Cyclooxygenase-2 expression in primary and recurrent pterygium

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PURPOSE. *Pterygium is a proliferative, inflammatory, and invasive ocular surface disease associated with excessive ultraviolet radiation exposure and has several tumor-like characteristics. Cyclooxygenase-2 (COX-2) is an inducible enzyme and recently increased expression of the enzyme was found in many cancers and premalign lesions. This study was conducted to identify the COX-2 expression in pterygium tissues.*
METHODS. *Immunohistochemical staining using a primary antibody for COX-2 was performed on 30 specimens with primary pterygium (20 pterygium without recurrence and 10 pterygium which recurred during a 12-month follow-up), 11 specimens with recurrent pterygium, and 8 specimens of conjunctival tumor. As a control we used 10 specimens of normal conjunctiva. Extent and intensity of cytoplasmic and membranous staining in epithelial cells were evaluated.*
RESULTS. *Higher expression of COX-2 was detected in conjunctival tumor (87.5%) specimens and recurrent pterygium specimens (72.7%) compared to the both normal conjunctiva (30%) and primary pterygium without recurrence (30%). COX-2 expression in primary pterygium tissues with recurrence (60%) was not different from primary pterygium without recurrence (p=0.114) and recurrent pterygium (p=0.537). However, recurrent pterygium tissues were found to express higher COX-2 than primary pterygium without recurrence (p=0.022).*
CONCLUSIONS. *COX-2 expression is increased in recurrent pterygium tissues and COX-2 expression may be a marker for the prediction of recurrence. (Eur J Ophthalmol 2007; 17: 879-84)*

KEY WORDS. *Pterygium, Primary, Recurrent, Cyclooxygenase-2*

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INTRODUCTION

Pterygium is a common ocular surface disease characterized by overgrowth of abnormal conjunctiva onto the cornea. Pterygium was considered as a degenerative disorder of conjunctiva, a relatively benign condition. But recently, tumorlike characteristics of pterygium were reported. Pterygium can exhibit abnormal histologic features ranging from mild dysplasia to carcinoma in situ (1). In addition, pterygium has an invasion capability, and a high recurrence rate despite the many treatment options, including a wide excision of the lesion, administration of adjuvant radiotherapy, and antimitotic chemotherapy which resembles the tumor treatment (2-4). Although the most widely accepted recognized etiologic factor is chronic ultraviolet (UV) radiation exposure (5, 6), pathogenesis of pterygium is not clearly understood. Data have provided evidence implicating a genetic component, antiapoptotic mechanisms, cytokines, growth factors, extracellular matrix remodeling, immunologic mechanisms, and viral infections in the pterygium pathogenesis (7). Recently, Chiang et al (8) reported that cyclooxygenase-2 exists in primary pterygium tissues and this may play a role in pterygium formation.

Cyclooxygenase (COX) enzymes convert arachidonic acid to prostaglandins (PG). Human cells contain at least two

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isoforms of COX. COX-1 is considered to be a housekeeping gene, and constitutively expressed in most tissues. In contrast, COX-2 is undetectable in most normal tissues (9) and is induced by cytokines, growth factors, oncogenes, tumor promoters, UV radiation, and therefore contributes to the synthesis of PG in inflamed and malignant tissues (10, 11). Recent reports explain that COX-2 expression is associated with many aspects of tumorigenesis such as transformation, cell growth and apoptosis, tumor angiogenesis, invasiveness and metastasis, and modulation of immune response (9, 12). Because of the tumorlike characteristics of pterygium, this study was designed to investigate COX-2 expression in primary and recurrent pterygium tissues.

MATERIALS AND METHODS

This study was conducted by the ophthalmology and pathology departments of Mersin University Hospital. The research adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the patients before the tissues were collected. Pterygium specimens were collected from 30 patients with primary pterygium and 11 patients with recurrent pterygium. Patients with primary pterygium were followed at least 12 months. Normal conjunctival tissues were excised during extracapsular cataract surgery from 10 patients without pterygium. All specimens were fixed in formalin and embedded in paraffin by standard methods. Also, eight paraffin-embedded limbal tumor tissues (six with conjunctival intraepithelial neoplasm and two with squamous cell carcinoma) were retrieved from the pathology archive of Mersin University.

Tissues were divided into five groups. Normal conjunctival tissues composed Group 1. Group 2 included 20 primary pterygium tissues from 20 patients who did not have recurrence. Group 3 included 10 primary pterygium tissues from 10 patients who had pterygium recurrence during 12 months follow-up. Group 4 included 11 recurrent pterygium tissues of 11 different patients from group 3. Group 5 included samples of limbal tumor tissues.

All patients with pterygium had corneal extension of at least 3 mm and graded as intermediate (T2) or fleshy (T3) pterygium according to the previously described system based on the assessment of pterygium translucency (13). None of the patients in Group 1 had any inflammatory signs or symptoms.

Paraffin tissue blocks were cut at 4 μm thickness, sections were deparaffinized in xylene and alcohols, and placed for 30 minutes in 5% H_2O_2 to block endogenous peroxidase. To reveal masked antigens, slides were placed in boiling 10 mM citrate buffer (pH 6.0) and in the pressure cooker for 15 minutes. After removing the container from the cooker and cooling for 20 minutes, slides were placed in phosphate-buffered saline (pH 7.6). Sections were then treated with Ultra V Block (LabVision, Cat. No: TA-125-UB) for 5 minutes to prevent background staining and incubated for 1 hour with the primary antibodies for COX-2 (COX-2 [SP21] Ab at 1:100 dilution, Neomarkers, LabVision, Cat. No: RM-9121-R7). Slides were rinsed in phosphate-buffered saline for 10 minutes and incubated with biotinylated-linked antibody (LabVision, Cat. No: TA-125-BN) for 30 minutes and with labeling reagent peroxidase conjugated streptavidin for 30 minutes (LabVision, Cat. No: TA-125-HR). After rinsing, the peroxidase label was demonstrated using 3-amino-9-ethyl carbazole (LabVision, Cat. No: TA-015-HA) for 10 minutes and counterstained with Mayer hematoxylin. 3-Amino-9-ethyl carbazole produces a red end product. A negative control was run using the same technique but omitting the primary antibody and adding the streptavidin-biotin complex. A known colon adenocarcinoma tissue was used as positive control for COX- 2.

The stained specimens were independently evaluated by two blinded pathologists using light microscopes without information of patients. For COX-2 assessment in epithelial cells, extent and intensity of cytoplasmic and membranous staining were scored according to the previously described criteria by Putti et al (14). The percentage of stained epithelial cells was graded as follows: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The intensity of staining was graded as follows: 0 (negative), 1(weak), 2 (moderate), and 3 (strong). The final staining score (FSS) for COX-2 was obtained by multiplying the extent and intensity scores for each specimen. In this study FSS of ≥3 is defined as positive staining or high expression and <3 as negative staining or weak expression of the COX-2 (Fig. 1).

For statistical analysis we used the chi-square test and frequency of the COX-2 positive stained specimens in groups were compared. A p value of less than 0.05 is considered significant. The statistical tests were performed using SPSS for Windows version 11.5.

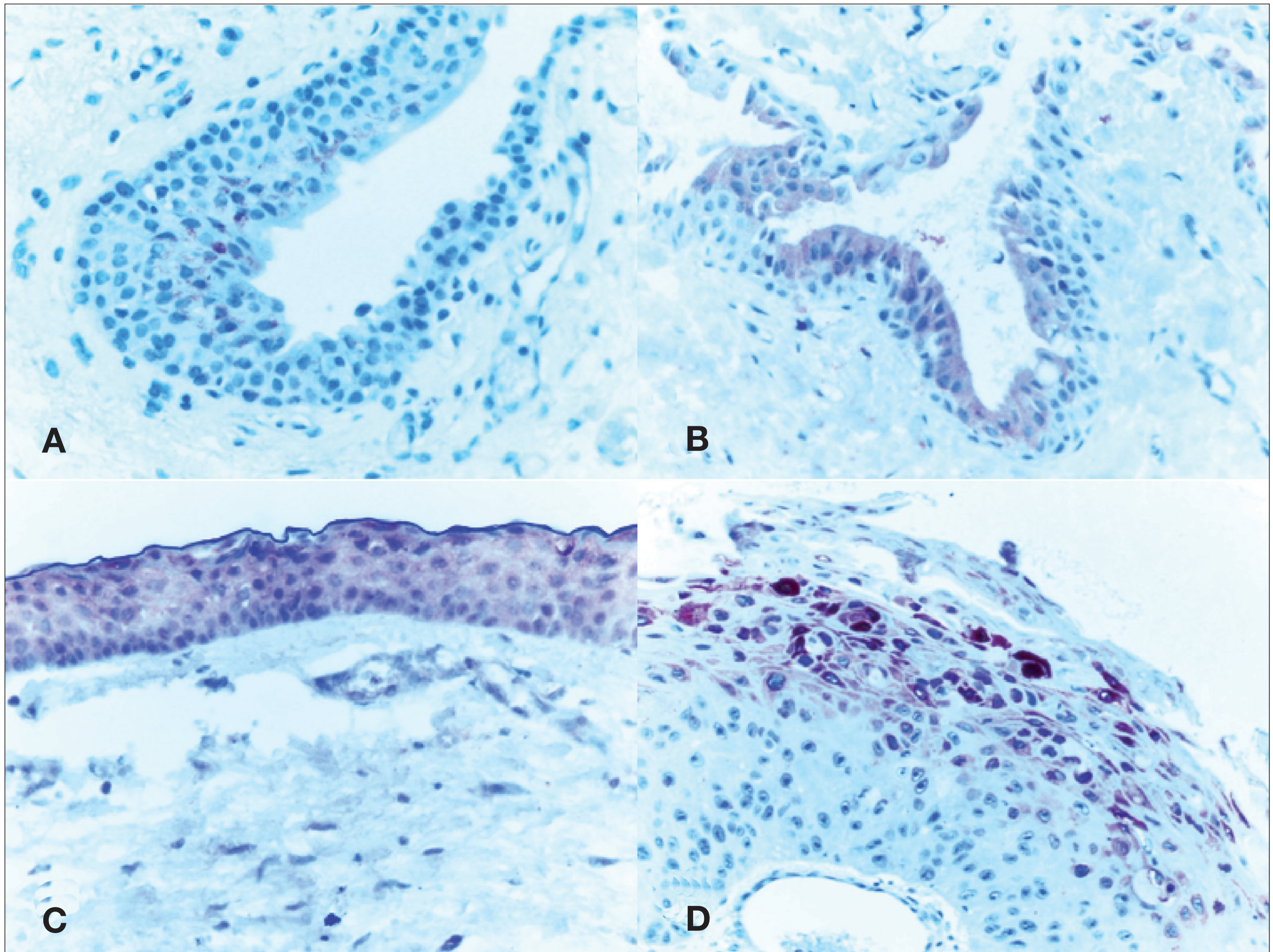

Fig. 1 - *Immunohistochemical staining with the primary COX-2 antibody: negative staining of primary pterygium without recurrence* **(A)***, positive staining of primary pterygium with recurrence* **(B)***, recurrent pterygium* **(C)***, and conjunctival tumor tissue* **(D)** *were seen (original magnification x200).*

RESULTS

In this study 6 of 10 normal conjunctiva and 4 of 20 primary pterygium tissues without recurrence had no staining, but all specimens in the other three groups were stained with COX-2 antibody. However, a total of 19 specimens in groups had negative staining (FSS < 3) (Tab. I).

The number and the percentages of the COX-2 positive stained specimens (FSS ≥ 3) in groups can be seen in Table I. COX-2 positive staining was the same and the lowest in Groups 1 and 2. Recurrent pterygium and conjunctival tumor groups were also similar (p=0.435) and express the highest COX-2. However, recurrent pterygium and conjunctival tumor groups expressed higher COX-2 than normal conjunctiva and primary pterygium tissues without recurrence (p=0.022 and p=0.006, respectively). Analyses of COX-2 positive staining in pterygium groups showed that Group 3 was an intermediate group between Groups 2 and 4 and they were not different from Group 3 (p=0.114 and p=0.537, respectively).

DISCUSSION

Both isoforms of COX (constitutive COX-1 and inducible COX-2) catalyze the production of prostanoids from

TABLE I - NUMBER (%) OF COX-2 STAINING OF SPECIMENS IN GROUPS

Groups	Final COX-2 staining			
	No staining (FSS = 0)	Negative (FSS < 3)	Positive* (FSS ≥ 3)	p*
Group 1 (n=10) (normal conjunctiva)	6 (60)	1 (10)	3 (30)	
Group 2 (n=20) (primary pterygium without recurrence)	4 (20)	10 (50)	6 (30)	1.000
Group 3 (n=10) (primary pterygium with recurrence)	0	4 (40)	6 (60)†	0.114
Group 4 (n=11) (recurrent pterygium)	0	3 (27.3)	8 (72.7)	0.022
Group 5 (n=8) (conjunctival tumor)	0	1 (12.5)	7 (87.5)	0.006

*Chi-square test result represents the difference of positive staining in groups from normal conjunctiva.
†Value was not statistically different as compared to other groups

arachidonic acid. COX-2 induced synthesis of PG is often implicated in inflammatory diseases such as rheumatoid arthritis (15), osteoarthritis (16), systemic lupus erythematosus (17), and Graves ophthalmopathy (18). Experimental studies demonstrated that ocular surface tissues can express COX-2 (19-25). COX-2 is also closely involved in the carcinogenesis process and is overexpressed in colon, gastric, esophageal, head and neck, breast, lung, prostate, and skin cancers (26, 27). COX-2 induced synthesis of PG stimulates cancer cell proliferation, promotes angiogenesis, inhibits apoptosis, increases metastatic potential, and modulates immune response. These functions of COX-2 are mediated by increased fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), anti-apoptotic bcl-2 protein, and matrix metalloproteinases (MMPs). Recently anti-tumor and chemoprotective properties of COX-2 specific inhibitors have been demonstrated by epidemiologic studies, clinical trials, and data from experimental animal models (9, 12, 28).

This study demonstrated that the conjunctival tumors (Group 5) and recurred pterygium (Group 4) tissues had the highest COX-2 positive staining. COX-2 expression in Group 4 was found higher than primary pterygium tissues involved in Group 2. However it was not different from primary pterygium tissues of patients who had pterygium recurrence during 12 months follow-up (Group 3). These results suggest that COX-2 expression may play a role in pterygium recurrence. In addition, overexpression of COX-2 in primary pterygium may be a risk for recurrence and may be a prediction marker for recurrence, because of the twofold COX-2 positive staining score of Group 3 despite the insignificant difference from Group 2 (p=0.114). However, equal COX-2 expression in normal conjunctiva and primary pterygium (Group 2) tissues showed that COX-2 could not be alone responsible for pterygium pathogenesis.

In the most recent study by Chiang et al (8), COX-2 expression was reported 83.3% in 90 primary pterygium tissues. They also reported no staining in 40 normal conjunctiva and 5 normal limbus. Although authors stated that COX-2 may play a role in pterygium formation, they accepted the staining of at least 1% cells as positive staining. In the present study, scoring system of staining includes extent and intensity and pterygium tissues were divided into three groups according to recurrence. Therefore our study is different from the report of Chiang et al and COX-2 expression in primary pterygium tissues seems underestimated.

Pterygium pathogenesis is complex and not completely understood. Presence of anti-apoptotic protein bcl-2, proinflammatory cytokines, growth factors, growth factor receptors, and MMPs in pterygium tissues were demonstrated (7). The widely accepted etiologic factor is chronic UV exposure (5, 6). Chiang et al mentioned the possible UV-ROS (radical oxygen species)–COX-2 and PGE2 pathway in the pterygium formation (8). Moreover, COX-2 is the major enzyme that is responsible for the UVB induced PG synthesis (11). In skin cancer, UVB irradiation increases both mRNA and protein levels of COX-2 in human keratinocytes (29). Besides the increased COX-2 expression by chronic UVB exposure in UVB induced premalign le-

sions and squamous cell carcinomas, recent studies have shown increased COX-2 expression in human skin in response to acute UVB exposure (26, 30, 31). Dissimilar to Chiang et al, this special feature can also explain the positive staining of normal conjunctiva in our study.

Furthermore, specific inhibitors of COX-2 such as celecoxib have been shown to decrease carcinogenesis and to decrease tumor growth with pre-existing UVB induced tumors (32-37). According to the results of this study, selective COX-2 inhibitors may decrease or limit the pterygium recurrence and also the pterygium growth.

We conclude that COX-2 expression is increased in recurrent pterygium tissues. COX-2 expression may play a role in pterygium recurrence and may be a marker for the prediction of recurrence. Further studies with larger samples are needed for utilizing COX-2 as a recurrence prediction marker and prevention of recurrence with selective COX-2 inhibitors.

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REFERENCES

1. Clear AS, Chirambo MC, Hutt MSR. Solar keratosis, pterygium, and squamous cell carcinoma of the conjunctiva in Malawi. Br J Ophthalmol 1979; 63: 102-9.
2. Frucht-Pery J, Siganos CS, Islar M. Intraoperative application of topical mitomycin C for pterygium surgery. Ophthalmology 1996; 103: 674-7.
3. Lam DS, Wong AK, Fan DS, Chew S, Kwok PS, Tso MO. Intraoperative mitomycin C to prevent recurrence of pterygium after excision: a 30-month follow-up study. Ophthalmology 1998; 105: 901-4.
4. Sanchez-Thorin JC, Rocha G, Yelin JB. Meta-analysis on the recurrence rates after bare sclera resection with and without mitomycin C use and conjunctival autograft placement in surgery for primary pterygium. Br J Ophthalmol 1998; 82: 661-5.
5. Moran DJ, Hollows FC. Pterygium and ultraviolet radiation: a positive correlation. Br J Ophthalmol 1984; 68: 343-6.
6. Taylor HR. Ultraviolet radiation and the eye: an epidemiologic study. Trans Am Ophthalmol Soc 1989; 87: 802-53.
7. Girolamo ND, Chui J, Coroneo MT, Wakefield D. Pathogenesis of pterygia: role of cytokines, growth factors, and matrix metalloproteinases. Progr Retin Eye Res 2004; 23: 195-228.
8. Chiang CC, Cheng YW, Lin CL, et al. Cyclooxygenase 2 expression in pterygium. Mol Vis 2007; 13: 635-8.
9. Dempke W, Rie C, Grothey A, Schmoll HJ. Cyclooxygenase-2: a novel target for cancer chemotherapy? J Cancer Res Clin Oncol 2000; 127: 411-7.
10. Fosslien E. Molecular pathology of cyclooxygenase-2 in neoplasia. Ann Clin Lab Sci 2000; 30: 3-21.
11. Zoumpourlis V, Solakidi S, Papathoma A, Papaevangeliou D. Alterations in signal transduction pathways implicated in tumour progression during multistage mouse skin carcinogenesis. Carcinogenesis 2003; 24: 1159-65.
12. Trifan OC, Hla T. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. J Cell Mol Med 2003; 7: 207-22.
13. Tan DT, Chee SP, Dear KB, Lim AS. Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. Arch Ophthalmol 1997; 115: 1235-40.
14. Putti TC, Teh M, Lee YS. Biological behavior of keratoacanthoma and squamous cell carcinoma: telomerase activity and COX-2 as potential markers. Mod Pathol 2004; 17: 468-75.
15. Kang RY, Freire-Moar J, Sigal E, Chu CQ. Expression of cyclooxygenase-2 in human and an animal model of rheumatoid arthritis. Br J Rheumatol 1996; 35: 711-8.
16. Siegle I, Klein T, Backman JT, Saal JG, Nusing RM, Fritz P. Expression of cyclooxygenase 1 and cyclooxygenase 2 in human synovial tissue: differential elevation of cyclooxygenase 2 in inflammatory joint diseases. Arthritis Rheum 1998; 41: 122-9.
17. Tomasoni S, Noris M, Zappella S, et al. Upregulation of renal and systemic cyclooxygenase-2 in patients with ac-

tive lupus nephritis. J Am Soc Nephrol 1998; 9: 1202-12.
18. Konuk EB, Konuk O, Misirlioglu M, Menevse A, Unal M. Expression of cyclooxygenase-2 in orbital fibroadipose connective tissues of Graves' ophthalmopathy patients. Eur J Endocrinol 2006; 155: 681-5.
19. Oka T, Shearer T, Azuma M. Involvement of cyclooxygenase-2 in rat models of conjunctivitis. Curr Eye Res 2004; 29: 27-34.
20. Biswas PS, Banerjee K, Kim B, Kinchington PR, Rouse BT. Role of inflammatory cytokine induced cyclooxygenase 2 in the ocular immunopathologic disease herpetic stromal keratitis. J Virol 2005; 16: 10589-600.
21. Sellers RS, Silverman L, Khan KN. Cyclooxygenase-2 expression in the cornea of dogs with keratitis. Vet Pathol 2004; 41: 116-21.
22. Miyamoto T, Saika S, Okada Y, et al. Expression of cyclooxygenase-2 in corneal cells after photorefractive keratectomy and laser in situ keratomileusis in rabbits. J Cataract Refract Surg 2004; 30: 2612-7.
23. Amico C, Yakimov M, Catania MV, Giuffrida R, Pistone M, Enea V. Differential expression of cyclooxygenase-1 and cyclooxygenase-2 in the cornea during wound healing. Tissue Cell 2004; 36: 1-12.
24. Miyamoto T, Saika S, Ueyama T, et al. Cyclooxygenase 2 expression in rat corneas after ethanol exposure. J Cataract Refract Surg 2006; 32: 1736-40.
25. Yamada M, Kawai M, Kawai Y, Mashima Y. The effect of selective cyclooxygenase-2 inhibitor on corneal angiogenesis in the rat. Curr Eye Res 1999; 19: 300-4.
26. Kuwano T, Nakao S, Yamamoto H, et al. Cyclooxygenase 2 is a key enzyme for inflammatory cytokine-induced angiogenesis. Faseb J 2004; 18: 300-10.
27. Buckman SY, Gresham A, Hale P, et al. COX-2 expression is induced by UV-B exposure in human skin: implications for the development of skin cancer. Carcinogenesis 1998; 19: 723-9.
28. Iniguez MA, Rodriguez A, Volpert OV, Fresno M, Redondo JM. Cyclooxygenase-2: a therapeutic target in angiogenesis. Trends Molec Med 2003; 9: 73-8.
29. Chen W, Tang Q, Gonzales MS, Bowden GT. Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. Oncogene 2001; 20: 3921-6.
30. An KP, Athar M, Tang X, et al. Cyclooxygenase-2 expression in murine and human nonmelanoma skin cancers: implications for therapeutic approaches. Photochem Photobiol 2002; 76: 73-80.
31. Athar M, An KP, Morel KD, et al. Ultraviolet B (UVB)-induced cox-2 expression in murine skin: an immunohistochemical study. Biochem Biophys Res Commun 2001; 280: 1042-7.
32. Fischer SM, Lo HH, Gordon GB, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. Mol Carcinog 1999; 25: 231-40.
33. Fischer SM, Conti CJ, Viner J, Aldaz CM, Lubet RA. Celecoxib and difluoromethylornithine in combination have strong therapeutic activity against UV-induced skin tumors in mice. Carcinogenesis 2003; 24: 945-52.
34. Pentland AP, Schoggins JW, Scott GA, Khan KN, Han R. Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. Carcinogenesis 1999; 20: 1939-44.
35. Orengo IF, Gerguis J, Phillips R, Guevara A, Lewis AT, Black HS. Celecoxib, a cyclooxygenase 2 inhibitor as a potential chemopreventive to UV-induced skin cancer: a study in the hairless mouse model. Arch Dermatol 2002; 138: 751-5.
36. Wilgus TA, Koki AT, Zweifel BS, Kusewitt DF, Rubal PA, Oberyszyn TM. Inhibition of cutaneous ultraviolet light B-mediated inflammation and tumor formation with topical celecoxib treatment. Mol Carcinog 2003; 38: 49-58.
37. Wilgus TA, Breza TS, Tober KL, Oberyszyn TM. Treatment with 5-fluorouracil and celecoxib displays synergistic regression of ultraviolet light B-induced skin tumors. J Invest Dermatol 2004; 122: 1488-94.