CASE STUDY

Evaluation of the Potentials of Autologous Blood Injection for Healing in Diabetic Foot Ulcers

Mohammed Al azrak, MSc^{a,*}, Taher Ismail, PhD^b, Olfat Shaker, PhD^c

^aBurn & Plastic Surgery Unit, Fayoum General Hospital & PhD researcher in Plastic Surgery Division, Surgery department, Faculty of Medicine, Cairo University, Cairo, Egypt

^bPlastic Surgery Division, Surgery Department, Faculty of Medicine, Cairo University, Cairo, Egypt; and

^cMedical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

KEYWORDS:

Autologous blood;
Blood injection;
Healing;
Diabetic ulcers

Abstract Healing is a complex multifactorial process, hence it is not easy to be studied accurately. In this paper we tried to demonstrate the potentials of application of autologous blood by injection into the raw areas and ulcers of three diabetic patients using their blood as an alternative to synthesized and cultured stem cells or growth factors. It was found that a natural easily obtained blood can be used to enrich the media of the wound. Also it was applicable in relation to its cost-effectiveness as well as availability. The healing process was accelerated in the injected side more than the non-injected one. © 2013 Elsevier Inc. All rights reserved.

Introduction

Accurate and rapid healing is an essential aim to surgeons. Certain medical conditions including diabetes mellitus affect the healing abilities of the body, requiring additional intervention to mend the damage.¹ Trials to improve healing in vulnerable patients using growth factors have been applied with promising results especially PDGF, TGF- β and FGF.^{2–5} Also beneficial effects of stem cells (MSC & EPC) therapy, have been observed in promoting angiogenesis and soft tissue repair.^{6,7} We have used blood as an essential source of growth factors to enrich the wound media aiming at triggering natural healing process in a series of cases with promising results. In this manuscript

we discuss three cases of feet ulcers in diabetic patients to evaluate the effects of autologous blood injection potentials.

Study Cases

Case (1)

A 50 years old female came to the clinic complaining of raw area on the dorsum of left foot since 10 weeks. She noticed that an area of skin on the dorsum of her left foot sloughed and claimed to be of traumatic etiology. The size of the wound raw area did not decrease despite using different topical agents and treatment. She was type 2 diabetes, and subjected to surgical operation for removal of a stone in left kidney more than 1 year before. After that, she complained of incisional hernia at the site of the operation, repaired 3 months ago and the patient had a long ugly left loin scar and lipedema at the pendulum of the lower abdomen. She could stand and move on feet.

Patient's laboratory results revealed: normal liver and renal functions, FBG was within normal with no leukocytosis

The authors have no conflict of interest/financial disclosure regarding this article. Also we confirm that the contained material in the article demonstrates our own work, no grants were involved and none disclaimers.

* Corresponding author. Beside Electricity Company, Sinures, Fayoum 63749, Egypt. Tel.: +20 (0)2 084 6906955.

E-mail address: mohammedazraq@yahoo.com

and hemoglobin was 10.5 g/dl. The patient fasting blood sugar was 140 mg/dl and she had been on oral anti-diabetic drugs (Gliclazide MR® [Servier], 120 mg/day).

Neurovascular assessment and duplex scanning of left lower limb excluded (Peripheral Arterial Disease, PAD) with felt distal peripheral pulses (dorsalis pedis a) and normal ABI, no sensory deficit was present but peripheral neuropathy was not excluded.

Local examination of the foot revealed elongated raw area at the medial aspect of the dorsum of left foot measured (9–10 cm length/1.5–2.5 cm width). It was covered by topical ointment (Fig. 1) and the patient was about to finish a 2 weeks course of broad spectrum antibiotic (Levofloxacin®, [Sanofi] 500 mg/day).

The plan of management was discussed with the patient, including surgical coverage (skin grafting) and non-surgical methods. Patient refused surgical methods and a consent was taken to use blood injection. The wound was dressed with saline soaked, closed without removal of the ointment for 24 h. After that, the raw area was washed with saline to remove the remnant of the ointment and the wound was exposed. Our observation about the wound that it was formed of granulation tissue-pink, with scattered areas of fibrin deposition and no evident infection (Wagner grade: 1). The wound was measured and divided into two approximate halves. The right side was injected (at the edge and base) with 1.5 ml of venous blood drawn from the patient and the left side was left without injection. The whole wound was covered with saline soaked closed dressing to be changed once daily (Fig. 2a). The patient was seen after 4 days and her examination revealed improvement and epithelialization of the right side compared to the left side which was still remaining in the phase of granulation with scattered areas of fibrin deposition (Fig. 2b and c).

Notice and Observations

The injected part of the wound was stimulated, enriched and reacted to heal more rapidly than the other side.

Figure 1 The persistent wound 10 weeks, as soon as seen with topical ointment applied.

Figure 2 (a) The wound raw area 24 h after removal of the ointment debris, divided into two approximate sides, the right one marked by dark arrow injected at (the edge/base) by 1.5 ml of venous blood drawn from the patient and the left side marked by white arrow was left as such. (b) and (c) The wound after 4 and 5 days of daily saline soaked closed dressing with noted epithelialization in the right injected side.

Case (2)

A male patient 52 years old with type 2 diabetes for 10 years, on oral anti-diabetic drug (Gliclazide MR® [Servier], 120 mg/day). He came to the clinic complaining of swollen left foot especially the big toe. Examination of the foot revealed a picture of infection and necrosis of the medial aspect of big toe. Peripheral pulsations were felt and duplex scan on lower limb vessels showed mild atherosclerosis, X ray foot showed no evidence of osteomyelitis. All patient's vital signs and general condition were accepted away from uncontrolled diabetes. Explanation of the medical status and options available for management were discussed with the patient and written consent was obtained (Fig. 3). Conservative management was the best approach and it consisted of drainage and keen surgical debridement of the necrotic tissues in addition to readjustment of medical condition and prescribing of sensitive antibiotic (Amikin® [Squib], 500 mg/tds) according to wound

Figure 3 Diabetic left foot with infected pre-gangrenous big toe.

swab/culture (Fig. 4). After removal of the dressing in the second day, the produced raw area in the big toe was assessed (Wagner grade: 2) and divided into two halves, proximal and distal part (Fig. 5). The distal one was injected with the patient's venous blood (2 ml). Injection was done into the interstitium of the ulcer edges and bed in variable depth. Sterile dressing after washing by saline was applied to be changed daily. The dressing was soaked by glycerin magnesia every day then every other day to help in decreasing the bacterial load in the media.

After 8 days the ulcer was re-assessed again and examination revealed difference between the injected and non-injected area (Fig. 6). The injected area was advanced in the cascade of the healing process than the non-injected area, as angiogenesis and healthy granulation tissue was formed to cover the bed and decrease the defect. No evident infection in addition to decrease in the discharge and exudates were observed. Another session of blood

Figure 4 The foot after surgical debridement with developed raw area.

injection was applied and repeated dressing to see the patient again and re-examined the ulcer after an additional 4 days.

A clear line of demarcation was formed between the distal injected part and proximal non-injected part (Fig. 7a). The patient was left on ordinary dressing for 2 days. After that, examination revealed encroachment of granulation tissue from distal to proximal. Then injection of the proximal part was done. Examination of the ulcer after the following 4 days from that, revealed filling of the whole ulcer with healthy granulation tissue and saving of the toe (Fig. 7b and c).

Notice and Observations

1. Early diagnosis and proper intervention is essential to save the foot and prevent progression to amputation.
2. Blood injection was observed to provide fuel to withstand periods of deprivation from essential elements of healing and may help in shortening the duration of healing in vulnerable wounds.

Case (3)

A female patient 47 years old, diabetic 9 years ago, on oral anti-diabetic drugs (Gliclazide MR® [Servier], 60 mg/day). Presented with two neuropathic ulcers on right and left big toes, persistent for 18 days. Laboratory examinations (CBC, LFT, U + E, Coag. profile) were done and all were within normal values except elevation of fasting blood sugar (205 mg/dl), which were adjusted medically. X ray and duplex examination of foot and lower limb were within normal in both sides. After complete history taking, general examination and local examination of both of feet and ulcers, the surgical status of the patient was determined and explained.

The management plan was the conservative approach to the ulcers and consisted of; debridement of covering crusts and sloughs then washing and cleaning the resultant raw area (right and left toes ulcers) which were assessed (Wagner grade: 1) and covered by sterile dressing soaked with glycerin magnesia (Fig. 8a and b). Sterile dressing was changed daily and the patient was re-examined after 4 days for re-assessment of the ulcers and getting an idea about the healing initials. Measurements of the average length and width of both ulcers were done. The right ulcer was measured 30 mm × 20 mm and the left ulcer was measured 17 mm × 11 mm (Fig. 8c). Autologous blood injection was considered for the right ulcer and started by withdrawing a 1.5 ml of patient's venous blood and re-injecting the blood into the interstitium of the ulcer edges and bed. Sterile dressing was applied and the left ulcer was covered by sterile dressing without prior injection. An instruction was given to the patient to change the dressing every other day as well as daily foot care. Ulcers care consisted of application of saline wash and glycerin magnesia solution in alternate. No evident infection was observed and both ulcers were clinically healthy during

Figure 5 The raw area divided into two parts proximal part indicated by black arrow and distal part indicated by transparent arrow, injection was done into the distal part first.

the course of treatment. Autologous blood injection was applied in the right ulcer in repeated sessions as indicated in (Table) and frequent measurement of both ulcers was done to observe the progress.

Notice and Observations

1. Blood injection helped to hasten the healing in the right ulcer.
2. The left (non-injected ulcer) got complete healing from a size of (17 × 10 mm) in 37 days, while the right ulcer (injected) got complete healing after reaching a nearly similar size of (18 × 10 mm) in 18 days. This means average half the time of the left ulcer.

Discussion

Healing is a complex process studied by many investigators. It is preferred to use the phases as described by

Figure 6 Eight days after the initial injection revealed difference in the status of the ulcer between injected and non-injected areas.

Jorgensen as healing encompasses; coagulation, inflammation, angiogenesis, fibroplasia, contraction, epithelialization and remodeling in an inclusive differentiation.⁸ It is clear that cascading is one of the characteristics of healing process, starting rapidly in coagulation then slow down in the following phases.⁹ Every phase deliver to the next one frame to build upon it. Also every phase is dependent on certain cells and cytokines. For example: coagulation depends on platelets plus PDGF & TGF- β ,¹⁰ inflammation depends on neutrophils, macrophages plus LIF & IL-6,^{11,12} angiogenesis depends on platelets & monocytes plus VEGF,^{13,14} fibroplasia depends on fibroblasts plus NO,^{10,15} contraction depends on myofibroblasts plus EGF & TGF- β & other kinases¹⁶⁻¹⁸ and epithelialization depends on keratinocytes plus KGF & FGF-7 & PDGF & EGF & TGF- β .^{19,20}

Exogenous application of such growth factors to stimulate normal as well as impaired healing was tried,^{2-5,21} despite controversies regarding degradation by proteases activity of wound fluids.^{22,23} Platelet rich plasma was used topically as well.²⁴ As growth factors controlling the process of healing is derived and released essentially from blood cells.²⁵ In addition to the importance of blood cells themselves especially macrophages, in proper wound healing.²⁶⁻²⁸ We hypothesized that, transferring the patient own blood by drawing it from the blood vessel and then re-injecting it as freshly into the interstitium of the wound area passing the natural barriers, may increase the body perception and response to this area. As well as deliver essential useful cells carried by the blood especially platelets, macrophages and peripheral stem cells. We aimed from this preliminary case studies to evaluate the potentials of autologous blood injection on the clinical outcome of the wounds. We have applied this method in management of various wounds and ulcers with good results. As diabetic patients are more vulnerable to healing impairment, we tried in these study cases to include ulcers with different etiologies as traumatic, infectious and neuropathic ulcers in order to clarify the healing potentials of autologous blood injection. We can say that a clear

Figure 7 (a) The ulcer after 12 days with two sessions of Blood injection shows a line of demarcation separating two parts; distal (injected) & proximal (non-injected). (b) After 15 days encroachment of granulation tissue from distal to proximal was noted, then injection done into the proximal part. (c) The ulcer is filled with healthy granulation within 19 days.

difference was observed during our application, and a detailed study is needed to provide more assessment as well.

Figure 8 (a) Two neuropathic ulcers in right and left big toes in 47y female diabetic pt. (b) The raw areas after excision of crusts and skin sloughs. (c) Both ulcers at 4 days left after debridement. (d) Both ulcers at day 32, right injected ulcer measured 14×6 mm, left non-injected ulcer measured 10×4 mm. (e) Epithelialization was observed in left ulcer at day 41, while right ulcer is still and measured 9×3 mm. (f) Complete epithelialization occurred at day 46 in both sides.

Conclusion

Healing process in the body is still not understood completely. Wound healing, being a dynamic process, it may slow down. Autologous blood injection into the interstitium of the wound or ulcer may hasten the healing

Table Comparative Measures of Right (Injected) Ulcers and Left (Non-Injected) Ulcer.

Day of Exam/Ulcers	Left Ulcer Mean Measures (mm)	Right Ulcer Mean Measures (mm)	Amount of Injected Blood (cc)
0 day	17×11	30×20	–
4 day	17×10	29×19	1.5
10 day	16×8	26×17	1.5
16 day	15×7	24×14	1.3
22 day	14×6	22×11	1
28 day	12×6	18×10	1
32 day	10×4	14×6	1
41 day	Complete heal	9×3	0.5
43 day	–	5×2	0.5
46 day	–	Complete heal	–

process in the injected side as observed in our study of clinical cases. Further studies are needed.

References

1. Steed DL, Donohoe D, Webster MW, Lindsley L: Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg*. 1996;183:61–64.
2. Mustoe TA, Pierce GF, Morishima C, Deuel TF: Growth factors induced acceleration of tissue repair through direct and indirect activities in a rabbit dermal ulcer model. *J Clin Invest*. 1991;87:694–703.
3. Jones SC, Curtsinger LJ, Whalen GD, et al: Effect of topical recombinant TGF-beta on healing of partial thickness injuries. *J Surg Res*. 1991;51:344–352.
4. Richard JL, Parer RC, Daures JP, et al: Effect of topical fibroblast growth factor on healing of chronic diabetic neuropathic ulcer of the foot. A pilot, randomized, double blind, placebo-controlled study. *Diabetes Care*. 1995;18:64–69.
5. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R: PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am J Pathol*. 1990;136:1235–1246.
6. Katritsis DG, Sotiropoulou PA, Karvouni E, et al: Transcatheter transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv*. 2005;65:321–329.
7. Kolvenbach R, Kreissig C, Ludwig E, Cagiannos C: Stem cell use in critical limb ischemia. *J Cardiovasc Surg (Torino)*. 2007;48:39–44.
8. Jorgensen LN: Collagen deposition in the subcutaneous tissue during wound healing in humans: a model evaluation. *APMIS Suppl*. 2003; (115):1–56.
9. Tran H, Anand SS: Oral antiplatelet therapy in cerebrovascular disease, coronary artery disease, and peripheral arterial disease. *JAMA*. 2004;292(15):1867–1874.
10. Diegelmann RF, Evans MC: Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci*. 2004;9:283–289.
11. Gadiant RA, Patterson PH: Leukemia inhibitory factor, interleukin 6, and other cytokines using the GP130 transducing receptor: roles in inflammation and injury. *Stem Cells*. 1999;17(3):127–137.
12. Schremmer-Danninger E, Naidoo S, Neuhof C, et al: Visualisation of tissue kallikrein, kininogen and kinin receptors in human skin following trauma and in dermal diseases. *Biol Chem*. 2004;385(11):1069–1076.
13. Schober A, Weber C: Mechanisms of monocyte recruitment in vascular repair after injury. *Antioxid Redox Signal*. 2005;7(9–10):1249–1257.
14. Sayan H, Ozacmak VH, Guven A, et al: Erythropoietin stimulates wound healing and angiogenesis in mice. *J Invest Surg*. 2006;19(3): 163–173.
15. Witte MB, Barbul A: Role of nitric oxide in wound repair. *Am J Surg*. 2002;183(4):406–412.
16. Eichler MJ, Carlson MA: Modeling dermal granulation tissue with the linear fibroblast-populated collagen matrix: a comparison with the round matrix model. *J Dermatol Sci*. 2006;41(2):97–108.
17. Lee AR: Enhancing dermal matrix regeneration and biomechanical properties of 2nd degree-burn wounds by EGF-impregnated collagen sponge dressing. *Arch Pharm Res*. 2005;28(11):1311–1316.
18. Chen Y, Shi-Wen X, van Beek J, et al: Matrix contraction by dermal fibroblasts requires transforming growth factor-beta/activin-linked kinase 5, heparan sulfate-containing proteoglycans, and MEK/ERK: insights into pathological scarring in chronic fibrotic disease. *Am J Pathol*. 2005;167(6):1699–1711.
19. Greenhalgh DG: The role of growth factors in wound healing. *J Trauma*. 1996;41:159–167.
20. Bennet NT, Schultz GS: Growth factors and wound healing: biochemical properties of growth factors and their receptors. [Review]. *Am J Surg*. 1993;165:728.
21. Slavin J, Nash JR, Kingsnorth AN: Effect of transforming growth factor beta and basic fibroblast growth factor on steroid impaired healing intestinal wounds. *Br J Surg*. 1992;79:69–72.
22. Yager DR, Nwomeh BC: The proteolytic environment of chronic wounds. *Wound Repair Regen*. 1999;7:433–441.
23. Bullen BC, Longaker MT, Updike DL, et al: Tissue inhibitor of metalloproteinases-1 is decreased and activated gelatinases are increased in chronic wounds. *J Invest Dermatol*. 1995;104:236–240.
24. Driver VR, Hanft J, Fylling CP, et al: A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. *Ostomy Wound Manage*. 2006;52(6): 68–70.
25. Singer AI, Clark RA: Cutaneous wound healing. *N Engl J Med*. 1999; 341:738–746.
26. Broughton G 2nd, Janis JE, Attinger CE: The basic science of wound healing. *Plast Reconstr Surg*. 2006;117(7 suppl):12S–34S.
27. Leibovich SJ, Ross R: The role of the macrophages in wound repair: a study with hydrocortisone and anti macrophage serum. *Am J Pathol*. 1975;78(1):71–100.
28. Maruyama Kazuichi, Asai Jun, Ii Masaaki, et al: Decreased macrophages number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. *Am J Pathol*. 2007;170(4):1178–1191.