Abstract: Objective. The purpose of this study was to reveal the effect of N-acetylcysteine (NAC) on random-skin flaps in rats. Introduction. N-acetylcysteine is an agent among free radical scavengers which is used primarily as a mucolytic agent. Experimental studies have demonstrated protective effects of NAC on hepatic, renal, lung, and intestinal injuries. Methods. Wistar female rats were divided into 2 groups (control and NAC group), and the NAC group received intramuscular injections for 7 days. Flaps were raised on day 2 and rats were sacrificed on day 7. Skin samples from the second cm and fifth cm of the skin flap were collected for biochemical and histopathological examinations. Results. The mean necrotic area ratios in the control and NAC group were 38% and 12%, respectively ($P < 0.001$). Malondialdehyde (MDA) levels were significantly lower in skin samples collected from the control group as compared to samples obtained from the NAC group ($P = 0.002$). Superoxide dismutase (SOD) activity was significantly higher in the NAC group ($P < 0.0001$). Histopathologically, a significant increase in macrophage and fibroblast activity was observed in the NAC group. Mononuclear cell infiltration and fibroblast activity had increased, especially in samples from the fifth cm of the skin flap in the NAC group. The histopathological evaluation in the NAC group revealed protective effects of NAC. Conclusions. Treatment of rats with NAC significantly reduced flap necrosis as well as MDA levels while increasing SOD levels. These data suggest that NAC has a protective role in flap survival and demonstrates preventive effects against flap necrosis.

The risk of flap necrosis after flap surgery is a major problem. In recent years, the pharmacological and surgical treatments of flap ischemia have become an area of interest. Experimental studies have been performed and the protective effects and beneficial roles of antioxidant drugs have been discussed. Numerous pharmacological agents, such as sympatholytics, vasodilators, hemorheologics, anticoagulants, prostaglandin inhibitors, and free radical scavengers, have been examined for their ability to prevent skin flap ischemia. An example of these agents are endothelial
growth factors, pentoxifylline, heparin, octreotide, streptokinase, allopurinol, magnesium sulphate, montelukast, erythropoietin, estradiol, dimethyl sulfoxide, and herbal medications. Although beneficial results were obtained by several agents experimentally, there is not a consensus about management in clinical practice.

N-acetylcysteine (NAC) is a pharmacological agent among free radical scavengers that reacts with hydroxyl-radical and hypochlorous acid, and is poorly reactive with hydrogen peroxide and the superoxide radicals. As a pharmaceutical drug and nutritional supplement, NAC is used primarily as a mucolytic agent, and in the management of acetaminophen overdose. Primary indications for NAC are mucolytic therapy as an adjuvant in respiratory conditions including emphysema, bronchitis, tuberculosis, bronchiectasis, amyloidosis, pneumonia, cystic fibrosis, and chronic obstructive pulmonary disease. It is also indicated for pulmonary complications of surgery and anesthesia. Moreover, experimental studies have demonstrated the protective effects of NAC on hepatic, renal, lung, and intestinal injuries. The purpose of this study was to determine the effects of NAC administration, a highly effective antioxidant, on the survival of random pattern skin flap in a rat model.

**Materials and Methods**

The procedures and animal protocols followed in this study complied with the Guide for the Care and Use of Laboratory Animals, and were approved by the Ethics Committee of the Ankara Oncology Training and Research Hospital, Ankara, Turkey.

Rats were randomly divided into 2 groups with 15 subjects in each. All subjects in the control (Group 1) and NAC (Group 2) groups were kept at room temperature and had free access to water and food.

**Keypoints**

- Rats were randomly divided into 2 groups with 15 subjects in each. All subjects in the control (Group 1) and NAC (Group 2) groups were kept at room temperature and had free access to water and food.
- Subjects in the NAC group received NAC (300mg/kg/day) by intramuscular injection at 24 hours before the operation, 15 minutes before flap elevation, and daily after the operation until postoperative day 5.

After 7 days of flap elevation, the rats were re-anesthetized. The ischemic necrotic area was well demarcated in the distal portion of the skin flap; identified easily by gross observation and the area of viable and nonviable skin in the flap was assessed using the paper template technique previously described. Skin that was rosy in color, and soft and warm to the touch, was considered viable. Conversely, hairless, stiff, darkened, and colder skin was considered necrotic. These areas were demarcated on the animal subjects and also on transparent paper. With the aid of a precision scale, the necrotic area of the flap was calculated from the weight of the paper template corresponding to the necrotic area in relation to the weight corresponding to the total flap area expressed as a percentage. After determination of the percentage of the necrotic area, skin samples were obtained from each animal at a distance of 2 cm and 5 cm from the cranial, and 0.5 cm from the lateral margins of the flap. Flap tissues were sampled from the second cm and fifth cm of the skin flaps to determine the effects of NAC proportional to the degree of ischemia/necrosis. The main reason the tis-
Sue samples were obtained from the second cm and fifth cm of the skin flaps is that, in earlier control studies, the ischemic areas were observed intensively between 2.4 cm - 5.3 cm. For evaluating the effects of NAC entirely, the second cm and fifth cm of a flap have been established to be the most appropriate. In this way, the possible favorable effects of NAC could be determined by the differentiations on these areas.

Skin specimens were divided into 2 equal pieces for pathological examination and biochemical analysis. All specimens taken for histopathological evaluation were fixed in 10% formaldehyde, stained with hematoxylin-eosin, and examined under a light microscope by the same pathologist in a random and blinded fashion. Four microscopic fields under 40x magnification were randomly picked in the second cm and fifth cm samples of the flap to count macrophages, fibroblasts, and mononuclear leukocytes. The counts were made in the dermis and subcutaneous planes, and mean values were calculated for each group of samples. The level of malondialdehyde (MDA) in tissue homogenate was determined using the Mihara and Uchiyama method. Half a milliliter of homogenate was mixed with 5mL H3 PO4 solution (1% v/v) followed by addition of 1 mL thiobarbituric acid solution (0.67% w/v). Then the mixture was heated in a water bath for 45 minutes. The resulting red-colored complex was extracted into n-butanol and absorption at 532 nm was measured using tetramethoxypropane as standard. Malondialdehyde levels were expressed as a nanomol per gram of protein (nmol/g protein). Superoxide dismutase (SOD) levels were determined as an index of oxidation state, as described by Sun Y et al and expressed as ug of protein.

The descriptive values of data were represented as means ± standard error of the mean (SEM). The statistical evaluation for necrosing flap areas were evaluated by using the Kruskal-Wallis test. The Independent samples t test was used for the determination of significant differences in the levels of MDA and SOD. Microscopic results of histopathological evaluation were assessed using the One-way analysis of variance (ANOVA), which evaluated whether there were differences between groups. A P-value less than 0.05 was considered to be statistically significant.

**Results**

One anesthesia-related death occurred in the control group in the immediate post-surgical period. No death occurred in the NAC group. Total flap necrosis area was 5.35 ± 1.00 cm² inches in the control group, and 1.75 ± 0.82 cm² inches in the NAC group. The necrotic area on the skin flaps was stabilized 5 days after surgery with
clear boundaries between the surviving and necrotic areas (Figures 1 and 2). The mean necrotic area ratios in the control and NAC groups were 38% and 12%, respectively. The flap necrosis area values between the 2 groups were significantly different ($P < 0.001$). Measurements of necrotic areas (cm$^2$) in skin flaps of both groups were shown in Figure 3.

As for histopathological evaluation, the quantification of macrophage and fibroblast showed a significant increase in the NAC group in comparison with that in the control group ($P < 0.002$). Mononuclear leukocyte infiltration and fibroblast activity had increased especially in the samples from the fifth cm of the skin flap in the NAC group ($P < 0.001$) (Figures 4 and 5).

The MDA levels of flap tissues were as follows: second cm of the skin flap of control group, 2.54 ± 0.67; second cm of NAC group 2.00 ± 0.45; fifth cm of NAC group, 2.06 ± 0.37. The tissue MDA levels in the NAC group were significantly lower than the levels of control group (second and fifth cm of the skin flap, $P = 0.017$ and $P = 0.002$, respectively).

The SOD levels of flap tissues were as follows: second cm of the skin flap of control group, 7.65 ± 1.47; fifth cm of control group 9.18 ± 2.30; second cm of NAC group 23.27 ± 2.60; fifth cm of the NAC group, 20.01 ± 2.30. The tissue SOD levels in the NAC group were significantly higher than the levels of the control group (second and fifth cm of the skin flap, $P < 0.001$ and $P < 0.001$, respectively). The results of biochemical analysis of tissue samples were shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. The results of biochemical analysis of tissue samples.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (Control)</strong></td>
</tr>
<tr>
<td>2nd cm</td>
</tr>
<tr>
<td>MDA (nmol/g)</td>
</tr>
<tr>
<td>SOD (u/g)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM  
* $P = 0.017$, significantly different from control group.  
† $P = 0.002$, significantly different from control group.  
‡ $P <0.001$, significantly different from control group.  
§ $P <0.001$, significantly different from control group.
Discussion

Flap survivability is the major concern in flap surgery. Distal ischemic necrosis is the most common clinical complication in pedicled skin flap surgery. Ischemic necrosis on random skin flaps occur because of venous flow insufficiency and oxygen deficiency. The most important factor that effects the survival of skin flaps in the ischemic process is the increase of oxygen radicals by reperfusion. N-acetylcysteine, which is an antioxidant and free radical scavenger, increases the survival of ischemic skin flaps in rabbit models.21 Although ischemic necrosis is a serious complication of skin flap surgery, incidence of flap failure remains low. Several studies have searched for an effective drug therapy for flap survivability; however, an ideal drug therapy is not currently available for skin flap necrosis.2-7 Although multiple pharmacological approaches have been used to enhance flap survival experimentally, none have found widespread clinical acceptance or utility. Systemic adverse effects, high cost, poor availability, complex dosing schemes, or invasive drug delivery are limiting their effectiveness. An ideal drug that is well tolerated, cost-effective, safe, and readily available should help prevent necrosis of skin flaps. Oxygen radical scavengers were shown to improve the survival in animal models of sepsis.9,22,23

Malondialdehyde is an end product of lipid peroxidation and was used to evaluate the severity of lipid peroxidation and quantified to allow an estimation of the aggression by reactive oxygen species (ROS).24,25 In addition, MDA level is a critical indicator of tissue damage. The results showed that the MDA levels in both samples from the second cm and fifth cm of the skin flap in the NAC group were significantly lower than of the control group. Also, since ischemia led to high levels of MDA in the skin samples, NAC reversed this undesirable action, reducing the MDA levels and confirming its antioxidant capacity. Although the MDA level is an indicator of free radicals, the levels of SOD indirectly reflect the body’s ability to eliminate free radicals and also plays an important role on the oxidation balance.26-28 In this study, SOD levels in the NAC group were significantly higher than the levels of control group. The percentage area of surviving flap correlated with high SOD levels in the NAC group, suggesting that NAC can improve flap survival by increasing the level of SOD in the flap. A significant increase in macrophage and fibroblast activity, and mononuclear cell infiltration in the NAC group was also found. This finding supports NAC’s proliferative effect, suggesting a protective effect against the vascular dysfunction by reducing ROS.29

N-acetylcysteine is a well known antioxidant agent, which is used extensively in pneumology with its low toxicity. N-acetylcysteine inhibits both the apoptotic process induced by ROS, and has an experimental protective effect against the vascular dysfunction, by reducing the ROS.30 Inadequate blood flow may induce chronic lesions to be formed, related to the stage of ischemia. Ischemia, caused by any reason, leads to increase of ROS that damage the tissues. Increased ROS triggers white blood cell infiltration and high metalloprotein levels as well as deficiency of protein synthesis in tissues. In a study, it has been showed that NAC provides a redox medium for fibroblasts, and also increases the fibroblast proliferation.30 N-acetylcysteine's protective effects on hepatic, renal, and intestinal injuries have been shown in several experimental studies.11,12,21,31 N-acetylcysteine may also improve the microcirculation, as reduced thiols are required for in vivo expression of nitric oxide, a vital mediator of organ perfusion, which is readily inactivated by ROS. Moreover, various studies have shown the important effects of NAC in microcircular blood flow and tissue oxygenation.32 Furthermore, NAC has beneficial effects on the inflammation process, such as suppression of cytokine expression, inhibition of adhesion molecule expression, and inhibition of nuclear factor kappa B.33-35 Its anti-inflammatory potential encompasses inhibition of neutrophil chemotaxis, activation and aggregation, suppression of macrophage activation, inhibition of leukocyte-endothelial cell adhesion, and attenuation of the release of tumor necrosis factor-α.36-38

The data in this study demonstrates that NAC treatment exerts an important protective effect against flap necrosis. Moreover, the present study provides evidence that NAC reduces the flap necrosis, morphological injury, neutrophil infiltration, and lipid peroxidation. Several

Keypoints

- N-acetylcysteine (NAC) is a well known antioxidant agent, used extensively in pneumology with its low toxicity.
- NAC has beneficial effects on the inflammation process, such as suppression of cytokine expression, inhibition of adhesion molecule expression, and inhibition of nuclear factor kappa B.33-35
- Its anti-inflammatory potential encompasses inhibition of neutrophil chemotaxis, activation and aggregation, suppression of macrophage activation, inhibition of leukocyte-endothelial cell adhesion, and attenuation of the release of tumor necrosis factor-α.36-38

The data in this study demonstrates that NAC treatment exerts an important protective effect against flap necrosis. Moreover, the present study provides evidence that NAC reduces the flap necrosis, morphological injury, neutrophil infiltration, and lipid peroxidation. Several
studies have pointed out similar results in other organs such as lung, liver, and intestine or in conditions like sepsis.\textsuperscript{31,12,22,31,39} These results indicate that the beneficial effects of NAC are related to its action on microcirculatory blood flow and tissue oxygenation. In addition, lower levels of MDA, which is the product of lipid peroxidation, suggests that NAC treatment has the capacity to abolish the lipid peroxidation products in surgical flaps.

Conclusion

Flap necrosis, which is a complication of flap surgery, can be prevented with NAC treatment due to its antioxidant effects, and the fact that it maintains better results with improved flap survival. N-acetylcysteine is also useful for its safety and cost-effectiveness as a therapeutic agent.

References


23. Harrison PM, Wendon JA, Gimson AE, Alexander GJ, Wil...


