Oxidative stress and cyclooxygenase activity in prostate carcinogenesis: targets for chemopreventive strategies

S.K. Pathak a,b, R.A. Sharma b, W.P. Steward b, J.K. Mellon a, T.R.L. Griffiths a, A.J. Gescher b,*

a Division of Urology, Clinical Sciences Unit, Leicester General Hospital, University of Leicester, Gwendolen Road, Leicester LE5 4PW, UK
b Department of Cancer Studies and Molecular Medicine, Leicester Royal Infirmary, University of Leicester, 5th Floor RKCSB, Leicester LE2 7LX, UK

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Abstract

Over the last decade, epidemiological, experimental and clinical studies have implicated oxidative stress in the development and progression of prostate cancer. Oxidative stress may be linked to the effects of androgens, anti-oxidant systems and the pre-malignant condition, high-grade prostatic intraepithelial neoplasia. Cyclooxygenase-2 activity has been linked with prostate carcinogenesis. Evidence suggests that oxidative stress and cyclo-oxygenase-2 activity may be mechanistically linked. Agents such as antioxidants and cyclo-oxgenase-2 inhibitors may be of value in the chemoprevention of prostate cancer. The feasibility of intervention with such agents will depend on the development and validation of biomarkers for clinical trials, particularly markers of oxidative damage caused by reactive oxygen species (ROS). A greater understanding of the molecular events associated with oxidative stress will enhance the development of such biomarkers and should result in better strategies for the chemoprevention of prostate cancer.

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1. Introduction

Cancer of the prostate is the most commonly diagnosed solid malignancy and the second leading cause of cancer-related deaths in men living in developed countries [1]. Government statistics show that in the United Kingdom (UK) over 20000 new cases of prostate cancer are diagnosed and approximately 9000 men die as a result of the disease per annum [2]. The incidence is predicted to double over the next 20 years in the UK, mainly due to a combination of improved overall longevity of the general population and the more widespread use of prostate-specific antigen (PSA) testing, as has already been noted in North America following prostate cancer screening [3,4]. Therefore there exists a strong need for a greater understanding of the molecular events in early prostate carcinogenesis and the potential interventional opportunities to prevent or slow the progression of the disease.

Over the last decade, it has become apparent that oxidative stress may be an important aetiological factor in the development and progression of prostate cancer. The impetus for experimental and clinical research into the role of oxidative stress and prostate cancer has increased following important secondary analyses of two clinical trials. The a-Tocopherol and b-Carotene study (ATBC study) was a double-blind, randomised, placebo-controlled trial of 29133 male smokers receiving a-tocopherol, b-carotene, ATBC in combination, or a placebo. Lung cancer incidence was the principal...
endpoint measured. Paradoxically, the trial showed an increased incidence of lung cancer amongst one subgroup of men given β-carotene supplements. However, secondary analysis of the data at six years follow-up demonstrated a 32% reduction in the incidence and a 41% reduction in mortality from prostate cancer among men who received supplementary α-tocopherol [5]. In a randomised controlled study of non-melanoma skin cancer prevention, 1312 patients were randomised to receive selenium supplements or placebo. The study did not show a protective effect on skin cancer incidence; however, secondary analysis at follow-up showed a 60% reduction in the incidence of prostate cancer [6].

Amongst the principal biological mechanisms of selenium and α-tocopherol (vitamin E) are anti-oxidant actions involving the quenching of reactive oxygen species (ROS) [7,8].

The concept that oxidants may play a role in the aetiology of prostate cancer or precursor lesions is relatively new. In this article, the evidence which links oxidative events with the disease process is reviewed. Furthermore, potential interventional strategies which exploit anti-oxidant activity in the prevention of prostate carcinogenesis are discussed.

2. Prostate carcinogenesis

Prostate cancer is rarely seen in men before the age of 50 years. On autopsy, the incidence of histological prostate cancer is 80% in men above 80 years of age, although the vast majority of these lesions are clinically insignificant. Prostate cancer is a global, multi-focal disease. Anatomically, greater than 70% of cancers occur in the peripheral zone of the prostate gland. The methods used in the diagnosis of prostate cancer include physical examination of the prostate (that might easily miss small or centrally placed tumours), serum PSA testing (that is not specific for prostate malignancy) and tissue biopsy (sampling error may miss malignancy). For example, prostate cancer has been detected in approximately 30–40% of men having repeat biopsies, having shown only high-grade prostatic intraepithelial neoplasia (HGPIN) on their initial biopsies [9]. The natural molecular history of prostate cancer suggests that HGPIN and, more recently, proliferative inflammatory atrophy (PIA) are pre-malignant lesions (see below).

3. Oxidative stress

Oxidative stress is defined as a disturbance in the equilibrium between ROS and detoxifying anti-oxidant systems; an excess of ROS leads to oxidative damage to cellular constituents (Fig. 1). The balance is normally maintained by two principal mechanisms which render ROS less harmful. Firstly, intracellular anti-oxidants, such as vitamin C, vitamin E and selenium quench ROS. Secondly, anti-oxidant enzymes, such as glutathione S-transferase (GST)-π catalyse the conjugation of ROS to glutathione. Common ROS of biological significance include hydrogen peroxide, superoxide ions and hydroxyl anions. Sources of ROS can be subdivided into endogenous and exogenous, both types causing oxidative damage to important cellular molecules, including lipids, proteins and DNA [10].

ROS may react with DNA bases to produce oxidative DNA adducts. Such adducts have been associated with mutagenesis and carcinogenesis [11]. ROS may directly damage DNA leading to the formation of the DNA adduct, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG). Indirect actions of ROS include lipid peroxidation of cellular membranes, resulting in the production of compounds (e.g. malondialdehyde) which are capable of forming DNA adducts such as a pyrimidopurinone adduct of deoxyguanosine [11]. ROS can also increase the expression of transcriptional factors including c-fos and c-jun oncogenes involved in neoplastic transformation [12–14]. It has been shown that oxidative stress can be measured in prostate cancer cells [15,16], and that DNA damage can be strongly linked to oxidative damage [17]. A pro-oxidant state, which can be achieved by either an increase in ROS...
or a disruption of the normally protective anti-oxidant systems, has been observed in both HGPIN and prostate cancer [18].

4. Proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia

The association of chronic inflammation with the development of human cancers is well recognised [19]. It is likely that ROS released by inflammatory cells during cycles of cellular damage and regeneration in these organs, result in permanent DNA damage [20].

The prostate gland is a common site for chronic inflammation. Although most focal prostatic atrophy lesions are considered quiescent, prostate epithelial cell proliferation is increased in some lesions, thus focal prostatic atrophy, which is associated with chronic inflammation, is considered to be proliferative [21]. Recently, it has been suggested that PIA may develop into HGPIN and/or directly into prostate cancer. Unlike diffuse atrophy which is seen in androgen blockade therapy, PIA represents focal atrophy of certain regions of the prostate gland. Lesions previously reported as sclerotic atrophy and post-atrophic hyperplasia may be reclassified as PIA [22]. PIA is not routinely evident with standard haematoxylin and eosin staining, but can be recognised by special immunohistochemical staining, such as Ki-67 [23] and 34bE12 [24], and may be recognised by groups of highly proliferative prostatic epithelial cells intermingled with inflammatory cells [23]. It has been hypothesised that PIA is a pre-malignant condition similar to pre-malignant inflammation observed in the liver and large bowel [21]. Oxidative stress may play a role in PIA and HGPIN [23].

HGPIN is a lesion that is now widely accepted as a pre-malignant condition for prostate cancer development [25,26]. It has been suggested that HGPIN may take 20–30 years to develop and that progression to clinically significant cancer may take between 3 and 15 years [27]. HGPIN may be a reversible condition, and the potential for reversibility has been shown by androgen deprivation therapy [28]. In this trial, men diagnosed with prostate cancer were randomised into two groups prior to their radical prostatectomies, a treatment group to receive androgen deprivation therapy for three months, and the control group received no therapy prior to surgery. Histological features showed a statistically significant decrease in the incidence of HGPIN and stage of prostate cancer in the treated group. Although this trial showed histological alterations in HGPIN lesions with anti-androgen therapy, there is no evidence in well controlled clinical trials that this equates to a decrease in clinical progression of the disease.

5. The role of cellular anti-oxidant enzymes

Cellular anti-oxidant enzymes include GSTs, dismutases, catalase, glutathione peroxidase and glutathione reductase. Most studies in prostate cancer have focused on the first three of these enzyme systems, especially the GST superfamily. The potential damage inflicted upon cellular proteins and DNA due to the generation of ROS may be prevented by a number of anti-oxidant enzyme systems detoxifying ROS. For example, GST catalyses the conjugation of ROS to glutathione, thus rendering the reactive species less capable of reacting with cellular structures such as DNA (Fig. 1).

5.1. Glutathione S-transferases

The most extensively studied anti-oxidant enzyme in prostate cancer is the π class GST. In particular, expression of GSTP1 has been extensively studied in human prostatic tissues. Two thirds of normal basal prostatic epithelial cells immunostain intensely for GSTπ, whereas acinar epithelia stain weakly for GSTα [29]. Immunopositivity for GSTπ is rare in HGPIN and incidental prostate cancer [30]. Interestingly, GSTπ expression is increased in PIA, possibly in response to oxidative stress [23]. It has been postulated that some clones within PIA lesions are incapable of expressing GSTπ, potentially as a result of a gene defect [28]. Lesions may then become repetitively damaged by ROS, subsequently developing into HGPIN or prostate cancer.

Even in the presence of a normal GSTP1 gene, it is known that hypermethylation of the GSTP1 gene promoter can result in failure to transcribe the gene [18]. Hypermethylation of GSTP1 gene CpG island is present in a subset of PIA lesions, but not in normal or hyperplastic epithelium of the prostate [31]. Indeed, hypermethylation is seen in more than 70% of HGPIN lesions [32], and more than 90% of prostate cancer [33]. This epigenetic alteration may explain the vulnerability of HGPIN lesions and prostate cancer to oxidative cellular damage. In a detailed study using laser-capture microdissection, hypermethylation of the GSTP1 gene was seen to be present in some PIA lesions. However, this was not present in normal or hyperplastic epithelia of the prostate [31], supporting the hypothesis that these subsets of PIA lesions may lead to HGPIN and/or prostate cancer development.

Other members of the GST superfamily have been less well studied in prostate carcinogenesis. Increases in the α class GST expression have been implicated in the pathogenesis of human cancers, including renal and bladder cancers [34]. Recently, in a study of radical prostatectomy specimens, high levels of GSTA1 immunoreactivity were reported in PIA lesions, whereas low levels were found in HGPIN and prostate cancer [35].
This finding further suggests a lack of detoxification activity in HGPIN and prostate cancer.

5.2. Dismutases

Dismutases catalyse the conversion of superoxide radicals to water. There are two major forms of the superoxide dismutase (SOD). Intracellular copper-zinc SOD1 is found in the nucleus and cytoplasm. Manganese superoxide dismutase (SOD2) is found in mitochondria. Studies have found SOD1 and SOD2 immunoreactivity to be lower in prostate cancer cells than in benign cells [36, 37]. The selective immunoreactivity for SOD1 was found to be more nuclear than cytoplasmic in benign cells and cytoplasmic in prostate cancer cells [37]. A further study showed that nuclear immunoreactivity to SOD2 was greater in metastatic than primary prostate cancer cells [38]. Since ROS induce antioxidant enzyme systems, a plausible explanation could be that, in general, the growth rates of metastatic prostate cancer cells tend to be much greater than primary prostate cancer cells; and the greater oxidative stress seen in the former cells reflects progression of the disease.

In contrast to these immunohistochemical studies, enzyme activity assays for SOD1 and SOD2 have shown similar levels of activity in benign and malignant prostatic epithelial cell cultures [39]. However, enzyme activity was assessed in cellular homogenates that were contaminated with normal cells, which may make it difficult to draw definite conclusions from the enzyme activity study.

5.3. Catalase

Catalase (CAT) catalyses the conversion of hydrogen peroxide to water and is located within peroxisomes and the cytoplasm of cells. Two studies have shown lower CAT immunoreactivity in prostate cancer than in benign prostate tissue [36, 37]. Lower CAT immunoreactivity has also been shown in HGPIN compared with benign prostate tissue [37]. By contrast, another study detected increased CAT activity in prostate cancer tissue compared with benign prostate tissue [40]. Another enzyme assay has detected similar levels of CAT activity in prostate cancer tissues and primary human prostate cells [39]. Conflicting results are not uncommon in the measurement of short-lived and highly reactive indices, such as assays that aim to directly measure ROS. An indirect, but more stable, and perhaps more biologically relevant, indicator of oxidative stress is the measurement of the levels of the products of oxidative damage, such as protein or oxidative DNA adducts. Such markers have been found to be elevated in metastatic prostate cancer cells compared with primary cancer cells [38].

5.4. Potential interventions

A number of interventional strategies are under development in an attempt to rectify deficiencies in the levels of anti-oxidant enzymes during the early stages of prostate carcinogenesis. Supplementary anti-oxidants may be given to patients as capsules or as part of the dietary matrix [41]. Alternatively, it may be possible to augment the expression of other anti-oxidant or detoxification enzymes, thus compensating, at least partly, for low levels of activity of enzymes such as GSTp. An agent which may be capable of favourable enzyme induction, oltipraz, is currently being developed in large-scale clinical trials in the chemoprevention of hepatocellular carcinoma in China [42], and its potential to prevent bladder cancer is also under scrutiny. Its effects on human prostate cells are not yet known.

6. The role of arachidonic acid metabolism

Diets rich in α-3 polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) found in fish oils may reduce the risk of prostate cancer [43]. α-6 Polyunsaturated fatty acids such as arachidonic acid and its precursor, linolenic acid, are major ingredients of vegetable oils: they are consumed in high quantities in the typical Western diet, and may be relevant to the pathogenesis of prostate cancer [44]. Oxidative lipid peroxidation accompanying the biosynthetic metabolism of arachidonic acid from membrane phospholipids to prostaglandins is a possible contributor to prostate carcinogenesis [45].

6.1. Cyclo-oxygenase enzymes

Cyclo-oxygenase is a rate-limiting enzyme in prostaglandin biosynthesis, a two-step enzymatic process in which ROS are generated as reviewed by Hussain et al. [46]. Firstly, arachidonic acid is converted to prostaglandin G2 by the oxygenase activity of cyclo-oxygenase, prostaglandin G2 then undergoes peroxidation to prostaglandin H2 (Fig. 2). Unlike cyclo-oxygenase-1 (COX-1), COX-2 is normally undetectable in most tissues, but it is inducible by a variety of stimuli, including mitogens, growth factors and cytokines [47]. Recent studies have shown that overexpression of COX-2 is sufficient to induce breast tumours in transgenic mice, subsequent inhibition of the COX-2 pathway resulted in a reduction in tumour incidence and progression [48]. In actual prostate tissue specimens, COX-2 expression remains controversial. Earlier data suggested COX-2 expression was significantly higher in cancerous than in benign prostate tissues when assessed by immunohistochemistry [49–51]. Western blotting [52] and reverse transcription-polymerase chain reaction (RT-PCR)
It has also been shown that the intensity of immunoreactivity of COX-2 correlates with tumour grade [54]. Of particular interest, increased immunostaining for COX-2 has been observed in most HGPIN [55] and PIA lesions [52]. Recent data suggests that there is no difference in expression between normal, HGPIN and cancerous lesions, except in high grade cancers and PIA lesions, in which COX-2 expression was found to be significantly increased [52]. This data emanates from the use of validated immunohistochemical assays and quantitative RT-PCR. With regard to preclinical comparison, COX-2 expression has been found to be significantly increased in HGPIN lesions compared with normal prostate tissue in the TRansgenic Adenocarcinoma of the Mouse model of Prostate cancer (TRAMP). Animals in this model invariably develop HGPIN lesions followed by malignant changes, thus it may represent a suitable pre-clinical model to test chemopreventive strategies using COX-2 inhibitors [56]. In contrast to actual prostate tissue specimens, human prostate cancer cell lines, commonly used in mechanistic studies, such as PC3, LNCaP and DU145 do not express COX-2 [57].

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, sulindac and indomethacin, inhibit the activity of both COX-1 and COX-2; consequently, they can cause platelet dysfunction, gastrointestinal ulceration and renal damage. For this reason, selective COX-2 inhibitors, such as celecoxib and rofecoxib, may be more attractive as potential chemopreventive agents, although their potential toxicities cannot be disregarded [58]. Epidemiological observations support the importance of NSAIDs in the chemoprevention of colon cancer [59]. However, epidemiological evidence for a protective effect of NSAIDs against prostate cancer is equivocal [60,61]. In vitro work using LNCaP and PC3 cell lines show that NSAIDs such as sulindac derivatives and etodolac decrease cell growth and induce apoptosis [62,63]. In contrast, apoptosis was not demonstrated in normal prostatic epithelial (PrEC) and stromal (PrSC) cell lines [62,63]. In vivo studies have shown that selective COX-2 inhibitors can induce apoptosis in PC3 cells grown in nude mice [64]. However, it is important to recognise that some of the effects of NSAIDs and selective COX-2 inhibitors are independent of their inhibition of COX-2 activity. Indeed, derivatives of celecoxib which lack COX-2 inhibitory activity can induce apoptosis in PC3 cells [65]. Multiple events independent of COX-2 include the induction of apoptosis, linked to the dephosphorylation of Akt and MAP kinase Erk2 [66]. In randomised trials, celecoxib has been shown to reduce the incidence of colonic and duodenal polyps in patients with familial adenomatous polyposis [67,68]. The clinical role of selective COX-2 inhibitors in the potential reversal or delay of progression of the pre-malignant lesion, HGPIN, is not yet known.

COX-2-derived metabolites may also have neoplastic promoting effects that are independent of ROS generation. COX-2 overexpression that may contribute to the malignant phenotype include decreased E-cadherin expression with consequent loss of cell-to-cell adhesion, matrix-metalloproteinase overexpression with an associated increase in invasiveness, and modulated production of angiogenic factors by cancer cells [69,70]. A correlation has been found between hypoxia-induced COX-2 expression and upregulation of vascular endothelial growth factor (VEGF) in PC3 and LNCaP cells [71]. Moreover, the COX-2-dependent effect of VEGF has been found to be inhibited by treatment with a specific COX-2 inhibitor, and this inhibitory effect was reversed by PGE2 treatment [72]. Since VEGF plays an important role in angiogenesis, its upregulation by COX-2 expression and inhibition suggests an important role for COX-2 in angiogenesis.

6.2. Lipo-oxygenase enzymes

Lipo-oxygenases (LOX) are lipid peroxidising enzymes. In mammalian cells, the three major families are 5-, 12- and 15-LOXs [73]. The metabolites of arachidonic acid generated as a result of LOX catalysis, have been linked to prostate cancer development and progression [74]. In particular, it has been shown that inhibition of 5-LOX triggers rapid and massive apoptosis in PC3.

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6.2. Lipo-oxygenase enzymes

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and LNCaP cells [75]. This effect was blocked by the treatment of these cells with the anti-oxidant, N-acetyl-
L-cysteine, but not by androgens, a powerful survival factor in prostate cancer cells.

15-LOX has two major forms, 15-LOX-1 and 15-LOX-2. These isozymes have different tissue distributions: the former are found in reticulocytes, macrophages and eosinophils; the latter are found in prostate, lung and skin [76,77]. The metabolites of these two enzymes have opposing effects, 15-LOX-1 is found to have increased expression in prostate cancer compared with normal tissue, while 15-LOX-2 is found to have increased expression in normal and benign prostate tissue and decreased levels in prostate cancer and HGPIN lesions, as determined by immunohistochemistry [78]. Reduced expression of 15-LOX-2 was also found to correlate with grade of prostate cancer [79]. 15-LOX-1 is capable of generating lipid peroxidation products e.g. the mechanism whereby it functions in reticulocytes. Thus, a shift in expression from predominantly 15-LOX-2 to 15-LOX-1 may serve as a biomarker for prostate cancer development and may represent a target for future chemoprevention strategies.

7. The role of androgens

Androgens play a key role in the development and progression of most prostate cancers [28,80]. Prostate cancer rarely develops in men castrated before puberty [81]. Indeed, it has been suggested that the increased risk of prostate cancer development and mortality among African Americans compared with Caucasian Americans may, in part, be related to elevated chronic androgen exposure in the former group [82].

There is increasing evidence to support the hypothesis that the role of androgens in prostate carcinogenesis is, at least in part, due to oxidative stress. The ROS induced by androgens may directly or indirectly result from their influence on mitochondria [83]. Experimental work carried out on the androgen-sensitive human prostate cancer cells, LNCaP, found that stimulation with physiological levels of androgens resulted in increased levels of ROS [15]. This hypothesis has been further supported by a failure to observe increases in ROS with androgens in the presence of the competitive androgen receptor antagonist, flutamide [16]. As one would expect, androgen stimulation of the cell lines, DU145 and PC3, which do not express functional androgen receptors, does not appear to influence the levels of ROS.

Increased COX-2 activity may lead to an increase in ROS [84]. Immunohistochemical studies, Western and Northern blots in human foetal ejaculatory ducts [85], and the distal vas of adult rats [86], have suggested that COX-2 expression is androgen-regulated. However, COX-2 was not induced in LNCaP and PC3 prostate cancer cell lines following treatment with androgens [87]. Although definite conclusions cannot yet be stated, COX-2 expression may be influenced by androgens.

Although the first results of the Prostate Cancer Prevention Trial (PCPT) are now available, the potential role of anti-androgen therapy in the chemoprevention of prostate cancer is still not clearly defined. The PCPT was a large randomised, placebo-controlled trial of androgen manipulation in men at risk of developing prostate cancer. The trial recruited 18882 men, aged 55 years and above with a normal digital rectal examination (DRE) of the prostate and a serum PSA < 3.0 ng/ml. Men in the treatment group received the 5-α reductase inhibitor, finasteride (5.0 mg daily) and participants in the control arm of the trial received a placebo. All men were screened annually by serum PSA level and DRE. The primary endpoints to be studied were survival and the histological diagnosis of cancer in sextant biopsies of the prostate gland. The trial was terminated earlier than scheduled due to a 25% reduction in the prevalence of prostate cancer in the treatment group [88]. However, the trial showed a marginal, but significant increase in high-grade tumours in the finasteride group. This trial highlights the need to develop biomarkers and better strategies for assessing the potential benefit or detriment of the intervention being studied in any given trial.

8. Dietary anti-oxidants

Epidemiological, experimental and clinical studies implicate the potential benefits of a number of dietary anti-oxidants in the chemoprevention of prostate cancer [89]. Of these, vitamin E, selenium, lycopene and isoflavones found in soy beans have anti-oxidant properties, and vitamin E and lycopene are free-radical scavengers in the extracellular environment. Moreover, long-term administration of vitamin E decreases serum androgen concentration, which may contribute to its mode of action in the prevention of prostate cancer [90]. Vitamin E has also been shown to mediate a G1/S-phase arrest as a consequence of the decreasing expression of cell cycle regulatory proteins, such as cyclin D1, D3 and E, cyclin-dependent kinases -2 and -4, and upregulation of p27 [91]. Although the biochemical functions of selenium remain largely unknown, the anti-oxidant enzyme glutathione peroxidase is selenium-dependent [92]. Recently, it has been shown that selenomethionine can activate p53 by a redox mechanism that is independent of DNA damage, thus leading to the augmentation of DNA repair [93]. As well as having anti-oxidant actions, genistein (the principal isoflavonoid component of soy) inhibits tyrosine kinases and increases cell adhesion
Genistein also induces glutathione peroxidase in LNCaP and PC3 cells [95].

9. ROS and chemoprevention for prostate cancer

There is a growing body of evidence suggesting a role for oxidative stress in the pathogenesis of early prostate cancer. The long latency period in the development of prostate cancer provides a large ‘window of opportunity’ for intervention during this process, ideally suited to dietary manipulation or chemoprevention using diet-derived agents [41]. Biomarkers for assessing the efficacy of agents, such as oxidative DNA adducts to study the pharmacodynamic effects of vitamin E, may be of value in reducing the time-frames required for chemoprevention trials and for providing measurable indicators of potential benefit [41].

An example of one such trial is that of tomato sauce supplements prior to radical prostatectomies (see below). Such trials will be of increasing value in the design of large-scale studies, such as the ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT). This double-blind, randomised, placebo-controlled trial is aiming to recruit 32400 men, into four study arms (daily selenium + placebo, vitamin E + placebo, selenium + vitamin E, or placebo + placebo). The trial is expected to take 12 years to complete. The principal endpoint measured will be the incidence of prostate cancer by routine clinical care [96].

Although, the PCPT and SELECT trials have clearly defined endpoints, such as prostate cancer incidence, the endpoints chosen as the primary goals require large samples sizes and long time-frames. There are a number of inherent problems encountered in designing trials over smaller time-frames. Intermediate endpoints must be used for such trials, such as changes in histological pre-malignant lesions, biochemical and molecular markers. Such intermediate markers need to have the following characteristics, detectable in small specimens (prostate biopsies), high sensitivity and specificity and low range of spontaneous change [97]. Unfortunately, no reliable biomarkers have so far been validated to the extent that they can be used in large-scale trials to complement or even replace definitive endpoints such as cancer incidence. For example, HGPIN lesions have been targeted using chemopreventive agents. However, the effect of sampling error has not been fully considered. If patients with such lesions are to be recruited to clinical trials then repeat biopsies should be obtained to reduce sampling bias to a statistically acceptable level [98]. Unfortunately, the natural history, and consequent sampling error associated with HGPIN lesions, make this endpoint marker more appropriate in trials lasting years rather than months.

10. Oxidative DNA adducts as potential markers of oxidative stress

Oxidative DNA adducts are potential biomarkers of oxidative stress [11]. Oxidative DNA adducts, such as 8-oxo-dG, are formed by the direct reaction of hydroxyl radicals with DNA. In contrast, lipid peroxidation initiated by less reactive free-radicals, generate genotoxic ROS, such as peroxy and alkoxyl radicals. Oxidative DNA adducts arising from lipid peroxidation include base adducts formed with malondialdehyde (MDA). Site-specific mutagenesis studies suggest that mutation frequency caused by indirectly formed oxidative DNA adducts, such as the MDA-guanosine adduct, is similar to that of directly formed oxidative DNA adducts, such as 8-oxo-dG [99].

A recent phase II trial was performed in 32 men undergoing radical prostatectomies for prostate cancer, who received supplements of tomato sauce (containing high levels of the anti-oxidant, lycopene) for three weeks prior to surgery [100]. Pre- and post-treatment levels of 8-oxo-dG were determined in blood leucocytes and prostate tissues. Post-treatment levels of 8-oxo-dG were significantly reduced in both prostatic tissue and leucocytes. At the study endpoint, levels of 8-oxo-dG were higher in prostatic tissue than in blood leucocytes, there was a correlation between prostate and leucocyte oxidative DNA damage. 8-oxo-dG levels were measured in prostate biopsies (not cancer cells specifically), so it is unclear whether levels are likely to be representative of normal, HGPIN or cancer cells. The results of this small study suggested that it may be possible to use blood leucocytes as surrogate markers for oxidative DNA damage in prostate tissue to monitor the efficacy of dietary anti-oxidant intervention over a short time-frame. An important issue to consider in the measurement of 8-oxo-dG levels is the artifactual oxidation of DNA both during the harvesting and preparation of tissue samples for analysis, which may lead to falsely high levels of 8-oxo-dG [101]. For discussion of these issues and the implications for validation, the reader is referred to a recent review [102].

11. Conclusions

Oxidative stress and COX-2 activity appear to play a role in the development and progression of prostate cancer. Further work carried out on ROS and COX-2 activity, particularly in the putative precursors of prostate cancer, such as PIA and HGPIN, will add to our understanding of the pathogenesis of prostate cancer and may allow these indices to be used as biomarkers of the efficacy of intervention in the chemoprevention of prostate cancer. Since agents currently exist that interfere with these cellular processes, such work may accelerate the
potential for the clinical use of anti-oxidants and/or COX-2 inhibitors in men with pre-malignant lesions, providing exciting opportunities for chemoprevention trials.

Conflict of interest statement

None declared.

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