The Effects of Montelukast on Random Pattern Skin Flap Survival: An Experimental Study in Rats

Kaan Gideroglu, MD[1]; Fahrettin Yilmaz, MD[2]; Fadullah Aksoy, MD[3]; Guler Bugdayci, MD[4]; Husamettin Cakici, MD[5]; and Onur Hapa, MD[6]

[1]Medical Faculty, Department of Aesthetic, Plastic, and Reconstructive Surgery, Abant Izzet Baysal University, Bolu, Turkey; [2]Medical Faculty, Department of Otorhinolaryngology and Head and Neck Surgery, Abant Izzet Baysal University, Bolu, Turkey; [3]Otorhinolaryngology and Head and Neck Surgery Clinics, Haseki Education and Research Hospital, Istanbul, Turkey; [4]Medical Faculty, Department of Biochemistry, Abant Izzet Baysal University, Bolu, Turkey; [5]Medical Faculty, Department of Orthopedics, Abant Izzet Baysal University, Bolu, Turkey; and [6]Orthopedic Clinic, State Hospital, Bolu, Turkey

ABSTRACT

BACKGROUND: A variety of methods to improve skin flap survival, including the use of pharmacologic agents, have been intensively investigated. Decreasing neutrophil-mediated inflammation and tissue injury has been reported to be effective in improving flap survival. Montelukast is a selective reversible cysteinyl leukotriene receptor-1 antagonist that has been found to have protective effects against renal ischemia reperfusion injury and burn-induced oxidative injury of the skin in rats. However, its effects on skin flap survival have not been previously reported.

OBJECTIVE: The aim of this study was to investigate the effects of montelukast on neutrophil-mediated random pattern skin flap survival.

METHODS: Male Sprague-Dawley rats weighing 230 to 250 g were randomly divided into 2 groups—the montelukast-treated group and the control group. Caudally based rectangular random pattern skin flaps 3 × 9 cm were elevated on the backs of the rats. The flaps were sutured into their original places. In the montelukast group, 1 mL of solution containing 10 mg/kg montelukast was administered intra-peritoneally (IP) 30 minutes before surgery and then daily for 6 days. In the control group, 1 mL of saline was administered IP 30 minutes before surgery and then daily for 6 days. To observe the effects of montelukast, myeloperoxidase (MPO) activity, an index of tissue neutrophil infiltration, was measured from extracted skin tissue 12 hours after flap elevation. Flap viability was evaluated 7 days after surgery by measuring necrotic flap area and total flap area.

RESULTS: Sixteen rats (mean [SD] weight, 240.6 [6.6] g) were equally divided between the 2 groups. All rats survived throughout the study period. Mean (SD) MPO activity in flap tissue was significantly lower in the montelukast group than in the control group (14.57 [2.33] vs 21.28 [4.86] U/g protein; $P = 0.005$). The percentage

of necrotic flap area was significantly lower in the montelukast group than in the control group (17.17 [7.95] vs 37.51 [10.72]; $P = 0.001$).

CONCLUSION: This small, experimental, in vivo animal study found that montelukast was associated with both lower MPO activity and a lower percentage of necrotic random pattern skin flap area. Future studies are needed to clarify these findings. (*Curr Ther Res Clin Exp*. 2008;69:459–465) © 2008 Excerpta Medica Inc.

KEY WORDS: montelukast, skin flap survival, necrosis.

INTRODUCTION

Random pattern skin flaps are commonly used to repair various skin defects in reconstructive surgery; however, necrosis is a major concern with these flaps.[1] Although the pathophysiology of skin flap necrosis is complex, 2 main mechanisms are suspected: neutrophil infiltration and inadequate blood supply due to vascular thrombosis.[2–6] The infiltration of polymorphonuclear leukocytes in tissue is characteristic of acute inflammation and indicates the collective action of chemotactic mediators. The 5-lipoxygenase metabolites of arachidonic acid, various chemokines, and lipid mediators (cysteinyl leukotrienes [CysLTs]) are potent inflammatory mediators associated with tissue injury.[1–7] Reactive oxygen species, proteases, elastase, myeloperoxidase (MPO), and various other mediators, all of which are involved in tissue injury, are released once neutrophils migrate into the ischemic area.[5–7] Skin blood flow initially decreases in the distal portion of random pattern skin flaps and then increases over time.[8] An incomplete ischemic state with tissue damage is found in the distal region of random pattern skin flaps.[9] Neutrophil-mediated microvascular dysfunction and tissue injury can be reduced dramatically by preventing neutrophil influx into tissues, either by depleting the number of circulating leukocytes or by preventing neutrophil adhesion. In this way, the surviving flap area can be increased.[1,2,9–11] MPO, which is specific to neutrophils, is deposited in neutrophil granules.[2] Measurement of MPO enzyme activity is one of the most commonly used techniques to determine neutrophil accumulation in tissues.[1,2,7,10] Preventing or decreasing neutrophil invasion in ischemic tissue by blocking any step of neutrophil activation also reduces tissue MPO enzyme activity.[2]

Montelukast is a selective reversible CysLT1 receptor antagonist used in the treatment of asthma.[7] It was found to reduce eosinophilic inflammation in the airways.[7,12] By modifying neutrophil function, montelukast was found to provide protection against burn-induced oxidative injury of the skin.[13]

The aim of this study was to investigate the effects of montelukast on neutrophil-mediated random pattern skin flap survival.

MATERIALS AND METHODS

This study was performed in the experimental animal laboratory of the Department of Histology at Abant Izzet Baysal University School of Medicine (Bolu, Turkey) and was approved by the local ethics committee of Haseki Education and Research Hospital (Istanbul, Turkey).

Male Sprague-Dawley rats weighing 230 to 250 g were purchased from the experimental animal production center of Düzce University (Düzce, Turkey). All animals

were housed in cages at room temperature under a 12-hour day/night lighting system and access to water and food was ad libitum. All experiments were carried out in accordance with the World Medical Association's Declaration of Helsinki on Animal Rights.[14]

STUDY DESIGN

The animals were randomly divided into 2 groups—the montelukast-treated group and the control group. Randomization was achieved using a computer-based number generation technique. The montelukast group received 1 mL of solution containing 10 mg/kg montelukast intraperitoneally (IP) 30 minutes before surgery and then daily for 6 days. In the control group, 1 mL of saline was administered IP 30 minutes before surgery and then daily for 6 days. Rats were anesthetized using a mixture of ketamine hydrochloride (50 mg/kg IP) and chlorpromazine (30 mg/kg IP). The surgeon was blinded to the treatment groups. Each animal's back was shaved with an electric clipper. A sterile technique was used in all surgical procedures. The animals were placed in the prone position and a 3 × 9-cm caudally based rectangular flap was drawn on the backs of the rats according to the method described by Khouri et al.[15] After assessment of skin flap survival on postoperative day 7, all rats were euthanized with IP injections of high-dose sodium pentothal.

SURGICAL TECHNIQUE

After the skin was shaved, each rat's back was rinsed with povidone solution. Asepsis was maintained by providing a local sterile environment. The caudally based dorsal flap was located in reference to anatomic landmarks and extended from the scapular tip to the hip joints. A dorsal random pattern flap 3 × 9 cm was elevated between these reference points. The flap consisted of skin, panniculus carnosus muscle, and submuscular areolar tissue. The flap was sutured back into its original location after meticulous hemostasis.

MYELOPEROXIDASE DETERMINATION

Twelve hours[2,16] after the elevation of the flap, a full-thickness 4-mm punch biopsy of the skin flap was retrieved from lightly anesthetized rats at the center of each flap by a second investigator who was blinded to treatment groups. Fifty milligrams of flap tissue was homogenized in ice-cold 50-mM potassium phosphate buffer (PB) (pH, 6) and centrifuged at 10,000g for 10 minutes; pellets were suspended in 50-mM PB containing 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical Corp., St. Louis, Missouri). After 3 freeze/thaw cycles, with sonication between cycles, the samples were centrifuged at 12,000g for 10 minutes, and 0.1 mL of the supernatant was added to 2.9 mL of 50-mM PB. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50-mM PB (pH, 6), o-dianisidine, and 20-mM hydrogen peroxide (H_2O_2) solution. H_2O_2 was used as a substrate for MPO. Oxidized o-dianisidine forms a stable chromophore absorption at a wavelength of 460 nm. One unit of *enzyme activity* was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 minutes. One unit of *MPO activity* was

defined as the amount required to degrade 1 μmol of H_2O_2 per minute at 25°C. MPO activity was recorded as activity per gram of tissue (U/g protein).[17]

ASSESSMENT OF SKIN FLAP NECROSIS

The primary end point in this study was to assess skin flap necrosis. A third investigator who was blinded to the study groups assessed skin flap survival on postoperative day 7. Gross observation was used to identify the line of demarcation between the viable and the necrotic tissue. The entire flap and the necrotic and viable portions were traced onto a transparent cellophane sheet and transferred to graph paper with a 1-mm^2 grid.[18] The area of necrosis was expressed as a percentage of the entire flap area (necrotic flap percentage = necrotic flap area/total area × 100). After assessment, the rats were euthanized using an overdose of sodium pentothal.

STATISTICAL ANALYSIS

Data were presented as mean (SD). SPSS for Windows version 11.0 (SPSS Inc., Chicago, Illinois) was used for statistical analysis. Because the results of the assessment of skin flap necrosis and biochemical analyses did not distribute normally according to the Kolmogorov-Smirnov test, they were compared using the nonparametric Mann-Whitney U test. $P < 0.05$ was considered significant.

RESULTS

Sixteen rats (mean [SD] weight, 240.6 [6.6] g) were randomly divided into 2 groups—the montelukast-treated group and the control group (received saline)—with 8 rats to each group. All rats survived throughout the study period.

FLAP TISSUE MYELOPEROXIDASE ACTIVITY

Twelve hours after surgery, mean (SD) flap tissue MPO activity was significantly lower in the montelukast group than in the control group (14.57 [2.33] vs 21.28 [4.86] U/g protein; $P = 0.005$).

FLAP SURVIVAL

On postoperative day 7, the percentage of necrotic flap area was significantly lower in the montelukast group than in the control group (17.17 [7.95] vs 37.51 [10.72]; $P = 0.001$).

DISCUSSION

In plastic surgery, random pattern skin flaps are used to reconstruct various defects.[18] Necrosis in random pattern skin flaps remains a serious problem, potentially leading to long hospitalization and high morbidity.[19] An ischemic zone that may undergo necrosis is likely to occur in the distal portion of a random skin flap. Although many studies have investigated ischemic skin necrosis, the pathogenic mechanism is still unclear. Tissue ischemia induced by leukocyte inflammation and inadequate blood flow is believed to be the principal factor predisposing a patient to flap tissue necrosis.[1–4]

Anti-inflammatory drugs, vasodilators, fibrinolytic enzyr ·s, energy-rich phosphate compounds, free radical scavengers, anticoagulants, vitamins, neutrophil function modulators, and immunosuppressants are among the most investigated pharmacologic agents to improve ischemic skin flap survival.[1,2,9–11,16,18,20–22] Montelukast significantly increased skin flap survival in this experimental study. We suggest that this finding may be related to the inhibiting effect of montelukast on neutrophil infiltration and its regulatory effect on generation of inflammatory mediators.

Microvascular disorders in an ischemic flap may be decreased by reducing neutrophil infiltration into the tissues by preventing neutrophil adhesion or decreasing circulating neutrophil count.[2,7,9–11] Neutrophil activation is a multistep process that involves a complex series of events.[2] After a prolonged ischemic period, hypoxia causes an elevation in cytosolic calcium concentration; phospholipase A_2 and lipoxygenase enzyme activities increase,[23] resulting in increased leukotriene activity. CysLTs are thought to be inflammatory mediators due to their potent chemotactic and chemokinetic properties.[24] These lipid mediators have also been suggested to enhance the vascular permeability and recruitment of neutrophils, which are associated with tissue injury.[25] CysLTs have also been reported to be produced during cerebral ischemia, which in turn, disrupts the blood–brain barrier and leads to brain edema.[26] Experimental studies found that CysLT1 receptor antagonists are protective against these effects of leukotrienes after ischemia.[27,28] The CysLT1 antagonist montelukast was found to have protective effects against burn-induced oxidative injury of the skin in rats.[13] We hypothesize that montelukast ameliorates tissue injury and flap necrosis by blocking CysLT receptors, which are responsible for increased permeability and recruitment of neutrophils and macrophages. In addition, we suggest that the decrease in neutrophil and macrophage recruitment leads to a decrease in production of proinflammatory mediators. However, montelukast may act as an antioxidant in ischemic tissues, not only by blocking the infiltration of neutrophils and macrophages, but also by interacting with receptors expressed on mesangial cells and/or macrophages.[7] However, we did not investigate this in our study. This theory should be investigated by further studies.

Sener et al[7] found that both renal tissue necrosis and tissue MPO enzyme activity were attenuated by blocking CysLT receptors with montelukast in renal ischemia-reperfusion in a rat model. Furthermore, skin MPO activity decreased with montelukast treatment after burn-induced oxidative injury.[13] MPO activity in both groups in the present study reflected tissue neutrophil counts and was believed to be directly related to flap tissue necrosis. Treatment with montelukast (10 mg/kg daily) 30 minutes before surgery and for 6 days postoperatively was associated with significantly decreased MPO activity and flap necrosis in this study.

Montelukast 10 mg/d is usually used in the treatment of asthma in adults.[29] However, we used montelukast in a 10-mg/kg daily regimen, since its protective effects against ischemia-reperfusion injury and burn-induced oxidative skin injury have been reported at this dosage.[7,13] In our opinion, lower doses may also be effective in increasing skin flap survival. However, this needs to be investigated in future studies that particularly focus on the dose-dependent properties of montelukast.

STUDY LIMITATIONS

The method of assessing the proportion of skin flap necrosis by tracing onto a transparent sheet and transferring onto graph paper was a limitation of this study, as the outcome was more subjective than using a computer program to analyze a digital picture. However, the method we used is among the methods commonly used for this assessment.[18]

CONCLUSION

In this experimental animal study, administration of montelukast was associated with significantly decreased MPO activity, an index of tissue neutrophil recruitment, and random pattern skin flap necrosis. Future studies are needed to clarify these findings.

ACKNOWLEDGMENT

REFERENCES
1. Törkvist L, Löfberg R, Raud J, Thorlacius H. Heparin protects against skin flap necrosis: Relationship to neutrophil recruitment and anti-coagulant activity. *Inflamm Res.* 2004;53:1–3.
2. Cetin C, Köse AA, Aral E, et al. Protective effect of fucoidin (a neutrophil rolling inhibitor) on ischemia reperfusion injury: Experimental study in rat epigastric island flaps. *Ann Plast Surg.* 2001;47:540–546.
3. Gute DC, Ishida T, Yarimizu K, Korthuis RJ. Inflammatory responses to ischemia and reperfusion in skeletal muscle. *Mol Cell Biochem.* 1998;179:169–187.
4. Lee C, Kerrigan CL, Tellado JM. Altered neutrophil function following reperfusion of an ischemic myocutaneous flap. *Plast Reconstr Surg.* 1992;89:916–923.
5. Ichikaea F, Miyazaki S. New biodefence strategies by neutrophils. *Arch Immunol Ther Exp.* 2005;53:226.
6. Kelly KJ, Williams WW Jr, Colvin RB, et al. Intercellular adhesion molecule-1–deficient mice are protected against ischemic renal injury. *J Clin Invest.* 1996;97:1056–1063.
7. Sener G, Sehirli O, Velioglu-Ogünç A, et al. Montelukast protects against renal ischemia/reperfusion injury in rats. *Pharmacol Res.* 2006;54:65–71.
8. Suzuki S, Miyachi Y, Niwa Y, Issihiki N. Significance of reactive oxygen species in distal flap necrosis and its salvage with liposomal SOD. *Br J Plast Surg.* 1989;42:559–564.
9. Akamatsu J, Ueda K, Tajima S, Nozawa M. Sulfatide elongates dorsal skin flap survival in rats. *J Surg Res.* 2000;92:36–39.
10. Törkvist L, Månsson P, Raud J, et al. Role of CD18-dependent neutrophil recruitment in skin and intestinal wound healing. *Eur Surg Res.* 2001;33:249–254.
11. Vedder NB, Bucky LP, Richey NL, et al. Improved survival rates of random flaps in rabbits with a monoclonal antibody that blocks leukocyte adherence. *Plast Reconstr Surg.* 1994;93:1035–1040.
12. Wenzel SE. Arachidonic acid metabolites: Mediators of inflammation in asthma. *Pharmacotherapy.* 1997;17:3S–12S.
13. Sener G, Kabasakal L, Cetinel S, et al. Leukotriene receptor blocker montelukast protects against burn-induced oxidative injury of the skin and remote organs. *Burns.* 2005;31:587–596.

14. World Medical Association. World Medical Association Statement on Animal Use in Biomedical Research. http://www.wma.net/e/policy/a18.htm. Accessed November 2, 2007.
15. Khouri RK, Angel MF, Edstrom LE. Standardizing the dorsal rat flap. *Surg Forum*. 1986; 37:590–594.
16. Aydogan H, Gurlek A, Parlakpinar H, et al. Beneficial effects of caffeic acid phenethyl ester (CAPE) on the ischaemia-reperfusion injury in rat skin flaps. *J Plast Reconstr Aesthet Surg*. 2007;60:563–568.
17. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *J Invest Dermatol*. 1982;78: 206–209.
18. Akan M, Cakir B, Misirlioglu A, et al. Effects of clopidogrel and high dose aspirin on survival of skin flaps in rats. *Scand J Plast Reconstr Surg Hand Surg*. 2005;39:7–10.
19. Campbell SP, Moss ML, Hugo NE. When does a random flap die? *Plast Reconstr Surg*. 1992; 89:718–721.
20. Atabey A, Im MJ, Akgur FM, et al. The effect of cobalt chloride on skin flap survival. *Br J Plast Surg*. 1996;49:321–324.
21. Bilgin-Karabulut A, Ademoglu E, Aydin I, et al. Protective effects of vitamins A and E pretreatment in venous ischemia/reperfusion injury. *J Reconstr Microsurg*. 2001;17:425–429.
22. Kawabata H, Knight KR, Coe SA, et al. Experience with calcium antagonists nitrendipine, diltiazem, and verapamil and beta 2-agonist salbutamol in salvaging ischemic skin flaps in rabbits. *Microsurgery*. 1991;12:160–163.
23. Kiang JG, Tsen KT. Biology of hypoxia. *Chin J Physiol*. 2006;49:223–233.
24. Krysztopik RJ, Bentley FR, Spain DA, et al. Free radical scavenging by lazaroids improves renal blood flow during sepsis. *Surgery*. 1996;120:657–662.
25. Takamatsu Y, Shimada K, Chijiiwa K, et al. Role of leukotriens on hepatic ischemia reperfusion injury in rats. *J Surg Res*. 2004;119:14–20.
26. Baba T, Black KL, Ikezaki K, et al. Intracaratoid infusion of leukotriene C4 selectively increases blood-brain barrier permeability after focal ischemia in rats. *J Cereb Blood Flow Metab*. 1991;11: 638–643.
27. Yu GL, Wei EQ, Zhang SH, et al. Montelukast, a cysteinyl leukotriene receptor-1 antagonist, dose- and time-dependently protects against focal cerebral ischemia in mice. *Pharmacology*. 2005;73:31–40.
28. Zhang W-P, Wei E-Q, Mei R-H, et al. Neuroprotective effect of ONO-1078, a leukotriene receptor antagonist, on focal cerebral ischemia in rats. *Acta Pharmacol Sin*. 2002;23:871–877.
29. Zeiger RS, Bird SR, Kaplan MS, et al. Short-term and long-term asthma control in patients with mild persistent asthma receiving montelukast or fluticasone: A randomized controlled trial. *Am J Med*. 2005;118:649–657.

ADDRESS CORRESPONDENCE TO: Kaan Gideroglu, MD, Medical Faculty, Department of Aesthetic, Plastic, and Reconstructive Surgery, Izzet Baysal Medical University, 14280, Gölköy, Bolu, Türkiye. E-mail: kaangideroglu@hotmail.com