# The Effect of Enalapril on Skin Flap Viability is Independent of Angiotensin II AT1 Receptors

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**Abstract:** Random pattern skin flaps are still widely used in plastic surgery. However, necrosis in the distal portion resulting from ischemia is a serious problem, increasing the cost of treatment and hospitalization. To enhance skin flap viability, a variety of pharmacologic agents have been intensively investigated. The aim of this study was to assess the effect of enalapril (an angiotensin-converting enzyme inhibitor) and losartan (an angiotensin receptor blocker) in skin flap viability.

Male rats of 200 to 250 g were used. Different doses of enalapril (5, 20, and 50 mg/kg) and losartan (5 mg/kg) were administrated 30 minutes prior to elevate the flap. Flap survival area was evaluated on the seventh postoperative day.

Enalapril improved survival area in a dose-dependent manner, but losartan failed to improve survival area, which suggested that the effect of enalapril was not mediated through AT1 receptors.

**Key Words:** angiotensin receptor, enalapril, losartan, skin flap

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Increasing use of extensive skin flap for the closure of surgery defects of reconstructive surgery seems unavoidable. Random pattern flaps are common type of skin flaps used for this purpose.[1] However, the utility of random pattern flaps may be limited by their restricted blood supply, which sometimes causes development of necrosis in distal area of flap.[2,3] Moreover restoration of normal vascular supply can results further damage by introduction of oxygen into the ischemic flap when blood flow is re-established and by activating of neutrophils.[3–6] Tissue necrosis subsequent to ischemia reperfusion (IR) in random pattern skin flap surgery is a dominant complication with unexpected tissue loss. Therefore prophylactic treatments, which prevent flap injury would be highly desirable in reconstructive surgery.[1]

In the heart, it has been shown that a brief period of ischemia followed by reperfusion results in improved myocardial muscle survival when the muscle is subsequently subjected to prolonged ischemia known as ischemic preconditioning (IPC).[7] Moreover, it is known that administration of drugs such as adenosine or ATP-sensitive potassium channel opener before global ischemia can mimic IPC called pharmacologic preconditioning.[8,9] Recent studies demonstrated that IPC can improve random pattern flap survival in rats[10,11] also in other studies pharmacologic treatments improved skin flap survival by mimicking IPC.[12,13]

Yet it has been shown that, various pharmacologic agents including sympatholytics, vasodilators, hemorheologic agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free radical scavengers are among the drugs thought to be beneficial for flap survival, and they have been investigated for their efficacy in preventing or reversing skin flap ischemia.[14–21] In a very recent study, it was shown that captopril, an angiotensin-converting enzyme (ACE) inhibitor, improved ischemia induced angiogenesis and increased viability and vascularity of skin flap in rats.[22] In spite of well-known improving effects of ACE inhibitors against IR injury in other tissues, there is not sufficient data on skin flap models.

The renin-angiotensin system plays an important role in the regulation of blood pressure and body fluid electrolytes. Renin acts on angiotensinogen to form angiotensin I, which is converted by ACE to angiotensin II (AII). Also many of nonpeptide AII receptor antagonists, with losartan being the first, are available.[23] The effects of ACE inhibitors and AT1 receptor blockers on different tissue IR models has been evaluated in previous studies include investigations on heart,[24,25] liver,[26,27] kidney,[28] and brain[29]; all of these studies has shown protective role of these agents in ischemic tissue preservation.

The role of enalapril as an ACE inhibitor in improvement of heart, liver, and kidney functions after IR injury has been well established in previous studies,[30–33] but there was not sufficient study on the effects of ACE inhibitors or AT1 receptor blockers on skin flap viability after ischemia. In the present study, we assessed the effects of different doses of enalapril and losartan, an AT1 blocker, on skin flap survival.

## MATERIALS AND METHODS

Forty-eight Sprauge-Dawley rats weighing 200 to 250 g were used in 6 groups each with 8 rats. All protocols were approved by the Institutional Animal Care Committee of Iran University of Medical Sciences accredited by Ministry of Health and Medical Education of Iran. The random pattern skin flap elevation procedure was performed under general anesthesia induced by intraperitoneal injections of ketamine (50 mg/kg, Parke-Davis Pharmaceutical Co., Cambridge, UK) and xylazine (10 mg/kg, Parke-Davis Pharmaceutical Co) mixture.[34] The model used was similar to that described by McFarlane et al.[35] The animals were shaved using clippers, scrubbed with povidone-iodine and isopropyl alcohol, and artificial tear was administered to their eyes. Saline or pharmacologic agents were administered subcutaneously. The total volume of 1 mL injected subcutaneously at points 5.5, 6.5, and 7.5 cm from caudal margin of flap 30 minutes before surgery. In control group, we injected saline, and in experimental group, we injected enalapril (Tehran-Chimi Pharmaceutical Co, Tehran, Iran) at 3 dose of 5, 20, and 50 mg/kg, losartan (Tehran-Chimi Pharmaceutical Co) 5 mg/kg and hydralazine (Alborz-Darou Pharmaceutical Co., Tehran, Iran) 9 mg/kg. Caudal to cephalad, 2 incisions 8 cm in length were made 3cm apart on the dorsal surface of the rat. These incisions began right below the scapula. Distal parts of these 2 incisions connected to each other with third incision 3 cm in length. This flap was then raised from the underlying fascia, and an impermeable plastic barrier cut in the same dimensions was placed between the flap and its donor bed.[36] After that, the rats were placed in individual cages, receiving food and water available, the percentage of skin flap

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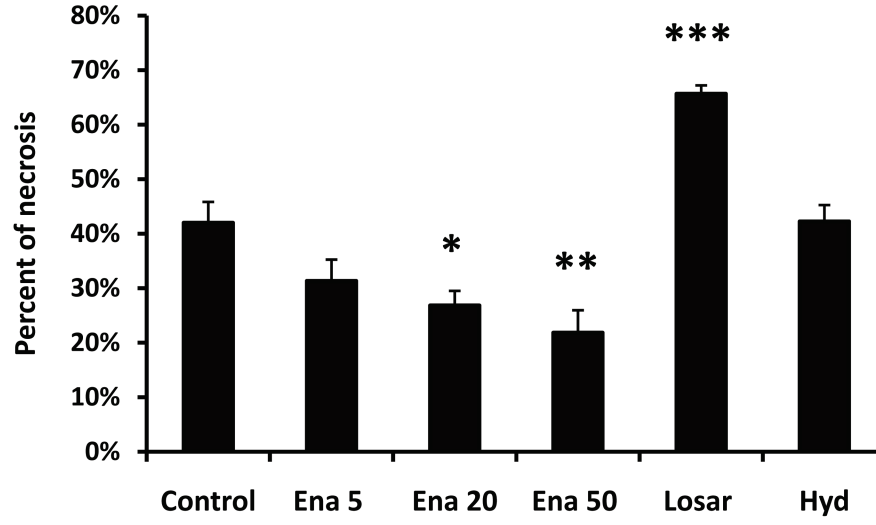


**FIGURE 1.** Effects of different doses of enalapril and losartan or hydralazine on skin flap survival. Data are showed as mean ± SEM of each group. *$P < 0.05$ versus control group, **$P < 0.01$ versus control group, ***$P < 0.001$ versus control group. Ena indicates enalapril; losar, losartan; hyd, hydralazine.

necrosis area was calculated on the seventh postoperative day via the paper template method; the limit between viable tissue characterized by soft skin, rosy, warm, and with hair and necrotic tissue by soft skin, dark, cool, and without hair was determined in animals.[35] A mold of entire flap was drawn and cut in transparent paper and weighed (±0.001 g error). The area corresponding to necrotic skin was cut from this paper template and weighed. The following equation was used to determine percentage of necrotic area:

$$\text{Percentage of necrosis area of the flap} = \frac{\text{Weights of paper temple of flap necrosis}}{\text{Weights of paper temple of total area of flap}} \times 100$$

The animals were then killed with an intracardiac administration of ketamine (150 mg/kg). All results are presented as means ± SEM. Means of groups were compared by 1-way analysis of variance then post-hoc analysis (Tukey test) was performed for assessing specific group comparisons. The level of statistical significance was accepted as $P < 0.05$. Calculations were performed using the SPSS statistical package (version 14).

## RESULTS

The protective effect of administration of different doses of enalapril (5, 20, and 50 mg/kg) on random pattern skin flap survival and the effect of losartan (5 mg/kg) and hydralazine 9 mg/kg is showed in Figure 1. The flap necrotic area was 42.06% ± 3.83% in control group, where in enalapril 10, 4, and 1 mg/flap treated groups, it was 21.83% ± 3.91% ($P < 0.01$), 26.80% ± 2.67% ($P < 0.05$), and 31.33% ± 4.12%. In the group that was treated with losartan, the necrotic area increased significantly (65.66% ± 1.54%) in comparison with control or enalapril (at all doses) ($P < 0.001$). Hydralazine 9 mg/kg could not change the necrotic area in comparison to control group (42.250% ± 3.01%).

As shown in figures that obtained from animals, in control group and in enalapril 5 mg/kg, the distal part of flap had sever necrosis and tissue damage (Figs. 2, 3), where in enalapril 20 and 50 mg/kg treated group, the necrosis reduced significantly (Figs. 4, 5) and a normal tissue in all parts of flap could be seen in (Fig. 5). Losartan at dose of 5 mg/kg caused severe necrosis that covered all parts of flap including proximal sections (Fig. 6).

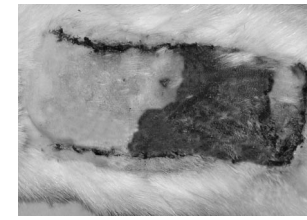

**FIGURE 2.** Skin flap in control group. Necrosis is shown in wide distal parts of flap.

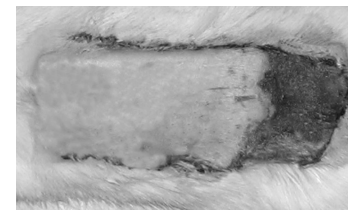

**FIGURE 3.** Enalapril 5 mg/kg has not significant effect on reducing necrotic area.

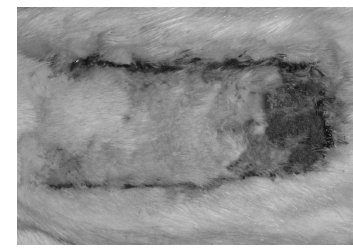

**FIGURE 4.** Effect of enalapril 20 mg/kg on the distal part necrosis of skin flap. Damaged area was reduced significantly ($P < 0.05$) in comparisons to control group.

## DISCUSSION

The main results of the present study are that the inhibition of ACE by enalapril prevented ischemia-induced distal necrosis area in skin flaps, and this effect was dose dependent. Conversely, losartan

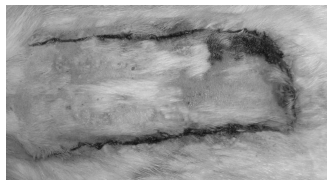

**FIGURE 5.** Enalapril 50 mg/kg significantly ($P < 0.01$) improved flap viability; distal part necrosis was limited effectively in this group.

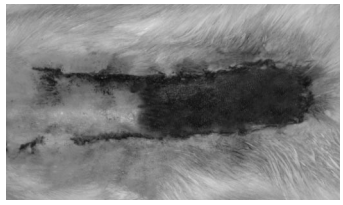

**FIGURE 6.** Losartan 5 mg/kg abolished the skin flap viability with severe necrosis in all part of skin flap. The rate of damage was significant ($P < 0.001$) when compared with control group.

failed to protect distal parts of flap from necrosis via blocking of angiotensin receptors. In addition, hydralazine could not preserve flap survival.

There are experimental and limited clinical evidence indicating a role for ACE inhibitors in limiting myocardial ischemia-reperfusion-induced injury. Although the sulfhydryl group-containing ACE inhibitor captopril has been extensively used in most of studies, the cardioprotective role of non-sulfhydryl group-containing ACE inhibitors, particularly ramipril and enalapril, recently has been identified.[37–39] ACE inhibitors reportedly limit infarct size, prevent ventricular remodeling and, more importantly, stabilize the electrical activity of the reperfused heart and prevent the occurrence of reperfusion arrhythmias. Preliminary, clinical trials indicate an antianginal role for ACE inhibitors. ACE inhibition has also been reported to improve left ventricular performance in patients with long standing infarcts and ischemic failure.[40] Meanwhile enalapril, an ACE inhibitor, reduced infarct size and augmented the preconditioning effect in the pig heart[38,41] In the case of hepatic ischemia reperfusion, ACE inhibition by enalapril is effective in reducing damage as assessed by the leukocyte-endothelium interaction.[42] Enalapril maleate protects the membrane integrity and thus plays a role in restoring ischemia-reperfusion injury.[43] In present study as shown in Figure 1, enalapril reduced necrotic area IR injury in skin flaps that was in accordance with findings on other tissues. Although pharmacologic effects of ACE inhibitors are well known, the mechanism of tissue protection at the molecular and cellular levels is elusive.

The results of the present experiment show that ACE inhibitors have an effect on flap survival that suggested being through inhibition of AII formation. The failure of losartan to prevent lesion in the present study suggested that the observed effect of enalapril was independent of AT 1 receptors. There are many studies that show use of selective antagonists of the bradykinin $B_2$-receptor completely abolished the beneficial effects of ACE inhibition against infarction and IR induced injury.[44–47] Moreover, in kinin-deficient animals, ACE inhibitors could not reduce infarct size and end-diastolic pressure demonstrating that these effects of the ACE inhibitor were mediated by the potentiating of bradykinin rather than the inhibition of AII formation.[47,48]

Reperfusion of ischemic tissue results in local and systemic damage associated with the release of oxygen free radicals, polymorph nuclear leukocytes, and such endothelial hormones as endothelin-1, EDRF (endothelial-derived relaxing factor), thromboxane, complement, and cytokines.[10] It was demonstrated that increased local concentrations of bradykinin reduced neutrophil activation and adhesion,[49] attenuated free radical production,[50] inhibited platelet aggregation and adhesion,[51,52] and enhanced glucose uptake.[53] Moreover, by activation of nitric oxide, which is one of the well-defined pathways for preconditioning state, bradykinin can protect against IR injury.[54] Therefore it may be suggested that protective effects of enalapril in skin flap model may be produce by B2 kinin receptor activation, where precise mechanisms involved required further studies.

Ischemia-reperfusion disrupts the delicate balance that maintains homeostasis in the microcirculation.[55] Failure of a flap to heal involves deficiencies in the inflow of arterial blood into the capillary system and/or retention of venous blood, resulting in edema.[56,57] Recently, it has been shown that sildenafil has the potential to enhance of blood flow to flaps through the vasodilator effects as phosphodiesterase inhibitors and enhancer smooth muscle relaxation (by increase of nitric oxide) and may play a more important role in early postoperative skin flap viability.[58] As protective effects of enalapril in present study, it may be suggested that increasing of blood flow to ischemic area enhanced skin viability in rats treated with ACE inhibitor. It must be mentioned that hydralazine is a vasodilator with mechanism of action independent of renin angiotensin system.[59] A recent study to assess the effect of hydralazine hydrochloride on the viability of random skin flaps showed iontophorese with topical administration of hydralazine hydrochloride was not efficacious in reduction of the necrotic area of random skin flaps in rats.[60] In accordance, the results of the present experiment showed that hydralazine had no effect on skin flap survival of rats. Although the vascular/microvascular diameter of the skin flap was not measured in the present experiment, one can speculate that the effect of enalapril on tissue injury is not through the vasodilator effect.

In conclusion, we suggest that enalapril improved skin flap survival and this effect was dose dependent. Improvement of survival area was not mediated through activation of AT1 receptors because losartan failed to protect against flap necrosis. We suggest that the effect of enalapril on skin flap survival may be through AT2 receptor or mechanisms independent of AII or other unknown mechanisms.

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