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Prolonged, acute suppression of cysteinyl leukotriene to reduce capsular contracture around silicone implants

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ABSTRACT

We hypothesize that periodically early, local suppression of cysteinyl leukotrienes (CysLTs), which are potent inflammatory mediators, can reduce the fibrotic capsular contracture around silicone implants. We tested this hypothesis with the silicone implants enabled with the sustained release of montelukast, a CysLT receptor antagonist, for 3 and 15 days. In this work, we inserted each of the distinct implants into the pocket of the subpanniculus carnosus plane of living rats and performed histological and immunofluorescent (IF) analyses of the tissues biopsied at predetermined periods for 12 weeks after implant insertion. The implants with montelukast exhibited significantly reduced polymorphonuclear leukocytes (i.e., PMNs), implying a concurrent reduction of CysLT. This effect was more prominent after long-term local montelukast exposure. Thus, fewer fibroblasts were recruited, thereby reducing transforming growth factor (TGF)-β and myofibroblasts in the tissue around the implant. Therefore, the fibrotic capsule formation, which was assessed using the capsule thickness and collagen density, decreased along with the myofibroblasts. Additionally, the tissue biopsied at the experimental end point exhibited significantly decreased mechanical stiffness.

Statement of Significance

Capsular contracture is troublesome, making the tissues hardened around the silicone implant. This causes serious pain and discomfort to the patients, often leading to secondary surgery for implant replacement. To resolve this, we suggest a strategy of long-term, local suppression of cysteinyl leukotriene, an important mediator present during inflammation. For this, we propose a silicone implant abled to release a drug, montelukast, in a sustained manner. We tested our drug-release implant in living animals, which exhibited a significant decrease in capsule formation compared with the intact silicone implant. Therefore, we conclude that the sustained release of montelukast at the local insertion site represents a promising way to reduce capsular contracture around silicone implants.

1. Introduction

In the field of cosmetic and reconstructive surgery, silicone implants are one of the widely used medical devices [1,2]. Although silicone implants are approved for clinical use, local complications, such as capsular contracture, implant rupture and gel bleeding, have not yet been fully resolved [3,4]. One of the most serious complications is capsular contracture, which has been reported to occur in up to 30% of patients after the insertion of silicone implants [5,6]. When a silicone implant resides in the body for a prolonged period, excessive fibrous connective tissue, which is mainly composed of collagen and fibroblasts, accumulates around it, isolating the implant from the local tissue environment [7,8]. Subsequently, a contractile force originating from the

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collagen and myofibroblasts, which are differentiated from fibroblasts, causes capsular contracture around the silicone implant. In serious cases, this can require a secondary surgery, which is highly inconvenient for patients [5,9].

This pathological phenomenon usually occurs because of a prolonged inflammation reaction, which leads to inflammation from acute to chronic stages [10]. In this case, the period of the acute inflammatory response can also be prolonged from days to weeks [11]. Once the silicone implant is inserted, acute inflammation initiates with infiltration of polymorphonuclear leukocytes (PMNs) from the blood vessels to the implant site [11]. Then, the PMNs secrete cysteinyl leukotrienes (CysLTs), which are potent inflammatory lipid mediators [12] and are also involved in the recruitment and survival of PMNs via autocrine and paracrine signaling [13,14]. The persistent presence of the silicone implant leads to chronic inflammation, in which CysLTs stimulate the migration and proliferation of fibroblasts [15,16]. During this chronic inflammation stage, fibroblasts differentiate into myofibroblasts and synthesize collagen, which is mediated by transforming growth factor (TGF)-β secreted by the fibroblasts themselves [17–19] and these lead to the capsule formation by fibrosis. Eventually, both the α smooth muscle actin (i.e., α-SMA) expressed on the myofibroblasts and the tension caused by the collagen fibrils are major causes of capsular contracture, i.e., a contractile force around the silicone implant [7,20,21].

Therefore, inhibiting CysLT production could be a strategy to prevent the formation of capsular contractures. Decreasing the number of CysLTs would reduce the recruitment and proliferation of fibroblasts, thereby decreasing the amount of myofibroblasts and collagen present during chronic inflammation. Montelukast is a potential therapeutic agent for the inhibition of CysLT production [22,23]. Montelukast, a specific leukotriene receptor antagonist, selectively blocks the production of leukotriene D_4 (LTD_4) by binding to the type 1 CysLT receptor (CysLT1) located on the outer plasma membrane of the PMNs [22–24]. This drug has been reported to inhibit the accumulation of fibroblasts/myofibroblasts and the deposition of collagen [25,26] and is already used clinically for the treatment of asthma or allergic diseases caused by CysLT overproduction [27–30].

Therefore, we suggest using the local, sustained release of montelukast around silicone implants to prevent capsular contracture. We hypothesize that the formation of the fibrous capsule, which occurs during chronic inflammation, can be reduced by inhibiting CysLT production even during the acute stage of inflammation, i.e., acute inflammation. Periodically early suppression of CysLT should decrease the amount of PMNs and thereby reduce the amount of CysLTs, which are secreted by the PMNs themselves. Decreasing the amount of CysLTs would result in less recruitment and proliferation of fibroblasts during chronic inflammation [15,31], leading to reduced TGF-β secretion and less myofibroblast differentiation and collagen synthesis and, eventually, diminished capsular contracture. Therefore, we also hypothesize that this effect should increase as CysLT production is suppressed for longer periods, i.e., long-term montelukast exposure, during prolonged acute inflammation.

To examine our hypothesis, two distinct silicone implants were prepared in this work. For this, we differently coated the shell of silicone implants, already clinically available (SFS-LP, Hans Biomed, Korea), to give a silicone shell coated with montelukast only (i.e., the MON_SI) and one coated with a mixture of montelukast and poly (lactic-co-glycolic acid) (i.e., the PLGA_MON_SI). For the MON_SI, drug release was relatively short. For the PLGA_MON_SI, a longer drug release was achieved with the poly(lactic-co-glycolic acid) (PLGA) as a barrier against drug-diffusion [32,33]. For controls without the drug, we also employed two different silicone implants: an uncoated, intact shell of a silicone implant (i.e., the SI) and a shell coated solely with PLGA (i.e., the PLGA_SI).

In this work, we assessed the *in vivo* effect of montelukast on anti-fibrosis as a function of the period of local drug release around the silicone implant. We inserted each of the different silicone implants in the subpanniculus plane of rats and biopsied the tissues around the implant at scheduled times for 12 weeks. The tissues were examined histopathologically with hematoxylin and eosin (H&E) staining, Masson's Trichrome (MT) staining and immunofluorescence (IF) staining. To examine the degree of the capsular contracture, we measured the tensile force at failure of the tissues (i.e., the maximum force needed to break the tissue) around the implants at the end point (12 weeks) of the experiments.

2. Materials and methods

2.1. Materials

The shells of the clinically used silicone implants (SFS-LP) were a generous gift from Hans Biomed (Seoul, Korea). Montelukast was kindly donated by Daewoong Bio (Seoul, Korea). PLGA (inherent viscosity = 0.41 dl/g; LA:GA = 50:50) was obtained from Lakeshore Biomaterials (Birmingham, USA). We obtained dimethylformamide (DMF) from JT Baker (NJ, USA). EPO-TEK® 301-2 medical epoxy was purchased from Epoxy Technology (Billerica, USA). Tween 80 was obtained from Sigma-Aldrich (ME, USA). Zoletil 50 was purchased from Virbac (Fort Worth, TX, USA), and Rompun was obtained from Bayer (Lerverkusen, Germany). Paraformaldehyde (4%) was purchased from KCFC (Korea). For H&E staining, xylene, ethanol and hydrochloric acid (35%–37%) were purchased from Duksan Pure Chemicals (Ansan, Korea). Ammonia solution (28%–30%) was obtained from Junsie Chemical (Tokyo, Japan). Modified Mayer's H&E Y solutions were supplied by Richard-Allan Scientific (MI, USA). For MT staining, acetic acid (1%) was obtained from Duksan Pure Chemicals (Ansan, Korea). Biebrich scarlet-acid fuchsin, phosphomolybdic acid, phosphotungstic acid and aniline blue solutions were purchased from Sigma-Aldrich (St. Louis, MO, USA). For IF staining, target-retrieval solution (10×) was purchased from Dako (Glostrup, Denmark). anti-TGF-β (sc-146) was acquired from Santa Cruz Biotechnology (Dallas, TX, USA). anti-vimentin (ab92547) and anti α-SMA (ab5694) were purchased from Abcam (Cambridge, MA, USA). anti-CD163; ED2 (MCA342R) was obtained from AbD Serotec (Raleigh, NC, USA). Paraffin was supplied by Merck (USA).

2.2. Preparation of silicone implant samples

In this work, we fabricated four different types of implant samples using the shells of clinically used silicone implants (SFS-LP, Hans Biomed, Korea): intact implant shells (SI), implant shells coated with PLGA only (PLGA_SI), implant shells coated with montelukast (MON_SI) and implant shells coated with both PLGA and montelukast (PLGA_MON_SI). To prepare the SI, an implant shell, 1.5 mm in thickness, was first cut into a circle with a diameter of 2 cm. Then, the inner surfaces of two circular samples were bonded with medical epoxy (EPO-TEK® 301-2). Thus, the two outer surfaces of the shells were exposed to the surrounding tissues after insertion.

The other three samples were prepared by the following procedures. First, 10% w/v PLGA, 0.1% w/v montelukast or both 10% w/v PLGA and 0.17% w/v montelukast was dissolved in DMF to prepare the coating solutions for PLGA_SI, MON_SI and PLGA_MON_SI, respectively. Then, we sprayed the solution on the outer surface of a circular sample (2-cm diameter and 1.5-mm thickness) cut from the silicone implant shell. The spraying conditions are in