Modification of Cysteinyl Leukotriene Receptor Expression in Capsular Contracture

Preliminary Results

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Abstract: The development of a fibrotic capsule around foreign material in the body is a physiologic reaction undertaken by the body to protect itself from a material it does not recognize. The periprosthetic capsule can pathologically contract, pressing on the implant; it can cause pain, firmness, and sometimes implant extrusion. The pathogenesis of capsular contracture is still unclear, but most reports indicate a multifactorial explanation. The aim of this study is to investigate the role of cysteinyl leukotriene receptors (cysLTR) on the inflammatory cells involved in the development of the capsular contracture.

We recruited 20 patients affected by severe capsular contracture (Baker III–IV) and a control group composed of normal patients who had undergone implant substitution. In both groups, we performed a semiquantitative analysis of mRNA encoding for cysLTR1, cysLTR2, tumor necrosis factor-α (TNF-α) and interleukin 10 (IL-10) on myofibroblasts and macrophages of the periprosthetic capsular tissue. The molecular analysis showed an increase in the cysLTR2, TNF-α gene expression but no change in the cysLTR1 and IL-10 genes in patients affected by capsular contracture. These preliminary findings suggest a primary role for cysteinyl leukotrienes in the activation and up-regulation of capsular contraction mechanisms.

Key Words: capsular contracture, cysteinyl leukotriene receptors, periprosthetic capsule, myofibroblast, macrophage, cysLTR1

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Periprosthetic capsular contracture is the most common problem related to breast implant augmentation and reconstruction, with a reported incidence ranging from 0.5 to 50%.[1] It is a frustrating problem for patients, surgeons, and manufacturers. Despite frequent clinical and laboratory investigations, no solution has been found to solve this problem.

The capsular contracture usually appears in the early postoperative period, but it may also appear many years later, causing pain and firmness as a result of increased intraluminal pressure. The origin and histopathogenesis of capsular contracture are still unclear. Many authors in their clinical studies agree on a multifactorial etiology, in which different mechanisms are involved: physical implant properties (textured versus smooth surface, filling materials), the inherent biocompatibility of the host or surgical reasons (hematoma, bacterial prosthesis contamination, talc inoculation, seroma).

Histologic studies have demonstrated the primary role of many inflammatory cells, such as macrophages, monocytes, and myofibroblasts, in the capsular contracture. These cells, reactivated by inflammatory cytokines and peptide growth factors, restart a subacute inflammatory response that leads to capsular contracture.[2–4]

The subinflammatory state is also sustained by the activation of the cyclooxygenasic and lipoxygenasic pathways; recent studies have demonstrated the presence of leukotriene receptors (cysLT1 and cysLT2) in the macrophages and fibroblasts and have suggested new functions and bioactivities of these substances in the activation and persistence of inflammatory state, inflammatory cell recruitment, fibrosis, and vascular permeability.[5–7]

Based on these assumptions, in our study we aim to analyze the change in gene expression of the cysLT receptors in the fibroblasts and macrophages present in the contracted capsule to demonstrate the primary role of cysLT receptors in capsular contracture pathogenesis.

MATERIALS AND METHODS

Patient Recruitment

Our study was conducted on 40 patients between the ages of 25 and 45, of whom 20 had capsular contracture of severe grade (III–IV Baker score), group A, and 20 control

patients who wanted to replace their implants with implants of a greater size, group B. Every patient had undergone breast augmentation in the last 5 years. The inclusion criterion for group A was the development of a capsular contracture 1 year after the operation (mean period, 6 months). The inclusion criterion for group B was the absence of clinical evidence of capsular contracture 5 years after breast augmentation. The same implants (textured surfaced, highly cohesive silicon gel) and surgical techniques (sub glandular placement) were used in every patient. In both groups, a capsule was partially removed and prepared to be homogenized and analyzed.

RNA Extraction and RT-PCR

Periprosthetic capsules were homogenized using a Diox 900 homogenizer (Heidolph, Nurnberg, Germany). Total RNA was extracted from homogenized periprosthetic capsules using an RNA Tri-Reagent (Molecular Research Center Inc, Cincinnati, OH) according to the manufacturer's protocol. The extracted RNA was subjected to DNase I treatment at 37°C for 40 minutes. The total RNA concentration was determined by UV spectrophotometer. The mRNA levels of the genes under analysis were measured by RT-PCR amplification.[8] Sequences for the human mRNAs from GenBank (DNASTAR Inc, Madison, WI) were used to design primer pairs for RT-PCRs (Oligo 4.05 software; National Biosciences Inc., Plymouth, MN). Each RT-PCR was repeated at least 4 times. Each sample was loaded and electrophoresed in a 2% agarose gel. A semiquantitative analysis of mRNA levels was carried out by the Gel Doc 2000 UV System (Bio-Rad, Hercules, CA). The measured mRNA levels were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), chosen as the housekeeping gene, and the gene expression values were expressed as arbitrary units ± SE.

Statistics

Molecular data are shown as means of arbitrary units ± SE. ANOVA, followed by the Student-Neuman-Keuls post hoc test, was used to determine the statistical significance between the groups. $P < 0.05$ was considered statistically significant.

RESULTS

The semiquantitative analysis of mRNA levels measured by RT-PCR amplification (Fig. 1), in the periprosthetic capsules, showed an increase in cysteinyl leukotriene receptor 2 (cysLTR2) gene expression, together with no change in the cysLTR1 gene in patients affected by capsular contracture (Table 1). The expression level of the tumor necrosis factor-α TNF-α was also increased in patients affected by capsular contracture (mean ± SE of arbitrary units: 1.72 ± 0.08 versus 1.11 ± 0.05 in patients affected by capsular contracture and control patients, respectively) (Table 1). Conversely, the mRNA levels of the anti-inflammatory IL-10 were not changed (mean ± SE of arbitrary units: 0.61 ± 0.04 versus 0.67 ± 0.09 in the patients affected by capsular contracture and the control patients, respectively) (Table 1).

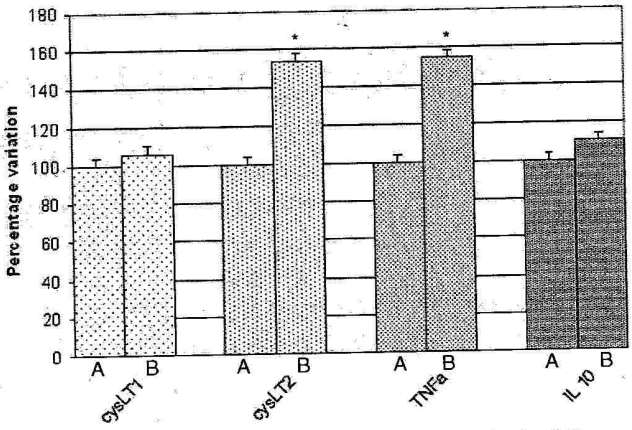

FIGURE 1. The mRNA levels (percentage variation) of the genes under analysis measured by RT-PCR amplification are reported. A, Control patients; (B) patients with capsular contracture. *$P < 0.05$.

TABLE 1. mRNA Levels (mean ± SE) of the Genes Under Analysis Measured by RT-PCR Amplification

Gene	Control	Patients With Capsular Contracture of III and IV Degree
cysLTR1/GAPDH	0.65 ± 0.02	0.69 ± 0.01
cysLTR2/GAPDH	0.11 ± 0.04	0.17 ± 0.04*
TNFα/GAPDH	1.11 ± 0.05	1.72 ± 0.08*
IL-10/GAPDH	0.61 ± 0.04	0.67 ± 0.09

Each RT-PCR was repeated at least 4 times. The semiquantitative analysis of mRNA levels was carried out by the Gel Doc 2000 UV System (Bio-Rad, Hercules, CA). The measured mRNA levels were normalized with respect to *GAPDH* (housekeeping gene), and gene expression values were expressed as arbitrary units ± SE.

*$P < 0.05$ versus the corresponding control, as analyzed by ANOVA, followed by Student-Neuman-Keuls test.

DISCUSSION

Cysteinyl leukotriene are peptide-conjugated lipids that are mainly produced by activated inflammatory cells such as eosinophils, basophils macrophages, and mast cells; they are now recognized as potent inflammatory mediators that start and regulate several biologic responses.[9–11]

In this period, cysteinyl leukotrienes are the subject of much investigation to clarify their role as inflammatory mediators and the possible clinical implications they may have related to cell receptor modulation. Recent studies have demonstrated the presence of cysLTRs and their positive modulation on the macrophages and fibroblasts involved in the inflammatory state of many tissues.[12,13]

In light of these findings, the possibility of using leukotriene receptor antagonists has been proposed to modulate the activity of cysLTRs in capsular contracture.[14] The possible effectiveness of these drugs is based on several investigations conducted on these mediators and on their involvement in subacute inflammation, endothelial activation, fibrosis, and cell recruitment. Nevertheless, the use of drugs with no specific indication has been severely criticized and discouraged for moral, medical, and legal reasons.[15]

The rationale for our investigations arises from the necessity to provide a biomolecular basis for a clinical hypothesis, which has never been scientifically demonstrated.

In inflammatory conditions, platelet activation causes a release of several proinflammatory molecules, such as TGF-β, epidermal growth factor (EGF), TNF-α.[16] These molecules are able to trigger the migration of inflammatory cells (eg, macrophages, mast cells, basophils, and neutrophils) to the site of the injury.

In our study, the fact that the patients subjected to the capsular contracture show high mRNA levels of TNF-α, together with no change in the anti-inflammatory IL-10 gene expression, confirms the presence of an inflammatory process associated with the capsular contracture formation.

In our study, we analyze the change in gene expression of the cysLT receptors and proinflammatory molecules on the fibroblasts and macrophages present in the contracted capsule. Our preliminary results demonstrate an important role for the cysLT2 receptors in the capsular contracture pathogenesis.

Based on these observations, the therapeutic application of agents that are able to modulate the expression or activation of cys-LT2 receptors is certainly going to increase, opening new frontiers for the therapeutic application of leukotriene receptor antagonists.

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