Cytotherapy, 2017; ■■: ■■–■■

International Society for Cellular Therapy ISCT®

The adipose tissue–derived stromal vascular fraction cells from lipedema patients: Are they different?

ELENI PRIGLINGER[1,2], CHRISTOPH WURZER[1,2,3], CAROLIN STEFFENHAGEN[1,2], JULIA MAIER[1,2], VICTORIA HOFER[4,5], ANJA PETERBAUER[2,6], SYLVIA NUERNBERGER[2,7,8], HEINZ REDL[1,2], SUSANNE WOLBANK[1,2,†] & MATTHIAS SANDHOFER[5,†]

[1]AUVA Research Center, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Linz, Austria, [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria, [3]Liporegena GmbH, Breitenfurt, Austria, [4]Faculty of Medicine/Dental Medicine, Danube Private University, Krems-Stein, Austria, [5]Austrian Academy of Cosmetic Surgery and Aesthetic Medicine, Linz, Austria, [6]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria, [7]Bernhard Gottlieb University Clinic of Dentistry, Universitätsklinik für Zahn-, Mund- und Kieferheilkunde Ges.m.b.H, Vienna, Austria, and [8]Department of Trauma Surgery, Medical University of Vienna, Vienna, Austria

Abstract

Background aims. Lipedema is a hormone-related disease of women characterized by enlargement of the extremities caused by subcutaneous deposition of adipose tissue. In healthy patients application of autologous adipose tissue–derived cells has shown great potential in several clinical studies for engrafting of soft tissue reconstruction in recent decades. The majority of these studies have used the stromal vascular fraction (SVF), a heterogeneous cell population containing adipose-derived stromal/stem cells (ASC), among others. Because cell identity and regenerative properties might be affected by the health condition of patients, we characterized the SVF cells of 30 lipedema patients in comparison to 22 healthy patients. *Methods.* SVF cells were analyzed regarding cell yield, viability, adenosine triphosphate content, colony forming units and proliferative capacity, as well as surface marker profile and differentiation potential in vitro. *Results.* Our results demonstrated a significantly enhanced SVF cell yield isolated from lipedema compared with healthy patients. In contrast, the adipogenic differentiation potential of SVF cells isolated from lipedema patients was significantly reduced compared with healthy patients. Interestingly, expression of the mesenchymal marker CD90 and the endothelial/pericytic marker CD146 was significantly enhanced when isolated from lipedema patients. *Discussion.* The enhanced number of $CD90^+$ and $CD146^+$ cells could explain the increased cell yield because the other tested surface marker were not reduced in lipedema patients. Because the cellular mechanism and composition in lipedema is largely unknown, our findings might contribute to a better understanding of its etiology.

Key Words: *adipogenesis, adipose tissue, adult stem cells, CD146, lipedema, stromal vascular fraction*

Introduction

Lipedema is a progressive disease characterized by subcutaneous bilateral deposition of adipose tissue in the extremities and buttocks [1,2]. In 60% of lipedema cases, genetic background with familial predisposition has been described [3]. In a recent study, we observed that 89% of lipedema patients had maternal and paternal predisposition over three generations [4]. The genetic background of lipedema was demonstrated in a clinical report with 330 family members, although the involved genes have not been completely identified [5]. Patients suffer for a long time because their symptoms, such as reduced joint mobility, hematoma, and edema, are frequently misdiagnosed with adipositas or lipohypertrophy [6–8]. In fact, the increase in fatty tissue is a consequence of adipocyte hypertrophy and hyperplasia [9] accentuated by alterations of the connective tissue [10]. Furthermore, the enhanced adipocyte growth leads to capillary permeability and insufficient lymphatic backflow associated with hematoma [11]. Lipedema symptoms have primarily been treated with compression [12] or lymph drainage [13–15]. Because lipedema does not respond

†These authors contributed equally.
Correspondence: **Eleni Priglinger**, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Krankenhausstraße 7, 4010 Linz, Austria.
E-mail: Eleni.Priglinger@trauma.lbg.ac.at

(Received 17 January 2017; accepted 21 March 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.03.073

to diets, drugs or physical activity, the only efficient treatment option with demonstrated long-term benefit to date [16] is micro-cannular liposuction in tumescent anesthesia [17–20]. Additionally, radial shockwave therapy is able to enhance lymphatic decongestion in lipedema after micro-cannular liposuction [19], and water-jet-assisted liposuction provides long-term improvement by preserving lymph vessels in lipedema [21].

Lipedema is a hormone-related disease mainly affecting women starting in puberty, after pregnancy or during menopause [5,22]. Estrogen contributes to decreased lipolysis in the gluteal, compared with the abdominal, region due to a distinct pattern of estrogen receptors [23]. Moreover, estrogen regulates bone morphogenetic protein (BMP)-2, which can stimulate adipogenesis but inhibits it by knockdown PPARgammma2; thus, PPARgamma2 may play a role in BMP-induced adipogenesis [24]. However, the exact etiopathogenesis of this disorder is still largely unknown. Examination of adipose tissue of lipedema patients exhibit differences in adipocyte morphology. Suga *et al.* analyzed adipose tissue of lipedema patients and observed infiltration of macrophages and the presence of necrotizing adipocytes. In parallel, enhanced proliferation capacity of adipose-derived stem/progenitor/stromal cells ($Ki67^{+}CD34^{+}$ cells) was described, which seems to promote adipogenesis [25]. Additionally, hypertrophy-induced hypoxia and subsequently enhanced angiogenesis of pathologic vessels might contribute to capillary permeability [26,27]. This would explain the fluid increase in the interstitium and consequently the emerging orthostatic edema.

All in all, little is known about the cell composition including the properties of adult stem and precursor cells in lipedema. The aim of this study was to investigate adipose tissue–derived cells obtained from lipedema patients by liposuction compared with healthy patients. Because adipose tissue is a highly vascularized tissue containing a broad variety of regenerative cells, we concentrated on the adipose-derived stromal vascular fraction (SVF) including adipose-derived stromal/stem cells (ASC). Cells isolated from liposuction material from healthy and lipedema patients were analyzed regarding phenotypic and functional criteria for the identification of adipose-derived cells as defined in the statement of the International Federation for Adipose Therapeutics and Science (IFATS) together with the International Society for Cellular Therapy (ISCT) [28].

Methods

Liposuction

The collection of human adipose tissue was approved by the local ethical board with patients' written consent. Dermal and subcutaneous white adipose tissue was obtained during routine outpatient liposuction procedures from the hips and outer thighs ("saddlebags") under local tumescence anaesthesia. Table I provides patient characteristics. Tumescence solution contained one vial Volon-A 10 mg (Dermapharm), three vials Suprarenin 1 mg/mL (Sanofi), 45 mL bicarbonate 8.4% (Fresenius Kabi) and 60 mL Xylocaine 2% including Lidocaine 0.04% (Astra Zeneca). The harvesting cannulas were triport and 4 mm in diameter (MicroAire System power-assisted liposuction).

Table I. Characteristics of healthy and lipedema patients.

Characteristics	Healthy	Lipedema
N	22	30
Sex	Female	Female
Age (years)	42 ± 10	41 ± 13
Weight (kg)	75 ± 17	91 ± 18
BMI (kg/m^2)	27 ± 6	33 ± 7
Type II	—	3%
Type III	—	5%
Type I–III	—	10%
Type I–IV	—	82%
Stage 2	—	55%
Stage 2–3	—	24%
Stage 3	—	21%

SVF/ASC isolation

SVF isolation was performed as modified from Wolbank *et al.* [29]. Briefly, 100 mL of liposuction material was transferred to a blood bag (Macopharma) and washed with an equal volume of phosphate-buffered saline (PBS) to remove blood and tumescence solution. Afterward, for tissue digestion PBS was replaced with 0.2 U/mL collagenase NB4 (Serva) dissolved in 100 mL PBS containing Ca^{2+}/Mg^{2+} and 25 mmol/L N-2-hydroxyethylpiperazine-N0-2-ethanesulfonic acid (HEPES; Sigma), and the blood bag was incubated at 37°C under moderate shaking (180 rpm) for 1 h. The digested tissue was transferred into 50-mL tubes. After centrifugation at 1200 *g* for 7 min, the cell pellet was incubated with 100 mL erythrocyte lysis buffer for 5 min at 37°C to eliminate red blood cells. The supernatant was aspirated after centrifugation for 5 min at 500 *g*. The pellet was washed with PBS and filtered through a 100-µm cell strainer (Greiner). After another centrifugation step at 500 *g* for 5 min, the supernatant was removed, and the isolated SVF cells were cultured in endothelial growth medium (EGM-2) at 37°C, 5% CO_2 and 95% air humidity or resuspended in EGM-2 for further analyses. After seeding the SVF on plastic surface in expansion medium (EGM-2), the adherent cell fraction including ASC could outgrow as an adherent monolayer and was cultured to a subconfluent state. Medium was changed