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REVIEW

The risks and benefits of cyclo-oxygenase-2 inhibitors in prostate cancer: A review

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Abstract Cyclo-oxygenase (COX), also referred to as prostaglandin (PG) endoperoxidase synthase, is a key enzymatic mediator in the production of arachidonic acids to PGs and eicosanoids. Two isoforms of COX exist, namely COX-1 and COX-2, which have distinct physiological functions and tissue distribution. Epidemiological studies suggest that regular consumption of aspirin and/or other non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX, could notably reduce the risk of developing many cancers. COX-2 expression has been shown to increase in many cancers and cancer cell lines, including human prostate adenocarcinoma. COX-2 may also be upregulated in proliferative inflammatory atrophy (PIA) of the prostate, a pre-neoplastic lesion. The COX-2 pathway may therefore be a useful target for chemoprevention of prostate cancer, and there is much interest in exploring this with the use of COX-2 inhibitor drugs such as celecoxib. While there is concern regarding the cardiovascular toxicities of coxibs, there is no evidence that there is any increased risk with the use of celecoxib in the short-term neoadjuvant setting.

Introduction

Prostate cancer has now become the most commonly diagnosed male malignancy amongst Western nations and its incidence is increasing.[1] It is the second leading cause of cancer-related death next to lung cancer in men in the USA[2] and, despite the increasing use of PSA, greater than 50% of patients still present with, or develop, metastatic disease.[3] Advanced prostate cancer initially responds to androgen ablation therapy, most probably due to the restriction of prostatic blood flow which impairs angiogenic growth; however, progression to an androgen-independent state with a further increase in tumour load is inevitable.[4] If prostate cancer is diagnosed and treated at an early organ-confined stage however, there is a >85% chance of disease-free survival at 10–15 years.[3] Hence it is vital to develop strategies to both prevent prostate cancer and delay its progression

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once diagnosed. It has recently become apparent that selective COX-2 inhibition offers huge promise in this regard.

The biochemistry of cyclo-oxygenases

Cyclo-oxygenase (COX) is a bi-functional rate limiting enzyme, containing a COX site, involved in the production of prostaglandins (PG),[5] in the conversion of arachidonic acid to prostaglandin endoperoxide synthases (PGG2), as well as reducing PGG2 to PGH2 at the peroxidase site[6] (see Fig. 1).

The PGs are a varied group of autocrine and paracrine hormones that mediate many cellular and physiological functions.[6] Many studies have showed that COX has two distinct isoforms, namely COX-1 and COX-2[7]; while both enzymes catalyze the same enzymatic reaction as well as have the same K_m and V_{max} values for arachidonic acid, significant differences exist with regard to their functions.[8] Pairet and Engelhardt[8] showed that COX-1 is widely expressed and produced in the great majority of mammalian cells, is localized on the human chromosome 9q32—q33.3, spans 25 kb in size, contains 11 exons, and synthesizes 2.8 kb mRNA which produces a 68 kDa protein. It performs the cell's ''house keeping duties,'' such as the immediate production of prostanoids which regulate the homeostatic vasculature, water reabsorption, gastric acid, renal blood flow and platelet aggregation.[8] In contrast COX-2 is an 8 kb gene with 10 exons located on the human chromosome 1q25.2—q25.3, and transcribes a 4.1—4.5 kb mRNA that encodes a protein of about 68 kDa.[8] It is widely regarded as pro-inflammatory which can be activated by cytokines, mitogens, growth factors and tumour promoters at both the transcriptional and post-transcriptional levels.[7] It is involved in the processes of inflammation, ovulation, and labour where only a transient PG production is required.[7] However, both COX-1 and COX-2 are also found on the luminal surfaces of the nuclear envelope and endoplasmic reticulum.[9,10]

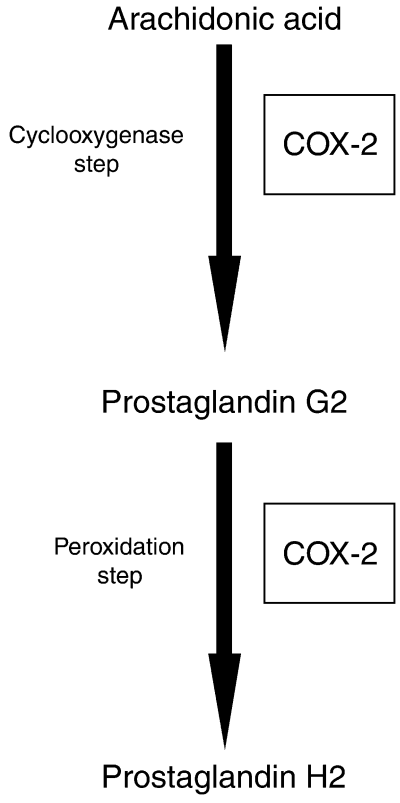

Figure 1 The catalytic actions of COX.

The role of NSAIDs in prostate cancer

It is widely acknowledged that NSAIDs have analgesic, anti-inflammatory, and antipyretic properties[11,12]; in recent years there has been huge interest in whether these drugs also have the ability to decrease the risk and progression of human cancers. The importance of these drugs in cancer chemoprevention has been supported by many epidemiological and experimental studies[13] showing a decreased risk of some cancers, most notably colorectal and breast, in those individuals who regularly consume aspirin or other NSAIDs.[13,14] The abnormal growth of tumour tissue is thought to cause an inflammatory reaction from the peripheral invading cells that is necessary for further growth, and this can be inhibited by NSAIDs.[14,15] Norrish et al.[15] reported a tendency towards a reduced risk of advanced prostate cancer following the regular administration of aspirin. Nelson and Harris[16] also correlated the regular daily administration of ibuprofen or aspirin with a 66% reduction in prostate cancer risk. Roberts et al.[17] found, in white men over 60 who regularly consumed NSAIDs, a lower incidence of prostate cancer compared to the general population over this age.

Pollard and Luckert[18] demonstrated in transplantable rat prostate adenocarcinoma III cells, which were treated with the NSAID piroxicam, a suppression of tumour growth, metastasis, and bone degeneration. Furthermore, tumour suppressor effects and a significant decrease in tissue PGE2 levels have been noted in a chemically induced prostate carcinoma F344 rat model supplemented with soluble indomethacin.[19] In prostate cancer-bearing rats treated with indomethacin, thromboxane synthase inhibitor and nafazatron, all of which are PG inducers, fewer pulmonary

metastases were seen compared to untreated controls.[19] Gupta et al.[20] using the TRAMP mouse model, demonstrated that celecoxib-fed mice at 16, 24 and 32 weeks of age had significantly lower prostate cancer volumes and fewer lung and lymph node metastases compared to controls. Furthermore, celecoxib supplementation led to down-regulation of COX-2 protein expression and increased in vivo apoptosis in the dorso-lateral prostates of these animals.[20]

However, non-selective NSAIDs such as aspirin, sulindac and indomethacin can cause platelet dysfunction, peptic ulceration and kidney damage, thought to be due to COX-1 inhibitory effects.[21–23] Several studies have revealed that the anti-tumorigenic action of NSAIDs is mediated by selective inhibition of COX, especially COX-2.[21–23] Hence, selective COX-2 inhibitors (e.g. melaxicam, celecoxib and rofecoxib), the so-called coxibs, should therefore have less side-effects, as well as exhibit increased anti-cancer effects, when compared to conventional NSAIDs.[24]

COX-2 expression and prostate cancer

Through the work of authors such as Dubois et al.,[25] Leahy et al.,[26] and Lipsky[27] it has become widely acknowledged that an enhanced expression of COX-2 is implicated in the progression of cancer in most sites in the human body. Prescott and Fitzpatrick,[28] using genetic and clinical studies, provide unequivocal evidence that the upregulation of COX-2 is one of the key steps in carcinogenesis; other studies have demonstrated that the increased expression of COX-2 is sufficient to instigate tumorigenesis in animal models.[29] Tiano et al.[30] have shown that skin tumorigenesis is significantly reduced in COX-2 knock out mice. Interestingly, the inhibition of COX-2 causes tumour regression.[29] However, there are some inconsistencies to this widely acknowledged observation. For example, Bol et al.[31] have recently demonstrated that COX-2 overexpression in the skin of transgenic mice resulted in the regression of cancer development.

O'Neill and Ford-Hutchinson[32] in 1993 reported that the highest levels of COX-1 and COX-2 were found in the prostate gland. Recently, Gupta et al.[33] demonstrated that COX-2 is overexpressed in human prostate adenocarcinoma. They confirmed that the mean levels of COX-2 mRNA and protein expressions were significantly increased in prostate adenocarcinoma compared with control samples.[33] Yoshimura et al.[34] found that COX-1 was very weakly expressed but that COX-2 was highly expressed in prostate cancer cells in comparison to control samples. In normal prostate tissues and benign prostatic hyperplasia (BPH) samples, the expression of both COX isoforms was found to be very weak,[34] whilst the extent and intensity of immunoreactive COX-2 polypeptides were significantly enhanced in prostatic tumour cells compared to BPH samples. Further studies using mRNA analysis have confirmed an increased expression of COX-2 but not COX-1 in prostate cancer cells.[34] These results suggest, therefore, that prostate cancer cells may generate COX-2 or that the expression of COX-2 leads to the development of malignancy.

Kirschenbaum et al.[35] analyzed 31 specimens of prostate carcinoma and 10 specimens of BPH.[35] They found that COX-1 was expressed in basal epithelial cells of BPH tissue, with a 90% positive staining, and in the smooth muscle cells of prostate cancer cells.[35] COX-2 was found to be less expressed in the basal epithelial cells of BPH tissue, but was highly expressed in areas of high grade prostate intraepithelial neoplasia (PIN), and even more expressed in areas of prostate cancer, with 87% of samples demonstrating immunoreactivity.[35] Therefore this study indicates that both COX-1 and COX-2 are upregulated in human prostate cancer compared to BPH and that expression of COX-2 is intermediate between BPH and cancer areas for PIN tissue.[35] Madaan et al.[36] also demonstrated both COX-1 and COX-2 expressions in 82 prostate cancer specimens as well as 30 BPH specimens, but with a significant overexpression of COX-2 in tumour cells in comparison to benign tissue. They found that COX-1 expression in tumour cells was equal to that of the benign specimens. The authors found a significant correlation between increasing tumour grade and COX-2 expression, suggesting that COX-2 may play a critical role in the development and/or progression of prostate cancer.[36] Lee et al.[37] also support the notion of increased COX-2 expression in prostate cancer with 15 out of 18 (83%) prostate cancer samples displaying immunoreactivity compared to 22% (4/18) of benign specimens. Uotila et al.[38] also demonstrated that the intensity of COX-2 was stronger in prostate cancer cells than in the normal surrounding epithelium. COX-2 was also found to be present in PIN lesions as well as in the muscular fibres of the BPH specimens.[38] Importantly with regards to COX-1 expression, no significant difference was noted.[38] Thus, Uotila et al.[38] concluded that COX-2 was overexpressed in both prostate cancer and PIN.

In contrast to the above observations, Zha et al.[39] noted, using immunohistochemical (IHC) analysis of 144 human prostate cancer cases, no

consistent overexpression of COX-2 in established prostate cancer or high grade PIN in comparison to normal prostatic tissue. In this study, positive staining was only observed on scattered cells in both the tumour and normal tissue regions, although much more consistently observed in areas of PIA, known to be pre-neoplastic tissue.[39] Other authors have even suggested that the expression of COX-2 in human prostate cancer cell lines is relatively down-regulated; in LNCaP, DU145, PC-3 and tumour necrosis factor (TNF) prostate cell lines, COX-2 expression has been found to be undetectable under baseline conditions, although it has been shown that it is temporarily induced, under phorbol ester treatment, in TNF and PC-3 cells.[39] Subbarayan et al.[40] have interestingly proposed that basal COX-2 mRNA was found to be higher in normal prostate epithelial cells when compared with other prostate carcinoma cell models (PC-3, LNCaP and DU145). In support of this Hong et al.[41] found COX-2 transcripts to be absent in LNCaP and PC-3 cells and in another study LNCaP and PC-3 prostate cancer cells have shown to display insignificant quantities of COX-1 and COX-2.[42]

COX-2 dependent and independent mechanisms of action for coxibs in prostate cancer

Tjandrawinata and Huges-Fulford[43] showed that increased PG synthesis has both growth-promoting and positive feedback effects in prostate cancer (see Fig. 2). They found that prostate carcinoma cells (PC-3) that were treated with exogenous PGE2 resulted in increased mitogenesis and COX-2 upregulation.[43] Furthermore, treatment of PC-3 cells with 5 μM flurbiprofen, in the presence of exogenous PGE2, resulted in enhanced expression of COX-2 and significant tumour growth.[43] Liu et al.[44] demonstrated that the increased expression of COX-2/PGE2 contributed to prostate cancer progression and development through the initiation of the IL-6 signalling pathway.

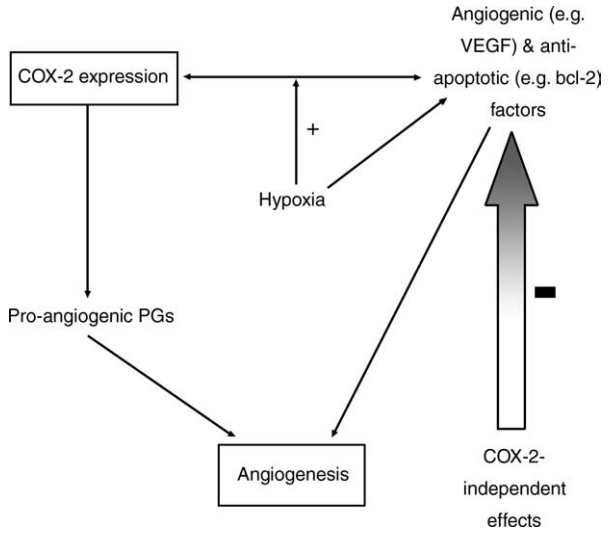

Figure 2 The COX-2 dependent and independent actions of coxibs.

In relation of prostate cancer apoptosis COX-2 overexpression has shown to upregulate Bcl2 expression in an attempt to decrease tumour apoptosis[45]; Liu et al.[46] have demonstrated, using human prostate carcinoma LNCaP cells that the overexpression of COX-2 causes apoptosis inhibition, and following treatment with a selective COX-2 inhibitor (NS398) Bcl2 down-regulation ensues causing apoptosis induction. Celecoxib, which inhibits COX-2 selectively, has shown to induce apoptosis in both androgen-responsive LNCaP as well as androgen-unresponsive PC-3 cells through the inhibition of Akt phosphorylation.[47] It has also been demonstrated that COX-2 expression is initiated by TNFα; this emphasizes the inducibility of COX-2 in response to a pro-inflammatory stimulus.[40] Cumulatively, the above data suggest a pro-inflammatory, anti-apoptotic, and growth stimulatory nature of COX-2 in the development and progression of prostate cancer.

Experimental data suggest that NSAIDs and COX-2 specific inhibitors (coxibs) exert their chemopreventive action via specific inhibition of this enzyme whilst several studies have proposed the existence of a COX-2 independent mechanism as a mode of action of these agents in their ability to prevent cancer. Sulindac derivatives (examples of coxibs) block cell growth and promote apoptosis in PC-3 and LNCaP prostate cancer cells to similar degrees despite these cell lines expressing COX-2 at varying levels,[42] suggesting the involvement of a COX-2 independent pathway. Another coxib, celecoxib, has been reported to initiate the process of apoptosis by interfering with functioning of Akt,[46] which may be one of the underlying mechanisms for the COX-2 independent actions of coxibs and NSAIDs.

Studies also indicate that induction and production of angiogenic factors (neovascularization), as well as inhibition of E-cadherin production (loss of cell-to-cell adhesion) and matrix metalloproteinase overexpression (increased invasiveness), are alternative methods by which COX-2 overexpression can lead to a potentially malignant phenotype.[48,49] Liu et al.,[50] using PC-3 and LNCaP prostate cancer cells, demonstrated a correlation between hypoxia-induced COX-2 expression and

the upregulation of the main angiogenic stimulus, vascular endothelial growth factor (VEGF). When treated with the coxib NS398, down-regulation of VEGF is seen; there is reciprocal upregulation following treatment with PGE2.[51] Masferrer et al.[51] confirmed VEGF upregulation by COX-2 overexpression and down-regulation following coxib administration. Liu et al.[52] also showed a significant decrease in VEGF production correlating with prostate cancer regression following the use of a coxib in PC-3 nude mice. Taken together, these findings suggest that COX-2 is important in tumour angiogenesis via a VEGF mechanism, and that coxibs may cause prostate cancers to regress by inhibiting this process.[41]

However, the exact mechanisms by which these pharmacological agents exert their anti-cancer effects are controversial and unclear; suffice to say, there appear to be both COX-2 dependent and COX-2 independent mechanisms of action, probably involving Akt interference (pro-apoptotic) and VEGF down-regulation (anti-angiogenic). The degree to which these mechanisms correlate with COX-2 expression is controversial, but COX-2 independent pathways may explain the conflicting evidence regarding COX-2 mRNA and protein overexpression in prostate cancer cells.

Adverse effects of coxibs

The major use of coxibs has been in the clinical management of chronic, painful conditions such as rheumatoid arthritis, where the prolonged use of conventional NSAIDs is made difficult due to resulting gastric and renal adverse effects. Clinical trials have shown that coxibs are as equally effective as non-selective NSAIDs for analgesia.[53,54] The Vioxx gastrointestinal outcomes research (VIGOR) trial[55] randomized patients with rheumatoid arthritis to rofecoxib 50 mg/day or naproxen 1000 mg/day and found a two-fold reduction in gastrointestinal events in the rofecoxib arm, but also showed a five-fold increase in the incidence of acute myocardial infarction in the rofecoxib arm when compared with the naproxen arm.[55] Because there was no placebo arm in the VIGOR trial these findings could suggest either an adverse cardiac effect of rofecoxib or a protective cardiac effect of naproxen.

Graham et al.[56] examined a cohort of patients treated with an NSAID between Jan. 1, 1999, and Dec. 31, 2001, using data from the Kaiser Permanente (a national integrated managed care organization providing comprehensive health care to more than 6 million Californians). Of 2,302,029 person-years of follow-up, there were 8143 cases of serious coronary heart disease, each of which was risk-set matched with four controls on age, sex, and health-plan region. The multivariate odds ratio was 1.59 (95% confidence interval 1.10—2.32) for all doses of rofecoxib, 1.47 (0.99—2.17) for 25 mg or less daily, and 3.58 (1.27—10.11) for doses greater than 25 mg daily, all compared with celecoxib. Celecoxib was not associated with any increased risk of cardiac events compared with remote (more than two months ago) NSAID use (odds ratio 0.84, 0.67—1.04).

The adenomatous polyp prevention on Vioxx (APPROVe) trial[57] examined the effects of rofecoxib on the incidence of benign sporadic colonic adenomas. The manufacturers of rofecoxib, Merck, were forced to withdraw Vioxx from the market after the finding that the group assigned to rofecoxib had a four-fold increased risk of serious thromboembolic events (mainly acute myocardial infarction and cerebrovascular accident) compared to the placebo group.[58]

Hudson et al.[59] undertook a retrospective cohort study of over 2000 patients prescribed celecoxib, rofecoxib, or a non-selective NSAID at their index admission for congestive cardiac failure (CCF). The combined risk of death and recurrent CCF was higher in patients prescribed rofecoxib or NSAIDs than in those prescribed celecoxib (hazard ratios 1.27 and 1.26, respectively). The celecoxib long-term arthritis safety study (CLASS)[60] compared celecoxib with either ibuprofen or diclofenac in patients with osteoarthritis or rheumatoid arthritis, and found no difference in the rates of myocardial infarction. However, the adenoma prevention with celecoxib (APC) trial[61] was suspended by the US National Cancer Institute (NCI) when it was found that patients taking 400 mg and 800 mg daily celecoxib had a 2.5-fold and 3.4-fold increase, respectively, in their risk of experiencing a major cardiovascular event compared to patients on placebo.

Further ongoing trials are awaited, but unfortunately they are mostly being performed in patients with rheumatoid arthritis (a known risk factor for cardiac disease) and hence these studies will not answer the question of the risk:benefit ratio of short-term use of these drugs in patients at little or no risk for adverse cardiac events. This is a crucial issue in determining whether the coxibs are safe in a chemopreventive role.

In summary, it appears that there are well justified cardiovascular safety concerns for coxibs in patients with arthritis and other chronic pain conditions. Current evidence provides some reassurance that celecoxib is safer than other coxibs

and at least as safe as other NSAIDs. However, the APC trial suggested that celecoxib is still not as safe as placebo, and further studies are thus needed to confirm its safety as well as that of the NSAIDs as a whole.

Current clinical trials of coxibs in prostate cancer

Clinical trials evaluating the effectiveness of coxibs in prostate cancer are very limited. A small trial of R-flurbiprofen in 23 patients with advanced prostate cancer, showed a moderate benefit in 52% of the patients' rate of PSA increase.[62] This positive result has led to a large, multicentric trial in men that have suffered biochemical recurrence following initial definitive therapy for prostate cancer.[63] Participants are randomized to 800 mg R-flurbiprofen, 600 mg R-flurbiprofen, or placebo. Primary endpoints are comparative effects on time to clinical disease progression and changes in PSA velocities. A small pilot study by Derksen and Pruthi[64] examined the effect of celecoxib 200 mg twice daily (bd) on PSA kinetics in 13 patients with biochemical recurrence post-definitive treatment. They found an inhibitory effect on serum PSA levels in most patients, with celecoxib significantly increasing PSA doubling time over the 12-month study duration. This has prompted the authors to initiate a larger phase 2 study, this time using celecoxib 400 mg bd in 100 patients with PSA-only recurrence. As well as PSA kinetics, this study will also assess time to clinical recurrence.

While the effects of coxibs in advanced prostate cancer are interesting, we feel that the real potential benefit of these drugs may be in the neoadjuvant setting. This limited exposure will almost certainly lead to no increase in adverse cardiac events. The NCI are currently performing a phase 1 trial of a 4-week neoadjuvant course of celecoxib versus placebo followed by prostatectomy for high-risk localized prostate cancer patients.[65] The investigators will evaluate the effects on angiogenic factors and PGs in surgical samples. We are ourselves conducting a similar randomized trial of 4-week neoadjuvant celecoxib 400 mg bd versus no drug in patients before radical prostatectomy for clinically localized disease. Our endpoints include counts of proliferative, angiogenic, apoptotic, and hypoxic factors, plus assessment of COX-2 expression, using immunohistochemical methods on pre- and post-operative samples. Also, peri-operative samples are taken for cDNA microarray analysis to investigate if celecoxib alters the gene expression profiles of these tumours.

Conclusions

There is an ever increasing body of epidemiological evidence for a chemopreventive effect of NSAIDs and coxibs in many human cancers, including prostate adenocarcinoma.[13–17] This has led to huge interest in the application of NSAID and coxibs in cancer chemoprevention and treatment. COX-2 appears to be of more importance in this regard than COX-1, an observation consistent with the different physiological functions of the two enzymes. Indeed, studies have shown increased COX-2 expression in prostate cancer in both in vivo and in vitro models.[25–38] The mechanisms of action of coxibs in the cancer setting have proven difficult to elucidate, but both COX-2 dependent and independent pathways appear to be involved.[43–52] Induction of apoptosis and inhibition of angiogenesis may be important mechanisms, and studies are currently under way to investigate this. While there are well justified concerns with the long-term use of coxibs in elderly patients with arthritis there is no evidence that this translates into a risk in the short-term chemopreventive setting in patients without cardiac risk factors. This is especially true of celecoxib, the coxib thought to have the least damaging risk profile and also the greatest anti-cancer effects. Further research will determine whether the risk:benefit ratio for celecoxib is favourable enough to recommend it in the prevention of prostate cancer.

Key points

- Coxibs have a real anti-cancer effect in both in vitro and in vivo studies.
- Celecoxib is the most potent coxib with regard to its anti-cancer properties.
- Celecoxib also appears to have the most favourable toxicity profile of the coxibs.
- The reasons for celecoxib's superiority is likely to be due to its lower selectivity for COX-2 but greater COX-2 independent mechanisms of action.
- Future studies focusing on celecoxib may further elucidate these mechanisms of action, but at present the anti-cancer effects are primarily thought to be due to an inhibition of angiogenesis and a stimulation of apoptosis.

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