

Exosomes from Mesenchymal Adipose Stem Cells of Homologous Origin; A Case Report on a Resolution of a Bedsore Mediated Through Exosomes from ADSC of Homologous-Allogenic Fat-First Observational Case

Erri Cippini*

Plastic Reconstructive and Aesthetic Surgery, Association of Medical Doctors and Surgeons of the Province of Brescia, Italy

***Corresponding author:** Erri Cippini, Specialist in Plastic Reconstructive and Aesthetic Surgery, Member (n° 3588) of the Association of Medical Doctors and Surgeons of the Province of Brescia, Italy. Tel: +39 0303758502; Email: cippini@daysurgeryclinic.it

Citation: Cippini E (2017) Exosomes from Mesenchymal Adipose Stem Cells of Homologous Origin. Plast Surg Mod Tech 2: 127. DOI: 10.29011/2577-1701.100027

Received Date: 2 August, 2017; **Accepted Date:** 16 August, 2017; **Published Date:** 24 August, 2017

Abstract

I forward an observational case; I think the first case in the world, of a bedsore healed through exosomes from fat derived stem cells of homologous-allogenic origin, i.e. from a relative of the affected patient (!!). Having been dealing since many years with healing of trauma and burn wounds by adipose tissue transferred from patient himself (autologous fat transfer), I have had the occasion to treat a bedsore (skin ulcer due to compression) by a new method. It was a chance, and it worked. Through a Swiss stem cell factory which developed exosomes from ADSC (Adipose Derived Stem Cells), I had the opportunity to treat the bedsore of a woman with exosomes from ADSC of her husband, achieving a resolution in few days after some months of unsuccessful traditional plastic surgery care. It was a chance based on well-established references on the matter of homologous effectiveness of exosomes from MSC (Mesenchymal Stem Cells). The aimed success of the procedure, always in condition of safety for the patient, who was throughout informed on it, was achieved. Presently, it is only a single case report, but it should be of some use in order to overcome and go over the present condition of believed. I could seem to have faced a risky business, especially for the present absence of guidelines on homologous use of microvesicles from [1] MSC, but in the present case report the homologous ADSC exosomes were applied topically (never injected) with no risk of general worsening of the whole-body health of the patient, and the patient was checked every day.

No special purpose is the aim of this observational case report, other than a suggestion for further studies on large number of patients after having well established preclinical evaluation. I think, in the present absence of specific guidelines on the use of microvesicles from homologous origin, that a peer to peer commission and a consensus conference would be set up, in order to avoid every attempt to forward a "Miracle Therapy" and every "Pirate Business", but also to deliver to clinical use a safe and clever way to treat wounds. This observational case report was only a chance that was offered to the patient and to medical researchers, and because I happened to be in the right place at the right moment, I tried and I succeeded. A future deep interest from researchers should be devoted to the healing effect, even if made easy and supported by homing to affected and inflamed target tissue, [2] in terms of a suggested (but not proven) induction by ADSC derived exosomes to any of the three germ layers derived stem cells, or in terms of a simple but important anti-inflammatory effect of these microvesicles on mesodermal layer only which then improves the whole bulk of affected tissue. In my point of view, only an improvement in anti-inflammatory potential of local tissue has happened, but this regenerative potential can be a new item in the never-ending history of medical matter. It could work also on wounds from war fields and from car traumas and in burns and in the loss of tissue (especially for its clear anti-inflammatory potential) and it means that fat tissue is far most more important, and easy and less expensive to harvest, than bone marrow (to say nothing on the importance of homologous tissue other than autologous tissue!!!). There had been a stop in the research trial because of items (maybe of economic business I don't know). The evidence of a business interest in this matter, should never make go lost the foremost importance of ethical issues connected with this homologous use of fat. Anyway, I have had the opportunity to observe this first case and I can witness it through the patient itself. As an observational case report, it comes from bedside to bench but, I hope for researchers, then from bench to bedside in future.

Keywords: ADSC (Adipose Derived Stem Cells); Allogenic ADSC Exosomes Transplant; Allogenic Exosomes Origin; Allogenic Fat Transplant; Autologous Fat Transplant; Exosomes from ADSC; Exosomes from MSC; Fat Transplant; Homologous ADSC Exosomes Transplant; Homologous Exosomes Origin; Homologous Fat Transplant; MSC (Mesenchymal Stem Cells)

Introduction

A very widespread use of autologous fat transplant is a common procedure, and it is a well-known way to treat bad healing wounds and to fill area of tissue loss. Fat is harvested from patient as autologous fat. Mesenchymal Stem Cells from autologous fat tissue (MSC), [3] as Adipose Derived Stem Cells (ADSC), are a new frontier in facing treatment of different diseases and to support regenerative medicine.

Joseph Murray, Nobel Prize in 1990, highlighted the idea of a change from the previous age of ablative surgery, through the present age of reconstructive surgery (by both autologous transplant and homologous transplant), to the future development of regenerative/inductive surgery.

The use of exosomes, microvesicles not carrying deoxyribonucleic acid (other than very little portion of DNA whose importance is not clear) but miRNA, could be a new frontier and foresee new therapy scenarios because of their possible use even from homologous origin.

MSC/ADSC multilineage differentiation potential into multiple mesenchymal lineages, even allowing the possibility of a differentiation in a different germ layer's cells, and their promotion of tissue repair together with a broad spectrum of immuno regulatory capabilities are widely well known MSC/ADSC effectiveness, their safety (even if some item is to be set out), and guidelines on their use in humans are also widely well known.

More information's are available for MSC/ADSC derived exosomes of autologous origin. Not the same for MSC/ADSC derived exosomes of homologous origin. The whole bulk of information on the matter is present in the widely available Literature. Here, a new situation (a very first clinical case) is presented with the only wish to stimulate future research.

Clinical use of exosomes from stem cells.

Mesenchymal Stem Cells [4] secrete many trophic factors and cytokines (through secretomes-microvesicles-exosomes) that have therapeutic relevance for the neurogenic, neuroprotective, angiogenic and anti-inflammatory/immuno regulatory activities. In addition, when in extracellular medium, such micro vesicles/exosomes are proposed as key mediators of information transfer between different cells for tissue repair and regeneration.

The underlying mechanism(s) of reparative action is not clear, but evidences suggest that MSC derived exosomes and mi-

cro vesicles can recapitulate the beneficial effects as their cellular counterparts in tissue regeneration. Thus, exosomes and micro vesicles represent a promising candidate for a novel cell -free therapy for neurodegenerative diseases, and much more widely for inflammatory diseases, that has many advantages in overcoming the limitations and risks associated with the cell-based therapeutics.

Factors secreted from mesenchymal stem cells derived exosomes can suppress local inflammation/immune responses, reduce oxidative stress, fibrosis and cell death, stimulate angiogenesis and induce the recruitment, proliferation and differentiation of endogenous stem cells.

In theory, secretome and micro vesicles from Autologous mesenchymal stem cells have some disadvantages. The procurement of tissues for MSC cultures from patients, donor age-related cellular capacity, and extended culture duration for the preparation of final products (secretome or micro vesicles) can be the obvious limitations. In contrast, secretome and micro vesicles from Allogenic donors have clear advantages. They can be prepared in advance from cultured MSCs that are pre-selected and screened by multiple parameters including exogenous pathogen, donor age, multi potency and secretome profiles with large quantity and controlled quality. Unlike cellular counterparts, exosomes and micro vesicles from allogeneic MSCs, in theory, may not induce immune responses as they lack the immunogenic antigens and are derived from highly immunosuppressive cells. The generation of this type of pre-manufactured "Off-the-Shelf" products from selected and screened allogeneic MSCs under compliant processing facility would lead to the acceleration of clinical translation.

With further experimental evidence of the medical use, exosomes and micro vesicles will be able to give rise to shift from utilizing cell-based therapy to taking advantage of non-cell based (cell-free) approaches in the treatment of neurological and inflammatory disorders.

While beneficial therapeutic effects with long-term safety of MSC-based clinical studies have been demonstrated, there are pitfalls to be considered for clinical application of MSCs. They include (1) local immune responses that can lead to long-term rejection of transplanted MSCs, (2) disruption of local tissue homeostasis causing inflammation, (3) increased risk of tumor formation due to long-term ex vivo expansion and/or local immuno suppression and (4) ectopic tissue formation of donor cell (MSC) origin. These concerns stress the importance of long-term studies for the safety of administering MSCs in neurodegenerative and inflammatory diseases. On the contrary, the use of MSC-derived exosomes and micro vesicles has many advantages over the benefits of parental cell-based therapy. The exosomes and micro vesicles can manipulate the microenvironment of damaged tissues through various mechanisms. They have a reparative potential that mirrors the role of parental cells and that has been established in a number of animal models and whose role is still underway.

Procedure

- Consultation and clerking of patient.
- Signature of patient in the informed consent.
- Fat Harvesting - Description of mini liposuction technique.

Perform the mini liposuction after disinfection of the area of skin (abdomen or hips, the abdomen has a better fat) where fat should be harvested: the harvesting area in as large as a hand. Administration of 50 cc of saline solution with anesthetic and epinephrine (the usual Klein solution) with a normal syringe needle, or better a Klein cannula, through a small incision of 1.2mm.

Then, a particular 1,2mm needle (a Coleman fat harvesting cannula) connected to a 30 cc syringe can run the aspiration of fat with the power of aspiration exerted only by the operator's hand on the plunger of the syringe (no need for a vacuum device, rather it is contraindicated because fat must be treated gently and not managed under excessive pressure).

Obtained 30 cc (or less) of fat, then closure of the little incision with a small plaster (steristrip) or a stitch 4/0 nylon or polyester thread. Apply antibiotic ointment. Patch Prescription of oral antibiotics to take at home for two days. The only contraindication is the use of anticoagulants (e.g.; Coumadin for patients in atrial fibrillation or history of pulmonary thromboembolism that are contraindications by themselves).

Attention, during consultation and clerking, must be paid for avoiding allergic reaction to disinfectant fluid during skin disinfection at the beginning of the procedure and to antibiotic ointment on skin under the dressing at the end of it, or to the antibiotic pills prescribed for use at home. Obvious exclusion if any previous infectious diseases. The harvested fat is distributed with great care and delicacy in three vials of sterile plastic, together with 8 mg of gentamicin and a few cc of saline, and placed in a polystyrene box for shipping by international courier to the stem cell factory (my referee was settled in Switzerland).

The content is declared in the waybill. In the box is also added a vial of blood of the patient for further control and testing for infectious diseases. It should be noted that all the tissues (fat and blood) are sealed in vials that are strictly checked and then put into plastic containers and then again in another plastic container and then again into the final box in polystyrene (also sealed and checked), to avoid any risk of contamination from "in or out". The polystyrene box is then inserted in a cardboard box for shipping. No ice or cooling devices in any part of the box. Harvested fat must be delivered to stem cell factory within forty-eight hours to grant for viability of cells.

Fat will then be processed in the stem cell factory to allow selection and banking of ADSC in three-week time. Then exosomes from ADSC will then be made available. This usual and standard procedure has been applied to thirty patients for autol-

ogous treatment with the aim to exploit ADSC exosomes' anti-inflammatory potential. Four patients authorized the homologous use of their own relatives ADSC exosomes (usually husband for wife or vice-versa).

Only one case happened to be in condition to be used in homologous setting (ADSC exosomes of a husband on a bedsore of his severely ill wife compelled to bed for ALS). The procedure was offered "Free" by the swiss stem cell factory we dealed with. Unfortunately, there had been a stop in the research trial because of items maybe of economic business we are not informed in. Anyway, I think this first and single clinical case has been a true situation to witness something which will support and stimulate future studies. The patient is still alive and the tissue local condition is still good.

Clinical Summary

Following an admission to an intensive care unit ward, in January 2014, (the name of the Hospital is not indicated) the patient A.A, a woman suffering from ALS and definitely lying in bed, did not receive any mobilization. This resulted in the formation of a pressure ulcer (a bedsore) in the right buttock area next to the sacrum bone. All this was discovered after about 10 day stay, when patient was moved to another hospital (after discharge from the former ICU).

Since then, she began to be treated daily with standard medical ointments as Iruroxol (collagenase plus chloramphenicol) and Fitostimoline (Triticum vulgare plus phenoxethanol) cream.

After about 3 months of standard treatment, the bedsore visibly worsened. Once at home, after having got some opinion from family doctor and other medical officers, each one with contrasting ideas, the patient was evaluated by a surgeon who suggested a surgical debridement and a check up for new debridement sessions to be done monthly. During time, the plague had been worsening more and more. From March 2014 to August 2014, three cleaning procedures of debridement had been performed in a private facility. At first a slight improvement seemed to be brought about, but after a few days the situation worsened again, and returned to a condition where, indeed, as in patient's husband words, the underlying tissue (gluteus maximus muscle) could be seen.

In September 2014, through a swiss stem cell factory, ten vials of a liquid solution based on exosomes from mesenchymal stem cells of the husband's adipose tissue was sent to be sprayed locally on the bedsore suffering area.

- Carboxyfluorescein Succinimidyl Ester (CFSE) 100% positivity, which highlights the presence of corpuscolated material of biological nature and cellular derivation, has been performed on the liquid solution of vials produced by the factory. But this test is not available for these ten vials.

The liquid solution was sent free of any charge, with the purpose to evaluate the effectiveness of its topical use, on my suggestion,

with the idea to sprinkle (with a syringe without the needle) directly on the wound, all for 10 days with about 10ml of solution each application, once a day. (Figure 1)



Figure 1: Pictures of wound.

Treatment began in late December 2014

After three applications, the regrowth of tissue and narrowing of the plaque could be clearly seen. After eight applications, the wound had reduced by 80%, and within a few days after the 10th and final application the wound was completely gone, raising the total amazement of all medical operators and nurses. The treatment deals with a whole often procedures of local (topical) application of 10 ml (once a day) of a liquid containing exosomes applied through sprinkling the solution at the time of the daily changing of dressing.

Medication technique (topical local application was the new idea!): - from one of the ten sealed vials sent by the stem cell factory and carefully kept in a household fridge, a total of 10 ml solution is taken and applied (after having cleaned with water and soap half an hour before, and then cleaned with sterile saline solution) directly by sprinkling on the wound, inside and outside the area of the bedsore, through a sterile syringe without the needle

The solution is left in contact with the bedsore area for five

minutes (on open air) and then a dressing is made through a no sticking material (Allevyn). NB During the treatment the patient has always kept the lying position in bed or on a wheelchair, and never has she been in other positions (for example lying on the other side).

Long Term Effect of the Therapy

Many diseases (in animal models, at the moment) may have a suggested improvement in treatment result by the use of MSC derived microvesicles as listed in dedicated medical literature.

Moreover, as highlighted in my very short bibliography, I wouldn't like to bother readers and researchers improving the whole bulk of words in this article of mine. I simply had the chance to witness a clinical result after a new treatment still out of available guidelines: homologous use of fat derived exosomes by, I wish to underline, topical application. And the patient, a single case, at the moment, is still in good clinical condition and never has had any adverse reaction.

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