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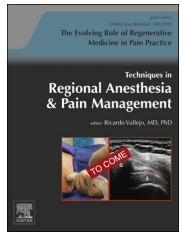

Safety of stromal vascular fraction cells applications in chronic pain

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ABSTRACT

Autologous stromal vascular fraction (SVF) can be enzymatically released from lipoaspirate obtained under local anesthesia. SVF is known to have regenerative, anti-inflammatory, pain mitigating, and immune-modulatory properties. Our translational research network has been studying the safety and efficacy of SVF since 2012. Almost 100 related physician teams around the world are applying the same institutional review board–approved methods of SVF production, which use a surgically closed SVF isolation system. During the same outpatient surgical procedure, procured SVF is administered according to strict investigative protocols to mitigate diseases associated with chronic pain including arthritis, autoimmune disease, neurodegenerative disease, and various inflammatory conditions. The shared research collaborative online database contains safety and efficacy data on more than 3500 patients. Our processed SVF contains valuable anti-inflammatory cytokine growth factors in addition to both adult mesenchymal and hematopoietic stem cells targeting damaged, or inflamed tissue. SVF administration may potentially play a large role in the outpatient treatment of pain. In this article, we describe our protocol for the production and administration of SVF, and its safety and efficacy in the treatment of pain associated with chronic conditions.

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Background

Stromal vascular fraction (SVF) isolated from connective tissue associated with subcutaneous fat and blood vessels is known to contain adult mesenchymal stem cells (MSC), T regulatory cells, endothelial precursor cells, preadipocytes, anti-inflammatory M2 macrophages, and numerous cytokine growth factors.[1] A large amount of veterinary experience with SVF has demonstrated its safety and efficacy.[2] There is an extensive anecdotal, and more recently, evidence-based information to suggest that adult MSCs may have a significant beneficial use for a variety of autoimmune, inflammatory, and degenerative conditions, and MSCs have been shown to accelerate healing as well as exhibit immunomodulatory effects.[3-10] MSCs also may be systemically effective at dampening overactive pain fibers signals, and in animal models for interstitial cystitis, injected MSCs activated the Wnt signaling cascade to alleviate pain.[11]

The most experience gained using SVF for the treatment of pain has been related to osteoarthritis. Published data on safety and outcomes using stem cells for arthritis are still scant. Recently, Michalek et al[12] from the Czech Republic studied 1856 joints in 1128 patients with grade 2-4 degenerative osteoarthritis, and demonstrated an excellent safety and very high efficacy using lipoaspirate predominantly processed with collagenase. A study by Centeno et al[13] using

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bone marrow-derived cells for osteoarthritis treatment showed similar safety and efficacy.

Our research network mainly focused on treatment of conditions involving the musculoskeletal system, but encompassing many degenerative conditions (article in preparation). Here, we describe the SVF treatment protocol adopted and its safety in the treatment of pain associated with chronic conditions.

Methods

This publication evaluates safety of treatment of 1524 patients. Overall, our research network treated more than 4000 patients with SVF under institutional review board (International Cell Surgical Society) approval for the investigational use of SVF (Clinicaltrials.gov #CSN111). Patients with neurodegenerative diseases, osteoarthritis, erectile dysfunction, autoimmune diseases, cardiomyopathies, and emphysema were included. Exclusion criteria in the study included patients younger than 18 years of age, severe coagulopathy, systemic infections (especially dental infections), and metastatic cancer. Many of the patients had already been treated with numerous medical (nonsteroidal anti-inflammatory drugs or opiates) and regenerative therapies including platelet-rich plasma and commercially available preparations containing amniotic stem cells. Patients signed institutional review board–approved informed consents, and underwent an additional history and physical examination before their procedure. To harvest fat, patients received instillation of local anesthetic (lidocaine 0.5% with epinephrine 1:400,000 and sodium bicarbonate 8.4%) using a nontumescent "subdermal" method into a small region of skin on the posterior flanks. After deployment of local anesthetic, a 3 mm puncture wound was created and a mini liposuction was performed using the negative pressure syringe technique.

Approximately 50 mL of lipoaspirate solution was obtained and condensed by centrifugation. Roche GMP grade collagenase was added to the condensed fat and incubated at 38 °C for 30 minutes to digest the collagen matrix to release the SVF in a closed fashion in the operating room. This yielded the "vascular" portion of the SVF as noncollagenase methods (lecithin or mechanical and "nutational" methods) of processing fat only yield the "stromal" component. Optimal SVF contains both elements. The Time Machine by Medikan International Inc., South Korea (Food and Drug Administration approved for fat preparation) was used to isolate the SVF product. The SVF was sequentially washed and then filtered through a 100-μm nylon filter. Photomicrography was performed to document lack of aggregation, and to allow for a basic cell count and quantification of cell viability.

Once SVF was prepared as a final sterile product (approximately 10 mL sterile fluid) patients received treatment in accordance with protocols for their specific conditions. Administration of SVF into soft tissue and joints was performed by experts working within their respective scope of practice and usually under radiographic guidance. Any short- and long-term complications were followed-up, as well as mild, moderate, or serious adverse events, and reported on our online database. Patient verbal response to treatment ("improvement" vs "no improvement") was documented as well. Most of the specialists involved in the project had expertise in sports medicine, orthopedics, anesthesia pain, or surgery. All outcome data were collected on an online database over a 5-year period.

Results

Autologous SVF of 10 mL produced from 50 cc of adipose tissue lipoaspirate in a sterile was isolated in surgically closed system within 2 hours in the operating room. One-half (5 mL) of the SVF was injected locally and another half (5 mL) was injected intravenously (IV). A small number of patients (3%) received only local SVF injection because of difficult IV or patient refusal. In patients treated with IV injection of the SVF, there were no cases of infection, pulmonary emboli, or any other complications reported. To the best of our knowledge, this is the largest reported series of stem cell-based systemic treatment to date and was associated with an excellent safety profile.

A number of conditions associated with chronic pain have been treated with SVF; osteoarthritis and musculoskeletal disease are the most common. Large weight-bearing (as well as small peripheral joints), cervical, and lumbar spine (facets, epidural, and disk targets) cases have also been treated. Most of the patients we have treated had chronic pain from osteoarthritis; approximately 25% of these showed radiographic evidence of cartilage formation with more than 80% showing significant reductions in pain. The Figure and Table present the treatment outcomes from our database. Most cases of successful arthritis treatment were sustained for well over 6 months and most responding patients appeared to have beneficial effects that persisted for several years.

Neurodegenerative diseases have also been treated with SVF, many cases of which are associated with painful neuropathy as well as sensory nerve abnormalities. Many other conditions associated with chronic pain were also treated successfully including temporomandibular joint syndrome, complex regional pain syndrome types 1 and 2, fibromyalgia, and vulvodynia. Of 53 patients treated for interstitial cystitis, more than 80% showed pain reduction.

Discussion

The stem cell therapy mechanism of action is still poorly defined. Some stem cells affect healing by engraftment and transdifferentiation whereas others do so through the paracrine effect, inducing damaged cells to heal using signaling molecules. Clinical cell therapy in painful joints appears to turn off the inflammatory signals of the arthritic joint milieu, reducing pain and inflammation, and allowing healing, with cartilage formation in some cases.

Response rates to SVF exceeded those expected with placebo. Furthermore, it is unlikely that patients experienced a significant placebo effect with SVF, as they had already failed numerous other therapies and then responded to SVF. No placebo trials with SVF for chronic pain have been conducted by our research group to date; however, a

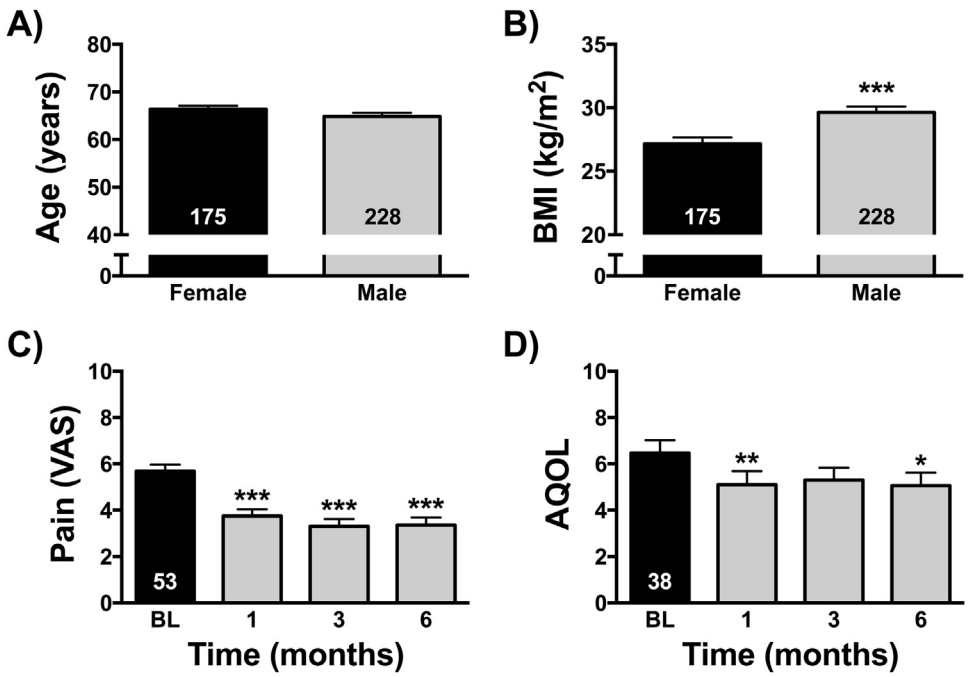

Fig – Baseline data, pain, and AQOL. In all patients with orthopedic diseases, (A) the age of female and male patients was not significantly different from each other, (B) although male patients had significant higher BMI as analyzed by unpaired student t-test. Time had an overall significant effect on (C) pain ($P < 0.001$) and (D) AQOL ($P < 0.006$) as analyzed by one-way ANOVA, whereas the post hoc comparison revealed a significant reduction in pain after 1, 3, and 6 months, and for AQOL after 1 and 6 months. Data are presented as mean ± SEM and were assumed significant when $P < 0.05$. ANOVA, analysis of variance; AQOL, assessment of quality of life; BMI, body mass index; SEM, standard error of the mean.

double-blind placebo-controlled trial for knee osteoarthritis is planned in the near future.

The advantages of using adipose tissue as a source of stem cells is that SVF contains at least 100-fold more adult stem cells than bone marrow-derived sources. Bone marrow stem cells have limited stem cell quantities (even when "concentrated"), and therefore have to be cultured and expanded to achieve numbers similar to that found in fat. Furthermore, bone marrow aspiration is considerably less comfortable for patients than mini liposuction using our surgical technique. It was developed especially for this procedure and administers a subdermal (not tumescent cytotoxic) anesthetic to protect cell integrity. Fat derived cells appear to be best suited for immunomodulation.[14] However, accurate cell source comparison data are conflicting and it is likely that on a cell by cell comparison, both fat and bone marrow sources of stem cells yield adequate functioning cells with good proliferative and differentiating potential, and the ability to heal damaged tissue.

Table – Treatment outcomes for selected orthopedic conditions.

Localization	Number of patients	% With improvement
Knee	381	81
Hip	53	89
Shoulder	70	84
Spine	58	81

Conclusion

SVF is expected to have an important role in medical healing and in dealing with chronic conditions associated with pain. Because this system is simple to use, safe, and cost effective, it may provide many patients with a preferential alternative for treating conditions that would otherwise require more invasive and expensive procedures (eg, total joint replacement). We can conclude that intravenous, intra-articular administration of SVF into soft tissue is generally safe and well tolerated for the treatment of various conditions associated with chronic pain. A limitation of this study was that there was no placebo arm used to evaluate efficacy. In the future, more stratified and long-term outcome data and controlled studies are necessary to further investigate treatment outcome for chronic pain conditions.

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