

The relevance of piroxicam for the prevention and treatment of nonmelanoma skin cancer and its precursors

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Abstract: Piroxicam (PXM), a nonsteroidal anti-inflammatory drug, is an enolic benzothiazine and a potent member of the oxicam series. The drug suppresses the synthesis of proinflammatory enzymes, such as cyclooxygenases-1 and -2 (COX-1 and 2), downregulates the production of prostaglandins (PGs) and thromboxanes, and inhibits polyamines production by blocking ornithine decarboxylase induction involved in nonmelanoma skin carcinogenesis. In addition, PXM is able to induce tumor cell apoptosis and suppresses metalloproteinase 2 activities. Skin carcinogenesis is a multistep process in which the accumulation of genetic events leads to a gradually dysplastic cellular expression, deregulation of cell growth, and carcinomatous progression. COX-1 upregulation plays a significant role in PG and vascular epidermal growth factor production supporting tumor growth. Increased level of PGs in premalignant and/or malignant cutaneous tumors is also favored by upregulation of COX-2 and downregulation of the tumor suppressor gene 15-hydroxy-prostaglandin dehydrogenase. Chemoprevention can be a hopeful approach to inhibit carcinoma occurrence before an invasive tumor develops. The chemopreventive effect of nonsteroidal anti-inflammatory drugs on nonmelanoma skin cancers has been established. In this study, we highlighted the different modalities of action of PXM on the pathogenesis of nonmelanoma skin cancer, analyzing and evaluating binding modes and energies between COX-1 or COX-2 and PXM by protein–ligand molecular docking. Our clinical experience about the local use of PXM on actinic keratoses and field cancerization is also reported, confirming its efficacy as target therapy.

Keywords: COXs inhibitor, actinic keratosis, tumor progression, binding mode

Introduction

Piroxicam (PXM), an enolic benzothiazine and potent member of the oxicam series, suppresses the synthesis of proinflammatory enzymes, reducing lipid mediators such as prostaglandins (PGs) and thromboxane (TXs), and also inhibits ornithine decarboxylase (ODC) induction,¹ both pathways participating in carcinogenesis. Further effects of PXM are the inhibition of metalloproteinase-2 activity and induction of apoptosis, determined to have an antiaging effect on skin texture.² PXM belongs to a class of nonsteroidal anti-inflammatory drugs (NSAIDs) indicated for the symptomatic treatment of inflammatory and degenerative rheumatic diseases (rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis). On the basis of the documented effect of PXM in skin tumorigenesis, through the block of cyclooxygenases-1 and -2 (COX-1 and -2) activity,³ we reported preliminary studies of its usefulness in the treatment of actinic keratoses (AKs).⁴ COX inhibitors, diclofenac and celecoxib, have also been used for the treatment of AKs and squamous cell carcinomas (SCCs).⁵ The effectiveness of NSAIDs is attributable to the inhibitory effect on the increased COX-1

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and -2 enzyme activity in skin tumors produced after chronic exposure to UVB (ultraviolet B) (accompanying skin inflammation). Aberrant overexpression of COX-2 in both AK and SCC upon UVB irradiation and UVB-induced *p53* tumor suppressor gene mutations are early events responsible for the progression of sun-exposed nonmelanoma skin cancers (NMSCs).⁵ Topical NSAID treatment works as a nonspecific COX inhibitor and is an effective and well-tolerated treatment for AK. COX inhibitors have been examined with respect to their role in cancer prevention and cancer treatment in both animal models and humans.⁶ In humans, topical application of NSAIDs is also potent in inhibiting the UV light-induced acute sunburn reactions such as erythema and peeling of superficial layers, if applied immediately after UVB exposure.^{7,8} An exhaustive meta-analysis on PXM made by Richy et al⁹ on controlled clinical trials pointed out a more favorable efficacy and safety profile of PXM compared to other topical NSAIDs (diclofenac, naproxen, tenoxicam, indomethacin, etodolac, meloxicam, ibuprofen, salicylates, nabumetone, aceclofenac, droxicam, flurbiprofen, ketoprofen, nimesulide, and diflunisal).

Here, we described the different modalities of action of PXM on the pathogenesis of NMSC. The inhibitory effects of PXM on COX-1 and -2 activity have been investigated by analyzing its binding mode. We also reported our clinical experience about the effectiveness of local use of PXM as chemopreventive agent on AKs and field cancerization.

Steps in skin tumorigenesis and role of COX enzymes in cancer

Skin cancer is the most common cancer in humans. The skin of the head and neck accounts for less than 10% of the body's surface area, but this region accounts for 70%–80% of skin cancer cases. Although mortality from NMSC is low, its high incidence leads to a significant public health burden, making them a suitable target for chemoprevention and long-lasting research. NMSC includes SCC and basal cell carcinoma (BCC).¹⁰ Carcinogenesis occurs in two steps: initiation and promotion.¹¹ The promotion phase, an important target for the design of potential chemoprevention studies, is temporally prolonged and potentially reversible.¹¹ A variety of model systems have demonstrated an increase in tissue polyamine levels, including putrescine, during tumor promotion.¹² Mammalian polyamine biosynthesis is at least partially controlled via induction of ODC, which makes the inhibition of this enzyme a potential target for chemoprevention.¹³ α -Difluoromethylornithine is an enzyme-activated irreversible inhibitor of ODC that has been shown to prevent tumors in experimental animal

systems.¹⁴ In addition, accumulation of genetic events within cells leads to a gradually dysplastic cellular manifestation, deregulated cell growth, and finally, carcinoma. An upregulation of COX-2 plays a significant role in PG and vascular epidermal growth factor (VEGF) production for the tumor proliferation.¹⁵ SCC of the head and neck showed little response to COX-2 inhibition. Therefore, a cotreatment of COX-1 and -2 determined a meaningful inhibition of VEGF, as observed by Park et al.¹⁵ Increased levels of prostaglandin E₂ and F₂ (PGE₂ and PGF₂) in pre-malignant and/or malignant cutaneous tumors are favored by upregulation of COX-2 and downregulation of tumor suppressor gene 15-hydroxy-prostaglandin dehydrogenase (15-PGDH).¹⁶ Chemoprevention can be a hopeful approach to inhibit carcinoma occurrence before an invasive tumor develops. The chemopreventive effect of NSAIDs on NMSC has been established in animals^{17–20} and in vitro studies.²¹ Experimental studies have proposed the topical or oral use of NSAIDs in human subjects for the regression of cutaneous tumors.²² Findings from observational cases studies or clinical trials documented a defensive effect of NSAIDs against colorectal cancer.^{23–25} The hypothesis that NSAIDs might prevent the occurrence of colorectal cancer arose from studies showing that PGE₂ levels were higher in colorectal cancer than in surrounding normal tissue. On the other hand, few epidemiological studies have investigated the efficacy of NSAIDs in the prevention of skin cancer.^{26–28}

PXM effects for the prevention of NMSC

It is well known that UV irradiation is the major cause of induction and development of AK and NMSC. Normally, sun-damaged cells undergo, through *p53* activation, apoptosis for self-destruction.⁵ Skin cells can acquire tumorigenic mutations by UV irradiation or chemical stimulation and loss of the normal ability of cell differentiation and apoptosis. The stepwise evolution of AK with an increased expression of antiapoptotic Bcl-2 favors the progression to SCC.²⁹ As a matter of fact, retinoid-induced modulation of apoptosis in NMSC is an efficacious therapeutic approach.^{30–32} In addition, the dermal response with inflammation, characterized by erythema and edema, mediated by PGs is a critical component of tumorigenesis, promoting tumor growth, tissue invasion, angiogenesis, and metastasis.²⁹ The action of PXM plays a role in early stages of carcinogenesis by blocking the phases of inflammation and thus angiogenesis; the evidence of its effectiveness for the treatment of AKs has been described in our previous study.⁴

Also, prolonged use of PXM acts on photodamaged areas which is expression of field cancerization.⁴ To date, a higher occupational sun exposure of men compared with women justified the different incidence of NMSC.³³ A study of Johannesdottir et al³⁴ provides an alternative elucidation, which attributes the reduced incidence to the chronic use of analgesics for menstrual cycle-related symptoms. Several epidemiological studies confirm that aspirin can act as a chemopreventive agent and can decrease the incidence of various cancers, including skin tumors. The major inhibitor effect of aspirin on NMSC and precursors is related to its targeting elevated COX-2 levels in SCC. Most studies also examined the use of PXM in different disorders, especially for analyzing absorbance, tolerance, and efficacy in animal models,³⁵⁻³⁷ but the efficacy of PXM in humans was analyzed considering the general effects of NSAIDs, as in colon rectal carcinoma^{38,39} or in NMSC.⁴⁰⁻⁴²

Rathore et al⁴³ recently reported that cotreatment of PXM with masitinib significantly inhibited cell proliferation of oral squamous cell carcinoma (OSCC) as compared to either drug alone, through the c-kit and AKT signaling pathways. PXM inhibited masitinib-induced COX-2 expression in all tested OSCCs. Therefore, targeting these two signaling pathways simultaneously was more efficient for inhibition of OSCCs across these species.⁴³ A few studies evidenced the chemopreventive use of PXM for NMSC,^{4,44} but its interaction with COX-2 is well known;⁴³ however, more studies provided evidences for efficacy of PXM in melanoma treatment.⁴⁵⁻⁴⁷

Cyclooxygenase pathway

In vertebrates, two isoforms of active COX enzymes have been identified, which display similar enzymatic properties, but differ in their expression and regulation.⁴⁸ The best-known mechanism of action of NSAIDs is the inhibition of COX isoforms, in particular, COX-1 and COX-2, both implicated in inflammation and promotion of tumorigenesis.¹⁶ Chronic inflammation correlates with the increased risk of developing cancer in the affected organ and can be caused by acute UV radiation and other factors.^{49,50} The metabolism of arachidonic acid, the predominant precursor of PGs and the major control point for their synthesis, can be triggered by UV effect on the skin cell membranes, thus starting the inflammatory process.⁵¹ Arachinoid acid metabolism and PG synthesis through the COX-related pathway promotes cancer progression. COX is the key enzyme in prostanoid biosynthesis, which catalyzes the conversion of arachidonic acid to prostaglandin H₂ (PGH₂), which is subsequently converted to biologically active lipids such as TX, PGE₂, and prostacyclin

(PGI₂) by different enzymes.⁵² The skin is a major site of PG synthesis. All skin cells are PGs producers: keratinocytes, melanocytes, Langerhans cells, mast cells, fibroblasts, and endothelial cells.⁵³ While COX-1 is constitutively expressed, COX-2 is induced by tumor promoters, growth factors, and cytokines.⁵⁴ COX-1 is responsible for maintenance of the gastric and platelet functions, vascular homeostasis, as well as renal blood flow, and it also seems to contribute to PG synthesis during tumor progression in various tissues, including skin.^{55,56} The relative contribution of the two COX isoforms to skin inflammation is evaluated by combining studies with genetic knockout models and pharmacological intervention with isozyme-selective inhibitors.^{57,58} The carcinogenic actions of COX-2 should be distinguished from those of COX-1 in the application of PG-blocking therapy for cancer treatment.⁵⁹ The proliferative effects of COX-2 are due primarily to the increased synthesis of PGs, which directly influence cell growth after binding to specific cell surface receptors, including PG E, F, and I classes of receptors.^{60,61} The protumorigenic effect of prostaciclins can be mediated by specific receptors as EP2, and the different forms of PGs mediated their biological effects through EP, FP, and IP receptors.⁶² PGs may also promote the retention of UV-damaged cells through the inhibition of apoptosis.⁶³ A study of Kuzbicki et al⁶⁴ demonstrated that differently from SCC, neither benign epithelial skin lesions nor BCC are associated with enhanced COX-2 expression. Both COX isoenzymes are strongly expressed in SCC, deriving from a more differentiated epidermal layer.⁶⁴ A high COX-2 expression could be considered a risk factor of BCC recurrence, determining a role in BCC prognosis.⁶⁵ In general, NSAIDs are effective chemopreventive agents for skin cancer, and they function by acting on COX pathway specifically during the treatment of AK and SCC.

PXM binding mode

The inhibitory effects of PXM on COX-1 and -2 activity have been investigated by analyzing its binding mode. Protein-ligand molecular docking has been used to evaluate binding modes and energies between COX-1 or COX-2 and the PXM drug (Figures 1 and 2). The docking simulations have been performed by using the AutoDock Vina 1.1.2 program,⁶⁶ through the AutoDock/Vina PyMOL plugin (<http://wwwuser.gwdg.de/~dseelig/adplugin.html>); the PyMOL Molecular Graphics System Version 1.5.0.4. Schrödinger, LLC). The PXM SDF file,⁶⁷ downloaded from the PubChem compound database (<https://pubchem.ncbi.nlm.nih.gov>), has been converted into mol2 file and filled with hydrogens using the Open Babel program.⁶⁸ In

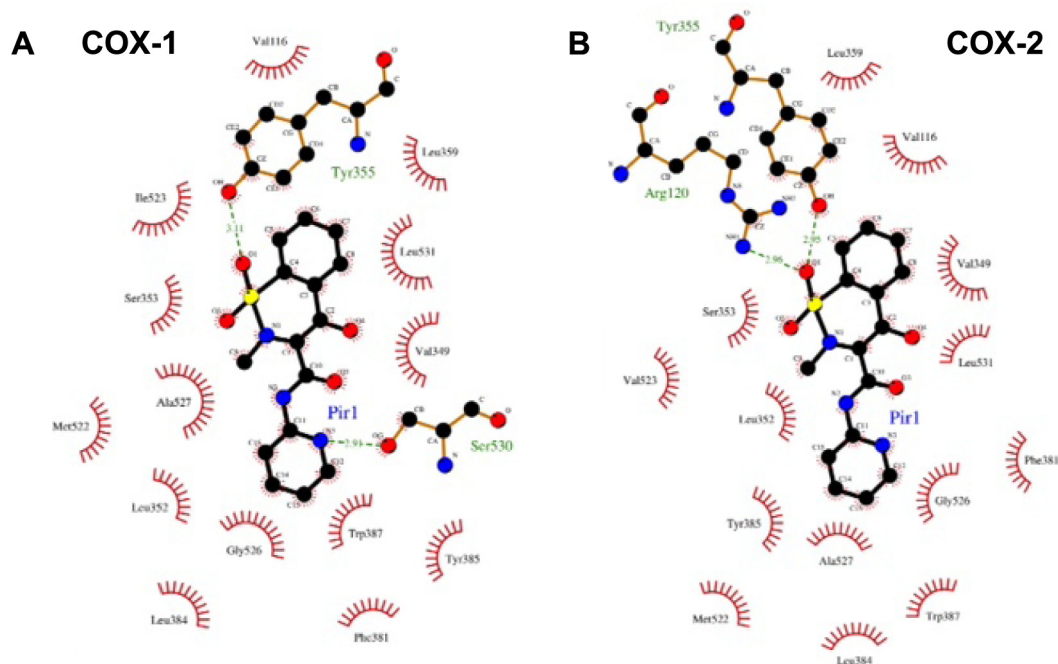


Figure 1 Schematic view of the interactions between COX-1 (A), COX-2 (B), and PXM (Pyr1).

Notes: The two-dimensional depictions show hydrogen bonds as green dashed lines between the interaction partners on either side. Residues comprising the active sites and in proximity of the drug are shown. The ligand itself is drawn using the two-dimensional draw engine according to chemical drawing conventions.

Abbreviations: COX-1 and -2, cyclo-oxygenases-1 and 2; PXM, piroxicam.

the absence of the human enzymes, the X-ray structures of *Ovis aries* COX-1, PDB ID code 1CQE,⁶⁹ and *Mus musculus* COX-2, PDB ID code 1PXX,⁷⁰ deposited in the Protein Data Bank (PDB, <http://www.rcsb.org/pdb>), have been selected as receptors since their sequence identity with human enzymes

is very high (~95%) and the residues comprising the active sites fully conserved. In COX-1 and COX-2, the active sites are composed of the same group of amino acids with the exception of the residue in position 523, which is located at the border of the substrate pocket: that in COX-1 is an isoleucine, while in COX-2 is a valine.⁷¹ The cocrystallized ligands flurbiprofen (1CQE) and diclofenac (1PXX) have been removed before starting the simulations that have been performed using the Genetic Algorithm with local gradient optimization.⁶⁶ The docking boxes (dimensions $X=22.5$; $Y=22.5$; $Z=22.5$ Å) have been centered according to the X-ray ligand positions and include all the residues forming the binding sites. As previously reported,⁴ the molecular docking analysis confirms that PXM is a nonselective drug that blocks the activity of COX-1 and -2, detecting the same binding energy (-7.6 kcal/mol) in both the enzymes, even if some slight differences in the binding mode are present. In COX-1 (Figures 1A and 2A), the Ser530 residue forms a hydrogen bond with the carboxamide group of the PXM, while a second hydrogen bond involves the Tyr355 hydroxyl group and the benzothiazine moiety of the drug. In COX-2 (Figures 1B and 2B), the same hydrogen bond occurs between Tyr355 and benzothiazine group, while the Ser530-carboxamide interaction is lost and a new hydrogen bond, involving the Arg120 side chain and the benzothiazine

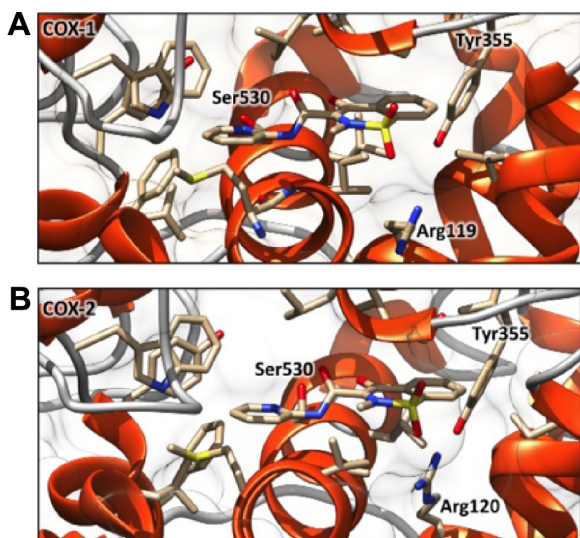


Figure 2 Best docking complexes between COX-1 (A), COX-2 (B), and PXM.

Notes: The α -helices are shown as orange spirals and the loops are represented by light gray wires. The PXM molecules, hosted in both the active sites in a very similar binding mode, are depicted by stick representations.

Abbreviations: COX-1 and -2, cyclo-oxygenases-1 and 2; PXM, piroxicam.

group, occurs. The remaining interacting residues show a similar distribution pattern, leading to a very comparable binding mode of the PXM molecule in the two binding sites. These results are in line with previous site-directed mutagenesis experiments that identified Arg120 as the key residue for selective inhibition of COX-2, while identifying Ser530 and Tyr355 as nonspecific binding determinants.⁷² Moreover, this structural analysis, determining the same binding energy, confirms that PXM is a nonselective COX-1 and COX-2 inhibitor.

Pathogenesis of AKs as therapeutic target of PXM

AK is a chronic, progressive disease, which is expected to develop into SCC at a rate of 10% over 10 years,⁷³ the latter potentially metastasizing in 5% of cases. Although it is not possible to predict which specific AKs will progress to SCC, histologic evidence indicates the majority (60%–70%) of SCCs arise from AKs lesions.⁷⁴ The entire sun-damaged skin is a result of UV-induced field cancerization with multiple visible and subclinical lesions.⁷⁵ AKs are typically seen on fair-skinned population on chronic sun-exposed areas such as the face, arms, dorsum of the hands, bald scalp, and upper back.⁷⁶ UVB radiation, considered the most important factor implicated in AK pathogenesis, cause mutations in the *telomerase* gene and tumor suppressor gene *p53*.⁵ Increased *telomerase* activity delays programmed cell death, making the mutated cells immortal. The tumor suppressor gene *p53* leads to the arrest of cell cycle, allowing the repair of damaged DNA. If mutated, the proliferation of damaged cells will give rise to potentially neoplastic cells.⁷⁷ Several other factors increase the prevalence of AK, like skin type I and II, advanced age, genetic predisposition, photodamage in outdoor workers, sun exposure in childhood, use of artificial tanning beds, PUVA (psoralens ultraviolet radiation) therapy, administration of X-rays, and immunosuppressive therapies.⁷⁶ Human papilloma viruses are also implicated in etiopathogenesis.⁷⁸ Many patients with epidermodysplasia verruciformis are immunosuppressed patients, ie, renal transplant recipients and develop HPV-associated AK and NMSCs. The viral E6 protein of HPV promotes proteolytic degradation of the proapoptotic BAK-protein, promoting skin cancerogenesis.⁷⁹ Other molecular markers that may indicate an increased likelihood of malignancy include the expression of p16^{ink4}, the CD95 ligand, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors, and loss of heterozygosis.^{80,81} Skin inflammation is due to chronic UVB irradiation, which promotes the

production of eicosanoids and COX-1 and -2 produce PGs from arachidonic acid. Inflammation is associated with the progression of AK to SCC. In fact, PGs promote tumor growth by stimulating cell proliferation, invasion, and angiogenesis.^{82,83} Increased levels of PGE₂ and PGF_{2α} in premalignant and/or malignant epithelial skin cancers are due to the constitutive upregulation of enzymes involved in PG biosynthesis, such as COX-2, and downregulation of tumor suppressor gene 15-PGDH.¹⁶ A schematic representation of PXM effect on AK after UVB radiation is shown in Figure 3: after chronic UVB exposure (290–320 nm), several genes underwent mutations, including *p53*, with alterations in the arrest of cell cycle. All the mutations and other factors determine the progression in AK. The local use of PXM blocked the action of COX, resulting in blocking the biosynthesis of PGs and in 15-PGDH increased expression. Finally, a reduction of proliferation, tumor progression, and angiogenesis occurred, whereas there was an increase in apoptosis. After topical treatment of AK with PXM, normal epidermal architecture was restored (Figure 3). In addition, COX-1 seems to contribute to tumor progression in various tissues including skin, and its inhibition lowers cell proliferation in some cancers, including ovarian and colon cancer.^{84,85} As recently reported, COX-1 and -2 are involved in VEGF production in SCC of head and neck.⁸⁶ The coinhibition of COX-1 and -2 could block VEGF synthesis. Treg-inducing VEGF reduction counteracted pathological angiogenesis.^{87,88} The efficacy of PXM is related to its activity on both COX enzymes.⁸⁹ In a preliminary open-label trial, we evaluated the efficacy and tolerability of PXM 1% gel in the treatment of patients affected by AKs. Ten patients affected by multiple AKs were treated twice daily for a period of 3 months.⁴

To measure the efficacy of the therapy, we evaluated the lesions clinically and by means ± standard error of mean of dermoscopy analysis at an initial baseline visit, at intermediate visits, and after 3 months.⁴ The modifications during treatment were evaluated using a new scoring system, named AKESA, based on the clinical presence of erythema, scale, and atrophy on a target lesion.⁴

We observed improvement either in the typical features of the AKs, as confirmed by the AKESA score, or in the perilesional photodamaged skin area in terms of skin softening and a significant reduction in dermal vessels, also documented by dermoscopic investigation.⁴ On the basis of our clinical experience, we observed a healing response in more than 50% in AKs with the use of PXM, a reduction in the appearance of new lesions in patients with multiple

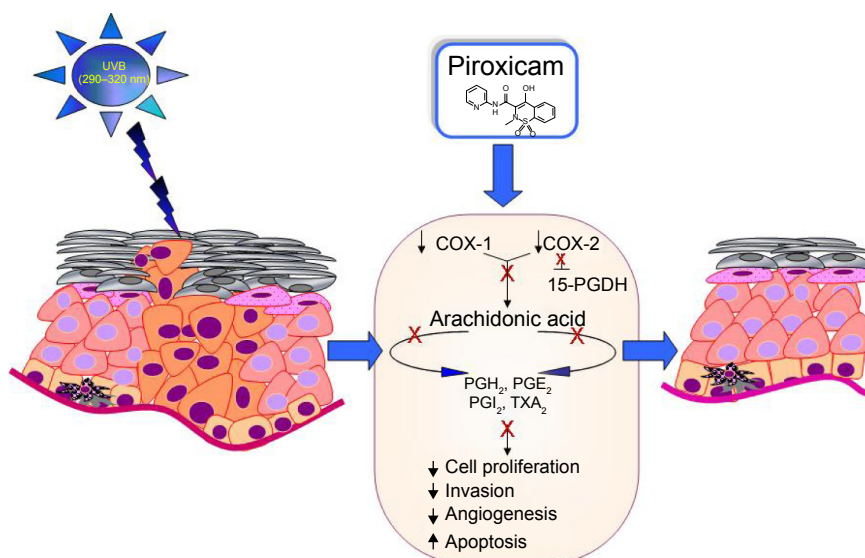


Figure 3 Schematic representation of PXM effect on AK UVB irradiation-induced mutations on keratinocyte dysplasia and AK progression.

Notes: PXM inhibits COX-1 and COX-2 activity, with the downregulation of prostaglandin (PGH_2 , PGE_2 , PGI_2 , and TXA_2) biosynthesis and the inhibition of the upregulated 15-PGDH. COX-1 and COX-2 activities are involved in skin tumor progression arrest and restoration of normal epidermal architecture (right).

Abbreviations: COX-1 and -2, cyclo-oxygenases-1 and 2; PXM, piroxicam; AK, actinic keratose; UVB, ultraviolet B; 15-PGDH, 15-hydroxy-prostaglandin dehydrogenase; PGH_2 , prostaglandin H_2 ; PGE_2 , prostaglandin E_2 ; PGI_2 , prostaglandin I_2 ; TXA_2 , thromboxan 2.

AKs. In the preliminary trial, we showed that PXM exerts a significant antitumorigenic effect, and new studies with increased number of patients validated our results.⁴ AK is considered as a precursor to SCC, and its eradication is mandatory in affected patients. The drug with a nonspecific COX-1 and COX-2 inhibitor action works on the early stages of skin carcinogenesis, and for this reason it is eligible for the treatment of AKs and the field cancerization. The lack of side effects and its efficacy allow the use of PXM for a longer time even up to 1 year.

Conclusion

PXM appears to be safe, effective, and well tolerated, although its use in AKs is still off-label. Further studies need to validate the use of PXM on NMSC, exploring its activity on different tumorigenesis pathways and on different histologic subtypes.

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Disclosure

The authors report no conflicts of interest in this work.

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