PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 495–499

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Cyclooxygenase-2 and chemoprevention of breast cancer☆

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Abstract

This article discusses the role of cyclooxygenase-2 (COX-2) in the aetiology and progression of breast cancer. Renewed interest in chemoprevention using non-steroidal antiinflammatory drugs (NSAIDS) has come from observations that regular NSAID use is associated with a reduced incidence of some cancers including that of the breast. There is an increasing body of evidence supporting a role for COX-2 in breast cancer development and progression via effects on angiogenesis and apoptosis as well as via effects on intratumoural aromatase. New selective inhibitors of COX-2 are currently licensed for use in the treatment of arthritis and more recently in the chemoprevention of familial adenomatous polyposis (FAP). Large clinical chemoprevention studies with COX-2 inhibitors are already underway in colorectal cancer. Their role in breast cancer prevention and treatment has yet to be fully characterised, but merits further investigation.

Keywords: Cyclooxygenase-2; Breast cancer; Angiogenesis; Aromatase; Chemoprevention

1. Non-steroidal anti-inflammatory drugs and cancer

There have been a number of studies over the past 25 years linking non-steroidal anti-inflammatory drug (NSAIDS) usage and reduced cancer incidence [1] including cancer of the breast. A study by Egan et al. found no association between regular aspirin use and the incidence of breast cancer [2]. By contrast, in their case-control study, Harris et al. [3] found a reduced risk of breast cancer associated with the use of any NSAIDS three times or more weekly for at least a year (relative risk, RR 0.66). The epidemiological data relating NSAID use and the incidence of breast cancer are summarised in Table 1.

2. Non-steroidal anti-inflammatory drugs—mechanisms of action

Arachidonic acid, a 20-carbon polyunsaturated fatty acid is the precursor for prostaglandin synthesis. The first step is the hydrolysis of phospholipids to produce free arachidonic acid catalysed by phospholipase A_2 (Fig. 1). The next step is catalysed by cyclooxygenase (COX) which inserts molecular oxygen into arachidonic acid. Each of the prostaglandins formed has a distinct biological function.

The main target of NSAID action is COX. Two isoenzymes exist in the human form, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The two isoenzymes are regulated independently: COX-1 is constitutively expressed, whereas the inducible isoenzyme COX-2 is expressed only in response to certain stimuli, such as tumour promoters, endotoxin, cytokines and hormones [4].

3. COX-2 and carcinogenesis

A number of studies have shown over-expression of COX-2 in a number of solid malignancies including breast tumours [5]. The most compelling data to support a causal relationship between over-expression of COX-2 and carcinogenesis have come from studies of animal models for human familial adenomatous polyposis (FAP), a condition caused by a germline mutation of the *Apc* gene in which individuals develop numerous adenomatous colorectal polyps, which predispose to colorectal carcinomas. Several strains of mice have been developed which carry mutations in one Apc allele, including the Min mouse [6] and Apc d716 [7]. Analysis of adenomatous polyps from Min mice show increased expression of COX-2 relative to normal mucosa [8]. The effect of the absence of COX-2 in the ApcΔ716 mouse has been studied by introduction of a knockout mutation of the *COX-2* gene. Removal of the *COX-2* gene in the mouse reduces the number and size of intestinal polyps [9].

☆ Presented at the VI International Aromatase Conference: AROMATASE 2002, Kyoto, Japan, 26–30 October 2002.

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0960-0760/$ – see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/j.jsbmb.2003.07.004

Table 1
NSAID use and the incidence of human breast cancer

Authors	Year of study	Study size	NSAID used	Relative risk (95% CI) comments
Prospective studies				
Paganini–Hill et al.	1989	13987	Aspirin	0.95–1.67
Thun et al.	1993	635031	Aspirin	0.98 (0.76–1.26)
Shreinemachers et al.	1994	12668	Aspirin	0.70 (0.50–0.96)
Egan et al. [2]	1996	89528	Aspirin	1.01 (0.80–1.27)
Case control studies				
Harris et al. [3]	1996	511	Aspirin Ibuprofen	0.66 (0.52–0.83)
Harris et al. [3]	1999	32505	Aspirin Ibuprofen	0.6
Coogan et al.	1999	6558	Aspirin	0.8 (0.7–1.0)
Sharpe et al.	2000	5882	Aspirin Ibuprofen	0.76 (0.63–0.92)

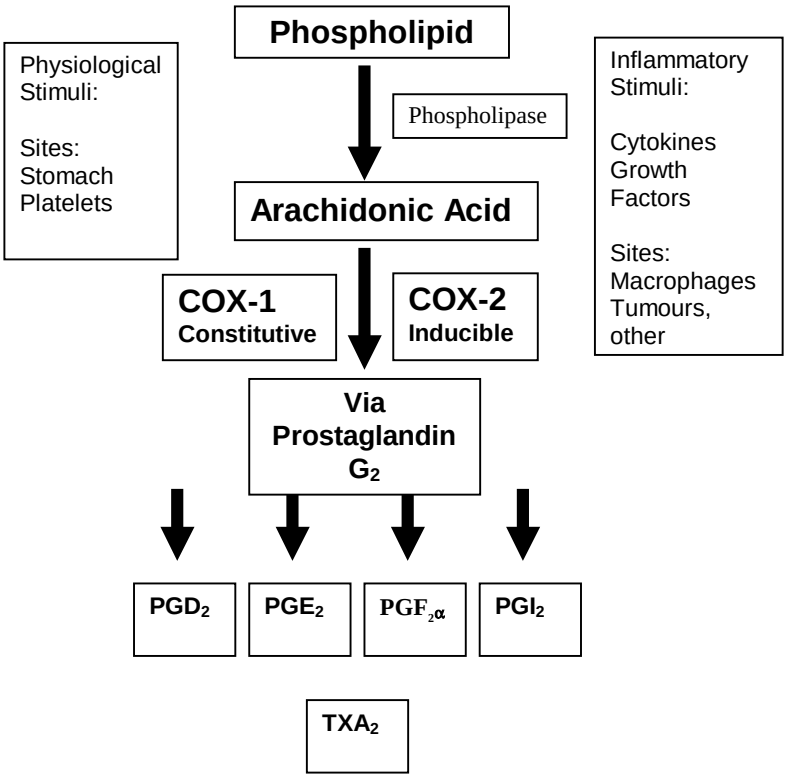

Fig. 1. Prostaglandin synthesis via arachidonic acid.

4. COX-2 and breast cancer

COX-2 has been implicated in mammary carcinogenesis in several ways. COX-2 expression was detected using reverse transcription polymerase chain reaction (RT-PCR) in 13/13 human breast tumours, with no detectable expression in normal breast tissue [5]. A further study of 21 human breast tumour specimens found no detectable COX-2 expression in normal breast tissue specimens, but detectable and heterogeneous *COX-2* gene expression in each of the 21 tumour specimens [10].

Indirect evidence linking COX-2 to breast cancer has come from studies of tissue prostaglandins. Early studies demonstrated a relationship between tissue prostaglandin levels in human breast tumours, poor post-operative survival and development of metastases [11]. The main product of COX-2, is PGE_2. PGE_2 is found in high levels in tumour cells [12], and is synthesised by several human breast cancer cell lines. In clinical studies, high PGE_2 concentrations have been associated with both high metastatic potential and a lack of oestrogen and progesterone receptors [13].

The inhibition of COX-1 and COX-2 has also been investigated in rat models. A 35-day course of ibuprofen in rats with induced mammary carcinomas resulted in significant reduction of tumour volume ($P < 0.05$) and a significant reduction in gene expression of both COX-1 and COX-2 [14]. The chemopreventive effect of a selective COX-2 inhibitor, Celecoxib, against induced mammary carcinogenesis in rats has been investigated [15]. Dietary administration of Celecoxib produced striking reductions in the incidence, multiplicity and volume of mammary tumours relative to the control group. More definitive evidence has now been provided by the recent demonstration that COX-2 over-expression is sufficient to induce mammary tumourogenesis in transgenic mice [16]. Over-expression of COX-2 in the mammary glands of transgenic mice was achieved by the use of the mouse mammary tumour virus promoter.

5. Correlations between COX-2 and clinicopathological variables in breast cancer

Significant relationships between COX-2 and a number of clinicopathological variables including tumour stage, hormone receptor status and HER-2 were found in a recent large series of 1576 breast tumours [17]. A positive correlation between increasing COX-2 expression and poorer disease-free survival in breast cancer patients provides supportive evidence for the prognostic validity of increased COX-2 expression.

6. COX-2 over-expression and mammary carcinogenesis-potential mechanisms

The expression of COX-2 in human solid cancers is not confined to the epithelial component of the tumour. The neovasculature associated with colonic adenomas, carcinomas and metastatic liver lesions also demonstrates COX-2 staining by immunohistochemical methods [18]. The effects of specific COX-2 inhibitors have been tested in animal models of angiogenesis and Celecoxib, a specific COX-2 inhibitor, has been shown to cause inhibition of the angiogenic response in FGF-induced corneal angiogenesis in rats [18].

In addition to angiogenic effects, COX-2 expression may have effects on apoptosis. The specific COX-2 inhibitor Celecoxib induces apoptosis in human prostate cancer cells, whereas the COX-1 inhibitor piroxicam has no appreciable effect [19].

7. Intratumoural aromatase and breast cancer

A number of laboratory studies have shown that hormones, and in particular oestrogens control the growth of breast epithelial cells and affect the course of established disease. In premenopausal women studies of serum oestradiol and its correlation with breast cancer risk have been inconsistent [20]. However, a number of studies [21,22] have reported an association between high serum concentrations of oestradiol and increased the risk of breast cancer in postmenopausal women. Some studies, e.g. Thomas et al. [21], report a steep gradient of association. If the association is causative, a small decrease in serum oestradiol concentrations would lead to a substantial reduction in breast cancer risk.

In postmenopausal women, plasma oestrogen production from androgen precursors principally results from peripheral aromatisation, particularly in adipose tissue. However, many breast cancers contain aromatase, and studies using radioactive isotopes indicate that both of these sources contribute to the oestrogen detectable in breast cancers [23]. The association between high levels of aromatase in the quadrant of normal tissue containing a breast carcinoma has been suggested as causative [24]. Intratumoural aromatase may therefore be of importance in breast cancer incidence and progression.

8. Regulation of breast aromatase gene expression and transcription

Transcriptional regulation of the aromatase gene is complex and differs between tissues, providing tissue-specific control of the enzyme. There is evidence that the promoters that drive aromatase expression in the breast differ between normal and malignant tissue [25,26]. The specific promoters detected in malignant tissue are under the control of cAMP [27]. Thus the concept has been developed of the promoter switching between normal and malignant breast tissues (Fig. 2). PGE_2, which is synthesised by many breast carcinomas, as noted above, is a known stimulant of cAMP in breast cancer cells [28], its production by breast cancer may be instrumental in the switching of aromatase promoters. If these mechanisms are important in breast cancer, a relationship between COX-2 and aromatase activity might be expected.

COX-2 expression has been found to correlate with aromatase expression within human breast cancer tissue. A study of 23 human breast tumours found that COX-2 and aromatase expression, as measured by semi-quantitative RT-PCR showed a significant positive correlation [10]. The COX-2 product prostaglandin E_2 (PGE_2) and cytokines, such as interleukin-6 (IL-6) or tumour necrosis factor α (TNFα) can also regulate aromatase activity [27].

9. Prospects for COX-2 inhibitors in breast cancer prophylaxis

There is increasing evidence to support a link between the over-expression of COX-2 and mammary carcinogenesis

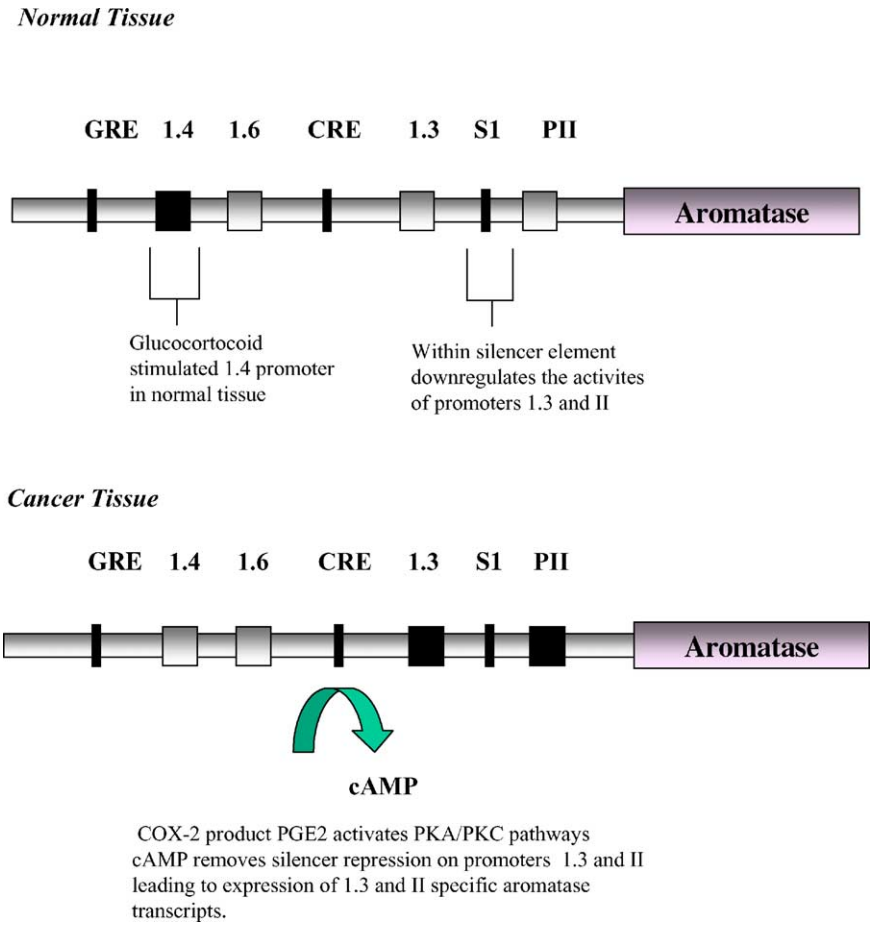

Fig. 2. Transcriptional regulation of aromatase via COX-2.

which may at least in part be dependent on the induction of aromatase by PGE_2. The selective inhibition of COX-2 may modulate a critical step in the initiation and promotion, as well as progression of breast cancer. COX-2 inhibitors are well tolerated and have minimal side effects and as such may be well suited to prophylactic use.

Their long-term usage has been evaluated in several large randomised trials including the Vioxx Gastrointestinal Outcomes Research Study (VIGOR) [28] and the Celecoxib Long-term Safety Study (CLASS) [29]. These studies confirm the improved gastrointestinal safety profile compared to conventional NSAIDS. However, recent concerns have been raised about the possible cardiovascular risks associated with COX-2 inhibitor usage [30], but comparisons between groups of patients, some of whom took aspirin, make direct comparisons of these large studies difficult. Further studies are needed to evaluate the presence of, and magnitude of any such risk.

Aromatase inhibitors are under evaluation for their possible utility in chemoprevention, but their profound suppression of oestrogen throughout the body may lead to unacceptable side effects, particularly on bone resorption. In contrast the possible link between the COX-2 products and breast aromatase could provide a mechanism for selective suppression of oestrogenic stimulation of the breast. It is likely that such targeted oestrogen suppression will lead to only partial withdrawal of oestrogen, but the epidemiological data indicate that there may be a steep dose relationship between oestrogen exposure and breast cancer incidence, suggesting that even partial oestrogen suppression may have profound effects on breast cancer incidence. COX-2 inhibitors therefore may have a role in chemoprevention, based in part on their anti-angiogenic and pro-apoptotic effects and in part on a tissue specific inhibition of oestrogen synthesis. Full scale multicentre trials on colorectal cancer prevention are already underway. Pilot studies of the chemopreventive use of COX-2 inhibitors are underway to test these concepts in the breast cancer setting. These will provide the platform for further large scale clinical trials with these compounds.

KEY POINTS

- There is epidemiological evidence linking non-steroidal anti-inflammatory drug use and reduced cancer incidence.
- Cyclooxygenase-2 is the inducible form of the enzyme.

- Cyclooxygenase-2 is over-expressed in breast cancer tissue.
- Cyclooxygenase-2 inhibitors cause regression of mammary carcinomas in rat models.
- Potential effects of cyclooxygenase-2 inhibitors include effects on angiogenesis, apoptosis and intratumoural aromatase expression.
- Cyclooxygenase-2 inhibitors are safe and well tolerated.
- Large multicentre trials of cyclooxygenase-2 inhibitors in colorectal cancer prevention are underway.
- Cyclooxygenase-2 inhibitors may have a role in breast cancer chemoprevention.

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