Cytotherapy, 2016; 18: 36–40

International Society for Cellular Therapy
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Novel bronchoscopic treatment for bronchopleural fistula using adipose-derived stromal cells

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Abstract

Background aims. In this report, we describe the successful bronchoscopic management of bronchopleural fistula in two patients, using autologous adipose-derived stromal cells. Cell therapy was considered for 2 cases of bronchopleural fistula refractory to conventional surgical treatment after control of the primary disease was confirmed and active pleural infection was ruled out. Briefly, adipose-derived stem cells were first isolated from lipoaspirate and used without cell expansion. In 24 months, we have not received more patients with bronchopleural fistula in our hospital and we have not been able to include more patients. *Methods.* Briefly, adipose-derived stem cells were first isolated from lipo-aspirate and used without cell expansion. A bronchopleural fistula was identified through bronchoscopy, and the mucosa surrounding the fistula was ablated with an argon plasma coagulator. Isolated stem cells were then endoscopically injected into the de-epithelialized area and fistulous tract. If an open thoracostomy was present at the time of the intervention, the same procedure was performed on the pleural side. Bronchoscopic follow-up was scheduled weekly during the first month, monthly during the first year, and then yearly. The underlying etiologies were left pneumonectomy and right lower video-assisted lobectomy for non–small-cell lung cancer. The sizes of the fistulas were 6 mm and 3 mm in diameter, respectively. *Results.* Both patients were discharged on the first postoperative day. The 3-year follow-up revealed a successful and maintained fistula closure, no treatment-related adverse reactions, nonlocal malignant recurrence and improved quality of life. *Conclusions.* This preliminary study showed that bronchoscopic application of autologous adipose-derived stem cells is a feasible, safe and effective procedure for treating bronchopleural fistula.

Key Words: *Bronchoscopy fistula, Cell transplantation, Stem cells, Wound closure*

Introduction

Bronchopleural fistula (BPF), a communication between the pleural space and bronchial tree, is a rare and potentially fatal complication that usually results from surgical procedures involving pulmonary resection. The reported incidence of BPF varies between 1.5% and 28% after pneumonectomy and 0.5% after lobectomy [1].

Management of BPF is still a therapeutic challenge because of a lack of scientific evidence [2]. Therapeutic options range from conservative treatment to aggressive surgical procedures, which are sometimes disabling. In recent decades, flexible bronchoscopy has gained acceptance as a diagnostic and therapeutic modality for patients with BPF, especially for high-risk surgical patients [3]. Multiple biological and artificial compounds have been applied through bronchoscopy, with varying degrees of success [3,4]. However, further studies are needed to elucidate the most effective sealant and the therapeutic approach that produces the most favorable patient outcomes.

Current studies have suggested that adult stem cells could participate in regenerating and repairing

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(Received 5 August 2015; accepted 1 October 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.10.003

diseased airways and lungs [5–8]. We have previously reported the potential application of adipose-derived mesenchymal stromal cells (ASCs) in the management of fistulous pathology of the airways and digestive system [9,10]. In light of our clinical experience, we developed a minimally invasive bronchoscopic treatment of BPF, using ASCs. The therapeutic protocol for the use of stromal vascular fraction of adipose tissue (SVF) was presented to and approved by the authorities of the La Paz University Hospital and the Spanish health authorities, and the patients signed the consent for treatment and for the publication of the results in scientific journals and congresses within the anonymity, according to the requirements of Spanish law and Declaration of Helsinki. The patients signed a detailed informed consent form prior to the procedure, and they were informed of the procedure by surgical team.

Methods

Isolation and characterization of autologous ASCs

We used cells from SVF of 80 to 100 mL of lipoaspirate, that is, ASCs without expansion. To extract the cellular fraction, the washed fat was digested with type I collagenase (GIBCO BRL) at a final concentration of 0.075% in saline solution at 37°C for 45 min. Collagenase was inactivated and tissue disaggregate were then centrifuged for 10 min at 250 *g*, and phosphate-buffered saline was used again to wash the pellet. Centrifugation was repeated, and the remaining erythrocytes were lysed. To end the cellular extraction, a final wash was performed and the product was filtered through a 40-µm nylon mesh. SVF cells containing approximately 1.5% to 2.5% ASCs were isolated after standard abdominal lipo-aspiration according to a previously published protocol [9–12]. Cell viability was confirmed with trypan blue (Sigma). Ten percent of the isolated cells were used for cell growth quantification and characterization. We determined the percentage of ASCs by counting pre-culture and post-culture cells. For characterization, cells were plated in culture dishes at a density of 5000 cells/cm in standard culture medium (Dulbecco's modified Eagle medium + 10% fetal bovine serum + 1% penicillin/streptomycin) at 5% CO_2 and 37°C for 7 days, with culture medium changed at 24 h and 96 h.

Bronchoscopic treatment and clinical follow-up

Prior to the application the Spanish Health authorities of compassionate use, patients had to meet certain inclusion and exclusion criteria (Table I). We performed a minimally invasive bronchoscopic treatment for BPF, using local application of ASCs. Briefly, a standard abdominal liposuction procedure was performed, and the SVF containing ASCs was isolated and implanted without cell culture or expansion. In the same anesthetic procedure, both rigid and flexible bronchoscopies were performed. After locating the BPF, the mucosa surrounding the endobronchial lumen of the fistula was ablated with the use of an argon plasma coagulator (50 W) followed by endoscopic injection of two-thirds of the ASCs under the submucosa of the d-epithelialized areas. Additionally, one-third of the ASCs were suspended in the thrombin component of a fibrin tissue adhesive (Tissucol Duo, Baxter) before combining the 2 components and endoscopically applying the mixture to fill the fistulous cavity. If an open thoracostomy was present at the time of the intervention, the same procedure was performed on the pleural side. The total duration of the process from liposuction to cell injection was less than 4 h. Patients were discharged on the first postoperative day. Bronchoscopic follow-up was performed on a weekly basis during the first month, monthly during the first year and then yearly.

Table I. Minimum criteria that patients should have to apply for compassionate use according to European and Spanish legislation.

Inclusion:
1. A prior diagnosis of BPF in which the usual surgical procedures have failed or are not applicable.
2. Accessibility of the endoscopic approach.
3. Older than 18 years of age.
4. Signed informed consent.
Exclusion:
1. Women who are pregnant or nursing or women of childbearing potential who are unwilling to maintain contraceptive therapy for the duration of the study.
2. Exposure to any investigational drug or procedure within 3 months prior to study entry.
3. Extreme malnutrition, which hinders the liposuction procedure.
4. Active pleuro-pulmonary infection.
5. Allergy to anesthetics or fibrin glue (Tissucol Duo).
6. Active infectious disease known to have tested positive for HIV, HTLV, HBV, HCV, CMV (IgM > IgG) and/or syphilis.
7. Known drug or alcohol dependence,
8. History of cancer in the last 5 years.
9. Any illness, which, in the investigator's judgment, can interfere with treatment.

Patient 1

A 64-year-old male former smoker (60 packs/year), age 57 years, underwent a left pneumonectomy for pT3N1 squamous cell lung carcinoma (stage IIIA) followed by adjuvant radiotherapy (23 Gy). A week after beginning chemotherapy, he was readmitted for fever, productive cough and purulent secretion through the surgical incision. A left pleural empyema