Differential expression of cyclooxygenases in hypertrophic scar and keloid tissues

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ABSTRACT

Hypertrophic scar (HS) and keloid (KL) are two forms of an abnormal cutaneous scarring process, mainly characterized by excessive extracellular matrix deposition and fibroblast proliferation. Despite the increased understanding of the molecular and cellular events leading to HS and KL, the pathogenesis of these lesions remains poorly understood. A pivotal role in the formation of abnormal scars has been ascribed to transforming growth factor-β, whose activity appears to be mediated through a link with pathways acting via cyclooxygenases (COX-1 and COX-2). To date, there is no report on the in vivo expression of COX-1 and COX-2 in human HS and KL tissues. Therefore, using immunohistochemistry and Western blot analysis, we investigated 36 cases of KL, 32 cases of HS, and 25 cases of normal skin in order to define the localization and distribution of COX-1 and COX-2 in the tissues of these scar lesions and the overlying epidermis. The results mainly show the following: (a) a significant overexpression of COX-1 in HS tissues and the overlying epidermis as compared with normal skin and KL tissues and (b) a significant overexpression of COX-2 in KL tissue and the overlying epidermis in contrast to normal skin and HS tissues. Our data support the hypothesis that both COXs are involved in the pathogenesis of scar lesions in different ways and, particularly, COX-1 in the formation of HS and COX-2 in the formation of KL. In addition, the overexpression of COX-1 and COX-2 in the epidermis overlying HS and KL tissues, respectively, underlines the importance of epithelial–mesenchymal interactions in the pathogenesis of scar lesions.

Hypertrophic scar (HS) and keloid (KL) represent two forms of an abnormal wound-healing process characterized by local fibroblast proliferation and excessive collagen production in response to a cutaneous injury. However, HS and KL show significant differences in their clinical features, histology, and pathogenesis. Clinically, by definition, KL exceeds the wound margin, often with claw-like extensions resembling "the pincers of a crab" involving the normal adjacent skin, whereas an HS remains confined to the site of the original injury.[1–3] The lesion appears as a firm, mildly tender, nodule or plaque more irregular in KL than HS, and is often pruritic and painful. Usually, HS shows signs of regression after a few months and may resolve spontaneously.

In contrast, KL tends to grow to a certain size. Subsequently, KL stops growing and may display some regression, usually partial and slow, but in rare case it may relentlessly progress.

To date, epidemiologic data on HS and KL are insufficient but suggest a greater susceptibility to KL formation in darker-pigmented races, Hispanics and Asians, as distinct from Caucasians and albinos, who are much less affected.[1,2] KLs may occur at any age but most reported cases have occurred in individuals between 10 and 30 years of age.[1]

From a histological viewpoint, unequivocal criteria for a definite distinction between KL and HS have not yet been established. Histologically, KL shows thickened, hyalinized, brightly eosinophilic-staining collagen bundles, classically described as "keloidal collagen,"[1,3] with an irregular orientation. This contrasts with an HS, where collagen bundles are oriented parallel to the epidermis. In addition, KL displays abundant mucin between the collagen bundles and tends to be less cellular than HS.

Nevertheless, the presence of "keloidal collagen" is rarely reported in HS and may be lacking in KL.[1,2] To clarify this differential diagnosis, Lee et al.[3] proposed other histological features characteristic for KL such as the absence of prominent vertically oriented blood vessels, the presence of a tongue-like advancing edge underneath a normal-appearing epidermis and papillary dermis, horizontal fibrous bands in the upper reticular dermis, and a prominent fascia-like band.

α-smooth muscle actin expression failed as a differentiating marker because it is present in both KL and HS.[3]

In an attempt to clarify the pathogenesis of HS and KL, several hypotheses and numerous etiological factors have been proposed, but to date the mechanism of formation of abnormal wound healing appears to be rather complex and multifactorial, and its complete understanding has not been achieved as yet.

Some etiological factors proposed are as follows: trauma or inflammation of the skin, infection, aberrant metabolism of the melanocyte-stimulating hormone, physiologic hyperactivity of the pituitary gland, genetic and familial disorders, nutritional deficiencies in Fas, and p53 gene mutation.[1–4]

The spontaneous onset of KLs reported occasionally by patients probably represents a trauma that was forgotten or unnoticed by the patient.[2]

The existence of at least some form of genetic susceptibility has been suggested by the description of families with multiple affected members from KLs[5] but to date no genes favoring the formation of KLs have been identified.

In addition, several studies have recently indicated that several factors such as interleukin-1β (IL-1β),[6] tumor necrosis factor-α (TNF-α), numerous cytokines,[7] and growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and transforming growth factor-β (TGF-β) may be involved in the pathogenesis of KL.[8,9]

In particular, TGF-β stimulates matrix proteins such as collagen, inhibits protease production, and enhances mitogenesis.[10] Recent in vitro studies have hypothesized the possible pivotal role of TGF-β_1 overexpression in KL fibroblasts in the pathogenesis of KL.[2–10]

Results from cell culture experiments support the hypothesis that TGF-β_1 has a function as an upstream regulator of the cyclooxygenase (COX) pathway, and also that the growth promotion by TGF-β is mediated through a link with the COX pathway.[2,11]

COXs catalyze the conversion of arachidonic acid, which is derived from membrane phospholipids by phospholipase, into prostaglandins.[12] There are two main COX isoforms, COX-1 and COX-2, which share the same substrates, generate the same products, and catalyze the same reactions using identical mechanisms. Nevertheless, COX-2 is significantly more efficient than COX-1 with respect to the enzymatic activity.[12]

COXs appear to be involved in tumorigenesis of numerous neoplasia such as carcinoma of the breast, prostate, skin, colorectum, lung, bladder, stomach, and Kaposi's sarcoma by several pathogenetic mechanisms, including neoangiogenesis, fibrosis, proliferative activity, regulation of expression of integrins, matrix metalloproteinases (MMPs), p53, and bcl-2.[12–15]

In a previous study,[15] we reported a significant overexpression of COX-1 and COX-2 not only in spindle cells of classic and epidemic Kaposi's sarcoma tissues but also in keratinocytes of epidermis overlying KS compared with control skin tissues.

In fact, a growing number of significant studies in the literature[2,8,9,16] indicate interactions between the epidermis and dermal tissue, which play an important role in the regulation of homeostasis, growth, and differentiation of epithelial and mesenchymal tissue. Besides COXs, keratinocytes synthesize and release a broad variety of factors, cytokines, and chemokines that modulate the activity of other epithelial cells as well as of dermal fibroblasts, endothelial cells (ECs), and macrophages in a paracrine and autocrine manner, as reported by various studies.[16–18]

These data strongly suggest that the overlying epidermis is not just a protective barrier, but that epidermal cells interact closely with the underlying dermis.

In addition, COXs also seem to be implicated in the pathogenesis of abnormal wound healing as also shown by the reduction of formation of KL and scar in patients using a nonsteroidal anti-inflammatory drug and a COX-2 inhibitor.[2,19]

Therefore, the aim of this study was to assess in vivo the distribution and localization of COX-1 and COX-2 by the immunohistochemical method in KL and HS tissues and also in the epidermis overlying these lesions.

MATERIALS AND METHODS

Our research complies with the ethical rules for human experimentation that are stated in the 1975 Declaration of Helsinki, and was approved by the appropriate Institutional Review Boards.

Sixty-eight untreated scar lesions were used for this investigation: 36 cases of KL and 32 cases of HSs. Patients ranged in age from 5 to 58 years (mean age of 42 years).

KLs had been present for at least 6 months and were located in the ear lobes and face (16 case), shoulders (8), arms (5), chest wall (3), neck (2), and abdominal wall (2).

HSs were located in the head and neck region (12 cases), chest wall (8), shoulders (7), knees (3), and abdominal wall (2). Twenty-five cases of normal skin were also used as control tissue.

All biopsy tissues were formalin fixed and embedded in paraffin using a routine histological procedure; 5-μm-thick sections were cut from each specimen. Afterwards, slides were deparaffinized and then rehydrated in graded alcohols according to the standard protocol.

Immunostaining was performed as reported previously in detail.[15] The following primary antibodies were used for immunohistochemistry: monoclonal mouse antibodies for CD34, CD45 (LCA), CD68 (Immunomarkers-Diapath, Martinengo, Italy), monoclonal mouse antibodies for COX-1 and COX-2 (Novocastra, Newcastle, UK), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). The sections were immunostained with a streptavidin–biotin system using an automated slide stainer (Benchmark, Ventana Medical System, Tucson, AZ). The omission, replacement, and inversion of the primary antibody were carried out as negative controls.

The double-labeling technique was performed using the Ultra-View Universal Alkaline Phosphatase Detection Kit (Ventana-Diapath, Tucson, AZ, USA) using Fast red and DAB as chromogens according to the procedure indicated by Ventana. For each section, the staining pattern of COX-1 and COX-2 was scored for the percentage of positive cells (A= < 10% of positive cells; B=from 11 to 30%; C= > 31% of cells).

A semi-quantitative scale (+=weak intensity; ++=moderate intensity; +++=intensive staining) was used only for the assessment of the intensity of staining pattern by COX-1 in the epidermis.

A comparison between different percentages of positive cells was not possible because COX-1 is always expressed

in almost all epithelial cells of both control normal skin and the epidermis overlying scar lesions.

Three observers (R.R., R.L., M.E.P.) separately estimated the staining pattern and percentages of positive cells of each section by counting the number of COXs-positive cells per viewing field at a magnification of ×400.

The level of concordance, expressed as the percentage of agreement between observers, was 91% (133 of 146 specimens). All specimens with a discordant score were reevaluated by investigators using a multiheaded microscope and a consensus was reached after collegial revision.

The level of concordance was 100% as regards the diagnosis of KL and HS.

The protein extraction was performed by modifying the previously described method.[20,21] In brief, four tissue sections obtained from nine cases of HS and eight cases of KL were deparaffinized with xylene three times for 10 minutes and placed in 100% ethanol for washing three times for 10 minutes. After air drying, protein from deparaffinized tissue was solubilized in 1% sodium dodecyl sulfate (SDS) and 100 mM $NaHCO_3$, incubated at 65 °C overnight, and sonicated for 2 minutes. Proteins were precipitated with 10% TCA before solubilization in 60 μL of a cross-linking reversal solution (tris-HCL 0.250 M pH 8.8; glycerol 10%, w/v; SDS 2%, w/v; 2-mercaptoethanol 0.5 M), from formaldehyde cross-link reverse, and incubated for 25 minutes at 99 °C.[21] Ten microliters of samples were run in SDS 10% polyacrylamide gels (bis/mono 1 : 37.5) on a Mini-Protean II apparatus (BioRad, Hercules, CA) according to the Leammli electrophoresis method.[22] Western blot analysis and peroxidase activity were performed according to the manufacturer's instructions (Amersham Bioscience, Buckinghamshire, UK) using an ECL-AdvancedTM chemiluminescence kit (GEHealthcare, Buckinghamshire, UK) with monoclonal antibodies anti-COX-1 (1/2000), and anti-COX-2 (1/600). Protein loading was controlled by anti-β-actin. Ten microliters of same samples were used to determine the amounts of protein (100–10 kDa) by Coomassie blue G-250 staining electrophoresis gel, compared with a total of 2 μg of a broad-range protein standard (BioRad) as described by Becker et al.[23] The amount of protein of each sample ranged between 2 and 5 μg for the electrophoresis lane. Densitometric analysis of tif scan images, both Western blot and stained slides, obtained using Arcus II Agfa, was performed using TotalLab 1D (Non-Linear Dynamics, Newcastle, UK).

Descriptive statistics were used to summarize the morphologic evaluation of immunohistochemical data. Comparisons between the groups were made by the Fisher exact test and Pearson chi-square when appropriate; p value < 0.05 was considered statistically significant.

RESULTS

As described by us previously,[15] COX-1 positivities in normal skin appeared to be uniformly distributed in all epithelial layers of the epidermis, showing faint or moderate staining; in contrast, COX-2 was absent or focally expressed in epithelial cells mostly of the upper layers (Table 1).

In the dermis, a variable percentage of ECs and fibroblasts showed COX-1 staining, whereas COX-2 positivities were sometimes detected in macrophages, lymphocytes, and fibroblasts (Table 1).

Table 1. COX-1 and COX-2 in normal skin

COX-1						COX-2			
Epidermis			Dermal cells*			Epidermis		Dermal cells*	
Value	*N*	%	Value	*N*	%	*N*	%	*N*	%
+†	20	80	0‡	9	36	17	68	11	44
++	5	20	A	16	64	8	32	13	52
+++	0	0	B	0	0	0	0	1	4
–	–	–	C	0	0	0	0	0	0

*Fibroblasts, endothelial cells, macrophages, lymphocytes.
†Semi-quantitative scale from +(weak staining), ++ (moderate staining), +++ (intensive staining).
‡Score for percentage of positive cells from 0 (no positive cells) to A (1–10% of positive cells), B (11–30% of positive cells), C (> 31% of positive cells).
COX, cyclooxygenase.

With regard to the cases of HS, immunostaining for COX-1 revealed intensive and diffuse positivities in epithelial cells of the epidermis overlying a scar lesion as well as in fibroblasts and ECs, whereas COX-2 expression appeared to be less intensive and diffuse as reported in Table 2.

The comparison between normal skin and tissues involved in HS revealed that more intensive and diffuse staining for COX-1 was detected in a scar lesion as compared with the normal epidermis and dermal tissue (differences statistically significant for both: $p < 0.001$).

With regard to COX-2, the immunohistochemical results showed less intensive and diffuse positivities in HS tissues as compared with the staining pattern displayed by COX-1 (Table 2).

The comparison between normal skin and HS indicated a minimal expression of COX-2 in both the epidermis and

Table 2. COX-1 and COX-2 expression in hypertrophic scar (HS) tissues and overlying epidermis

COX-1						COX-2			
Epidermis			Dermal cells*			Epidermis		Dermal cells*	
Value	*N*	%	Value	*N*	%	*N*	%	*N*	%
+†	0	0	0‡	0	0.0	21	65.6	15	46.9
++	17	53.1	A	3	9.4	10	31.3	14	43.7
+++	15	46.9	B	12	37.5	1	3.1	3	9.4
–	–	–	C	17	53.1	0	0.0	0	0.0

*Fibroblasts, endothelial cells, macrophages, lymphocytes.
†Semi-quantitative scale from + (weak staining), ++ (moderate staining), +++ (intensive staining).
‡Score for percentage of positive cells from 0 (no positive cells) to A (1–10% of positive cells), B (11–30% of positive cells), C (> 31% of positive cells).
COX, cyclooxygenase.

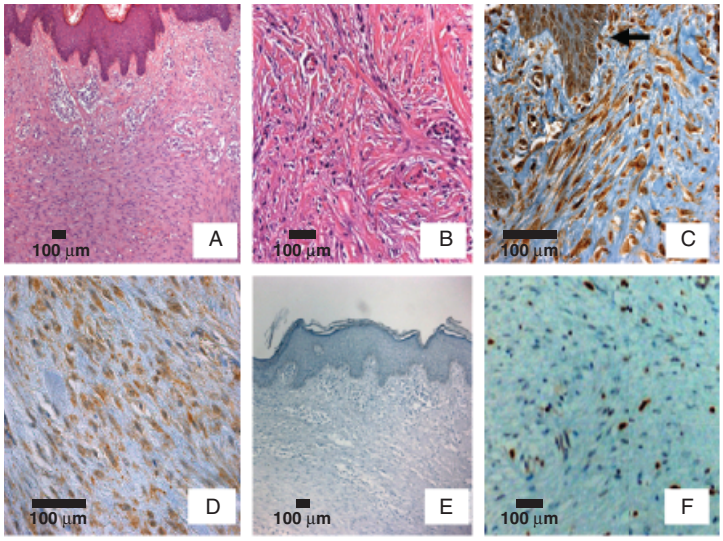

Figure 1. Hypertrophic scar (HS). (A) Morphological picture of HS: nodular fibroblastic proliferation within the dermis (hematoxylin–eosin, ×100). (B) Higher magnification of the previous picture: the lesion is composed of fibroblasts with a variable collagenous stroma (hematoxylin–eosin, ×200). (C) Intensive staining for cyclooxygenase-1 (COX-1) in both the epidermis (arrow) and dermal spindle cells (×400). (D) Intensive staining for COX-1 in dermal spindle cells (×400). (E) Lack of COX-2 staining in both the epidermis and dermal tissue (×100). (F) Few and faint COX-2 positivities in dermal spindle cells and lymphocytes (×200).

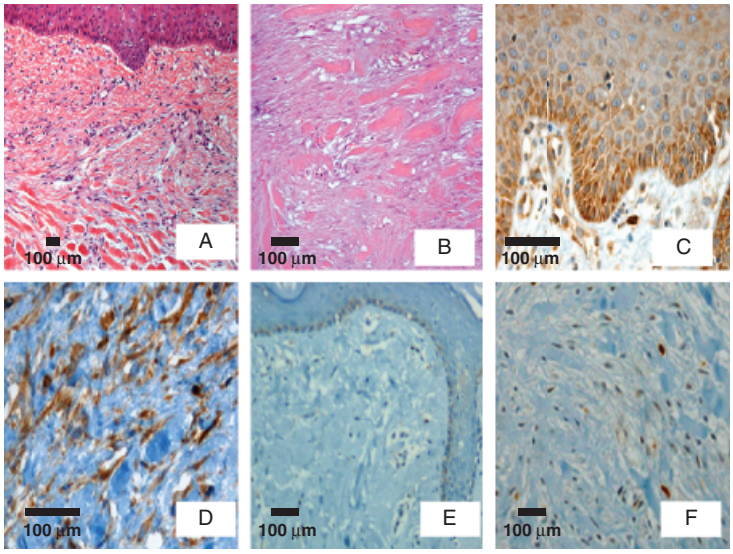

Figure 2. Keloid (KL). (A) Morphological appearance of KL also showing broad bundles (keloidal collagen) of eosinophilic hyalinized collagen (hematoxylin–eosin, ×100). (B) Keloidal collagen at a higher magnification (hematoxylin–eosin, ×200). (C) Intensive cyclooxygenase-2 (COX-2) staining in the epidermis (×400). (D) Intensive COX-2 staining identifiable in dermal fibroblasts (×400). (E) Lack of COX-1 staining in both the epidermis and dermal tissue (×200). (F) Few and faint positivities for COX-1 in dermal cells (×200).

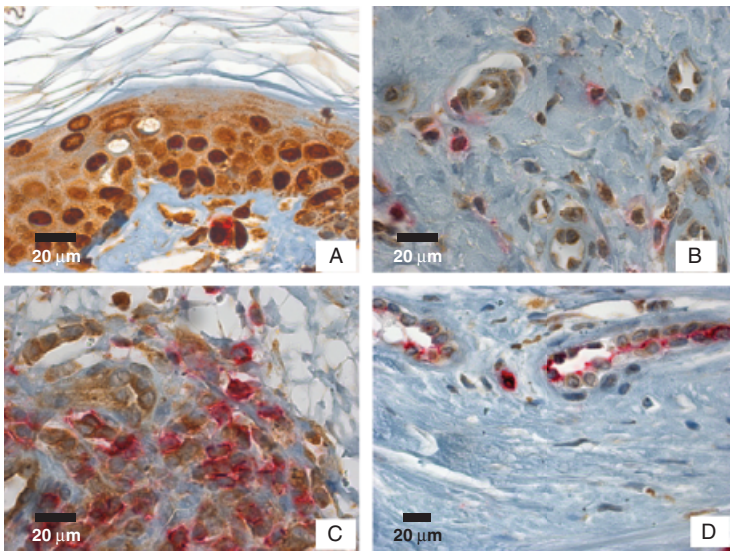

Figure 3. Double labeling technique (chromogens: AEC-red, DAB-brown) in keloid (KL) lesions. (A) Coexpression of CD68 (red) and cyclooxygenase-2 (COX-2; brown) occurs in two dermal macrophages, whereas the epidermis only shows an intense expression of COX-2 (×1000). (B) Coexpression of CD68 (red) and COX-2 (brown) occurs in some macrophages, whereas endothelial cells (ECs) show staining only for COX-2 (×1000). (C) Coexpression of LCA (red) and COX-2 (brown) in lymphocytes (×1000). (I) Coexpression of CD34 (red) and COX-2 (brown) in ECs of a dermal vessel (×630).

Table 3. COX-1 and COX-2 expression in keloid (KL) tissues and overlying epidermis

COX-1						COX-2			
Epidermis			Dermal cells*			Epidermis		Dermal cells*	
Value	*N*	%	Value	*N*	%	*N*	%	*N*	%
+†	24	66.7	0‡	8	22.2	0	0.0	0	0.0
++	11	30.5	A	23	63.9	2	5.6	3	8.3
+++	1	2.8	B	5	13.9	22	61.1	16	44.5
–	–	–	C	0	0.0	12	33.3	17	47.2

*Fibroblasts, endothelial cells, macrophages, lymphocytes.
†Semi-quantitative scale from + (weak staining), ++ (moderate staining), +++ (intensive staining).
‡Score for percentage of positive cells from 0 (no positive cells) to A (1–10% of positive cells), B (11–30% of positive cells), C (> 31% of positive cells).
COX, cyclooxygenase.

the dermal cells (Figure 1). The differences were not statistically significant (normal epidermis/epidermis overlying HS tissues: $p=0.12$; dermal cells of normal skin/HS tissues: $p=0.066$).

The staining patterns of COX-1 and COX-2 in KLs and the overlying epidermis are summarized in Table 3.

The comparison between normal skin and KL tissues did not show remarkable variations in the expression of COX-1 in the epidermis ($p=0.087$), whereas a weak significant difference ($p=0.01042$) was found between dermal cells of normal skin and KL tissues.

In contrast, COX-2 expression (Figure 2) was more significantly increased in the epidermis and dermal cells (fibroblasts and ECs) of KL tissues in comparison with normal epidermis and dermis (normal epidermis/epidermis overlying KL tissues: $p < 0.0001$; demal cells of normal skin/KL tissues: $p < 0.0001$).

Particularly in KL tissues, ECs and fibroblasts often displayed moderate/strong positivities for COX-2 (Figure 2), as well as macrophages and lymphocytes, often distributed around dermal vessels, whereas focal or weak staining was observed for both COXs in the dermal inflammatory cells of HS tissues.

Indeed, the double-staining technique revealed in KL tissues coexpression of COX-2 and CD34, CD68, and CD45 in ECs, macrophages, and lymphocytes, respectively (Figure 3).

The expression of COX-1 and COX-2 in scar lesions as well as in the overlying epidermis showed remarkable and significant differences between HS and KL, as reported in Figures 4 and 5.

COX-1 was significantly overexpressed in HS and the overlying epidermis in comparison with KL tissues. In contrast, COX-2 was significantly overexpressed in KL tissue and the overlying epidermis as compared with HS tissues.

The expression of COX-1 and COX-2 molecules in HS and KL lesions was analyzed by a Western blot assay (Figure 6B). An expected molecular weight band of 69 kDa, detected by the anti-COX-2 antibody in the entire KL

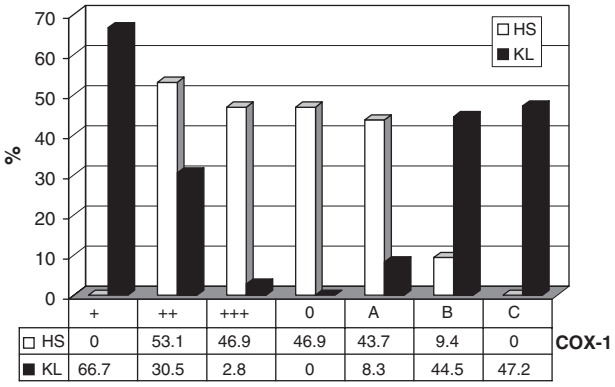

Figure 4. Cyclooxygenase-1 (COX-1) expression in hypertrophic scar (HS) and keloid (KL) tissues by immunohistochemistry. The staining pattern of COX-1 and COX-2 was scored for percentage of positive cells (A= < 10% of positive cells; B=from 11 to 30%; C= > 31% of cells). A semi-quantitative scale (+=weak intensity; ++=moderate intensity; +++=intensive staining) was used only for the assessment of the intensity of the staining pattern by COX-1 in the epidermis.

sample group and in four out of nine in the HS sample group, is shown (Figure 4). Instead, the anti-COX-1 antibody weakly shows a molecule of 68 kDa present in both groups of samples. The different expression of two molecules was highlighted by densitometric analysis. In fact, the analysis of the means of single sample volume density showed a COX-1 prevalence in the first group (HS) and COX-2 in the KL group (Figure 6A left). The measure of a specific β-actin band (43 kDa) present on the same samples did not show differences between the HS and the KL group. Densitometric Western blot data were normalized

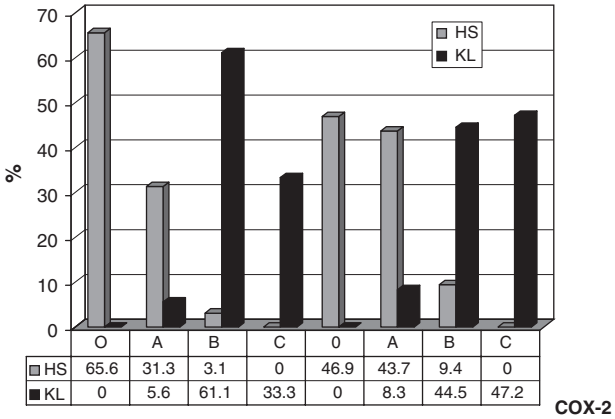

Figure 5. Cyclooxygenase-2 (COX-2) expression in hypertrophic scar (HS) and keloid (KL) tissues by immunohistochemistry. The staining pattern of COX-1 and COX-2 was scored for percentage of positive cells (A= < 10% of positive cells; B = from 11 to 30%; C= > 31% of cells). A semi-quantitative scale (+=weak intensity; ++=moderate intensity; +++=intensive staining) was used only for the assessment of the intensity of the staining pattern by COX-1 in the epidermis.

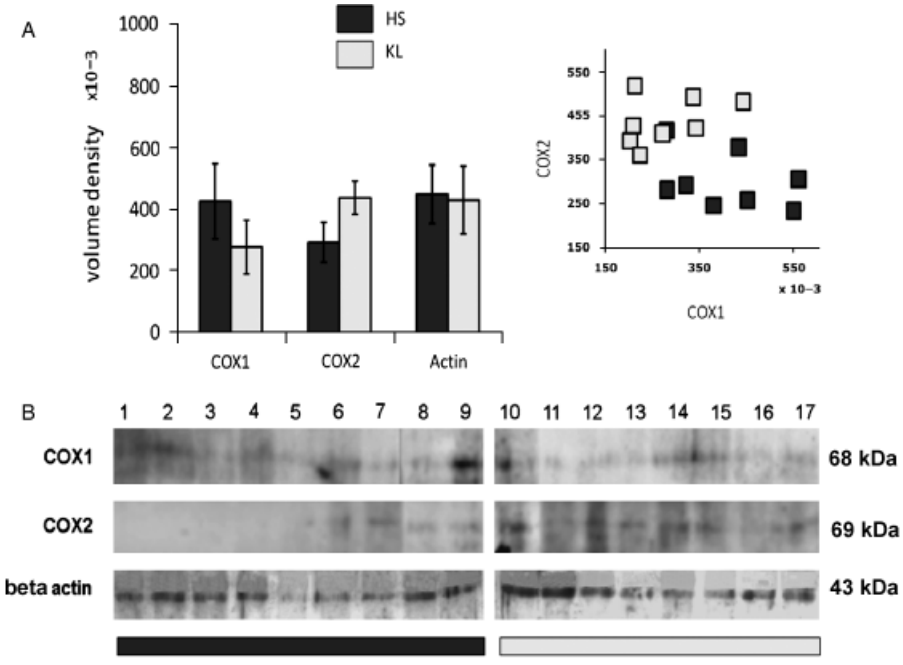

Figure 6. Western blot analysis of hypertrophic scar (HS) (dark gray) and keloid (KL) (light gray) cell extracts. A left densitometric analysis of Western blot samples with anti-cyclooxygenase-1 (COX-1), anti-COX-2, and anti-β-actin antibodies. The bars indicate the mean of sample groups HS and KL with relative SD normalized for loaded proteins. (A) Right dispersion graph of single sample density for anti-COX-1 in the *x*-axis and anti-COX-2 in the *y*-axis. (B) Western blot analysis of HS and KS cell extracts using polyclonal anti-COX-1, polyclonal anti-COX-2, and anti-β-actin antibodies. Lanes from 1 to 9 samples are of the HS group while lanes from 10 to 17 are those of the KL group.

for a loaded amount of proteins. The COX-1 and COX-2 values of each sample, represented by the dispersion graph in Figure 6A, showed a different scattering of groups in two specific *areas*. Only one HS sample was present in the KL group area.

DISCUSSION

The results lead to the following main conclusions:

1. COX-1 is significantly overexpressed in HS tissues and in the overlying epidermis in comparison with normal skin.
2. COX-2 expression in HS tissues and in the epidermis overlying a scar lesion displays no significant differences as compared with normal skin.
3. KL tissues are characterized by a remarkable overexpression of COX-2 in a dermis scar lesion and in the overlying epidermis in comparison with HS tissues as well as with normal skin.

Despite the expected difficulties of the procedure used for protein extraction (from fixed tissue sections) and the limitations of the Western blot, we may state that the results obtained appear to be consistent with the immunohistochemistry observations.

Our results might indicate a role for COX-1 in the pathogenesis of HS, whereas COX-2 only appears to be implicated in the pathogenetic mechanisms involved in KL scarring.

Cultured keloidal fibroblasts have been found to have an increased production of collagen and MMPs, lower rates of apoptosis and down-regulation of apoptosis-related genes, and a high proliferation rate as compared with normal dermal fibroblasts.[2,6–8,10] Several studies have indicated that certain cytokines frequently produced by these fibroblasts, acting as autocrine or paracrine factors, participate in abnormal wound scarring[6–8,24–26] such as some transcription factors, TNF-α, interleukins, VEGF, PDGF, CTGF, insulin-like growth factor-1 (IGF-1) and, chiefly, TGF-β.

Indeed, TGF-β plays a major role in metabolic pathways and gene domains associated with scar and KL formation. Particularly, TGF-β induces prostaglandin production in cultured fibroblasts by overexpression of COX-1, as demonstrated by some investigators.[27,28]

Nevertheless, up-regulation of TGF-β expression may be considered necessary but not sufficient for excessive scarring because HS and KL formation is a multistep process that requires complex interactions of multiple factors.[2,25]

In addition, other factors might be implicated in the pathogenesis of KL to explain the overexpression of COX-2 in KL, because TGF-β mainly induces COX-1 expression. Among these, an important factor involved in the wound-healing process is TNF-α, which induces nuclear factor-kβ (NF-kβ) activation in fibroblasts; NF-kβ is a transcription factor involved in signaling transduction pathways regulating proliferation, angiogenesis, and apoptosis.[26,28,29]

NF-kβ and its target genes, especially the antiapoptotic genes, are up-regulated in KL fibroblasts.[26,29] Recent findings[26] also show that activation of NF-kβ is responsible for TNF-α–induced COX-2 overexpression in KL fibroblasts.

In addition, several experimental and clinical investigations[2,19,30] have shown that down-regulation of COX-2 may reduce scar and KL formation and also that various COX-2 inhibitors have shown some beneficial influence on KL management.

Moreover, it has been shown[2,3,8,31] that occlusion of microvessels and decreased vascular density frequently occur

in KL compared with HS and normal scars. These findings suggest that hypoxia in KL tissues[8] may play an important role.

The significant differences in relation to the expression of COX-1 and COX-2 in abnormal wound tissues suggest different molecular mechanisms involved in the pathogenesis of HS and KL. In contrast to certain authors,[2,31] who have stated that a precise distinction between HS and KL is not always possible and that the two lesions represent a continuous spectrum, HS and KL display remarkable clinical, morphological, and molecular differences.[32,33]

Indeed, KL represents abnormal wound healing showing characteristics similar to tumors such as a tendency to grow beyond the site of original injury and to recur, whereas HS remains within the boundaries of the original lesion, does not recur, and may spontaneously regress.[1,3,31] In addition, HS is histologically characterized by dermal nodular proliferation of fibroblasts and myofibroblasts, oriented parallel to the epidermis, whereas KL tissues show abundant extracellular matrix, eosinophilic acellular bands of collagen, thickened hyalinized collagen bundles with an irregular orientation, and relatively few fibroblasts.[1,3,31]

Also, molecular characteristics display some remarkable differences because KL fibroblasts show higher upregulation of VEGF, higher volume density of elastin, overexpression of protease-activated receptors and mutation of p53, a lower level of connexin 43, and a higher resistance to FAS-mediated apoptosis as compared with HS fibroblasts.[34–36]

Our findings confirm these remarkable molecular differences between the two lesions. The behavior of normal skin and epidermis overlying scar lesions should also be emphasized as regards the expression of COX-1 and COX-2.

As reported by us previously,[15] immunohistochemistry reveals that COX-1 is generally present throughout all layers of the epidermis of normal skin, whereas COX-2 may be absent or expressed in more differentiated suprabasal keratinocytes.

In the epidermis overlying scar lesions, COXs expression parallels the staining pattern of COX-1 and COX-2 in scar lesions: COX-1 is mainly overexpressed in both HS dermal tissues and the overlying epidermis, whereas COX-2 is overexpressed in both KL tissues and the overlying epidermis. These findings strongly underline the involvement of keratinocytes in the multistep process related to HS and KL formation. Indeed, the epidermis overlying dermal fibrotic lesions is no longer considered solely as a protective barrier, because epidermal cells contribute to normal and abnormal wound-healing processes by regulating dermal matrix accumulation and degradation of collagen, proliferation, and apoptosis of fibroblasts as well as angiogenesis through epithelial–mesenchymal signaling in a paracrine fashion.[8,9,16–18,36] It has been clearly shown that keratinocytes secrete several proteins and cytokines such as TGF-β, CTGF, EGF, FGF-2, IGF-1, VEGF, PLGF, KGF, and IL-1, which have an important paracrine effect on underlying fibroblasts.[8,9,16–18,37,38]

Our results reinforce the importance of epithelial/mesenchymal interaction in stimulating an abnormal wound-healing multistep process and the remarkable role of keratinocytes in the behavior of dermal fibroblasts.

Furthermore, COX-2 has also been detected in macrophages and lymphocytes of KL tissues other than in fibroblasts and ECs as shown by the double immunostaining technique. In a previous study,[15] we also found similar results revealing COX-2 expression in macrophages and lymphocytes, in contrast to COX-1, which was mainly detected only in fibroblasts and ECs. Experimental studies[25,39] and morphological observations[2,26,31] suggest that during scar formation, inflammation plays a remarkable role that results in an increases of macrophages, leukocytes, and collagen deposition. Indeed, infiltrating inflammatory cells may be detected in normal and abnormal scarring tissues[2,3,29,40] and these cells may also contain several cytokines.[29,40] These data and our findings suggest that inflammatory cells may contribute to the development of KL also by COX-2 expression. Moreover, COX-2's remarkable positivities in KL tissues, in spite of intensive staining of COX-1 in HS tissues, might be used as a further criterion for a definite histological distinction between KL and HS.

There is little understanding of the molecular and cellular mechanisms leading to the formation of KL and HS at present, and no adequate animal model is available because these scar lesions only occur in humans.

On the other hand, these lesions are still extremely challenging, particularly in their variable response to treatment. Nevertheless, our in vivo data support the hypothesis that COX-1 and COX-2 may be implicated in different ways in the molecular mechanisms involved in HS and KL formation, respectively.

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