Cyclooxygenase in Cancer Prevention and Treatments for Actinic Keratosis

Gareth J. Thomas · Colin A. Morton

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are a chemically diverse class of drugs that target the cyclooxygenase (COX) pathway and have anti-inflammatory, analgesic, and antipyretic properties. Elevated expression of COX-2 has been associated with tumor progression in skin cancer through multiple mechanisms. We present evidence for a chemoprotective effect of NSAIDs and discuss potential mechanisms of action of COX-2 in cancer. We also discuss the challenges associated with the treatment of actinic keratosis and the factors that should be taken into consideration when selecting a treatment regimen. A range of treatments are reviewed, with an emphasis on combination therapies.

Keywords: Actinic keratosis; Cancer prevention; Cyclooxygenase; Non-steroidal anti-inflammatory drugs; Prognosis; Treatment

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), a chemically diverse class of drugs with anti-inflammatory, analgesic, and antipyretic properties, target the cyclooxygenase (COX) pathway [1]. Two major isoforms of COX exist: COX-1 and COX-2 [2]. While both catalyze an identical enzymatic reaction, the two are independent and are implicated in distinct physiologic and pathogenic processes, and they differ in their pattern of expression: COX-1 is constitutively expressed in most tissues [3], whereas COX-2 is usually induced by numerous physiologic stimuli [4]. COX-1 and COX-2 are involved in the synthesis of prostaglandins (PGs), catalyzing the conversion of arachidonic acid to PGH2. PGH2 is subsequently converted to a number of eicosanoids, including PGD2, PGE2, PGF2a, PGI2, and thromboxane (TX). Both COX-1 and COX-2 are capable of synthesizing PGs from arachidonic acid. However, it has been shown that PGE2 and PGI2 are mainly derived from the COX-2 pathway [5]. The variety of PGs produced depends on the downstream enzymatic machinery present in a particular cell type. For
example, platelets predominantly produce TXA2.

**COX-2 AND SKIN CANCER**

Elevated expression of COX-2 occurs in many cancers, where it contributes to tumor progression through multiple mechanisms [6, 7]. There is strong evidence from epidemiologic studies and animal models that COX-2 is involved in the development and progression of cutaneous squamous cell carcinoma (SCC) and may represent a target for prevention and/or therapy [8–11].

COX-2 expression is upregulated in human and murine SCC, accompanied by a high level of PG synthesis [6]. Genetic and pharmacologic approaches have been used to demonstrate the contribution of COX and PGs to murine skin cancer: COX-2 knockout mice are significantly protected from UV-induced skin cancer compared with wild-type mice [8]. Conversely, transgenic mice overexpressing COX-2 in the epidermis develop many more tumors in response to UV treatment compared with wild-type mice [8]. UV-induced COX-2 expression increases PGE2 production, and overexpression of the PGE2 receptors EP1, 2, and 4 is also associated with increased numbers of UV-induced tumors [12–14]. Selective COX-2 inhibitors have been shown to suppress UV- and carcinogen-induced SCC [10, 11, 15]. For example, application of celecoxib has been shown to reduce UV-associated skin damage in mice and the number and size of new skin tumors [10]. The nonselective NSAIDs naproxen, aspirin, and sulindac have also been found to significantly reduce the number of UV-induced skin tumors [2].

While COX-1 and COX-2 are present in non-melanoma skin cancer in humans [9], COX-2 is the primary UV-responsive COX isoform in human skin. UV radiation is a known stimulus for COX-2 expression in the epidermis, and single doses of UV (1–2 minimal erythema dose) results in a substantial increase in COX-2 expression, with no change in COX-1 expression [16]. While COX-2 is not detectable in normal skin, it is upregulated in SCC and actinic keratosis (AK) [17].

Epidemiologic evidence supports a preventive effect for NSAIDs in the development of SCC. An Australian case-control study on a cohort of 1621 individuals found that people who used NSAIDs twice a week or more for at least a year had a significantly lower incidence of AKs and SCCs than those who did not [18]. A Danish population-based case-control study also found a decreased risk of developing SCC and malignant melanoma in individuals taking NSAIDs, especially with long-term (≥7 years) or high-intensity use (>25% of prescription coverage during total duration of use) [19]. Although several studies have not found a significant association between NSAIDs and SCC protection [20, 21], a recent meta-analysis of published studies revealed that aspirin and other oral NSAIDs are associated with a significant reduction in the risk of SCC compared with non-use of NSAIDs, and this association is particularly evident among individuals with a history of AK or SCC (Fig. 1). The analysis was not able to determine whether the dose or duration of NSAID administration impacted on SCC prevention [22]. A meta-analysis of topical NSAID treatment of AKs with the COX-2 inhibitor diclofenac in 2.5% hyaluronic acid found that target lesions were completely resolved in 40% of cases after a mean 75 days of treatment [23]. There is evidence indicating that diclofenac, which has been approved for the treatment of AK in the form of diclofenac/hyaluronic acid, acts by inducing cell death. Topic treatment of four SCC cell lines (acting as a model for AK because of a general lack of AK cell lines) with diclofenac/hyaluronic acid was found to induce apoptosis, possibly by sensitizing neoplastic keratinocytes to death ligand-induced apoptosis [24].

**THE MECHANISM OF ACTION OF COX-2 IN MALIGNANCY**

The mechanism by which COX-2 promotes SCC development is unclear; however, the
enzyme has been shown to promote several functions associated with malignant transformation and progression. PGE2 stimulates the proliferation of keratinocytes [10], and this effect can be suppressed by NSAIDs, which also act to promote keratinocyte apoptosis. COX-2 expression in SCC has also been shown to correlate with epithelial-to-mesenchymal transition, suggesting that COX-2 may promote cell motility. Consistent with this, COX-2 has been associated with invasion in squamous carcinomas of the head, neck, and esophagus [25, 26]. While PGE2 is involved in the inflammatory process, paradoxically, it also has an immunosuppressive role [27]. The COX-2/PGE2/EP pathway can suppress activity of dendritic cells, NK cells, and T-lymphocytes and promote tumor immune evasion [28]. Zelenay and colleagues recently demonstrated a synergistic effect between COX inhibitors and immune checkpoint inhibitors in a murine cancer model, suggesting that NSAIDs may have utility as an immunotherapeutic adjunct [29].

When Should Actinic Keratosis be Treated?

The decision to treat a patient with AK lesions is made on the basis of clinical factors, such as a history of AK lesion persistence, the age of the patient, presence of discomfort, the extent of co-existing photodamage, expected tolerance to the side effects of therapy, and history of skin cancer [30].

Further support for the decision to treat AK lesions comes from the finding that similar genes are differentially expressed in AK and SCC, confirming that AK is a precursor lesion of SCC [31]. Elucidation of the process by which SCC develops from AK has revealed that the classical progression from AK I to II to III, and subsequently SCC, occurs in a substantial proportion of cases, while the direct progression from AK I to SCC (the so-called differentiated pathway) is also common [32]. (See ‘Skin cancer—Epidemiology, Disease Burden, Pathophysiology, Diagnosis and Therapeutic Approaches,’ by Zoe Apalla, Dorotheé Nashan, Richard Weller, and Xavier Castellsagué,
SELECTING THE MOST APPROPRIATE TREATMENT

There are increasing numbers of treatment options for AK and, correspondingly, a greater choice for patients. However, the range of available options can be limited by healthcare costs and/or reimbursement complications, in addition to the preferences of the physician (and patient) for procedural, topical, or combination treatment. Consequently, delivering the most appropriate therapy may be a challenge. Given the scope of the current article, we summarize below those treatment options that are applicable to the European market.

Selecting the most appropriate treatment should take into account the clinical presentation of AK: the type, distribution, and location of lesions and whether field or lesional treatment is necessary. Also, data on the efficacy and tolerability of treatments, patient preference, and cost-effectiveness (prescriber preference and price) should be considered. Several evidence-based treatment guidelines are available to assist clinicians in navigating the field, including those from the Primary Care Dermatology Society (Fig. 2).

Physically Destructive Methods

Lesion- or field-directed treatment options aimed at physically destroying AK lesions include cryotherapy and laser treatment. Cryotherapy is non-specific and uses liquid nitrogen to freeze and promote necrosis of lesions, although both atypical and normal cells are at risk of destruction. It is widely used for small, or a low number of, AK lesions [33–35]. A single application of cryotherapy may be sufficient, or treatment can be repeated several times [35]. Laser (either CO₂ or Er:YAG) treatment destroys skin to a controlled depth [34]. It is useful when there is co-existent photodamage or where lesions are recalcitrant, although hypopigmentation may be a problem [36].

Surgical Removal

Surgical removal of AK lesions involves excision or curettage and is suitable for single, discrete, hyperkeratotic lesions [35]. Surgery is unlikely to be the first line of treatment, unless diagnosis is uncertain [34]. While there are no clinical trials evaluating the efficacy of surgery for AK lesions, it is likely to be effective [34].

Topical Treatments

A number of topical treatments are available for the treatment of AK lesions. Diclofenac 3% in hyaluronic acid [37] is administered twice daily for 60–90 days for the treatment of AK lesions in all body areas. Although the mechanism of action of diclofenac in AK is not known, it may be related to the inhibition of the cyclooxygenase pathway leading to reduced PGE2 synthesis. Common side effects include usually mild erythema, crusting, scaling, and pruritus.

5-Fluorouracil 5% is a chemotherapeutic agent that destroys tumor cells via blocking the methylation reaction of deoxyuridylic acid to thymidylic acid. Treatment is administered once or twice daily for 3–4 weeks, and a normal response is associated with an early and severe inflammatory phase (usually week 2), followed by a necrotic phase and then healing. Other common side effects include erythema, crusting, scaling, and pruritus [38].

5-Fluorouracil 0.5% plus 10% salicylic acid (5-FU/SA) comprises an antimetabolite combined with a keratolytic agent to reduce hyperkeratoses. This is administered once daily for 6–12 weeks for slightly palpable and/or moderately thick hyperkeratotic AK (grade I/II) lesions on the face, forehead, or balding scalp (epidermal thickness must be considered when administering to other sites). Common side effects of 5-FU/SA include erythema, inflammation, irritation, pain, and pruritus [39–41].

Imiquimod 5%, a Toll-like receptor 7 agonist, disrupts tumor proliferation and induces cytokines with indirect anti-tumor

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It is applied three times per week for 4 weeks on typic, non-hyperkeratotic, non-hypertrophic AK lesions on the face/scalp; this is done prior to normal sleeping hours and washed off after approximately 8 h. In Europe, this treatment cycle can be repeated after 4 weeks if required [42]. Common side effects include erythema, crusting, and scaling (treatment can be interrupted if intense local inflammatory reactions occur). Flu-like symptoms including headaches, fever, and chills may also be experienced. Imiquimod 3.75% is applied once daily for 2 weeks for AK lesions on the face/scalp (Europe). This is followed by a 2-week break without treatment and then a further 2 weeks with treatment [43].

Ingenol mebutate was isolated from *Euphorbia peplus* and is involved in rapid targeted cell necrosis and an immune response. It is applied on non-hyperkeratotic, non-hypertrophic AK lesions to the trunk/extremities (500 μg gel) once daily for 2 days and to the face/scalp (150 μg gel) once daily for 3 days. Erythema, flaking/scaling, and crusting typically occur within 1 day of initiation and peak in intensity up to 1 week following completion of treatment [44, 45].

Conventional methyl aminolevulinate-photodynamic therapy (MAL-PDT) is administered to thin or non-hyperkeratotic and non-pigmented AK lesions on the face/scalp. After topical application of methyl aminolevulinate, porphyrins accumulate intracellularly [including protoporphyrin IX (PpIX) as photoactive, fluorescing compounds]; light activation leads to a photochemical reaction and thereby phototoxicity to the light-exposed target cells. During light exposure, common side effects include burning/stinging pain, whereas post-therapy swelling and erythema are often followed by crusting [46]. Conventional aminolevulinic acid-photodynamic therapy (ALA-PDT), involving the application of 5-aminolevulinic acid, is administered to patients with mild-to-moderate AK lesions on the face/scalp (Olsen grade I–II; refer to Fig. 2) [47–49]. For conventional ALA-PDT, common side effects are similar to those observed with conventional MAL-PDT. Daylight MAL-PDT [46, 50, 51] may be used to treat patients with mild-to-moderate AK lesions and is suitable for most weather conditions (avoiding rainy days) above 10°C. Pain is a common side effect but minimal compared with standard PDT.
Combination Therapy

Field therapies have often been combined (or used sequentially) with each other or with lesion-targeted therapies in an effort to improve results by taking advantage of the synergy of the individual mechanisms of action [35]. There is increasing interest in the use of combination or sequential therapy in an effort to improve outcomes.

In a small study of patients with multiple grade I/II AK lesions (including recurrence), diclofenac in hyaluronic acid was administered twice daily for 12 weeks, followed by a 2-week treatment-free interval, and then 5-fluorouracil (5-FU) + salicylic acid once daily for up to 6 weeks, as required. Complete clinical and histologic clearance occurred in 83.3% of 12 patients [52].

The combination of diclofenac/hyaluronic acid therapy with cryotherapy, using different freeze times and order of therapy, resulted in higher lesion clearance rates than single-agent therapy [53–55].

An enhanced clinical effect was observed when AK lesions were pre-treated with diclofenac/hyaluronic acid or 5-FU before photodynamic therapy (PDT), sequential PDT and imiquimod, with patients having fewer lesions at follow-up compared with those who were not pre-treated [56–58]. The combination of PDT with other treatments, such as diclofenac/hyaluronic acid, 5-FU, imiquimod, or ingenol mebutate, appears to improve outcomes with PDT [59]. However, most of the studies have included small numbers of patients, with clinical evaluation only, and no histologic confirmation of results. Further studies with larger numbers of patients and longer follow-up are needed to evaluate these combinations more fully [59].

ECONOMIC CONSIDERATIONS

In the outpatient setting, AK is among the most commonly treated skin conditions [33, 60]. As AK is a disease with increasing incidence, which may evolve to SCC, the willingness by payers to cover the cost of AK therapy has increased. As with all treatments, the National Health Service and payers will consider clinical data, unmet needs, the burden of illness, and the cost-effectiveness and budgetary impact of potential therapies.

More research is needed on epidemiologic data, evidence-based standards, delineating cost drivers in immunocompetent and immunocompromised patients, and on health outcomes.

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