Comparison of Topical Anti-ischemic Agents in the Salvage of Failing Random-Pattern Skin Flaps in Rats

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Objective: To determine the efficacy of topical anti-ischemic drug therapy in the salvage of failing, random-pattern skin flaps.

Design: Prospective, randomized, placebo-controlled, therapeutic trial.

Setting: Academic medical center.

Subjects: Sixty-one adult male Sprague-Dawley rats.

Intervention: Each experimental rat underwent a caudally based random-pattern skin flap using the modified McFarlane technique. Rats were randomized to 1 of 6 treatment groups: topical nifedipine, topical trolamine salicylate, topical nitroglycerin, topical trolamine salicylate-nitroglycerin combination, topical nifedipine-trolamine salicylate-nitroglycerin combination, or inert carrier ointment (control). Treatment was initiated immediately following flap closure and continued every 6 hours for 7 days. At the end of the treatment period, animals were euthanized and flap survival was determined for each one.

Results: Topical anti-ischemic drug therapy resulted in a statistically significant reduction in ischemic flap necrosis for each drug (or combination) tested relative to the 44.2% mean necrosis observed in control animals. Treatment with the combination of topical nitroglycerin and topical trolamine salicylate resulted in the best salvage response (25.2% mean necrosis) with a statistically significant improvement in flap survival relative to both controls and nitroglycerin alone.

Conclusions: Topical anti-ischemic agents are effective in reducing ischemic necrosis of failing, random-pattern skin flaps in the rat model. Although nitroglycerin, trolamine salicylate, and nifedipine possess unique pharmacologic mechanisms of action, each drug produced a statistically significant improvement in flap survival. The results of this study suggest that topical drug therapy may play an important role in clinical salvage of the failing skin flap. Further studies are needed to explore the potential of combination drug therapy.

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Because of the growing prevalence of skin malignancy, reconstructive surgeons are confronted by an increasing number of complex skin defects. Most of these defects arise on the face where cosmetic concerns are of prime importance. Because random-pattern skin flaps typically enjoy excellent color, texture, and thickness match, they remain a valuable asset in facial reconstruction. Unfortunately, the susceptibility of these flaps to ischemic necrosis is difficult to predict owing to variations in flap design and intrinsic tissue vascularity. As a consequence, unforeseen skin flap necrosis remains a rare but serious complication of facial reconstruction. A safe, convenient, and cost-effective treatment for failing skin flaps is currently needed.

As the pathophysiologic characteristics of random-pattern skin flap necrosis become more clearly defined, pharmacologic treatment of flap ischemia has become an area of increasing interest. To date, numerous pharmacological agents have been examined for their ability to prevent or reverse skin flap ischemia. These drugs include sympatholytics, vasodilators, calcium channel blockers, hemorheologics, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free radical scavengers. Unfortunately, few of these drugs have proven unequivocally effective. Of those agents with proven experimental efficacy, most have been limited by undesirable adverse effects, high cost, or restricted availability. Moreover, to use many of these drugs, extensive preoperative treatment or direct vascular infusion is required, making their use in postoperative ischemic flap salvage either impractical or impossible. As a consequence, the ideal agent for salvage of the failing, random-pattern skin flap does not currently exist.

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MATERIALS AND METHODS

Sixty-one adult male Sprague-Dawley rats (weighing 250-275 mg) were used as experimental subjects in full accordance with the protocols established by the Institutional Animal Care and Use Committee (IACUC) at the University of Miami, in Florida. Isoflurane inhalational anesthesia was selected to approximate routine surgical conditions. Anesthetic induction was achieved with 5% isoflurane and subjects were maintained intraoperatively on a 1.5% isoflurane regimen administered through a snout cone by spontaneous ventilation.

Following induction, animals were shaved, prepared, and uniformly immobilized in the prone position. Restrictive collars were then placed to prevent unwanted movement during self-grooming. After adequate depth of anesthesia was confirmed using the pinch flexion/withdrawal test, caudally based, random-pattern skin flaps, each measuring 3 x 10 cm, were raised along the dorsal midline by sharp dissection. Flaps were designed according to the Khouri modification of the original McFarlane technique,33,34 and care was taken to elevate the panniculus carnosus with the overlying skin flap. After hemostasis was secured with electrocautery, the flaps were sutured in the native position using 4-0 interrupted silk sutures (Ethicon Inc, Somerville, NJ). To prevent postoperative flap cannibalization, all animals were returned to solitary housing following emergence from anesthesia. Subjects were allowed water and rat chow ad libitum throughout the study period, and acetaminophen with codeine was added to the drinking water for 5 days postoperatively.

Immediately following flap closure, each flap pedicle was uniformly coated with topical carrier ointment containing active drugs according to 1 of 6 randomized treatment groups. The control group received only inert (placebo) carrier ointment, while the remaining groups received either nifedipine (5 mg), trolamine salicylate (30 mg), nitroglycerin (30 mg), trolamine salicylate-nitroglycerin combination (30 mg each), or trolamine salicylate-nitroglycerin-nifedipine combination (30 mg, 30 mg, 5 mg, respectively). Care was taken to distribute ointment evenly along the entire flap and dosing was repeated every 6 hours for 7 days postoperatively.

At the conclusion of the study period, all of the animals were euthanized by rapid carbon dioxide asphyxia in accordance with approved IACUC protocols. Comparisons of flap survival were then determined for each cohort by calculating ratios of ischemic to healthy flap tissue using the acetate weight technique previously described.35 Data were statistically evaluated using 1-way analysis of variance (ANOVA) to detect differences between treatment groups. Pairwise comparisons were performed at the 95% confidence level using the least significant difference criterion.

Although the mechanisms for random-pattern skin flap ischemia have yet to be fully elucidated, surgically induced adrenergically mediated vasoconstriction is believed to play a major role in the reduction of nutrient capillary blood flow.2,7-9 Various pharmacologic agents that diminish sympathetic tone or promote arteriolar smooth muscle relaxation have been shown to improve skin flap perfusion and survival, lending support to this proposed pathophysiologic mechanism.10 One such group of vasoactive drugs is the calcium channel blockers. Nifedipine is a clinically available calcium channel blocker believed to block adrenergically mediated vasoconstriction by inhibiting calcium ion flux into vascular smooth muscle cells.10 Other potential benefits of calcium channel blockers, such as inhibition of platelet activation and cell membrane stabilization, may also contribute to improved skin flap survival.10,12

To date, 3 animal studies using oral nifedipine have demonstrated improved random-pattern skin flap survival with postoperative drug administration.10,13,14 Pal and coworkers13 noted a reduction in random-pattern skin flap necrosis from 37% to 12% in rats given 0.3 to 0.5 mg/kg per day of postoperative oral nifedipine. Only a slight additional benefit was observed when treatment was initiated preoperatively. Similarly, Bailet and coworkers10 showed a 25% reduction in ischemic flap necrosis in the rodent model when nifedipine administration was begun intraoperatively by intraperitoneal injection (0.75 mg/kg) and continued orally for 7 days postoperatively with rat chow containing 3000-ppm nifedipine.10 Pretreatment with oral nifedipine reduced necrosis further to 50% of controls. Hypotension was not reported in either experiment. Finally, Hira et al14 observed improved flap survival from 56% in the control group to 77% in animals treated with 1 mg/kg per day of postoperative oral nifedipine. Interestingly, in the same study, animals given much higher doses of oral nifedipine (10 mg/kg per day) also demonstrated an improved mean flap survival of 72%. The failure of this much higher dose to produce systemic hypotension and flap hypoperfusion is difficult to explain. Bailet and coworkers10 have theorized that other pharmacological properties of nifedipine, such as platelet deactivation and cell membrane stabilization, may have been responsible for the beneficial effects of nifedipine at higher doses.

In contrast to these reports, 2 additional studies have shown no beneficial effects of nifedipine on flap survival. Miller et al13 reported no improvement in random-pattern skin flap survival using pigs treated postoperatively with 1.5 mg/kg of oral nifedipine. However, the authors reported significant hypotension following nifedipine administration and concluded that drug-related hypotension may have negated any beneficial effects on flap survival. Similarly, Emery et al16 observed no improvement in flap survival in rats treated with 2.5 mg/kg of oral nifedipine. In their study, blood pressure monitoring was conducted in only a single test animal, and although hypotension was not observed at the 2.5-mg/kg dose, this dose has been previously shown to produce hypotension in normotensive rats.17 Thus, unrecognized hypotension may have also been a factor in this experiment. Moreover, Fuma9 has suggested that vasoconstricting agents contained within the drug vehicle (caffeine, theobromine, and theophylline) may have further negated any beneficial effects of nifedipine on flap survival.
From these data, it seems likely that nifedipine improves ischemic random-pattern skin flap survival if therapeutic tissue levels can be achieved without significant reductions in systemic blood pressure. Moreover, since nifedipine is safe, widely available, and inexpensive, it is potentially well suited to clinical use in ischemic flap salvage.

Another commonly recognized vasoactive drug that relaxes smooth muscle tone in peripheral arteries and veins is nitroglycerin. Nitroglycerin acts predominantly through venous dilation and is commonly prescribed in the treatment of angina pectoris due to coronary artery disease. The vasodilatory effect of nitroglycerin is believed to stem from the release of prostacyclin, a potent endogenous vasodilator found in vascular endothelium. In addition, nitroglycerin also appears to preferentially dilate spastic vessels within ischemic tissue. Recent animal studies have shown a reduction in random-pattern skin flap necrosis following treatment with nitroglycerin, suggesting a beneficial effect on nutrient blood flow. Like nifedipine, nitroglycerin is widely available, inexpensive, and generally well tolerated, making it potentially suitable for clinical use in flap salvage.

In addition to adrenergic vasoconstriction, another proposed mechanism for ischemic skin flap necrosis is microvascular compromise resulting from platelet aggregation. Surgically induced platelet aggregation is believed to stem from release of thromboxane A₂ (TXA₂), a platelet-derived prostaglandin that behaves as a potent vasoconstrictor and promoter of platelet aggregation. Under normal circumstances, TXA₂ is held in check by its more abundant antagonist, prostacyclin (PGI₂), which is selectively synthesized and released by vascular endothelium. However, following surgical trauma there seems to be an accelerated synthesis of TXA₂ and a corresponding reduction in PGI₂ synthesis, both of which lead to increased platelet aggregation.

Trolamine salicylate (Aspercreme) is a topical anti-inflammatory analgesic hydrolyzed to salicylic acid, a potent inhibitor of prostaglandin synthesis. Like other aspirin-containing compounds, trolamine salicylate is an irreversible inhibitor of platelet aggregation. Because experimental results using either nonspecific TXA₂ inhibitors or selective TXA₂ antagonists have shown improvements in random-pattern skin flap survival, trolamine salicylate may also prove efficacious in the salvage of failing random-pattern skin flaps. Like both nifedipine and nitroglycerin, trolamine salicylate is readily available, inexpensive, and generally well tolerated.

In an effort to enhance the target tissue response, reduce systemic adverse effects (such as hypotension), and avoid cumbersome intravascular drug delivery systems, we chose to evaluate the efficacy of anti-ischemic agents administered through topical application. Topical drug therapy enjoys several potential advantages over oral or intravascular methods of drug delivery. Foremost among these advantages is the direct targeting of ischemic tissue. Direct application of anti-ischemic drugs to the target tissue optimizes local drug concentrations while minimizing systemic drug distribution. Hypotension or other adverse effects produced by systemic drug distribution are thus reduced or avoided. Previous studies of salicylate levels following topical application of trolamine salicylate have confirmed intra-articular drug levels comparable to those achieved with oral aspirin administration, while serum levels remained hundreds of times lower. Studies using the adrenergic blocking agents phentolamine and phenoxybenzamine applied in a high-dose topical paste to random-pattern skin flaps in rats have shown increased postoperative flap survival relative to treatment with intramuscular injection. Despite the topical application of doses considerably higher than that recommended for parenteral use, animal mortality was not observed, suggesting that the normally potent adverse effects of phentolamine or phenoxybenzamine were significantly reduced by topical application. Animal experiments with topical nitroglycerin ointment have also demonstrated augmented random-pattern skin flap survival. Rohrich and coworkers observed statistically significant improvements in flap survival using 2% nitroglycerin ointment applied to skin flaps raised in both the rat and pig. Again, no lethal adverse effects were reported despite comparatively large topical doses. Similarly, Blomain et al studied random-pattern skin flap necrosis in pigs treated post-operatively for 4 days with 2% nitroglycerin ointment. Survival improved to 125% of controls in dorsally based flaps and 176% of controls in ventrally based flaps, and no lethal adverse effects were reported. Based on these studies, it appears that direct tissue targeting through topical drug application may improve ischemic flap survival while simultaneously minimizing dose-limiting adverse effects. Topical drug delivery also appears to be particularly well suited to skin flap ischemia where flap hypoperfusion often prevents effective intravascular drug distribution, particularly in the presence of co-existing hypotension.

In addition to improving the therapeutic ratio, topical drug administration is also convenient, cost-effective, and noninvasive. Because of its simplicity, topical administration is well suited to outpatient use, and drug administration may continue without interruption despite impaired or restricted oral intake. Finally, because nitroglycerin and trolamine salicylate are commercially available as topical ointments, and because nifedipine is largely lipophilic, all 3 drugs are likely to achieve therapeutic tissue levels via topical application. The purpose of this study is to establish the efficacy of anti-ischemic agents when administered through the transdermal route. The 3 drugs selected for this study, nitroglycerin, trolamine salicylate, and nifedipine, have widely differing mechanisms of action, and each drug has either a theoretical or proven potential for the salvage of ischemic random-pattern skin flaps. We have chosen to evaluate the efficacy of topical anti-ischemic therapy in the setting of postoperative flap salvage using an animal model. The model selected in this study has been previously standardized to produce consistent levels of ischemic flap necrosis in untreated (control) animals and serves as a statistically valid method for assessing the prevention of surgically induced ischemic flap changes.
RESULTS

Sixty-one animals were enrolled in the study and 59 animals were available for data collection at the conclusion of the study period. Two animals died intraoperatively from anesthetic overdose. Postmortem examination of all animals revealed no evidence of hematomas or seroma formation.

The mean percentage of flap necrosis was compared for each treatment group. Control animals treated with inert petrolatum ointment exhibited the highest percentage of flap necrosis (mean = 44.1% ± 1.6% SE). By comparison, all animals treated with topical anti-ischemic agents exhibited a statistically significant reduction in ischemic flap necrosis at the 95% confidence level (Figure). Treatment with topical nitroglycerin produced the weakest salvage response with 31.6% ± 3.1% flap necrosis. However, relative to control animals, this reduction in flap necrosis was statistically significant (P < .05). Similarly, in comparison to control subjects, both topical nifedipine and topical trolamine salicylate demonstrated an improved salvage response with 26.2% ± 1.5% and 26.2% ± 2.5% flap necrosis, respectively (P < .05). However, the trend toward reduction in flap necrosis relative to nitroglycerin was not statistically significant. Combining all 3 agents failed to improve the salvage response relative to any single agent alone, although the salvage response of 26.2% ± 1.5% flap necrosis was statistically significant relative to the control group (P < .05).

Overall, the best salvage response was produced from the combination of topical nitroglycerin and topical trolamine salicylate with 25.2% ± 1.9% flap necrosis. This reduction in flap necrosis was statistically significant relative to both the control group (P < .05) and the nitroglycerin treatment group (P < .05). In contrast, this combination failed to produce a statistically significant improvement over either the nifedipine or the trolamine salicylate treatment groups.

COMMENT

Because ischemic necrosis is difficult to predict, unforeseen flap loss remains a serious complication of random-pattern skin flap surgery. Although the incidence of flap failure remains comparatively low, the morbidity associated with flap necrosis has fueled the search for an effective drug therapy. To date, there is no ideal drug therapy for random-pattern skin flap necrosis.

Although some drugs have demonstrated experimental reductions in ischemic flap necrosis, these benefits have usually been offset by systemic adverse effects, high cost, or poor availability. In addition, administration of many of these agents requires lengthy pretreatment dosing schemes or invasive drug delivery systems for therapeutic success, limiting their effectiveness in the treatment of unexpected flap ischemia or acute skin trauma.

Moreover, since most random-pattern skin flap procedures are not associated with flap ischemia, it is difficult to justify the routine use of anti-ischemic prophylaxis for low-risk patients, particularly if drug therapy is associated with increased cost or significant adverse effects. Consequently, a safe, inexpensive salvage drug is currently needed.

To be most effective, the ideal salvage drug would need to prevent tissue necrosis even when drug administration was withheld pending the development of early ischemic signs or symptoms. Ideally, such a drug would also be well tolerated, cost-effective, readily available, and suitable for outpatient administration. Because such a salvage agent would obviate the need for global prophylaxis or expensive flap delay procedures, it would also be well suited for use in acute skin trauma. Currently, the ideal salvage drug does not exist.

To date, drug therapy for the salvage of failing skin flaps has focused largely on systemic and sometimes invasive routes of drug delivery. Although these approaches have occasionally met with experimental success, dose-limiting adverse effects from systemic drug distribution have often been an obstacle to satisfactory flap salvage. Ironically, the adverse effect most frequently encountered, systemic hypotension, likely serves to increase ischemic tissue loss by further reducing perfusion pressure in the already compromised skin flap. Consequently, many potentially efficacious drugs, including sympatholytics, vasodilators, and calcium channel blockers, have likely been rendered ineffective in flap salvage as a consequence of systemic drug distribution.
In an effort to circumvent the problem of systemic drug distribution, we chose to evaluate the efficacy of flap salvage using topical drug administration. Topical anti-ischemic therapy offers numerous advantages over conventional oral or parenteral therapy, the foremost of which is the ability to achieve therapeutic target tissue levels without significant systemic drug distribution. In spite of drug levels far in excess of recommended parenteral or oral doses, results of initial investigations using topical salvage agents have shown reductions in ischemic necrosis without dose-limiting systemic adverse effects.\(^{22,23,32}\) In contrast to oral or parenteral administration, topical drug therapy may also continue uninterrupted despite postoperative nausea, oral intake restrictions, or patient discharge, making them ideal for postoperative ambulatory use.

In this study we chose to evaluate 3 different topical drugs, each with the potential to prevent or reverse ischemic injury in the failing, random-pattern skin flap. The results of this study suggest that all 3 drugs are effective in reducing impending ischemic tissue loss when applied topically to the target tissue. Using a carefully standardized animal model, topical nifedipine and topical trolamine salicylate therapy improved survival from 56% in control animals to 74% in treated animals. This reduction in ischemic necrosis was observed without the need for pretreatment or systemic drug delivery. Moreover, the magnitude of tissue salvage observed following topical nifedipine administration compares favorably with studies using oral drug administration. To our knowledge, this is also the first published report of statistically significant flap salvage using a topically applied platelet inhibitor (trolamine salicylate). Although topical nitroglycerin demonstrated the weakest salvage response (68% flap survival), these results are similar in magnitude to those previously reported by Rohrich and coworkers\(^{22}\) or Blomain et al\(^{23}\) and serve to confirm the efficacy of topical nitroglycerin in ischemic flap salvage. As with previous experimental studies of topical anti-ischemic agents, we also observed no visible evidence of adverse effects, suggesting that systemic effects of topical drug therapy were negligible. Unlike many drugs previously evaluated in flap salvage, the drugs used in this study are widely available, inexpensive, and well tolerated. Based on the results of this preliminary animal study, topical drug therapy may eventually provide an effective clinical means for the salvage of failing random-pattern skin flaps.

It is worth emphasizing that this study did not confirm a true salvage drug response because drug therapy was initiated prior to clinical signs of tissue ischemia. Rather, these data suggest only that trolamine salicylate, nifedipine, and nitroglycerin are effective for increasing tissue tolerance to surgical ischemia. Whether topical drug therapy would augment flap survival if treatment were withheld pending the onset of visible signs of flap ischemia was not assessed in this study. However, because both nitroglycerin and nifedipine presumably improve impaired nutrient blood flow through vasodilation, these drugs may also have efficacy as legitimate salvage drugs. Further studies will be necessary to define their precise efficacy as salvage agents.

Because nifedipine, trolamine salicylate, and nitroglycerin presumably work by distinct pharmacological mechanisms of action (ie, inhibition of calcium ion flux into vascular smooth muscle, inhibition of TXA\(_2\) synthesis, and stimulation of endothelial PGI\(_2\) release, respectively), the results of this study support the notion that the origin of random-pattern skin flap necrosis is complex and multifactorial. Hence, combining drugs with differing mechanisms of action should theoretically result in an additive or even synergistic salvage response. The failure of combination drug therapy to produce such a response in this study is difficult to explain. Perhaps the absence of additive or synergistic drug interactions reflects a single common mechanism of action for the 3 drugs tested. Another possible explanation is that the combination of potent vasodilators may have negated any beneficial effects of topical drug therapy through sublethal reductions in systemic blood pressure. Although the combination of trolamine salicylate and nitroglycerin did produce a statistically significant reduction in flap necrosis relative to nitroglycerin alone, the response was not statistically significant relative to trolamine salicylate alone, suggesting that the observed improvement may have been from the isolated effect of trolamine salicylate. Because these 3 drugs are believed to work through separate and complementary mechanisms, further study using variations in drug dosages and drug combinations will be necessary to assess the potential for improved flap salvage through combined (synergistic) drug therapy.

**CONCLUSIONS**

The results of this animal investigation indicate that topical drug salvage of ischemic random-pattern skin flaps is safe, efficacious, and potentially well suited to clinical application. Further studies of this potential treatment modality are needed to better define optimal drug doses, application intervals, and drug combinations. Ultimately, topical anti-ischemic drug therapy may provide a needed pharmacologic rescue in the clinical management of failing skin flaps.

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