Original Article

"EFFECT OF AMLODIPINE AND ENALAPRIL ON WOUND HEALING IN DIABETIC *WISTAR* ALBINO RATS"

HIREN N. HIRAPARA[1], VISHAL M. GHORI[2], ASHISH P. ANOVADIYA[3], C. B. TRIPATHI[3]

[1]Tutor, GMERS Medical College, Junagadh, Gujarat, India, [2]Billev Pharma East, Parmova ulica 14, 1000 Ljubljana, Slovenia, [3]Government Medical College, Bhavnagar 364001, Gujarat, India
Email: dr.hirenhirpara@gmail.com

ABSTRACT

Objective: A number of structural and functional mechanisms have been identified in the pathogenesis of impaired wound healing in diabetes. Diabetes promotes endothelial dysfunction as evidenced by decreased nitric oxide (NO) production. NO deficiency and resultant impaired angiogenesis have been implicated in impaired wound healing in diabetes. The objective of this study was to evaluate the effects of amlodipine and enalapril on wound healing in streptozotocin induced diabetic rats based on previous observations that amlodipine increases NO bioavailability and enalapril promote angiogenesis.

Methods: Four groups for each wound model (n=6 in each group; total 8 groups) were used and served as diabetic control, active control (glibenclamide), amlodipine, and enalapril groups. Wound closure rate and re-epithelialization were studied in the excision wounds. Incision wounds were studied for wound breaking strength while dead space wounds were studied for granulation tissue weight, hydroxyproline content, and histological changes in granulation tissue.

Results: Amlodipine and enalapril significantly (*P*<0.05) increased re-epithelialization in excision wound model. Amlodipine significantly improved incision wound breaking strength while enalapril increased granulation tissue formation. None of the study agents had a significant effect on wound granulation tissue histology.

Conclusion: Amlodipine and enalapril enhance the re-epithelialization in the diabetic wound. Choosing amlodipine or enalapril as antihypertensive in diabetic patients may help to improve impaired wound healing in these patients. Further human trials are needed to demonstrate similar benefits in diabetic patients with wounds.

Keywords: Antihypertensive drugs, Diabetic ulcers, Re-epithelization, Streptozotocin

INTRODUCTION

The wound healing is a highly dynamic and complex process of an integrated series of cellular, physiological and biochemical events leading to the re-establishment of structural integrity and functional restoration of the injured tissue. Impaired wound healing in diabetes mellitus (DM) accounts for high morbidity and health care cost [1, 2]. Micro and macrovascular abnormality, impaired epithet–lialization, and reduced angiogenesis have been described as some of the important causative mechanisms for impaired wound healing in diabetes [3]. Nitric oxide (NO) is one of the major factors regulated by endothelium that maintains vascular homeostasis [4]. Diabetes-related endothelial dysfunction and resultant decreased NO production/bioactivity [5] could plausibly play an important role in impaired wound healing in diabetes. Moreover, it has been demonstrated that impaired wound healing in diabetes is attributable to reduced wound NO synthesis [6]. In addition, topical application of NO donor has been shown to improve re-epithelialization in wound healing [7]. A third-generation calcium-channel blocker, amlodipine has been observed to enhance endothelial NO bioavailability by increasing NO production and prolonging NO half-life through its antioxidant property [8]. Formation of new blood vessels (angiogenesis) in wound bed is necessary to sustain the newly formed granulation tissue. Diabetes significantly hampers angiogenesis and thereby wound healing [9]. Angiogenesis stimulating property of angiotensin-converting enzyme (ACE) inhibitors has been proved previously and patented [10-12].

As amlodipine and enalapril were previously studied for their wound healing activities in dexamethasone suppressed and normal wound respectively [13-15], we designed present study to evaluate effects of amlodipine, and enalapril on diabetic wound healing in *Wistar* albino rats.

MATERIALS AND METHODS

This study was approved by institutional animal ethics committee (IAEC), government medical college, Bhavnagar (GMCB), Gujarat (India). (Approval no.–26/2012; Pharmacology no.–24/2012.) Adult, *Wistar* albino rats of either sex (weighing 200–350 gm) were procured from the central animal house, GMCB. They were acclimatized to animal holding room/laboratory environment and placed in individual polypropylene cages. They were maintained on normal food and water *ad libitum*, under controlled room temperature (25 °C±2 °C, 60–70 % humidity) and 12–12 h light-dark cycle.

Streptozotocin (STZ)-induced diabetes

A single dose of streptozotocin 50 mg/kg (Alfa Aesar, A Johnson Matthey Company, MA/USA, CAS: 18883-66-4) was given by intraperitoneal route to produce diabetes. [7] Four days after, random blood sugar (RBS) was measured from a blood drop, drawn from the tail vein, using glucometer (Accu–Chek Go, Roche Diagnostic, Germany). The rats showing RBS>300 mg/dl were used in subsequent experiments. Intermittent-acting neutral protamine hagedorn (NPH) insulin (5 IU/kg; Wosulin, Wockhardt Limited, Aurangabad, India) was given subcutaneously, once a day, to maintain RBS between 250–350 mg/dl.

Experimental design, and wound models

Forty-eight diabetic *Wistar* albino rats were divided into eight groups (n=6 in each group). Group I (Diabetic control), Group II (Glibenclamide–active control), Group III (Amlodipine), and Group IV (Enalapril) served as the excision wound groups. Group 1 received 1 ml distilled water, group 2 received 0.5 mg/kg glibenclamide (SIGMA Life science, New Delhi, India), group 3 received 3 mg/kg amlodipine,

and group 4 received 15 mg/kg enalapril (Cadila Pharmaceuticals Pvt. Ltd., Dholka, Ahmedabad, Gujarat, India) orally daily till complete healing of the excision wounds. Group V (Diabetic control), Group VI (Glibenclamide–active control), Group VII (Amlodipine), and Group VIII (Enalapril) served as incision and dead space wound groups and received the same doses of respective treatment agent as described above for 11 d. All the wounds were inflicted under aseptic precautions and anesthesia induced by ketamine (75 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally.

Excision wound

A full-thickness circular skin patch (measuring approximately 500 mm²) was excised from the nape of the neck to produce excision wound [16]. Wound contraction and re-epithelialization were evaluated by tracing wound margin on a transparent plastic sheet. Wound area was measured soon after wounding and 3, 7, and 11 d later without scab removal. Re-epithelialization was measured on 11th day of wounding as by 11th day scabs fell off the wound, and epithelium was visible. The plastic sheet was scanned, and the wound area was measured using a UTHSCA image analyzer (version 3.00, The University of Texas Health Science Center, San Antanio, USA). Wound closure rate was calculated using the formula below [7]:

$$\text{Wound closure rate (\%)} = \frac{\text{Area}_{day0} - \text{Area}_{dayn}}{\text{Area}_{day0}} \times 100$$

Where Area_{day0} = initial wound area at day 0,

Area_{dayn} = area on nth post wounding day

The wound re-epithelialization was calculated using the formula mentioned below:

Re–epithelialization (%) on 11th day =

Total wound area (%) – Wound area not covered with epidermis (%)

Re-sutured incision and dead space wound

Two 5 cm long, para-vertebral, full-thickness incisions were made on either side of the vertebral column and sutured [17]. In the same rats, the dead space wounds were created by inserting and suturing sterile grass pith (measuring 2.5 cm×0.3 cm) in the loose areolar tissue of the groins on either side. A sterile cotton pellet (weighing 10 mg) was inserted and sutured in both the axillary regions. The sutures were removed on 7th day of wounding in incision wounds and on 11th day in dead space wounds. The incision wound breaking strength was measured on 11th post wounding day by constant water flow technique described by Lee, under anesthesia [18]. After measuring wound breaking strength, the rats were sacrificed with a high dose of ketamine and xylazine, and the granulation tissue formed on grass piths were harvested. This granulation tissue were subjected to hydroxyproline estimation and histological examination [19]. The hydroxyproline content was expressed as µg/100 mg of granulation tissue. The axillary cotton pellets were excised and dried overnight at 60ºC in the hot air oven. The weight of dried granulation tissue with cotton pallet was measured and expressed as mg/100 gm body weight [20]. A pathologist, semi-quantitatively, analyzed the haematoxylin and eosine (H & E) stained sections of granulation tissues and graded (grade 1–4) them for the presence of polymorphonuclear cells, macrophages, fibroblasts, and neoangiogenesis [21].

Statistical analysis

Statistical analysis was done by using Graphpad instat demo version number 3.0. The data were expressed as mean±standard error of mean (SEM). One way analysis of variance (ANOVA) followed by Tukey-Kramer test for parametric variables and Kruskal-Wallis followed by Dunn's multiple comparison tests for non-parametric variables were used to compare mean differences between different groups. $P<0.05$ was considered statistically significant.

RESULTS

Excision wound

The percentages of re-epithelialization on 11th post wounding day were significantly higher in amlodipine, and enalapril-treated groups as compared to control, and glibenclamide groups (table 1; $P<0.05$). There was no significant difference in wound closure rates among the groups (table 1). Wound closure was initially rapid because of wound contraction followed by slow closure through re-epithelialization (fig. 1). The dynamics of wound closure in different groups are illustrated in the wound healing curve (fig. 2). Initially, the wound healing was slow in amlodipine and enalapril group followed by relatively rapid healing through re-epithelialization as compared to glibenclamide group (table 1).

Table 1: Effect of amlodipine and enalapril on excision wound model

S. No.	Groups (n=6)	Wound closure (%)			Re-epithelialization (%) 11th day
		3rd Day	7th Day	11th Day	
1	Diabetic control	15.3±1.4	37.3±3.8	56.3±3.4	56.01±2.5
2	Glibenclamide	15.7±1.2	46.7±1.8	57.6±2.5	63.2±1.8
3	Amlodipine	15.3±2.1	41.9±3.9	69.6±5.8	78.6±1.5*#
4	Enalapril	22.4±1.2	41.9±3.0	69.7±2.6	79.1±2.7*#

n=6 in each group, values are represented as mean±SEM, *$P<0.05$ as compared to diabetic control and #$P<0.05$ as compared to glibenclamide (Tukey Krammer multiple comparison tests)

Re-sutured incision and dead space wound

Mean incision wound breaking strength on 11th post wounding day was significantly higher in amlodipine and glibenclamide groups as compared to control group. In the enalapril group, the mean incision wound breaking strength was numerically higher than the diabetic control group. However, it did not reach predefined statistical significance (table 2). Granulation tissue dry weight for enalapril and glibenclamide groups was significantly higher than the control group. There was no statistically significant difference in hydroxyproline contents among all the four groups (table 2).

Table 2: Effect of amlodipine and enalapril on incision and dead space wound model

S. No.	Group	Incision wound breaking strength (gm)	Granulation tissue dry weight (mg/100 gm body weight)	Hydroxyproline (µg/100 mg granulation tissue)
1	Diabetic control	265.8±10.4	26.1±0.6	19.3±0.5
2	Glibenclamide	369.2±8.9*	37.5±0.9*	24.4±0.9
3	Amlodipine	337.5±17.1*	29.6±1.2	23.6±0.7
4	Enalapril	310.8±9.9	30.9±1.1*	22.6±1.4

n=6 in each group, values are represented as mean±SEM. * $P<0.05$ as compared to diabetic control. (Tukey-Krammer multiple comparison tests)

A semi-quantitative histological analysis of 11th post-wounding day granulation tissue did not show any difference in any of the parameters examined (table 3).
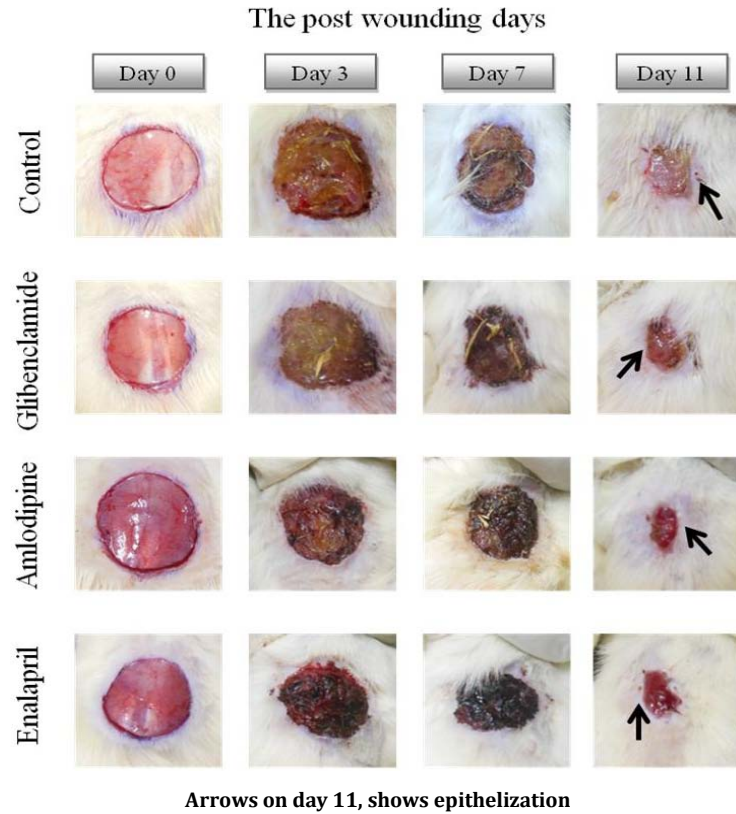
Arrows on day 11, shows epithelization

Fig. 1: The excision wound healing time course noted on 0, 3, 7 and 11 post-operative day

Table 3: Semi-quantitative evaluation of histological changes in granulation tissue in dead space wound model

Histological change	Groups			
	Diabetic control	Glibenclamide	Amlodipine	Enalapril
Neutrophils	1.0±0.36	1.5±0.55	1.3±0.21	1.5±0.56
Macrophages	0.3±0.21	0.8±0.3	0.8±0.16	0.2±0.16
Fibroblasts	2.5±0.42	1.4±0.2	1.6±0.21	3±0.63
Neo-angiogenesis	1.8±0.54	1.2±0.16	1.5±0.56	1.1±0.23

n=6 in each group, values are represented as mean±SEMs. (Dunn multiple comparison test, $P<0.05$)
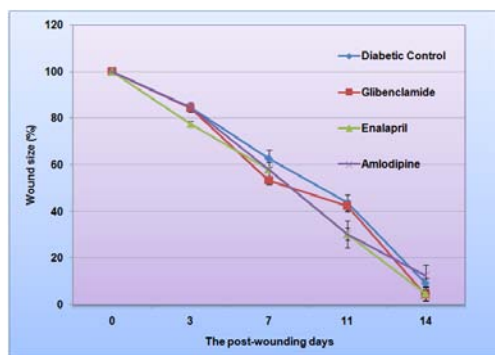

Fig. 2: Wound healing curve. The graph shows percentage of excision wound closure over time period

DISCUSSION

This study evaluated wound healing activity of amlodipine, and enalapril in diabetic *Wistar* rats based on previous observations that amlodipine increases endothelial NO bioavailability, and enalapril promotes angiogenesis [8, 10-12]. Angiogenesis and endothelial NO play an important role in enhancing wound healing, especially in diabetic wound healing, where they are negatively affected [6, 9]. NO promotes angiogenesis in addition to its other wound healing promoting properties [22]. Furthermore, topical NO donor, glyceryl trinitrate, has been demonstrated to promote wound healing in diabetic *Wistar* rats [7]. Amlodipine and enalapril have been studied for wound healing activity in non-diabetic wounds, however, at the best of our knowledge; present study is the first to evaluate wound healing activity of amlodipine, and enalapril in diabetic wounds [13-15].

In the present study, STZ-induced diabetes model was used, which is well-established model to study diabetic wound healing [23]. STZ-induced diabetes model has been shown to exhibit decreased cutaneous endothelial nitric oxide synthase (eNOS) expression, constitutive NOS activity, and increased superoxide levels [24]. Furthermore, it has been demonstrated that in STZ-induced diabetes, angiogenesis is impaired. [25] Thus, STZ-induced diabetes model provides suitable opportunity to study effects of present study agents on diabetic wound healing. Glibenclamide was used as active control as it has been demonstrated that lowering of blood glucose in diabetes helps to promote wound healing [26]. This active control group helps differentiate if the study agents have any additional advantage in promoting diabetic wound healing over blood glucose lowering agents that could promote wound healing by merely improving glucose homeostasis in diabetes.

In the present study, at day 11, re-epithelialization in the excision wound model was significantly higher in both the treatment groups

as compared to diabetic and active control groups. Wound contraction, on 11th post wounding day, in both treatment groups was observed non-significantly higher than diabetic control, and active control group. In a previous study of dexamethasone suppressed wound healing, amlodipine enhanced rate of wound contraction and re-epithelialization [13]. Another study showed that enalapril improves wound healing by enhancing re-epithelialization, wound breaking strength, and granulation tissue collagen content [15]. In accordance with observations in the previous studies, in the present study, amlodipine and enalapril increased re-epithelisation in the diabetic wound. However, in contrast to previous studies, in present study amlodipine and enalapril did not enhance wound contraction. This contradictory observation can be possibly explained by the fact that wound contraction is already hampered in diabetes and as a result wound contraction promoting the effect of study agents could not reach statistical significance. The re-epithelialization enhancing the effect of amlodipine is plausibly brought about by its effect on NO bioavailability. Amlodipine has been shown to enhance NO production from endothelium and prolong its half-life and thereby increase NO bioavailability [8].

NO treatment has been observed to promote re-epithelialization in healing wounds [7, 27]. Another report suggests that inducible nitric oxide synthase (iNOS) inhibitor N^{ω}-imino ethyl L-lysine (L-NIL) decreases proliferation of keratinocytes and delays re-epithelialization with atrophied hyperproliferative epithelium at the wound edge [28, 29]. The re-epithelialization enhancing effect of enalapril is plausibly explained by its angiogenesis stimulating property. The angiogenesis stimulating property of ACE inhibitors is patented with US Patent No. 6,191,144 B1, 2001. [10] Diabetes is known to affect the angiogenesis process negatively by decreasing serum NO and vascular endothelial growth factor (VEGF) concentration. Enalapril has been shown to increase serum NO and VEGF levels in diabetic rats [12]. High level of VEGF mRNA was found in keratinocytes present in wound surface [30]. In addition to stimulating angiogenesis, VEGF has also been found to accelerate epithelialization in the wound healing process [31-33].

Both amlodipine and enalapril significantly increased re-epithelisation in excision wound compared to glibenclamide. This observation suggests that amlodipine and enalapril have an additional advantage in terms of increased re-epithelialization over merely blood glucose lowering agents. In human, the skin over the foot, which is commonly involved in the diabetic wound, is stretched and closely tethered to the dermis. It heals predominantly by re-epithelialization due to lack of loose areolar tissue. Thus, enalapril and amlodipine may help to enhance wound healing in the diabetic foot.

In incision wound model, amlodipine significantly increased incision wound breaking strength compared to diabetic control. Calcium channel blockers (CCBs), including the amlodipine, are considered potential etiological factor for drug-induced gum hypertrophy [34, 35]. The hallmark of gum hypertrophy is increased amount of connective tissue dominated by collagen fibers [36]. Underlying proposed mechanism are up-regulation of cytokines like transforming growth factor β1 (TGF β1), and interleukin-6 (IL6), which increase the synthesis and reduce the proteolytic activities of fibroblasts, the source of collagen [35, 37-39].

Glibenclamide also significantly increased incision wound strength compared to diabetic control; however, there was no significant difference in incision wound breaking strength between amlodipine and glibenclamide. This observation suggests that amlodipine has a similar magnitude of effect in increasing incision wound breaking strength as could be achieved by glucose lowering agents in diabetes. Enalapril did not have any significant effect on incision wound breaking strength compared to diabetic control. During first three weeks of wound healing, the wound strength correlates with scar collagen content, while in the late phase, organized (architecturally rearranged) collagen contributes to increased wound strength [40]. In experimental models, enalapril treatment has been shown to reduce collagen deposition in rat heart ventricles or large/small arteries, which explain the non-significant effect of enalapril on wound breaking strength [41].

In dead space wound model, enalapril significantly increased dry granulation tissue weight as compared to diabetic control; however, amlodipine did not have a significant effect on dry granulation tissue weight. Angiogenesis supports growth and contents of granulation tissue components and thereby improves granulation tissue weight in enalapril group [15, 42]. There was no significant difference in hydroxyproline content among all the groups. In the present study, the colorimetric technique was used to measure hydroxyproline content in granulation tissue, which is less sensitive, and may have missed minute differences in hydroxyproline content between groups.

Histological examination of granulation tissue did not reveal any significant difference among the groups in polymorph nuclear cell infiltration, macrophage, fibroblast, or neo-angiogenesis in this particular study setup.

The limitations of this study are that the methods used in quantifying re-epithelialization, and hydroxyproline estimation are less sensitive. Furthermore, for evaluation of angiogenesis in granulation tissue, specific immunostaining using a blood vessel marker such as CD31 would have been more revealing. However, due to unavailability of resources, such specific immunostaining could not be performed.

CONCLUSION

This study demonstrates that amlodipine and enalapril enhance the re-epithelialization in the diabetic wound. In addition, amlodipine increases wound tensile strength while enalapril increases granulation tissue formation in diabetic wounds. Hypertension is a common co-morbidity in patients of diabetes, and amlodipine and enalapril are commonly used anti-hypertensive agents. Choosing amlodipine or enalapril as antihypertensive in such diabetic patients may help to improve impaired wound healing in these patients. Further human trials are warranted to demonstrate similar benefits in improving impaired wound healing in patients of diabetes.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Singer AJ, Clark RAF. Cutaneous wound healing. N Engl J Med 1999;341:738-46.
2. Ramsey SD, Newton K, Blough D, McCulloch DK, Sandhu N, Reiber GE, *et al.* Incidence, outcomes, and cost of foot ulcers in patients with diabetes. Diabetes Care 1999;22:382-7.
3. Falanga V. Wound healing and its impairment in the diabetic foot. Lancet 2005;366:1736-43.
4. Anderson TJ. Nitric oxide, atherosclerosis and the clinical relevance of endothelial dysfunction. Heart Failure Rev 2003;8:71-86.
5. Traub O, Bibber VR. Role of nitric oxide in insulin-dependent diabetes mellitus related vascular complication. West J Med 1995;162:439-45.
6. Schaffer MR, Tantry U, Efron PA, Ahrendt GM, Thornton FJ, Barbul A. Diabetes-impaired healing and reduced wound nitric oxide synthesis: a possible pathophysiologic correlation. Surgery 1997;121:513-9.
7. Ghori V, Mandavia DR, Patel TK, Tripathi CB. Effect of topical nitric oxide donor (0.2 % glyceryl trinitrate) on wound healing in diabetic Wistar rats. Int J Diabetes Dev Countries 2014;34:45-9.
8. Berkels R, Taubert D, Bartels H, Breitenbach T, Klaus W, Roesen R. Amlodipine increases endothelial nitric oxide by dual mechanisms. Pharmacology 2004;70:39-45.
9. Silvestre JS, Lévy BI. Molecular basis of angiopathy in diabetes mellitus. Circ Res 2006;98:4-6.
10. Isner JM. Method of using angiotensin-converting enzyme inhibitor to stimulate angiogenesis. U. S. Patent No. 6,191,144; 2001.
11. Fabre JE, Rivard A, Magner M, Silver M, Isner JM. Tissue inhibition of angiotensin-converting enzyme activity stimulates angiogenesis *in vivo*. Circulation 1999;99:3043-9.

12. Fallahzadeh AR, Khazaei M, Sharifi MR. Restoration of angiogenesis by enalapril in diabetic hindlimb ischemic rats. Biomed Papers 2011;155:137–42.
13. Bhaskar HN, Udupa SL, Udupa AL. Effect of Nifedipine and amlodipine on wound healing in rats. Indian J Physiol Pharmacol 2004;48:111–4.
14. Bhaskar HN, Udupa SL, Udupa AL. Effect of nifedipine and amlodipine on dead space wound healing in rats. Indian J Exp Biol 2005;43:294-6.
15. Torgal SS, Hiremath SV, Majagi SI, Gouripur VV, Patil PA, Hogade AP. Evaluation of wound healing activity of angiotensin converting enzyme inhibitors in wistar rats. Recent Res Sci Technol 2010;2:76-80.
16. Morton JJP, Malone MH. Evaluation of vulneray activity by an open wound procedure in rats. Arch Int Pharmacodyn Ther 1972;196:117-26.
17. Ehrlich HP, Hunt TK. The effects of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann Surg 1969;170:203-6.
18. Lee KH. Studies on the mechanism of action of salicylate. II. Retardation of wound healing by aspirin. J Pharm Sci 1968;57:1042-3.
19. Woessner Jr JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961;93:440-7.
20. Dipasquale G, Meli A. Effect of body weight changes on the formation of cotton pellet-induced granuloma. J Pharm Pharmacol 1965;17:379-82.
21. Abramov Y, Golden B, Sullivan M, Botros SM, Miller JJR, Alshahrour A, *et al.* Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model. Wound Repair Regeneration 2007;15:80-6.
22. Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, *et al.* Nitric oxide mediates angiogenesis *in vivo* and endothelial cell growth and migration *in vitro* promoted by substance P. J Clin Invest 1994;94:2036–44.
23. Sumi Y, Ishihara M, Kishimoto S, Takikawa M, Hattori H, Takikawa M, *et al.* Effective wound healing in streptozotocin-induced diabetic rats by adipose-derived stromal cell transplantation in plasma-gel containing fragmin/protamine microparticles. Ann Plast Surg 2014;72:113-20.
24. Luo, JD, Wang YY, Fu WL, Wu J, Chen AF. Gene therapy of endothelial nitric oxide synthase and manganese superoxide dismutase restores delayed wound healing in type 1 diabetic mice. Circulation 2004;110:2484–93.
25. Teixeira AS, Caliari MV, Rocha OA, Machado RDP, Andrade SP. Aminoguanidine prevents impaired healing and deficient angiogenesis in diabetic rats. Inflammation 1999;23:569-81.
26. McMurry JF Jr. Wound healing with diabetes mellitus. Better glucose control for better wound healing in diabetes. The Surg Clin North Am 1984;64:769-78.
27. Zhu H, Ka B, Murad F. Nitric oxide accelerates the recovery from burn wounds. World J Surg 2007;31:624-31.
28. Stallmeyer B, Anhold M, Wetzler C, Kahlina K, Pfeilschifter J, Frank S. Regulation of eNOS in normal and diabetes-impaired skin repair: implications for tissue regeneration. Nitric Oxide 2002;6:168-77.
29. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 2000;117:1162-72.
30. Werner S, Peters KG, Longaker MT, Fueller-Pace F, Banda MJ, Williams LT. Large induction of keratinocyte growth factor expression in dermis during wound healing. Proc Natl Acad Sci USA 1992;89:6896-900.
31. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. J Surg Res 2009;153:347-58.
32. Ondrick K, Samojla BG. Angiogenesis. Clin Podiatric Med Surgery 1992;9:185-202.
33. Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, *et al.* Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. Am J Pathol 2004;164:1935–47.
34. Seymour RA, Ellis JS, Thomson JM, Monkman S, Idle JR. Amlodipine-induced gingival overgrowth. J Clin Periodontol 1994;21:281-3.
35. Joshi S, Bansal S. A rare case report of amlodipine-induced gingival enlargement and review of its pathogenesis. Case Reports Dentistry 2013. Doi.org/10.1155/2013/138248. [Article in Press]
36. Brown RS, Sein P, Corio R, Bottomley WK. Nitrendipine-induced gingival hyperplasia. First case report. Oral Surg Oral Med Oral Pathol 1990;70:593-6.
37. Eickelberg O, Roth M, Block LH. Effects of amlodipine on gene expression and extracellular matrix formation in human vascular smooth muscle cells and fibroblasts: implications for vascular protection. Int J Cardiol 1997;62:S31–7.
38. Martelli-Junior H, Cotrim P, Graner E, Sauk JJ, Coletta RD. Effect of transforming growth factor-β1, interleukin-6, and interferon-γ on the expression of type i collagen, heat shock protein 47, matrix metalloproteinase (MMP)-1 and MMP-2 by fibroblasts from normal gingiva and hereditary gingival fibromatosis. J Periodontol 2003;74:296-306.
39. Robertson WV. Metabolism of collagen in mammalian tissues. Biophys J 1964;4:93-106.
40. Madden JW, Peacock EE Jr. Studies on the biology of collagen during wound healing: rate of collagen synthesis and deposition is cutaneous wounds in rats. Surg 1968;64:288-94.
41. Keeley FW, Elmoselhi A, Leenen FH. Enalapril suppresses normal accumulation of elastin and collagen in cardiovascular tissues of growing rats. Am J Physiol 1992;262:H1013-21.
42. McGrath MH, Emery III JM. The effect of inhibition of angiogenesis in granulation tissue on wound healing and the fibroblast. Ann Plast Surg 1985;15:105-22.