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EVALUATION OF WOUND HEALING ACTIVITY OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS IN WISTAR RATS

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Abstract

Angiotensin converting enzyme (ACE) inhibitors are known to increase the level of bradykinin by preventing its breakdown and also promote prostaglandin synthesis by direct and indirect methods which in turn may promote wound healing. However there is paucity of scientific information in this regard. Therefore in the present study we have investigated the effect of ACE inhibitors like Captopril and Enalapril on different wound models in Wistar rats. Excision, resutured incision and dead space wounds were inflicted in male Wistar rats under light ether anaesthesia, taking aseptic precautions. Control animals received vehicle and other groups received Captopril (10mg/kg) and Enalapril (10mg/kg) orally for a period of 10 days in incision and dead space wound models, whereas similar treatments were continued in excision wound models till complete closure of wounds. On the 11th day, after estimating breaking strength of resutured incision wounds (under anaesthesia), granulation tissue was removed from dead space wounds to estimate breaking strength, hydroxyproline content as well as quantification of granulation tissue and histological studies were carried out in control and treated groups. Captopril and Enalapril significantly increased the rate of wound healing, reduced the number of days required for complete epithelialization and final area of scar in excision wounds. Both the ACE inhibitors significantly increased breaking strength of resutured incision wounds and granulation tissue. Also these two drugs significantly enhanced both granulation tissue formation and granulation tissue hydroxyproline content. Histological studies confirmed these findings. Captopril and Enalapril significantly promoted the healing process in all the three wound models studied. These results indicate the wound healing property of ACE inhibitors and clinical studies in this regard are worthwhile.

Keywords: Captopril, Dead space wounds, Enalapril, Excision wounds, Healing, Incision wounds

Introduction

Wound has been defined as disruption of anatomic or functional continuity of living tissue (Schilling,1968) produced by physical, chemical, electrical or microbial insult to the tissue and wound healing refers to the restoration of the continuity of living tissue. The healing of wound follows a general scheme in which a sequence of processes takes place in an orderly way viz., inflammatory phase, proliferative phase and remodelling phase (Clark and Henson,1988; Kanzler *et al.,*1986; Goslen,1988; Wahl *et al*.,2004; McKay and Leigh,1991). The inflammatory phase is marked by vasodilatation, increased vascular permeability, platelet accumulation and coagulation and leucocytic migration (Kumar,2004). Bradykinin, an important mediator of inflammation, apart from causing vasodilatation and oedema is known to stimulate angiogenesis (Hu and Fan,1993) and proliferation of fibroblasts by interacting with inflammatory cytokine interleukins-I (Kimball and Fisher,1988) and also it has been reported that kinins are known to release cytokines, interleukin – I and tumour necrosis factor (Tiffany and Burch,1989) which are known to stimulate fibroblast proliferation (Kahaleh *et al*.,1988). Kinins are known to stimulate release of histamine from mast cells which in turn is known to stimulate gastric parietal cells (Cambel and Sgouris,1952) and hair follicle cells (Butcher,1940). It has been also reported that kinins act directly as mitogens stimulating DNA synthesis and thereby promote cell proliferation (Whitfield *et al*.,1970; Owen and Villereal,1983).

Angiotensin converting enzyme (ACE), an enzyme discovered serendipitously in plasma as a factor responsible for conversion of angiotensin – I to angiotensin – II is known to inactivate bradykinin and other potent vasodilator peptides. This enzyme is known to be present throughout the vascular system particularly it is abundant in lung and skin(Regoli and Barabe,1980). Thus ACE inhibitors are known to increase bradykinin levels by inhibiting its breakdown (Yang *et al*.,1970) and also directly stimulate prostaglandin release (Zusman,1981; Stephen,1982) thereby increasing the levels of bradykinin and prostaglandin. These drugs in turn may favour the process of wound healing, as described earlier. As ACE inhibitors are the drugs used for prolonged periods in chronic diseases like hypertension,ischemic heart disease and congestive heart failure, it is desirable to know the influence of these drugs on wound healing. However there is paucity of literature regarding the influence of ACE inhibitors on wound

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healing. Hence the present study was undertaken to investigate the influence of commonly used ACE inhibitors like captopril and enalapril in their clinically equivalent doses on healing of three different models of wounds in Wistar rats.

Materials and Methods

Animals and drugs

Healthy male Wistar rats weighing 150-250 g were housed individually, on standard pellet diet, with water *ad libitum* and were starved overnight before the day of experimentation. Depilation at wounding site was done a day before wounding. After wounding, the animals were divided into control and treatment groups (n = 6 in each) for each wound model to receive various treatments. The clinical doses of the drugs were converted into rat equivalent doses, as calculated with the help of the conversion table devised by 'Paget and Barnes' (Paget and Barnes,1964) The dose of Captopril (10 mg/kg) [Lupin laboratories] and Enalapril (10 mg/kg) [Dr. Reddy's laboratories] were dissolved into distilled water and were administered orally once daily at 8:00 a.m., while control groups received equal volume of the vehicle. The duration of the treatment was 10 days for animals inflicted with incision and dead space wounds, whereas it was continued in animals bearing excision wound till their complete closure.

Wound models

Excision wound

Excision wounds were prepared by excising the full thickness circular skin (approximately 500 mm^2) from the nape of neck under ether anaesthesia as described by Morton and Malone (1972). Wound-closure rate and epithelialization time were assessed by tracing the wound on polythene paper from wounding day, followed by 4, 8, 12, 16 and 18^{th} day and subsequently on consecutive days till complete epithelialization (fall of scab without any raw area). Similarly, scars were traced on complete epithelialization to assess wound contraction by noting scar size and shape.

Incision wound

Resutured incision wounds were inflicted with two 6 cm long paravertebral parallel incisions under light ether anaesthesia as described earlier (Ehrlich and Hunt,1969). Sutures were removed on 7^{th} day and on 10^{th} post wounding day, breaking strength was measured by the continuous water flow technique as described by Lee (1968).

Dead space wound

Infliction of dead space wounds were done by implanting sterile cotton pellets (10 mg) and cylindrical grass piths (2.5 cm × 0.3 cm) s.c. in the groin and axilla alternatively by the technique of D'Arcy et al. as described by Turner (1965). All the granulation tissues were removed under light ether anaesthesia on 10th post-wounding day and dried at 60°C overnight to record the dry weight which was expressed as mg per 100g, body weight as suggested by Dipasquale and Meli (1965). As described earlier, one of the granulation tissues over the grass piths was opened and trimmed to a rectangular piece for estimation of breaking strength and subsequently colorimetric estimation of hydroxyproline content (Woessner, 1961). Histopathological studies were conducted on the tissue covering the other grass pith. After removal, the tissue was fixed in 10% formalin and processed to prepare paraffin blocks. Sections were taken from these blocks and stained with haematoxylin and eosin. The slides were studied under microscope.

All the procedures were performed in accordance with the CPCSEA guidelines ['Committee for the Purpose of Control and Supervision on Experiments on Animals' under Ministry of Animal Welfare Division, Government of India] and the study was approved by IAEC [Institutional Animal Ethics Committee].

Statistical Analysis

Data were expressed as Mean ± SEM and analysed by one way ANOVA followed by Dunnet's test. $p<0.05$ was considered as significant.

Results

Percentage closure of excision wound area was significantly ($p<0.01$ and $p<0.001$) enhanced at 4, 8, 12, 16 and 18 hours in both captopril and enalapril treated groups as compared to that of control group (Table 1). Number of days required for complete epithelialization were reduced significantly ($p<0.001$) in captopril and enalapril treated groups as compared to that of control group (Table 1). On complete epithelialization the scar shapes in captopril and enalapril treated groups were significantly ($p<0.001$) smaller as compared to that of control group (Table 1).

The breaking strength was significantly ($p<0.001$) increased in captopril and enalapril treated animals both in resutured incision wound as well as granulation tissue as compared to distilled water treated control group (Table 2). Both captopril and enalapril significantly ($p<0.001$) enhanced the granulation tissue formation (Table 2). The granulation tissue hydroxyproline content was significantly ($p<0.001$) more in captopril and enalapril treated animals as compared to that of control animals (Table 2).

Haematoxylin and eosin stained sections of tissues over the grass piths when observed under light microscope revealed more number of macrophages, fibroblasts and blood vessels (indicating granulation tissue) in enalapril and captopril treated groups when compared with vehicle treated control group (Fig 1).

Table 1. Effect of ACE inhibitors on excision wound healing

Groups (n=6)	Drugs & Dose (mg/kg)	Percentage closure of wound area					Time for complete epithelialization (days)	Scar area (mm^2)
		Mean ± SEM						
		Day - 4	Day - 8	Day - 12	Day - 16	Day - 18		
1	Control	17.86 ±1.38	49.83 ±2.43	63.80 ±1.89	76.96 ±1.81	92.67 ±1.80	19.83 ±0.40	43.83 ±1.51
2	Captopril (10)	27.77 ±1.84*	62.20 ±2.04*	82.43 ±2.19**	94.20 ±1.89**	99.13 ±0.63*	17.33 ±0.21**	31.17 ±1.08**
3	Enalapril (10)	26.40 ±2.10*	60.47 ±2.15*	81.63 ±2.12**	93.16 ±1.84**	98.83 ±0.57*	17.05 ±0.22**	32.00 ±1.75**

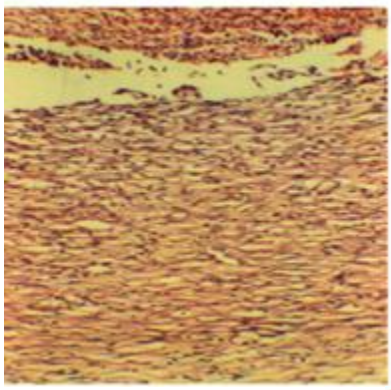
ANOVA followed by Dunnet's test, p<0.01*, p<0.001**

Table 2. Effect of ACE inhibitors on incision and dead space wounds

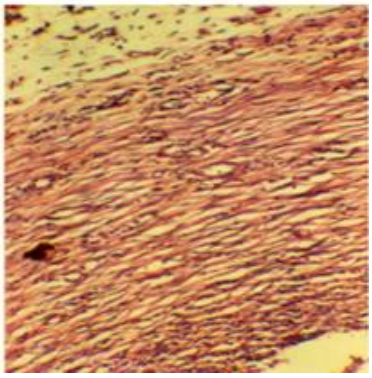
Groups (n=6)	Drugs and Dose (mg/kg)	Breaking strength (G)		Granulation tissue dry weight (mg% b.w)	Granulation tissue hydroxyproline content (mg/gm of tissue)
		Resutured incision wounds	Granulation tissue		
		Mean ± SEM			
1	Control	203.33±8.13	329.17±9.26	43.33±1.59	0.99±0.02
2	Captopril(10)	288.33±10.05*	434.17±11.64*	63.28±2.63*	1.42±0.02*
3	Enalapril(10)	282.50±9.97*	422.50±8.33*	60.52±2.27*	1.37±0.02*

ANOVA followed by Dunnet's test, p<0.001*

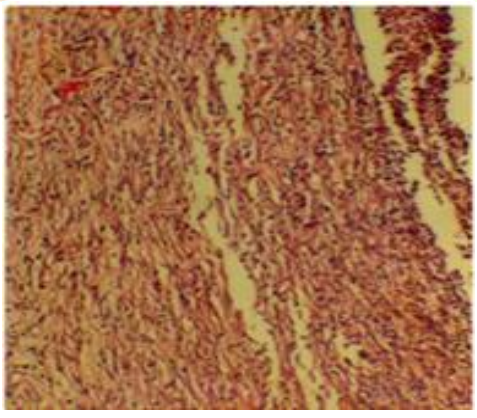
Figure 1: Microphotographs of granulation tissues stained with H&E (100 X)



i) Control (vehicle) [H&E – 100 X]



ii) Captopril (10 mg/kg) [H&E – 100 X]



iii) Enalapril (10 mg/kg) [H&E – 100 X]

Note: Abundant granulation tissue is seen in Enalapril treated group (iii) while moderate increase in granulation tissue is seen in Captopril treated group (ii) when compared to vehicle treated control group (i).

Discussion

In the present study two structurally different ACE inhibitors were investigated in their therapeutic equivalent doses for their possible influence on wound healing in different wound models in Wistar rats.

In excision wound study, both captopril and enalapril have significantly promoted wound contraction as evidenced by their effect on wound area throughout the study. The mean scar area on complete epithelialization which indicates the extent of wound contraction was significantly decreased in both captopril and enalapril treated groups as compared to that of control group. Both captopril and enalapril enhanced the epithelialization as evidenced by the significant decrease in the time required for complete epithelialization.

The incision wound model which represents surgical wound in clinical practice, where the contraction and epithelialization contribute least to the healing process in contrast to excision wound. Both captopril and enalapril have enhanced the healing of incision wounds significantly as evidenced by the increase in breaking strength of resutured incision wound indicating that both ACE inhibitors have promoted collagenation significantly. Both captopril and enalapril treatment groups have significantly increased granulation tissue breaking strength as well as dry weight as compared to those of vehicle treated control indicating enhanced fibroblast proliferation and collagen synthesis. The hydroxylation of proline and lysine is required for synthesis of collagen chain which provides strength and integrity of all tissue repair cells (Buffoni, 1993). The significant increase in hydroxyproline content in the present study indicates an increase in collagen content of the granulation tissue.

The histopathological studies revealed more amount of granulation tissue both in captopril and enalapril treated groups when compared with vehicle treated control group. These histological findings support the observation of increased dry weight, breaking strength and hydroxyproline content in captopril and enalapril treated groups.

It is quite clear from the present observations that both captopril which contain sulfhydryl group and enalapril containing carboxy alkyl group have significant prohealing effect on all aspects of different wound models. There is paucity of information in this regard.

The present study does not reveal the exact mechanism of prohealing effect of both ACE inhibitors. The initial event in wound healing process is the inflammatory phase, which is due to the release of inflammatory mediators like bradykinin, prostaglandins and other factors. It has been reported that ACE inhibitors are known to increase bradykinin levels by inhibiting its breakdown (Yang,1970) and directly or indirectly stimulate PGE_2 synthesis (Zusman, 1981; Stephen, 1982). As mentioned earlier, bradykinin plays an important role in inducing inflammation by causing vasodilatation and oedema is also known to stimulate angiogenesis (Hu and Fan, 1993) and proliferation of fibroblasts by interacting with inflammatory cytokine interleukin-I (Kimball and Fisher, 1988) and tumour necrosis factor – I (Tiffany and Burch, 1989) and bradykinin acts as mitogen stimulating DNA synthesis there by promoting cell proliferation (Whitfield, 1970; Owen and Villereal, 1983). As described earlier, kinins are known to stimulate release of histamine from mast cells which inturn stimulates proliferation of certain cells (Cambel and Sgouris, 1952; Butcher, 1940).The angiogenesis which is induced by bradykinin is an important physiological process involved in wound healing as it induces better nutritional conditions for fibroblasts and epidermal cells to eventually close the wound. NSAIDs (Lee, 1968; Francis and Marks, 1977) and glucocorticoids (Francis and Marks, 1969) which inhibit PG synthesis and release, respectively are known to suppress wound healing suggesting the key role of PGs in wound repair process.

Thus prohealing effect of captopril and enalapril could be explained on the basis that these ACE inhibitors increase the bradykinin level by preventing its break down and increase prostaglandin levels by stimulating their synthesis and release. They may act by stimulating the release of histamine, as these autocoids are known to be involved in the process of wound repair.

Conclusion

ACE inhibitors are the most appropriate antihypertensive drugs in patients with diabetes, nephropathy, left ventricular hypertrophy, congestive heart failure, angina and post myocardial infarction cases. They are also considered to be safe in patients suffering from asthma, diabetes mellitus and peripheral vascular disease. The present findings of the study suggest that whenever these patients undergo surgery or sustain injuries the ACE inhibitors can promote wound healing.

The findings of the present study also suggest that apart from the well established indications of these ACE inhibitors they can also be promising drugs in promoting wound healing, which is often a problem in clinical practice. However, further clinical studies in this regard are really worthwhile.

Annexure

Author Contributions: *Study concept and design:* V.V. Gouripur, P.A. Patil. *Acquisition of data:* Dr. S.S. Torgal. *Analysis and interpretation of data:* Dr. S.S. Torgal. *Drafting of the manuscript:* Dr. Suneel I. Majagi. *Critical revision of the manuscript for important*

intellectual content: Dr. Suneel I. Majagi. *Statistical analysis:* Dr. A.P. Hogade. *Administrative, technical or material support:* Dr. S.V. Hiremath. *Study supervision:* Dr. S.V. Hiremath, Dr. A.P. Hogade.

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