EGT022 accelerates pressure ulcer wound healing

Ji Won Lee

Department of Medicine

The Graduate School, Yonsei University

EGT022 accelerates pressure ulcer wound healing

Ji Won Lee

Department of Medicine

The Graduate School, Yonsei University

EGT022 accelerates pressure ulcer wound healing

Directed by Professor Duk-Chul Lee

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Ji Won Lee

December 2012

This certifies that the Doctoral Dissertation of Ji Won Lee is approved.

Thesis Supervisor: Duk-Chul Lee

Thesis Committee Member#1: Min-Geol Lee

Thesis Committee Member#2: Myoung Hee Kim

Thesis Committee Member#3: Jae Yong Shim

Thesis Committee Member#4: Yang Je Cho

The Graduate School
Yonsei University

December 2012

ACKNOWLEDGEMENTS

I owe my utmost gratitude to my supervisor, Prof. Duk-Chul Lee, for his encouragement, guidance and support from the initial to the final level of this dissertation. It has been an honor for me to be his student and I will never forget his sincerity and enthusiasm as a doctor and a professor that inspired me as an excellent role model. One simply could not wish for a better supervisor.

I would like to express my gratitude to the thesis committee members: Prof. Min-Geol Lee, Prof. Myoung Hee Kim, Prof. Jae Yong Shim and Prof. Yang Je Cho for their devoting guidance, advice and insight. I am also heartily grateful to Prof. Hyang-Kyu Lee and Jin-Wook Jang, Hyong-Joon Lim in the EyeGene Inc. and Tae-Gyun Kim who supported me with their valuable assistance.

Lastly, I would like to thank my parents who raised me with love and supported me throughout becoming a doctor, my husband Ki Deok Park and my in-laws and my son Chan Hyuk Park for all their love, support and understanding.

Ji Won Lee

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

<ABSTRACT>

EGT022 accelerates pressure ulcer wound healing

Ji Won Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Duk-Chul Lee)

Background

EGT022 is a recombinant protein, having a human-derived Arg-Gly-Asp (RGD) motif. We investigated the wound healing effects of EGT022 in a pressure ulcer mouse model induced by magnetic plates.

Materials and Methods

For study 1, healthy Institute of Cancer Research (ICR) mice were randomized into three groups: control, treated with EGT022, and treated with basic fibroblast growth factor (bFGF). For study 2, ICR mice were randomized into four groups (healthy control, diabetic control, diabetic mice treated with EGT022, and diabetic mice treated with bFGF). Each mouse was put through three ischemia-reperfusion cycles by the external application of magnets. EGT022 and bFGF were administered topically for 5 days from 2 days after the ischemia-reperfusion cycle. Wound area was measured using a microscope and a histological examination was

performed to assess reepithelialization, granulation, and inflammation status.

Results

The wound area treated with EGT022 was significantly attenuated compared with healthy controls and diabetes control mice. EGT022 had a similar therapeutic effect on wound healing to bFGF treatment. Topical application of EGT022 accelerated tissue reepithelialization and reduced inflammation in both healthy ICR mice and diabetic mice.

Conclusions

EGT022 treatment had an effect on facilitating and improving pressure wound healing in healthy ICR mice and diabetic mouse model. EGT022 may be a good therapeutic candidate for treating chronic wounds.

Key words: EGT022, pressure ulcer, wound healing

EGT022 accelerates pressure ulcer wound healing

Ji Won Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Duk-Chul Lee)

I. INTRODUCTION

A pressure ulcer (PU) is a localized injury to the skin and/or underlying tissue, usually over a bony prominence, as a result of pressure, or pressure in combination with shear and/or friction.[1] PUs cause pain, infection, and result in significant morbidity and mortality, with a huge financial burden for patient care.[2-3] These wounds are commonly associated with elderly, bedridden, and debilitated patients and diabetics.[4] Despite efforts to prevent PU and a substantial number of emerging technologies of potential value in treating PUs, PU incidence is still increasing and limited clinical evidence is available regarding curative treatments for PU.[5] For this reason, there is a continuing need to develop new drugs for PU, and, in recent efforts, several biologics, such as basic fibroblast growth factor (bFGF) and platelet-derived growth factor have been approved for treating PU.[6-7]

EGT022 is a 58-amino-acid recombinant protein, originating in ADAM15 ("a disintegrin and metalloproteinase") and having the human-derived Arg-Gly-Asp (RGD) motif. The RGD peptide sequence has been identified as a recognition motif used by extracellular matrix proteins and several previous reports suggest the possibility that RGDs alters systemic inflammation.[8-10] We hypothesized that

EGT022 would accelerate wound healing and investigated the effects of EGT022 on PUs using a reproducible murine model of ischemia-reperfusion injury using the external application of magnets. Although conditions leading to the formation of PUs are considered multifactorial, the occurrence of cycles of ischemia-reperfusion (I/R) has been considered to be a significant contributor to the pathogenesis of pressure ulcers.[11] I/R injury has been defined as cellular injury resulting from the reperfusion of blood to previously ischemic tissue.[12] Ischemia induces a proinflammatory state in the vascular endothelium that serves not only as a vascular barrier, but also orchestrates polymorphonuclear leukocyte trafficking, and the subsequent restoration of blood flow initiates tissue necrosis and the development of ulcers due to excessive free radicals and severe derangement of the cell recruitment process to the site of injury.[13-15] Additionally, an I/R injury model induced by applying and removing a magnet to a dorsal region of mouse skin is known as a simple, non-invasive, clinically relevant model of PUs.[11] Thus, we investigated the effects of RGD-containing EGT022 on wound healing compared with bFGF, a positive control, in a pressure ulcer mouse model induced by magnetic plates.

II. MATERIALS AND METHODS

1. EGT022

EGT022 was produced by EyeGene Inc. (Seoul, Korea). An EGT022-expressing recombinant pEG022 vector was inserted into yeast (*Pichia pastoris*) and fermented using glycerine (Daejung, Korea) and methanol (Daejung). EGT022 was expressed and secreted into the fermentation medium. After the fermentation process, the culture medium was centrifuged and filtered. Ammonium sulfate (Merck, Germany) was added to the filtered supernatant and dissolved. Then, the supernatant was primary-purified using a phenyl Sepharose

column (GE Healthcare, USA). After primary purification, the purified solution was concentrated by ultrafiltration/diafiltration. The concentrated solution was secondarily purified using a gel filtration column (GE Healthcare, USA) and the purified active pharmaceutical ingredient (API) was used to make an EGT022 solution. EGT022 API was prepared by freeze-drying with mannitol as the vehicle.

2. EGT022 preparation for treatment

EGT022 API was redissolved in water for injection before use (50 μg/mL, 0.005% w/w). The prepared EGT022 solution was syringe-filtered.

3. Animals and treatment

A. Animal for Study 1: Verification of wound healing effects of EGT022 in induced pressure ulcers in healthy ICR mice

This study was approved by EyeGene IACUC. All animal care and procedures were performed following the standard operating procedure (SOP) of the EyeGene Research Center.

Nine-week-old male Institute of Cancer Research (ICR; 38 g) mice were kept on a 12/12-h light/dark cycle with food and water available *ad libitum*. Room temperature was set at 22±2℃ and 50±10% humidity. Mice were randomized into three groups (control mice, mice treated with EGT022, and mice treated with bFGF [brand name "Fiblast"]).

In total, 60 mice were evaluated over the study and five mice per group were analyzed and sacrificed at each time point. EGT022 was administered at 0.5 μg/10 μL phosphate-buffered saline (PBS)/cm^2 wound/day and bFGF, as a positive control, was administered at 1.0 μg/10 μL PBS/cm^2 wound/day. Control mice received equivalent doses of PBS.

B. Animal for Study 2: Verification of wound-healing effects of EGT022 in induced pressure ulcers in streptozotocin-induced diabetic mice

Ten-week-old male ICR mice were kept on a 12/12-h light/dark cycle with food and water available *ad libitum*. Room temperature was set at 22±2℃ and at 50±10% humidity. Mice were randomized into four groups (healthy control and diabetic control mice, diabetic mice treated with EGT022, and diabetic mice treated with bFGF [brand name Fiblast]). In total, 60 mice were evaluated over the study and five mice per group were analyzed and sacrificed at each time point.

Diabetes was induced by a single bolus intraperitoneal injection of streptozotocin (130 mg/kg, Sigma) mixed in 0.1 M sodium citrate buffer (pH 4.5) during the fasting state and was verified using an Accu-Check Active glucometer (Roche, Lyon, France). Animals to be used as healthy controls received equivalent doses of PBS. Serum glucose via sampling through the caudal vein was checked 3 days after the induction of diabetes. Diabetes was diagnosed when the result showed more than 400 mg/dL and polyuria. Although it does not indicate complete β cell destruction, blood glucose greater than 400 mg/dL provides a scenario close to the human situation with little interference from endogenous insulin while decreasing the likelihood of endogenous islet recovery.[16] Serum glucose levels in healthy control mice were typically 120 mg/dL, on average. EGT022, the experimental drug, and bFGF, the positive control, were administered at 0.5 μg/10 μL PBS/cm^2 wound/day and 1.0 μg/10 μL PBS/cm^2 wound/day, respectively.

4. I/R model of PUs in the mouse

The dorsal area was cleansed with 70% isopropanol, and dorsal hair was shaved using clippers (ER-541, Voguers, Korea) and shaving cream (Veet, Reckitt Benkiser). The skin was gently pulled up and placed between two round neodymium magnets (LG Magnet, Korea; diameter 11 mm, thickness 1.0 mm

average, weight 0.7 g, magnetic force 1000 Gauss). The resulting "pinch" procedure was designed to leave an 11-mm skin bridge and two 11-mm skin wounds (Fig. 1). Magnets were placed on the same position each time after locating the wounds. Each mouse was put through three ischemia-reperfusion cycles, consisting of a 12-h period of magnet placement, to initiate stage II-III pressure ulcer (partial skin loss involving epidermis and dermis-full thickness loss with damage and necrosis of subcutaneous tissue) formation. Necrotic and eschar tissue were debrided and removed from the ulcerative site.

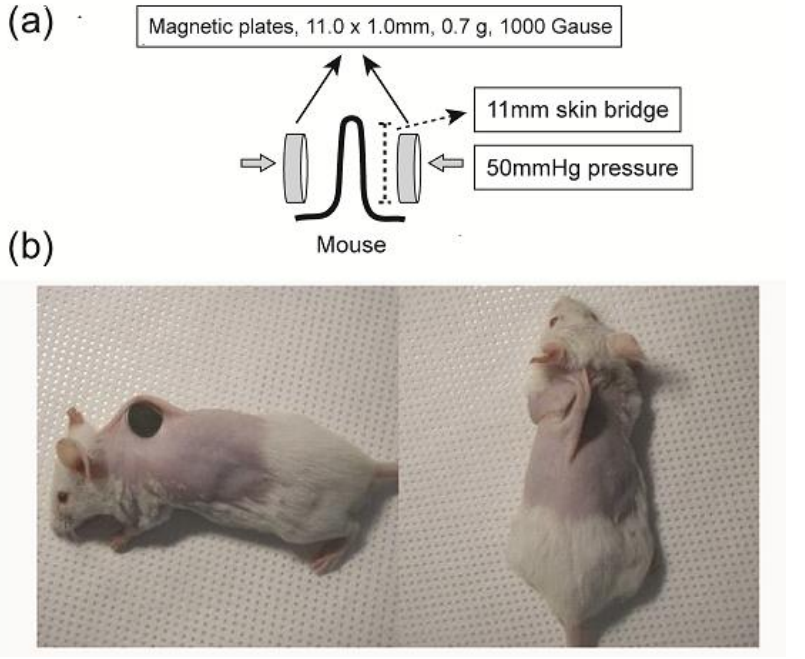

Figure 1. Schematic drawing and photographs of the experimental procedure demonstrating magnetic plate placement and technique for the creation of pressure ulcers on the mouse dorsum. (a) Schematic. (b) Final position of the magnetic plates to initiate the ischemic cycle.

EGT022 solution was prepared, containing 50 μg/mL EGT022, before treatment and stored at 4℃. The bFGF (Fiblast, 500 μg/5 mL, Daewoong Inc., Korea) was purchased from a commercial vendor for use as a positive control. EGT022 (0.5 μg/10 μL PBS/cm^2 wound/day) or bFGF (1.0 μg/10 μL PBS/cm^2 wound/day) was administered topically for 5 days from 2 days after the ischemia/reperfusion cycle.

5. Morphometric analysis of wound closure

Mice were anesthetized by intraperitoneal injection with a combination of anesthetics, Rompun (Bayer, Korea) and ketamine (Yuhan, Korea); the ratio was Rompun: ketamine = 0.2 mL/kg:1 mL/kg. Wounds were observed at 6-day intervals after injection (days 0, 6, 12, 18) for study 1, and at 3-day intervals after injection (days 0, 3, 6, 9, 12) for study 2, and were photographed with a digital camera (Olympus, Japan) and measured using the Image J program.

6. Histological and morphometric analyses

For histological analyses, the wound tissue samples, including full thickness skin layers (epidermis, dermis, and hypodermis) and the underlying muscle layer, were fixed in 4% formaldehyde, and embedded in paraffin. Then, 5-μm tissue sections, stained with hematoxylin and eosin (H&E), were examined and photographed with a BX40 microscope and digital camera (Olympus, Japan).

All specimens were evaluated by an independent pathologist with no knowledge of previous treatments or the time from wounding. The main histological outcome measures included reepithelialization, granulation tissue formation, and inflammatory cell infiltration (Table 1), modified from previous reports.[17-18]

Table 1. Criteria for reepithelialization, granulation, and inflammation scores

Scale	Reepithelialization	Granulation tissue formation	Inflammatory cell infiltration
0	Absent	Absent	Absent
1	Thickness of cut edges	Mild-surrounding tissue	Minimal
2	Migration of cells	Mild-granulation tissue	Mild
3	Bridging	Moderate-granulation tissue	Moderate
4	Keratinization	Marked-granulation tissue	Marked

7. Statistics

All results are expressed as means ± standard deviations. The non-parametric Wilcoxon rank sum test was used to test for differences between control mice and mice treated with EGT022 or bFGF for study 1, and between diabetic control mice and diabetic mice treated with EGT022 or diabetic mice treated with bFGF for study 2. Statistical significance was set at $P < 0.05$ or $P < 0.01$, as indicated.

III. RESULTS

1. Study 1: Verification of wound healing effects of EGT022 in induced pressure ulcers in healthy ICR mice

The open wound area was measured on days 0, 6, 12, and 18 using a microscope. The wound area of mice treated with EGT022 was significantly attenuated compared with that of control mice on days 6, 12, and 18. In the EGT022 group versus the control, the hole diameter was 15.59±8.36 mm^2

versus 30.48±10.63 mm^2 on day 6 ($P < 0.01$), 4.23±1.58mm^2 versus 19.22±5.40 mm^2 on day 12 ($P < 0.01$), and 1.30±0.48 mm^2 versus 3.74±1.52 mm^2 on day 18 ($P < 0.01$; Fig. 2). Wound area in the bFGF-treated group was also significantly reduced, compared with the controls, on days 12 and 18.

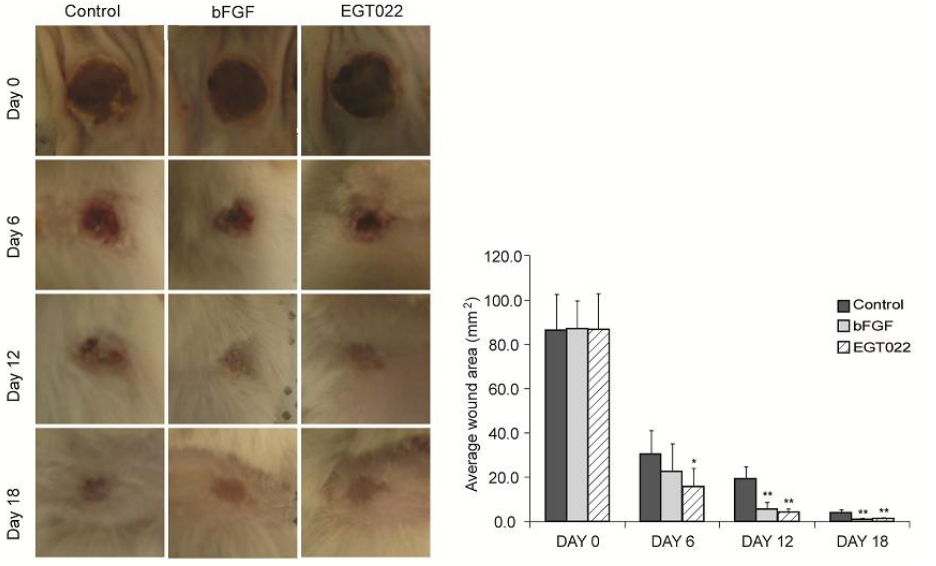

Figure 2. Wound area of pressure ulcer induced in normal ICR mice. The open wound area was measured on days 0, 6, 12, and 18. The open wound areas in mice treated with EGT022 or bFGF were significantly attenuated, compared with controls. *, $P < 0.05$; **, $P < 0.01$.

Furthermore, for histological confirmation and comparison the healing effects of EGT022, the damaged tissue was sampled, H&E-stained (Fig 3), and analyzed in terms of reepithelialization, granulation tissue formation, and inflammation status, following the scoring system in Table 1. Under the microscope, the EGT022-treated group showed a more organized reepithelialization over the granulation tissue, compared with control group, on day 6. The EGT022-treated group score was 2.40±0.89 for reepithelialization

status, which was significantly increased compared with the control group (0.20±0.45, $P < 0.01$). Such accelerated reepithelialization was also observed in the bFGF-treated group (1.20±0.84, $P < 0.05$). On day 12, all groups showed completion of reepithelialization. Granulation status was analyzed in the same tissue slides. The EGT022-treated group (2.60±0.55) showed a tendency for accelerated granulation tissue formation compared with the control (1.80±0.45, $P < 0.05$) on day 6. The bFGF-treated group score was 2.20±0.84 on day 6 and showed a similar tendency for accelerated granulation tissue formation; however, neither group showed a statistically significant difference compared with the control group. Finally, the degree of inflammation was observed on day 6. The EGT022-treated group score was 2.60±0.55, which was significantly decreased compared with the control group (4.00±0.00, $P < 0.01$). Like the EGT022-treated group, the bFGF-treated group showed a similar effect in inflammation (3.00±0.00). However, there was not a statistically significant difference compared with the control group. On days 12 and 18 after treatment, inflammation scores among the EGT022- and bFGF-treated groups and the control group were similar.

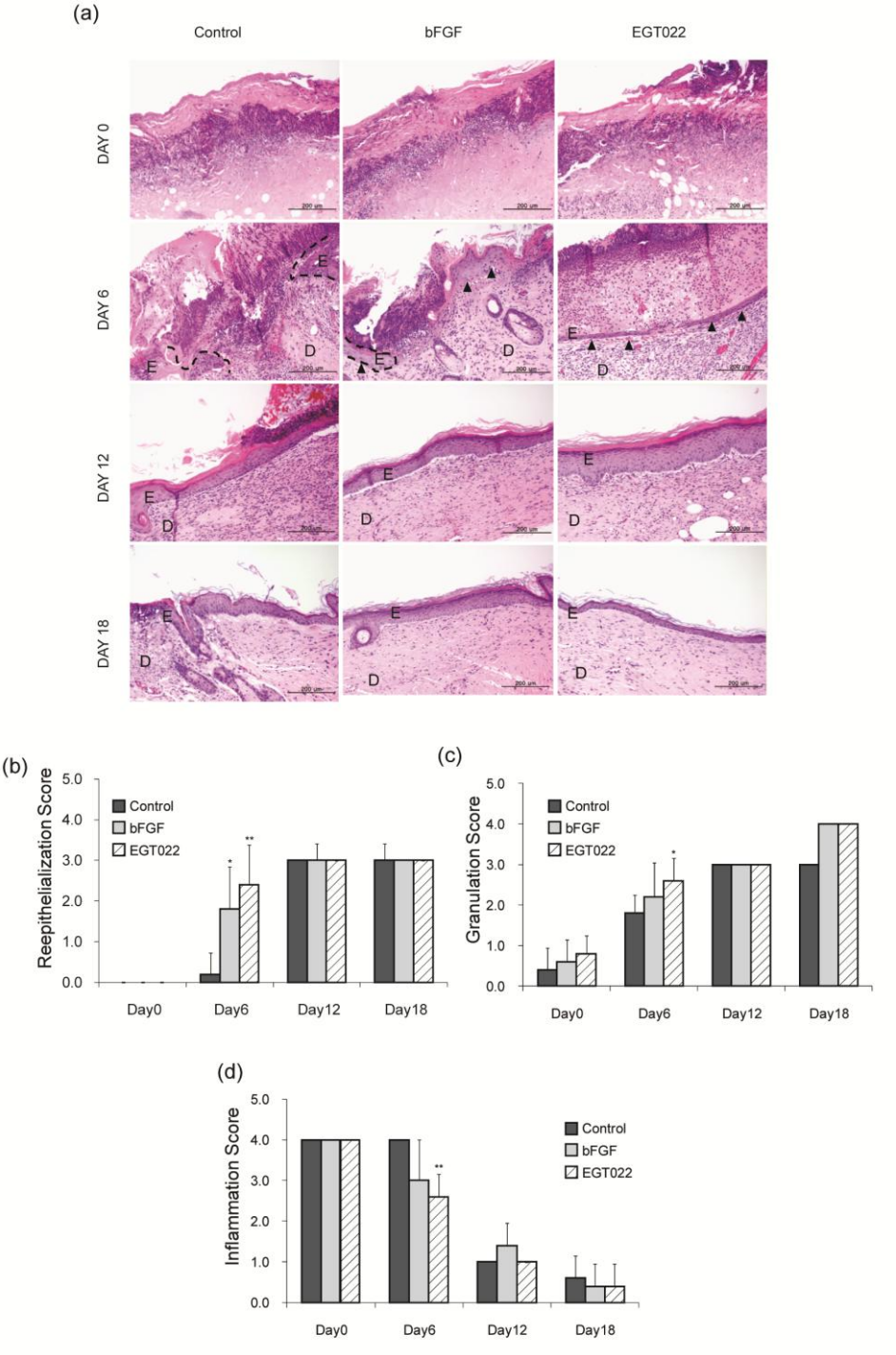
(a)
Control
bFGF
EGT022
DAY 0
DAY 6
DAY 12
DAY 18
E
D
(b)
Reepithelialization Score
Control
bFGF
EGT022
Day0
Day6
Day12
Day18
(c)
Granulation Score
(d)
Inflammation Score

Figure 3. H&E-stained microscopic images and reepithelialization, granulation, and inflammation scores of pressure ulcers in normal ICR mice

Paraffin-embedded wound tissues were stained with hematoxylin and eosin and microscopic images show pressure wound lesions (a). (original magnification: ×20, bar = 200 μm). On day 6, the EGT022-treated wound showed more organized reepithelialization over the granulation tissue compared with the control wound. On day 18, no significant histological difference in terms of epidermal thickness or epidermal/dermal cellularity was found among the control, EGT022-, and bFGF-treated wounds. E: epidermis; D: dermis; ▲arrow head: reepithelialization site. Reepithelialization (b), granulation tissue formation (c), and inflammation (d) were scored on days 0, 6, 12, and 18. Reepithelialization scores of mice treated with EGT022 or bFGF were significantly increased, compared with the control, on day 6 ($P < 0.05$ and $P < 0.01$, respectively). Granulation scores in wounded tissue treated with EGT022 were also increased significantly, compared with the control, on day 6 ($P < 0.01$). The inflammation score of mice treated with EGT022 was significantly reduced, compared with the control, on day 6 ($P < 0.05$). However, no significant difference in the granulation or inflammation scores of mice treated with bFGF were observed on the same day. *, $P < 0.05$; **, $P < 0.01$.

2. Study 2: Verification of wound-healing effects of EGT022 in induced pressure ulcers in streptozotocin-induced diabetic mice

Diabetes-induced mice showed significant weight loss after treatment with streptozotocin, and blood glucose levels of mice in the three groups injected with streptozotocin were increased significantly compared with the control untreated with streptozotocin in all experimental periods ($P < 0.01$; Fig. 4).

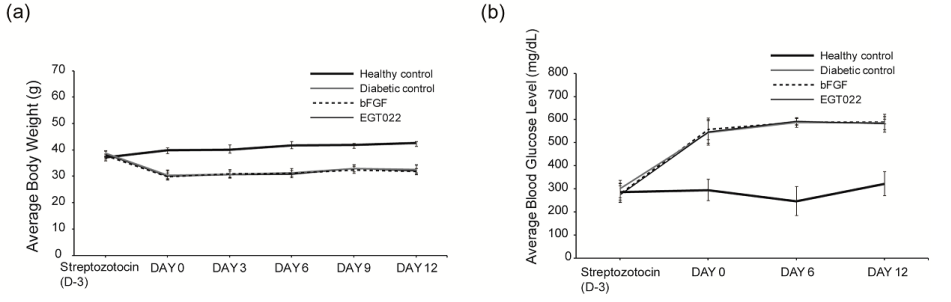

Figure 4. Body weights and blood glucose levels of pressure ulcer-induced normal and diabetic mice (a) Body weights of mice were measured before and after days 0, 3, 6, 9, and 12 of streptozotocin administration. Body weights of mice in three groups injected with streptozotocin were significantly ($P < 0.01$) reduced, compared with non-diabetic controls during the entire study period. (b) Blood glucose levels were measured before and after days 0, 6, and 12 of streptozotocin administration. Diabetes was defined as blood glucose over 400 mg/dl on day 0. Blood glucose levels of the mice in the three groups injected with streptozotocin were significantly ($P < 0.01$) increased, compared with non-diabetic controls in all experimental periods.

The open wound area was measured on days 0, 3, 6, 9, and 12 with a microscope. Wound areas of mice treated with EGT022 were significantly attenuated compared with the diabetes wound control group on days 3, 6, and 9. In EGT022 solution versus diabetes controls, the hole diameter was 12.5±3.8 mm^2 versus 34.5±5.3 mm^2 on day 3 ($P < 0.01$), 5.7±1.9 mm^2 versus 10.1±4.0

mm^2 on day 6 ($P < 0.05$), and 3.3±0.5 mm^2 versus 4.3±1.8 mm^2 on day 9 ($P < 0.05$; Fig. 5). The wound area in the bFGF-treated group was also significantly reduced, compared with the diabetes control wound group, on day 3 (12.1±3.98 mm^2).

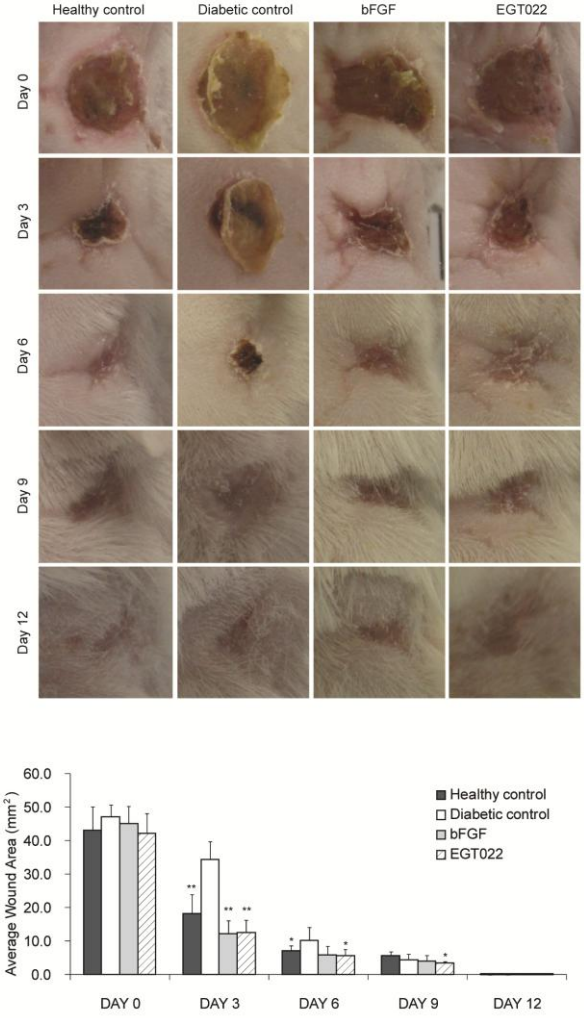

Figure 5. Wound areas of pressure ulcers in control and diabetic control mice, diabetic mice treated with EGT022, and diabetic mice treated with basic fibroblast growth factor (bFGF)

The open wound area was measured on days 0, 3, 6, 9, and 12. The EGT022-treated wound area was significantly attenuated, compared with the diabetes control wound, on days 3, 6, and 9. The wound area of the bFGF-treated group was also significantly reduced, compared with the diabetes control wound, on day 3. *, $P < 0.05$; **, $P < 0.01$.

To histologically confirm and compare the healing effects of EGT022, the damaged tissue was sampled and H&E-stained on days 0, 6, and 12. On day 6, incomplete reepithelialization with poorly formed and immature tissue was observed in the healthy control and diabetic control wound groups. On the other hand, EGT022- and bFGF-treated wounds showed bridging reepithelialization with some hyperplasia of the epidermis over the granulation tissue. On day 12, remodeling of epidermis and dermis were complete in EGT022- and bFGF-treated wounds. However, remaining inflammatory cells overlying granulation tissue were observed in healthy control and diabetic control wounds.

The damaged tissue was scored regarding reepithelialization, amount of granulation, and inflammation status on days 0, 6, and 12. On day 6, the EGT022-treated group score was 4.0±0.00 for reepithelialization status, which was significantly increased, compared with the diabetes control (3.4±0.55, $P < 0.05$). This accelerated reepithelialization was also observed in the bFGF-treated group (3.6±0.55), but was not statistically significant when compared with the diabetes control. On day 12, all groups showed completion of reepithelialization. Granulation status was analyzed in the same tissue slides. EGT022- (2.40±0.55) and bFGF (2.40±0.55)-treated groups showed a tendency for accelerated granulation tissue formation compared with the diabetes control group (2.20±0.45) on day 6; however, the differences were not statistically significant. Finally, the degree of inflammation was observed on day 12; the EGT022- and bFGF-treated group scores were 0.80±0.84 and 1.40±0.55, respectively, which were significantly decreased compared with the diabetes

control group (2.20±0.45) ($P < 0.01$ and $P < 0.05$, respectively; Fig. 6).

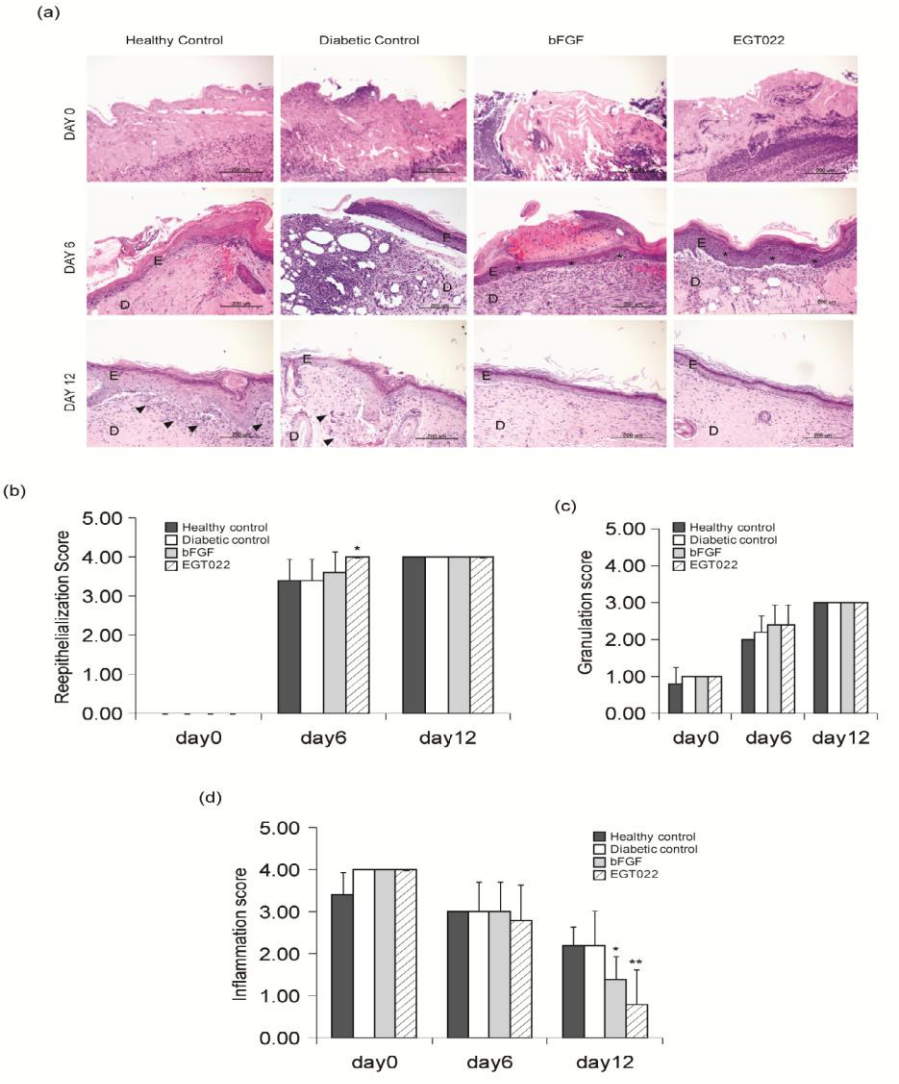

Figure 6. H&E-stained microscopic images and reepithelialization, granulation, and inflammation scores of pressure ulcer-induced normal and diabetic mice. Paraffin-embedded wound tissues were stained with hematoxylin and eosin and microscopic images show pressure wound lesions (a). (original magnification: ×20, bar = 200 μm)

On day 6, incomplete reepithelialization with poorly formed and immature tissue was observed in healthy control and diabetic control wounds. On the other hand, EGT022- and bFGF-treated wounds showed bridging reepithelialization with some hyperplasia of the epidermis over the granulation tissue (* reepithelialization site). On day 12, complete remodeling of the epidermis and dermis were observed in EGT022- and bFGF-treated wounds. However, remaining inflammatory cells overlying granulation tissue (arrow heads) were observed in normal control and diabetic control wounds. E: epidermis, D: dermis.

Reepithelialization (b), granulation tissue formation (c) and inflammation (d) were scored on days 0, 6, and 12. The reepithelialization scores of mice treated with EGT022 were increased compared with other groups on day 6 ($P < 0.05$). The granulation grade of bFGF- and EGT022-treated wounds tended to increase; however, no significant difference was found compared with the diabetic control wound in any experimental period. The inflammation scores of mice treated with EGT022 and bFGF were significantly reduced, compared with the diabetic control wound, on day 12 ($P < 0.01$ and $P < 0.05$, respectively). *, $P < 0.05$; **, $P < 0.01$.

IV. DISCUSSION

We studied the therapeutic efficacy of EGT022 using the PU mouse model, induced by I/R cycles using magnetic plates. Topical application of EGT022 promoted healing processes, leading to accelerated tissue reepithelialization and reducing inflammation in healthy ICR mice and in streptozotocin-induced diabetic mice. Indeed, EGT022 had a similar therapeutic effect on wound healing to bFGF, a growth factor that has been approved for use in treating chronic wounds.

EGT022 is a 6.1 kDa protein containing the human-derived RGD motif. RGD peptides are widely used to increase cell attachment to non-adhesive materials.[19] Previously, an RGD-containing peptide was shown to effectively inhibit collagen-triggered activation of leukocytes and platelets.[9] Moreover, the RGD peptide inhibits expression of the inflammatory cytokines iNOS, and MMP-9 in rat liver after cold ischemia/reperfusion injury.[10] On this basis, we hypothesized that clinical application of topical EGT022 may be a suitable choice for alleviating pressure ulcers related to I/R injuries.

I/R injury is regarded as a major contributing factor in the formation of chronic skin wounds, including diabetic foot ulcers.[20] The pathogenesis of I/R injury begins with an inflammatory influx to ischemic vascular endothelium and the subsequent reperfusion event causes tissue damage in a variety of ways, including the secretion of proteolytic enzymes, leading to the degradation of growth factors and structural proteins of the extracellular matrix and the generation of oxygen-derived free-radicals that can directly damage cells or extracellular matrix.[21-22] Thus, blockade of the inflammatory cascade has been considered a potential strategy for preventing I-R injury.[21] In this study, we found that EGT022 decreased inflammation, which might improve wound closure in both healthy and diabetic mice. Although it remains unclear as to whether the reduced inflammation was specifically related to I-R injury-induced inflammation, it at least suggests that EGT022 plays an important role in modulating inflammation in the wound healing process.

Additionally, EGT022 accelerated reepithelialization in the early stages of the healing process in both healthy and diabetic mice. Recent evidence indicates that the epidermis has an important role in dermal scar formation as well as wound closure. Modulation of the inflammatory state of the epidermis—especially through restoration of barrier function—is a key target in

the control of hypertrophic scar formation and delayed reepithelialization could cause an increased dermal scar.[23] Thus, it is possible that the accelerating effect of EGT022 on reepithelialization may be a promising new target for continued therapeutic efforts at scar reduction. Further studies are needed to define the pathophysiological mechanism underlying our hypothesis.

In addition to inflammation and reepithelialization, microvascular dysfunction and impaired angiogenesis are major causes of poor wound healing in I/R-induced PUs and reestablishment of structural and functional microvasculature could be beneficial in promoting wound healing in these patients.[24-25] Indeed, several growth factors, including bFGF, are known to promote angiogenesis, leading to accelerated tissue repair.[26] Thus, in future studies, we must define whether EGT022 is involved in vascular remodeling, maturation, and stabilization processes.

V. CONCLUSION

Based on the findings presented here, topical EGT022 treatment had an effect on facilitating and improving pressure wound healing in healthy ICR mice and a diabetic mouse model. EGT022 may be a therapeutic candidate for treating chronic wounds.

REFERENCES

1. National Pressure Ulcer Advisory Panel. Pressure ulcer stages revised by NPUAP. http://www.npuap.org/pr2.htm. Accessed June 2, 2009.
2. Duncan KD. Preventing pressure ulcers: the goal is zero. Jt Comm J Qual Patient Saf 2007; 33(10):605–10.
3. Thomas D. The prevention and management of pressure ulcers. Rev Clin Gerontol 2007; 17:241–57.
4. O'Brien S P, Gathan V, Wind S, Kerstein M D. What is the paradigm: Hospital or home health care for pressure ulcer? Am Surg 1999; 65: 303–6.
5. Walker Sewill DK, Van Sell S, Kindred C. Pressure ulcer prevention: utilizing unlicensed assistive personnel. Crit Care Nurs Q 2010;33(4):348-55.
6. Cross KJ, Mustoe TA. Growth factors in wound healing. Surg Clin N Am 2003;83:531–45.
7 Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003;83:835–70.
8. Mousa SA. alphav Vitronectin receptors in vascular-mediated disorders. Med Res Rev 2003;23(2):190–9.
9. Bengtsson T, Grenegård M. Leucocyte activation by collagen-stimulated platelets in whole blood. Scand J Clin Lab Invest 2002;62:451–61.
10. Fondevila C, Shen XD, Moore C, Busuttil RW, Coito AJ. Cyclic RGD peptides with high affinity for $\alpha_5\beta_1$ integrin protect genetically fat Zucker rat livers from cold ischemia/reperfusion injury. Transplantation Proceedings 2005;37:1679–81.
11. Stadler I, Zhang RY, Oskoui P, Whittaker MS, Lanzafame RJ.Development of a simple, noninvasive, clinically relevant model of pressure ulcers in the mouse. J Invest Surg 2004;17(4):221–7.
12. Wolfson R G, Millar C G, Neild G H. Ischemia reperfusion injury in the

kidney: Current status and future direction. Nephrol Dial Transplant 1994; 9: 1529–31.

13. Kong T, Eltzschig HK, Karhausen J, Colgan SP, Shelley CS. Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of beta2 integrin gene expression. Proc Natl Acad Sci USA 2004;101:10440–5.
14. Robson MC, Hill DP, Smith PD, Wang X, Meyer-Siegler K, KO F, et al. Sequential cytokine therapy for pressure ulcers: Clinical and mechanistic response. Ann Surg 2000;231(4):600–11.
15. Peirce SM, Skalak TC, Rieger JM, Macdonald TL, Linden J.Selective A(2A) adenosine receptor activation reduces skin pressure ulcer formation and inflammation. Am J Physiol Heart Circ Physiol 2001;281(1):H67-74.
16. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, et al. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab Anim. 2011;45(3):131-40.
17. Lee YH, Chang JJ, Chien CT, Yang MC, Chien HF. Antioxidant sol-gel improves cutaneous wound healing in streptozotocin-induced diabetic rats. Exp Diabetes Res 2012;2012:504693.
18. Sevimli-Gür C, Onbaşılar I, Atilla P, Genç R, Cakar N, Deliloğlu-Gürhan I, et al. In vitro growth stimulatory and in vivo wound healing studies on cycloartane-type saponins of Astragalus genus. J Ethnopharmacol 2011;134(3):844-50.
19. Schmedlen RH, Masters KS, West JL. Photocrosslinkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. Biomaterials 2002; 23: 4325–32.
20. Chen WY, Rogers AA. Recent insights into the causes of chronic leg ulceration in venous diseases and implications on other types of chronic wounds. Wound Repair Regen 2007;15(4):434-49.

21. Anaya-Prado R, Toledo-Pereyra LH, Lentsch AB, Ward PA. Ischemia/reperfusion injury. J Surg Res 2002; 105: 248–58.
22. Vajdovich P. Free radicals and antioxidants in inflammatory processes and ischemia-reperfusion injury. Vet Clin N Am 2008; 38: 31–123.
23. Mustoe TA, Gurjala A. The role of the epidermis and the mechanism of action of occlusive dressings in scarring.Wound Repair Regen 2011;19 Suppl 1:s16-21.
24. Sivan-Loukianova E, Awad OA, Stepanovic V, Bickenbach J, Schatteman GC. CD34+ blood cells accelerate vascularization and healing of diabetic mouse skin wounds. J Vasc Res 2003;40(4):368-77.
25. Cho CH, Sung HK, Kim KT, Cheon HG, Oh GT, Hong HJ, et al. COMP-angiopoietin-1 promotes wound healing through enhanced angiogenesis, lymphangiogenesis, and blood flow in a diabetic mouse model. Proc Natl Acad Sci U S A 2006;103(13):4946-51.
26. Kawai K, Suzuki S, Tabata Y, Nishimura Y. Accelerated wound healing through the incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis using a pressure-induced decubitus ulcer model in genetically diabetic mice. Br J Plast Surg 2005 ;58(8):1115-23.

< ABSTRACT (IN KOREAN)>

EGT022의 압박궤양 상처치유의 효과

<지도교수 이 덕 철 >

연세대학교 대학원 의학과

이 지 원

연구배경

EGT022 은 인간에서 유래된 Arg-Gly-Asp (RGD) motif 를 포함하고 있는 재조합 단백질이다. 자석을 이용하여 마우스에서 압박궤양 모델을 만든 후 EGT022가 상처 치유에 미치는 효과에 대해 알아보았다.

연구방법

연구 1에서는 ICR 마우스를 대조군, EGT022 투여군, basic fibroblast growth factor (bFGF) 투여군의 세 그룹으로 무작위배정을 하였고 연구 2에서는 건강대조군, 당뇨병유도 대조군, 당뇨병 유도 후 EGT022 투여군, 당뇨병 유도 후 bFGF 투여군의 네 그룹으로 무작위배정을 하였다. 각각의 마우스에서 자석을 이용하여 3번의 허혈-재관류 손상 과정을 거친 뒤 압박궤양이 생성되었다. EGT022와 bFGF는 허혈-재관류 손상 과정 2일 후부터 5일 동안

연속하여 국소 투여 되었다. 현미경을 이용하여 상처 크기를 측정하였고 조직학적 검사를 통해 상피재생, 육아조직 형성, 염증 상태를 관찰하였다.

연구결과

정상 마우스와 당뇨병이 유도된 마우스 모두에서 EGT022 투여는 압박궤양 상처치유의 효과가 있었고 bFGF 투여와 비슷한 치료 효과를 나타내었다. 정상 마우스와 당뇨병이 유도된 마우스 모두에서 EGT022 국소투여는 압박궤양 상처의 상피재생을 촉진하고 염증반응을 감소시켰다.

결론

EGT022 국소투여는 정상 마우스와 당뇨병이 유도된 마우스 모두에서 압박궤양 상처치유를 빠르게 하고 호전시키는 효과가 있었으며, 향후 만성 상처의 치료제로 사용될 후보물질로써의 가능성을 나타낸다.

핵심되는 말: EGT022, 압박궤양, 상처치유