Treatment of actinic keratosis through inhibition of cyclooxygenase-2: Potential mechanism of action of diclofenac sodium 3% in hyaluronic acid 2.5%

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Abstract
Cyclooxygenase-2 (COX-2) and its metabolic product prostaglandin E2 (PGE2) are induced in response to growth factors, inflammatory cytokines, tumor promoters, activated oncogenes, and, in the skin, ultraviolet (UV) radiation. Accumulating evidence suggests a role for the COX-2/PGE2 pathway in tumorigenesis in various tissue types including cutaneous squamous cell carcinoma. There is also strong evidence for a role in the development of actinic keratoses (AKs) — common dysplastic lesions of the skin associated with UV radiation overexposure — considered as part of a continuum with skin cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory, analgesic, and antipyretic effects by reversibly or irreversibly acetylating COX isoforms, inhibiting downstream prostaglandins, and may have a chemopreventive role in malignancies, including skin cancer. Topical treatment of AK lesions with the NSAID diclofenac sodium 3% in combination with hyaluronic acid 2.5% has been shown to be effective and well tolerated, although the mechanism of action remains to be elucidated.

KEYWORDS
actinic keratosis, cyclooxygenase-2 inhibitors, diclofenac

1 | INTRODUCTION

Cyclooxygenase (COX) exists as two isoforms (COX-1 and COX-2) that mediate the conversion of arachidonic acid into the precursor molecule prostaglandin (PG) H2 (PGH2; Figure 1) (Williams & Buvanendran, 2011; Zhan & Zheng, 2007). COX-1 is expressed constitutively throughout the body, whereas COX-2 is generally undetectable until it is induced in response to growth factors, inflammatory cytokines, tumor promoters, activated oncogenes, and, in the skin, ultraviolet (UV) light (Williams & Buvanendran, 2011; Zhan & Zheng, 2007). PGH2 is converted by different synthases to prostacyclin, PGD2, PGE2, PGF2α, and thromboxane A2, which sustain homeostatic functions and also mediate various pathogenic mechanisms (Fitzpatrick, 2004; Menter, Schilsky, & DuBois, 2010). Of note, PGE2 is closely associated with inflammation and carcinogenesis as a result of the activity of various cytosolic and membrane-bound PGE synthases (PGES); in particular, membrane-bound PGES-1 drives increased levels of PGE2 during inflammation and tumorigenesis (Menter et al., 2010). Deregulation of the COX-2/PGE2 pathway impacts all hallmarks of cancer, with pleiotropic effects on cell growth and survival (Greenhough et al., 2009), making inhibition of this pathway an attractive prospect for cancer therapy. Here, the present authors review the evidence for a chemopreventive role of non-steroidal anti-inflammatory drugs (NSAIDs) through inhibition of the COX-2/PGE2 pathway. In particular, the present authors discuss the evidence for a chemopreventive role of NSAIDs in the continuum of skin cancer from actinic keratosis (AK) to squamous cell carcinoma (SCC). The evidence presented may inform treatment strategies and highlights future areas of research to improve our understanding of the underlying mechanistic pathways.

2 | NSAIDs AS CHEMOPREVENTIVE AGENTS

NSAIDs are a structurally diverse drug class that have anti-inflammatory, analgesic and antipyretic properties (Williams & Buvanendran,
Aspirin (acetylsalicylic acid) exerts its anti-inflammatory, analgesic, and antipyretic effects via irreversible acetylation of COX, which inhibits production of PGs from arachidonic acid. Initial evidence from pharmaco-epidemiological studies supported an association between the regular use of NSAIDs that non-selectively block COX (mainly aspirin) and reductions in the risk of some cancers (Harris, Beebe-Donk, Doss, & Burr Doss, 2005). These NSAID-related reductions in relative risk were observed in the colon, breast, lung, and prostate cancer and were of the magnitude of 36–63% (Harris et al., 2005). However, a more recent appraisal of the biomedical literature highlighted conflicting reports regarding the potential beneficial effects of regular NSAID intake on reduced risk of malignant melanoma (Goodman & Grossman, 2014).

Aspirin (acetylsalicylic acid) exerts its anti-inflammatory, analgesic, and antipyretic effects via irreversible acetylation and inactivation of both COX-1 and COX-2 isoforms, making it a unique form of NSAID drug (Alfonso, Ai, Spitale, & Bhat, 2014). Regular use of aspirin in individuals without prior cardiovascular disease is not recommended for the primary prevention of vascular or non-vascular outcomes, based on nonsignificant reductions in the risk of death from cardiovascular disease or cancer mortality (odds ratio [OR] = 0.99 [95% confidence interval (CI), 0.85–1.15] and OR = 0.93 [95% CI 0.84–1.03], respectively) and concerns over an increased risk of non-trivial bleeding events (OR = 1.31 [95% CI 1.14–1.50]) (Seshasai et al., 2012). In patients diagnosed with colorectal cancer (CRC), several studies have demonstrated an association between the regular use of aspirin and improvements in CRC-specific and overall survival (Bastiaanet et al., 2012; Chan, Ogino, & Fuchs, 2009; McCowan, Munro, Donnan, & Steele, 2013; Reimers et al., 2012). Findings from a prospective study of individuals with a diagnosis of CRC (n = 1,279; median follow-up, 11.8 years) showed that regular aspirin users had a lower risk of CRC-specific and overall mortality versus aspirin nonusers (multivariate hazard ratio [HR] = 0.71 [95% CI 0.53–0.95] and multivariate HR = 0.79 [95% CI 0.65–0.97], respectively) (Chan et al., 2009). Moreover, the effect of aspirin on risk of CRC-specific mortality was even greater among new aspirin users (i.e., those who had not taken aspirin regularly before CRC diagnosis; n = 719) compared with nonusers (multivariate HR = 0.53 [95% CI 0.33–0.86]). In a subgroup of individuals with primary tumors that over-expressed COX-2 (n = 314), regular aspirin use post-diagnosis was associated with a lower risk of CRC-specific mortality than in aspirin nonusers (multivariate HR = 0.39 [95% CI 0.20–0.76]). In contrast, no such benefit was observed among those with primary tumors featuring weak or absent COX-2 expression (n = 145) (multivariate HR = 1.22 [95% CI 0.36–4.18]) (Chan et al., 2009).

Besides aspirin, other traditional NSAIDs and selective COX-2 inhibitors are also prescribed to patients at a high risk of developing CRC (Cuzick et al., 2009). However, unlike low-dose aspirin, neither traditional NSAIDs nor COX-2 inhibitors have cardioprotective effects.

In the development of skin cancer, there has been conflicting evidence regarding the chemopreventive role of NSAIDs (Asgari, Chren, Warton, Friedman, & White, 2010; Elmets, Ledet, & Athar, 2014; Muranushi, Olsen, Pandeya, & Green, 2015; Zhang, Liang, Ye, & Wang, 2014). A case-control study of 415 patients (43–85 years of age) diagnosed with SCC in the Kaiser Permanente Northern California population matched on age, gender, and race with 415 control subjects found no associations between the use of aspirin or other NSAIDs (self-reported regular use or pharmacy data) and SCC risk (Asgari et al., 2010). A subsequent meta-analysis of data from eight studies (seven observational studies and one randomized controlled trial) investigating the effects of NSAIDs on nonmelanoma skin cancers (NMSCs) found no significant differences between users and nonusers of NSAIDs in their risk of developing SCC (relative risk [RR] = 0.86 [95% CI 0.73–1.02], p = .085) or basal cell carcinoma (BCC) (RR = 0.94 [95% CI 0.85–1.04], p = .266) (Zhang et al., 2014). In contrast, evidence from several preclinical, epidemiological, and translational studies has suggested that COX-2 inhibitors could have potential in preventing NMSC development (Elmets et al., 2014). Additionally, a systematic literature review identified nine epidemiological studies with sufficiently robust data to be included in a
meta-analysis evaluating the effects of aspirin and other NSAIDs in reducing the risk of cutaneous SCC (Muranushi et al., 2015). Users of any NSAIDs and nonaspirin NSAIDs had significantly reduced risks of developing cutaneous SCC (RR = 0.82 [95% CI 0.71–0.94] and RR = 0.85 [95% CI 0.78–0.94], respectively). Individuals with ever-use of aspirin showed a trend toward a slightly reduced risk of developing cutaneous SCC versus nonusers, although this was not statistically significant (RR = 0.88 [95% CI 0.75–1.03]). The authors concluded that not only do NSAIDs reduce the risk of developing cutaneous SCC but also that these NSAID effects (any aspirin/non-aspirin use) are more pronounced among people who have a high prevalence of AK or a history of keratinocyte cancers (RR = 0.70 [95% CI 0.57–0.86]) compared with the general population (RR = 0.91 [95% CI 0.78–1.05]), although statistical significance was lacking. Of note, the conclusions of this SCC-specific meta-analysis were based on a more comprehensive dataset compared to that of Zhang et al. (2014).

3 | ACTINIC KERATOSIS AND SCC

AK is a common dysplastic lesion of keratinocytes that is seen in around one in seven patients who consult a dermatologist (Kirby, Scharnitz, Seiverling, Ahms, & Ferguson, 2015). AK develops almost exclusively as a consequence of overexposure to environmental UV radiation; lesions occur more frequently among fair-skinned individuals and are more common among Caucasian individuals (≥40 years of age) in Australia than in the United States and Europe (up to 60% vs. up to 26%, respectively) (Dodds, Chia, & Shumack, 2014; Ulrich, Pellacani, Ferrandiz, & Lear, 2014). Although some AK lesions will regress or remain stable, with focal epidermal dysplasia, hyperkeratosis, and vascular ectasia, other AK lesions will become malignant and can progress to SCC in situ or to invasive SCC (Arciniegas et al., 2015; Dodds et al., 2014). The estimated 10-year risk of AK lesion progression ranges from 1 to 10%; however, it has been reported that in individuals with multiple AK lesions the annual risk of developing invasive cutaneous SCC ranges between 0.15 and 80% (Dodds et al., 2014). Given the risk of progression, AK can be considered as part of a continuum between photo-damaged skin and invasive SCC. Of note, the global burden of disease attributable to UV radiation in people with AK was estimated to be 8,000 disability-adjusted life years (DALYs); however, SCC was associated with a much higher disease burden (59,000–83,000 DALYs) (Lucas, McMichael, Armstrong, & Smith, 2008).

4 | ROLE OF COX-2 AND SCC TUMORIGENESIS

COX-2 expression and PG production are induced following skin exposure to UV radiation (Zhan & Zheng, 2007). These events play a key role in inflammatory processes in the skin that are believed to be associated with progression from AK to SCC (Campione et al., 2015).

Several studies in murine models have demonstrated the importance of COX-1, COX-2, PGE2, and PGE2 receptors for skin tumor development and growth (Fischer, Pavone, Mikulec, Langenbach, & Rundhaug, 2007; Morita, 2002; Simper et al., 2014; Sung, He, Hwang, & Fischer, 2006; Tiano et al., 2002; Tober et al., 2006; Zelenay et al., 2015). Targeted disruptions of the genes encoding either COX-1 or COX-2 led to altered epidermal keratinocyte differentiation and reduced skin tumorigenesis in a multistage mouse skin model (Tiano et al., 2002). An association between COX-2 inhibition and chemopreventive activity against both chemical- and UV-induced skin cancer has been reported, based on observations in transgenic mice with COX-2 overexpression that were found to be sensitized to skin carcinogenesis, whereas those with COX-2 deficiency had markedly lower tumor numbers (Zhan & Zheng, 2007).

Further evidence for the importance of COX-2 in the development and progression of human skin carcinomas comes from immunohistochemical studies (Buckman et al., 1998; Kuzbicki, Lange, Stanek-Widera, & Chwior, 2011). COX-2 expression was measured in epithelial tissues from AK, SCC, and BCC biopsies taken from volunteers 24 hours after they were exposed to different doses of UV light and compared with normal non-sun-exposed control skin biopsy samples (Buckman et al., 1998). Prominent COX-2 immunostaining was observed in SCC and AK biopsies, whereas minimal and less intense staining was identified in BCC and normal skin biopsies, respectively. Western blot analysis confirmed increased expression of COX-2 in SCC samples compared with controls (Buckman et al., 1998). In contrast, a similar COX-2 immunostaining pattern was seen when normal skin and benign epithelial lesions were compared (Kuzbicki et al., 2011). However, in comparison to BCCs where expression of COX-2 was lower than that of normal skin, significantly increased expression of COX-2 was detected in precancerous lesions, including AK, and in SCCs (Kuzbicki et al., 2011). In the skin of a subset of individuals, the response to UV radiation 24 hours post-exposure, assessed by the maximal intensity of patients’ erythema response and corresponding cellular proliferation, measured by immunohistochemical staining for proliferating cell nuclear antigen, was suppressed by pretreatment with a selective COX-2 inhibitor (Rodriguez-Burford et al., 2005). This effect was not found in patients receiving placebo and was independent of skin type, age, sex, or serum levels of the COX-2 inhibitor. The difference in erythema response could potentially be extrapolated to patients’ susceptibility to skin cancer and highlights the importance of identifying the subset of patients who are likely to benefit from treatment with COX-2 inhibitors (Rodriguez-Burford et al., 2005).

5 | DICLOFENAC SODIUM 3% IN HYALURONIC ACID 2.5% FOR THE TREATMENT OF AK

Diclofenac is an established NSAID belonging to the phenylacetic acid class that has been widely used as an anti-inflammatory analgesic since its introduction in 1973 (Altman, Bosch, Brune, Patrignani, & Young, 2015). Diclofenac sodium 3%, in combination with hyaluronic acid 2.5% (diclofenac 3%/HA 2.5%; Solaraze®, Almirall Ltd., Uxbridge, UK), is the only NSAID approved in the United States and Europe for the topical treatment of AK lesions. In an early phase II, open-label study in 29 patients with AK, among 27 patients reevaluated 30 days after treatment was discontinued, 22 (81%) showed a complete response,
and a marked clinical improvement was shown in 4 (15%) patients (Rivers & McLean, 1997). The treatment was well tolerated, with adverse events relating to skin irritation at the treatment site reported in seven patients (24%) (Rivers & McLean, 1997). Results of two subsequent double-blind placebo-controlled phase III trials confirmed that treatment was generally well tolerated and effective (Rivers et al., 2002; Wolf Jr, Taylor, Tschen, & Kang, 2001). A significantly greater proportion of patients receiving diclofenac 3%/HA 2.5% gel had target lesion number scores and cumulative lesion number scores of zero compared with placebo after 60 (Rivers et al., 2002) and 90 (Wolf Jr et al., 2001) days of treatment. Investigator and patient global improvement indices (IGII and PGII, respectively) were also significantly better in those on active treatment versus placebo (Rivers et al., 2002; Wolf Jr et al., 2001). At follow-up, 30 days after treatment cessation, the proportion of patients rated as completely improved (IGII or PGII score of 4) on active treatment versus placebo was 47% versus 19% for IGII and 41% versus 17% for PGII (p < .001 for both) (Wolf Jr et al., 2001). A similar incidence of adverse events was reported in both groups (Rivers et al., 2002; Wolf Jr et al., 2001).

Beyond evidence from phase III clinical studies, there is a substantially broader evidence base that supports the use of diclofenac 3%/HA 2.5% in this setting (Martin & Stockfleth, 2012). A systematic literature review published in 2012 identified 18 clinical studies (comprising a mixture of blinded and open-label study designs and case series in a total of 1,779 patients) investigating the role of diclofenac 3%/HA 2.5% at various body sites (Martin & Stockfleth, 2012). In most studies, the duration of diclofenac 3%/HA 2.5% treatment was 3–6 months; evaluated outcome measures included reductions in number of target/cumulative lesions, reduction in lesion size, and complete clinical/histological clearance. Diclofenac 3%/HA 2.5% demonstrated efficacy at difficult-to-treat and sensitive body locations such as the lips and the periorcular region and was also efficacious in immunesuppressed patients (Ulrich et al., 2014). In comparative studies, diclofenac 3%/HA 2.5% had similar efficacy to and a better safety profile than the active comparator, 5-fluorouracil 5% cream (Segatto, Dornelles, Silveira, & Frantz Gde, 2013; Smith, Morhenn, & Piacquadio, 2006), and a similar efficacy and tolerability profile to imiquimod 5% cream (Akarsu, Aktan, Atahan, Koç, & Özkan, 2011; Kose, Koc, Erbil, Caliskan, & Kurumlu, 2008).

Dermatologists and General Practitioners should advise all patients with AK to protect themselves from sunlight and regularly use a high sun protection factor (≥50) broad-spectrum sunscreen (Dirschka et al., 2017). In the trials evaluating the efficacy of diclofenac in AK, subjects were advised to concomitantly also use sunscreens (Dirschka et al., 2017). In the trials evaluating the efficacy of diclofenac 3%/HA 2.5% led to a 78% reduction overall in the number of AK lesions (from 165 to 36 after 3 months), with AK reductions observed continuously over the study period in each patient (Maltusch et al., 2011). The mechanisms of action underlying the observed effects of diclofenac 3%/HA 2.5% reported in this study were investigated via histological and immunohistochemical analysis of skin biopsies. Treatment with diclofenac 3%/HA 2.5% led to a significant reduction in the expression of the inflammatory markers COX-2 (epidermis), CD3, and CD8 compared with pretreatment levels (p = .006, .005, and .013, respectively). Furthermore, posttreatment expression of markers of apoptosis and/or cell cycle arrest (p53 and p21) were significantly reduced compared with healthy skin (p53 [p = .011]) and versus pretreatment levels (p21 [p = .003]). Posttreatment expression of CD31, a marker of angiogenesis, was also significantly lower compared with pretreatment levels (p = .015). A similar but statistically nonsignificant trend was observed for the proliferation marker Ki67. The authors concluded that diclofenac 3%/HA 2.5%-related clinical improvements in AK are driven primarily by its anti-inflammatory and anti-angiogenic effects but that effects on proliferation and apoptosis also contribute. Other research groups have confirmed the anti-angiogenic effects of diclofenac 3%/HA 2.5% and have found effects on apoptotic activity (Eberle et al., 2007; Fecker et al., 2010; Kaur & Sanyal, 2011; Rodust, Fecker, Stockfleth, & Eberle, 2012). These apoptotic effects appear to be exerted in SCC cell lines via activation of the mitochondrial/intrinsin apoptosis pathways and the extrinsic death ligand-mediated apoptosis pathway (Eberle et al., 2007; Fecker et al., 2010; Rodust et al., 2012).

Another COX inhibitor that has been used for the treatment of AK is piroxicam, an enolic benzothiazine and potent member of the oxicam series, which has been demonstrated to exert a significant...
antitumorigenic effect (Babino et al., 2016; Campione et al., 2015; Puviani et al., 2017). Piroxicam is a nonspecific COX-1 and COX-2 inhibitor, with higher inhibitory activity (10-fold) for COX-1 (Meade, Smith, & DeWitt, 1993), which inhibits the early stages of skin carcinogenesis. The mechanism of action of piroxicam includes the down-regulation of prostaglandins and thromboxanes, and the inhibition of polyamine production by the blockade of ornithine decarboxylase induction, which is involved in nonmelanoma skin carcinogenesis. In addition, piroxicam is able to induce tumor cell apoptosis and suppresses metalloproteinase 2 activities (Campione et al., 2015).

Diclofenac also appears to have multiple mechanisms of action besides its established inhibition of COX (Gan, 2010). Several putative effects have been described such as inhibition of synthesis of leukotrienes and phospholipase A2, modulation of free arachidonic acid levels, stimulation of peripheral nitric oxide cyclic guanosine monophosphate-potassium channel pathways, and centrally mediated and neuropathic effects (elevated plasma β-endorphin levels and N-methyl-d-aspartate pathway inhibition). Emerging mechanisms of action include inhibition of peroxosome proliferator activated receptor-γ, reduction of levels of inflammatory mediators (e.g., substance P and interleukin-6 in the plasma and synovial fluid), inhibition of the thromboxane-prostanoid receptor, and inhibition of acid-sensing ion channels (Gan, 2010). Although established mechanisms are well supported by clinical data, further research is needed to validate the proposed putative and emerging mechanisms of action. Further insights into how diclofenac 3%/HA 2.5% exerts its anti-AK effects were generated by an open-label, multicenter, phase IV trial (NCT00204542) in 65 Caucasian patients (51–81 years of age) with face or scalp AK lesions randomized to 3 or 6 months’ treatment (Dirschka, Bierhoff, Pflugfelder, & Garbe, 2010). Following 3 months’ treatment, 16.9% of patients (n = 11) achieved complete clinical resolution and 23.1% (n = 15) achieved full histological resolution. Furthermore, posttreatment AK grades appeared to be significantly improved versus pretreatment gradings, based on the AK grade I–III classification scheme (Röwert-Huber et al., 2007). In addition, a significantly lower number of mitoses per high-power field were counted posttreatment (p < .001 vs. pretreatment); semiquantitatively evaluated inflammatory infiltrate levels were also significantly lower posttreatment (p = .005 vs. pretreatment). Semiquantitative evaluation of biopsy specimens using immunohistochemical staining identified significant posttreatment reductions in expression of the anti-p53-antibody (directed against the p53 tumor suppressor gene [p = .009 vs. pretreatment]) and the anti-MIB-1 antibody (directed against the proliferation marker Ki-67 [p = .021 vs. pretreatment]). The authors concluded that diclofenac 3%/HA 2.5% gel induces regression of signs of cancerous transformation in AK (Röwert-Huber et al., 2007).

**7 CONCLUSIONS**

In summary, AK is a common dysplastic lesion of keratinocytes that is associated with exposure to UV radiation and has the potential to transform to invasive SCC. As discussed, COX-2, the main COX isofrm in human skin, and its principal metabolic product PGE2 are induced in response to UV radiation and have been implicated in the processes involved in the development of AK and SCC, in addition to tumorigenesis in various other tissue types. Evidence from epidemiological, preclinical, and translational studies in skin cancer reviewed herein suggests that the inhibition of COX-2 and PGE2 production with NSAIDs may have antitumor effects. In particular, in patients with existing AK lesions, topical treatment with diclofenac 3%/HA 2.5% is effective, causing regression of signs of cancerous transformation, and well tolerated, with most adverse events relating to skin irritation. Although the exact mechanism of action remains to be elucidated, there is evidence to suggest that the effects of diclofenac 3%/HA 2.5% are mediated via inflammation, angiogenesis, and apoptosis pathways. The evidence presented may inform treatment strategies and highlights future areas of research to improve our understanding of the underlying mechanistic pathways.

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**CONFLICT OF INTEREST**

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