JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2020, VOL. 54, NO. 6, 382–387
https://doi.org/10.1080/2000656X.2020.1814312

ARTICLE

The effects of valsartan on scar maturation in an experimental rabbit ear wound model

Muzaffer Kurt[a], Funda Akoz Saydam[b], Mehmet Bozkurt[c], Merdan Serin[d] and Aysel Caglar[e]

[a]Department of Plastic Surgery, Tekirdag State Hospital, Tekirdag, Turkey; [b]Department of Plastic Surgery, Medicine Hospital, Istanbul, Turkey; [c]Department of Plastic Surgery, Bagcilar Training and Research Hospital, Istanbul, Turkey; [d]Department of Plastic Surgery, Istanbul Training and Research Hospital, Istanbul, Turkey; [e]Department of Pathology, Bagcilar Training and Research Hospital, Istanbul, Turkey

ABSTRACT

Introduction: In our study, we aimed to search and compare the effects of valsartan and enalapril on the pathological scar formation on the basis of histomorphological parameters.

Materials and Methods: Nine New Zealand albino male rabbits, which were divided into three groups, were included in the study. A previously described rabbit ear wound model was used. Enalapril was administered 0.75 mg/kg/day on the first group and valsartan was administered 10 mg/kg/day on the second group for 40 days. The third group was the control group. Results were evaluated on the 40th day with scar elevation index calculation and histological studies. Histological studies were done by using Hematoxylin-eosin, Masson trichrome and Sirius Red stains.

Results: Enalapril and valsartan groups were both significantly effective on the prevention of pathological scar formation when compared to the control group in terms of fibroblast count, capillary count, type 1/3 collagen ratio, collagen organization, and epithelial thickness. There was no significant difference between the enalapril and control group on the scar elevation index. Valsartan group was more efficient than the enalapril group on the reduction of fibroblast count and epithelial thickness.

Conclusion: Both Valsartan and Enalapril are found to be effective for the prevention of pathological scar formation.

ARTICLE HISTORY
Received 16 January 2020
Revised 7 August 2020
Accepted 17 August 2020

KEYWORDS
Valsartan; pathological scar; rabbit

Introduction

Pathological scars are characterized by proliferation of the dermal tissue, with excessive deposition of fibroblast-derived extracellular matrix proteins, chronic inflammation and fibrosis. Multiple methods have been used for the treatment of abnormal scars, but to date, the optimal treatment method has not been established.

Despite decades of research regarding molecular and cellular processes of pathological scar formation, its exact mechanisms remain unclear [1]. Experimental and clinical studies in the recent years revealed the presence of angiotensin II molecules in hypertrophic scar tissue samples and fibroblast cell cultures [2,3]. Angiotensin II receptor mRNA expression has been shown in human keratinocytes cell cultures with RT-PCR techniques [4]. In addition fibroblast cell cultures from human scar tissues have been shown to contain angiotensin II receptors [5]. Experimental studies in recent years have shown that angiotensin converting enzyme (ACE) inhibitor enalapril leads to a decrease in pathological scar formation. The cytokines which belong to the transforming growth factor (TGF)-β family plays an important role in the wound healing process and hypertrophic scarring. Dermal fibroblasts, which are responsible for the synthesis of collagen type I and extracellular matrix proteins, are stimulated by TGF-β. Angiotensin II increases TGF-β expression so ACE inhibition also inhibits TGF-β stimulation ACE inhibition causes a non-selective decrease of TGF-β including β1, β2 and β3 [6].

The tissue renin angiotensin system (tRAS) is a locally active system and acts as o modulator for tissue homeostasis and regeneration. Due these mechanisms, it has been suggested as a target of the treatment for pathologies involving hemostasis and wound healing. The tRAS can have both pro-inflammatory/pro-fibrotic as wells as anti-inflammatory and anti-fibrotic effects. Since tRAS shares receptors with systemic RAS, drugs designed to act on systemic RAS, such as antihypertensives, can also be used to regulate tRAS.

The aim of this study was to show the efficiency of Valsartan in reducing the pathological scar formation in a previously defined pathological scar formation model in rabbit ear [7].

Materials and methods

This study was approved by the Istanbul Bagcilar Training and Research Hospital Ethical Comity (approval number: 2015/99). Nine New Zealand White male rabbits (6–8 months, 2500–3500 g) were divided into three groups, with three rabbits in each group. The rabbits were individually housed in 12–12 h night day cycle, temperature regulated room (21 ± 2 C°), fed twice a day and had access to water *ad libitum*.

Group 1: Enalapril: 0.75 mg/kg/day
Group 2: Valsartan: 10/mg/kg/day
Group 3: Control

Surgical technique

A previously defined rabbit ear hypertrophic scar model was used for the study [8,9]. Surgical anesthesia was performed with intramuscular injection of 10% Ketamine HCL (35 mg/kg) (Ketalar, Eczacıbasi, Turkey) and Xylazine HCL (5 mg/kg) (Rompun, Bayer,

CONTACT Merdan Serin merdanserin@gmail.com Department of Plastic Surgery, Istanbul Research and Teaching Hospital, Istanbul, Turkey

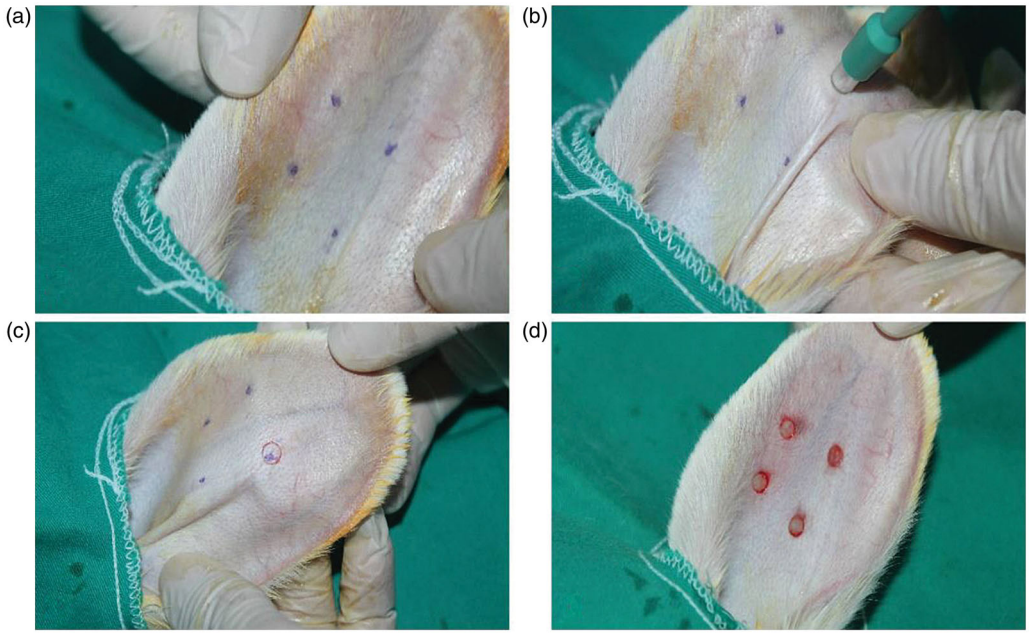

Figure 1. (a) Four points are marked on the ventral surface of both ears. (b–d) Four 5 mm defects were created with a circular punch biopsy tool.

Germany). Prior to the surgical procedure depth of anesthesia was confirmed with the control of corneal and extremity reflexes. The surgical procedures were performed by the same surgeon under sterile conditions under operating microscope. After surgical cleaning of the area four 5 mm defects were created on the ventral surface of the both ears. Under magnification, all of the epidermis, dermis and perichondrium within the defect area was removed. Wound closure of the defects was performed with Tegaderm (3 M Co., St. Paul, MN) dressing material following hemostasis (Figure 1). Following the procedure, the animals were monitored and kept in separate cages, the wounds were cleaned daily with an antiseptic solution and the tegaderm dressings were replaced daily (Figure 1).

First group received 0.75 mg/kg/day of Enalapril following the surgery for 40 days with oral gavage. Second group received 10 mg/kg/day of Valsartan for 40 days with oral gavage. The tablets were prepared into a solution in 2 ml of distilled water. Third group did not receive any treatment. Standardized digital photographs of the scars were taken at 20th and 40th postoperative days. At the 40th day following the surgery the animals were sacrificed with high dose intracardiac Ketamine injection. Scar tissues including complete thickness of dermis and epidermis were excised along with 1 mm of normal skin for histopathological analysis.

Histopathological analysis

All of the histopathological examinations were performed by the same pathologist. The tissue samples were fixed in 10% formaldehyde solution. The samples were embedded in paraffin blocks. 4 μm thick slices were obtained. Staining with Hematoxylin and Eosin, Mason Trichrome, Elastic Van Gieson and Sirius Red was performed. Olympus BX51 light microscope was used for the evaluation. The number of fibroblasts was counted with 400× magnification in 3 randomized fields. The number of capillary formations was counted under 40× magnification. Type 1 and 3 collagen formations patterns were evaluated Sirius Red staining with 400× magnification. Type 1 mature collagen fibers were thicker with orange-red staining while type 3 immature collagen fibers were thinner with green staining. Collagen structure was evaluated with a scale from 0 to 3 in which '0' being normal organized collagen structure and '3' being the least organized collagen structure (Figure 2).

Scar elevation index

Scar elevation index (SEI) is a previously defined measurement method which correlates with wound healing and scar evaluation methods. This method calculates the ratio of normal wound zone inside the whole scar area. SEI measurement with occulometric evaluation was performed. Epithelial thickness was measured in millimeters. SEI takes into account the cellular proliferation, matrix structure and epithelial thickness [6]. This index is defined as the ratio of the total wound area and tissue height to the area of normal tissue under the hyper-trophic scar (Figure 3).

Statistical analysis

Statistical analysis was performed with Prism 7 software (Version 7.00 for Windows, GraphPad Software, La Jolla, CA, USA). Statistical evaluation was performed to establish a descriptive and comparative analysis of the results. The data was not in normal distribution. A non-parametric Kruskal–Wallis test was used to compare the groups. Multiple comparisons were performed using Dunn's test.

Results

The mean number fibroblasts were 432, 340 and 729 in the Enalapril, Valsartan and Control groups, respectively. The difference between enalapril and control group was significant ($p < 0.001$). The difference between valsartan and control group was significant ($p < 0.0001$). The difference between enalapril and valsartan group was significant ($p < 0.001$).

The mean number capillary formations were 6.33, 5.67 and 10.67 in the Enalapril, Valsartan and Control groups, respectively. The difference between enalapril and control group was significant ($p < 0.001$). The difference between valsartan and control

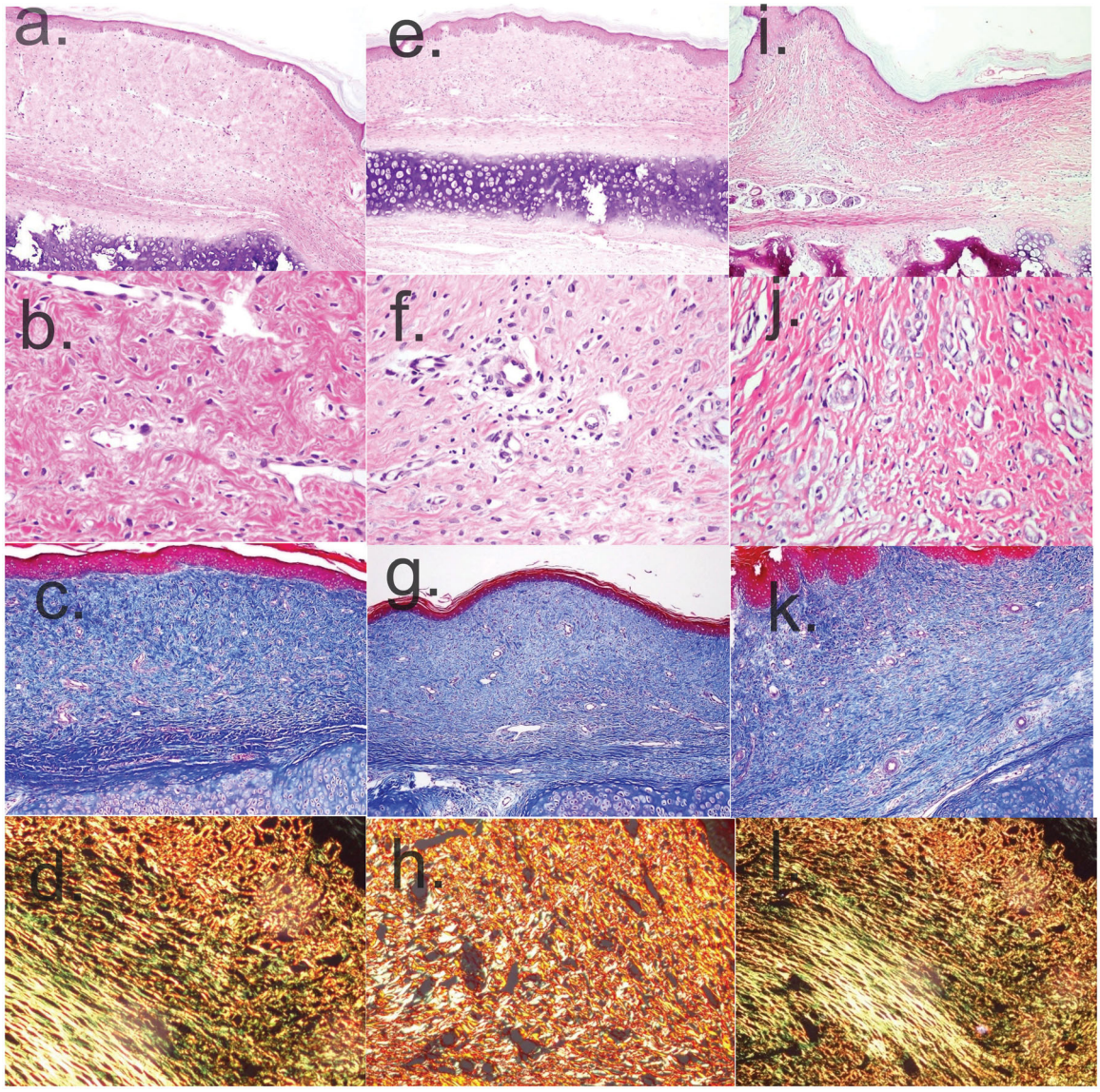

Figure 2. Sample images from histopathological evaluations of the scar tissues. (a) Group 1: Increased epithelial thickness and regular collagen fibers [Hematoxylin and Eosin (H&E), ×100]. (b) Group 1: Fibroblast cells and capillary formations (H&E, ×400). (c) Group 1: Regular collagen fibers (Mason Trichrome, ×100). (d) Group 1: Type 1 collagen fibers (Sirius Red, ×200). (e) Group 2: Epithelial layer can be seen (H&E, ×100). (f) Group 2: capillary formations (H&E, ×400). (g) Group 2: Regular collagen fiber formations (Mason Trichrome, ×100). (h) Group 2: Type 1 and 3 collagen fibers (Sirius Red, ×200; Sirius Red, ×200). (i) Group 3: Irregular collagen fibers (H&E, ×100). (j) Group 3: Fibroblast cells and capillary formations (H&E, ×400). (k) Group 3: Irregular collagen fiber formations (Mason Trichrome, ×100). (l) Group 3: Type 1 and 3 collagen fibers (Sirius Red, ×400).

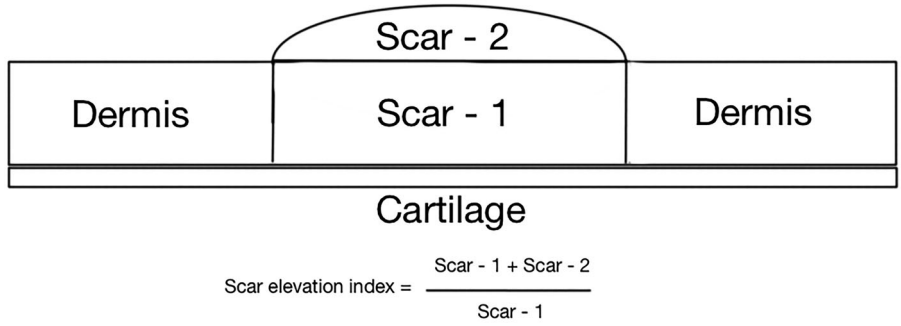

Figure 3. Calculation of scar elevation index.

group was significant ($p < 0.001$). The difference between enalapril and valsartan group was insignificant.

The mean ratio of type 1/type 3 collagen was 1.13, 1.29 and 2.88 in the Enalapril, Valsartan and Control groups, respectively. The difference between enalapril and control group was significant ($p < 0.001$). The difference between valsartan and control group was significant ($p < 0.001$). The difference between enalapril and valsartan group was insignificant.

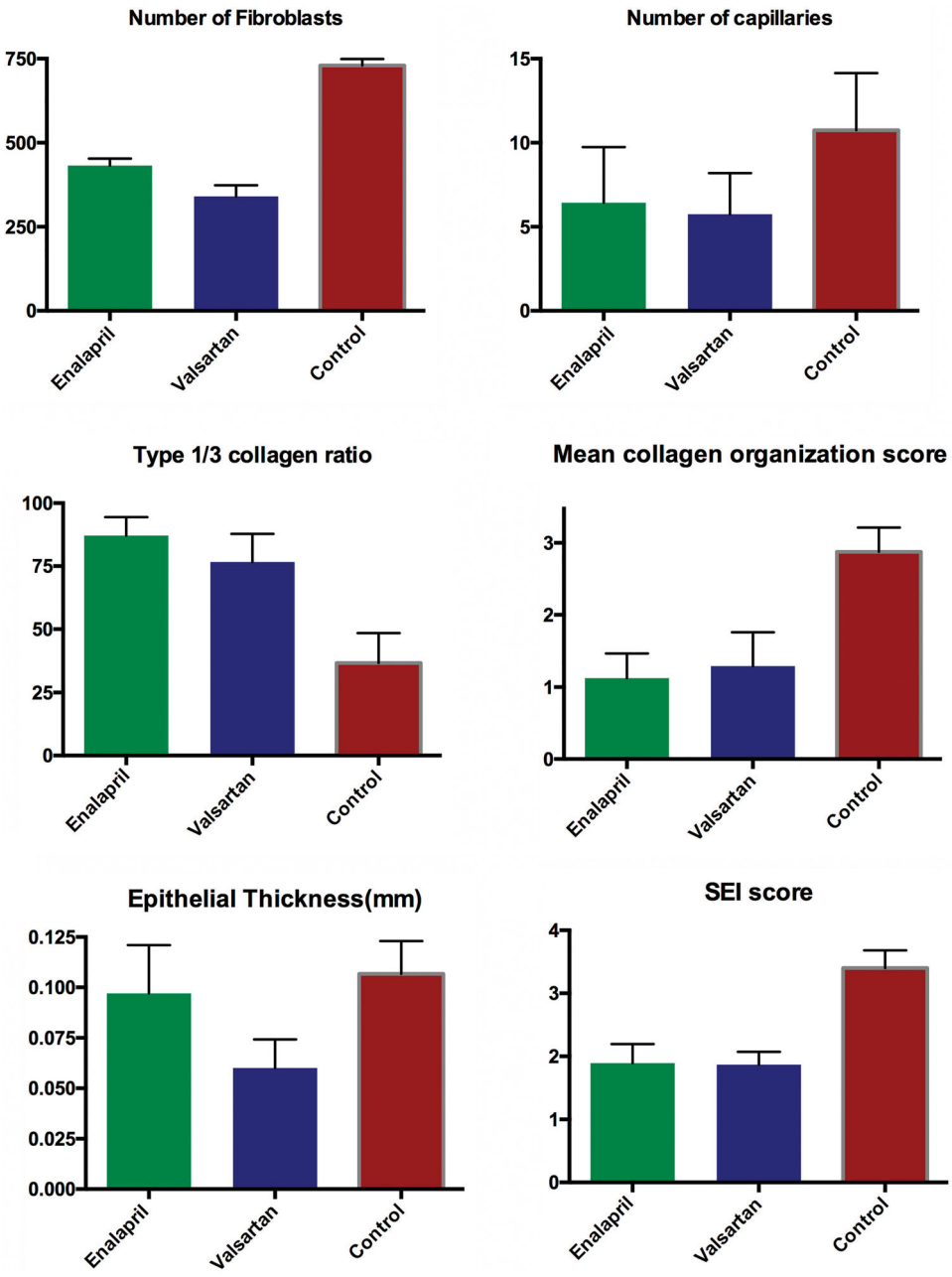

Figure 4. Summary of histopathological results. (a) Mean number of fibroblasts in each group. (b) Mean number of capillary formations in each group. (c) Mean of type 1/3 collagen ratio for each group. (d) Mean collagen organization score for each group. (e) Mean value of epithelial thickness for each group in millimeters. (f) Mean value of SEI score.

The mean of collagen organization score was 1.125, 1.292 and 2.875 in the Enalapril, Valsartan and Control groups, respectively. The difference between enalapril and control group was significant ($p < 0.001$). The difference between valsartan and control group was significant ($p < 0.001$). The difference between enalapril and valsartan group was insignificant.

The mean epithelial thickness in millimeter was 0.0967, 0.06 and 0.1067 in the Enalapril, Valsartan and Control groups, respectively. The difference between enalapril and control group was insignificant. The difference between valsartan and control group was significant ($p < 0.001$). The difference between enalapril and valsartan group was significant ($p < 0.001$).

The mean value of SEI score was 1.867, 1.867 and 3.4 in the Enalapril, Valsartan and Control groups, respectively. The difference between enalapril and control group was significant ($p < 0.001$). The difference between valsartan and control group was significant ($p < 0.001$). The difference between enalapril and valsartan group was insignificant (Figure 4).

Discussion

Hypertrophic scar presents itself as an elevated scar with increased collagen production. Dermal tissue proliferation resulting from increased extracellular matrix proteins from fibroblasts, increase in inflammation and fibrosis have been previously shown [10].

Various treatment modalities for the treatment of abnormal pathological scars have been defined. Despite this, there are no particular treatment algorithms for their treatment [11]. These methods include surgical excision, pressure treatment [12],

intradermal IFN treatment, topical and intradermal corticosteroid treatment [13], intradermal bleomycin [14], laser therapy [15], silicone gel dressings [16] and other topical agents [17].

Shin et al. [18] have compared intramarginal and extramarginal excision with skin grafting techniques for the treatment of hypertrophic scars in a retrospective study. They have found that intramarginal excision has lower recurrence rate. In our study, Valsartan has been given for 40 days and significant improvement has been demonstrated when compared with the control group. Despite this, we have no data regarding the possibility of recurrence in scar tissue following the cessation of the treatment. Future studies could be planned to answer this question.

Huang et al. [19] have previously shown that pressure treatment increases the metalloproteinase activity and reduces pathological scar formation. We have found in our study that type 1 collagen, which is major component of a healthy scar tissue was significantly higher in the enalapril group. The exact mechanism of how valsartan or enalapril effects the remodeling phase of wound healing remains unclear. Further studies with specific immunohistochemical staining might shed a light on these mechanisms.

Uzun et al. [6] have shown that intradermal corticosteroid treatment was only superior to early enalapril treatment in terms of number of fibroblasts and SEI score. In our study, Valsartan treatment was also superior to Enalapril in terms of fibroblast number. On the other hand, the difference between the Valsartan and Enalapril in terms of SEI score was insignificant. Further studies are required to clarify these effects. In Uzun's study Enalapril treatment was found to be superior to corticosteroid injection. In the light of this result and in order to avoid unnecessary sacrifice of animals we have not included a corticosteroid group in our study.

Valsartan is an angiotensin (AT) receptor blocker which selectively blocks AT1 receptors. It has previously been shown that AT II increases the pathological scar formation by binding to AT1 receptor [5]. Enalapril has previously been shown to have positive effect scar maturation by regulating the synthesis of various cytokines and the number of fibroblast [20]. In our study, we have shown that the number of fibroblasts was significantly lower in the enalapril and Valsartan groups. These effects might be related to the regulation of migration and proliferation of keratinocytes and fibroblasts.

Rodgers et al. [21], have demonstrated that angiotensin II can increase neovascularization and endothelial proliferation by increasing VEGF and PDGF. Uzun et al. have shown that enalapril and corticosteroids have similar effects on the reduction number of capillaries in hypertrophic scars. In our study the number of capillaries in Enalapril and Valsartan contained significantly lower number capillaries.

In previous studies it has been shown that number of type 3 collagen increases in pathological scars [22,23]. We have found a significant increase in the ratio of type 1/type 3 collagen in the enalapril and valsartan groups. In the remodeling phase of wound healing collagen fibers are organized by the actions of metalloproteinases [24]. On the contrary in pathological wound healing the collagen fibers are less organized and are formed into nodules surrounded by myofibroblasts. In our study the collagen fibers in enalapril and valsartan groups were significantly more organized. These results suggest an effect on the remodeling phase of the wound healing process. SEI is a scar evaluation measurement which can give high predictive values regarding clinical correlation. In our study we have found both enalapril and valsartan SEI values were significantly improved when compared to the control group.

Uzun et al. compared the efficacy of early stage enalapril, late stage enalapril and corticosteroid treatments on scar maturation. They have found that early enalapril treatment was more efficient in preventing pathological scar formation. In our study we found that early stage enalapril treatment was effective when compared to the control group. Valsartan group revealed better results when compared to enalapril in terms of epithelial thickness and number of fibroblasts. These results might indicate the longer efficiency of ACE inhibitor treatment when compared with ARB treatment.

In previous studies, nanofat grafting techniques have been proposed as method for treatment of scars. Gentile et al. [25] have shown that supercharged-modified nanofat grafts give the best results and the most stromal vascular fraction yield. Gentile et al. [26] has also shown that autologous fat grafting can be effectively used for the correction of scars on the face. In another study Cervelli et al. [27,28] have shown that platelet rich fat transfer can promote natural healing in soft tissue defect caused by various disorders. Stromal vascular fraction which is rich with adipose-derived stem cells (ADSCs), mesenchymal and endothelial progenitor cells play a critical role in the effectiveness of fat grafting. Platelet-derived growth factors, cytokines, chemokines and adipokines secreted from these cells seem to be playing an important role in the mechanism of action of both fat grafting and Valsartan.

There are number of shortcomings related to the study. Further investigation of the effects of Valsartan on scar formation using polymerase chain reaction, immunohistochemical staining and western blots techniques are essential to shed light on the subject. In addition, we have several concerns regarding the clinical translation of these results. Valsartan is a very well-known and is a relatively safe drug for the treatment of high blood pressure. But despite this there are various side effects of this drug including dizziness, lightheadedness, and headache caused by hypotension and hyperkalemia. Another matter in terms of clinical translation is the dosage of the drug to be used for human subjects. In current clinical practice, 80–320 mg/day of Valsartan are used for the treatment of high blood pressure. Further studies are required to determine the required doses of valsartan for the prevention of pathological scars.

In conclusion, both Valsartan and Enalapril can be effectively used to prevent pathological scar formation. Valsartan might be preferred in patients in whom enalapril might cause intolerance due to its effects on kinin-kallikrein metabolism. Further clinical and experimental studies are required to shade a light on unanswered questions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Kryger ZB, Sisco M, Roy NK, et al. Temporal expression of the transforming growth factor-beta pathway in the rabbit ear model of wound healing and scarring. J Am Coll Surg. 2007;205:78–88.

[2] Morihara K, Takai S, Takenaka H, et al. Cutaneous tissue angiotensin-converting enzyme may participate in pathologic scar formation in human skin. J Am Acad Dermatol. 2006;54:251–257.

[3] Zhang GY, Li X, Yi CG, et al. Angiotensin II activates connective tissue growth factor and induces extracellular matrix changes involving Smad/activation and p38 mitogen-activated protein kinase signalling pathways in human dermal fibroblasts. Exp Dermatol. 2009;18:947–953.
[4] Steckelings UM, Henz BM, Wiehstutz S, et al. Differential expression of angiotensin receptors in human cutaneous wound healing. Br J Dermatol. 2005;153:887–893.
[5] Liu HW, Cheng B, Yu WL, et al. Angiotensin II regulates phosphoinositide 3 kinase/Akt cascade via a negative crosstalk between AT1 and AT2 receptors in skin fibroblasts of human hypertrophic scars. Life Sci. 2006;79:475–483.
[6] Uzun H, Bitik O, Hekimoğlu R, et al. Angiotensin-converting enzyme inhibitor enalapril reduces formation of hypertrophic scars in a rabbit ear wounding model. Plast Reconstr Surg. 2013;132:361e–371e.
[7] Morris DE, Wu L, Zhao LL, et al. Acute and chronic animal models for excessive dermal scarring: quantitative studies. Plast Reconstr Surg. 1997;100:674–681.
[8] Saulis AS, Mogford JH, Mustoe TA. Effect of mederma on hypertrophic scarring in the rabbit ear model. Plastic Reconstr Surg. 2002;110:177–183.
[9] Wang H-j, Gao W-c, Ma S-l. Effect of Abnormal Savda Munziq on hypertrophic scar formation in a rabbit ear model. Chin J Integr Med. 2015;21:537–541.
[10] Atiyeh BS. Nonsurgical management of hypertrophic scars: evidence-based therapies, standard practices, and emerging methods. Aesthetic Plast Surg. 2007;31:468–492.
[11] Rabello FB, Souza CD, Farina Jr JA. Update on hypertrophic scar treatment. Clinics (Sao Paulo). 2014;69:565–573.
[12] Anzarut A, Olson J, Singh P, et al. The effectiveness of pressure garment therapy for the prevention of abnormal scarring after burn injury: a meta-analysis. J Plast Reconstr Aesthet Surg. 2009;62:77–84.
[13] Jalali M, Bayat A. Current use of steroids in management of abnormal raised skin scars. Surgeon. 2007;5:175–180.
[14] Naeini FF, Najafian J, Ahmadpour K. Bleomycin tattooing as a promising therapeutic modality in large keloids and hypertrophic scars. Dermatol Surg. 2006;32:1023–1030.
[15] Leclère FM, Mordon SR. Twenty-five years of active laser prevention of scars: what have we learned? J Cosmet Laser Ther. 2010;12:227–234.
[16] O'Brien L, Jones DJ. Silicone gel sheeting for preventing and treating hypertrophic and keloid scars. Cochrane Database Syst Rev. 2013;CD003826.
[17] Shih R, Waltzman J, Evans GRD, Plastic Surgery Educational Foundation Technology Assessment Committee. Review of over-the-counter topical scar treatment products. Plast Reconstr Surg. 2007;119:1091–1095.
[18] Shin JU, Park J, Lee JH, et al. Extramarginal excision is preferable for hypertrophic scars. Int J Dermatol. 2014;53:1138–1144.
[19] Huang D, Shen KH, Wang HG. Pressure therapy upregulates matrix metalloproteinase expression and downregulates collagen expression in hypertrophic scar tissue. Chin Med J (Engl). 2013;126:3321–3324.
[20] Lee JYY, Yang CC, Chao SC, et al. Histopathological differential diagnosis of keloid and hypertrophic scar. Am J Dermatopathol. 2004;26:379–384.
[21] Rodgers K, Abiko M, Girgis W, et al. Acceleration of dermal tissue repair by angiotensin II. Wound Repair Regen. 1997;5:175–183.
[22] Friedman DW, Boyd CD, Mackenzie JW, et al. Regulation of collagen gene expression in keloids and hypertrophic scars. J Surg Res. 1993;55:214–222.
[23] Zhang K, Garner W, Cohen L, et al. Increased types I and III collagen and transforming growth factor-beta 1 mRNA and protein in hypertrophic burn scar. J Invest Dermatol. 1995;104:750–754.
[24] Gauglitz GG, Korting HC, Pavicic T, et al. Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. Mol Med. 2011;17:113–125.
[25] Gentile P, Scioli MG, Bielli A, et al. Comparing different nanofat procedures on scars: role of the stromal vascular fraction and its clinical implications. Regen Med. 2017;12:939–952.
[26] Gentile P, De Angelis B, Pasin M, et al. Adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical evaluation for cell-based therapies in patients with scars on the face. J Craniofac Surg. 2014;25:267–272.
[27] Cervelli V, Bocchini I, Di Pasquali C, et al. P.R.L. platelet rich lipotransfert: our experience and current state of art in the combined use of fat and PRP. Biomed Res Int. 2013;2013:434191.
[28] Cervelli V, Gentile P. Use of cell fat mixed with platelet gel in progressive hemifacial atrophy. Aesthetic Plast Surg. 2009;33:22–27.