The expression of cyclooxygenase-1, cyclooxygenase-2 and 5-lipoxygenase in inflammatory muscle tissue of patients with polymyositis and dermatomyositis

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Abstract

Objective

To describe cyclooxygenase–1 (COX-1), cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) expression in muscle tissue in patients with idiopathic inflammatory myopathies (IIM) – dermatomyositis (DM) and polymyositis (PM) – and to find out if any differences between affected and non-affected muscles detected by MRI exist.

Methods

Samples of muscle tissue from 7 patients with dermatomyositis (DM) and from 4 with polymyositis (PM) were obtained by needle biopsy from affected and non-affected sites distinguished by magnetic resonance imaging. In situ *hybridization with antisense mRNA probes was employed to detect COX-1, COX-2 and 5-LOX mRNA.*

Results

Expression of COX-1, COX-2, and 5-LOX mRNA was found in all samples – in the muscle cells, inflammatory cells and in vessels. COX-1 mRNA expression predominated in the inflammatory cells and vessels and was higher in affected than in non-affected sites detected by MRI (mean intensity 3.22 ± 0.67 vs. 2.0 ± 0.87; p = 0.0006). The expression of COX-2 mRNA was high mainly in inflammatory cells and/or vessels and was increased in MRI-detected affected tissues (3.5 ± 0.8; 1.9 ± 1.1; p = 0.003), as was the expression of COX-2 mRNA in muscle cells (2.1 ± 1.0 vs. 1.3 ± 1.0; p = 0.021). 5-LOX mRNAwas largely expressed in muscle cells from MRI-detected affected sites and the signal intensity was higher in comparison with samples taken from non-affected tissues detected by MRI (3.22 ± 0.7 vs. 1.67 ± 0.7; p = 0.0007).

Conclusion

Expression of COX-1, COX-2 and 5-LOX mRNA was observed for the first time in muscle tissues from IIM patients. This expression was increased in affected tissues detected by MRI, which may suggest a role of COX-1, COX-2, and 5-LOX in the pathogenesis of IIM.

Key words

Polymyositis, dermatomyositis, COX-1, COX-2, 5-LOX.

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Introduction

The idiopathic inflammatory myopathies (IIM) include dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM) (1-3), which are clinically characterized by symmetrical proximal muscle weakness. The etiology and pathogenesis are unknown, although immunological mechanisms appear to be involved (1). The hallmark and basic histopathological criterion is the infiltrate with inflammatory cells. This inflammation of muscle is often focal and heterogeneous. Not all of the muscles are affected at the same time in the same patient. Cases with persistent muscle weakness exist, while at the same time the inflammatory infiltrate is only minimal or can no longer be found. The cause of the weakness appears largely unknown.

Apart from the cellular infiltration, sarcolemmal hyper-expression of HLA molecules, the increased expression of TNFα, IL-1 and adhesion molecules in endothelial cells, and the lack of apoptosis of muscle cells have all been implicated in the pathogenesis of IIM (4-6).

Cyclooxygenases (COX) and lipoxygenases (LOX) are enzymes that catalyze a key step in the conversion of arachidonate to eicosanoids. Cell injury is a signal for the release of arachidonic acid (AA) from the membrane double layer of phospholipids by the activity of lipases such as phospholipase A_2 (7-9). Then, by the intrinsic cyclooxygenase and peroxidase activities of COXs, arachidonic acid is metabolized to prostaglandin-H_2 (PGH_2), the immediate substrate for the synthesis of a series of cell-specific prostanoids including the prostaglandins (PG) and tromboxanes (Tx) (7,9). On the other hand, LOX enzymes convert liberated AA to leukotrienes or lipoxines (8, 10, 11).

Prostaglandins, tromboxanes and leukotrienes play critical roles in numerous biologic processes from which the regulation of immune function and hemodynamics, as well as the induction of pain and fever may have an important effect on the inflammatory reactions in IIM (8, 12-14).

There are two isoforms of the COX enzyme, which differ mainly in their pattern of expression. COX-1 is expressed in many different tissues under physiological conditions, and it is the constitutive "housekeeping" enzyme, which generates a pool of prostanoids that maintain cellular homeostasis. The induction of COX-2 expression usually accompanies various pathophysiological situations. Most tissues do not express COX-2 constitutively, but its expression is induced by mitogenic and proinflammatory stimuli such as cytokines, growth factors, oncogenes, and also by arachidonic acid. Although COX-2 is typically known as the inflammatory and inducible isoform of COX, it may have some constitutive functions as well. It is a novel finding that COX-2 is also expressed in striated muscle under physiological conditions. COX-2 activity in striated muscle represents a possible explanation for the hitherto unknown localization of prostanoid synthesis under physiological conditions. (7, 9, 14-16).

The first step in the 5-LOX metabolism of AA is the formation of 5-hydroperoxy-6-trans-8, 11, 14-cis-eicosatetraenoic acid (5-HPETE), which is further converted into LTA_4 – the direct substrate of all other leukotrienes. 5-LOX is predominantly expressed in lymphocytes; however, it can also be expressed in numerous other cells and tissues, including neutrophils, monocytes, macrophages, mast cells, keratinocytes and fibroblasts in the lung, spleen, brain, heart and skin (8,10,11,17).

The exact role of COX and LOX in inflammation still remains unclear. In addition, the relative contribution of each COX isoform (COX-1 or COX-2) to inflammatory responses is not completely resolved (12,13). Prostanoids appear to contribute to the formation of edema, a classical feature of acute inflammation. Prostanoids also exert various effects on cells of the immune system and modify its function in different ways. For example PGE_2, a major product of COX, induces immature thymocytes and B-lymphocytes to differentiate and acquire the functional characteristics of mature cells (12, 18,19). PGE_2 has also been shown to inhibit apoptosis and increase the expression of Bcl-2 in human colon cancer (20).

Table I. Clinical and laboratory characteristics of patients with PM/DM.

Pt. no.	PM/ DM	Male/ female	Age	Disease duration	Disease activity	MRI	CK	Autoantibodies	Treatment¶
1	DM	F	62	1 year	3	4	1	negative	prednisone, methotrexate
2	PM	M	55	2 years	2	4	2	ANA1:1280*	no therapy
3	PM	M	51	2 years	1	3	1	negative	NSAID
4	PM	F	45	1 year	2	ND	1	negative	methylprednisolone, methotrexate
5	DM	F	35	1 year	2	4	2	anti-Jo-1	no therapy
6	DM	F	51	3 month	2	3	2	negative	no therapy
7	DM	F	56	5 month	3	5	2	ANA1:2560*	no therapy
8	DM	F	44	2 years	2	2	0	anti-Jo-1	methylprednisolone
9	DM	F	43	1 month	3	ND	2	ANA1:320*	prednisone
10	DM	F	60	2years	2	1	0	anti-PM-Scl	prednisone, NSAID
11	PM/SSc	F	64	2 years	3	5	1	negative	methylprednisolone, cyclophosphamide

The evaluation of disease activity, MRI and CK are explained in the Materials and methods section.
*ANApositivity represents the highest titer by indirect imunoflurescence on Hep-2 cells, while the specificity of the autoantibodies could not be determined.
¶At the time of biopsy
ND: not done; PM: polymyositis; DM: dermatomyositis; SSc: systemic scleroderma.

Leukotrienes, mainly LTB_4, have strong chemotactic effects on immune cells (21). Moreover, the low and the high concentration of prostanoids can have opposite effects on the tonus of vessels – vasoconstriction or vasodilatation. Eicosanoids are metabolically unstable *in vivo*. They are enzymatically degraded immediately after their production. It is believed that they work only locally, on the site of their production (19).

In the present study we searched for the contribution of COX and LOX enzyme production in the muscle during the evolution of myositis in order to understand the role that COX, LOX and their products might play in the pathogenesis of this disease. We observed an elevated expression of mRNA for these enzymes in MRI-detected affected tissues in comparison with MRI-detected non-affected parts and this was seen not only in inflammatory or vascular cells, but also in muscle cells. The fact that COX and LOX enzymes are induced in myositis suggests their contribution to pathologic processes during the disease.

Patients and methods

Eleven patients with IIM, 4 patients with PM and 7 patients with DM were examined. Nine patients were female and 2 were male. Their average age was 51.5 years. PM and DM were diagnosed on the basis of valid diagnostic criteria (2, 3). Patients with inclusion body myositis were excluded. The basic clinical and laboratory characteristics of patients are presented in Table I.

The disease activity at the time of the first biopsy was semiquantitatively assessed and divided into four categories (0 – 3): 0 = no activity, 1 = low activity, 2 = medium activity, 3 = high activity. This assessment took into consideration particularly muscle weakness as evaluated by the manual muscle strength test (22), muscle endurance and the level of creatinkinase (CK). Creatinkinase was measured using commercial kits (Boehringer Mannheim, Germany) and the upper normal limit was 2.84 nkat/l. The levels of CK were split into three groups and scored on a point system from 0 to 2 (0 = normal CK level; 1 = CK level was above normal but less than 15 nkat/l; and 2 = CK level greater than 15 nkat/l).

Autoantibodies in the serum were determined by indirect immunofluorescence on Hep-2 cells, and with counterimmunoelectrophoresis and immunoblotting using reference sera (23).

Magnetic resonance imaging (MRI) using the short tau inversion recovery (STIR) technique was employed to distinguish between affected and non-affected muscles (24). This was based on the presence of edema in the skeletal muscle (Fig. 1 A and B). MRI images were scored semiquantitatively and defined as follows: 5 = very strong signal; 4 = strong signal; 3 = medium signal; 2 = mild signal; 1 = weak signal; and 0 = no inflammation.

The Ethics Committee at the Institute of Rheumatology in Prague approved the tissue sampling procedure and all patients gave their informed consent.

Nine patients were biopsied from the thigh muscles using a bioptic needle (Quick Core Biopsy Needle, 14G, Cook, Denmark or Somatex, Germany) while 2 patients underwent an open biopsy. The needle biopsy was guided by MRI-STIR (Fig. 1Aand B). In all 9 needle-biopsied patients, samples from both affected and non-affected sites as detected by MRI were taken. Samples obtained by open biopsy from the 2 additional patients were considered as having been removed from MRI-detected affected sites. In 2 cases the biopsy was repeated at intervals of 2 and 6 months, when clinical improvement had taken place (at least 30% improvement in the muscle function or significant reduction of CK). One of these patients had been treated with glucocorticoids and the other with glucocorticoids in combination with methotrexate.

The muscle samples were immediately covered with Tissue Tek embedding medium (Miles, Elkhart IN, USA) and frozen in propane-butane cooled in liquid nitrogen. One part of the muscle sample was processed for routine histopathology (staining with hematoxylin-eosin, trichrome, for ATPases and electron microscopy). The second part was used for *in situ* hybridization. Cryostat sections (6 mm thick) were mounted on glass slides (Superfrost Plus, Menzel

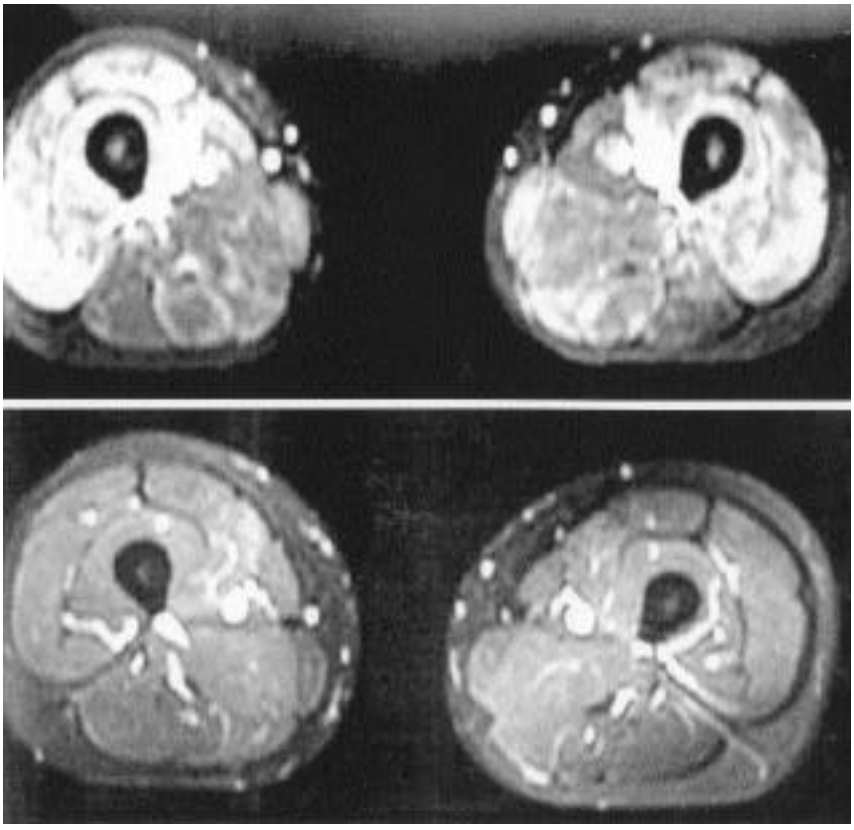

Fig. 1. Muscle MRI-STIR of a patient with polymyositis (**A**) and control scan taken 3 months later (**B**). Uneven distribution of inflammatory changes in different parts of the thigh. Anterior parts of thigh muscles are severely involved, whereas posterior parts are minimally affected (A).

Glaser, Germany) and fixed in 4% paraformaldehyde in DEPC water buffered with RNases free phosphate buffer.

Oligonucleotide cDNA sequences designed specifically for human COX-1, COX-2 and 5-LOX were used for the *in situ* hybridization. Anti-sense and sense oligonucleotide probes corresponding to the COX-1 (625 base pairs), COX-2 (564 bp) and 5-LOX (630 bp) sequences were selected and prepared.

The Qiagen MidiPrep or MaxiKit were used for the large-scale preparation of COX-1, COX-2, 5-LOX inserts containing plasmids from successfully transfected clones, and templates were prepared by linearization with BamH I or Not I (Life Technologies, GIBCO). Plasmid sequences were checked by automated sequencing, which confirmed the 100% identity of the COX-1, 2 and 5-LOX fragments to the published GenBank sequences. COX-1 fragment was complementary to nucleotides 725 - 952 of the Homo sapiens prostaglandin-endoperoxide synthase 1 (ref. NM 080591.1); COX-2 fragment to nucleotides 591 – 1041 of the Homo sapiens prostaglandin-endoperoxide synthase 2 (ref. XM 051899.1) and 5-LOX to nucleotides 95- 535 of Homo sapiens arachidonate 5-lipoxygenase (ref. XM 165564.1).

Antisense and sense RNA probes were then obtained by *in vitro* transcription using T3 and T7 RNA polymerase (Boehringer-Mannheim, Germany) with a commercially available transcription kit (Stratagene, California, USA). The probes were labeled with digoxigenin-UTP (Boehringer-Mannheim, Germany).

In situ hybridization was performed as described by Kriegsmann *et al*. (25). Briefly, after fixation tissue sections were hybridized with the digoxigenin-labeled riboprobes (either antisense or sense) in hybridization buffer containing 50% formamide, 1 x Denhardt's solution, 10% dextran sulphate, 25 g/ml herring sperm DNA (Boehringer-Mannheim, Germany), and 40 mg/ml yeast transfer RNA (Sigma Chemical Co, St Louis, Missouri, USA). First, the probes were denatured at 80ºC for 10 minutes, and then 15 µl of the probe was applied to each slide, covered with cover slide and then incubated overnight at 52°C.

The slides were washed several times in 2x SSC buffer and STE buffer. Unbound probes were digested at 37°C for 45 min with 10 g/ml RNase A (Boehringer-Mannheim, Germany), then washed. These washing steps were performed at 50°C at the following stringencies: 50% formamide/2 x SSC (5 min); 1 x SSC + 1% sodium dodecyl sulphate (SDS; 15 min); 0.25 x SSC + 1% SDS (15 min); and 0.1% SSC + 1% SDS (15 min). Immunological detection was performed after blocking non-specific binding sites with 4% horse or goat serum (30 min at room temperature) by incubation with alkaline phosphatase-conjugated antidigoxigenin Fab fragments (Boehringer-Mannheim, Germany) for 1 h at room temperature, diluted 1/500 in Tris-NaCl, pH 7.6, containing 1% normal horse or goat serum. The NBC/BCIP method was used for color development after washing with Tris-NaCl. The sections were incubated with 5-bromo-4-chloro-3-indolyl-phosphate/4-nitro blue tetrazolium chloride color substrate solution (Boehringer Mannheim, Germany) containing 1 mmol/l levamisole (DAKO, Zug, Switzerland), and developed at room temperature in darkness. Color development was stopped with Tris-NaCl (pH 7.6).

Stained sections were coded and then evaluated by two independent observers. Samples were analyzed using a light microscope (Olympus, Hamburg, Germany). The grading for COX-1, COX-2 and 5-LOX mRNA expression was performed semiquantitatively using 5 randomly selected high power fields and a 5-point scale. The grades were defined as follows: 0=none, (+) = mild, (++) = moderate, (+++) = obvious, and (++++) = strong. The results were calculated as percentages of the maximally attainable expression (maximum number of +) for each group separately for MRI-detected affected tissue, MRI-detected non-affected tissue, and tissue after treatment. The location of positive staining in the muscle cells and in the peri- and endomysium (containing

mainly inflammatory and/or endothelial cells) was assessed and recorded. The intensity of the inflammatory infiltrate was assessed on H-E stained samples and a 5-point grading system was used: 0 = none, (+) = mild, (++) = moderate, (+++) = obvious, and (++++) = strong.

Statistical analysis

For the paired test the plus signs were replaced with numbers (0 = 0, + = 1, ++ = 2, +++ = 3, ++++ = 4). The Wilcoxon matched pairs test and Spearman's rank correlation test were used for the statistical analysis. P values less than 0.05 were considered significant.

Results

Histopathology

The inflammatory infiltrates evaluated on sections stained with hematoxyline and eosine revealed variable inflammatory changes in all muscle samples (Tables II and III). Increased amounts of inflammatory infiltrates were found in samples taken from MRI-detected affected tissues in comparison with those biopsied from MRI-detected non-affected sites (p = 0.008).

Expression of COX-1, COX-2 and 5-LOX mRNAin affected and non-affected muscle tissue detected by MRI

In-situ hybridization with digoxigenin-labeled antisense RNA probes specific for COX-1, COX-2, and 5-LOX revealed signals in all the investigated muscle sections taken from tissues that were either affected or non-affected as detected by MRI, as well as in sections taken from tissues after treatment (Fig. 2A-C). The signal was detected in muscle cells and in inflammatory cells and/or vessels.

Expression of COX-1 mRNA

COX-1 mRNA was expressed mainly within inflammatory infiltrates and/or vessels and was higher in all samples taken from MRI-detected affected tissues in comparison with MRI-detected non-affected tissues and tissues after treatment (81.8%, 50.0%, and 25.0% of maximally attainable expression, mean expression intensity 3.27 ± 0.53; 2.0 ± 0.67; 1.0±0.0, respectively). Only 1 sample from MRI-detected affected tissue and 5 samples from MRI-detected non-affected sites displayed weak positive staining on muscle cells (4.5% and 13.9% of maximally attainable expression; mean expression 0.18 ± 0.33 and 0.56 ± 0.49, respectively). The total expression of COX-1 within the infiltrates, vessels and muscle cells was 43.2% of the maximally attainable expression for MRI-detected affected tissues, 31.9% for MRI-detected non-affected sites, and 12.5% for tissue after treatment (mean expression intensity 1.73 ± 1.57, 1.28 ± 0.81 and 0.50 ± 0.50, respectively) (Tables II and III, Figs. 2Aand 3A).

Table II. Expression of COX-1, COX-2, and 5-LOX mRNA in muscle tissues of patients with IIM, biopsied from MRI-recognized affected tissues. *In situ* hybridization with specific riboprobes.

Pt. no.	Inflammatory infiltrate	COX-1 mRNA		COX-2 mRNA		5-LOX mRNA	
		Muscle cells	Vessels, inflammatory cells	Muscle cells	Vessels, inflammatory cells	Muscle cells	Vessels, inflammatory cells
1	+++	0	+++	NA	NA	+++	+
2	+++	0	+++	+++	++++	++++	+++
3	+++	++	+++	+++	+++	+++	++
4	+++	0	+++	0	+++	++	0
5	++++	0	++++	++	++++	++++	++++
6	+++	0	+++	0	++	+++	+++
7	++++	0	++++	++	++++	+++	+++
8	+++	0	++	++	+++	++	0
9	+++	0	++++	0	++	+	++
10	++++	0	++++	++	++++	++++	++++
11	+++	0	+++	+++	++++	+++	++
Mean	3.27 ± 0.40	0.18 ± 0.33	3.27 ± 0.53	1.70 ± 1.02	3.30 ± 0.70	2.91 ± 0.68	2.18 ± 1.11
		1.73 ± 1.57		2.50 ± 1.05		2.55 ± 0.99	

Staining: 0 none, + mild, ++ moderate, +++ obvious, ++++ strongly positive. NAnot available.

Table III. Expression of COX-1, COX-2, and 5-LOX mRNAin muscle tissues of patients with IIM, biopsied from MRI-recognized non-affected tissues and from 2 patients after treatment. *In situ* hybridization with specific riboprobes.

Pt. no.	Inflammatory infiltrate	COX-1 mRNA		COX-2 mRNA		5-LOX mRNA	
		Muscle cells	Vessels, inflammatory cells	Muscle cells	Vessels, inflammatory cells	Muscle cells	Vessels, inflammatory cells
1	++	+	++	++	++	+	+++
2	++	+	+	++	++	++	+
3	++	+	+	+++	+	+++	++
4	NA	NA	NA	NA	NA	NA	NA
5	+++	0	++	+	+++	++	+++
6	++	+	+++	0	++	+	+++
7	+++	0	+++	++	+++	++	+++
8	++	0	+	0	+	+	+
9	NA	NA	NA	NA	NA	NA	NA
10	+++	+	+++	+	+++	+	+++
11	+++	0	++	+	0	++	++
Mean	2.44 ± 0.49	0.56 ± 0.49	2.00 ± 0.67	1.33 ± 0.81	1.89 ± 0.81	1.67 ± 0.59	2.33 ± 0.74
		1.28 ± 0.81		1.61 ± 0.88		2.00 ± 0.67	
Tissues after treatment							
2	++	0	+	++	++	++	0
5	++	0	+	+	0	+	+
Mean	2.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.50 ± 0.50	1.00 ± 1.00	1.50 ± 0.50	0.50 ± 0.50
		0.50 ± 0.50		1.25 ± 0.75		1.00 ± 0.50	

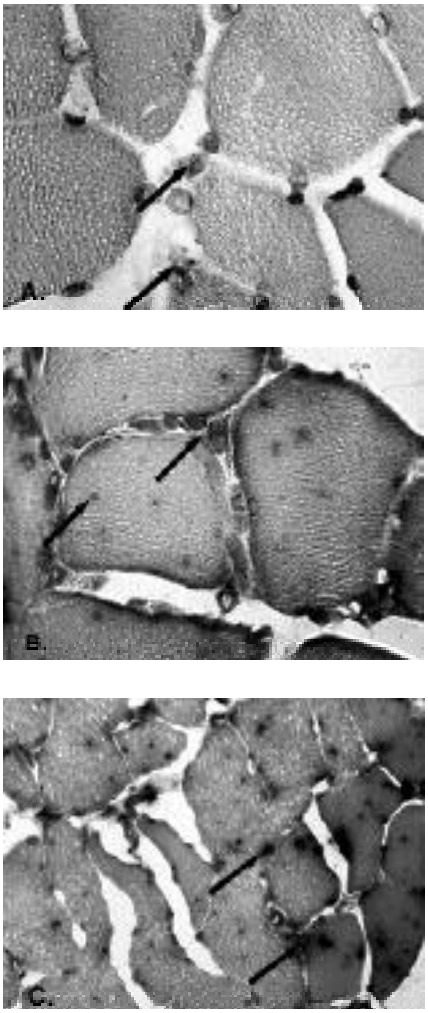

Fig. 2. Expression of COX-1 (**A**), COX-2 (**B**) and 5-LOX (**C**) mRNA in affected muscle tissues from patients with IIM (*in situ* hybridization, cross-sections, magnification x40 A and B, x20 C). Semiquantitive analysis of 9 paired samples taken from affected and non-affected tissues distinguished by MRI showed statistical difference in the intensity of COX-1 mRNA expression within inflammatory cell and/or vessels ($p = 0.0006$) (**A**, both arrows); in COX-2 within muscle cells ($p = 0.021$) (**B**, left arrow) and in inflammatory cell and/or vessels ($p = 0.003$) (**B**, right arrow); and in 5-LOX within muscle cells ($p = 0.0007$) (**C**, both arrows).

Semiquantitative analysis of 9 paired samples that had been taken simultaneously from MRI-detected affected and non-affected tissues showed statistically significant differences in the intensity of COX-1 mRNA expression within inflammatory cells and/or vessels in the two biopsied sites (mean intensity 3.22 ± 0.67 vs. 2.0± 0.87; p=0.0006). As the MRI-detected affected tissue also showed significantly larger infiltrates, the increased expression of COX-1 mRNA was most likely due to the increased number of inflammatory cells producing COX-1 (Fig. 2A). In fact, a correlation was found between the size of the infiltrate and the intensity of COX-1 mRNAexpression ($r= 0.76$; $p = 0.009$).

Expression of COX-2 mRNA

In contrast to COX-1, COX-2 mRNA was not only expressed in inflammatory cells and/or blood vessels but also in muscle cells. Quantitative comparison of total COX-2 mRNA expression showed higher signals in MRI-detected affected tissues (62.5% of maximally attainable expression; mean expression intensity 2.50 ± 1.05) than in MRI-detected non-affected tissues (40.3%; 1.61 ± 0.88) and tissues after treatment (31.3%; 1.25 ± 0.75). Generally higher expression of COX-2 mRNA was seen in inflammatory cells and/or vessels than in muscle cells; the maximally attainable expression was 82.5% and 42.5% (mean expression intensity 3.30± 0.70 and 1.70±1.02) for MRI-detected affected tissues; 47.2% and 33.3% (1.89 ± 0.81 and 1.33±0.81) for MRI-detected non-affected tissues; and 25.0% and 37.5% (1.0±1.0 and 1.5±0.5) for tissues after treatment, respectively. (Tables II and III, Figs. 2B and 3B)

In 9 paired samples that were taken from MRI-detected affected and non-affected sites, the semiquantitative rating showed statistically significant differences between the two sites in the intensity of COX-2 mRNA expression in muscle cells (MRI-detected affected tissues 2.1 ± 1.0; MRI-detected non-affected tissues 1.3 ± 1.0; $p = 0.021$); and in inflammatory cells and/or vessels (MRI-detected affected tissues 3.5 ± 0.8; MRI-detected non-affected tissues 1.9 ± 1.1; $p = 0.003$). The lack of any correlation between the number of inflammatory cells and the expression of COX-2 mRNA suggests either a variable induction of COX-2 mRNA in these cells or a significant contribution from endothelial cells.

Expression of 5-LOX mRNA

5-LOX mRNA was produced mainly by muscle cells and less positive staining was seen in inflammatory cells and/or vessels (72.7% of maximally attainable expression in muscle cells in comparison with 54.5% in inflammatory cells and/or vessels for MRI-detected affected tissues; 41.7% in muscle cells and 58.3% in inflammatory cells and/or vessels for MRI-detected non-affected tissues; 37.5% in muscle cells and 12.5% in inflammatory cells and/or vessels for tissues after treatment; mean expression 2.91 ± 0.68 and 2.18 ± 0.11; 1.67 ± 0.59 and 2.33± 0.74; 1.50 ± 0.50 and 0.50±v0.50, respectively). Total expression of 5-LOX mRNA was 63.6% for MRI-detected affected tissues, 50.0% for MRI-detected non-affected tissues, and 25.0% for tissues after treatment (mean intensity expression 2.55 ± 0.99, 2.0 ± 0.67 and 1.0 ± 0.5, respectively) (Tables II and III, Figs. 2C and 3C).

In 9 paired samples from MRI-detected affected and non-affected tissues, the semiquantitative evaluation showed statistically significant differences in the intensity of 5-LOX mRNA expression in muscle cells. The expression for MRI-detected affected tissues was 3.22 ± 0.7 and for MRI-detected non-affected tissues 1.67 ± 0.7 ($p = 0.0007$). No significant difference in LOX-5 mRNA was found between MRI-detected affected and non-affected tissues in inflammatory cells and/or vessels (2.44 ± 1.3 and 2.33 ± 0.9). The predominance of 5-LOX mRNAexpression in muscle cells from MRI-detected affected sites suggests an induction of 5-LOX associated with the inflammatory process and might indicate a direct role of the skeletal muscle cell in a pathogenesis of IIM.

Correlation of COX-1, COX-2 and 5-LOX expression with clinical and laboratory parameters

No correlation was found between disease activity and the expression of mRNAfor any of the enzymes. The intensity of inflammation and edema detected by MRI did not correlate with the magnitude of COX and LOX expression. Among the 3 enzymes, the best but still non-significant correlation was with expression of COX-2 ($r = 0.566$).

Discussion

To assess the contribution of COX-1, COX-2 and 5-LOX to pathogenic mech-

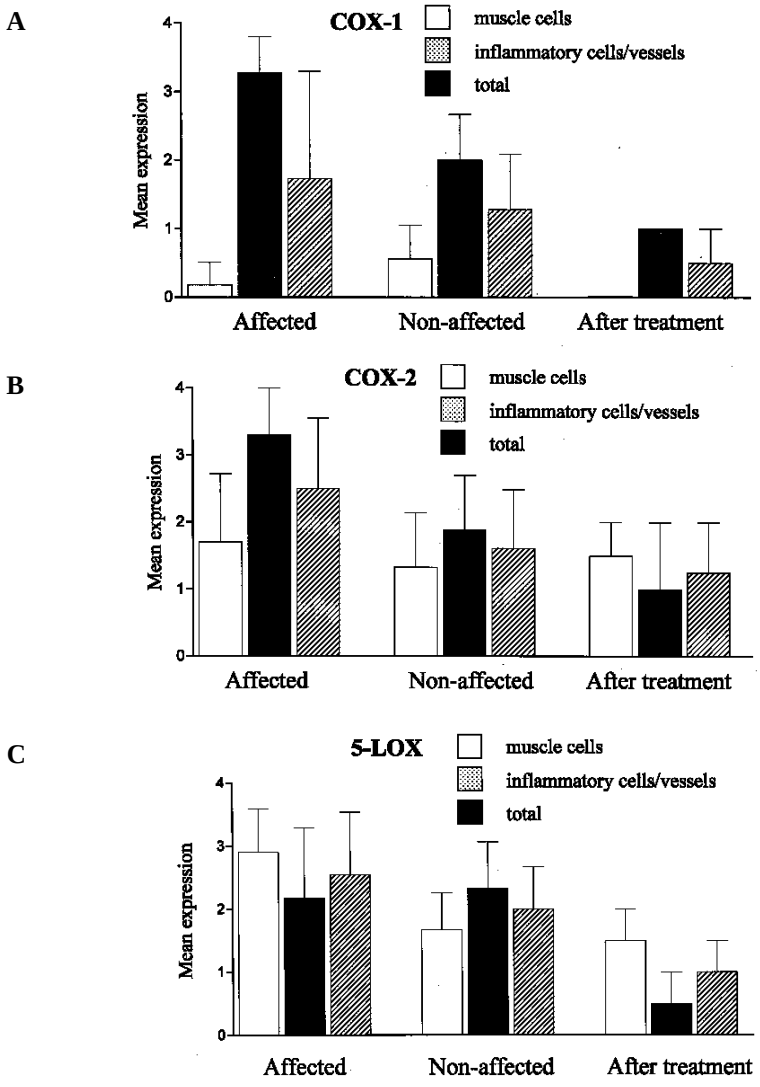

Fig. 3. Comparison of mean intensity of COX-1 (**A**), COX-2 (**B**) and 5-LOX (**C**) mRNAexpression in samples excised from MRI-recognized, affected and non-affected tissues and tissues after treatment.

anisms in IIM, we evaluated samples biopsied from the MRI-detected affected and non-affected parts of thigh muscles. Such a distinction is possible for two reasons: 1) the inflammatory process in IIM is frequently focal and limited to some groups of muscles, and 2) the MRI-STIR technique can distinguish between edematous and non-edematous tissues. However, it does not appear to be possible to find a complete association between the edema, as detected by MRI, and inflammation, although significantly less, but still a few, inflammatory cells could be found in those samples that appeared non-edematous on the MRI scan. This suggests that the difference is rather quantitative and that a sufficient level of inflammation and production of various mediators must be present in order to trigger an edema in muscles detectable by MRI. The two-site biopsy, with different stages of inflammation, might therefore help to understand the evolution of myositis.

Enzymatic conversion of arachidonic acid can produce several families of lipid mediators that include the prostaglandins, tromboxanes and leukotrienes (8). Products of COX and LOX function as important players in the inflammatory cascade. Their exact role in the pathophysiology of inflammation in chronic connective tissue diseases such as in polymyositis and dermatomyositis has not been investigated. It is well established that there is a direct effect of prostaglandins on the tonus of the vessels regulating vasodilatation and vascular permeability, which both contribute to the formation and maintenance of edema (8). The observed increase in mRNA expression for COX and LOX in MRI-recognized affected edematous tissue supports their role in the formation of edema in myositis.

The principal producers of eicosanoids are thought to be macrophages from the inflammatory infiltrates. However, it is suggested that a significant contribution to myositis comes from the endothelial cells and muscle cells. The evidence for this notion comes from the observed lack of correlation between the size of inflammatory infiltrates and the expression of COX-2 mRNA and the increased expression of 5-LOX mRNA in muscle cells from MRI-recognized affected muscles. No known prostanoids are produced by lymphocytes, although both COX-1 and COX-2 have been detected in these cells (16). Therefore, a discrepancy might occur between the intensity of COX mRNA expression and the actual production of proteins, which could be associated with a higher representation of macrophages in the inflammatory infiltrates.

COX and LOX are most likely stimulated in their expression by cytokines produced in inflammatory cells, such as by IL-1 and TNFα. Overexpression of both cytokines has been found in endothelial and inflammatory cells in muscle samples from patients with myositis and was observed to be decreased after successful treatment (26). In two samples taken after treatment we detected lower levels of all of the investigated enzymes. The latter observation suggests that the decrease of COX and LOX mRNA expression in muscle cells could be associated with a diminution of cytokine production by endothelial and inflammatory cells as a response to therapy.

Interestingly, TGFβ1, which has been detected in myositis and implicated in the pathogenesis of inflammation (27, 28) upregulates the activity of 5-LOX in HL-60 cells during their differentiation and is enhanced by TNFα (28). This may be relevant to the initiation of inflammatory infiltration in early disease as well as to the excessive fibrosis that is seen in the advanced stages of

myositis, particularly in view of the role that 5-LOX might have in systemic scleroderma, where strong 5-LOX mRNA expression in fibroblasts has recently been described (29).

Another role which COX and LOX may play in the evolution of not only acute but also chronic inflammation is a direct modulation of cells of the immune system, including a potent chemotactic effect and stimulated adherence of leukocytes to the vascular endothelium (21, 30, 31).

Not much is known about the factors that contribute to the persistence of inflammatory cells in muscle tissues. One mechanism by which inflammatory cells are removed from inflamed tissues includes programmed cell death. Eicosanoids acting at the sites of their production appear to be involved in the regulation of the apoptosis-inhibiting molecule Bcl-2. It is well established that LTB_4 and LTD_4 products of LOX increase the expression of Bcl-2 and, similarly, expression of COX-2 and production of PGE_2 enhance the expression of Bcl-2 (20, 32-34). Although the overexpression of Bcl-2 has been described in the muscle tissue of IIM patients, the role of Bcl-2 in the pathogenesis of IIM appears to be controversial (35, 36).

In summary, we have demonstrated for the first time the expression of COX-1, COX-2 and 5-LOX mRNAin the muscle tissues of patients with polymyositis and dermatomyositis. This expression was significantly enhanced in samples taken from MRI-recognized affected edematous sites. The role of arachidonic acid metabolites in myositic tissue might be the initiation of inflammation and formation of edema. It needs to be established what initiates and/or maintains COX and LOX production in IIM.

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