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The effect of adipose derived stromal vascular fraction on stasis zone in an experimental burn model

Atilla Adnan Eyuboglu [a,*], *Cagri A. Uysal* [a], *Gonca Ozgun* [b], *Erhan Coskun* [a], *Nilgun Markal Ertas* [a], *Mehmet Haberal* [c]

[a] *Baskent University Faculty of Medicine, Department of Plastic and Reconstructive Surgery, Ankara, Turkey*
[b] *Baskent University Faculty of Medicine, Department of Pathology, Ankara, Turkey*
[c] *Baskent University Faculty of Medicine, Department of General Surgery, Ankara, Turkey*

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ABSTRACT

Background: Stasis zone is the surrounding area of the coagulation zone which is an important part determining the extent of the necrosis in burn patients. In our study we aim to salvage the stasis zone by injecting adipose derived stromal vascular fraction (ADSVF).

Methods: Thermal injury was applied on dorsum of Sprague–Dawley rats (n=20) by the "comb burn" model as described previously. When the burn injury was established on Sprague–Dawley rats (30 min); rat dorsum was separated into 2 equal parts consisting of 4 burn zones (3 stasis zone) on each pair. ADSVF cells harvested from inguinal fat pads of Sprague–Dawley rats (n=5) were injected on the right side while same amount of phosphate buffered saline (PBS) injected on the left side of the same animal. One week later, average vital tissue on the statis zone was determined by macroscopy, angiography and microscopy. Vascular density, inflammatory cell density, gradient of fibrosis and epithelial thickness were determined via immunohistochemical assay.

Results: Macroscopic stasis zone tissue viability (32±3.28%, 57±4.28%) ($p<0.01$), average number of vessels (10.28±1.28, 19.43±1.72) ($p<0.01$), capillary count (15.67±1.97, 25.35 ±2.15) ($p<0.01$) vascular density (1.55±0.38, 2.14±0.45) ($p<0.01$) epithelial thickness (0.014 ±0.009 mm, 0.024±0.0011 mm) were higher on ADSVF side. Fibrosis gradient (1.87±0.51, 1.50 ±0.43) ($p<0.01$) and inflammatory cell density (1.33±0.40, 1.20±0.32) ($p<0.01$) were higher on the PBS side.

Conclusion: Macroscopic and microscopic findings determined that ADSVF has a statistically significant benefit for salvaging stasis zone on acute burn injuries.

* *Corresponding author at*: Baskent University, Faculty of Medicine, Department of Plastic and Reconstructive Surgery, Fevzi Cakmak Cad. 5. Sok. No: 48, 06490 Bahcelievler, Ankara, Turkey. Fax:+ 90 312 203 05 26.
E-mail addresses: atillaeyuboglu@yahoo.com, aeyuboglu@hotmail.com (A.A. Eyuboglu).
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1. Introduction

Thermal injuries can be severe and life threatening. In 1947 Jackson described three zones of burn [1]. The stasis zone, the surrounding area of the coagulation zone is an important part that determines the irreversible tissue loss. The damage in this zone is progressive in 24h which can be potentially salvageable by decreasing the amount of inflammatory mediators released from burned tissues which culminates in cell death due to ischemia [2,3]. Several treatment modalities for stasis zone in addition to sufficient electrolyte and fluid replacement were described in the literature [4,5].

Stem cell treatments opened a new era for medical therapies which has shown that capacity of regeneration and differentiation capacity of stem cells can prevent further tissue loss [6–8]. Sinan et al. described the affectivity of bone marrow derived stem cell (BSCs) on stasis zone for tissue viability [6]. Atalay et al. [9] have indicated that the stromal vascular fraction improved the deep partial thickness burn wound healing. Cardoso et al. [10] have found out that adipose stromal vascular fraction helped in the treatment of full thickness burns in rats. Foubert et al. [11] have reported that adipose derived stem cells have modulated the inflammation and improved wound epithelialization and angiogenesis in a burn model. Adipose derived stromal vascular fraction (ADSVF) have been introduced to bare low morbidity with the same efficacy as BSC [12,13]. In this study, we aimed to find out the effects of ADSVF on stasis zone in an experimental burn model.

2. Materials and methods

Twenty five male, 15 weeks old Sprague Dawley rats (350–450g) (Baskent University Experimental Animal Center, Ankara, Turkey) were used. Five rats were used for stem cell harvesting procedures. The “comb burn” model as described previously were preformed on twenty rats [4,6].

2.1. Isolation and preperation of stromal vascular fraction

Five Sprague Dawley rats were anesthetized with 40mg/kg ketamine hydrochloride (Ketosal; Interhas Co. Ltd., Ankara, Turkey) and 5mg/kg xylazine hydrochloride (Xylazin Bio; Interhas Co. Ltd., Ankara, Turkey) via intraperitoneal injection and shaved. Stromal vascular fraction were gathered and treated according to our established protocol [14–16]. Phosphate-buffered saline (PBS) (Gibco BRL, Grand Island, NY) were used to wash inguinal fat pads extensively. Then adipose tissue were minced delicately on 100mm^2 tissue culture plates (Marienfield, Konigshofen, Germany) until it becomes ungraspable by tissue forceps. Tissue was rinsed for 5min three times in phosphate-buffered saline and then transferred to 50cc centrifuge tube. 0.15% collagenase (Serva, Heidelberg, Germany) was added to centrifuge tube for enzymatic digestion. Then 30min of vigorous shaking at 37°C was applied. Later that, control medium (Dulbecco’s Modified Eagle Medium (DMEM)) (Gibco BRL) with the same amount mixed with 1% antibiotic-antimycotic (Bilim ilac, Istanbul, Turkey) and 10% foetal bovine serum (FBS) (Gibco BRL) were added. This procedure neutralized the collagenase. The cell suspension was centrifuged for 5min at 1300rpm (260g) and the cell stack was re-suspended with the control medium. 0.1cc of suspension was taken and dyed using metyhlene blue for counting stem cells. Nucleated and non-nucleated cells were counted on light microscope on hemacytometer (Neubauer Thoma, Germany). Another 0.1cc of suspension was taken and cultured on 100mm^2 tissue culture plates (Marienfield, Konigshofen, Germany) with the control medium for further characterization of the adipose SVF and post-processing cell viability as described previously [16–19]. Rest of the suspension was prepared for Dil labeling.

2.2. 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine labelling of stromal vascular fraction cells

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (Dil) (Invitrogen; Medsantek, Life Technologies, Carsbad, Ca) was dissolved in 99% ethanol at a 25% concentration and stored at −20°C for use. Cells were labelled with flourescent Dil according to manufacturer’s recommendations [20,21]. Cells in suspension were incubated in phosphate-buffered saline with Dil at 2.5μg/ml concentration at 4°C for five minutes.

2.3. Experimental model

Twenty Sprague–Dawley rats(350–450g) were used in this study. They were kept in 23°C with 12h of light and dark cycles. Rats consumed standard raw chow and water ad libitum. Rats were anesthetised with 40mg/kg ketamine hydrochloride (Ketosal; Interhas Co. Ltd., Ankara, Turkey) and 5mg/kg xylazine hydrochloride (Xylazin Bio; Interhas Co. Ltd., Ankara, Turkey) via intraperitoneal injection and shaved. The comb burn injury model was performed to the dorsum of the twenty Sprague–Dawley rats with a brass probe containing 4rows(1×2cm) and 3 interspaces (0.5×2cm) as described by Regas [22]. Brass probe was dipped in the boiling water for 5min and was kept for 20s without applying pressure. The interspaces were the unburned area clinically named, stasis zone (Fig. 1). The histological analysis of the burned area was performed and the necrosis was confirmed depending on tissue viability and vascularization (Fig. 2). 0.5cc of ADSVF (4×10^6 cells) were injected to the zone of stasis the right side while same amount of PBS injected in the stasis zone of the left side of the same animal subcutaneously 30min after the burn injury.

2.4. Evaluation of stasis zone

Standard photos of rats were taken on postoperative first and seventh day for evaluating the length and the area of viable tissue. To standardize the photographic measurements, all photographs were taken from the same distance (60cm) and a ruler was placed near the rats. The calibration of the photographs were done and Adobe Photoshop CS 5 (Adobe Systems, Inc., San Jose, CA) program was used for calculating the percentage of tissue viability. The viable area was marked on

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