Improved Dorsal Random-Pattern Skin Flap Survival in Rats with a Topically Applied Combination of Nonivamide and Nicoboxil

Georg M. Huemer, M.D., Gottfried Wechselberger, M.D., Angela Otto-Schoeller, M.D., Raffi Gurunluoglu, M.D., Hildegunde Piza-Katzer, M.D., and Thomas Schoeller, M.D.

Innsbruck, Austria

The effects of a topically applied combination of nonivamide and nicoboxil in improving skin perfusion and preventing distal flap necrosis were tested in a random-pattern dorsal skin flap model. Forty male Wistar rats were randomized into two groups ($n = 20$), and a standardized dorsal random-pattern skin flap was raised on each rat. Animals in the experimental group were treated with the topically applied drug combination four times per day for 6 days, whereas in the control group only a placebo ointment was applied each time. Skin flap viability was evaluated on day 7, and the extent of skin flap necrosis was compared between the two groups. The topically applied combination of nonivamide and nicoboxil resulted in a statistically significant decrease in skin flap necrosis, compared with the control group (mean percentage of skin flap necrosis in the nonivamide/nicoboxil-treated group, 22.6 ± 6.0 percent; control group, 36.8 ± 4.3 percent; $p < 0.05$). The topical combination of nonivamide and nicoboxil was effective in reducing ischemic necrosis in failing random-pattern skin flaps in this rat model. The results of this study suggest that such a topical drug application might have significant effects in the reduction of ischemic necrosis in the distal parts of skin flaps, and this treatment might also have applications as prophylactic therapy for risky skin flaps. (*Plast. Reconstr. Surg.* 111: 1207, 2003.)

Random-pattern skin flaps are still widely used as a reconstructive option in plastic surgery. An adequate blood supply is crucial for survival of these flaps, and any injury to flap vascularity or a too-risky flap design may lead to partial or complete flap necrosis. To overcome this potential problem, investigators have focused on the improvement of flap survival, especially among high-risk patients.

Surgical delay, which is known to enhance flap viability, is an effective technique that is often used for this purpose.[1] However, this approach has the disadvantage of involving a two-stage procedure. Various pharmacological agents have been investigated for their efficacy in preventing or reversing skin flap ischemia. Sympatholytics, vasodilators, calcium channel blockers, hemorheological agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free radical scavengers are among the drugs thought to be beneficial for flap survival.[2–8] Although they are beneficial to some extent, the major drawback associated with these substances is the need for systemic application, at relatively high doses, to achieve significant improvements in flap survival, with increased possibilities of potential systemic side effects.

The unwanted side effects associated with systemic drug application could be reduced if a pharmacological agent could be used topically to enhance skin flap viability. Two substances (nonivamide and nicoboxil) that are known to be angiogenic[9–11] and to increase blood flow to tissues[12–15] when administered systemically are also commercially available as a Food and Drug Administration-approved ointment (Finalgon; Bender and Co., Vienna, Austria), which is often used for various indications (such as pain relief) among patients with sport injuries or rheumatic disease. On the basis of this knowledge, an experimental study was conducted to investigate whether topical application of this ointment, which includes a combination of

From the Department of Plastic and Reconstructive Surgery, Leopold Franzens University. Received for publication September 14, 2001; revised May 20, 2002.

DOI: 10.1097/01.PRS.0000047404.28025.C5

nonivamide and nicoboxil, would improve the survival of random-pattern skin flaps in a rat model.

Materials and Methods

Animals and Treatments

A total of 40 male Wistar rats, weighing between 250 and 350 g, were used in this experimental study. The rats were housed individually after surgery, to prevent skin flap cannibalism. Standard laboratory food for rats and water were provided ad libitum. All animals received humane care according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 86-23, revised 1985). The animals were randomized into two groups of 20 rats each. All rats were anesthetized with intraperitoneal injections of sodium pentobarbital (35 mg/kg body weight). The skin of the dorsal trunk was shaved with electric clippers and then prepared with Betadine (Purdue Frederick Co., Norwalk, Conn.) and alcohol. During the surgical procedure, asepsis was maintained by providing a local sterile environment. After adequate anesthesia depth was confirmed with the pinch flexion/withdrawal test, a random-pattern skin flap, which was a modification of the dorsal skin flap described by McFarlane et al.,[16] was designed on the dorsal trunk of the rat. Then, a skin flap measuring 2 × 6 cm was elevated with sharp dissection; care was taken to include the panniculus carnosus in the skin flap. With meticulous hemostasis, the flap was sutured back into place with 4-0 running nylon sutures. The rats were then randomized into two groups according to the type of topical treatment. In group I ($n = 20$), the surface of each dorsal flap was uniformly anointed with 1 g of topical carrier ointment containing 25 mg of nicoboxil and 4 mg of nonivamide, as well as 2 mg of sorbic acid as carrier (Finalgon). Topical application of this ointment was repeated every 6 hours for 6 days. Group II ($n = 20$) served as the control group, and the rats received a placebo ointment [Vaseline (Chesebrough-Ponds USA, Greenwich, Conn.) without vasoactive ingredients], in the same manner as the Finalgon-treated group. Vaseline was chosen as the control ointment because it does not have any known pharmacological properties. 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. On day 7, the rats were reanesthetized for evaluation of flap viability. First, digital images of the dorsal skin flaps were recorded on a computer. Then, the necrotic skin (defined by the necrotic skin borders) and total flap (defined by the surgical borders) areas were delineated, and surface areas were calculated (in square centimeters) by using a computer software program. The necrotic surface area was divided by the total flap area, and the results were expressed as percentages of skin necrosis. The animals were then killed with an overdose of intracardiacally administered ketamine (150 mg/kg).

Statistical Analyses

The results (expressed as mean ± SD) were analyzed by using the two-tailed t test. Statistical significance was presumed at $p < 0.05$.

Results

The 40 animals that underwent surgery survived throughout the follow-up period of the study. There was a certain amount of skin necrosis in the distal parts of all dorsal skin flaps, regardless of treatment. Hyperemia was consistently noted in the skin flaps of all rats treated with Finalgon ointment, compared with control rats.

The mean surviving skin flap area in the Finalgon-treated group was significantly larger than that in the control group (Finalgon, 9.3 ± 0.7 cm^2; range, 8.2 to 10.4 cm^2; control, 7.5 ± 0.5 cm^2; range, 7 to 9.0 cm^2; $p < 0.05$). Figure 1 presents five samples from each group, to demonstrate the differences in the extent of viable and necrotic skin areas between the two groups. There was a significant reduction in the amount of skin necrosis in the skin flaps treated with Finalgon ointment, compared with control flaps (22.6 ± 6.0 percent versus 36.8 ± 4.3 percent, $p < 0.05$). Numerical data on necrotic flap areas and percentages of necrosis for individual rats are presented in Table I.

Discussion

Unpredicted flap loss remains a serious problem after random-pattern skin flap surgery. Although the incidence of flap failure remains comparatively low, the resulting morbidity is almost always high in cases with this complication. In addition to surgical methods, several pharmacological measures are being tested for their possible use in improving flap

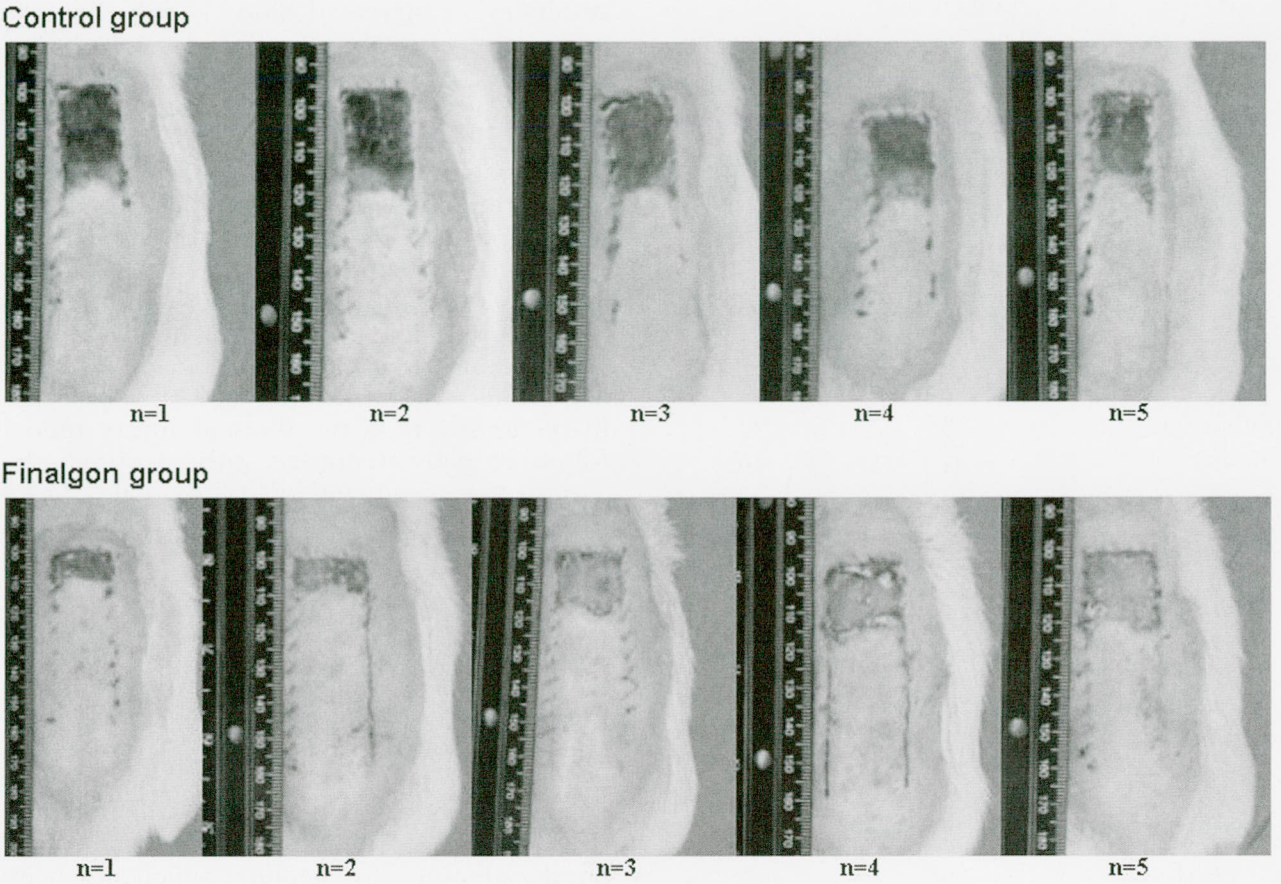

FIG. 1. Comparison of viable and necrotic skin regions in random-pattern dorsal skin flaps on day 7 in the control and Finalgon groups.

viability. However, the majority of experimental studies involved parenteral drug application, and only a few articles described topical uses for enhancing flap survival.

In this experimental study, a commercially available ointment with proven clinical safety was tested for its effect on the survival of random-pattern skin flaps in a rat model. Topical application was chosen as the mode of drug delivery, because we think that systemic delivery is associated with too many detrimental side effects and is thus of little use in salvaging local skin flaps in a clinical setting.

According to Rohrich et al.,[17] an ideal pharmacological agent for improving flap survival would have the following features: (1) clinical availability, (2) easy administration, (3) a high therapeutic index (safety), (4) reproducibility of effective results, (5) feasibility of only postoperative treatment, (6) cost-effectiveness, (7) known mechanism of action, (8) established bioavailability, and (9) protective effects on flap necrosis. To date, no substance has fulfilled all of the requirements and an ideal substance has not yet been found. However, we think that a topical agent would be preferable to a parenterally administered agent for the treatment of skin flaps, because such application would lead to effective therapeutic tissue levels without significant systemic drug distribution, with a high therapeutic index (safety). Furthermore, such an agent could be easily administered postoperatively (even as an outpatient treatment in clinical settings). The topically administered ointment used in this study seems to meet all of the noted requirements except for total prevention of flap necrosis (although this depends on the appropriateness of the chosen flap dimensions).

The use of the combination of nonivamide and nicoboxil as an ointment (Finalgon) significantly enhanced random-pattern skin flap survival in this experimental model. Nonivamide is a synthetic capsaicin analogue derived from the Spanish peppercorn, whereas nicoboxil is an ester of nicotinic acid.

The mechanism of action of the combination of these two agents seems to be threefold.

TABLE I
Necrotic Flap Areas and Percentages of Flap Necrosis in the Control and Finalgon Groups*

Rat No.	Necrotic Flap Area (cm^2)	% Necrosis
Control group		
1	5.0	41.7
2	5.0	41.4
3	4.6	36.0
4	5.0	41.7
5	3.6	30.4
6	4.6	38.5
7	4.7	39.5
8	3.6	30.4
9	4.9	40.7
10	3.0	25.0
11	4.3	36.2
12	4.5	37.5
13	4.4	36.4
14	4.8	40.0
15	4.4	37.0
16	4.7	39.2
17	4.4	36.4
18	3.9	32.4
19	4.7	39.4
20	4.4	37.8
Mean ± SD	4.4 ± 0.5	36.8 ± 4.3
Finalgon group		
1	3.8	31.7
2	3.3	27.8
3	1.7	14.3
4	3.0	24.7
5	2.8	23.7
6	1.6	13.3
7	1.6	13.3
8	3.1	25.7
9	3.7	30.2
10	3.4	28.2
11	2.5	21.0
12	2.7	22.2
13	3.5	29.5
14	1.9	15.3
15	1.8	14.7
16	3.2	26.8
17	3.5	29.0
18	2.3	19.5
19	2.5	20.8
20	2.6	22.0
Mean ± SD	2.7 ± 0.7	22.6 ± 6.0

* Control versus Finalgon, $p < 0.05$.

First, the nonivamide component of the ointment leads to arterial vasodilation[12–15] through a direct axonal reflex, thus opposing one of the main causes of flap failure, namely arterial insufficiency.[18] Nonivamide binds to the capsaicin-sensitive pain receptors of the skin, which leads to a release of substance P and other neuropeptides[19] that are known to promote the growth of different cell lines and to accelerate wound healing. The skin flap hyperemia that is observed after topical treatment can also be attributed to nociceptor stimulation, which subsequently elicits a cutaneovisceral reflex. Excitation of such cutaneovisceral reflex arcs results in an increased blood supply, because of spasmolysis in the skin flap circulation.

Another mechanism with a significant role in the enhancement of skin flap survival in this study involves stimulation of neoangiogenesis. Neoangiogenesis (the process of new capillary formation) is an essential part of the wound healing process that leads to increased tissue perfusion and thus increased flap survival. There is strong evidence in the literature that nicotinic acid and its ester nicoboxil have angiogenic properties.[9–11] Smith et al.[20] reported accelerated wound healing and increased capillary density in a rat thermal injury model following daily treatment with nicotinamide. Two studies conducted by Collins et al.[14,21] provided additional evidence that nicotinamide enhances flap survival through new capillary ingrowth.

A third mechanism involved in the enhancement of flap survival could be the amelioration of a metabolic derangement, such as the loss of nicotinamide coenzymes that occurs in ischemic portions of flaps.[22–24] Nicotinamide, which can be produced from the nicotinic acid ester nicoboxil by the enzyme nicotinamide deaminase,[25] replenishes the pool of nicotinamide adenine dinucleotide, which has been demonstrated to decrease the damage observed in ischemic heart preparations.[24,26] Nicotinamide adenine dinucleotide, the physiologically active form of nicotinic acid, plays a vital role in metabolism, as a coenzyme for a wide variety of proteins that catalyze oxidation-reduction reactions essential for tissue respiration.

It is not yet known exactly which mechanism is responsible for the distal necrosis observed in skin flaps. However, we think that it is a decided advantage to have a topical agent such as the ointment used in this study, which improves survival by targeting three possible causes of skin flap necrosis.

Conclusions

In this experimental study, we demonstrated increased skin flap survival following topical treatment with an ointment that contains a combination of nonivamide and nicoboxil. Although it was not totally preventive, the tested substance combination effectively reduced ischemic necrosis in random-pattern skin flaps. Because this ointment is safe, practical, noninvasive in use, inexpensive, and widely available, it seems to be appropriate for clinical applica-

tion in the field of plastic surgery. On the basis of our findings in this study, we think that this clinically approved ointment might have important therapeutic potential in improving skin flap survival in clinical settings. However, further studies are needed to better define optimal drug doses and application intervals.

Thomas Schoeller, M.D.
Department of Plastic and Reconstructive Surgery
Leopold Franzens University
Anichstrasse 35
6020 Innsbruck, Austria
Thomas.Schoeller@uibk.ac.at

ACKNOWLEDGMENT

This study was supported by the Ludwig Boltzmann Institute for Quality Control in Plastic and Reconstructive Surgery (Innsbruck, Austria).

REFERENCES

1. Morris, S. F., and Taylor, G. I. The time sequence of the delay phenomenon: When is surgical delay effective? An experimental study. *Plast. Reconstr. Surg.* 95: 526, 1995.
2. Kerrigan, C. L., and Daniel, R. K. Pharmacologic treatment of the failing skin flap. *Plast. Reconstr. Surg.* 70: 541, 1982.
3. Davis, R. E., Wachholz, J. H., Jassir, D., Perlyn, C. A., and Agrama, M. H. Comparison of topical anti-ischemic agents in the salvage of failing random-pattern skin flaps in rats. *Arch. Facial Plast. Surg.* 1: 27, 1999.
4. Emery, F. M., Kodey, T. R., Bomberger, R. A., and McGregor, D. B. The effect of nifedipine on skin-flap survival. *Plast. Reconstr. Surg.* 85: 61, 1990.
5. Shalom, A., Herbert, M., and Westreich, M. Effect of aspirin on random pattern flap survival in rats. *Eur. J. Plast. Surg.* 23: 21, 2000.
6. Miyawaki, T., Jackson, I. T., Bier, U. C., Andrus, L., Williams, F., and Bradford, M. The effect of capsaicin ointment on skin for the survival of a cutaneous flap. *Eur. J. Plast. Surg.* 24: 28, 2001.
7. Nakanishi, Y., Nakajima, T., Yoshimura, Y., Okamoto, Y., and Yamada, T. The transepidermal absorption of prostaglandin E_1 as a topical ointment: An experimental study of application over a rat skin flap. *Ann. Plast. Surg.* 40: 44, 1998.
8. Karacaoğlan, N., and Akbaş, H. Effect of parenteral pentoxifylline and topical nitroglycerin on skin flap survival. *Otolaryngol. Head Neck Surg.* 120: 272, 1999.
9. Folkman, J., and Klagsbrun, M. Angiogenic factors. *Science* 235: 442, 1987.
10. Folkman, J. Tumor angiogenesis. *Adv. Cancer Res.* 43: 175, 1985.
11. Kull, F. C., Jr., Brent, D. A., Parikh, I., and Cuatrecasas, P. Chemical identification of a tumor-derived angiogenic factor. *Science* 236: 843, 1987.
12. Lo, Y. C., Wu, J. R, Wu, S. N., and Chen, I. J. Glyceryl nonivamide: A capsaicin derivative with cardiac calcitonin gene-related peptide releasing, K^+ channel opening and vasorelaxant properties. *J. Pharmacol. Exp. Ther.* 281: 253, 1997.
13. Suzuki, T., Tomizawa, N., Kamata, R., et al. A possible role of nitric oxide formation in the vasodilatation of rabbit ear artery induced by a topically applied capsaicin analogue. *J. Vet. Med. Sci.* 60: 691, 1998.
14. Collins, T. M., Caimi, R., Lynch, P. R., et al. The effects of nicotinamide and hyperbaric oxygen on skin flap survival. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* 25: 5, 1991.
15. Weiner, M., and Van Eys, J. *Nicotinic Acid: Nutrient Cofactor Drug.* New York: Marcel Dekker, 1983.
16. McFarlane, R. M., DeYoung, G., and Henry, R. A. The design of a pedicle flap in the rat to study necrosis and its prevention. *Plast. Reconstr. Surg.* 35: 177, 1965.
17. Rohrich, R. J., Cherry, G. W., and Spira, M. Enhancement of skin-flap survival using nitroglycerin ointment. *Plast. Reconstr. Surg.* 73: 943, 1984.
18. Kerrigan, C. L. Skin flap failure: Pathophysiology. *Plast. Reconstr. Surg.* 72: 766, 1983.
19. Manzini, S., Perretti, F., De Benedetti, L., Pradelles, P., Maggi, C. A., and Geppetti, P. A comparison of bradykinin- and capsaicin-induced myocardial and coronary effects in isolated perfused heart of guinea pig: Involvement of substance P and calcitonin gene-related peptide release. *Br. J. Pharmacol.* 97: 303, 1989.
20. Smith, Y. R., Klitzman, B., Ellis, M. N., and Kull, F. C. The effects of nicotinamide on microvascular density and thermal injury in rats. *J. Surg. Res.* 47: 465, 1989.
21. Collins, T. M., Denish, A., Sheffield, J., Mitra, A., Stueber, K., and Smith, Y. R. Nicotinamide enhances skin flap survival. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* 23: 177, 1989.
22. Im, M. J., and Hoopes, J. E. Improved skin flap survival with nicotinic acid and nicotinamide in rats. *J. Surg. Res.* 47: 453, 1989.
23. Nunez, R., Calva, E., Briones, E., and Lopez-Soriano, F. Nicotinamide coenzymes in heart and coronary blood during myocardial infarction. *Am. J. Physiol.* 226: 73, 1974.
24. Klein, H. H., Schaper, J., Puschmann, S., Nienaber, C., Kreuzer, H., and Schaper, W. Loss of canine myocardial nicotinamide adenine dinucleotides determined the transition from reversible to irreversible ischemic damage of myocardial cells. *Basic Res. Cardiol.* 76: 612, 1981.
25. Gholson, R. K. The pyridine nucleotide cycle. *Nature* 212: 933, 1966.
26. Seifart, H. I., Delabar, V., and Siess, M. The influence of various precursors on the concentration of energy rich phosphates and pyridine nucleotides in cardiac tissue and its possible meaning for anoxic survival. *Basic Res. Cardiol.* 75: 57, 1980.