

Cytokine mRNA levels in human fat tissue after injection lipolysis with phosphatidylcholine and deoxycholate

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Abstract Injections with Lipostabil®, a phosphatidylcholine and deoxycholate containing substance, have become a popular technique to treat localized fat accumulation and lipomas. It is believed that the injected substance leads to fat cell destruction with subsequent acute panniculitis followed by a repair process of the treated fat tissue. We sought to investigate the mRNA expression of cytokines within the acute stage of panniculitis following Lipostabil® injections. Tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-8, and IL-10 mRNA expression were determined using quantitative real-time reverse transcriptase polymerase chain reaction in treated and adjacent non-treated fat tissue of lipomas 48 h after injection in seven patients. Following injection lipolysis with Lipostabil®, TNF- α , IL-6, IL-8, and IL-10 mRNA levels were significantly elevated in treated fat tissue compared to non-treated fat. No significant differences were found for IL-4. Both in treated and non-treated fat tissue, mRNA of IFN- γ , IL-2, and IL-5 was not expressed. Injection lipolysis induces acute panniculitis within the treated fat tissue, associated with changes of the cytokine profile. However, their pathogenetic relevance with respect to clinical dissolving of fat needs further investigation.

Keywords Fat tissue · Lipoma · Cytokine · Lipodissolve · Injection lipolysis · Inflammation

Injection lipolysis (syn. lipodissolve, fat dissolve) describes a procedure in esthetic medicine, which aims for reduction of localized fat accumulations by intralesional injection of substances that induce destruction of adipocytes [11]. A frequently used product is Lipostabil®, mainly consisting of phosphatidylcholine (PC) and sodium-deoxycholate (DC) [6]. It is unknown which ingredient of Lipostabil® is causing the destruction of adipocytes. Some authors believe that DC leads to adipocyte destruction by a detergent reaction, whereas others believe PC is responsible for lysis of fat cell membranes [5, 13]. A recent study on human lipomas showed that injection of Lipostabil® leads to destruction of fat cells with a subsequent panniculitis that changes during time after treatment [1]. In the beginning, a suppurative inflammation is present mainly consisting of neutrophils. This infiltrate is subsequently replaced by lymphocytes followed by lipophage granuloma, foam cells, and fibrosis [1, 12]. However, until today, no data are available on the characteristics of the acute panniculitis occurring after injection of Lipostabil®. We therefore aimed to investigate the expression of different pro-inflammatory cytokines within the fat tissue of human lipomas after injection lipolysis in order to establish the mechanism of action of Lipostabil®.

Seven subjects (median age 37.7 years, 2 ♂, 5 ♀) were enrolled in the study. A total of 18 biopsy specimens were evaluated, with 9 specimens from treated lipomas and 9 from untreated fat tissue surrounding the lipoma. Lipomas were treated with intralesional injection of Lipostabil® (Nattermann, Cologne, Germany) (volume range 1–3.5 cc) 48 h before extirpation. All subjects gave their informed consent. The study was conducted according to the Declaration of Helsinki. Total cellular RNA was isolated from fat tissue samples using RNeasy® Lipid Tissue Kit (QIAGEN, Chatsworth, CA, USA) following the manufacturer's

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protocol. Prior to cDNA synthesis, mRNA was digested with RNase-free DNase I (Roche Applied Science, Mannheim, Germany). cDNA was synthesized by reverse transcription from DNase I treated RNA using MultiScribe™ reverse transcriptase enzyme and random hexamers primers (TagMan® Reverse transcription reagents, Applied Biosystems, Forster City, CA, USA). Quantitative real-time RT-PCR was performed using a Power SYBR Green PCR Master Mix (Applied Biosystems) and GeneAmp® Sequence Detection System (Applied Biosystems). PCR primer of cytokines and the housekeeping gene (RPL 38) were designed using the computer program Primer Express (PE Applied Biosystems) and produced by the custom oligonucleotide synthesis service (TIB MolBiol, Berlin, Germany). The primers for TNF- α , IFN- γ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and RPL 38 are detailed in Table 1. Target gene mRNA levels were quantified based on standards and normalized to RPL 38. PCR amplifications were performed in

a total volume of 25 μ l, containing 5 μ l cDNA sample, 0.5 μ M of each primer, and 12.5 μ l SYBR Green PCR Master Mix (Applied Biosystems). PCR was started with 2 min at 50°C and an initial 10 min denaturing temperature of 95°C, followed by a total of 40 cycles of 15 s of denaturing and 1 min of annealing and elongation at 60°C. The reaction products were separated by 2% agarose gel electrophoresis. Relative mRNA expression levels were calculated using the comparative $\Delta\Delta C_t$ method as previously suggested by Livak and Schmittgen [9]. Quantities of all targets in the test samples were normalized to the corresponding RPL-38 RNA transcript in the skin samples. The quantities of mRNA levels were determined using the median log transform of the gene expression [9]. Non-parametric tests were used to analyze RT-PCR data as non-normal distribution was observed in subsets of the results (D'Agostino–Pearson test). The Wilcoxon test was applied to test for significant differences of data between treated

Table 1 Cytokine primers for RT-PCR and mRNA levels of cytokines observed in Lipostabil® treated and non-treated fat tissue

	Lipostabil® treated sites [median mRNA (range)]	Non-treated sites [median mRNA (range)]
TNF- α		
Forward 5'-CCCAGGCAGTCAGATCATCTTC-3'	0.86 (0–7.38)*	0.44 (0–7.08)
Reverse 5'-GCTTGAGGGTTTGCTACAACATG-3'		
IFN- γ		
Forward 5'-CAGGTCATTCAGATGTAGCGGATA-3'	No expression	No expression
Reverse 5'-TCTGTCACTCTCTCTTTCCAATTC-3'		
IL-2		
Forward 5'-CCCAAGAAGGCCACAGAACT-3'	No expression	No expression
Reverse 5'-TGCTGATTAAGTCCCTGGGTCTTA-3'		
IL-4		
Forward 5'-CACAGGCACAAGCAGCTGAT-3'	0 (0–0.015)	0 (0–0.021)
Reverse 5'-AGGACAGGAATTCAAGCCCG-3'		
IL-5		
Forward 5'-CCACAAGTGCATTGGTGAAAGA-3'	No expression	No expression
Reverse 5'-GGAATCCTCAGAGTCTCATTGGC-3'		
IL-6		
Forward 5'-CCTGAGAAAGGAGACATGTAACAAGA-3'	2.84 (0.54–65.98)*	1.3 (0.3–64.17)
Reverse 5'-GGCAAGTCTCTCATTGAATCC'		
IL-8		
Forward 5'-TGTGTGTAAACATGACTTCCAAGCT-3'	49.65 (1.78–280.89)*	0 (0–253.15)
Reverse 5'-GCAAAACTGCACCTTCACACAG-3'		
IL-10		
Forward 5'-GGCGCTGTCATCGATTTCTT-3'	4.3 (1.29–15.39)*	1.19 (0.04–3.67)
Reverse 5'-CTCTTGAGCTTATTAAAGGCATTCT-3'		
RPL 38		
Forward 5'-TCACTGACAAAGAGAAGGCAGAGA-3'		
Reverse 5'-TCAGTGTGTCTGGTTCATTTCAGTT-3'		

RPL 38, housekeeping gene

* Statistically significant difference

and non-treated sites. Spearman's coefficient of rank correlation was assessed to assess the relationship between cytokine expression. A P value < 0.05 was considered significant.

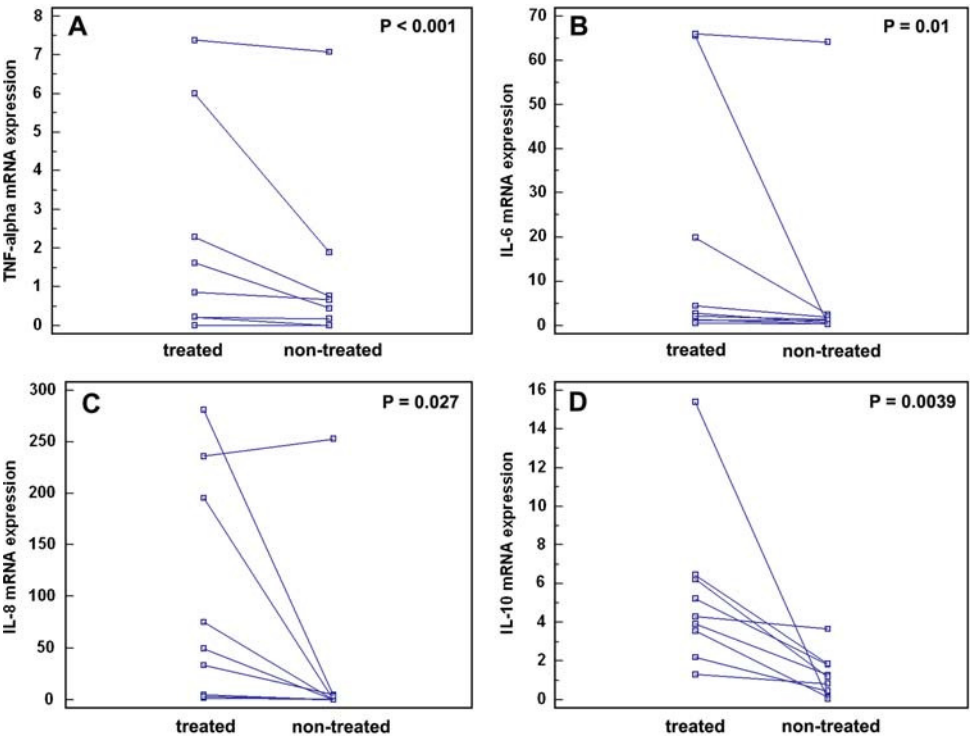
In Lipostabil[®] treated sites significantly up-regulated mRNA levels of TNF- α [0.86 (range 0–7.38) vs. 0.44 (range 0–7.08), $P < 0.001$], IL-6 [2.84 (range 0.54–65.98) vs. 1.3 (range 0.3–64.17), $P = 0.01$], IL-8 [0 (range 0–253.15) vs. 49.65 (range 1.78–280.89), $P = 0.0273$], and IL-10 [4.3 (range 1.29–15.39) vs. 1.19 (range 0.04–3.67), $P = 0.0039$] were observed when compared to adjacent non-treated sites. mRNA expression of TNF- α significantly correlated with IL-6 mRNA expression ($r = 0.95$, $P = 0.007$) but not with IL-8 and IL-10 expression ($P > 0.05$). There was no significant difference in IL-4 mRNA between treated and non-treated fat tissue [0 (range 0–0.015) vs. 0 (range 0–0.021), $P = 0.375$]. Both in treated and non-treated fat tissue, there were no mRNA expression of IL-2, IL-5, and IFN- γ (Figs. 1, 2).

The off-label use of PC and DC containing substances (e.g., Lipostabil[®]) for intralesional application in subcutaneous fat is a popular and widely used technique to dissolve localized fat accumulations [6, 11]. Lower lid fat pads, “buffalo hump” lipodystrophy, fat accumulations on the waist and the hip as well as lipolysis of lipoma have also been reported as possible indications for dissolution with Lipostabil[®] [2, 5, 8, 11, 14]. Despite its broad use, the mechanism of action of Lipostabil[®] is not clear. In fact, even no consent has been found, whether PC or DC is the

major active ingredient, or if a synergistic effect between both substances exists [1, 5, 13]. Recent studies on lipomas could show that a volume reduction is possible using injection lipolysis [2, 8]. An investigation evaluating the histological and immunohistochemical changes of fat tissue after injections with Lipostabil[®] could show that destruction of fat cells followed by an acute suppurative inflammatory response is caused by the injected substance [1]. During the time course after treatment, the neutrophilic infiltrate shifts towards an inflammation characterized by CD4-positive lymphocytes, which is subsequently replaced by lipohage granuloma, foam cells, and fibrosis [1, 12]. Although these histological observations suggest that the panniculitis with following repair process represents the mode of action of injection lipolysis, little is known about the characteristics of the acute inflammatory phase.

The data obtained from the present pilot study indicate that pro-inflammatory cytokines such as IL-4, IL-6, IL-8, IL-10, and TNF- α are involved in the early inflammation phase following treatment with Lipostabil[®]. Functions of IL-4 include the stimulation of activated B-cell and T-cell proliferation, and the differentiation of CD4+ T-cells into Th2 cells. The pro-inflammatory cytokine IL-6 is secreted by T cells, macrophages, fibroblasts, endothelial cells, and adipocytes to stimulate immune response to trauma and tissue damage. It is an important mediator of the acute phase response [3, 4]. TNF- α is mainly produced not only by macrophages, but also by a broad variety of other cell types

Fig. 1 In Lipostabil[®] treated sites, significantly up-regulated mRNA levels of TNF- α ($P < 0.001$), IL-6 ($P = 0.01$), IL-8 ($P = 0.0273$), and IL-10 ($P = 0.0039$) were observed when compared to adjacent non-treated sites



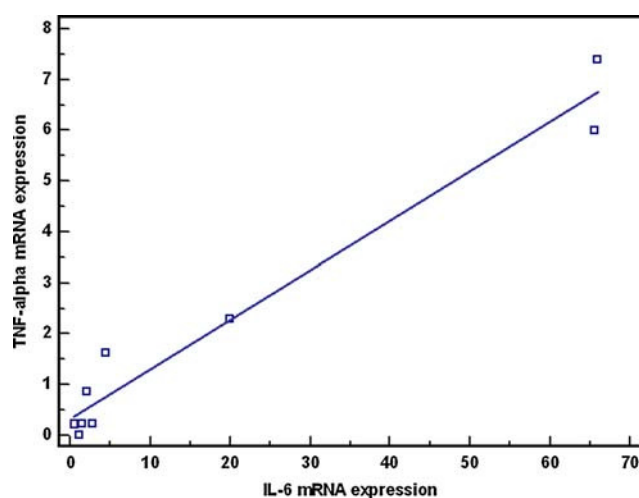


Fig. 2 mRNA expression of TNF- α and IL-6 significantly correlated in Lipostabil[®] treated sites ($r = 0.95$, $P = 0.007$)

including adipocytes and fibroblasts [7]. It attracts neutrophils very potently, and helps them to stick to the endothelial cells for migration. A locally increasing concentration of TNF- α will cause the cardinal signs of inflammation such as heat, swelling, redness, and pain. Interleukin-8 (syn. neutrophil chemotactic factor) is expressed by fibroblasts, neutrophils, macrophages, and adipocytes [16, 17]. Its primary function is to recruit neutrophils at the site of inflammation. Interleukin-10 is an anti-inflammatory cytokine and is capable of inhibiting synthesis of pro-inflammatory cytokines like IFN- γ , IL-2, IL-3, TNF- α , and granulocyte-macrophage colony-stimulating factor.

The characteristics of the above-mentioned cytokines underline the findings of previous histological and immunohistochemical findings on Lipostabil[®] injections [1]. Indeed, the latter seems to induce acute suppurative panniculitis with expression of pro-inflammatory cytokines. In particular, levels of TNF- α and IL-6 are increased after Lipostabil[®] injections. These cytokines also showed a very strong correlation in mRNA expression indicating that the latter has been commonly up-regulated. The expression of IL-10 may be seen as reactive to the initiated suppurative panniculitis. The fact that non-treated surrounding fat tissue also expressed pro-inflammatory cytokines may be due to a certain diffusion of the injected Lipostabil[®]. Whether injection of local anesthesia and/or the surgical procedure itself may have caused expression of pro-inflammatory cytokines remains speculative. It has been demonstrated that both TNF- α and IL-6 levels are significantly up-regulated in human fat tissue following minimal trauma [10].

No relevant expression in treated and non-treated fat tissue was found for IL-2, IL-5, and IFN- γ . The main functions of IL-2, IL-5, and IFN- γ may explain why they were not detectable in the treated and non-treated fat tissue at the early phase after injection. Interleukin-2 stimulates the

growth, differentiation, and survival of antigen-selected cytotoxic T cells. Interleukin-5, which stimulates B-cell growth, increases immunoglobulin secretion, and serves as a mediator in eosinophil activation, is produced by Th2 cells and mast cells. Taken together, cytotoxic T cells, B cells, and eosinophilic granulocytes are not involved in the early inflammatory process of fat tissue after injection lipolysis [1].


The limitations of the present study are as follows: as it is known that lipomas may biologically differ from normal fat tissue, the found data cannot be transferred to regular subcutaneous fat, which is mostly treated by injection lipolysis [15]. Ideally, treated as well as non-treated lipomas should have been compared in order to exclude the effect of diffusion of Lipostabil[®]. The influence of the injection of anesthesia and the surgical procedure itself on the production of pro-inflammatory cytokines was not evaluated in the present study. Moreover, we only investigated biopsies that were taken at the early stage of inflammatory process after injection of Lipostabil[®]. A previous study has shown that chronological changes of the induced panniculitis may be observed. Therefore, the cytokine profile of the present study only reflects the stage of suppurative panniculitis and possibly the beginning of a lymphocytic infiltration. Further studies evaluating the chronological changes of cytokines after injection lipolysis are needed. Additionally, it might be interesting to evaluate the expression of cytokines following the injection of different Lipostabil[®] volumes or of separated DC. Unfortunately, until today only combination of PC and DC are available for injection, meanwhile pure PC substances are missing [13]. Future injections with the separated compounds may deliver further information about the active ingredient. Finally, single cell separation by means of laser microdissection would be useful to distinguish which cells do actually express the pro-inflammatory cytokines. In conclusion, injection lipolysis induces acute panniculitis within the treated fat tissue, associated with changes of the cytokine profile. However, their pathogenetic relevance with respect to clinical dissolving of fat needs further investigation.

Conflict of interest statement None

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