A comparison of pretreatment with a topical combination of nonivamide and nicoboxil and surgical delay in a random pattern skin flap model

Georg M. Huemer\textsuperscript{a,c,*}, Stefan M. Froschauer\textsuperscript{a}, Thomas Pachinger\textsuperscript{a,b}, Oskar Kwasny\textsuperscript{a,b}, Harald Schoffl\textsuperscript{a,b}

\textsuperscript{a} MAZ Mikrochirurgisches Ausbildungs- und Forschungszentrum, Garnisonstrasse 21, 4020 Linz, Austria
\textsuperscript{b} Department of Trauma Surgery, General Hospital Linz, Krankenhausstrasse 9, 4020 Linz, Austria
\textsuperscript{c} Department of Plastic Surgery, Sisters of Mercy Hospital, Seilerstaette 4, 4020 Linz, Austria

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\textbf{Summary} Delay procedures are intended to increase flap safety in otherwise risky flaps. In general they are of surgical nature, making an additional operation necessary. To overcome this drawback, non-surgical alternatives that may be as effective as the surgical procedure are constantly sought. We have previously shown an effective postoperative method to augment flap viability by topical application of a combination of nicoboxil and nonivamide. The goal of this study was to investigate whether this combination is also effective in inducing a delay effect in experimental skin flaps. Thirty male Wistar rats were randomised into three groups (\(n=10\)), and a standardised dorsal random pattern (\(6 \times 2\) cm) skin flap was raised on each animal. In rats of group 1, a surgical delay procedure was carried out 1 week prior to full flap harvest by incising the two longitudinal borders of the flap. In group 2 rats, the whole flap area was treated by the topical ointment once a day in order to induce a chemical delay effect for 7 days prior to flap harvest. In group 3 rats, the flap was harvested without any prior intervention and this group served as a control. Skin flap viability was assessed on postoperative day 7, and the extent of the viable skin flap area was compared between the three groups.

The surgical delay procedure resulted in a significant increase in the viable area of the skin flaps compared to the chemical delay group and the control group. Additionally, there was a significant increase in skin flap viability between the chemical group and the control group (mean percentage of viable skin flap area in surgical delay group, 80.9 ± 15.6; nonivamide/nicoboxil pre-treated group, 71.8 ± 4.9; control group, 60.7 ± 2.1; \(p<0.05\)).

Although not as effective as the surgical delay procedure, the topical combination of nicoboxil and nonivamide proved to be of significant value in order to ameliorate ischemic necrosis in...
The delay procedure denotes a phenomenon in which a flap is partially elevated and reset in a separate procedure before definite flap elevation and transfer.\textsuperscript{1–3} The technique of surgical delay has been used for centuries as a strategy for making the survival of flap tissue more reliable. The benefits of delay are thought to be due to changes in sympathetic tone, increased angiogenesis within the flap, the dilation of choke vessels and metabolic changes within the flap which increase its tolerance against ischemia. The time recommended between delay procedures varies, but usually 7 to 14 days are sufficient. The major disadvantage of this useful procedure in daily clinical practice is the need for an additional surgical intervention. Thus, research has focused on finding alternative, non-surgical methods for increasing flap viability preoperatively. One possibility explored was laser therapy, in which the subdermal plexus on the flap border was selectively occluded.\textsuperscript{4} Another approach to this problem was chemical delay.\textsuperscript{5} Chemical delay procedures mainly focused on increasing angiogenesis on the planned flap by systemic or local administration of various substances, such as vasodilating agents\textsuperscript{6,7} or vascular endothelial growth factor (VEGF).\textsuperscript{8,9} However, all of these proposed techniques require invasive administration of the beneficial substance. Previously, we were able to show that a topically employed ointment was able to enhance skin flap viability in ischemically challenged skin flaps in rats.\textsuperscript{10} The goal of this study was to show that this topically administered combination of nicoboxil and nonivamid is able to effectively delay skin flaps. To the best of our knowledge, there is no previous report about a chemical delay procedure using topically administered vasoactive ointment.

Materials and methods

A total of 30 male Wistar rats, weighing between 250 g 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 ad libitum were provided. All animals received humane care according to the 'Guide for the Care and Use of Laboratory Animals' (NIH publication 86-23, revised 1985). The animals were randomised into two groups of 20 rats each. Animals were housed in groups of three. Anaesthesia was performed by intraperitoneal injection of 50 mg/kg ketamine (Ketamidor 100 mg/ml; Richter Pharma Ag, Austria) and 1.3 g/kg bw Xylazine (Rompun 20 mg/ml; Bayer Corp., KS) with periodic supplementation as needed.

Delay procedure

The rats were anaesthetised and the dorsum was shaved with a commercially available hair clipper. The skin was then prepared using aqueous iodine solution and wiped with isopropanol swabs. A caudally based 6 × 2 cm McFarlane flap was outlined on the back of the rat.\textsuperscript{11} As an anatomical reference, the base of the flap was situated between the posterior iliac crests. In the next step, two longitudinal incisions were made, in all rats of group 1 (n = 10), along the entire length of the flap and the flap was completely undermined to include the panniculus carnosus (Figure 1). Thus, group 1 rats were designated the surgical delay group. In group 2 (n = 10), no surgical intervention was carried out, but the whole flap area was covered with a topical combination of nicoboxil and nonivamid (Finalgon\textsuperscript{\textregistered} ointment, Boehringer Ingelheim, Vienna, Austria; Figure 2). The ointment was applied postoperatively once daily for the next 6 days.

Flap harvest

On the seventh post delay procedure day, rats of group 1 and 2 were re-anaesthetised again and the flap was now completely incised except for its base and elevated by sharp dissection\textsuperscript{12} (Figure 3). Additionally, the same flap was raised in another 10 rats (group 3), who did not receive any delay procedure and this group served as a control group. After 7 days, full-thickness necrosis was fully developed in each rat. All rats were re-anaesthetised so that digital images of their McFarlane flaps could be taken. After skin flaps were harvested for histological examination,
animals were sacrificed with an overdose of intraperitoneal pentobarbital (100 mg/kg). Digital images of each flap were recorded with a Sony DSC-F828 camera (Sony Corporation, Tokyo, Japan), and the total area of necrosis was determined using the Image J (downloaded from http://rsb.info.nih.gov/ij/download.html) image processing program.

Statistical analysis

The results were presented as percentages of skin survival areas (mean ± SD). Statistical testing was performed using a one-way ANOVA test, which is a parametric variance technique. Pairwise comparisons were made with a Scheffe test. No correction was made for multiple testing. Results were expressed as mean ± SD and considered significant when \( p < 0.05 \).

Results

Thirty animals underwent surgery and no animals died from the drug treatment or the surgical procedure. There was a certain amount of skin necrosis in the distal parts of all dorsal skin flaps, regardless of treatment (Figure 4). The mean surviving skin flap area for the surgical delay group (group 1) was significantly larger than that in the control group (group 3) (Surgical delay, 80.9 ± 15.6%, range 54.8 to 96.6%; control, 60.7 ± 2.1%, range 58.3 to 64.0%; \( p < 0.05 \)). Also, the mean surviving skin flap area for group 1 was significantly larger than that in the chemical delay group (group 2) (Chemical delay, 71.8 ± 4.9%, range 66.1 to 82.8%; \( p < 0.05 \)). Moreover, there was a significant increase between the mean surviving skin flap area of group 2 compared to the control group (\( p < 0.05 \)). Figure 5 shows a comparison of the mean percentages of the surviving area between all three groups.

Discussion

In the present study we were able to induce a delay effect in ischemically challenged skin flaps by topical application of a vasoactive ointment. Although not as effective as surgical delay, chemical delay was able to ameliorate ischemic necrosis to a significant extent compared to the control group.

Currently, surgical delay is the most reliable and effective method in order to enhance vascularity and survival of random pattern skin flaps. However, there are several major drawbacks when applying this useful method in a clinical setting, such as an additional surgical procedure, increased costs and prolonged treatment time. Several investigators have thus focused on alternative methods for inducing a delay effect without the need for invasive surgery. Examples include laser therapy, pharmacological delay, stress conditioning, suturing, clamping and chemical delay. One problem with the designation ‘chemical delay’, in this context, is the fact that most substances that have
already been used successfully are not delaying the tissue but are instead prefabricating it. However, this problem is of rather semantic nature since the effects induced by these substances are very similar to the ones observed during classic surgical delay.

From a pathogenetic point of view, the delay phenomenon is induced by sublethal ischemia in the target tissue. Due to the experimental work of numerous investigators, the underlying mechanisms leading to an improved tolerance to ischemia have been elaborated. According to these studies, the effects of vascular delay can be divided chronologically into early and late effects. The three main contributors to the early effects include an alteration in sympathetic tone, dilation of choke vessels and early changes in tissue metabolism. Both prolonged changes in tissue metabolism and neovascularisation constitute the most important late effects in delay, with neovascularisation being of crucial importance. As is currently known, neovascularisation is thought to occur by way of two different mechanisms: angiogenesis and vasculogenesis. Whereas angiogenesis denotes the sprouting of new vessels from a pre-existing capillary network, vasculogenesis indicates the blood vessel formation from bone marrow-derived endothelial progenitor cells that form new vessels in situ.

Any procedure, such as the above mentioned procedure, that prevents blood flow to the flap will be able to delay the flap to a certain amount. Due to this reduction in blood flow, hypoxia in the tissue will ensue, leading to an instant increase to hypoxia-inducible factor-1 (HIF-1). HIF-1 is

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**Figure 4** Comparison between animals of each study group with representative samples of viable and necrotic skin regions in random pattern dorsal skin flaps on day 7. In the upper row, four animals of the surgical delay group are shown (group 1; 1a–d), whereas in the middle row, animals of the chemical prefabrication are depicted (group 2; 2a–d) and in the lower row, animals of the control group are shown (group 3; 3a–d).

**Figure 5** Mean percentage survival area for all groups.

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Table 1 Summary of actions elicited by nonivamide and nicoboxil

<table>
<thead>
<tr>
<th>Active agent</th>
<th>Action</th>
<th>Response in flap tissue</th>
</tr>
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<tbody>
<tr>
<td>nonivamide</td>
<td>capsaicin</td>
<td>vasodilation</td>
</tr>
<tr>
<td>nicoboxil</td>
<td>nicotinic acid/nicotinamide</td>
<td>angiogenesis vasodilation NAD donator</td>
</tr>
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a key regulator\textsuperscript{19–21} of angiogenesis by activating transcription of genes encoding angiogenic growth factors including vascular endothelial growth factor (VEGF), angiopoietin 1, placental growth factor (PGF), and platelet-derived growth factor (PDGF). Several of these growth factors\textsuperscript{22} have been used successfully in experimental plastic surgery to enhance survival of skin flaps. Ultimately, the goal is increased angiogenesis with enhanced vascularity in the three-dimensional flap tissue in order to meet the demands once the three sides of the flap have been cut. We have previously shown that techniques capable of activating more than one key factor of angiogenesis, such as shock wave therapy, are probably superior to techniques employing only one of these factors.\textsuperscript{23–25}

The topical combination of nicoboxil and nonivamide (Finalgon\textsuperscript{®}) used in this experiment also exhibits more than one action in the target tissue. Originally, this topical remedy was used for pain relief, either after sports injuries or for rheumatic diseases. Nonivamide is a synthetic capsaicin analogue derived from the Spanish peppercorn, whereas nicoboxil is an ester of nicotinic acid. Both of these substances have been shown to be very effective in experimental skin flap surgery.\textsuperscript{26–29} The combined actions of these two active agents are listed in Table 1. Capsaicin (nonivamide) rapidly produces local neurogenic inflammation, characterised by oedema and erythema, which leads to an instant vasodilatation and increase in blood flow by binding to capsaicin-sensitive nerve fibres.\textsuperscript{30,31} This action leads to an increased blood flow, and blood pressure, to the flap tissue during the 7 days of chemical flap delay. Furthermore, binding of nonivamide to capsaicin-sensitive pain receptors elicits a release of substance P and other neuropeptides that are known to promote the growth of different cell lines and to accelerate wound healing.\textsuperscript{32,33} Nicotinic acid/nicotinamide (nicoboxil) exhibits angiogenic properties leading to neovascularisation within the flap tissue.\textsuperscript{34,35} This action is potentiated by the increased blood flow elicited by the nonivamide component. Secondly, nicotinamide also displays vasodilatory action.\textsuperscript{36} Moreover, nicoboxil is able to replenish the pool of nicotinamide adenine dinucleotide (NAD), which has been demonstrated to decrease the damage observed during an ischemic cell injury. Nicotinamide can facilitate DNA repair by inhibiting poly(ADP-ribose) polymerase, increasing NAD levels and adjusting other related enzyme activities leading to an amelioration of a metabolic derangement seen in tissue ischemia.\textsuperscript{37,38} Although all of the above mentioned mechanisms and actions may have a more powerful effect postoperatively, we strongly believe that topical treatment with the combination of nonivamide/nicoboxil mimics some of the effects encountered during classical surgical delay such as neovascularisation and metabolic changes, even in the preoperative period, making it suitable for chemical delay.

We currently use this ointment in daily clinical practice in order to augment blood flow in cases of critically perfused tissues, such as the nipple/areola-complex in large reduction mammaplasties, local random pattern flaps if they show signs of marginal perfusion and abdominoplasty wound edges if they appear bluish during the first change of dressing. The main problem with all experimental papers on skin flap perfusion is their very limited value for clinical practice. Randomised studies comparing a certain substance in humans would be highly unethical and, thus, the benefit of this substance is hard to define. To date there are only a few clinical studies testing the efficacy of a certain substance in a randomised manner.\textsuperscript{39–41} Since our topical combination has been in clinical use for many years and due to promising results in our animal study,\textsuperscript{42} we began to use this compound in clinical practice.

Although Kerrigan\textsuperscript{43} and Rohrich\textsuperscript{44} postulate that the ideal pharmacological agent should only be administered postoperatively, an ointment that is capable of producing a certain delay effect preoperatively may be of clinical value, particularly when considering that other prerequisites of an ’ideal’ pharmacological agent\textsuperscript{45} are fulfilled, such as easy administration, clinical availability, high therapeutic index and cost-effectiveness. The compound could be administered daily by the patient a few days before the scheduled surgical procedure on the area of interest. The potential risks are minimal, the costs reasonable and the procedure not very time consuming.

We were able to induce a considerable non-surgical delay effect in experimental skin flaps in rats with a topically applied ointment. Due to the ease of administration and high therapeutic safety, this treatment regimen may be adoptable for clinical application in the high risk patient and, for increased survival rates, could be combined with prolonged treatment in the postoperative period.

References


