



Cyclooxygenase/ prostaglandin signalling pathway: A novel target for managing breast carcinoma

Ruchika Nandha^{1*}, Harpal Singh² and Kamlesh Garg³

1, Department of Pharmacology, Dr Harvansh Singh Judge Institute of Dental Sciences, Panjab University, (Chandigarh) - India

2, Department of Critical Care, Max Superspeciality Hospital, Mohali, (Punjab) - India

3, Department of Clinical Research, Jamia Hamdard University, New Delhi, India

Abstract

Considering that cyclooxygenase -2(COX-2) is over expressed in 40% of invasive and pre-invasive breast cancer cell lines with a substantial role of prostaglandin E₂(PGE₂) in cancer initiation, progression, invasiveness and metastasis, COX-2 inhibitors have been recently found to be suitable options as anticancer agents. Various studies have demonstrated promising activity of this readily accessible, affordable and well tolerated class of drugs for their off the label use in pre invasive as well as invasive breast carcinomas but cardiotoxicity observed by them in trials has put a big question mark on their use. Though use of coxibs for cancer management has dampened in between because of worries about their safety, targeting COX/PG pathway can hence represent a radical step away from current conventional treatment and prevention modalities for expanding the armamentarium of anti-cancer therapeutics.

Key-Words: Breast cancer, COX-2 inhibitors, Celecoxib

Introduction

Breast cancer, a most common malignancy in females of developed countries is a second leading cause of cancer related mortality. It affects about 40,000 females each year with a rising trend in last fifteen years. [1] Conventionally breast carcinoma is managed according to the stages with chemotherapy, radiotherapy and surgery as the standard modalities. Several large phase III trials have demonstrated that tamoxifen and more recently, raloxifene can effectively reduce the incidence of invasive breast cancer by 50% but their use has been found to be associated with several rare, but serious, adverse events.[2] Newer chemotherapeutic agents and monoclonal antibodies have though paved a new direction to improve cancer mortality but these medications are very costly demanding the introduction of newer agents which are effective, safe, simple and affordable too.[1]

Cyclooxygenase -2(COX-2) association with breast cancer has been explored with interest few years back, suggesting the role of this key enzyme of prostaglandin(PG) synthesis in proliferation of breast cancer.[3,4]

COX is a central component of PG synthesis pathway converting arachidonic acid to prostaglandins, prostacyclin and thromboxane. COX occurs in two isoforms: COX -1 enzyme (PG-endoperoxidase synthase-1) is constitutive and ubiquitously present in body where as COX -2 (PG-endoperoxidase synthase 2) is inducible whose production in body is regulated by variety of signals like cytokines, mitogens, growth factors as well as by early response genes.[1,4,5]. Identifying that COX-2 has an effective role in tumour angiogenesis, invasion and metastasis, COX-2 inhibition as a potential mechanism for cancer treatment has been studied in past few years, demonstrating promising results along with minimal toxicity of coxibs in various preclinical and clinical trials.[6,7]

Studies confirmed that specific COX -2 inhibition is better than non selective inhibition as significant reduction of 71% in cancer risk was observed with celecoxib use as compared to non selective COX inhibitors underscoring their strong potential for breast cancer chemoprevention. [8] Though COX -2 inhibition is primarily implicated in managing breast cancer but significant additive effects by the combination of COX-1 and COX-2 inhibitors have also been demonstrated.[9]

* Corresponding Author

Email: rnandha23@yahoo.co.in
Telephone : +91-9872211016

Role of COX-2 in breast carcinoma

Most important triggering clue for initiating research with coxibs in breast carcinoma was the finding that COX -2 is over expressed in breast tumour as well as highly invasive, metastatic cell lines. [10] 40 to 80 % of neoplastic cells in human carcinoma possess over expression of COX -2 as compared to the normal tissue. [11,12] 37% of invasive breast cancer patients were found to possess moderate to strong expression of COX-2 in a study enrolling 1576 patients.[13] COX-2 over expression in breast cancer correlates with poor prognosis markers like large tumour size, more proliferation, hormone receptor negative status, increase in metastasis and over expression of human epidermal growth factor receptor 2(HER2).[3,14] COX- 2 expression has been demonstrated to be 80% in ductal in situ carcinoma suggesting the role of COX 2 inhibition in not only invasive but pre invasive breast cancer too. [1,15]

COX-2 is likely to be a key player in a number of biologic pathways leading to cancer as it modulates the critical cellular and molecular steps involved in initiation, promotion and progression of breast cancer. [11]COX- 2 has been found to be directly and indirectly involved in proliferation of tumour through the production of PGE2. Direct action, being stimulation of mitogenesis through a direct effect on fibroblasts, osteoblasts and mammary cells and indirect action involves targeting mutagenesis, angiogenesis, apoptosis and cell migration. [1] Inhibition of angiogenesis has been attributed to decrease in vascular endothelial growth factor [VEGF] by a study where significant decrease in VEGF was observed in celecoxib group as compared to control group at early time of treatment. [16] COX-2 activity also modulates the expression of matrix metallo proteases (MMPs) hence promoting cell invasion and migration. [17]Not only these mechanisms are responsible for progression and prognosis of tumour but PGE2 has also been found to be associated to enhance aromatase [Cyp450 enzyme] transcription ultimately increasing estrogen production. [1,3, 18] Recently a non-cyclooxygenase effect of COX-2 inhibitors, which combines the peroxisome proliferator activated receptor [PPAR] delta and the adenomatous polyposis coli [APC] tumour suppressor activity, was also demonstrated. [19, 20]

Various in vitro and in vivo studies have confirmed the regression of tumour tissues by inhibition of COX-2 mediated direct and indirect effects leading to decrease in mitogenesis, proliferation, cell migration, new vessel formation along with enhanced apoptosis in cancer tissue. [3] Epidemiological studies also have

shown that the use of non steroidal anti inflammatory drugs (NSAIDs) is associated with a reduced risk of several malignancies. In a meta analysis results of 13 reported studies, chemoprevention of breast cancer was found to be associated with the use of specific COX-2 inhibitors. [8] Combination therapeutic strategy of COX-2 with conventional anti cancer medications has additional therapeutic paradigm. Trials on combination therapy of celecoxib with aromatase inhibitors have demonstrated reduced overall disease by inhibiting the common target aromatase explicating their synergistic pharmacodynamic effect. [15]

Studies demonstrating the effectiveness of COX-2 inhibitors in breast cancer

The past few years have borne witness to many studies suggesting that COX represents a bona fide therapeutic target for cancer prevention and possibly treatment. Animal Studies have been done to demonstrate the mechanism behind basic carcinogenesis, expression of COX- 2 in tumour tissue and role of COX inhibitors in regression and chemoprevention of breast cancer. A study demonstrated PG synthesis and formation of oxygen and nitrogen free radicals are responsible for tumour initiation where as induction of aromatase expression and estrogen synthesis are responsible for sustained mitogenesis and tumour progression. VEGF expression due to PGE2 was also found to add to angiogenesis and tumour metastasis. [8]Role of enhanced expression of COX-2 in inducing tumorigenesis was confirmed by a study showing delay in mammary gland involution and decrease in apoptotic index in COX-2 transgenic mice. [21]

Study on HER2/neu induced experimental breast tumour mouse model resulted in significant reduction in incidence of tumour by celecoxib 500 parts per million (ppm); $p=0.003$ along with 50% reduction in the mammary PGE2 levels. [22]

Nimesulide, a preferential COX-2 inhibitor when given as 400 ppm with normal diet resulted in decreased carcinoma incidence in female rats with experimentally induced breast cancer as compared to control group on normal diet (51% versus 71%).Also significantly more reduction in tumour size and average multiplicity was observed with celecoxib administration($p<0.05$) demonstrating chemoprophylactic activity of COX-2 inhibitors. [23] Ibuprofen, a non selective COX inhibitor could also resulted in decrease in tumour volume($p<0.05$), when used for 35 days in another study.[24]

Celecoxib has been found be chemopreventive in a dose response study where 4 escalating doses of celecoxib were given to 7, 12 -dimethylbenzanthracene [DMBA] induced mammary cancer in rats. Control

groups were given normal diet where as experimental groups got 250,500, 1000 and 2000 ppm doses of celecoxib. Decrease in tumour incidence was 100%,80%,50%,45%,25% ($p < 0.001$), multiplicity (number of tumours per rat was 3.46,1.80,1.00,0.75 and 0.50 ($p < 0.001$), where as tumour volume was 1.29 cm³,0.42 cm³,0.34 cm³,0.31 cm³ and 0.16 cm³ ($p < 0.001$) in control rats, 250,500, 1000 and 2000 ppm groups respectively, after 122 days of therapy demonstrating dose dependent effect of celecoxib in mammary tumour with 250 ppm as the lowest effective dose.[25]

Clinical trials have also demonstrated the role of COX-2 inhibitors in chemoprophylaxis, neoadjuvant, adjuvant and metastatic treatment modalities. In a randomized phase 2 trial, enrolling 111 postmenopausal patients with hormone-sensitive breast cancer who had progressive disease after treatment with tamoxifen, control group received exemestane 25 mg/day alone where as drug group received celecoxib 400mg twice a day in combination with exemestane 25mg/day. Results of study demonstrated similar efficacy of combination in terms of clinical benefit [48.98% versus 47.06%], median time to tumour progression [20weeks versus 23.4 weeks] and median survival time [74.1 weeks versus 73.9weeks] where as median duration of clinical benefit was higher with combination as compared to exemestane alone[96.6 versus 49.1 weeks]. [26]

In another phase 2, neoadjuvant trial, 22 postmenopausal women with estrogen receptor (ER) and/or progesterone (PR) positive stages II-III breast cancers received 8 weeks of exemestane 25 mg daily, followed by 8 weeks of exemestane 25 mg daily and celecoxib 400 mg twice daily. There were statistically significant decreases in ER ($P = .003$), PR ($P = .002$), Ki-67 (cellular marker for proliferation) ($P < .001$), and COX-2 ($P = .004$) expression. [27]

In another trial enrolling a total of 111 postmenopausal women with advanced breast cancer who had progressed on tamoxifen, patients were randomized to receive 25 mg daily exemestane with or without celecoxib 400 mg twice a day. A lower rate of disease progression (30% vs. 40.5%) was observed in patients with visceral disease after combination therapy as compared to exemestane alone. In addition, ER and PR positive patients had a higher response rate (27.0% vs. 14.3%) and a lower rate of disease progression (29.7% vs. 35.7%) with the combination therapy.[28] Results of a multicentre, phase III randomized double-blind, placebo-controlled trial; Randomized Europe An Celecoxib Trial [REACT trial] are still awaited. [29]

Safety concerns for coxibs use in breast cancer

Recently safety of COX 2 inhibitors for clinical use has been questioned. Though earlier studies confirmed improved gastric tolerance with COX-2 inhibitors as compared to non selective ones but worries surrounded their use due to observed cardiovascular toxicity. [30] Cardiotoxic effects were attributed to increase in prothrombotic effects due to thromboxane A2 [TXA2] and decrease in cardio protection offered by PGI2 resulting in thrombogenesis, hypertension and atherogenesis. [31] As a result, rofecoxib was banned by regulatory authorities for clinical use in 2004 in India followed by valdecoxib a year later. Banning of these drugs has halted the research associated with the use of coxibs in breast cancer. Suddenly a new hope for cancer patients ended up in non desirability for using this affordable group of drugs.

Nevertheless the efficacy of coxibs as anticancer agents has paved a path to identify drugs which can target COX/PG signalling pathway and to define and explore alternate components of this pathway so that safer drugs with comparable efficacy can be searched for cancer treatment.[4]

New strategies targeting COX/PG pathway

Data suggest that PGE2 is the main protumour component which participates in tumour proliferation, angiogenesis and tumour survival. Hence another approaches which can be explored to inhibit its actions are decreasing its synthesis, its receptor mediated action and increasing its inactivation.

- Attention is now focused on microsomal PGE synthase 1 enzyme which is up regulated in human cancer.[32] Thus inhibiting this enzyme can lead to decrease in PGE2 synthesis hence associated effects which contribute to progression of tumour.
- Expression of all the four types of PGE2 receptors have been demonstrated in mouse mammary tumour with EP1,EP2 and EP4 having substantial protumourigenic activity. Thus another target for decreasing PGE2 mediated activity can be blockade of PGE2 receptors. [4]
- PGE2 is inactivated by enzyme hydroxyl prostaglandin dehydrogenase which is found to be decreased in breast cancer. Hence targeting this enzyme by reversing the epigenetic inactivation of its locus can also offer a novel approach to affect COX/PG signalling.[33]

Conclusion

In conclusion, COX-2 is over expressed in both early and late stages of carcinogenesis and coxibs have been

shown to be efficacious as monotherapy and in combination with conventional chemotherapeutics in relevant animal models. Though substantial role of coxibs in breast cancer as demonstrated in various studies has not been practically considered due to cardio toxicity associated with their use, but it has certainly directed us to explore new targets in COX/PG signalling pathway to identify therapeutically useful drugs with minimal toxicity.

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