Venous and arterial coronary artery bypass grafts in a pharmacological perspective
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Long-term prevention of graft spasm by exposure of human internal mammary artery or saphenous vein to a lipophilic calcium antagonist

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Submitted for publication.
Introduction

Graft spasm in the perioperative period increases the morbidity and mortality associated with aortocoronary bypass surgery (CABG). The internal mammary artery (IMA) may develop spasm during grafting to the coronary circulation and may contribute to perioperative infarction. Spasm of the saphenous vein (SV) may be provoked during its removal from the leg. To overcome SV contraction high-pressure distension is often applied. The exposure to high-pressure distension and the spasm itself are detrimental for the integrity of the vascular wall, and may reduce the patency of the graft. Postoperative spasm of venous grafts may also be a problem, as demonstrated in several case reports.

The underlying mechanism of graft spasm remains to be elucidated. Most probably spasm is the result of the sum of several factors such as the exposure to cold, circulating vasoconstrictor substances and endothelial dysfunction. Literature data showed differences in the responsiveness of SV and IMA to vasodilators, that proved dependent on the spasmogenic agent used. This observation may indicate functional dissimilarities between arterial and venous grafts.

At present in our clinic, prevention of spasm of IMA is achieved by the substernal storage of the vessel in a gauze submerged with papaverine solution (5 mg/mL) and/or by the intraluminal application of papaverine. Postoperatively, all patients with arterial grafts receive nitroglycerine via continuous IV infusion for 24 hours. For the prevention of SV spasm no particular measures are applied at the moment. It has been submitted that the use of papaverine might impair the endothelial function and/or integrity. The systemic application of nitroglycerine has been shown to improve general hemodynamics in patients subjected to CABG, but the systemic vasodilation provoked by this agent may be problematic. In this connection the systemic application of calcium antagonists (CA) proved to prevent ischemic and arrhythmic complications better than nitroglycerin, and when given in appropriate doses the CA did not influence the systemic circulation. Accordingly, the selection of an appropriate spasmolytic agent should be determined by its effectiveness against multiple mechanisms of vasocontraction, the duration of action, possible detrimental effects on the vessel wall integrity and adverse systemic effects. At present most agents are applied during the operation topically and/or intraluminally. The time span available to submerge the segments already dissected, and/or during harvesting is limited. Consequently, the onset of action of the spasmolytic agent must be rapid as well.
The aim of the present study was to investigate whether a long acting CA may prevent graft spasm for a longer period of time after short-term exposure of the isolated vessel segments during the surgical procedure.

In recent years new calcium antagonists have been developed with a more favourable kinetic profile than classical drugs. Owing to their slow onset of action reflex tachycardia is avoided, whereas they may display a prolonged duration of action and vasoselectivity. These characteristics are in part determined by physicochemical properties like the lipophilicity.¹⁴,¹⁵ This parameter, expressed as membrane partition coefficient (log P), denotes the proportion of substance solved in a lipophilic membrane relative to the amount in aqueous medium.

The lipophilic CA selected for the present study was lacidipine. This CA proved to possess a very long duration of action compared to other CA of the 1,4-dihydropyridine-class in various isolated vessels. The long duration of action can be explained by the high lipophilicity of the compound (Table 1).¹⁴,¹⁵ In the present study the inhibitory action of lacidipine was studied in isolated human IMA and SV preparations with respect to contractile responses induced by a high concentration of potassium (KCl) or by the α₁-adrenoceptor agonist phenylephrine. The effect of lacidipine at contractile responses was compared to that of nifedipine, a classical short acting CA, with a low lipophilicity (Table 1, Figure 1). For the evaluation of the effect of lacidipine in comparison to the currently applied spasmolytic measure, responses to papaverine were established as well. Since patients with arterial grafts for myocardial revascularisation receive nitroglycerine IV for 24 hours, this time period was selected to observe the effect of the three different spasmolytic compounds.

**Table 1: lipophilic properties of the compounds tested, expressed as log P.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>log P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papaverine</td>
<td>3.69</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>2.5</td>
</tr>
<tr>
<td>Lacidipine</td>
<td>5.39</td>
</tr>
</tbody>
</table>
Materials and Methods

*Human vessel preparations*

Saphenous vein and mammary artery remnants were obtained from 15 patients subjected to CABG. The patient’s characteristics are summarised in Table 2. Written informed consent was received from all patients, and the study was approved by the
Ethics Committee of the Academic Medical Center, according to the principles outlined in the Declaration of Helsinki.

All preparations were immediately stored in University of Wisconsin solution at 4°C for 24-48 hours. In a previous study it was demonstrated that functional properties of vessels stored in University of Wisconsin are preserved.\(^5\)

**Experimental protocol**

For the experiments adhesive tissue was removed and each blood vessel segment was cut into rings of approximately 5-mm length. The rings were mounted between two L-shaped stainless steel hooks in 5-mL organ baths continuously superfused with oxygenated Krebs-Henseleit solution of 37°C (pH 7.4). The rate of superfusion was 2-mL.min\(^{-1}\), and this was obtained by using a multichannel roller pump (Masterflex, Cole-Palmer Instrumental Company, Chicago, Illinois, U.S.A.). Each preparation was fixed, via a silk thread, to an isometric force transducer (A.D. Instruments, Castle Hill, Australia) and force was recorded via a MacLab/8 computer system (A.D. Instruments, Castle Hill, Australia). The SV and IMA preparations were subjected to a pre-tension of 20 mN and 40 mN respectively, which was maintained throughout the experiment. After an equilibration period of 60 minutes the vascular rings were primed and tested for viability, by exposing them twice to a potassium chloride solution.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td>11/4</td>
</tr>
<tr>
<td><strong>Age (mean ± SEM)</strong></td>
<td>65.2 ± 1.6</td>
</tr>
<tr>
<td><strong>Angina Pectoris (New York Heart Association classification)</strong></td>
<td>≥ III/IV</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td>15/15</td>
</tr>
<tr>
<td>(≥1 of hypertension, hyperlipidaemia, smoking, and/or positive family history)</td>
<td></td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
</tr>
<tr>
<td>nitrates</td>
<td>6/15</td>
</tr>
<tr>
<td>calcium channel blockers</td>
<td>9/15</td>
</tr>
<tr>
<td>β-blockers</td>
<td>12/15</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>6/15</td>
</tr>
<tr>
<td>diuretics</td>
<td>3/15</td>
</tr>
<tr>
<td>platelet aggregation inhibitors</td>
<td>11/15</td>
</tr>
<tr>
<td>lipid lowering drugs</td>
<td>10/15</td>
</tr>
</tbody>
</table>
(KCl) (60 mM) with 30 minute intervals. Subsequently a single concentration of the \( \alpha_1 \)-adrenergic receptor agonist phenylephrine (0.3 mM) was added and once the vascular contraction had reached a plateau, endothelium-dependent vasodilation was tested by exposure to methacholine (1\( \mu \text{M} \)). The priming procedure was completed by a third KCl-induced contraction. The responses induced by the third potassium challenge were taken as maximal (100%), and the following contractions to KCl in the presence of one of the agents tested were expressed as a percentage of the maximum. Preparations showing less than 10 mN contraction provoked by KCl were discarded.

Immediately after the last KCl-induced response the ring preparations were submerged with solutions of an equal concentration of lacidipine, nifedipine or papaverine (0.1 mM) for 30 minutes, respectively. Directly after the incubation period contractions evoked by KCl or phenylephrine were established. The preparations were flushed three times with fresh buffer and during the next 24 hours contractions mediated by KCl or phenylephrine were repeated every 30 minutes for 24 hours. after each KCl-induced contraction, followed by continuous superfusion.

Appropriate controls were run simultaneously in different ring preparations obtained from the same vascular segments.

**Drugs used**

Acetyl-\( \beta \)-methylcholine chloride, nifedipine, papaverine hydrochloride and phenylephrine hydrochloride were obtained from Sigma (St. Louis, MO, U.S.A.); lacidipine from Boehringer Ingelheim (Alkmaar, The Netherlands).

All drugs were dissolved in distilled water, except nifedipine and lacidipine. Nifedipine was dissolved in 67% dimethyl sulfoxide (DMSO) to a stock solution of 10 mM and lacidipine was dissolved in 100% DMSO to a stock solution of 20 mM. The maximal concentration of the vehicle in the organ bath was 0.7% and it did not affect the results as studied in separate control experiments. All experiments were performed under the exclusion of light.

**Solutions used**

The Krebs-Henseleit solution used for the experiments had the following composition (mM): NaCl 118.0; KCl 4.7; NaHCO\(_3\) 25.0; MgSO\(_4\) 1.2; CaCl\(_2\) 2.5; KH\(_2\)PO\(_4\) 1.1; and glucose 5.6.

The KCl solution had the same composition as the Krebs-Henseleit solution used, except for the NaCl (118 mM) which had been partially replaced by an amount of KCl corresponding to a concentration of 60 mM.
The composition of the University of Wisconsin solution (ViaSpan, Du Pont, Wilmington, DE, U.S.A.) has been described previously.\textsuperscript{16}

\textit{Statistical analysis}

The data were expressed as means ± S.E.M. for \( n \) observations. The results for the compounds investigated were analysed by using a computer program (Graph Pad, Institute for Scientific Informatics, San Diego, CA, U.S.A.). The statistical significance of the differences was analysed by means of two-sided Student's \( t \)-test for unpaired data. Values of \( P < .05 \) were considered significant.

\textbf{Results}

\textit{Internal mammarian artery}

In human IMA preparations the absolute contractile force induced by 60 mM KCl-solution amounted 18.1 ± 0.8 mN. In the control preparations this response could be maintained during the experiment and was 112.5 ± 14.1\% of the initial value after 24 hours of superfusion (Figure 2A).

The incubation of IMA ring preparations with lacidipine, nifedipine or papaverine (0.1 mM) for 30 minutes resulted in the inhibitions of KCl-induced responses that were similar for the three compounds. Contractile force produced after incubation was 34.6 ± 11.1\%, 20.1 ± 4.4\% and 9.6 ± 4.4\% of the initial value for lacidipine, nifedipine and papaverine, respectively (P n.s.) (Figure 3).

After a washout period of 30 minutes the inhibitory effect of papaverine was less than that shown for lacidipine or nifedipine, and completely absent after another 30 minutes (Figure 2A). The duration of action of nifedipine was longer when compared to papaverine. Nifedipine reached its maximal effect (85.1 ± 1.3\% inhibition) 30 minutes after the end of the incubation period. Following 1.5 hour of superfusion the inhibitory effect of nifedipine was smaller than that of lacidipine. The blockade by nifedipine of KCl-induced contractions was attenuated at 8.5 hours, and the contractile force evoked by depolarisation was comparable to that of control preparations (Figure 2A).

Immediately after exposure to lacidipine the inhibition of responses to KCl amounted 65.4 ± 11.1\% and increased during the next 1.5 hour to its maximum of 89.4 ± 3.1\%. The inhibition by lacidipine of potassium-evoked contraction was maintained after 1.5 hour and persisted throughout the 24 hours experiment (Figure 2A).
Figure 2: Time course of the inhibitory effect on KCl-induced contractile force by lacidipine(■), nifedipine(●) and papaverine(▼) compared to control(∗) after incubation of isolated (A) IMA preparations or (B) SV preparations. Values are given as mean ± SEM, n = 6-7.
In isolated IMA-preparations the maximal effect at contractions elicited by depolarisation was identical for the three compounds tested, thus only the time necessary to reach the maximal effect was different.

**Saphenous vein**

In isolated human SV preparations the exposure to KCl produced contractions of 22.4 ± 1.7 mN. In control SV rings the responses induced by potassium were reproducible during the experiment and amounted to 73.8 ± 15.8 % of the initial value after 24 hours of superfusion (P = n.s.) (Figure 2B).

In contrast to the IMA preparations, the direct inhibition in SV caused by the three compounds was significantly different (Figure 3). The contraction evoked by depolarisation immediately after incubation was 61.3 ± 2.1% of the basic value in preparations exposed to lacidipine, 21.6 ± 3.3% to nifedipine and 5.4 ± 1.3% to papaverine, respectively (n = 6-7, P < .05). The time required to reach the maximal effect with the concentration of 0.1 mM was different between the three compounds. For lacidipine 3.5 hours were necessary to reduce contractions to KCl by 76.6 ± 3.1%, for nifedipine 0.5 hour for 79.4 ± 3.5%, whereas papaverine reached its maximal action (94.6 ± 1.3%) directly after incubation (Figure 2B). Furthermore, the maximal inhibition by papaverine was stronger than that by lacidipine or nifedipine, whereas the

**Figure 3:** The KCl-induced contractions immediately after incubation of IMA and SV preparations with lacidipine, nifedipine or papaverine. Data are expressed as percentage of the maximal response to 60 mM of KCl in the absence of the agents. Values are given as mean ± SEM, n = 6-7. *P < .05 lacidipine, nifedipine and papaverine compared in SV, #P < .05 IMA compared to SV.
Figure 4: Time course of the inhibitory effect on phenylephrine-induced contractile force by lacidipine (□), compared to the inhibitory effect at KCl-induced responses (■) after incubation of isolated (A) IMA preparations or (B) SV preparations. Values are given as mean ± SEM, n = 6-7. *P < .05 for phenylephrine-, compared to KCl-induced responses.
antagonistic effect of lacidipine and nifedipine was the same. In SV lacidipine exhibited less inhibition on depolarisation-produced contractile force than in IMA-preparations. With respect to the duration of action of the three compounds continuously superfused, the following rank order was obtained in isolated SV and IMA preparations: lacidipine > nifedipine > papaverine. In isolated SV the effect of lacidipine was maximal after 3.5 hours and persisted for 24 hours. The effect of nifedipine was abolished after 4.5 hours of superfusion, and that of papaverine after 1.5 hour (Figure 2B). The duration of action of lacidipine was equal in SV and IMA rings. However, the duration of action of nifedipine was shorter in the venous preparations (4.5 compared to 8.5 hours in IMA), and that of papaverine longer (1.5 compared to 1 hour in IMA).

Phenylephrine-induced contractile responses

In both preparations, IMA as well as SV, phenylephrine (0.3 mM) could induce contractile responses which amounted to 13.4 ± 2.0 mN in IMA and 28.8 ± 5.0 mN in SV, respectively (P < .05, n = 4). Lacidipine (0.1 mM) inhibited responses to phenylephrine in IMA maximal by 74.7 ± 8.4%, after 2.5 hours of superfusion (Figure 4A). In SV the maximal effect (± 50 %, P < .05 compared to IMA, n = 4) was reached after 2 hours of superfusion (Figure 4B). The inhibition of phenylephrine-induced contractions was less pronounced when compared to KCl-induced contractions, independent of the preparation studied (Figure 4).

Discussion

To prevent spasm of internal mammary artery and saphenous vein grafts several pharmacological agents have been tested in vitro and in vivo. Besides papaverine and nitroglycerine, phosphodiesterase inhibitors, calcium antagonists, β-blockers and potassium channel openers have been evaluated. In the present study the calcium antagonist lacidipine was selected because of its very long duration of action. This property of the lacidipine may be caused by a specific physicochemical property, the lipophilicity. The lipid solubility of lacidipine implies that the compound dissolves very easily in lipid rich compartments such as the cell membrane. From these compartments it is slowly released and subsequently reaches the calcium channels. In IMA, SV as well as coronary artery, CA proved potent spasmolytic agents when applied topically or systemically. The pathogenesis of vasospasm is unclear yet, however, the recently found increased number of L-type calcium channels in vascular smooth muscle cells within the spastic site of coronary arteries, might explain the
successful treatment of coronary artery spasm with CA. Furthermore, CA might improve grafts’ long-term patency as a result of their effect on gene expression and extracellular matrix formation in human vascular smooth muscle cells. The effect of the compounds was evaluated with respect to depolarisation-induced and $\alpha_1$-adrenoceptor-mediated contractions. Depolarisation is the result of vascular smooth muscle cell stimulation by a multitude of agents. Phenylephrine was selected since it has been demonstrated that in patients subjected to CABG high levels of catecholamines are circulating and that $\alpha$-adrenergic responses in IMA are predominantly mediated by postjunctional $\alpha_1$-adrenoceptors. The comparison of the maximal contractile force induced by phenylephrine showed that SV developed stronger responses than IMA, which is consistent with the findings in other relevant studies.

In this model of superfusion it was demonstrated that the effect of incubation with papaverine was abolished after 1 and 1.5 hours of wash out in IMA and SV, respectively. In previous studies the effect of papaverine was maintained during 2 hours, but was not evaluated for a longer period time. The shorter duration of action of papaverine compared to nifedipine and lacidipine cannot be explained by the lipophilicity of the three compounds solely, since the logP-value of papaverine was higher than that of nifedipine (Table 1). Other physicochemical properties might play a role, like the presence of a basic nitrogen present in the chemical structure of papaverine (Figure 1), which leads to a lower $pK_B$-value of papaverine compared to the other two agents, implicating a higher water solubility for papaverine. In vivo the submersion of the pedicle enclosing the IMA in a papaverine solution might prolong the effect of the agent because of its slow release from the fat tissue. However, because of its high lipophilicity this effect must be present for lacidipine as well. The onset of action should, as mentioned above, be rapid. Only then peroperatively occurring spasm can be prevented or counteracted. The onset of action of lacidipine in arteries was demonstrated to be slow. To overcome this problem we used a high concentration of lacidipine, since in the clinical setting only limited time is available for incubation during the operation. Indeed in IMA the effect on KCl-induced contractile force reached immediately after 30 minutes of incubation with the three compounds was equal. After reaching this effect the spasmolytic action of lacidipine continued to increase up to 1.5 h, whereas the effects of papaverine and nifedipine started to diminish. In isolated SV, however, the effect of lacidipine immediately after submersion was less when compared with the other compounds, and needed 3.5 hours to achieve its maximal effect. The inhibition by lacidipine of depolarisation-mediated contractions, reached immediately after incubation amounted up to 39% in SV and 65% in IMA, respectively. However, for the clinical application an inhibition of 39% of the SV spasm
will probably increase the lumen diameter sufficiently. In SV lacidipine needed more
time for its onset of action when compared to IMA, and the maximal effect reached
with the single concentration applied was less for KCl-induced as well as phenylephrine-
induced contractions. Several factors might contribute to these dissimilarities found
between the artery and the vein. For example the wall thickness, distribution and
properties of calcium channels, and/or the underlying mechanism of contraction
induced.
The excessively long duration of action of lacidipine in IMA and SV exceeded the
boundaries of our protocol. Because of its comparable mechanism of action with
nifedipine (both 1,4-dihydropyridines) the prolonged effect of lacidipine when
compared to nifedipine, is most probably explained by its lipophilicity.
In summary, the present study demonstrated that after the relatively short exposure to a
specific concentration of a highly lipophilic calcium antagonist, spasm induced by
depolarisation as well as by \( \alpha \)-adrenergic stimulation of IMA and SV could be inhibited.
The onset of the spasmolytic effect was rapid, and/or sufficient to suppress spasm, and
sustained for more than 24 hours. The demonstrated difference in the arterial and
venous preparations of the effect of lacidipine is determined by vessel specific
properties. The application of papaverine appears to prevent graft spasm only during a
few hours and might also impair endothelial function, according to literature data. The
systemic application of nitroglycerin can be associated with adverse effects. Before
clinical application of lacidipine, further experimental investigations in vivo will be
required to determine the effect of slow release of the compound on the myocardium
and the systemic circulation.
In conclusion, the present study may offer a new approach to prevent graft spasm
utilising a CA with a very long duration of action. The long spasmolytic effect of
lacidipine is likely to be caused by its lipophilic character. The incubation of the isolated
vessel segments during CABG with this compound, proved promising and beneficial
compared with the currently applied method with papaverine and nitroglycerine.

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Prevention of graft spasm and lipophilic calcium antagonists

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