creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

계명대학교
KEIMYUNG UNIVERSITY

박 사 학 위 논 문

The Role of Platelet Rich Plasma in Wound Healing

계 명 대 학 교 대 학 원

의 학 과

박 지 은

지도교수 김 상 현

2 0 2 0 년 2 월

박 지 은 2020년 2월

계명대학교
KEIMYUNG UNIVERSITY

The Role of Platelet Rich Plasma in Wound Healing

지도교수 김 상 현

이 논문을 박사학위 논문으로 제출함

2020년 2월

계 명 대 학 교 대 학 원
의 학 과

박 지 은

계명대학교
KEIMYUNG UNIVERSITY

박지은의 박사학위 논문을 인준함

주 심 황 재 석

부 심 김 상 현

부 심 김 준 형

부 심 김 성 애

부 심 문 성 수

계 명 대 학 교 대 학 원

２０２０년 ２월

계명대학교
KEIMYUNG UNIVERSITY

감사문

이번 논문을 제출하기까지 바쁘신 가운데에도 많은 가르침을 주신 김상현 지도교수님과 심사위원을 맡아주신 황재석 교수님, 김준형 교수님, 김성애 교수님, 문성수 교수님께 진심으로 감사의 마음을 드립니다.

부족한 시간을 쪼개어 논문에 대한 고민과 조언을 아끼지 않았던 교수님들과 연구팀에게도 고마운 마음을 전합니다.

항상 저에게 힘이 되어 주시고 든든한 지원군이 되어 주는 가족들에게 이 논문을 바칩니다.

2020년 2월

박 지 은

계명대학교 KEIMYUNG UNIVERSITY

Table of Contents

계명대학교 KEIMYUNG UNIVERSITY

List of Tables

계명대학교 KEIMYUNG UNIVERSITY

List of Figures

계명대학교 KEIMYUNG UNIVERSITY

1. Introduction

The prevalence of diabetes among adults aged 30 years or older was 13.7% (4.8 million adults), in 2014, and incidences of diabetes-related complications were also found to be on the rise (1). Diabetic foot ulcer (DFU) is one of among the major complications in diabetes, and it is predicted that DFU affect about 3% of all diabetic patients (2). The conventional treatment methods for DFUs such as glucose control, infection control, pressure redistribution and local wound care are costly and require patients to be hospitalized for long periods of time, in several departments (3). Therefore, there is a necessity for new and effective treatment strategies to heal ulcerous lesions. As DFUs are often refractory to conventional treatments and have recently been tried to treat with various growth factors and low-level light therapy (4-6).

Platelet-rich plasma (PRP) containing predominantly platelets and various growth factors has increasingly been used to heal chronic ulcerous wounds including burn injury and chronic lower leg ulcers (7-9). Therefore, we enrolled 10 patients with DFU and added PRP treatment to conventional treatment for ulcer lesions. We investigated the effect of PRP as an alternative treatment for DFU, and evaluated the correlation between the various factors affecting DFU treatment.

계명대학교 KEIMYUNG UNIVERSITY

2. Materials and Methods

2.1. Study design and subjects

We recruited 10 patients with DFU, from April 2013 to August 2015. The institutional review board of our hospital approved the study protocol (2012-12-285). Among the Wagner's classification of grade 1 DFU patients, to analyze the correlation between ulcer depth and treatment effect, we divided the patients into four grades, according to the ulcer depth.

2.2. PRP preparation and application for DFU

PRP was prepared from 10-12 ml volume of the patients' own blood collected using a commercial kit (MyCells Autologous Preparation kit®, Holon, Israel) and centrifuged at 2500 rpm for 7 minutes. Prior to the application of PRP, necrotic tissues or eschars were debrided using a scalpel. Activated PRP was applied to the wound in a liquid or gel form, according to the wound condition, followed by a foam dressing (Allevin®, Smith and Nephew, Huntingdon, UK) placed on the ulcer for 48h every twice a week or once a week.

2.3. Efficacy and safety assessment

The primary end-point was the time taken for the wound size to reduce by half, while the secondary end-points were the presence of side effects such as pain, burning sensation, and local infection. The

계명대학교 KEIMYUNG UNIVERSITY

ulcerous wound area was quantified by using Image J®software (National Institutes of Health, Bethesda, MD, USA)

2.4. Statistical analysis

The mean and median values were calculated for the pretreatment period, PRP treatment period, and hospitalization treatment period, as well as the number of PRP treatments. Spearman's correlation tests were performed to determine the correlations between DFU grade, diabetic severity, and risk factors, and pretreatment period, DFU treatment period and PRP treatment period, using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

계명대학교 KEIMYUNG UNIVERSITY

3. Results

3.1. Patients' characteristics

A total of 10 patients (3 women and 7 men) were enrolled, and their mean age was 56.5 years (Table 1). All the patients had type 2 diabetes, and the mean HbA1c level was 8.52 %. Six of the 10 patients had a history of foot ulcer with amputation.

3.2. Efficacy and safety assessment

The mean and median pretreatment periods (time from the detection of the DFU to the start of DFU treatment) were 106.5 days and 45 days, respectively (Figure 1). The pretreatment period and ulcer grade were negatively correlated but not statistically significantly ($p > 0.05$). That is, shallow wounds tended to be left unattended for long periods. The mean time taken to start PRP treatment after DFU treatment was 15.7 days, and the median time was 5 days. The mean hospitalization period was 26.3 days and the DFU treatment period was 45.1 days. The mean duration of PRP treatment was 29.4 days, and the median was 32 days. PRP treatment was administered a mean of 3 (1-6) times. Through PRP treatment, the mean duration for a 50% reduction in the wound area to be achieved was 16 days. After the start of PRP treatment, the depth of the wound reduced and it underwent epithelialization from the margin (Figure 2&3). No side effects, such as pain, irritation or itching, were observed during PRP treatment. The PRP treatment period tended to be longer with a higher number of PRP

계명대학교 KEIMYUNG UNIVERSITY

treatments ($p < 0.01$), older age, longer duration of diabetes, higher HbA1c level, and higher number of risk factors, but this was not statistically significant.

The hospitalization period was significantly longer when the duration of diabetes was longer ($p < 0.05$, Spearman's correlation 0.628). The hospitalization period tended to increase as the age, depth of ulceration, pretreatment period, and duration of PRP treatment increased, but not significantly.

3.3. Histopathologic analysis of DFU on before and after PRP treatment

After PRP treatment, inflammatory cell infiltration was significantly decreased and multiple vessels of upper dermis were proliferated (Figure 4). Angiogenesis was confirmed by cluster of differentiation (CD)31 and hypoxia-inducible factor 1-alpha (HIF-1α) staining. CD31 staining endothelial cells were prominently increased after treatment. However, in the case of HIF-1α, there was no significant difference in the degree of staining before and after treatment (Figure 5).

계명대학교 KEIMYUNG UNIVERSITY

Table 1. Clinical Characteristics of Study Participants

	Mean	Median
Total number (Male/Female)	10 (7/3)	
Age (years)	56.5 (37-74)	
Duration of diabetes mellitus (years)	14.7 (3-40)	
HbA1c	8.52 (6.2-13.6)	
Retinopathy	4 (40%)	
Neuropathy	7 (70%)	
Nephropathy	2 (20%)	
Number of PRP treatments (times)	3 (1-6)	
DFU treatment period (days)	45.1 (4-90)	41
Hospitalization period (days)	26.3 (7-79)	16
PRP treatment period (days)	29.4 (4-42)	32
Pretreatment period (days)	106.5 (7-420)	45
Duration of 50% reduction of the wound area (days)	16.0 (7.5-32.6)	11.7

DFU: diabetic foot ulcer; HbA1c: glycosylated hemoglobin; PRP: platelet rich plasma.

계명대학교
KEIMYUNG UNIVERSITY

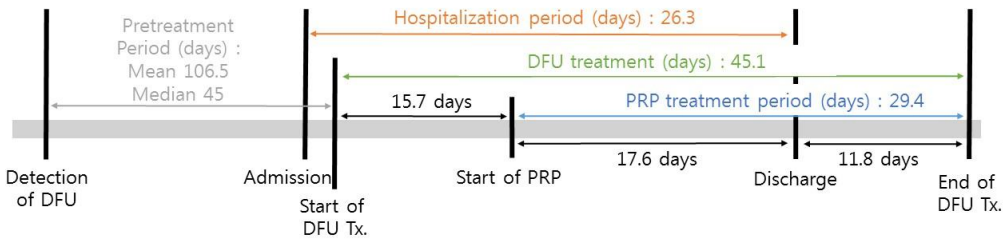

Figure 1. Diagram of the duration and timeline associated with DFU treatment. DFU Tx: diabetic foot ulcer treatment; PRP: platelet rich plasma.

계명대학교
KEIMYUNG UNIVERSITY

Figure 2. Complete epithelialization after PRP treatment, in patient number 3. PRP: platelet rich plasma.

계명대학교
KEIMYUNG UNIVERSITY

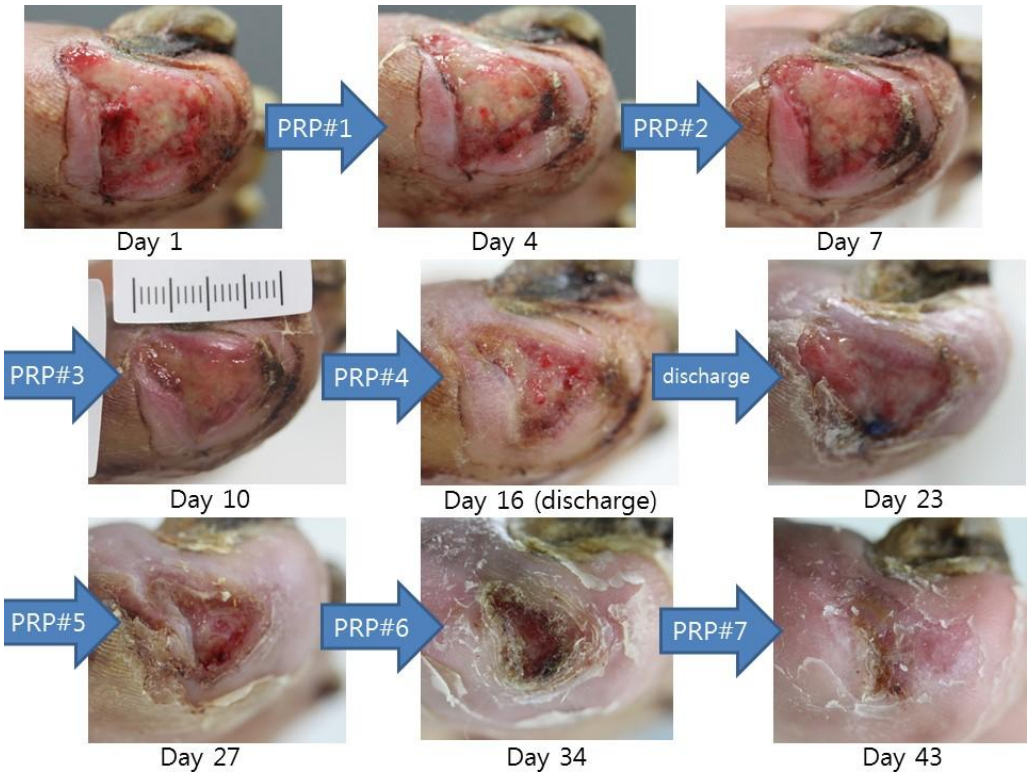

Figure 3. Complete epithelialization after PRP treatment, in patient number. 7. PRP: platelet rich plasma.

계명대학교
KEIMYUNG UNIVERSITY

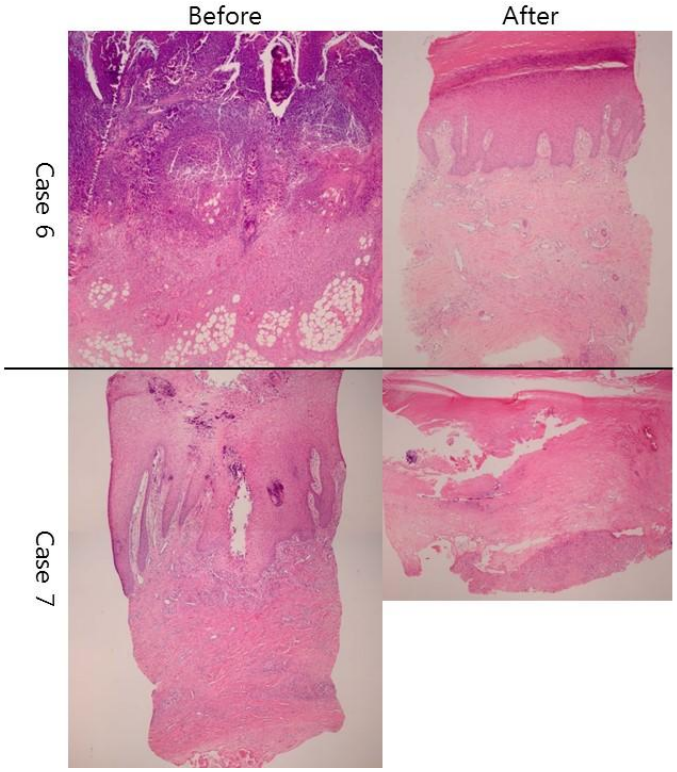

Figure 4. Histopathologic changes before and after PRP treatment(H&E stain, × 40). PRP: platelet rich plasma.

계명대학교
KEIMYUNG UNIVERSITY

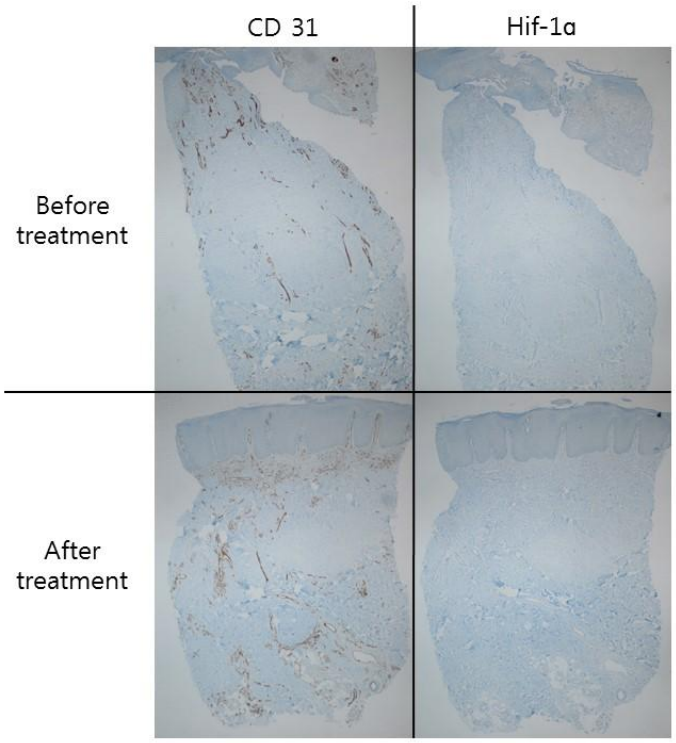

Figure 5. Immunohistochemistry changes of CD31 and HIF-1α before and after PRP treatment(H&E stain, × 40). CD31: cluster of differentiation 31; HIF-1α: hypoxia-inducible factor 1-alpha; PRP: platelet rich plasma.

계명대학교 KEIMYUNG UNIVERSITY

4. Discussion

PRP is highly concentrated in platelets (1 million / mm^3) which have tissue regeneration functions, such as chemotaxis, cell proliferation, differentiation, angiogenesis, intracellular accumulation, immune control, antimicrobial activity, and remodeling (10,11). The growth factors released from the platelets include the platelet-derived growth factor, transforming growth factor alpha, vascular endothelial growth factor (VEGF), and epidermal growth factor, and insulin-like growth factor-1 (10,11). Some advantages of PRP treatment are that it does not cause allergic reactions, relatively inexpensive and can be used for patients who do not want to experience pain or discomfort, and who are reluctant to undergo aggressive treatment (12). Whereas, PRP treatment can be easily administered, and prepared at any time using a centrifugal separator in local clinics.

In particular, diabetic patients usually have combined cormobidity and are known to be interfered with neovascularization including angiogenesis, arteriogenesis, and vasculogenesis (13). Therefore, we investigated the effects of PRPs on neovascularization of DFU wound healing in 10 patients.

In 10 cases, PRP was predicted to reduce the size of the lesion by 50% after 16 days. Most of the treatment was terminated after 11.8 days after discharge. In patients with a callus, the duration of the lesion without treatment was significantly longer due to delayed discovery of DFU, but the duration of treatment was short. Usually, The one of barriers for treatment of DFU is the long term hospitalization, which may increase the socioeconomic burden and decrease quality of life. Previously, Korean population studies reported the mean hospitalization

계명대학교 KEIMYUNG UNIVERSITY

period of DFU was 61 days (14). In our cases with PRP, the mean hospitalization period was 26.3 (7-79) days and the DFU treatment period was 45.1 (4-90) days. Therefore, this results mean PRP treatment may shorten the hospitalization period by promoting wound healing.

In histopathologic anaylsis, before treatment, there were irregular epidermal hyperplasia of the epidermis, diffuse dense inflammatory cell infiltration and RBC extravasation throughout the dermis. After PRP treatment, inflammatory cells were reduced but many blood vessels were proliferated in the dermis. It was confirmed by CD31 (15) that it was specifically expressed in endothelial cells. This result means PRP treatment may help healing ulceration with neovascularization. HIF-1α plays an important role in angiogenesis by promoting the differentiation and function of vascular endothelial cells and is known as an important transcriptional factor on the expression of VEGF (16). HIF-1α is known to be a master regulator of genes that are expressed by hypoxia and regulated by oxygen concentration and is involved in over 60 gene expression and plays an important role in the expression of several hypoxia inducible genes (17). In particular, the expression of HIF-1α is lower in diabetic ulcer than non-diabetic ulcer (16,18), and activation of HIF-1α in diabetic mouse model has stimulated wound healing (19). However, there was no significant change in HIF-1α before and after PRP treatment. And the absence of HIF-1α in the tissues of this lesion suggests that PRP does not increase HIF-1α and PRP itself induces angiogenesis directly but not through Hif-1α.

The advantage of PRP is that it does not cause allergic reaction with its own blood, it is easy to perform, and it is not painful. However, as a limitation of PRP, blood collection volume is rather high, so blood collection may be difficult if vein condition is bad.

A dirty DFU is debridement followed by various wound healing

계명대학교 KEIMYUNG UNIVERSITY

preparations. It can be treated with topical variable growth factors, for example recombinant human epidermal growth factor, but most of them are expensive (6). A single growth factor defect will be only part of the complex process of impaired healing, therefore diverse growth factor will be more helpful to complex wound healing process (20). PRP is relatively inexpensive and has the advantage of providing various growth factors. Therefore, In the case of chronic ulcers with small ulcers and shallow depths, prophylactic PRP can be performed in the outpatient setting unless active surgical treatment is needed. The procedure itself can be used for patients who do not want suffering from pain or discomfort in daily life and who are reluctant to aggressive treatment. In particular, PRP can be easily treated at local clinic because it can be easily applied to patient at any time by centrifugal separator. In summary, PRP treatment in addition to conventional therapy for DFU may reduce the duration of hospitalization by promoting wound healing.

There were several limitations in our study. First, we had no control group such as only conventional treatment without PRP treatment treatment because of skip-flap or graft operations recently. Therefore, we used the DFU data in Korean population as control data. Second, debridement or topical treatments might have affected the healing of the ulcers. Third, the number of enrolled patients was small.

In conclusion, patients with DFU were successfully treated by PRP in this study and PRP with various growth factors is expected to be another effective option for wound treatment in DFU. Additional studies are necessary to confirm these hypotheses of PRP for treatment in DFU in a larger population.

계명대학교 KEIMYUNG UNIVERSITY

5. Summary

Diabetic foot ulcers (DFUs) are among the major complications in diabetic patients, and in this study, we investigated the adjuvant wound-healing effect of platelet-rich plasma (PRP) in 10 patients with DFU using PRP prepared from the patients' blood. The mean hospitalization period was 26.3 days, and the DFU treatment period was 45.1 days. PRP was administered a mean of 3 times. The time taken for a 50% reduction in the wound area was 16 days. After the start of PRP treatment, the depth of the wound reduced and it underwent epithelialization from the margin. PRP treatment shorten the hospitalization period by promoting wound healing compared to conventional treatment. In summary, patients with DFU were successfully treated by PRP in this study and PRP with various growth factors is expected to be another effective option for wound healing in DFU.

계명대학교 KEIMYUNG UNIVERSITY

References

1. Won JC, Lee JH, Kim JH, Kang ES, Won KC, Kim DJ et al.: Diabetes Fact Sheet in Korea, 2016. An Appraisal of Current Status. Diabetes Metab J 2018; 42: 415-24.

2. Bae JI, Kim JS, Kim MD, Yoon CJ, Cho YG: Prevalence and Current Status of Treatment of Diabetic Foot in South Korea. J Korean Soc Radiol 2016; 74: 169-76.

3. Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, et al.: Diabetic foot ulcers: Part II. Management. J Am Acad Dermatol 2014; 70: e2-4.

4. Yang S, Geng Z, Ma K, Sun X, Fu X: Efficacy of Topical Recombinant Human Epidermal Growth Factor for Treatment of Diabetic Foot Ulcer: A Systematic Review and Meta-Analysis. Int J Low Extrem Wounds 2016; 15: 120-5.

5. Tchanque-Fossuo CN, Ho D, Dahle SE, Koo E, Isseroff RR, Jagdeo J: Low-level Light Therapy for Treatment of Diabetic Foot Ulcer: A Review of Clinical Experiences. J Drugs Dermatol 2016; 15: 843-8.

6. Game FL, Jeffcoate WJ: Dressing and Diabetic Foot Ulcers: A Current Review of the Evidence. Plast Reconstr Surg 2016; 138: 158s-64s.

계명대학교 KEIMYUNG UNIVERSITY

7. Pallua N, Wolter T, Markowicz M: Platelet-rich plasma in burns. Burns 2010; 36: 4-8.

8. BGhafoor R, Anwar MI: Successful Treatment of Haemoglobinopathy Associated Chronic Leg Ulcer with Platelet Rich Plasma. J Coll Physicians Surg Pak 2016; 26: 545-6.

9. Martinez-Zapata MJ, Marti-Carvajal A, Sola I, Bolibar I, Angel Exposito J, Rodriguez L, et al.: Efficacy and safety of the use of autologous plasma rich in platelets for tissue regeneration: a systematic review. Transfusion 2009; 49: 44-56.

10. Arora NS, Ramanayake T, Ren YF, Romanos GE: Platelet-rich plasma: a literature review. Implant Dent 2009; 18: 303-10.

11. Eppley BL, Pietrzak WS, Blanton M: Platelet-rich plasma: a review of biology and applications in plastic surgery. Plast Reconstr Surg 2006; 118: 147e-59e.

12. Latalski M, Walczyk A, Fatyga M, Rutz E, Szponder T, Bielecki T et al.: Allergic reaction to platelet-rich plasma (PRP): Case report. Medicine (Baltimore) 2019; 98: e14702.

13. Lin CJ, Lan YM, Ou MQ, Ji LQ, Lin SD: Expression of miR-217 and HIF-1α/VEGF pathway in patients with diabetic foot ulcer and its effect on angiogenesis of diabetic foot ulcer rats. J Endocrinol Invest. 2019; 42: 1307-17.

계명대학교 KEIMYUNG UNIVERSITY

14. Chung CH, Kim DJ, Kim J, Kim H, Kim H, Min KW, et al.: Current Status of Diabetic Foot in Korean Patients Using National Health Insurance Database. J Korean Diabetes Assoc 2006 ; 30: 372-6.

15. Khan I, Bhardwaj M, Shukla S, Lee H, Oh MW, Bajpai VK, et al.: Carvacrol encapsulated nanocarrier/nanoemulsion abrogates angiogenesis by downregulating COX-2, VEGF and CD31 in vitro and in vivo in a lung adenocarcinoma model. Colloids Surf B Biointerfaces 2019; 181: 612-22.

16. Ruthenborg RJ, Ban JJ, Wazir A, Takeda N, Kim JW: Regulation of wound healing and fibrosis by hypoxia and hypoxia-inducible factor-1. Mol Cells 2014; 37: 637-43.

17. Duscher D, Maan ZN, Whittam AJ, Sorkin M, Hu MS, Walmsley GG, et al.: Fibroblast-Specific Deletion of Hypoxia Inducible Factor-1 Critically Impairs Murine Cutaneous Neovascularization and Wound Healing. Plast Reconstr Surg 2015; 136: 1004-13.

18. Catrina SB, Okamoto K, Pereira T, Brismar K, Poellinger L: Hyperglycemia regulates hypoxia-inducible factor-1alpha protein stability and function. Diabetes 2004; 53: 3226-32.

19. Botusan IR, Sunkari VG, Savu O, Catrina AI, Grunler J, Lindberg S, et al.: Stabilization of HIF-1alpha is critical to improve wound healing in diabetic mice. Proc Natl Acad Sci USA 2008; 105: 19426-31.

계명대학교 KEIMYUNG UNIVERSITY

20. Nwomeh BC, Yager DR, Cohen IK: Physiology of the chronic wound. Clin Plast Surg 1998; 25: 341-56.

계명대학교 KEIMYUNG UNIVERSITY

The Role of Platelet Rich Plasma in Wound Healing

Park, Ji Eun

Department of Internal Medicine
Graduate School

Keimyung University

(Supervised by Professor Kim, Sang Hyon)

(Abstract)

With the increase in the prevalence of diabetic foot ulcer (DFU), the demand for optimal treatment and management strategies has risen. Our study investigated the adjuvant wound-healing effect of platelet-rich plasma (PRP) on DFU. Ten diabetic patients with grade 1 DFU, as per Wagner's classification, were treated using PRP prepared from the patients' blood. The mean hospitalization period was 26.3 (7-79) days, and the DFU treatment period was 45.1 (4-90) days. PRP was administered a mean of 3(1-6) times. The time taken for a 50% reduction in the wound area was 16 days. After the start of PRP treatment, the depth of the wound reduced and it underwent epithelialization from the margin. In addition, PRP treatment may shorten the hospitalization period by promoting wound healing compared to

계명대학교 KEIMYUNG UNIVERSITY

conventional treatment. In conclusion, patients with DFU were successfully treated by PRP in this study and PRP with various growth factors is expected to be another effective option for wound healing in DFU.

계명대학교 KEIMYUNG UNIVERSITY

상처 치유에 대한 혈소판 풍부 혈장(PRP)의 역할과 효과

박 지 은
계명대학교 대학원
의학과 내과학 전공
(지도교수 김 상 현)

(초록)

당뇨병 유병률의 증가에 따라 합병증 중 하나인 당뇨병성 족부 궤양의 적절한 치료에 대한 요구도 증가되고 있다. 본 연구에서는 당뇨병성 족부 궤양의 치료에 대해 혈소판 풍부 혈장(PRP)을 이용한 상처 치유에 대한 효과를 확인하고자 하였다. 와그너 분류 상 1단계에 해당되는 당뇨병성 족부 궤양 환자들 10명에 대해 PRP를 이용한 치료를 시행하였다. 평균 입원 기간은 26.3 일 이었고, 족부궤양 치료 기간은 45.1 일 이었으며, PRP 치료는 평균 3회 시행하였다. 치료 16일 후, 상처의 50 %가 치유되었다. PRP 치료 후, 상처의 깊이가 줄어들었고, 상피화가 진행되었다. 또한, 기존의 족부 궤양 치료에 PRP 치료를 추가하면 상처 치유의 가속화와 함께 입원 기간이 줄어들었다. 결론적으로, 본 연구에서는 당뇨병성 족부궤양의 환자에게 다양한 성장인자를 함유하고 있는 PRP 치료를 시행하여 성공적으로 치료함으로써, PRP 시술이 상처 치유의 좋은 치료 도구가 될 수 있음을 보여준다.