The Protective Effect of Aminoguanidine on Random Pattern Skin Flap Survival: An Experimental Study in Rats

AMİNOGUANİDİNİN RANDOM PATERNLİ CİLT FLEBİ YAŞAYABİLİRLİĞİ ÜZERİNE ETKİSİ: SIÇANLARDA DENEYSEL ÇALIŞMA

Hakan AYDOĞAN, MD,[a] Ali GÜRLEK, MD,[a] Hakan PARLAKPINAR, MD,[b]
Nilay AYDOĞAN, MD,[c] Ahmet ACET, MD,[b]

Departments of [a]Plastic, Reconstructive and Aesthetic Surgery, [b]Pharmacology, [c]Institute of Health Sciences, Biochemistry, İnönü University Faculty of Medicine, MALATYA

Abstract

Objective: Distal flap necrosis resulting from ischemia is a serious problem, and increases the cost of treatment. Reactive oxygen radicals (ROS) play an important role in tissue injury and ischemia, and may lead to partial or complete flap necrosis. Aminoguanidine (AG), a potent antioxidant, prevents ROS formation and lipid peroxidation. Besides, AG inhibits inducible nitric oxide synthase (iNOS) leading to decreased generation of nitric oxide (NO).

Material and Methods: Rats were randomly divided into three groups: Control, flap elevated saline group, and AG treated group. A caudally based rectangular flap, 3 x 10-cm was elevated on the back of the rats. Flap viability was evaluated 7 days after the initial operation, measuring necrotic areas and total flap areas by computer-assisted planimetry. Malondialdehyde (MDA), NO, glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels were measured in flap skin tissue to observe the effects of AG.

Results: Rate of flap necrosis and MDA, NO levels were higher in the saline group compared to the control group, while GSH, GSH-Px, and SOD enzyme activities were reduced. AG administration reduced lipid peroxidation, NO generation and increased GSH, GSH-Px, SOD enzyme activities. Furthermore, it significantly reduced the rate of flap necrosis when compared with the saline group.

Conclusion: We believe that AG, a potent antioxidant and iNOS inhibitor, has beneficial effects to improve skin flap viability when distal flap necrosis is a potential complication in longer flaps.

Key Words: Aminoguanidine; surgical flaps; reactive oxygen species; malondialdehyde

Özet

Amaç: İskemiye bağlı distal flep nekrozu tedavi masrafını arttıran ciddi bir sorundur. Doku hasarında ve iskemide önemli rol oynayan serbest oksijen radikalleri kısmi veya total flep nekrozuna neden olabilirler. Aminoguanidin (AG), potansiyel bir antioksidan olup serbest oksijen radikali oluşumunu ve lipid peroksidasyonunu engellemektedir. Ayrıca, AG indüklenebilir nitrik oksit sentazı (iNOS) inhibe ederek nitrik oksit (NO) oluşumunu azaltmaktadır.

Gereç ve Yöntemler: Bu çalışmada sıçanlar rastgele 3 gruba bölündü: Kontrol grubu, flep kaldırılan salin grubu ve AG uygulanan grup. 3 x 10 cm ebatlarında kaudal bazlı dikdörtgen flep sıçanların sırtından kaldırıldı. Flep yaşayabilirliği 7. gün değerlendirildi. Nekroz alanı bilgisayar destekli planimetrik yöntemle hesaplandı. Flep cilt dokusundan alınan doku örneklerinde malondialdehit (MDA), NO, glutatyon (GSH), glutatyon peroksidaz (GSH-Px) ve süperoksid dismutaz (SOD) düzeylerine bakıldı.

Bulgular: Flep nekroz oranları ve MDA, NO düzeyleri salin grubunda kontrol grubuna göre daha yüksek bulundu. GSH, GSH-Px ve SOD düzeyleri ise daha düşük bulundu. AG uygulanması lipid peroksidasyonunu ve NO oluşumunu azaltırken, GSH, GSH-Px ve SOD enzim aktivitelerini arttırdı. Ayrıca, AG uygulaması flep nekroz oranını salin grubuna göre istatistiksel olarak anlamlı düzeyde azalttı.

Sonuç: Potansiyel bir antioksidan ve iNOS inhibitörü olan AG'nin uzun fleplerin distalinde nekroz oluşumunu azaltacağını düşünmekteyiz.

Anahtar Kelimeler: Aminoguanidin; cerrahi flep; serbest oksijen radikalleri; malondialdehit

Turkiye Klinikleri J Med Sci 2007, 27:36-43

Geliş Tarihi/*Received:* 07.05.2006 **Kabul Tarihi/*Accepted:*** 12.10.2006

Yazışma Adresi/*Correspondence:* Hakan AYDOĞAN, MD
İnönü University Faculty of Medicine,
Department of Plastic, Reconstructive and
Aesthetic Surgery, MALATYA
haydogan@inonu.edu.tr

Skin flap necrosis is still a controversy in the field of plastic and reconstructive surgery, although mechanisms of flap necrosis were investigated. Skin flap necrosis results from alterations in the hemodynamics of the flap:

Insufficient arterial flow, inadequate venous drainage, or combination of the two. An adequate blood supply is crucial for survival of these flaps, and any injury to flap vascularity or a too-risky flap design causes ischemia and may lead to partial or complete flap necrosis.[1] To increase skin flap viability, many pharmacologic agents, such as sympatholytics, vasodilators, calcium channel blockers, hemorheological agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free oxygen radical scavengers have been utilized.[2]

One leading mechanism of skin flap necrosis is believed to involve reactive oxygen radical species (ROS) generation related to tissue ischemia-reperfusion (I/R) injury.[3,4] Also, excessive iNOS production plays an important role in flap injury. NO is generated in biological systems from L-arginine, O_2, and nicotinamide adenine dinucleotide phosphate (NADPH) by a group of enzyme-denominated nitric oxide synthases (NOSs) and an iNOS originally described in macrophages.[5] Local oxidative stress induces excessive production of NO.[6] Based on this relationship, treatment of the flaps with exogenous SOD, catalase, allopurinol, vitamin C, glucocorticoids, anti-inflammatory agents (etodolac and etofenamate), heparin, and deferoxamine significantly increase survival.[7-15]

To date, there is no study that investigates the effect of AG on flap survival. AG, a selective inhibitor of iNOS is structurally similar to L-arginine (the substrate for NO). It inhibits NO formation, and interferes with peroxynitrite ($ONOO^-$).[16,17] Previous studies pointed to the beneficial antioxidant effects of AG and scavenger effects of $ONOO^-$, which is a reactive oxidant produced from NO and superoxide (O^-) in various forms of tissue injury.[18-20] It was also shown to possess antioxidant potential and decrease the severity of pathophysiological changes attributed to excess NO production.[18,21] Additionally, Courderot-Masuyer et al. demonstrated that AG was a free radical scavenger against O^- and hydroxyl radicals (OH^-).[22] They revealed it to be an effective OH^- scavenger, by using electron paramagnetic resonance.

Since iNOS production and oxyradicals, especially $ONOO^-$ generation are the main causes of skin flap I/R injury, this experimental study was designed to investigate the effects of AG, a highly effective antioxidant and free radical scavenger, on the survival of random pattern skin flap in a rat model.

Materials and Methods

Experimental conditions. Female Wistar rats weighing 150-200 g were housed in temperature (21 ± 2°C) and humidity (60 ± 5%) controlled room in which a 12:12 hr light:dark cycle was maintained. Twenty-four female Wistar rats were used, and randomly divided into three groups (n= 8 rats each group): The control group (Group 1), saline group (Group 2), and AG treated group (Group 3). Flap was elevated in the latter two groups but not in the control group to determine the effects of flap elevation. The rats were anesthetized with intraperitoneal (i.p.) injections of a mixture composed of ketamine (50 mg/kg) and xylazine (5 mg/kg). The surgeons were blinded to the treatment groups. Each animal's back was shaved and sterile technique was employed in all surgical procedures. The animals were placed in a prone position and a caudally based rectangular flap, 3 x 10 cm was drawn onto the backs of the rats, according to the method described by Khouri and colleagues.[23] All the rats were sacrificed with intracardiac injections of pentobarbital, after the observations were completed.

Surgical Technique. Asepsis was maintained by providing a local sterile environment. A dorsal random pattern skin flap with a size of 10 x 3 cm was elevated on the dorsal trunk of the rats with meticulous hemostasis; the flap was sutured back into its original place (Figure 1 a). Morbidity and mortality were not observed during our experimental study. Saline was administered i.p. 0.2 ml/day to the control and saline groups for 6 days. One hour before flap elevation, AG, a selective iNOS inhibitor with antioxidant property, was administrated 100 mg/kg/day; administration continued for 6 days. AG (Aminoguanidine Hemisulfate Salt; Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) was dissolved in saline (0.9% NaCl wt/vol) to obtain a final concentration of

100 mg/mL which was determined according to relevant literature.[24] Intraperitoneal route was selected due to its ease of application and accuracy of dosing in rats.

Flap viability was evaluated 7 days after the initial operation, at which time a certain amount of necrosis in the distal part of all dorsal flaps was noted.[25,26] The evaluators were blinded to the treatment groups. All groups were photographed (Figure 1 b, c) for demonstration and then, the necrotic skin (defined by the necrotic skin borders) and total flap (defined by the surgical borders) areas were delineated on to an acetate sheet; surface areas were calculated (in square centimeters) by using computer-assisted planimetry. The necrotic surface area was divided by the total flap area, and the results were expressed as percentages of skin necrosis. Skin biopsy was taken from an area 3-4 cm proximal of the flap to determine the levels of MDA, NO and GSH, GSH-Px, SOD enzyme activities.[26]

All experiments in this study were performed in accordance with the guidelines for Animal Research from the National Institutes of Health and were approved by the Committee on Animal Research at İnönü University, Malatya, Turkey.

Biochemical determination. One hundred milligrams (mg) of frozen flap tissue biopsy specimens, cut into pieces with blades on dry ice, were homogenized in 1.15% KCl buffer (1:9, w/v) using a manual glass homogenizer for approximately 5 minutes and flushed by centrifugation for approximately 10 seconds to remove large debris. The supernatant was used for analysis.

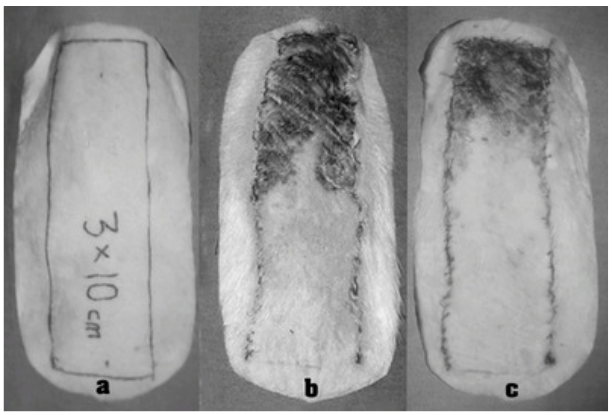


Figure 1. a) Design of our standard rat dorsal skin flap elevation technique.
b) Flap elevated saline group; notice the tissue necrosis.
c) Flap elevated group was treated with i.p. AG 100 mg/kg/day; a significant enhancement of flap survival in this group.

The MDA content of homogenates was determined spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances.[27] Results are expressed as nmol/g tissue.

Since tissue nitrite (NO_2^-) and nitrate (NO_3^-) levels may be used to estimate NO production, we measured the concentration of these stable NO oxidative metabolites. Quantization of NO_2^- and NO_3^- was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of NO_2^- with a mixture of naphthlethylenediamine and sulfanilamide.[28] Results are expressed as μmol/g tissue.

GSH was determined by the spectrophotometric method, which was based on the use of Elman's reagent.[29] Results were expressed as nmol/mg tissue.

GSH-Px activity was measured by the method of Paglia and Valentina.[30] In the presence of GSH reductase and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. GSH-Px activity was expressed as U/g protein.

SOD enzyme activity determination was based on the production of H_2O_2, from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium as previously described.[31] The product was evaluated spectrophotometrically. Results were expressed as U/g protein.

Statistical analysis

Flap tissue MDA, NO, GSH, GSH-Px and SOD levels were analyzed by using one-way ANOVA. Post-hoc comparisons were done using Tukey's test. Infarct size/flap size ratio was analyzed by Student's t-test. Differences were considered significant for $p< 0.05$. All results wee expressed as mean ± SEM.

Results

Evaluation of tissue enzymes. Figure 2 summarizes the data of MDA, NO, GSH, GSH-Px and SOD levels obtained from the skin tissues of rats in all groups.

MDA, the end product of lipid peroxidation, and NO levels were higher in the saline group compared to the control group while GSH, GSH-Px, SOD enzyme activities were significantly reduced.

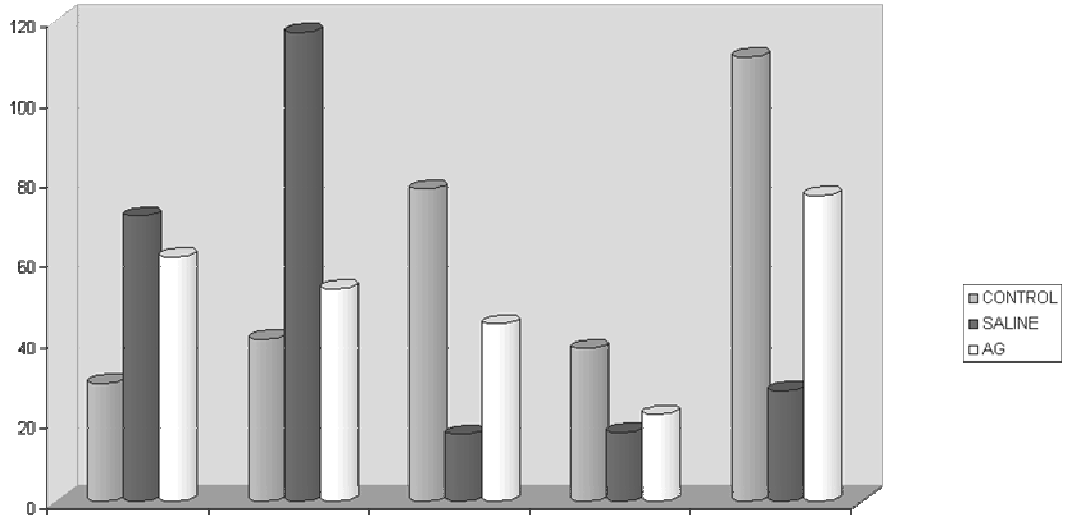
AG administration significantly reduced lipid peroxidation, NO generation and increased GSH, GSH-Px and SOD enzyme activities when compared with the saline group.

Gross Observations. No rats died during the experimental study. The regions of survival and necrosis were clearly demarcated in every flap since the flaps did not shrink on post-operative day 7. The surviving skin appeared pink-white, tender, and normal in its texture and it bled when cut, whereas the necrotic skin was black, rigid and did not bleed (Figure 1 a-c).

Evaluation of necrotic area/total flap area ratio. Figure 3 presents the summary of necrotic area/flap area ratio. Saline group caused a significant increase in necrotic area expressed as the percentage of risk zone (43.14 ± 4.10). AG administration significantly reduced the necrotic area (30.00 ± 3.57) ($p< 0.05$.).

Discussion

Random pattern flaps are widely used for the closure of skin and subcutaneous defects since flap elevation and transfer are technically simple. However, due to the unpredictable circulation of these flaps, partial flap necrosis is often encountered in the distal portion of the flap.[32] In the distal region of random pattern skin flap, cutaneous blood flow decreases initially, but increases significantly 24 hours after flap elevation.[33] The distal region of random pattern flap represents an incomplete ischemic state with tissue damage resulting from oxygen derived free radicals.[3] An adequate blood supply is crucial for the survival of these flaps, and any injury to flap vascularity or a too-risky flap



	MDA (nmol/g tissue)	NO (µmol/g tissue)	GSH (10xnmol/mg tissue)	GSH-Px (1/10xU/g protein)	SOD (U/g protein)
CONTROL	29,44	40,39	77,9	38,118	110,81
SALINE	71,14 [a]	116,9 [a]	16,7 [a]	17,158 [a]	27,57 [a]
AG	60,99 [b]	52,91 [b]	44,2 [b]	21,653 [b]	76,28 [b]

Figure 2. The effects of aminoguanidine (AG) on tissue content.
a: $p< 0.05$ vs control group, b: $p< 0.05$ vs saline group.

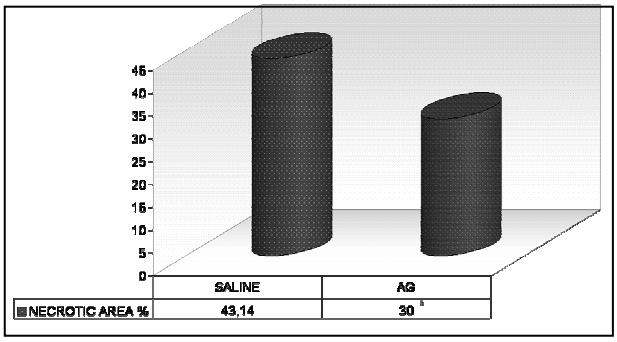


Figure 3. The effects of AG administration on infarct size/flap size ratio in flap elevated 2 groups. a: $p < 0.05$ vs. saline

design or I/R injury may lead to partial or complete flap necrosis.[34]

One leading mechanism of skin flap necrosis is believed to involve ROS generation related to tissue I/R injury.[3,4] Although ROS were implicated in a wide range of biological functions, they may have both beneficial and highly toxic effects on cellular homeostasis.[35] Several conditions are known to disturb the balance between the production of ROS and cellular defenses resulting in dysfunction and cellular destruction. An imbalance between pro and antioxidant factors plays an important role in many disease processes including I/R injury pathogenesis.[36] The formation of oxygen free radicals begins when oxygen is reintroduced to tissues after ischemia. In other words, the fresh supply of oxygen, which accompanies reperfusion, is necessary for the production of oxygen free radicals, which are toxic to all biologic substances.[37]

The effect of NO on the microcirculation in the periphery of a flap remains unclear; besides, its effect on flap survival is unknown because NO has a dual action. NO, on one hand, relaxes vascular tone, which is potentially protective against ischemia-induced flap necrosis and on the other hand, in the presence of O^- produces a powerful oxidant, $ONOO^-$, that may be cytotoxic to flap tissue.[38] NO is generated in biological systems from L-arginine, O_2, and NADPH by a group of enzymes denominated NOS that include two constitutive isoforms (endothelial and neuronal) and an iNOS originally described in macrophages.[5] Excessive iNOS production plays an important role in flap injury.[39] Plasma levels of NO are altered during experimental and clinical ischemia, hypoxia, sepsis and bacteremia, suggesting that NO may mediate important responses of the regional vascular beds in such conditions.[40]

Previous studies have shown NO-mediated modulation of free radical generation from polymorphonuclear leukocytes (PMNs), following hypoxic-reoxygenation as well as in the normoxic cells.[41] Also, Willemart et al. demonstrated that vasoconstriction, edema formation, accumulation and activation of leukocytes which is related to production of ROS contributed to formation of skin flap necrosis.[42]

AG acts as an antioxidant and free radical scavenger, preventing ROS formation and lipid peroxidation in cells and tissues. During the last decade, two important effects of AG were discovered, which caused this molecule to attract a great deal of interest. First, AG inhibits in vitro and in vivo formation of highly reactive advanced glycosylation end products (AGEs) associated with the pathogenesis of secondary complications of diabetes.[43] Second, AG inhibits NOS, particularly the iNOS isoform which is associated with production of large quantities of NOS, in response to various stimulants, e.g. cytokines, rendering AG an important pharmacological tool. AG is endowed with many other activities that together account for its beneficial effects. AG inhibits diamine oxidase, which catalyzes degradation of biologically active diamines such as histamine and putrescine.[44]

However, to date there is no study that investigates any effect of AG on flap survival. The protective effects of AG were previously addressed in other models of cell damage induced by drugs.[45] The beneficial effects of AG in various experimental models of inflammation have also been reported.[46] Previous studies pointed to the beneficial antioxidant, and $ONOO^-$ scavenger effects of AG.[18,19] Recently, Al-Shabanah et al. showed that AG protected mice against hepatotoxicity induced by carbon tetrachloride and also Mansour et al. reported that AG protected against nephrotoxicity

induced by cisplatin in rats.[24,47] Mostafa et al. showed that AG prevented the cardiotoxic effects of chronic doxorubicin administration.[48] Yildiz et al. also found that AG had direct scavenging activities against.[49] Finally, Giardino et al. reported that AG acted as an antioxidant in vivo, preventing ROS formation and lipid peroxidation in cells and tissues preventing oxidant-induced apoptosis.[50]

Based on this relationship, the present study was planned to investigate the possible protective effect of AG, a selective iNOS inhibitor and scavenger of oxyradicals, on ischemia related necrosis in rat random pattern skin flap. Since many studies are present concerning the role of ROS in the pathophysiology of flap necrosis, we decided to determine the levels of MDA, a lipid peroxidation end product, NO generation and GSH, GSH-Px and SOD enzyme activities in extracted skin tissue.[3,4]

In our study, biochemical evaluation of MDA and NO levels is correlated with previous literature findings (Table I). Flap elevation significantly increased MDA and NO levels. In addition, AG administration significantly reduced lipid peroxidation and NO generation. The beneficial effects of AG probably include its iNOS inhibitor, antioxidant, free radical scavenger and protecting lipid peroxidation effects. Although this protective mechanism is not clear, AG may directly eliminate free oxygen radicals such as $ONOO^-$ or directly increase the antioxidant enzyme activity and prevent the inhibition of these enzymes. Besides, inhibition of iNOS may contribute to this beneficial effect. The results of the present study with AG pre-treatment confirm the findings of Jakus et al. who demonstrated that AG had the potential to inhibit MDA in vitro in erythrocytes.[51]

Lipid peroxidation as well as altered levels of some endogenous scavengers are taken as indirect in vivo reliable indices for the contribution of free radical generation and in turn oxidative stress.[52] It was already demonstrated that depletion of GSH preceded the induction of lipid peroxidation.[53] Lipid hydroperoxides may be reduced and detoxified to lipid alcohols and these reactions are catalyzed by GSH-Px oxidizing GSH to GSSG.[54,55]

In the present study, flap elevation significantly reduced GSH, GSH-Px and SOD enzyme activities according to the control group. AG administration had a significantly beneficial effect on these enzymes (Figure 2).

Considering the reduced oxidative damage due to AG treatment, all investigators attributed protective actions of AG to its iNOS inhibitor, free radical scavenger and preventing ROS formation activity. This data is consistent with our results that AG administration reduced MDA and NO levels and increased GSH, GSH-Px, SOD enzyme activities. In addition, AG administration significantly reduced the ratio of skin flap necrosis, which was determined by using planimetry and supported by photography (Figure 1).

These findings strongly suggest that AG is important in preventing ischemic injury due to random pattern dorsal skin flap tissue. Based on the reduction of lipid peroxidation and NO generation by AG, which yielded parallel planimetric results, it is important to inhibit lipid peroxidation to prevent necrosis of skin flap (Figure 3).

In conclusion, we believed that the AG had beneficial effects to improve skin flap viability when distal flap necrosis is a potential complication of longer flaps. However, further investigations with larger animal models are needed to determine the optimal dose, effects of prolonged use and clarification of mechanism of action as well as new routes of administration such as topical application for the protective effect of AG on flap survival.

REFERENCES

1. Morris SF, Taylor GI. The time sequence of the delay phenomenon: When is a surgical delay effective? An experimental study. Plast Reconstr Surg 1995;95:526-33.
2. Karacaoglan N, Akbas H. Effect of parenteral pentoxifylline and topical nitroglycerin on skin flap survival. Otolaryngol Head Neck Surg 1999;120:272-4.
3. Akamatsu J, Ueda K, Tajima S, Nozawa M. Sulfatide elongates dorsal skin flap survival in rats. J Surg Res 2000;92:36-9.
4. Askar I, Bozkurt M. Protective effects of immunosuppressants and steroids against ischemia-reperfusion injury in cremaster muscle flap at microcirculatory level. Microsurgery 2002;22:361-6.

5. Arima H, Nishimoto Y, Motoyama K, Hirayama F, Uekama K. Inhibitory effects of novel hydrophilic cyclodextrin derivatives on nitric oxide production in macrophages stimulated with lipopolysaccharide. Pharm Res 2001;18:1167-73.
6. Tanaka Y, Shimizu H, Sato N, Mori M, Shimomura Y. Involvement of spontaneous nitric oxide production in the diabetogenic action of streptozotocin. Pharmacology 1995;50:69-73.
7. Sagi A, Ferder M, Levens D, Strauch B. Improved survival of island flaps after prolonged ischemia by perfusion with superoxide dismutase. Plast Reconstr Surg 1986;77:639-44.
8. Hardy SC, Homer-Vanniasinkam S, Gough MJ. Effect of free radical scavenging on skeletal muscle blood flow during postischaemic reperfusion. Br J Surg 1992;79:1289-92.
9. Im MJ, Manson PN, Bulkley GB, Hoopes JE. Effects of superoxide dismutase and allopurinol on the survival of acute island skin flaps. Ann Surg 1985;201:357-9.
10. Zaccaria A, Weinzweig N, Yoshitake M, Matsuda T, Cohen M. Vitamin C reduces ischemia-reperfusion injury in a rat epigastric island skin flap model. Ann Plast Surg 1994;33:620-3.
11. Tang-Liu DD, Neff J, Zolezio H, Sandri R. Percutaneous and systemic disposition of hexamethylene lauramide and its penetration enhancement effect on hydrocortisone in a rat sandwich skin-flap model. Pharm Res 1988;5:477-81.
12. Askar I, Saray A, Gurlek A, Sevin K, Sabuncuoglu BT. Effects of some pharmacological agents on the survival of unipedicled venous flaps: An experimental study. Microsurgery 2001;21:350-6.
13. Wright JG, Kerr JC, Valeri CR, Hobson RW 2nd. Heparin decreases ischemia-reperfusion injury in isolated canine gracilis model. Arch Surg 1988;123:470-2.
14. Gurlek A, Schusterman MA, Evans GR, Amin AA, Wang B, Gherardini G. Venous flap ischemia: Microcirculatory changes in experimental flaps in a rabbit model. J Reconstr Microsurg 1998;14:121-6.
15. Morris SF, Pang CY, Lofchy NM, et al. Deferoxamine attenuates ischemia-induced reperfusion injury in the skin and muscle of myocutaneous flaps in the pig. Plast Reconstr Surg 1993;92:120-32.
16. Misko TP, Moore WM, Kasten TP, et al. Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. Eur J Pharmacol 1993;16:119-25.
17. Suzuki N, Sakamoto A, Ogawa R. Effect of L-canavanine, an inhibitor of inducible nitric oxide synthase, on myocardial dysfunction during septic shock. J Nippon Med Sch 2002;69:13-8.
18. Ihm SH, Yoo HJ, Park SW, Ihm J. Effect of aminoguanidine on lipid peroxidation in streptozotocin-induced diabetic rats. Metabolism 1999;48:1141-5.
19. Szabo C, Ferrer-Sueta G, Zingarelli B, Southan GJ, Salzman AL, Radi R. Mercaptoethylguanidine and guanidine inhibitors of nitric-oxide synthase react with peroxynitrite and protect against peroxynitrite-induced oxidative damage. J Biol Chem 1997;272:9030-6.
20. Pacher P, Liaudet L, Bai P, et al. Potent metalloporphyrin peroxynitrite decomposition catalyst protects against the development of doxorubicin-induced cardiac dysfunction. Circulation 2003;107:896-904.
21. Corbett JA, Tilton RG, Chang K, et al. Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. Diabetes 1992;41:552-6.
22. Courderot-Masuyer C, Dalloz F, Maupoil V, Rochette L. Antioxidant properties of aminoguanidine. Fundam Clin Pharmacol 1999;13:535-40.
23. Khouri RK, Angel MF, Edstrom LE. Standardizing the dorsal rat flap. Surg Forum 1986;37:590-4.
24. Mansour MA, Mostafa AM, Nagi MN, Khattab MM, Al-Shabanah OA. Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp Biochem Physiol C Toxicol Pharmacol 2002;132:123-8.
25. Huemer GM, Wechselberger G, Otto-Schoeller A, Gurunluoglu R, Piza-Katzer H, Schoeller T. Improved dorsal random-pattern skin flap survival in rats with a topically applied combination of nonivamide and nicoboxil. Plast Reconstr Surg 2003;111:1207-11.
26. Gurlek A, Aydogan H, Parlakpinar H, et al. Protective effect of melatonin on random pattern skin flap necrosis in pinealectomized rat. J Pineal Res 2004;36:58-63.
27. Sahna E, Parlakpinar H, Ozturk F, Cigremis Y, Acet A. The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. Urol Res 2003;31:188-93.
28. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 1990;36(8 Pt 1):1440-3.
29. Parlakpinar H, Ozer MK, Sahna E, Vardi N, Cigremis Y, Acet A. Amikacin-induced acute renal injury in rats: Protective role of melatonin. J Pineal Res 2003;35:85-90.
30. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-69.
31. Aladag MA, Turkoz Y, Sahna E, Parlakpinar H, Gul M. The attenuation of vasospasm by using a sod mimetic after experimental subarachnoidal haemorrhage in rats. Acta Neurochir 2003;145:673-7.
32. Kerrigan CL. Skin flap failure: Pathophysiology. Plast Reconstr Surg 1983;72:766-77.
33. Suzuki S, Miyachi Y, Niwa Y, Isshiki N. Significance of reactive oxygen species in distal flap necrosis and its salvage with liposomal SOD. Br J Plast Surg 1989;42:559-64.
34. Gurlek A, Schusterman MA, Evans GR, Gherardini G. Blood flow and microcirculatory changes in an ischemia-reperfusion injury model: Experimental study in the rabbit. J Reconstr Microsurg 1997;13:345-9.
35. Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants and human disease: Where are we now? J Lab Clin Med 1992;119:598-620.
36. Yagmurca M, Fadillioglu E, Erdogan H, Ucar M, Sogut S, Irmak MK. Erdosteine prevents doxorubicin-induced cardiotoxicity in rats. Pharmacol Res 2003;48:377-82.
37. Darley-Usmar V, Halliwell B. Blood radicals: Reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. Pharm Res 1996;13:649-62.

38. Um SC, Suzuki S, Toyokuni S, et al. Involvement of nitric oxide in survival of random pattern skin flap. Plast Reconstr Surg 1998;101:785-92.
39. Kane AJ, Barker JE, Mitchell GM, et al. Inducible nitric oxide synthase (iNOS) activity promotes ischaemic skin flap survival. Br J Pharmacol 2001;132:1631-8.
40. Chen JC, Chen HM, Shyr MH, et al. Selective inhibition of inducible nitric oxide in ischemia-reperfusion of rat small intestine. J Formos Med Assoc 2000;99:213-8.
41. Sethi S, Sharma P, Dikshit M. Nitric oxide- and oxygen-derived free radical generation from control and lipopolysaccharide-treated rat polymorphonuclear leukocyte. Nitric Oxide 2001;5:482-93.
42. Willemart G, Knight KR, Morrison WA. Dexamethasone treatment prior to reperfusion improves the survival of skin flaps subjected to secondary venous ischaemia. Br J Plast Surg 1998;51:624-8.
43. Sugimoto K, Yagihashi S. Effects of aminoguanidine on structural alterations of microvessels in peripheral nerve of streptozotocin diabetic rats. Microvasc Res 1997;53:105-12.
44. Tilton RG, Chang K, Hasan KS, et al. Prevention of diabetic vascular dysfunction by guanidines. Inhibition of nitric oxide synthase versus advanced glycation end-product formation. Diabetes 1993;42:221-32.
45. Aoki K, Ohmori M, Takimoto M, Ota H, Yoshida T. Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species. Eur J Pharmacol 1997;336:43-9.
46. Shiomi M, Wakabayashi Y, Sano T, et al. Nitric oxide suppression reversibly attenuates mitochondrial dysfunction and cholestasis in endotoxemic rat liver. Hepatology 1998;27:108-15.
47. Al-Shabanah OA, Alam K, Nagi MN, Al-Rikabi AC, Al-Bekairi AM. Protective effect of aminoguanidine, a nitric oxide synthase inhibitor, against carbon tetrachloride induced hepatotoxicity in mice. Life Sci 2000;66: 265-70.
48. Mostafa AM, Nagi MN, Al Rikabi AC, Al-Shabanah OA, El-Kashef HA. Protective effect of aminoguanidine against cardiovascular toxicity of chronic doxorubicin treatment in rats. Res Commun Mol Pathol Pharmacol 1999;106:193-202.
49. Yildiz G, Demiryurek AT, Sahin-Erdemli I, Kanzik I. Comparison of antioxidant activities of aminoguanidine, methyl-guanidine and guanidine by luminol-enhanced chemiluminescence. Br J Pharmacol 1998;124:905-10.
50. Giardino I, Fard AK, Hatchell DL, Brownlee M. Aminoguanidine inhibits reactive oxygen species formation, lipid peroxidation, and oxidant-induced apoptosis. Diabetes 1998;47:1114-20.
51. Jakus V, Hrnciarova M, Carsky J, Krahulec B, Rietbrock N. Inhibition of nonenzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity. Life Sci 1999;65:1991-3.
52. El-Khatib AS, Moustafa AM, Abdel-Aziz AA, Al-Shabanah OA, El-Kashef HA. Effects of aminoguanidine and desferrioxamine on some vascular and biochemical changes associated with streptozotocin-induced hyperglycaemia in rats. Pharmacol Res 2001;43:233-40.
53. Raza M, Al-Bekairi AM, Ageel AM, Qureshi S. Biochemical basis of sodium valproate hepatotoxicity and renal tubular disorder: time dependence of peroxidative injury. Pharmacol Res 1997;35:153-7.
54. Raza M, Ahmad M, Gado A, Al-Shabanah OA. A comparison of hepatoprotective activities of aminoguanidine and N-acetylcysteine in rat against the toxic damage induced by azathioprine. Comp Biochem Physiol C Toxicol Pharmacol 2003;134:451-6.
55. Higo N, Hinz RS, Lau DT, Benet LZ, Guy RH. Cutaneous metabolism of nitroglycerin in vitro. I. Homogenized versus intact skin. Pharm Res 1992;9:187-90.