International Journal of Stem Cells Vol. 7, No. 2, 2014 http://dx.doi.org/10.15283/ijsc.2014.7.2.158

BRIEF REPORT

Combining Autologous Peripheral Blood Mononuclear Cells with Fibroblast Growth Factor Therapy Along with Stringent Infection Control Leading to Successful Limb Salvage in Diabetic Patient with Chronic Renal Failure and Severe Toe Gangrene

Hiroshi Osawa[1], Kouan Orii[1], Hiroshi Terunuma[2], Samuel JK Abraham[3,4]

[1]*Shimada General Hospital, Division of Cardiovascular Surgery, Chiba,* [2]*Biotherapy Institute of Japan, Tokyo, Japan,*
[3]*The Mary-Yoshio Translational Hexagon (MYTH), Nichi-In Centre for Regenerative Medicine (NCRM), Chennai, India,*
[4]*Yamanashi University- School of Medicine, Chuo, Japan*

Peripheral arterial disease (PAD) is a common complication of Diabetes Mellitus (DM) and often culminates in amputation of the affected foot. *Pseudomonas aeruginosa* infections associated with PAD are difficult to treat due to their multi-drug resistance. Herein we report a 38 year old male who reported with DM, chronic kidney disease (CKD) and rest pain of the right second toe in October 2011. He underwent percutaneous transluminal angioplasty (PTA) which was unsuccessful. The gangrene of the toes worsened and amputation of the right second toe was done. Bacteriological examination showed presence of *P. aeruginosa* which during the course of antibiotic therapy became multi-drug resistant. Gangrene and abscess of the foot worsened and amputation of the right third toe was performed. Then autologous peripheral blood mononuclear cell (PBMNC) therapy was performed but as infection control could not still be achieved, the fourth toe was amputated. A protocol of foot bath using carbonic water, local usage of antibiotics (Polymyxin-B), and basic fibroblast growth factor (b-FGF) spray was then employed after which the infection could be controlled and improvement in vascularity of the right foot could be observed in angiography. This combined approach after proper validation could be considered for similar cases.

Keywords: Diabetes mellitus (DM), peripheral arterial disease (PAD), Pseudomonas aeruginosa, Cell therapy, Infection control

Introduction

Diabetes mellitus (DM) associated peripheral arterial disease (PAD) affects approximately 4～5% of the patients and the incidence of foot amputations in diabetic patients range from 5 to 24 per 1000 patients per year (1). Every 30 seconds, a lower limb is amputated due to diabetes (2). *Pseudomonas aeruginosa* infections are commonly associated with the diabetic foot and they are difficult to treat due to their multi-drug resistance. Such *P. aeruginosa* infections lead to prolonged hospitalization duration and failure of the treatment (3-5). Another complicating factor is the association of chronic kidney disease (CKD) in such diabetic patients with PAD as there will impaired wound healing from the uremia due to CKD leading to increased morbidity and mortality (6). Herein we report the successful limb salvage in a diabetic patient with PAD, chronic kidney disease and severe *P. aeruginosa* infection by employing a combined therapy protocol.

Accepted for publication July 25, 2014, Published online November 30, 2014
Correspondence to **Samuel JK Abraham**
The Mary-Yoshio Translational Hexagon (MYTH), Nichi-In Centre for Regenerative Medicine (NCRM), PB 1262, Chennai 600034, Tamil Nadu. India
Tel: +91-44-28174743, Fax: +91-44-24732186
E-mail: drsam@nichimail.jp

Case Report

A 38 year-old male with Diabetes mellitus (DM) (Type 2) and chronic renal failure diagnosed when he was 30 years old and on routine hemodialysis reported with rest pain of the right second toe in October 2011. His glycemic levels were 90～170 mg/dl. He was under medication with Aspirin, Triclopidine hydrochloride, Beraprost sodium, Ethyl icosapentate, Pravastatin sodium and Barnidipine hydrochloride. On clinical examination a gangrene of the right second toe was noticed (Fig. 1A). Angiography showed an occlusion of the anterior and posterior tibial arteries of the right leg (Fig. 2A). Skin perfusion pressure (SPP) of the dorsal and plantar of the right foot was 42 mmHg and 50 mmHg respectively. The treatment procedures described below were done in accordance with the institutional and regulatory guidelines, laws that prevailed at the time of treatment. The patient was recommended for percutaneous transluminal angioplasty (PTA). PTA for anterior and posterior tibial artery was performed on December 2nd 2011. However PTA was unsuccessful. The gangrene of the toes worsened and abscess of the foot occurred. On December 17th 2011, the second toe was amputated and drainage of the abscess was performed (Fig. 1B). Bacteriological examination showed *P. Aeruginosa* that did not have multi-drug tolerance. The patient received intravenous drip of sensitive antibiotics but later the *P. Aeruginosa* acquired multi-drug tolerance. Hence the infection was uncontrolled and gangrene became worse. In January 2012, amputation of the third toe and wound debridement was performed (Fig. 1C). In order to prevent the further spread of gangrene and to avoid amputation of the other parts of the affected limb, the patient was referred for autologous peripheral blood mononuclear cells (PBMNC) implantation in February 2012. 60 ml of the PBMNC concentrate was extracted (PBMNC count was 2.6×10^9 cells)

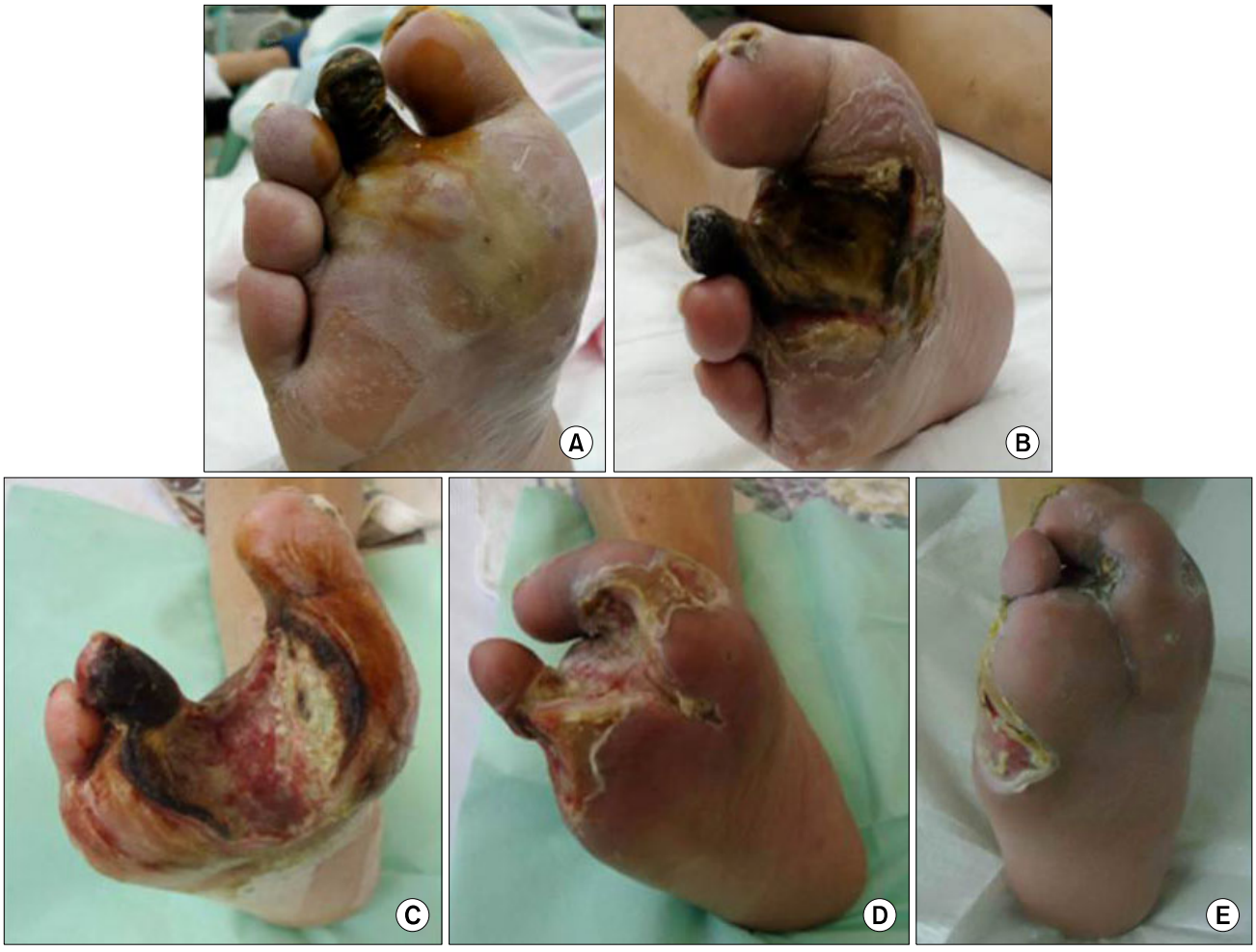

Fig. 1. (A) Gangrene of right 2nd toe. (B) Amputation of the right 2nd toe. (C) Amputation of the right 3rd toe and wound debridement. (D) Improvement in granulation and infection control. (E) Right foot at the time of discharge showing successful limb salvage.

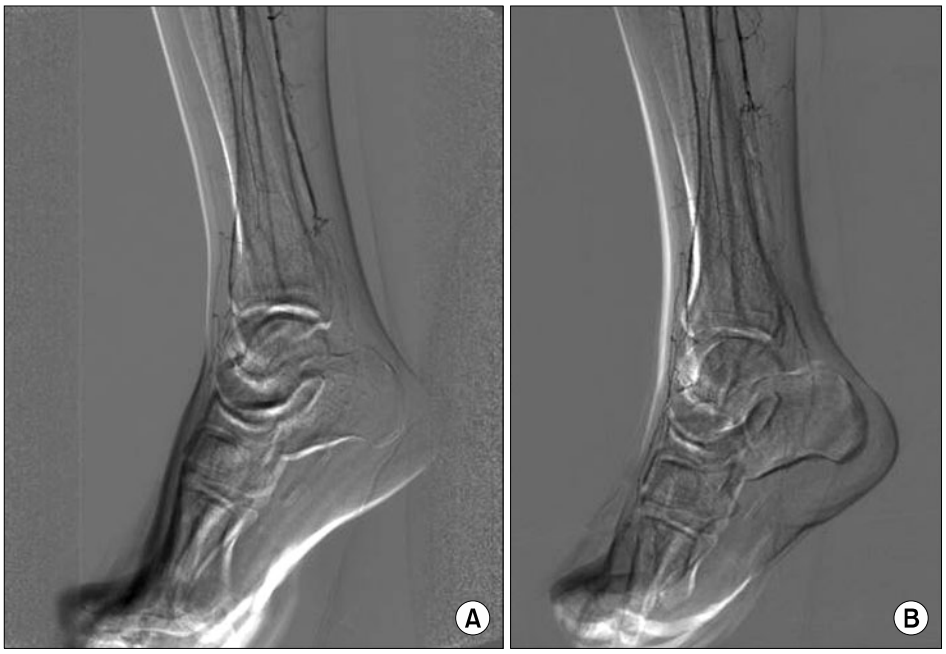

Fig. 2. Digital subtraction angiography (DSA) images of the right limb. (A) At the time of admission showing the occlusion of the anterior and posterior tibial arteries. (B) After the combined therapy protocol showing worsening of occlusion in the main route of posterior tibial artery but development of the collateral arteries.

and injected in various sites of right foot and lower limb. However the infection control was still difficult. In May 2012 the fourth toe amputation and debridement was done. In addition, foot bath using carbonic water, local usage of antibiotics (Polymyxin-B), and basic fibroblast growth factor (b-FGF) spray were also used. From July 2012, the granulation improved gradually and infection could be controlled (Fig. 1D). Angiography showed worsening of occlusion in the main route of posterior tibial artery, but the collateral arteries grew and tissue contrast changed dark in October 2012 (Fig. 2B). However the SPP did not improve. The patient became ambulatory and he was discharged from the hospital by foot (Fig. 1E showing the foot at the time of discharge) in December 2012.

Discussion

PBMNC implantation is considered a strategy with high treatment potential for critical limb ischemia and there are several studies which have reported significant outcome after cell therapy (7-9). Mononuclear cell implantation has been proven to result in successful limb salvage even in Fontaine Stage IV Critical limb ischemia (CLI) patients (10). b-FGF therapy when given synergistically with cell therapy has been reported to enhance therapeutic recovery (11). However in most of these studies, the associated infection could be well controlled and hence the outcomes are significant. Severe uncontrolled infections in CLI even after cell therapy has shown to lead to limb amputations (12). Thus infection control is the foremost factor for successful limb salvage. *P. aeruginosa* infections in diabetic CLI are very difficult to treat due to their multi-drug resistance (3-5). The compounding factor in this present case is associated CKD. Inspite of these hurdles, combined therapy of infection control, cell therapy and b-FGF therapy has resulted in successful limb salvage in this patient. Angiography showed that despite the fact that occlusion in the main route of the posterior tibial artery worsened, collateral arteries grew and tissue contrast changed dark after the combined therapy. Thus this is one of its kind reports wherein a combined approach involving cell therapy with b-FGF and stringent infection control measures have prevented the limb amputation in a case of severe gangrene associated with DM. However, it is difficult to comment which treatment applied was effective and which not. The total algorithm used in the case, although successful finally, needs larger studies for validation before application in another similar patient.

Conclusion

Infection of the ischemic region is a major threat in CLI patients with DM. A combined approach involving cell therapy and b-FGF administration along with stringent infection control could result in limb salvage in this patient who had DM and CKD. A beneficial outcome with this protocol makes us suggest this therapy for similar cases after thorough validation.

Acknowledgements

M/s Department of Clinical Research, Yamanashi University, Japan for their assistance with the publication of the manuscript.

Dr. Senthilkumar Preethy for her assistance with the drafting of the manuscript.

Potential conflict of interest

The authors have no conflicting financial interest.

References

1. Gazzaruso C, Coppola A, Collaviti S, Saluzzo CM, Furlani F, Gallotti P, Clerissi J, Solerte BS, Giustina A. Percutaneous transluminal angioplasty for critical limb ischemia in very elderly diabetic patients. Aging Clin Exp Res 2013;25:225-228
2. Apelqvist J. Diagnostics and treatment of the diabetic foot. Endocrine 2012;41:384-397
3. Boyanova L, Mitov I. Antibiotic resistance rates in causative agents of infections in diabetic patients: rising concerns. Expert Rev Anti Infect Ther 2013;11:411-420
4. Ertugrul BM, Oncul O, Tulek N, Willke A, Sacar S, Tunccan OG, Yilmaz E, Kaya O, Ozturk B, Turhan O, Yapar N, Ture M, Akin F. A prospective, multi-center study: factors related to the management of diabetic foot infections. Eur J Clin Microbiol Infect Dis 2012;31:2345-2352
5. Söderström M, Vikatmaa P, Lepäntalo M, Aho PS, Kolho E, Ikonen T. The consequences of an outbreak of multidrug-resistant Pseudomonas aeruginosa among patients treated for critical leg ischemia. J Vasc Surg 2009;50:806-812
6. Lewis S, Raj D, Guzman NJ. Renal failure: implications of chronic kidney disease in the management of the diabetic foot. Semin Vasc Surg 2012;25:82-88
7. Mohammadzadeh L, Samedanifard SH, Keshavarzi A, Alimoghaddam K, Larijani B, Ghavamzadeh A, Ahmadi AS, Shojaeifard A, Ostadali MR, Sharifi AM, Amini MR, Mahmoudian A, Fakhraei H, Aalaa M, Mohajeri-Tehrani MR. Therapeutic outcomes of transplanting autologous granulocyte colony-stimulating factor-mobilised peripheral mononuclear cells in diabetic patients with critical limb ischaemia. Exp Clin Endocrinol Diabetes 2013;121:48-53
8. Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care 2005;28:2155-2160
9. Ozturk A, Kucukardali Y, Tangi F, Erikci A, Uzun G, Bashekim C, Sen H, Terekeci H, Narin Y, Ozyurt M, Ozkan S, Sayan O, Rodop O, Nalbant S, Sıldıroglu O, Yalnız FF, Senkal IV, Sabuncu H, Oktenli C. Therapeutical potential of autologous peripheral blood mononuclear cell transplantation in patients with type 2 diabetic critical limb ischemia. J Diabetes Complications 2012;26:29-33
10. Subrammaniyan R, Amalorpavanathan J, Shankar R, Rajkumar M, Baskar S, Manjunath SR, Senthilkumar R, Murugan P, Srinivasan VR, Abraham S. Application of autologous bone marrow mononuclear cells in six patients with advanced chronic critical limb ischemia as a result of diabetes: our experience. Cytotherapy 2011;13:993-999
11. Layman H, Rahnemai-Azar AA, Pham SM, Tsechpenakis G, Andreopoulos FM. Synergistic angiogenic effect of code-livering fibroblast growth factor 2 and granulocyte-colony stimulating factor from fibrin scaffolds and bone marrow transplantation in critical limb ischemia. Tissue Eng Part A 2011;17:243-254
12. Skóra J, Barć P, Pupka A, Dawiskiba T, Korta K, Albert M, Szyber P. Transplantation of autologous bone marrow mononuclear cells with VEGF gene improves diabetic critical limb ischaemia. Endokrynol Pol 2013;64:129-138