytically inactive uPAR ligands, prevents cell surface plasminogen activation and consequently prevents angiogenesis in mouse model (Goodson et al. 1994). It was emphasized that the uPAR focuses uPA activity and initiates proteolytic activity on the vascular capillary cell surface, which is required for angiogenesis. Others observed inhibition of angiogenesis in the rabbit cornea, while treating animals with amiloride, one of competitive inhibitors of uPA (Ignjatovic and Nikolic 1996). Unlike most malignancies, prostate cancer metastasizes preferentially to the skeleton. Its ability to invade and grow in bone marrow stroma is thought to be due in part to degradative enzymes. The formation of prostate skeletal metastases has been reproduced *in vitro* by growing co-cultures of prostatic epithelial cells in bone marrow stroma. Expression of urokinase plasminogen was identified to be responsible for this process (Achbarou et al. 1994). It was proposed that osseous metastatic prostate cancer cells must be osteomimetic in order to

metastasize, grow, and survive in the skeleton. The reciprocal interaction between prostate cancer and bone stromal growth factors, including hepatocyte growth factor/scatter factor (HGF/SF), among the others, initiates bone tropism, and is enhanced by uPA (Wilson and Sinha 1993). HGF/SF bears sequence and structural homology with plasminogen. HGF/SF exists in both an inactive single-chain form, and an active two-chain form. It was proposed that plasminogen activators could properly cleave single-chain of hepatocyte growth factor to generate active two-chain. It was suggested that uPA is a natural biological regulator of HGF (Mars et al. 1993). Moreover, in a positive feedback manner HGF stimulation of cancer cells result in overproduction of proteases including uPA stimulating further activation of HGF (Sparks et al. 1983; Billstrom et al. 1995; Koenen et al. 1999; Kermorgant et al. 2001; Hart et al. 2002). Therefore, eradication of unwanted uPA activity expressed by cancer could result in inhibition of metastasis and in inhibition of uPA driven angiogenesis.

NATURAL PRODUCTS COULD REDUCE UROKINASE ACTIVITY

It has been reported that a decrease in delta 6-insaturated essential fatty acid (EFA) metabolites occurs in malignant cells and that γ linolenic acid (GLA) and eicosapentaenoic acid (EPA) exert antimutagenic effects. Both GLA (n-6) and EPA (n-3) acted as competitive inhibitors of urokinase with $K_i = 120$ and $96 \mu\text{M}$, respectively, (du Toit et al. 1994; Van Aswegen and Du Plessis 1994).

Waghray and Webber tested effects of all-trans retinoic acid (RA) on the net enzymatic activity of secreted, extracellular urokinase-type plasminogen activator (u-PA) in DU-145 human prostatic carcinoma cells. After 48 h treatment with $1.0 \mu\text{M}$ RA a 50% reduction in free u-PA antigen level was demonstrated as compared to control. These results show that RA can decrease the net extracellular urokinase activity produced by prostatic carcinoma cells (Waghray and Webber 1995).

Onesti et al. investigated inhibitory activity of the Kunitz-type trypsin inhibitor DE-3 from *Erythrina caffra* seeds (ETI) to several serine proteases including β -trypsin, α -chymotrypsin, the human tissue plasminogen activator, human

α -, β - and γ -thrombin, as well as the human urinary plasminogen activator. They found that proteinase:ETI complex formation characteristics vary between proteases but could inhibit urokinase in physiologically important concentrations (Onesti 1992).

Ushiro et al. purified compound from "Thunder God vine" (*T. wilfordii*) that have been used in China to treat rheumatoid arthritis. Rheumatoid arthritis, as well as solid tumors, is closely associated with neovascularization. Anti-arthritis drugs therefore may modulate tumor growth as well as neovascularization. He found that a compound purified from, the nortriter-penoid, demethylzeylasteral (TZ-93), inhibited expression of urokinase-type plasminogen activator (uPA) mRNA and uPA activity. TZ-93 significantly reduced the growth of well-vascularized tumors with volumes of more than 500 mm^3 and the density of microvessels in the tumors (Ushiro et al. 1997).

Contrary to above findings an unknown synergism of retinol (vitamin A) and L-ascorbic acid (vitamin C) was discovered using endothelial cells. Retinol stimulated the extracellular and intracellular activities of plasminogen activator up to approximately 8- and 4-fold from the control values, respectively. L-Ascorbic acid enhanced the extracellular and intracellular activities up to approximately 1.5-fold. However, administration of these two vitamins together enhanced the extracellular activity up to a 20- to 50-fold (Inada et al. 1985). Also, neutrophils isolated from cows that received vitamin E had significantly higher u-PA mRNA and total cell-associated and membrane-bound u-PA activity compared with non-treated control cows (Politis et al. 2001).

Transforming growth factor- β 1 (TGF- β 1) increases urokinase expression/secretion, and urokinase could be inhibited indirectly by restraining TGF- β 1. Genistein and curcumin, decrease u-PA levels induced by TGF- β 1 and TGF- β 1 stimulated cell migration and invasiveness (Santibanez et al. 2000).

LIPOXYGENASE

Lipoxygenase enzymes are found in a wide variety of plant and animal tissues. These enzymes have a non-heme iron serving as a catalytic center for the stereo and regiospecific dioxygenation of select carbon atoms in

polyunsaturated fatty acids containing a 1,4-pentadiene motif. Eighteen carbon chain fatty acids (*e.g.* linoleate) are the primary substrates of the plant lipoxygenases while the mammalian isozymes mainly catalyze the metabolism of fatty acids of carbon length 20 (*e.g.* arachidonate). Nomenclature for the lipoxygenases found in mammals arises from the positional oxygenation along the carbon chain of arachidonic acid and are described as 5-, 8-, 12-, and 15- type LOXs (Lanefelt and Martnsson 1985).

The amino acid sequences between plant and mammalian LOX enzymes show considerable homology. The soybean lipoxygenases isoenzymes, L1 (Boyington et al. 1993) and L3 (Skrzypczak-Jankun et al. 1997), are 72% identical in their amino acid sequences, but share only 25% sequence homology to any mammalian 15-LOX. Overall, sequence identity between plant and mammalian pairs of lipoxygenase isozymes is 21-27%, while plant pair sequence identity ranges from 43-86%, with mammalian pair sequences at 39-93% identity (Prigge et al. 1996). The highest level of sequence identity between lipoxygenases from plants and mammals lies in the area of the catalytic domain containing the non-heme iron atom. Lipoxygenases play physiological roles in processes such as growth, development, wound healing and senescence. Mammalian LOXs use arachidonic acid as the primary substrate which, once released from the mammalian membrane through the action of PLA₂ or a combination of other phospholipases (Needleman et al. 1986), can be metabolized into leukotrienes (LT), lipoxins, or into eicosanoids including hydroxyeic- osatetraenoic (HETE) acids. As a whole, mammalian LOXs and products produced by substrate metabolism plays significant roles in cancer cell growth, metastasis, invasiveness, and cell survival (Skrzypczak-Jankun et al. 2000).

Arachidonic acid is metabolized by one of two enzyme pathways, cyclooxygenase (COX) or lipoxygenase (LOX) generating biologically active metabolites that are involved in carcinogenesis. It has been shown that the LOXs in particular are key regulators of cell survival and apoptosis in cells. It has been shown also that 12-LOX is a regulator of human cancer development and it is overexpressed in a variety of tumors including breast, colorectal, and prostate cancer, and cancer cell lines (Pidgeon et al. 2002).

12-LOX expression was detected in human prostatic tumors and correlated to the clinical stage of disease. Also human prostate cancer cell lines express the platelet-type isoform of 12-LOX at both the mRNA and protein levels. The enzyme was detected by immunohisto chemistry in non-metastatic cells (PC-3 nm) and in metastatic cells (DU-145). After injection of tumor cells into SCID mice, the metastatic prostate carcinoma cells (DU-145) expressed 12-LOX at a significantly higher level compared with the non-metastatic counterparts, PC-3nm. Additionally, when cells were treated with the 12-LOX inhibitors N-benzyl-N-hydroxy-5-phenylpentamide (BHPP) or baicalein, it significantly inhibited lung colonization (Walker 2002). It has been shown also that PC3 and LNCaP convert arachidonic acid to the 5-lipoxygenase product, 5-HETE and when the formation of it is inhibited, prostate cancer cells enter apoptosis (Myers and Ghosh 1999).

The overexpression of 15-lipoxygenase-1 in the PC-3 human prostate cancer caused increased frequency of tumor formation in athymic nude mice and sizes of the tumors formed compared with parental PC-3 cells. Addition of a specific 15-LOX-1 inhibitor, PD146176, caused a dose-dependent inhibition of proliferation *in vitro* (Kelavkar et al. 2001).

In addition expression of 12-lipoxygenase, and 15-lipoxygenase-1 are up regulated during human prostate cancer progression. It has been reported that inhibition of oxidative enzymes such as 5-lipoxygenase and 12-lipoxygenase trigger tumor cell apoptosis, reduce tumor cell motility and invasiveness, or decrease tumor angiogenesis and growth (Nie et al. 2001).

NATURAL PRODUCTS COULD REDUCE LIPOXY GENASE ACTIVITY

Phenols and polyphenols are one of the major groups of nonessential dietary components appearing in vegetable, fruits and teas. More than 8,000 different compounds are included in this group and many of them are antioxidants. There is an increased interest in these compounds because they have been associated with the inhibition of atherosclerosis and cancer (Martinez-Valverde et al. 2000).

Schewe et al. reports on cocoas and chocolates which are rich in (-)-epicatechin and its related oligomers, the procyanidins. These compounds isolated from the seeds of

Theobroma cacao, trigger concentration-dependent inhibition of isolated rabbit 15-lipoxygenase-1. (-)-Epicatechin and procyanidin nonamer also inhibited the formation of 15-hydroxy-eicosatetraenoic acid (Schewe et al. 2001). The polyphenols of green and black tea inhibit lipoxygenase dependent arachidonic acid metabolism also. At a concentration of 30 µg/mL, (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), and (-)-epicatechin-3-gallate (ECG) from green tea and theaflavins from black tea inhibited LOX-dependent activity by 30-75%. The formation of 5-, 12-, and 15-LOX metabolites were inhibited to a similar extent (Hong et al. 2001). The polyphenols extracted from pomegranate (*Punica granatum*) fermented juice and seed oil showed strong antioxidant activity close to that of green tea (*Thea sinensis*), and significantly greater than that of red wine (*Vitis vitifera*). Flavonoids extracted from seed oil showed 81% inhibition of soybean lipoxygenase. Flavonoids extracted from fermented juice showed 21-30% inhibition of soybean lipoxygenase though no significant inhibition of sheep cyclooxygenase (Schubert et al. 1999).

Curcumin is yet another ingredient of food with antioxidant properties. It is a major component of the *Curcuma* species, which is commonly used as a yellow coloring and flavoring agent in foods in Asia. This chemical is a naturally occurring polyphenolic phytochemical isolated from the powdered rhizome of the plant *Curcuma longa*. Curcumin has known anti-inflammatory property and was used for generation in folk medicine. It is a potent inhibitor of oxidative enzymes, such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase and inducible nitric oxide synthase (Li and Lin-Shia 2001). By use of molecular modeling methods, X-ray diffraction and mass spectrometry, we have found that curcumin binds to active site of soybean lipoxygenase and when illuminated by X-rays it degrades to 4-hydroxyperoxy-2-methoxyphenol (Skrzypczak-Jankun et al. 2000).

Belman et al. investigated the inhibition of soybean lipoxygenase by onion and garlic components. He found that the di- (1-propenyl) sulfide was the only irreversible inhibitor with $K_i = 59 \mu\text{M}$. Diallyl trisulfide, allyl methyl trisulfide, and diallyl disulfide were competitive inhibitors, while 1-propenylpropyl sulfide and (E, Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (ajoene) were mixed inhibitors. Nordihydroguaiaretic acid

(NDGA), the most potent lipoxygenase inhibitor, was a competitive inhibitor with $K_i = 0.29 \mu\text{M}$. (Belman et al. 1989). Sendl et al. studied lipoxygenase inhibitory activity of garlic also. He used extracts of wild garlic (*Allium ursinum*) and garlic (*A. sativum*) with defined chemical compositions to assess their inhibitory potential on 5-lipoxygenase. The inhibition rated as IC50 values of these extracts showed a good correlation with the %-content of the major S- containing compounds (thiosulfinates and ajoenes) (Sendl et al. 1992). In a separate study nine thiosulfinates (TS) and four "Cepaenes" (CS) isolated from onions demonstrated dose dependent (0.25 to 100 µM) inhibition of 5-lipoxygenase activity (tested on porcine leukocytes). They observed the following rank order of activity: saturated aliphatic TS > aromatic TS approximately α , β unsaturated TS > CS (Wagner et al. 1990).

Interest in the health-promoting effects of 'Mediterranean diet' and more specifically virgin olive oil, impelled de la Puerta et al. to investigate the anti-eicosanoid and antioxidant effects in leukocytes of the principal phenolic compounds from the 'polar fraction': oleuropein, tyrosol, hydroxytyrosol, and caffeic acid. All these compounds inhibited leukotriene B4 generation at the 5-lipoxygenase level with effectiveness: hydroxytyrosol > oleuropein > caffeic acid > tyrosol (IC50 values: 15, 80, 200, and 500 µM) (de la Puerta et al. 1999).

Takahashi et al. found terphenyl compounds, named BI-I, BI-II, BI-III, BI-IV and BI-V, showing 5-lipoxygenase inhibitory activity. These have been isolated from the mushroom *Boletopsis leucomelas*. On the basis of physico-chemical and spectral evidence he concluded that these are to be a series of cycloleucomelone-leucoacetates (Takahashi et al. 1992).

An old folk remedy anti-inflammatory herb from *Isatis tinctoria* L. used as an old European and Chinese dye plant is a source of potent cyclooxygenase-2 and 5-lipoxygenase inhibitor tryptanthrin (indolo-[2,1-b]-quinazoline-6,12-dione) (Danz et al. 2002). Additionally, in different study methanolic extracts of 25 different Nepalese medicinal plants were tested for their activity to inhibit the biosynthesis of leukotriene B4 in bovine polymorphonuclear leukocytes. Many selected indigenous plants are used in traditional folk medicine as herb remedies to treat inflammatory diseases. The leaves of *Zanthoxylum nepalensis* shown the highest LOX

inhibitory activity with an IC(50) of 11 µg/ml. Other extracts from *Astercantha longifolia* and *Hedychium ellipticum* showed less inhibitory activity of IC50 between 20 and 22 µg/mL (Kumar et al. 2000).

The above short review indicates strong potential of the compound from the Nature pharmacy to be useful in preventing disease. However, many natural remedies are nonspecific and target broad ranges of enzymes. For this reason a future extensive studies might be required to learn about their action and cross interaction with other enzymes and compounds.

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