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Short title: autologous adipose-derived stromal vascular fraction in scleroderma

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ABSTRACT

Introduction

Hand involvement confers a substantial handicap in work and daily activities in patients with Systemic sclerosis (SSc). Autologous adipose-derived stromal vascular fraction is as an easily accessible source of cells with regenerative effects. We previously performed a phase I open-label clinical trial (NTC01813279) assessing the safety of subcutaneous injection of autologous adipose-derived stromal vascular fraction. Six and 12 month data have been reported. As patients were followed in our medical centre, we report their longer term outcome beyond the end of the trial.

Patients and method

Twelve females, mean age 54.5±10.3 years, initially enrolled in the clinical trial were assessed during a scheduled medical care, which took place between 22 and 30 months after treatment. **Results**

Multiple patient-reported outcomes showed sustained improvement, in comparison with the assessment performed just before surgery: 62.5% in the Cochin Hand Function Scale, 51.1% in the Scleroderma Health Assessment Questionnaire, 33.1% in hand pain, and 88.3% in the Raynaud Condition Score. A decrease in the number of digital ulcers number was noted. Mobility, strength and fibrosis of the hand also showed improvement. None of the 8 patients who had previously received iloprost infusion required new infusion.

Conclusion

Despite the limits of an open label study, the data are in favour of the long-term safety of the adipose-derived stromal vascular fraction injection. Two randomized double blind, placebo-controlled trials of this therapeutic agent are ongoing in the USA (NCT02396238) and in France (NCT02558543) and will help determine the place of this innovative therapy for SSc patients.

KEYWORDS: systemic sclerosis, stromal vascular fraction, adipose tissue

Introduction

Hand involvement is frequent in Systemic sclerosis (SSc) and confers a substantial handicap in work and daily activities [1]. To date, clinical care for the hand relies on vasodilators, cold and trauma protection, and regular physiotherapy. No antifibrotic therapy has proven effective.

The regenerative properties of cells derived from adipose tissue have been explored for over a decade. In 2002, Zuk identified and described a putative population of multipotent stem and progenitor cells within the stromal vascular fraction (SVF) cell population derived by enzymatic digestion of adipose tissue [2]. The SVF is composed of blood cells, fibroblasts, endothelial cells and their progenitors, pericytes, adipose stromal/stem cells (ASC) and preadipocytes. This population has been reported to possess multiple angiogenic, anti-inflammatory, immunomodulatory and regenerative properties [3].

We previously performed a phase I open-label single center clinical trial, called SCLERADEC (NTC01813279) assessing the safety and efficacy of adipose-derived stromal vascular fraction (ADSVF) in 12 SSc patients followed for 6 months [4], and later reported their extending outcome at 12 months [5]. We took advantage of routine medical follow-up of these patients to assess their longer term outcome.

Patients and methods

Patients: Twelve SSc patients, all female, were enrolled from December 2012 through May 2013. The population was composed of 8 subjects with limited cutaneous SSc and 3 with diffuse disease. Three patients were classified as early SSc disease (< 4 years). Subjects had mean age of 54.5±10.3 years and body mass index of 22.0±2.1 kg/m^2. As part of the inclusion criteria all subjects had hand disability of at least 20 points using the Cochin Hand Function Scale (CHFS).

Procedures: Adipose tissue collection, ADSVF extraction and quality controls have previously been described [4-6]. ADSVF was obtained within 2 hours after lipoaspiration using the automated processing Celution800/CRS system (Cytori Therapeutics, San Diego, USA). An average of 3.76±1.85 $x10^6$ cells was injected into each finger as previously described [3-5, with video of the surgical technique in 6].

Outcome: After completion of the trial patients continued their routine medical appointments in the internal medicine department. This allowed us to assess their hand function and other parameters using the same evaluators and same tools as those applied within the trial [4]. This was performed during a single visit occurring within the range of 22 to 30 months from the date of the ADSVF injection.

Data analysis: Continuous data were summarized by mean±standard deviation and median [minimum-maximum]. CHFS, Scleroderma Health Assessment Questionnaire (SHAQ), Raynaud's Condition Score (RCS) and hand pain visual analogue scale (VAS) were analyzed as co-primary outcomes. Mean changes from baseline were analyzed using mixed model with cutaneous form (diffuse or limited) as fixed effect and time (2, 6, 12 and 24 months) as repeated effect. Least square mean differences form baseline were tested with Tukey adjustment. Significance was set at $p<0.05$ level after adjustment.

Results

Consistent with prior reports [4,5], no evidence of treatment-related adverse events was noted in any patient. Results of functional assessments are shown in Table 1 and Figure 1. Results show that the benefit reported at the 6 month time point of the trial is sustained at 22-30 months. For example, the long-term follow-up data for CHFS, SHAQ, and RCS endpoints showed 62.5%, 51.1% and 88.3% improvement over baseline respectively. It is worth noting that the decrease of the VAS for hand pain which had lost statistical significance at 12 months post-surgery [5] regained significance at 24 months (33.1% decrease from baseline) (Figure

1). Improvement in objective endpoints such as Jamar grip strength reported at 6 months was sustained in the current assessment while pinch strength showed continued improvement. The modified Rodnan Skin Score focused on hands decreased from 10.9±4.9 at baseline to 8.8±5.9 although this difference did not achieve statistical significance. Lateral range of motion of the fingers assessed with the first corner distance and the sum of the 2-4th corner distances did not change significantly. The sum of pad to distal palmar line distances decreased over time for both hands without reaching significance p-value. The Kapandji score was high at baseline and did not change over time. The total number of digital ulcers (DU) number was 15 at entry and 6 at last visit.

Mean global disease severity score (Medsger scale) remained stable: 2.5 [1-3] at baseline and 2.5 [0-3] at last clinical assessment. Comparison of changes in medical drugs from baseline to last visit showed that none of the 8 patients who had previously received iloprost infusion required new infusion. One patient with diffuse cutaneous SSc was prescribed an immunosuppressive medication due to progression of skin fibrosis and another patient with diffuse cutaneous SSc received sildenafil for recurrent ischemic digital ulcer. Three patients previously treated with a calcium channel blocker stopped this treatment due to side effect without worsening of Raynaud's phenomenon.

Discussion

While the current report is limited by the repetition of hand assessment in an extended follow-up from an original phase I open label study and the potential bias related to natural course of the disease and the seasonal effect, the long-term assessment is in favour of safety and durability of the effect. Given the costs associated with preparation of an autologous autologous cell therapy product to standards required by local, national, and European regulatory authorities it is important to assess the apparent durability of the response in order to begin to build a pharmaco-economic argument for or against this therapy. The current study, with evidence of apparently sustained—possibly progressive—improvement and the reduction in need for costly medications such as iloprost, represents the first step in building the data set needed for a cost-benefit assessment. Due to the lack of a placebo group, we are aware that we cannot be definitive about the causal link between the long-term hand

improvement and ADSVF injection. However, it appears somewhat unlikely that the substantial magnitude of change over such a prolonged period could be attributed to a placebo effect and is unlikely to represent spontaneous sustained positive changes in the natural history of the disease. Rather, from a physiopathological point of view, we believe that the combined and synergistic pro-angiogenic, anti-inflammatory, anti-fibrotic and immunomodulatory effects possessed by the ASC and other progenitor cells inside the ADSVF [3] could explain these clinical results. Of course, the final determination of this question will depend upon the results of the robust randomized, placebo-controlled trials such as the two trials currently underway in France and in the USA.

Conclusion

Although interpretation of these results is hampered by the lack of a control group, the use of autologous ADSVF is an innovative therapy which appears to provide benefit for patients suffering from SSc who have hand dysfunction. Two randomized double blind, placebo-controlled clinical trials with this form of ADSVF are ongoing: Scleroderma Treatment with Celution Processed Adipose Derived Regenerative Cells (STAR) in USA and SCLERADEC 2 in France. Such evidence will help guide health care professionals on the place of this therapy for SSc patients.

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Table 1: Outcome of assessed parameters from baseline to last complete evaluation

	Mean±SD Median [min-max]			Least square mean difference	Test of LSM difference at M24	
	Baseline	**6 months**	**22-30 months**	**[adjusted 95% CI]**	**raw p-value**	**p-value Tukey adjustment**
SHAQ Score /3	1.4 ± 0.3 1.4 [0.8 - 2.1]	0.8 ± 0.4 0.8 [0.0 - 1.5]	0.7 ± 0.5 0.7 [0.0 - 1.9]	-0.6 [-1.1 ; -0.1]	**0.0006**	**0.0051**
CHFS total /90	48.5 ± 10.8 48.5 [30.0 - 69.0]	21.2 ± 15.4 20.0 [0.0 - 48.0]	18.6 ± 13.8 13.0 [0.0 - 45.0]	-28.6 [-43.0 ; -14.2]	**<.0001**	**<.0001**
RCS /10	7.2 ± 0.9 7.5 [6.5 - 8.0]	2.9 ± 1.4 3.0 [2.5 - 3.0]	0.8 ± 0.9 0.5 [0.0 - 2.5]	-6.3 [-7.7 ; -5.0]	**<.0001**	**<.0001**
Hand pain VAS /100	59.4 ± 17.2 58.5 [50.0 - 72.5]	17.8 ± 15.3 13.6 [9.0 - 26.0]	29.5 ± 25.2 32.5 [0.0 - 72.0]	-26.6 [-50.1 ; -3.0]	**0.0025**	**0.02**
Jamar score (kg) Dominant hand	16.0 ± 5.8 15.0 [9.0 - 26.5]	19.4 ± 7.4 20.0 [5.0 - 30.0]	19.0 ± 6.1 19.0 [8.0 - 29.0]	3.2 [-3.5 ; 10.0]	0.1793	0.6512
Jamar score (kg) Non-dominant hand	14.9 ± 6.1 14.0 [8.0 - 30.0]	17.6 ± 8.0 20.0 [3.5 - 29.0]	18.6 ± 6.3 18.0 [6.0 - 30.0]	4.3 [-1.6 ; 10.2]	**0.0421**	0.2398
Pinch score (kg) Dominant hand	1.3 ± 1.1 0.9 [0.2 - 4.1]	2.3 ± 1.3 2.0 [0.9 - 5.4]	5.7 ± 1.8 5.0 [3.5 - 9.0]	4.2 [2.7 ; 5.8]	**<.0001**	**<.0001**
Pinch score (kg) Non-dominant hand	1.3 ± 0.9 0.9 [0.2 - 3.2]	2.1 ± 1.0 2.0 [0.7 - 3.6]	5.5 ± 1.5 5.0 [4.5 - 6.0]	3.9 [2.6 ; 5.2]	**<.0001**	**<.0001**
mRSS applied to hand /18	10.9 ± 4.9 11.5 [3.0 - 18.0]	9.9 ± 6.0 12.0 [1.0 - 18.0]	8.8 ± 5.9 9.5 [0.0 - 16.0]	-1.5 [-4.7 ; 1.7]	0.1828	0.6587
1st corner distance (mm) Dominant hand	105.6 ± 24.7 112.0 [57 - 142]	112.9 ± 29.2 118.5 [57 - 154]	121.4 ± 27.9 126.0 [56 - 155]	15.2 [-4.7 ; 35.2]	**0.0353**	0.2084
1st corner distance (mm) Non-dominant hand	115.8 ± 24.5 118.5 [65 - 152]	122.3 ± 20.9 121.5 [88 - 155]	128.7 ± 23.4 130.5 [90 - 174]	13.4 [-6.0 ; 32.7]	0.0554	0.2974
Sum of corners distances [mm] Dominant hand	133.9 ± 18.5 130.5 [110 - 168]	131.2 ± 20.7 131.0 [94 - 169]	130.4 ± 30.4 134.0 [80 - 1179]	2.7 [-17.8 ; 23.3]	0.7057	0.9953
Sum of corners distances [mm] Non-dominant hand	132.1 ± 24.6 139.0 [73 - 158]	133.7 ± 29.4 139.5 [64 - 166]	135.0 ± 24.2 138.0 [100 - 186]	5.7 [-10.2 ; 21.5]	0.3136	0.8442
Sum of Pad/DPL distance [mm] Dominant hand	52.0 ± 46.5 49.5 [0 - 160]	47.3 ± 43.8 46.0 [0 - 115]	25.9 ± 45.0 0.0 [0 - 110]	-29.8 [-62.3 ; 2.7]	**0.0124**	0.0858

Sum of Pad/DPL distance [mm]Non-dominant hand	48.1 ± 54.5 32.0 [0 - 144]	46.8 ± 52.0 38.5 [0 - 160]	30.2 ± 57.0 0.0 [0 - 165]	-22.7 [-57.7 ; 12.4]	0.0719	0.3612
Kapandji score/10 Dominant hand	8.0 (± 1.4) 8.0 [7.0 - 9.0]	8.4 ± 1.7 9.3 [5.0 - 10.0]	8.5 (± 1.6) 9.0 [7.5 - 10.0]	0.6 [-0.6 ; 1.7]	0.1574	0.6050
Kapandji score/10 Non-dominant hand	8.5 (± 1.2) 9.0 [8.0 - 9.5]	8.8 ± 1.3 9.0 [6.0 - 10.0]	8.8 (± 1.3) 9.0 [8.0 - 10.0]	0.4 [-0.7 ; 1.5]	0.3068	0.8371

SD: Standard Deviation; SHAQ: Scleroderma Health Assessment Questionnaire, *score ranged from 0=no disability, to 30=severe disability*; CHFS: Cochin Hand Function Scale, *0=performed without difficulty, to 5=impossible to do. Disability was recorded as the total score (range 0–90)*; VAS: Visual Analogue Scale for hand pain (*0-100*); RCS: Raynaud's Condition Score *recording the frequency and severity of the attack on a scale from 0 to 10*; Grip and pinch strength was assessed using a Jamar and pinch dynamometers; mRSS: Modified Rodnan Skin Score applied to the hands assessed skin thickening on the dorsal hand and the first and second phalanges of the most affected finger, *scale 0=no skin fibrosis, to 18= maximum fibrosis (3 tested sites for each hand)*; Lateral range of motion of the fingers was performed by measuring the distance between the thumb and index finger (1^{st} corner) and the sum of the distances between the four fingers (2^{nd}, 3^{rd}, and 4^{th} corners) upon maximum stretch; Finger pad to distal palmar line (DPL) assessed fingers' flexion (mm); Kapandji Score assessed opposition of the thumb, *scale 0=impossible to 10=complete.*

Figure 1: Evolution of the 4 main parameters after 2 years of the procedure