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Chemoprevention of carcinoma prostate: A review

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Abstract. *Purpose:* Chemoprevention of prostate cancer is the administration of agents to prevent, inhibit, or delay progression of prostate cancer. Opportunities exist for testing various types of chemopreventive interventsion. *Material and methods:* The authors reviewed the relevant articles published in the last twenty years and studied the biology of the prostate cancer. An attempt is made to identify intermediate markers and surrogate endpoint markers. The various interventions and initial clinical trial results are described. End points for evaluation are mainly based on changes in PSA, changes of histological precursors, or time of onset of clinical disease. *Results:* Nutritional factors such as reduced fat intake, vitamin A, vitamin E, vitamin C, vitamin D, Lycopene and selenium may have a protective effect against prostate cancer. *Conclusion:* Numerous studies implicate dietary and nutritional factors in the onset and progression of prostate cancer. Hence, it is possible that bioactive compounds (anti-oxidants) like vits. A, C, D, E, minerals like selenium and carotenoids like lycopene can be a part of chemopreventive strategies for prostate cancer. Ongoing studies on nutrition and prostate cancer may bring the required evidence to support what is still only a hypothesis at present. However, absolute recommendation will have to await the results of long term prospective clinical trials.

Introduction

Prostatic carcinoma, in the male population ranks first as incidence and second as cause of oncologic mortality. The present trends of the clinical research in this field are directed towards the identification of factors involved in the onset of this neoplasm and the possibility of decreasing its incidence with programs of chemoprevention, the identification of new biological markers able to assess the biological potential of the disease. An overview of the prevention of this important condition is given in this article, and diagnostic markers along with possible experimental models for prevention or prolongation of carcinogenesis are presented in brief. The large variability in the incidence of the tumor in different geographical regions suggests the possibility of nutritional influences regarding the stimulation and/or inhibition of clinical cancer, as there is a similar prevalence worldwide of the precursor lesion. Epidemiological studies have shown that the Asian men have a much lower incidence of prostate cancer than men in Euorpe or the USA. Asian food includes low-fat, high-fiber diets, which provide a rich supply of weak dietary estrogens. These entrogens have been proposed as chemopreventive agents. In addition to their estrogenic activity, many of these plant compounds can interfere with steroid metabolism and bioavailability and can also inhibit enzymes, such as tyrosine kinase or topoisomerase, which are important for cellular proliferation [41]. A great number of publications have dealt with a number of nutritional factors such as reduced fat intake, phytoestrogens, vitamin E, vitamin D, and selenium and inferred that these may have a protective effect against prostate cancer. The fact was proven in large epidemiological studies as well as experimental observation. In the animal model, the progression of established tumors can be inhibited by these agents.

Discussion

Over the last decade, the Chemoprevention Branch, Division of Cancer Prevention and Control, National Cancer Institute, USA; has been developing drugs that will slow or stop the progression to invasive cancer of precancerous (pre-invasive) lesions generally termed ‘intraepithelial dysplasia’ or ‘dysplasia’. Over 40 short-term clinical trials are in progress [5]. Cancer

prevention refers to the prevention or prolongation of the onset of carcinogenesis by intervention with the agents to prevent, suppress, or reverse malignant transformation [3]. Why carcinoma prostate has been chosen for chemoprevention strategies? Carcinoma prostate is considered to be ideal for chemoprevention because of the following reasons.

i. It has got a protracted course (long latency time).
ii. A high incidence rate.
iii. Availability of an effective marker like serum PSA.
iv. Possible availability of a genetic marker (P53) that can be used as an end point of prostate cancer prevention.
v. Hormone dependency.

The study of prostate carcinogenesis and tumor progression is made difficult by the lack of appropriate in vitro and in vivo models. High prevalence intra-epithelial neoplasia and latent prostatic carcinoma, representing multiple steps in carcinogenesis to invasive carcinoma, are relevant targets for cancer prevention. Webber et al. (2001) derived four tumorigenic cell lines with progressive malignant characteristics. The MNU cell lines, in order of increasing malignancy were; WPE1-NA22, WPE1-NB14, WPE1-NB11, and WPE1-NB26. Development of effective chemopreventive agents for human consumption requires conclusive evidence of their efficacy in animal models that have relevance to human diseases. Transgenic adenocarcinoma mouse prostate (TRAMP) is an excellent model of prostate cancer that mimics progressive forms of human disease inasmuch as 100% of males develop histological PIN by 8–12 weeks of age that progress to adenocarcinoma. In these animals, ornithine decarboxylase (ODC) activity (> 3-fold) as well as protein expression (> 4-fold) was found to be markedly higher in the dorsolateral prostate as compared with the nontransgenic littermates, suggesting their suitability to determine the chemopreventive effect of alpha-difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ODC, against prostate cancer [14].

Prostatic intraepithelial neoplasia: A marker for high-risk groups and a potential target for chemoprevention

The search for the precursor of prostatic adenocarcinoma has focused in recent years on two histopathologic findings: high grade prostatic intraepithelial neoplasia (PIN) and atypical adenomatous hyperplasia (AAH). Clinical studies suggest that PIN predates carcinoma by 10 years or more, with low grade PIN first appearing in men in their 30s. Unlike PIN, AAH is weakly linked to carcinoma. It was stated that this family of cell lines with a common lineage represents a unique and relevant model which mimics stages in prostatic intra-epithelial neoplasia (PIN) and progression to invasive cancer, and can be used to study carcinogenesis, progression, intervention, and chemoprevention [39]. Lopaczynski et al. (2001), in preprostatectomy model observed that IGF system appears to play an important role in the development of prostate cancer by modulation of paracrine pathways, and also by modulation of the concentrations of different stromal and epithelial IGFBP, which are differentially expressed in the epithelium and stroma. Nerve growth factor is capable of stimulating a proliferative response via a high affinity Trk receptor present in normal and malignant prostate epithelia, and alternatively can mediate apoptosis via the low affinity p75NTR receptor that is progressively lost from the malignant prostate. As the role of each stromal element involved in carcinogenesis becomes further defined, these elements offer promising targets for new chemopreventive strategies [21]. There is a tremendous need for exposure biomarkers, which need to function as intermediate end-points in cancer chemoprevention studies. Imbalance between cell proliferation and cell apoptosis has been considered a key factor in carcinogenesis. Prostatic intraepithelial neoplasia (PIN) is the most likely precancereous lesion and represents the major target for chemoprevention of prostate cancer [41]. High-grade prostatic intraepithelial neoplasia (HGPIN) has also been investigated as an intermediate biomarker for prostate cancer. Endocrine therapy changes the morphology of PIN, hampering its identification by making it more closely resemble the normal benign glands. Androgen deprivation therapy decreases the prevalence and extent of PIN, suggesting that this form of treatment may play a role in chemoprevention [8, 9]. Cessation of endocrine therapy is likely to lead to renewed expansion of PIN, since PIN continues to express androgen receptors and the cell-cycle protein MIB-1 under conditions of low androgen levels. HGPIN has the advantage that it appears to be quite highly proximal to the development of cancer and to be modifiable [18, 23]. Loss of expression of the pi-class glutathione S-transferase enzyme GSTP1, which is associated with the hypermethylation of dexoycytidine residues in the 5′-regulatory CG island

region of the GSTP1 gene, is a near-universal finding in human prostate cancer. Likewise high-grade PIN is completely devoid of GSTP1. Hypermethylation of the 5′-regulatory region of the GSTP1 gene may serve as an important molecular genetic biomarker for both prostate cancer and PIN [10].

Animal models in defining efficacy of chemoprevention agents against prostate cancer

Animal models are crucial in preclinical efficacy testing of chemoprevention agents. The most feasible, realistic, and potentially effective target for prostate cancer chemoprevention is progression from prostatic intraepithelial neoplasia (PIN) to histologic cancer and from histologic to clinically manifest cancer. There are transgenic mouse models for prostate cancer and models for PIN, but these have not yet been fully developed and evaluated for chemoprevention studies. Human prostate cancer xenografts in mice and transplantable Dunning rat prostate carcinomas can be used to assess tumor growth inhibition. PIN occurs mostly in these two models, and metastases are frequent in some transgenic models and the MNU-testosterone rate model [7]. The three most widely used carcinoma cell liens, DU-145, PC-3, and LNCaP, developed between 1977 and 1980, have greatly contributed to our present understanding of prostate cancer. These cell lines will further serve as useful models for investigating tumor progression, invasion, metastasis, new therapeutic strategies, drug resistance and its reversal and chemoprevention [38].

Chemopreventive strategies

Chemopreventive agents (CPA) are classified as (i) Inhibitors of initiation, (ii) Anti- promotional agents and (iii) Inhibitors of progression. The various bioactive compounds which act as anti-oxidants and scavengers combine to the target tissues and protect the body against the harmful effect of free radicals which would otherwise combine to these tissues (Figure 1). Free radical is an atom or molecule that has one or more unpaired electrons its consequent tendency to acquire an electron makes it highly reactive. Antioxidant is defined as any compound, which breaks the free radical reaction chain. Different mechanisms have been proposed for various kinds of chemopreventive agents. Recommended chemoprevention strategies based on these mechanisms are (i) the development of better technology for early diagnosis, (ii) the development of multiple agents that block intralesional proliferation at steps along the signal pathway of mitotic signal transduction and along the signal pathway of sythnesis of daughter cell components, (iii) the development of nontoxic anti-inflammatory agents, antioxidants, antimutagens, and proapoptotics, (iv) the avoidance of 'clonal escape' through use of drug combinations, and (v) the use of computer-assisted quantitative image analysis to assay modulation of surrogate end points in chemoprevention clinical trials [6]. Ideal chemopreventive agent should be nontoxic, efficacious, easily available and inexpensive. CPA can be provided as a part of modified diet or synthetic derivatives (Pills). The various possible chemopreventive agents and measures for prostate cancer are:

- Vitamins: A, D, C and E
- Minerals: Selenium
- Carotenoids: Lycopenes
- Dietary fat
- Hormonal manipulation: Flutamide, Dehydroepiandrosterone, 5alpha-reductase inhibitor
- Miscellaneous compounds: Nonsteroidal anti-inflammatory drugs, Green tea.

Vitamin A

Fat soluble vitamin, which occurs in nature as retinal and dehydroretinol. Synthetically it is derived from carotenoids (betacarotene). Carotenoids retinal and retinoic acid interact with specific intracellular receptors and affect protein synthesis finally controlling cell chromatin, cell growth and cell differentiation [20]. There are over six human retinoid receptors (RAR [α, β and χ] and PXR [α, β and χ]) and all six belong to the steroid receptor superfamily. Vitamin A and its analogues modulate the growth and differentiation of cancer cells presumably by activating gene transcription via the nuclear retinoic acid receptor (RAR) alpha, beta, and gamma and retinoid X receptor (RXR) alpha, beta, and gamma [35]. Fenretinide (N-4-hydroxyphenyl retinamide) (4HPR) a vitamin A analogues has been found to be relatively nontoxic in preclinical experiments and early clinical trials. Experimental studies have shown in Mouse prostate reconstitution model system, Fenretidine showed a lowered incidence of tumor by 49%

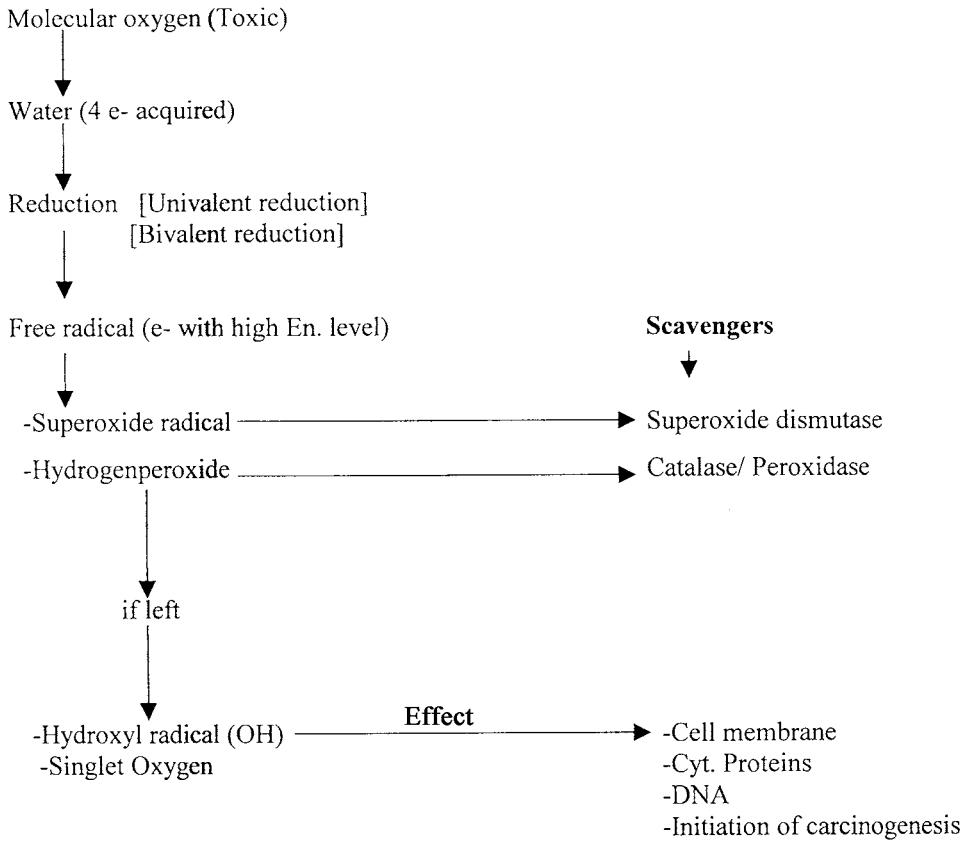

Figure 1. Mitochondrial cytochrome oxidase system.

and tumor mass by 52% as compared to normal fed animals and in LN Cap cell line culture. Fenretidine produced 82–95% suppression of cell growth, partial arrest in G1 phase of cell cycle and marked increase in the number of apoptic cells [34]. The chemopreventive effect of retinoids is most likely exerted at the tumor-promotion phase of carcinogenesis. Retinoids block tumour promotion by inhibiting proliferation, inducing apoptosis, inducing differentiation, or a combination of these actions [25]. It has been proposed that in prostate cancer, urokinase-type plasminogen activator (u-PA) is the key enzyme which occupies a place at the apex of the proteolytic cascade and initiates the degradative process needed for ability to invade and metastasize. All-trans retinoic acid (RA) reduced the ability of u-PA-mediated degradation of fibronectin and laminin [37]. Animal studies proved that prostate carcinoma tissue contains 5–8 times less retinoic acid than normal prostate or BPH. The lower retinoic acid content may contribute to cancer development or just be a marker of cellular transformation possibly explained by a more rapid degradation. However, the Alpha-tocopherol Beta-carotene Cancer Prevention study in a placebo controlled, randomized trial showed that betacarotene treatment resulted in increase in cancer at the lungs, prostate and stomach. This effect was more evident in alcoholics, which might shift the dose response curve. Another possibility could be that an excess administration may reduce the efficacy or even promote tumors [3].

Vitamin D

Calcitriol (1,25-D3) is the active form of vit. D which is produced in the skin ultraviolet radiation of 7-dehydrocholestol. Epidemiological studies have shown that a lower 1,25-D3 is associated with increased incidence of carcinoma prostate and an inverse relationship was observed between skin exposure and mortality rate for carcinoma prostate [31, 33]. Animal studies have proved that vit. D3

inhibit cell proliferation, promote cell differentiation and selectively decreases level of type IV collagen in carcinoma prostate tumor cells [32]. Human trials have shown that receptors for vitamin D (1alpha, 25-dihydroxyvitamin D3) exist in human prostate cancer cell lines like, LN Cap, PC-3 and DU 145 [33]. The ubiquitous presence of vitamin D receptor and 24-hydroxylase activity in human prostatic carcinoma cells suggests new alternatives for the pharmacological treatment of advanced prostatic cancer and implies that chemoprevention strategies could also make use of this endocrine axis [24]. It has been observed that vitamin D and its analogoues induce cell cycle arrest and apoptosis in the premalignant adenoma cells [11].

Vitamin E

It occurs in the nature as a mixture of several closely relative compounds called as tocopherols. It is an important natural antioxodant and scavenger. Due to its lypophilic character it accumulates in circulating lipoproteins, cellular membranes and fat deposits, where it reacts very rapidly with molecular oxygen and free radicals. It acts as a scavenger for these compounds, protecting unsaturated fatty acids from peroxidation reaction and protects cellular respiration by stabilizing coenzyme-Q. Animal studies have shown that dietary vit. E produces 30–60% inhibition of induced carcinogenesis due to its ability to inhibit synthesis of nitrosamine compounds. In human alpha-Tocopherol Bet-Carotene Cancer Prevention Study (1995) showed that man receiving vit. E had a 34% lower incidence of carcinoma prostate [1, 36]. Eichholzer et al. (1996) in their study observed that men with low plasma levels of vit. E had an increased risk of carcinoma prostate [12].

Vitamin C

Vitamin C is a six carbon organic acid with structural similarity to glucose. It is a potent reducing (anti-oxidant) in several hydroxylation reaction making it capable of reducing compounds like molecular oxygen and nitrates (scavenger). It inhibit malignant transformation, decreases chromosomal damage in the cell [4]. Diets mainly comprising of fruits and vegetables rich in vitamin C are associated with low incidence of cancer of oesophagus, stomach, colon, skin and lung. A decreased risk of lower urinary tract cancer has been shown with increased vitamin C intake in a study of patients matched for age, sex and ethnic groups to two population based controls [26]. Animal studies have shown that when treated with vit. C both tumor cell lines showed reduced viability for both DU145 (androgen independent) and LN Cap (androgen dependent). Vitamin C induces these changes through production of hydrogen peroxide, which in turn produces free radicals that damage the cells [22].

Selenium

It is an important component of metalloenzyme glutathione peroxidase, which destroys peroxidase in cytosol. It acts as a synergistic anti-oxidant with vitamin E. Epidemiological studies have shown that the risk of cancer for patients with low serum selenium levels reported up to twice that of subjects with high levels [19, 40]. A significantly low risk of developing prostate cancer has been observed in men receiving supplemental selenium.

Lycopene

Lycopene is a carotenoid which are found in high levels in some fruits and vegetables (cooked or raw tomatoes and watermelon). Lycopene acts as an anti-oxidant by preventing damage to DNA by protecting 2′-deoxy-guanosine against singlet oxygen damage. It suppresses insulin like growth factor-1-stimulated cell proliferation. An important study showed that lycopene is found in very high concentration in the prostate, adrenal and testes. Lycopene reduced levels of serum protein thiol (an oxidative damage biomolecular marker) levels among prostate cancer patients [19].

Prospective randomized controlled trials have shown lower prostate cancer risk in men with elevated plasma lycopene levels. Research results presented at 90th annual meeting of American Association for Cancer Research (AACR) described the different effects of lycopene like reduction in tumor size, lowering of S-PSA levels and reduction in grade of PIN.

Dietary fat

A study by the American Cancer Society has shown that obesity increased the risk of prostatic cancer [29]. Epidemiological studies done to compare different populations around the world revealed the relation of dietary fat and risk of prostate cancer [16]. Analysis has revealed that mortality rate of prostate cancer is correlated with the estimated intake of dietary fat. Essential fatty acids influence cellular proliferation, tissue invasiveness, metastatic spread of tumors and immune response as well as cell surface receptors [28]. Long chain n-3 fatty acids have inhibitory effect and n-6 fatty acids have stimulatory effect. Likwise men with increased levels of cholesterol have more risk of carcinoma prostate.

Hormonal manipulation

Flutamide

High-grade prostatic intraepithelial neoplasia (HGPIN) is believed to be a precursor for prostatic adenocarcinoma. The prevalence of prostatic intraepithelial neoplasia (PIN) increases with advancing age. Autopsy studies suggest that PIN may precede the development of prostatic adenocarcinoma by up to 10 years. Autopsy studies reveal that HGPIN is found in association with cancer in 63% to 94% of malignant and 25% to 43% of benign prostates [30]. As such, HGPIN is believed to be marker of increased risk. This provides a potential opportunity for chemoprevention. Flutamide is one agent with potential activity and limited side effects that may act to prevent or delay the onset of prostatic adenocarcinoma in men with HGPIN. A clinical trial is currently underway to assess the efficacy of flutamide [2].

Dehydroepiandrosterone (DHEA)

In male Wistar-Unilever rats after experimentally induction of prostate adenocarcinomas, it was seen that nontoxic doses of DHEA confer significant protection against prostate carcinogenesis in rats. The efficacy of delayed administration of DHEA suggests that the compound confers protection against later stages of prostate cancer induction and can suppress the progression of existing preneoplastic lesions to invasive disease [27].

5 alpha-reductase inhibitor

This compound is again under scrutiny as it has been observed that in male ACI/Seg rats, which spontaneously develop prostate cancer, the 5alpha-reductase inhibitor FK143 may, at specific doses, reduce the incidence of spontaneously developing prostate cancer [15].

Miscellaneous compounds

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) play potential roles in chemoprevention of colon cancer and others by inhibiting prostaglandin synthesis. Western and northern blot analyses demonstrated that flufenamic acid (FA) inhibited the androgen receptor (AR) expression at mRNA and protein levels when used on LNCaP cells, an androgen-responsive human prostate carcinoma cell line. Suppressed AR expression may be the cause of FA-mediated inhibition of the androgen inducible gene expression. FA and other similar NSAIDs may be potential candidates for chemoprevention of human prostate cancer by modulating the expression of AR [42].

Green tea

Ornithine decarboxylase (ODC), a rate-controlling enzyme in the polyamine biosynthetic pathway, is overexpressed in prostate cancer (PCA) and prostatic fluid in humans, modulation of ODC could be effective against prostate cancer. Green tea polyphenols (GTPs) posses strong chemopreventive properties against a variety of animal tumor models and in some human epidemiological studies. Similar effects of GTPs were observed in anchorage-independent growth assay of LNCaP cells where pretreatment of the cells with GTP was found to result in dose-dependent inhibition of colony formation. Similar effects of GTPs were observed in anchorage-independent growth assay of LNCaP cells where pretreatment of the cells with GTP was found to result in dose-dependent inhibition of colony formation [13]. In another study EGCG [(-)-epigallocatechin-3-gallate], the major constituent of green tea, was examined to understand mechanisms of action. Effects of EGCG on the cell population were examined with growth assays, cell cycle analysis, and western blots for retinoblastoma protein (pRB). In each cell type,

EGCG inhibited growth, with a decrease in efficacy as cells progressed from normal to cancer [17].

Conclusion

Numerous studies implicate dietary and nutritional factors in the onset and progression of prostate cancer. Hence, it is possible that bioactive compounds (anti-oxidants) like vits. A, D, C, and E, mineral like selenium and carotenoids like lycopene can be a part of chemopreventive strategies for prostate cancer. Besides the dietary fat modulation the various other compounds and measures proposed to have a chemopreventive effect are nonsteroidal anti-inflammatory drugs, green tea and hormonal manipulation (Flutamide, Dehydroepiandrosterone, 5alpha-reductase inhibitor). Ongoing studies on nutrition and prostate cancer may bring the required evidence to support what is still only a hypothesis at present. However, absolute recommendation will have to await the results of long term prospective clinical trials.

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