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Botulinum toxin A and lidocaine have an impact on adipose-derived stem cells, fibroblasts, and mature adipocytes in vitro

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Summary Lipofilling with autologous fat tissue is widely used in plastic and reconstructive surgery to treat soft-tissue deficiency. Unfortunately, implanted cells disappear gradually and make it difficult to predict the resorption rate. Several adjuvants are used to improve the success of fat tissue grafting. In this study, the effect of botulinum toxin (BoNT) on mature adipocytes, as well as adipose-derived stem cells (ASC) and fibroblasts was evaluated. As lidocaine is the most prevalent drug to anesthetize the donor site as well as the area to be treated with autologous fat, this local anesthetic was examined too.

Primary ASCs, fibroblasts, and mature adipocytes were exposed to 1, 10, and 20 IU/ml BoNT A and 1% lidocaine. Cells were tested on proliferation, viability, and LDH release. Adipogenic differentiation potential was evaluated by quantitative real-time PCR analyzing the expression of FABP4.

BoNT had no significant influence on the proliferation or viability of tested cells. By trend, low concentrations of BoNT improved adipogenic potential of ASCs. Lidocaine had a strong diminishing effect on the proliferation of ASCs and fibroblasts and on the viability of these cells. Mature adipocytes show no significant inferior viability after BoNT or lidocaine treatment.

BoNT has no negative effect on ASCs, mature adipocytes, or fibroblasts in vitro. Lidocaine (1%) negatively influences the proliferation and viability of fibroblasts and partly of ASCs but not of mature adipocytes.

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Introduction

In 2012, 6.1 million cosmetic procedures were performed with Botox (botulinum toxin) according to The American Society of Aesthetic Plastic Surgery.[1] Every year, autologous fat transfer is performed in about 60,000 patients for cosmetic soft-tissue augmentation.[1] The combination of both seems to be beneficial, especially when botulinum toxin (BoNT) is applied some days prior to fat injection. In the region of reduced muscle movement, the injected adipose tissue can connect to the vascular system more easily. Consequently, the outcome of the treatment will be more successful than with autologous fat alone.[2,3]

Autologous fat transfer is a well-established technique for soft-tissue augmentation on aesthetic and reconstructive surgery. Through liposuction, not only an aspirate consisting of mainly adipocytes but also adipose-derived stem cells (ASCs) and fibroblasts can be harvested. The success of autologous fat transfer is influenced by a variety of factors such as harvesting techniques,[4] vascularization, and condition of the recipient site.[5,6] Yet there are no in vitro studies that analyzed the effect of BoNT on ASCs, adipocytes, and fibroblasts in vitro. The knowledge about possible effects on viability, proliferation, and the ability of ASCs to differentiate into adipocytes could make the combination of both a safer procedure and make its results more predictable.

Adipose tissue is a connective tissue containing stored cellular fat and a reserve of mesenchymal stem cells (ASCs) that can divide indefinitely, potentially producing various cellular lines (e.g., fat, bone, cartilage, nerve).[7–9] Because they are able to replace cells that have been lost entirely in atrophied tissues,[10] stem cells offer the hope for restoration of the skin's full mechanical and biological properties. A considerable disadvantage in fat grafting is that implanted cells disappear gradually resulting in unpredictable resorption rates. Long-term studies reported on 10—50% remaining amount of injected fat tissue.[11] These results could be improved by using adjuvants which can be injected together with the tissue on one hand as scaffold or on the other hand as stabilizing agent, for example, injectable PLGA spheres,[12] hyaluronic acid,[13] or bFGF in combination with fibrin[14] or gelatin.[15] In recent in vivo studies, the application of BoNT lead to higher fat graft survival.[16]

The major compounds of BoNT are two polypeptide chains,[17] which induce chemo-denervation of muscles by preventing the release of neurotransmitters such as noradrenaline and acetylcholine at the neuromuscular junction.[18] BoNT is classified into seven toxin types according to immunological properties.[19] It is used and approved for several clinical applications, for example, to treat chronic myofascial pain,[20] headache,[21] urinary incontinence,[22] hyperhidrosis,[23] and — cosmetically — resurfacing of the cheek.[24,25] Recently, BoNT was shown to even enhance wound healing.[26]

In this study, we investigated the effect of BoNT A on mature human adipocytes, ASCs, and fibroblasts.

Materials/patients and methods

Patients and tissue harvesting

Skin and fat tissues were gained from otherwise healthy patients undergoing abdominoplasty. All persons gave written informed consent before participating in the study which had been approved by the ethics committee of the Medical University of Vienna and the General Hospital Vienna (EK no. 1949/2012).

Isolation and cultivation of mature adipocytes, ASCs, and fibroblasts

Adipose tissue was washed in phosphate-buffered saline (PBS, PAA Laboratories GmbH, Pasching, Austria), minced and digested with 2 mg/ml Collagenase Type IV (Sigma—Aldrich, St. Louis, MO, USA) in Hanks' buffered salt solution (HBSS, PAA Laboratories GmbH) for 1 h at 37 °C in a water bath with constant shaking. Digested tissue was filtered through cotton gauze and centrifuged for 5 min at 380 G.

Mature adipocytes were collected carefully and washed in PBS three times. For experiments, 250 μl of cells were cultivated in ThinCert™ cell culture inserts (pore size 8 μm, Greiner bio-one, Frickenhausen, Germany) in adipocyte nutrition medium (PromoCell GmbH, Heidelberg, Germany).

For isolation of ASCs, erythrocytes in the stromal vascular fraction were lysed in 2 ml Red Blood Cell Lysing Buffer (Sigma—Aldrich). Medium was added and suspension was filtered through a 70-μm cell filter. Cells were centrifuged for 5 min at 380 G and the cell pellet was resuspended in Dulbecco's modified Eagle medium (DMEM, Gibco, Life Technologies Ltd., Paisley, UK) supplemented with 10% fetal calf serum (Hyclone, Fisher Scientific GmbH, Schwerte, Germany), 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies Ltd, Paisley, UK). ASCs were cultured at 37 °C in supplemented proliferation medium in a humidified atmosphere with 5% CO_2.

Fibroblasts were isolated from diced human donor skin. Small pieces of tissue were put in 6-well plates and incubated for 2 weeks in DMEM supplemented with 10% fetal calf serum, 1% glutamine (Gibco, Life Technologies Ltd.), 100 units/ml penicillin, and 100 μg/ml streptomycin until fibroblasts migrated out. Cells were then trypsinized and cultivated in tissue culture flasks in a humidified atmosphere with 5% CO_2 at 37 °C.

Treatment of cells with BoNT and lidocaine

ASCs and fibroblasts were seeded into 96-well plates; freshly isolated mature adipocytes were cultivated in ThinCert™ cell culture inserts. Cells were incubated for 30 min with 1 IU/ml, 10 IU/ml, and 20 IU/ml BoNT A (subtype onabotulinumtoxin A, Botox, Allergan Inc., Irvine, CA, USA), or with 1 IU/ml + 1% lidocaine (Xylanaest purum, Gebro Pharma, Fieberbrunn, Austria) and 10 IU/ml + 1% lidocaine, respectively. To obtain different concentrations, a stock solution of BoNT (100 IU/ml) was diluted with physiologic salt solution (Fresenius Kabi Austria GmbH,