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Adipose derived Stromal Vascular Fraction (SVF) cells in the treatment of rheumatoid arthritis of the hand: a 2-year follow-up study

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Abstract

Objective: **Rheumatoid arthritis of the hand constitutes a debilitating aspect of this chronic autoimmune disease. Conventional management with medication is difficult with many side effects. The anti-inflammatory properties of autologous stromal vascular fraction (SVF) cells present an attractive alternative to drug therapy. In a previous small safety and efficacy study we documented therapeutic effect of SVF cells administered intra-articularly and periarticularly for rheumatoid arthritis of the hand in 5 patients. Six-month results reported showed a strong reduction in joint paint, improved function and increased grip strength. These finding were consistent with known anti-inflammatory properties of SVF cells in soft tissues affected by burns, ischemia, and fibrosis. This follow-up study of the previous series is intended to ascertain the longevity of results achieved by SVF treatment in RA of the hand at 12 and 24 months.**

Patients and Methods: **5 female patients with RA greater than 5 years duration were chosen for treatment and long-term follow-up. Lipoaspirate was used to process and generate SVF cells as previously described. Each joint was injected with a total of 1 cc distributed intra-articularly and around the joint capsule. Serial measurements were taken at 6 months, 12 months, and 24 months post-implantation using a functional hand score (FHS: pain, stiffness and activities); Visual Analog Score (VAS: pain intensity) and dynamometry (grip strength).**

Results: **Percentage changes at six months for FHS and VAS remained essentially unchanged from 6 months to 12 months to 24 months, showing durability of the response. Average grip strength was preserved at 12 and 24 months as well, with a slight decline relative to the 6-month values.**

Conclusions: **Engraftment of SVF cells to a niche in the highly vascularized synovium with survival of cells may possibly explain the longevity of the results. Despite systemic disease affecting other joints SVF at the treatment sites appears to play a localized and long-term protective function.**

Introduction

The effects of rheumatoid arthritis (RA) on the diarthrodial joints of the hand are particularly debilitating, causing pain with even the most routine of activities. Medical therapy is fraught with side effects[1]. The pathology of RA is biphasic beginning with an autoimmune response to collagen in the joints followed by inflammation and erosive destruction of both cartilage and bone. Helper T cells Th1 and Th17 are implicated as the reactive agents, producing chemokines that attract inflammatory cells into the synovium causing tissue destruction[2,3].

Stromal vascular fraction (SVF) cells are a heterogeneous cell population obtained from the enzymatic digestion of adipose tissue. SVF contains adipose-derived stem cells (ADSCs), pericytes, endothelial progenitor cells (EPCs), and fibroblasts[4,5]. Acting via paracrine factors[6] SVF cells demonstrate that can enhance the immunosuppressive effects of Treg cells in a murine model of collagen-induced arthritis[7]. The Tregs produce the anti-inflammatory immunoregulator Il-10. Given the safety profile of SVF cells transplanted subcutaneously and intra-articularly, we undertook a first-in-man open-label, non-randomized proof-of-concept trial using non-culture expanded adipose-derived SVF cells administered into and around the diarthrodial joints of the hand with surprising results at six months, including the changes of 91.3% and 91% from the baseline for functional hand score (FHS)[8]. Given the significant therapeutic effects observed for an arthritic pain in the hands, we wanted to determine the durability of these results over time.

Patients and Methods

Ethics Statement

This follow-up study was approved by the Medical Ethics Committee of UNAN-Leon and by the Ministry of Health of Nicaragua. The procedures followed in this study were purely observational and in accordance with the Ethical Standards of the Responsible Committee on Human Experimentation (Institutional and National, Universidad Nacional Autonoma de Nicaragua, León and the Helsinki Declaration of 1975, as revised in 2000). Informed consent was obtained from all participants in accordance with MINSA and the World Health Organization; this included the consent to publish this follow-up study.

Study Design and Patient Selection

This was a follow-up to a previously published small safety and efficacy study[6]. The study examined the effect of the stromal vascular fraction for the treatment of rheumatoid arthritis of the hand.

Patient Characteristics

All patients were right-handed between the ages of 41-74 years. Prior to injection, all patients have had rheumatoid arthritis affecting both hands for more than five years. Two patients had concomitant arthritis of both shoulders with significant limitation in range of motion. One patient had concomitant arthritic involvement of both ankles. None were smokers nor had concomitant chronic diseases.

Procedures

Collection and processing of SVF and the means of administration were previously described[9]. The tissue was collected and processed in a GID SVF-2 device and system (The GID Group, Louisville, CO, USA). An enzyme was used to dissociate the tissue. SVF cell viability ranged from 75% to 95% with SVF yield ranging from 61.8 x 10^6 to 410 x 10^6 SVF cells. These initial instruments, (FHS), visual analog scale (VAS), and grip strength as measured by dynamometry, were administered for each hand (Figure 1).

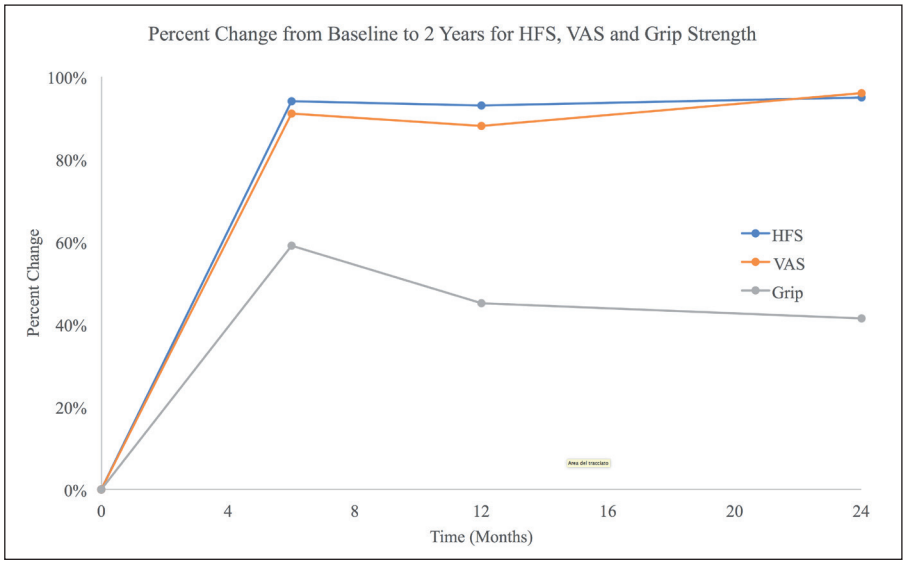

Figure 1. Percent Change from Baseline to 2 Years for HFS, VAS and Grip Strength.

Table 1. Statistics.

p*-values** ***Baseline comparison to	**1 year**	**2 years**
VAS	$p<0.0001$	$p<0.0001$
FHS	$p=0.00$	$p=0.00$
Grip strength	$p=0.003$	$p=0.002$
Cohen's d ***Baseline comparison to***	**1 year**	**2 years**
VAS	$p<0.0001$	$p<0.0001$
FHS	$p=0.00$	$p=0.00$
Grip strength	$p=0.003$	$p=0.002$

Cohen's definition of the effect size for VAS and FHS is huge and for grip strength very large.

Measurement Instruments

Functional hand score (FHS) was described previously. This is a patient-rated instrument with a total of 12 questions, each one scored with a 5-point Likert scale with 5-point Likert scale rating symptoms as: 1 = None, 2 = Mild, 3 = Moderate, 4 = Severe, and 5 = Extreme. Questions are in three categories: pain (5 questions), stiffness (2 questions), and function (5 questions). Pain questions are specific to pain opening a jar, pain with handwriting, pain that disturbs sleep, pain on turning a key or knob, and pain on opening a door. Stiffness distinguishes between morning stiffness and evening stiffness. Function questions assess difficulty with: carrying heavy objects (purse), using scissors, cutting up food with a knife, buttoning/unbuttoning a shirt, and difficulty tying a knot. In FHS, the minimum score possible is 12 and the maximum score was 60. Adjustment of the raw score to 100 points was done with a multiplier of 1.66.

Visual analog scale (VAS) is a patient-rated instrument to measure pain on a scale of 0 (no pain) to 10 (extreme pain).

Grip strength was measured with a dynamometer using the average of five attempts for each hand. The attempts have been alternated to allow the hand to rest and not have consecutive fatigue.

Statistical Analysis

A follow-up of a small safety and efficacy study was carried out. A baseline comparison to 6 months, 12 months and 24 months was evaluated using a 2-tailed paired *t*-test, Cohen's % confidence intervals, and Cohen's d test to quantify the size of the effect (Table 1). As reported in the previous study, there was no control group.

Results

Efficacy

All scores at 12 and 24 months maintained similar results to 6 months. We reported an average percent difference of 94% for FHS from baseline at 6 months. We found similar results at 12 months and 24 months (93% and 95%, respectively) (Table 2). All timepoints showed significant differences from baseline at $p<0.000$ and huge effect size (Table 3,4]. The VAS showed a 91% relief of pain at 6 months. At 12- and 24-months follow-up, this relief was maintained at 83% and 96%, respectively (Table 5). All time points showed significant

Table 2. Hand Function Scores from Baseline to 24 months *(100 point total with descending score indicating improvement).*

Subject	**Baseline**	**6 months**	**12 Months**	**24 Months**
1-L	91.5	2.1	0.0	2.1
1-R	79.0	12.5	0.0	2.1
2-L	22.9	0.0	2.1	0.0
2-R	41.6	2.1	4.2	0.0
3-L	31.2	2.1	2.1	2.1
3-R	33.3	2.1	2.1	2.1
4-L	12.5	0.0	0.0	0.0
4-R	43.7	0.0	6.2	2.1
5-L	85.3	10.4	10.4	16.6
5-R	99.8	10.4	10.4	8.3
Mean	54.1	4.2	3.7	3.5
Std Dev	31.7	4.9	4.0	5.2
% Change from Baseline		94%	93%	95%

Table 3. Hand Function Score, VAS and Grip Strength showing significance and Cohen's d (effect size) based on percent change from baseline at 12 and 24 months.

	HFS	VAS	Grip Strength
p-value at 12 months	0.000	<0.001	0.003
p-value at 24 months	0.000	<0.001	0.002
Cohen's d at 12 months	2.23	4.42	0.99
Cohen's d at 24 months	2.22	4.74	0.89

Table 4. Cohen's d Effect Size.

d	0.1	0.2	0.5	0.8	1.2	2.0
Effect Size	Very Small	Small	Medium	Large	Very Large	Huge

differences from baseline at $p<0.001$ and a huge effect size (Tables 3,4).

Grip strength was only measured in 4 patients (8 hands). One subject was unable to grip the dynamometer at baseline, due to metacarpophalangeal joint deformity. The gain in average grip strength of the 4 subjects decreased over time from 59% at 6 months to 45% at 12 months and 41% at 24 months (Table 6). All subjects maintained a significant increase in grip strength relative to baseline $p<0.003$ for all time points and a large effect size (Tables 3,4).

Safety

There were no device-related adverse events. Two subjects reported flaring of RA symptoms in multiple joints necessitating increase medication; however, during these flares, those joints treated with SVF remained asymptomatic.

Discussion

Rheumatoid arthritis affects 0.5% to 1% in developed countries and it is 2 times more prevalent in women than men. RA is an inflammatory systemic autoimmune disease and there is no known cure[10-12]. Conservative therapies include orthosis, exercise, joint protection, adaptive equipment, and medication. Medications only work to slow down or temporarily decrease inflammation and come with substantial side effects. Progression can result in severe disability if not managed and in the risk of other concomitant diseases. Our previous paper describes a first-in-man, small, open-label feasibility study using SFV cells at the point of care for diarthrodial joints; however, in a small study it was showed a very strong therapeutic effect at 6 months. The current study followed up with these patients, to determine if this treatment could effectuate a longer remission.

Table 5. VAS Pain Score from Baseline to 24 months *(0-10 point scale with descending score indicating less pain).*

Subject	Baseline	6 months	12 Months	24 Months
1-L	9	1	1	1
1-R	7	1	1	0
2-L	4	0	1	0
2-R	7	0	1	0
3-L	8	2	2	2
3-R	5	1	1	0
4-L	5	1	0	0
4-R	8	0	1	0
5-L	8	0	0	0
5-R	10	0	0	0
Mean	7.1	0.6	0.8	0.3
Std Dev	1.9	0.7	0.6	0.7
% Change from Baseline		91%	88%	96%

Table 6. Grip Strength from Baseline to 24 months *(Subject 5 was unable to grip the dynamometer at baseline).*

Subject	Baseline	6 months	12 Months	24 Months
1-L	17.0	25.0	22.7	20.9
1-R	17.5	36.0	27.7	25.0
2-L	20.0	27.0	20.9	22.7
2-R	21.0	28.1	27.2	23.6
3-L	24.0	25.0	25.0	29.5
3-R	24.0	34.0	34.1	34.7
4-L	0.0	17.0	12.7	13.6
4-R	8.0	17.0	20.5	15.9
5-L				
5-R				
Mean	16.4	26.1	23.8	23.2
Std Dev	8.4	6.9	6.3	6.8
% Change from Baseline		59%	45%	41%

It shows that a single injection of SVF cells for the treatment of RA in the hand has been effective in all patients for 2 years. The two most striking observations in this study are (1) the durability of the response to 24 months and (2) the near-complete elimination of pain (>90%) in the treated joints. The therapeutic effect of SFV cells may perhaps relate to engraftment in the inflamed synovium which in turn relates their natural perivascular niche. The action of SVF cells in RA is based on their ability to inhibit the pro-inflammatory response in the joints. The longevity of these effects in the treated joints is remarkable, the absence of relapse in these hands has continued despite the presence of on-going chronic disease. These results warrant additional studies as for the mechanisms involved.

Conclusions

The original study and this two-year follow-up study constitute, to the best of our knowledge, the first reports documenting the effects of fresh, non-fractionated, non-culture-expanded adipose-derived SVF cells for the treatment of rheumatoid arthritis of the hand. The improvement in hand function achieved argues for further study of this treatment modality. We anticipate follow-up of these patients in the future to observe if the treated joints continue to resist the assault of other active systemic diseases.

Declaration of funding interests:
Devices and enzymes for this study were donated by The GID Group, Louisville, CO, USA.

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Informed Consent:
Informed consent was obtained from all participants in accordance with MINSA and the World Health Organization; this included the consent to publish this follow-up study.

Ethical Committee:
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Conflict of interest:
Michael Carstens consults for the GID group. The remains authors declare that they have no conflict of interests.

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