Pharmacological characterization of vasomotor activity of human musculocutaneous perforator artery and vein

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Failure to close these wounds and achieve wound heal-
ing may destroy the exposed tissues, such as nerves, blood vessels, tendons, or bones, resulting in loss of for-

m and/or function in that part of the body. Autoge-

nous skin or skin and muscle transplantation (i.e.,
cutaneous or musculocutaneous free flap surgery) is
routinely used for wound coverage. Specifically, a
cutaneous or musculocutaneous flap is harvested from a
distant donor site of the body and is transferred to
cover the wound, with vascular anastomosis performed
at the recipient site to reestablish blood supply (8).
Despite advances in microsurgical technique, patient
selection, judicious donor site selection, and recipient
site preparation, flap failure associated with vaso-
spasm and thrombosis still occurs at a rate of 5–10%,
even in large medical centers (3). Vasospasm can occur
intraoperatively and shortly after release of the vascu-
lar clamp after vascular anastomosis, but vasospasm
can also occur within 48–72 h postoperatively. Vaso-
spasm plays an important role in the pathogenesis of
thrombosis, causing partial or total flap ischemic ne-
crosis (33, 57). Flap failure is time consuming and
costly because it requires additional surgery and a
prolonged period of hospitalization. In the United
States, the operating room costs range from $44,000
to $68,000 for each total free flap failure (19, 52), and
the additional surgeon reimbursements range from $5,000
to $35,000 (52). Furthermore, repeated reconstructive
surgery may increase donor site deformity and morbidity,
which may have a devastating effect on the patient.
Therefore, there is the need to understand the patho-
ophysiology of vasospasm in free flap surgery to develop
an effective pharmacological intervention for preven-
tion and/or mitigation of vasospasm in flap surgery.
The mechanism for vasospasm in free flap surgery is
unclear. It seems that mechanical stretch or trauma
may induce a myogenic response, causing vasospasm.
In addition, surgical trauma may induce local vaso-
spasm by stimulating the sympathetic nerve ending
to release norepinephrine (NE). The synthesis and re-
lease of endothelium-derived relaxing factors (EDRFs),
such as prostacyclin (PGL2) and nitric oxide (NO), may

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be reduced, or synthesis and release of endothelium-derived contracting factors, such as thromboxane A₂ (TxA₂) and endothelin-1 (ET-1), may be augmented (4, 8, 41, 42). Last but not least, reduced local blood flow due to vasospasm may also increase the thrombogenic nature of the suture lines of the vascular anastomosis. This may in turn promote platelet release of vasoconstrictive and prothrombotic substances such as NE, TxA₂, and serotonin [5-hydroxytryptamine (5-HT)] (29). Venous congestion, i.e., “blue flap,” is a common pathology in skin flap failure and is likely induced by venous spasm. There is the clinical impression among surgeons that veins appear to be more susceptible to vasospasm than arteries in flap surgery and, once established, venous spasm seems to be more difficult to resolve than arterial vasospasm (23). In addition, there is experimental evidence to indicate that venous ischemia is more injurious in flap surgery (26). These observations imply that venous vasospasm is an important pathogenic mechanism in free flap surgery. This study was designed to investigate whether indeed the human musculocutaneous perforator (MCP) vein is more susceptible to vasospasm than the MCP artery and whether this susceptibility is related to differences in sensitivity to vasoactive agents. The MCP artery and vein were chosen for this study because the MCP artery supplies blood to the muscle and skin from the segmental artery and this pattern of blood supply is relevant to musculocutaneous pedicled and free flap surgery.

MATERIALS AND METHODS

Source of human blood vessels. The skin pannus excised from patients undergoing abdominoplasty serves no purpose to the patient and is normally disposed of by incineration. A clinical protocol was approved to obtain MCP arteries and veins from the skin pannus after it was excised from the patient. Another protocol was also approved to obtain MCP artery and vein specimens (~1.0 cm in length) from patients undergoing transverse rectus abdominis musculocutaneous flap surgery. Obtaining these vascular specimens did not affect the procedure or outcome of the surgery. The blood vessel specimens were wrapped in gauze soaked with isotonic saline. The specimens were transported to the laboratory at room temperature. This mimicked the clinical situation in which the free flap is subjected to ischemia in room temperature during anastomosis at the recipient site. The vascular specimens were used for experimentation within 45–60 min after excision. This ischemic period may be slightly shorter than the ischemic time for free flap surgery in some cases. The subjects were female (40–60 yr of age) and were not known to smoke or have any systemic disease.

Preparation of vascular rings and tension recording. The MCP artery and vein specimens were placed in oxygenated Krebs-bicarbonate solution at room temperature, cleared of loose connective tissue, and cut into 4-mm-long rings. The outer diameter of these vascular rings was 1–2 mm. Two stainless steel wires were placed through the lumen of each ring. An arterial and a venous ring were used in each experiment. Each ring was placed in an organ chamber of 25-ml volume. One wire was anchored to the bottom of the organ chamber, whereas the other was connected to a Grass FT 0.03 force transducer (Grass Instrument, Quincy, MA) for the measurement of isometric force. Isometric contractions were recorded on a Grass model 75 polygraph. The ring was equilibrated in the organ chamber in Krebs-bicarbonate solution containing (in mM) 118.4 NaCl, 4.7 KCl, 2.25 CaCl₂, 1.2 MgSO₄, 1.2 KH₃PO₄, 25 NaHCO₃, and 11 glucose. The Krebs-bicarbonate solution was gassed with 95% oxygen-5% carbon dioxide and maintained at 37°C and pH 7.4. Arterial and venous rings were equilibrated under resting tensions of 2 and 0.5 g, respectively, which were determined, in a preliminary experiment, to be optimal for maximal tension development by 50 mM KCl. The endothelium was intentionally preserved by cautiously mounting the vascular rings, and the endothelium-dependent relaxation response of vascular rings to acetylcholine was tested in each experiment. Clinically, vasospasm in microvascular surgery is probably caused by a myogenic response induced by mechanical stretch or trauma and local release of vasoconstrictor substances. In the present studies, vascular contraction was induced by exogenous vasoconstrictors, which are known to be associated with experimental skin flap vasospasm.

Protocol 1. Each arterial and venous ring was equilibrated in Krebs-bicarbonate solution for ~30 min with continuous readjustment of resting tension to 2 g for arterial and 0.5 g for venous rings. At the end of the equilibration period, the absolute contract to 50 mM KCl was obtained. After three washings with Krebs-bicarbonate solution, the ring was allowed to stabilize for at least 30 min before an experiment was started. Vascular contractions to various vasoconstricting agents were expressed as a percent increase in tension induced by 50 mM KCl.

Cumulative concentration-response curves for MCP arteries and veins were obtained to the following agents: ET-1 (10⁻⁸ to 5 × 10⁻⁸ M), the stable TxA₂ mimetic U-46619 (10⁻⁹ to 5 × 10⁻⁶ M), and NE (10⁻⁴ to 5 × 10⁻⁵ M). Cumulative concentration-response curves were constructed by step addition of the constrictor substances to the organ chamber solution. Each increment was made only after the response for the preceding concentration of drug had stabilized (40 min for ET-1, 15 min for U-44619 and NE). Each ring was used to obtain the concentration-response curve for only one drug. At the end of each experiment, the presence of functional endothelium in the vascular ring was determined by testing the relaxation response to 10⁻⁵ M acetycholine.

Protocol 2. For relaxation experiments, each MCP artery and vein was preconstricted with a submaximal dose of ET-1 (3 × 10⁻⁹ M). Preliminary experiments indicated that the onset of contraction to ET-1 was slow and the maximal contraction was reached at ~30 min and sustained for >140 min. Immediately after the maximum contraction was achieved, the cumulative concentration-dependent relaxation effect of nitroglycerin, papaverine, nifedipine, or lidocaine (Xylocaine) was studied in arterial and venous rings. Cumulative concentration-response curves were constructed by addition of the vasodilator drugs to the organ chamber solution in 0.5-log unit steps. Each step was made only after the relaxation response had stabilized (~15 min). Each arterial and venous ring specimen was used to obtain the relaxation concentration-response curve for one drug only.

Biochemicals. Unless otherwise stated, all chemicals, except for the following, were purchased from Sigma Chemical (St. Louis, MO): ET-1 from Peptide International (Louisville, KY), U-46619 from Caymen (Ann Arbor, MI), NE and nitroglycerin from SABEX (Boucherville, Quebec), and lidocaine from Abbott Laboratory (St. Laurent, Quebec).

ET-1 was dissolved in 0.1% acetic acid and was stored at −70°C for no longer than 30 days before use. To make stock solutions, NE was dissolved in 5 ml of 5% dextrose at the
concentration of $6 \times 10^{-3}$ M. Papaverine and nifedipine were dissolved in 0.5 ml of DMSO at the concentration of $10^{-2}$ M. Injectable lidocaine was dissolved in perfusion solution. Krebs-bicarbonate solution and all drug stock solutions were made in the morning of each experiment day and were kept at 4°C. Various concentrations of drugs were made with 37°C Krebs-bicarbonate solution during the experiment. The quantity of acetic acid, dextrose, and DMSO used for dissolving drugs did not affect the isometric tension of the arterial and venous rings tested ($n = 3$).

Data processing and statistical analysis. Vascular contractions were expressed as a percentage of the tension induced by 50 mM KCl. Vascular relaxations were expressed as a percentage of contraction induced by $3 \times 10^{-9}$ M ET-1. The concentration of a drug exhibiting 50% of the maximal contraction or relaxation ($EC_{50}$) was calculated for each arterial or venous ring. Apparent affinity ($pD_2$) was calculated as negative log molar concentration of $EC_{50}$.

All values are expressed as means ± SE. One-way analysis of variance followed by Duncan’s multiple-range test was used for comparison of mean values. Student’s $t$-test was used for comparison of two mean values. Statistical significance was set at $P < 0.05$ for all tests. The number ($n$) of observations indicates the number of blood vessels obtained from different patients.

RESULTS

Contractions of the MCP artery and vein. ET-1, U-46619, and NE elicited cumulative concentration-dependent contraction in MCP arteries and veins (Figs. 1-3, respectively). The order of vasoconstrictor potency in both MCP arteries and veins as judged by the $pD_2$ values was ET-1 > U-46619 > NE (Table 1), and the differences were significant ($P < 0.05$). The order of maximal contraction induced by these vasoconstrictors was U-46619 > ET-1 = NE for MCP arteries and U-46619 = ET-1 > NE for MCP veins (Table 2), and the differences were also significant ($P < 0.05$).

Comparison of contractions between the MCP artery and vein. The vasoconstrictor potency of ET-1 and U-46619 as judged by $pD_2$ values (Table 1) was similar between the MCP artery and vein. However, the maximal contraction elicited by ET-1 was 56% higher in the MCP vein compared with the MCP artery and for U-46619 was 38% higher in the MCP vein than MCP artery (Figs. 1 and 2; Table 2). The vasoconstrictor potency of NE was twofold higher ($P < 0.05$) in the MCP vein compared with the MCP artery (Table 1), but the maximal contraction induced by NE was similar between the MCP artery and vein (Table 2).

Relaxations of the MCP artery and vein. Nitroglycerin, papaverine, nifedipine, and lidocaine elicited concentration-dependent relaxation in MCP arteries and veins preconstricted with $3 \times 10^{-9}$ M ET-1 (Fig. 4). The order of relaxation potency as judged by the $pD_2$ values was nitroglycerin > papaverine = nifedipine = lidocaine for MCP arteries and nitroglycerin > papaverine > nifedipine = lidocaine for MCP veins (Table 3), and the differences were significant ($P < 0.05$). Next to nitroglycerin, papaverine was the most effective vaso-
dilator observed in the present study. Specifically, the relaxation potency of papaverine was similar between nifedipine and lidocaine in the MCP artery but was significantly higher \((P < 0.05)\) than nifedipine and lidocaine in the MCP vein (Table 3). The maximal relaxation effect was similar among nitroglycerin, papaverine, and nifedipine in that they all completely mitigated ET-1-induced contraction in both MCP arteries and veins (Fig. 4). However, the maximal relaxation achieved by lidocaine was only 48% in the MCP artery and 49% in the MCP vein (Fig. 4).

**Comparison of relaxation between the MCP artery and vein.**

MCP veins preconstricted with ET-1 were less sensitive to the relaxation effect of vasodilators than MCP arteries preconstricted with the same concentration of ET-1. Specifically, the relaxation potency of nitroglycerin, nifedipine, and lidocaine, as judged by their \(pD_2\) values, was lower \((P < 0.05)\) in the MCP vein than in the MCP artery by 2.4-, 4.7-, and 5.8-fold, respectively (Table 3). However, the relaxation potency of papaverine was similar between the MCP artery and vein.

**DISCUSSION**

The present study demonstrated for the first time some differences in the responsiveness of the human peripheral artery and vein to vasoactive agents. Specifically, we observed that the vasoconstrictor potency of NE was higher in the MCP vein than in the MCP artery. Although the vasoconstrictor potency of ET-1 and U-46619 was similar between the MCP artery and vein, their maximal contraction effect was higher in the MCP vein than in the MCP artery. More importantly, MCP veins preconstricted with a submaximal concentration of ET-1 were less sensitive to the relaxation effect of nitroglycerin, nifedipine, and lidocaine than MCP arteries preconstricted with the same concentration of ET-1.

**Explanation for differences in contraction between the human MCP artery and vein.** In this study, we did not plan to investigate the mechanism responsible for the differences in sensitivity to the vasoconstrictor effects of NE, ET-1, and U-46619 between the human MCP artery and vein. These vasoconstrictors were used because there is experimental evidence to indicate that they most likely contribute to the pathogenesis of vasospasm in flap surgery (4, 41–43). Other investigators have observed that large canine veins were more sensitive to the vasoconstrictor effect of ET-1 than were their paired arteries (7, 35) and the human internal mammary vein was more sensitive to the vasoconstrictor effect of NE (31) and ET-1 (6, 32, 41).

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**Table 2. Maximal contraction effect of endothelin-1, U-46619, and norepinephrine in the human musculocutaneous perforator artery and vein**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Maximal Contraction Effect (% response to 50 mM KCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artery</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>104 ± 2b</td>
</tr>
<tr>
<td>U-46619</td>
<td>123 ± 4a</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>112 ± 3b</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 5\) experiments. \(a, b\) Within-group means without a common letter are significantly different \((a > b)\), \(P < 0.05\) (1-way ANOVA followed by Duncan’s multiple comparisons of means). \(*\) Venous mean value significantly different from arterial in endothelin-1 and U-46619 treatment, \(P < 0.05\) (Student’s \(t\)-test).
59) than was its corresponding artery. It was suggested that the greater vasoconstrictor effect of ET-1 and NE in the human internal mammary vein than the artery may be related to a greater vasoconstrictor effect of these vasoconstrictors on venous smooth muscle cells, less release of EDRFs, or less responsiveness to EDRFs in the vein than in the artery (31, 32). Further study is required to determine which of these factors is responsible for the difference in responsiveness to vasoconstrictors between the MCP artery and vein.

Relaxation mechanism. Nitroglycerin, papaverine, nifedipine, and lidocaine are common spasmolytic drugs (15–17, 51). Nitroglycerin releases NO, which interacts with soluble guanylate cyclase to raise the intracellular concentration of cGMP, which in turn mediates vascular smooth muscle relaxation (44, 45). There is evidence to indicate that cGMP may act to reduce intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) (1, 22) and modulate the Ca$^{2+}$ contractile apparatus in smooth muscle cells (2, 25).

Papaverine is known to inhibit oxidative phosphorylation (50) and increase intracellular accumulation of cAMP and cGMP in vascular smooth muscle cells by inhibition of cAMP and cGMP degradation by cyclic nucleotide phosphodiesterases (10, 28, 37). The accumulated cAMP and cGMP decrease [Ca$^{2+}$]$_i$ and the sensitivity of contractile activity in vascular smooth muscle cells (5).

Fig. 4. Cumulative concentration-dependent relaxations to nitroglycerin, papaverine, nifedipine, and lidocaine in the human MC perforator artery and vein with intact endothelium and preconstricted with $3 \times 10^{-9}$ M endothelin-1. Relaxations are expressed as a percentage of the contraction induced by $3 \times 10^{-9}$ M endothelin-1: 100% = 1.40 ± 0.05 g in MC perforator arteries and 0.67 ± 0.02 g in MC perforator veins. Values are means ± SE; $n$ = 4 experiments (nitroglycerin and papaverine) and $n$ = 5 experiments (nifedipine and lidocaine).
Nifedipine causes vasodilation by blocking entry of extracellular Ca\(^{2+}\) from L-type Ca\(^{2+}\) channels (20).

The mechanism of action of lidocaine is unclear. Its vasodilator effect may be partly due to its inhibitory effect on local sympathetic nerves and blocking of Na\(^{+}\) channels, thus leading to a decrease in intracellular Na\(^{+}\) concentration, which in turn reduces [Ca\(^{2+}\)], resulting in vasodilation (27).

**Limitations of the present findings.** Experimental evidence presented thus far seems to indicate that ET-1-preconstricted human MCP veins are less sensitive to vasodilators such as nitroglycerin, nifedipine, and lidocaine than are MCP arteries. However, further studies are required to demonstrate that these differences between human MCP arteries and veins in their response to the relaxation effect of these spasmylytic drugs are not limited to ET-1-induced vasoconstriction. Specifically, Miller and Vanhoutte (36) observed that the ability of NO donors to inhibit contractions in canine femoral arteries and veins varied with the agent used to activate the contraction. For example, it was reported that, when ET-1 was used to contract the canine femoral arteries and veins, 3-morpholinosydnonimine was more potent in relaxing arteries than veins, but this difference was not seen when these blood vessels were contracted with NE. It has been demonstrated previously that 5-HT and TxA\(_2\) (18, 40), blood vessels were contracted with NE. It has been reported that, when ET-1 was used to contract the canine femoral arteries and veins, 3-morpholinosydnonimine was more potent in relaxing arteries than veins, but this difference was not seen when these blood vessels were contracted with NE. It has been demonstrated previously that 5-HT and TxA\(_2\) (18, 40), but not NE (12), are likely to be involved in the pathogenesis of vasospasm and thrombosis in skin flap surgery. Therefore, future study with human MCP arteries and veins is required to clarify the relaxation effect of vasodilators on 5-HT- and TxA\(_2\)-induced vascular contractions.

**Clinical perspectives.** The effective agents for prevention and/or treatment of flap vasospasm remain elusive. Results obtained from this study certainly indicate that nitroglycerin is a potent vasodilator for resolving vasospasm in both MCP arteries and veins intraoperatively. However, it is of interest to investigate whether nitroglycerin can also be used as a prophylactic agent for the prevention of perioperative vasospasm. More importantly, it is well known that vasospasm and thrombosis can occur within 24–48 h postoperatively, and the effectiveness of nitroglycerin for prevention and/or treatment of postoperative vasospasm is unclear. Specifically, there are positive (9, 13, 43, 47, 51, 56) and negative (14, 39, 54) results in the therapeutic effect of nitroglycerin in augmentation of skin flap viability in laboratory animals. These controversial results may be attributed to differences in flap models, dosage, and method of drug administration. Nitroglycerin is a safe and inexpensive drug that can be administered topically in cutaneous and musculocutaneous flaps. Therefore, there is the need to elucidate the efficacy of nitroglycerin for the prevention and/or treatment of intraoperative and postoperative vasospasm in flap surgery.

The relaxation mechanism of nitroglycerin and an L-type Ca\(^{2+}\) channel blocker are different; therefore, the combined use of nitroglycerin and an L-type Ca\(^{2+}\) channel such as nifedipine may potentially produce an additive effect against vasospasm (15, 17). Theoretically, topical nitroglycerin in combination with oral treatment of an L-type Ca\(^{2+}\) channel blocker would provide optimal prevention and/or treatment against intraoperative and postoperative vasospasm in flap surgery. However, similarly to nitroglycerin, the experimental evidence concerning the efficacy of L-type Ca\(^{2+}\) channel blockers for augmentation of skin flap viability is equivocal at the present time (21, 24, 34, 38, 56). The dose-response effect of L-type Ca\(^{2+}\) channel blockers on skin hemodynamics and viability in flap surgery has yet to be investigated. It has been demonstrated that the vasoconstrictor effect of ET-1 is mediated by ET\(_A\) receptors, with little participation from ET\(_B\) receptors (30). Therefore, selective ET\(_A\)-receptor antagonists may also be used as a combined treatment for skin flap vasospasm. Papaverine is also a potent vasodilator in human MCP arteries and veins. However, there is the suspicion that papaverine may not be a drug of choice because commercially available papaverine solution is highly acidic (pH 3.0–4.5) and can be potentially damaging to endothelial cells. Papaverine is relatively unstable in nonacidic solution (17, 49). In addition, papaverine is not used systemically; thus the use of papaverine for the prevention and/or treatment of postoperative vasospasm in flap surgery may be limited.

The effect of cyclooxygenase products on skin flap viability is unclear. It was observed that intravenous infusion of prostacyclin (PGI\(_2\)) did not increase skin flap distal perfusion or viability in the pig (11). There were two clinical cases in which aspirin and iloprost (a stable analog of PGL\(_2\)) were used as antithrombotic drugs for salvage of failing flaps (46, 53), but the efficacy of these drugs for prevention and/or treatment of skin flap thrombosis has yet to be documented clinically. It is important to point out that there are potential complications of postoperative bleeding and hematoma formation with the use of antithrombotic agents in flap surgery.

In summary, using the vascular ring perfusion technique, we demonstrated for the first time that the vasoconstrictor potency of NE is higher in the human MCP vein than artery and the maximal contractions

### Table 3. Relaxation potency of nitroglycerin, papaverine, nifedipine, and lidocaine in the human musculocutaneous perforator artery and vein preconstricted with 3 × 10\(^{-9}\) M endothelin-1

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Artery</th>
<th>Vein</th>
</tr>
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<tbody>
<tr>
<td>Nitroglycerin</td>
<td>4</td>
<td>5.852 ± 0.063(^{a})</td>
<td>5.442 ± 0.015(^{a})</td>
</tr>
<tr>
<td>Papaverine</td>
<td>4</td>
<td>5.330 ± 0.017(^{b})</td>
<td>5.270 ± 0.049(^{a})</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>5</td>
<td>5.470 ± 0.103(^{b})</td>
<td>4.757 ± 0.044(^{a})</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>5</td>
<td>5.547 ± 0.094(^{b})</td>
<td>4.768 ± 0.043(^{a})</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. \(^{a,b,c}\)Within-group means without a common letter are significantly different (\(a>b>c\)), \(P < 0.05\); 1-way ANOVA followed by Duncan’s multiple comparisons of means. *Venous mean value is significantly different from arterial in nitroglycerin, nifedipine, and lidocaine treatments, \(P < 0.05\); Student’s t-test.
REFERENCES


