

Evaluation of the effects of losartan on a random pattern skin flap model in rats

Sıçan sırt deri flebinde losartanın etkisinin değerlendirilmesi

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BACKGROUND

Losartan, a drug with antiinflammatory properties, has been shown to prevent ischemic injury in various organs. The purpose of the present study was to investigate the effects of losartan on ischemic skin flaps and on flap survival.

METHODS

A 3x9 cm dorsal flap was elevated in 24 Sprague-Dawley rats. Rats received the following treatment for seven days: Group I (n=8): 10 mg/kg losartan; Group II (n=8): 40 mg/kg losartan; and Group III (n=8): nonionized water. At the end of the seventh day, the viable flap areas were calculated, and histological analysis was conducted to count cells and capillaries in microscopic fields.

RESULTS

Mean flap survival was 61%, 56% and 60% in Groups I, II and III, respectively. Comparison of flap survival between groups did not yield any significant difference ($p>0.05$). Ischemic zones of the flaps in all groups demonstrated an increase in number of neutrophils, fibroblasts and capillaries ($p<0.05$), whereas no difference was seen in mast cells. The cell counts in the viable areas of the flaps showed a significant decrease in fibroblasts in the group treated with 40 mg/kg losartan ($p<0.05$). The number of neutrophils, mast cells and capillaries was not influenced by treatment.

CONCLUSION

Losartan does not improve skin flap survival but it has a significant antiproliferative effect on fibroblasts.

Key Words: Angiotensin; AT1; fibroblast; flap survival; ischemic flap; ischemia-reperfusion; losartan; reperfusion injury.

AMAÇ

Losartanın enflamasyonu baskılayarak çeşitli organlarda iskemik-reperfüzyon hasarını azalttığı ve enfarkt alanını küçülttüğü ortaya konmuştur. Bu çalışma, losartanın deri fleplerindeki iskemik bölgeye ve flep süresine etkisini ortaya koymak amacıyla planlandı.

GEREÇ VE YÖNTEM

Sprague-Dawley sıçanlarda (n=24) 3x9 cm boyutlarında kaudal pediküllü sırt flebi kaldırıldı. Sıçanlar 3 gruba ayrılarak 1. gruba 10 mg/kg, 2. gruba 40 mg/kg losartan ve 3. gruba distile su verildi. Yedi günlük tedavinin sonunda fleplerdeki canlı doku alanı hesaplanarak gruplar arasında karşılaştırıldı. Ek olarak fleplerin proksimal ve iskemik bölgelerinde nötrofiller, fibroblastlar, mast hücreleri ve kapillerler sayıldı.

BULGULAR

Fleplerdeki canlı doku alanları ortalaması 1., 2. ve 3. gruplarda sırasıyla %61, %56 ve %60 olarak hesaplandı. Gruplar flep sağkalımı açısından karşılaştırıldığında anlamlı fark bulunmadı ($p>0,05$). Tüm gruplarda fleplerin iskemik bölgesinde nötrofil, fibroblast ve kapillerlerde anlamlı artış saptanırken ($p<0,05$), mast hücre sayısında fark bulunmadı. 40 mg/kg losartan ile tedavi alan grupta fleplerin yaşayan bölgesinde fibroblast sayısı anlamlı olarak azaldı ($p<0,05$). Nötrofil, mast hücreleri ve kapiller sayıları ise tedaviden etkilenmedi.

SONUÇ

Losartan sıçan sırt deri flebinin süresini arttırmamakta ancak fibroblastlar üzerine anlamlı antiproliferatif etkisi bulunmaktadır.

Anahtar Sözcükler: Anjiyotensin; AT1; flep süresi, fibroblast; iskemik flep; iskemik reperfüzyon; losartan; reperfüzyon hasarı.

Random flaps are frequently used in plastic surgery to reconstruct tissue defects. Skin necrosis that may occur at the distal portion of the flap is a main concern when designing such flaps. Various prophylactic and therapeutic measures have been utilized to enhance flap survival, but the search for a reliable and efficient technique continues.

Random pattern flaps undergo a gradient ischemia, which causes diminished blood flow at the distal end of the flap, and a cascade of events that are similar to ischemia-reperfusion injury are triggered.^[1,2] Neutrophils accumulate at the distal portions of random flaps, and they have important contributions to ischemic damage.^[1-3] Surgical techniques such as flap delay procedures^[4,5] have been employed to improve flap survival, and many drugs have been tested in the search for decreasing ischemic damage.^[2,6-11]

The renin angiotensin system (RAS) is a regulator of blood pressure with well-known effects on the kidneys and vessels. Recently, local angiotensin receptors have been demonstrated in many organs including the skin,^[12-15] which are upregulated during ischemia.^[12,14] Activation of these receptors causes an increase in inflammatory response.^[12,16-19] Losartan, which is an angiotensin type 1 (AT1) receptor blocker, has anti-inflammatory properties via acting on local systems. Zhu and Sato et al. showed this drug's ability to reduce the area of infarction in the myocardium.^[20,21] Moreover, losartan prevents ischemia-reperfusion injury in many organs including the heart,^[16,22] liver,^[12] lungs,^[23] endothelium,^[24] brain,^[25] and large intestine.^[19]

We hypothesize that ischemic damage in the skin may also be decreased if proinflammatory properties of angiotensin were inhibited. In this study, the effects of losartan on ischemic skin flaps and its possible role in improving flap survival were investigated. Additionally, the influence of the drug on individual cell lines was researched.

MATERIALS AND METHODS

Twenty-four male Sprague-Dawley rats weighing between 270-350 g were used in this study. The rats were fed with rat chow and drinking water and kept in individual cages during the experiment. Study approval was obtained from the ethical committee responsible for work with laboratory animals at Marmara University Faculty of Medicine.

The rats were divided into three groups with eight rats in each group (n=8). Group I received 10 mg/kg losartan, Group II received 40 mg/kg losartan and Group III received nonionized water (control group). The doses were determined based on previous studies, which calculated the amount of drug that inhibited the RAS without causing hypotension.^[20,26] Losartan was dissolved in nonionized water and administered via an

orogastric tube, with the first dose being 24 hours prior to elevation of the flap. The treatment was continued for seven days postoperatively.

The rats were anesthetized using ketamine (75 mg/kg) and xylazine (10 mg/kg) administered by intraperitoneal injection. Following induction of anesthesia, the dorsal regions were shaved and 3x9 cm caudally based McFarlane flaps^[5,27] were elevated with the panniculus carnosus muscle underneath. Iliolumbar arteries at the pedicle of the flap were cauterized to make the flap pure random pattern.^[28] The flaps were sutured back to the wound bed immediately.

Evaluation Methods

Flap survival

Animals were followed for a total of seven days, at which time viable and necrotic portions of the flaps were evident. Rats were sacrificed and the surviving surface area of the flaps was measured with a centimeter grid. Calculating the viable areas instead of contracted necrotic portions was preferred because it yields a more accurate result.^[2,4,29]

Histological analysis

The flaps were excised and a longitudinal strip 2 mm in width from the middle part of the flap (thereby avoiding incision lines) was removed for histopathologic examination. The specimens were fixed in 10% formaldehyde, stained with hematoxylin-eosin and toluidine blue and examined under light microscope by the same pathologist in a random and blinded fashion. Five microscopic fields under 40X magnification were randomly picked in the proximal (adjacent to pedicle) and ischemic (adjacent to necrotic area) zones of the flap to count neutrophils, fibroblasts, mast cells, and capillaries. The counts were made in the dermis and subcutaneous planes, and mean values were calculated for each group and each zone.

Statistical analysis

Mean values were compared between groups with paired-t, unpaired-t, Wilcoxon, and Mann-Whitney U tests using computer software. The data are presented as mean \pm standard deviation in the figures. Statistical significance was set at $p < 0.05$.

RESULTS

On the seventh day, the regions of survival and necrosis were clearly demarcated in all groups. The necrotic portions (black scab-like tissue with no bleeding) covered approximately 1/3 of the distal portion of the flaps. Microscopic examination revealed coagulation necrosis in this area with no viable cells and almost completely obstructed vessels. An ischemic zone with epidermal irregularity, dermal cellular infiltration and vascular proliferation was seen just proximal to the necrotic area (Fig. 1). Neutrophils, which were the

Table 1. Mean area and percent survival of flaps in the three groups

Group	Mean area of survival in cm ² (total flap area = 27 cm)	Mean survival (%)
10 mg Losartan (n=8)	16.43±2.50	61
40 mg Losartan (n=8)	15.23±1.64	56
Control (n=8)	16.31±1.25	60

dominant cell type, were accumulated in the dermis, while degranulated mast cells were observed mostly in the subcutaneous plane.

Flap Survival

At the end of the seventh day, mean viable flap percentages for Groups I, II and III were 61%, 56% and 60%, respectively. The survival areas were compared between study groups and no significant difference was found. The results are summarized in Table 1.

Histological Evaluation

Comparison of proximal and ischemic zones of flaps

Ischemic zones of the flaps in both the treatment and control groups demonstrated an increase in the numbers of neutrophils, fibroblasts and capillaries ($p<0.05$), whereas no change was seen in mast cells (Fig. 2).

Comparison of proximal zones of flaps between the three groups

The numbers of neutrophils, mast cells and capillaries in the proximal zone were compared between treatment and control groups, and no statistical difference was found (data not shown). The number of

fibroblasts in the proximal zone was lowest in Group II (40 mg losartan) in comparison to Group I (10 mg losartan) and the control group ($p<0.05$) (Fig. 3).

Comparison of ischemic zones of flaps between the three groups

The numbers of neutrophils, mast cells, capillaries, and fibroblasts in the ischemic zone were compared between the treatment and control groups, and no statistical difference was found (data not shown).

DISCUSSION

In random pattern flaps, flap survival depends on the subdermal plexus in the first postoperative days.^[1] If the blood supply is compromised during this period, an incomplete ischemia develops in the distal part of the flap that allows for accumulation of neutrophils.^[1-3] Neutrophils play a pivotal role in ischemic injury by causing direct cellular damage, forming microvascular plugs and releasing mediators such as cytokines, proteases and oxygen-derived free radicals.^[2,3,6,10,16] Inhibition of neutrophil functions has been shown to improve flap survival.^[2,4,6,10] We hypothesized that AT1 blockers may improve flap survival by inhibiting adhesion of neutrophils to the endothelium,^[16,17,19] decreasing tumor necrosis factor (TNF) alpha and interleukin (IL)-8 levels,^[12,15] modifying nitric oxide production,^[16] inhibiting platelet aggregation,^[30,31] and preventing apoptosis.^[21,24,25] Despite our expectations, comparison of flap survivals did not yield any differences between the study groups. There might be several reasons for the failure to show a beneficial effect of the drug on skin flaps. Previous studies that showed losartan's ability to reduce ischemic damage were done on organ models such as the myocardium,^[16,22] endothelium,^[24] liver,^[12] brain,^[25] and large intestine.^[19] Although AT1 receptors are present on rat skin,^[32,33] the literature is devoid of any studies about losartan's effect on these receptors. Also, the studies mentioned above were done on ischemia-reperfusion models. The gradient ischemia of random flaps constitutes a state similar to ischemia-reperfusion injury because the diminished blood flow at the distal end allows accumulation of inflammatory cells. However, the flap model we chose might have failed to fully represent ischemia-reperfusion injury, and this may be another reason for the lack of positive results. Additionally, the doses selected might have been insufficient to exert

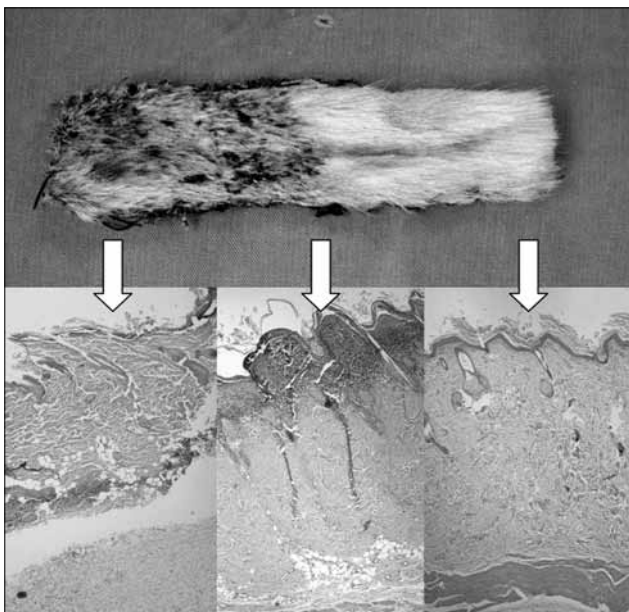


Fig. 1. The distal, middle and proximal zones of the flap in 4X magnification stained with hematoxylin-eosin.

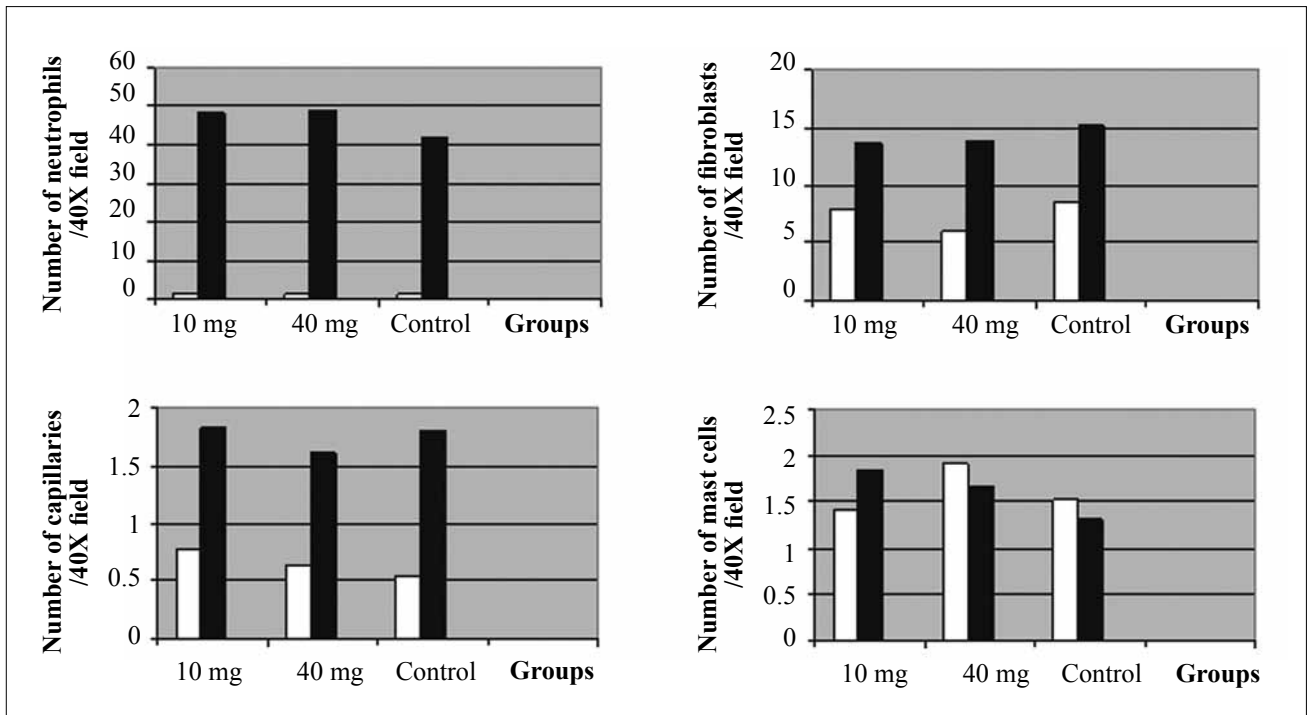


Fig. 2. Comparison of proximal (□) and ischemic zones (■) of flaps in the three groups.

an effect to improve skin flap survival. Finally, there are issues related with the wound bed. Hammond et al.^[4] demonstrated that a portion of random flaps may survive as a graft, which is a reason for the unreliable and inconsistent results in many random flap studies. Insertion of a silicone sheet under the flap is a method that prevents contact of flap with the wound bed, but this method is known to cause foreign body reactions and increase inflammation, which in turn could have biased the results.^[34]

Further histological analysis was conducted to investigate the effect of losartan on cell lines. The results showed that the drug had no influence on the number of neutrophils, mast cells or capillaries, but there was a significant decrease in the number of fibroblasts in Group II (treated with 40 mg/kg losartan). These results will be discussed individually.

The decrease in the number of fibroblasts is consistent with previous studies that demonstrated an antiproliferative effect of losartan on fibroblasts,^[35-37] but its confinement to the proximal zone of the flaps is interesting. This zone-limited effect is a proof of perfusion of the drug to the viable tissues; however, perfusion to the ischemic area remains questionable. Moreover, Viswanathan and Steckelings et al. have shown an upregulation of only AT2 receptors on rat skin fibroblasts during wound healing.^[13,32] Therefore, it is possible that subtype AT2 receptors were the only receptors increased in the ischemic zone, which explains losartan's inability to exert an effect. Thus, this study supports the antiproliferative effect of losartan on fibroblasts, but more studies should be carried out to test whether the drug is effective on various models of fibrosis or scar formation.

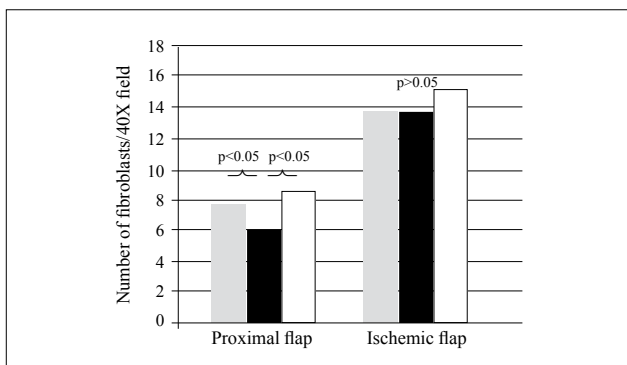


Fig. 3. Comparison of fibroblasts between the three groups. [(●) 10 mg, (■) 40 mg, (□) Control].

Losartan has well-known inhibitor actions on neutrophils in various organs such as the heart,^[16] liver,^[12] mesenteric venules,^[17] large intestine,^[19] and stomach.^[15] The drug acts via suppression of neutrophil functions (rolling, adhesion, migration) and causes an eventual decrease in extravascular infiltration. In the study presented, the neutrophil accumulation in the dermis and subcutaneous planes was evaluated, and there was no difference between the treatment and control groups. Losartan's failure to limit the extravascular neutrophil flux is an explanation for the unimproved flap survival. In the studies cited above, the dose and duration of therapy and method of administration of losartan were varied, which can also ex-

plain the contradictory results. The dose of the drug we selected might have been insufficient for inhibiting inflammation in a skin flap model.

Another finding in our study is about the role of mast cells in ischemia. Viable and ischemic areas of flaps were compared to evaluate cellular changes. An extravascular accumulation of neutrophils and fibroblasts was observed in ischemic areas, but there was no change in the number of mast cells. Although mast cells have been reported as contributors to ischemic insult,^[38] such a relation could not be shown here.

This study has several results concerning angiogenesis. Comparison of treatment and control groups yielded no significant difference in the number of capillaries, and losartan was found to be ineffective on angiogenesis. In the literature, there are controversial reports about the timing of angiogenesis after flap surgery or wound healing. Some of the studies claim an increase in angiogenesis after the first week^[39,40] while others report an onset as early as the second day.^[11,38] Our findings advocate an early onset of angiogenesis since the increase in the number of capillaries was already evident on the seventh day.

In studies investigating ischemia of flaps, a drug can be found to be effective in one study but ineffective in another. This could result from dissimilar laboratory environments, different administration methods, varying doses of drugs, various models of ischemia, insufficient perfusion of the drug to the flap, or survival of a part of the flap as a graft.^[4,38] Subsequent experiments with different methods or flap models can be conducted to see whether the results will support our findings. Additionally, it may be useful to evaluate the effects of losartan simultaneously in skin flaps and viscera, thereby investigating the tissue specificity of the drug.

In conclusion, administration of losartan in the doses and methods selected in this study did not improve random flap survival, but the drug was found to be effective in reducing the number of fibroblasts.

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