Vasorelaxant properties of nicorandil on human radial artery

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Abstract

Objective: The radial artery is becoming popular as a conduit for coronary artery surgery but there is concern about its tendency to vasospasm. Diltiazem is used clinically in an effort to prevent vasospasm but there are suggestions that it is relatively ineffective. The first aim of the study was to test the effectiveness of Ca²⁺ antagonists against vasospasm evoked by vasoconstrictor agonists. Because a large component of vasospasm was resistant to Ca²⁺ antagonists, the second aim was to test if a different class of vasodilator, nicorandil, might relax the residual tone. Methods: Isometric tension was recorded in human radial artery segments harvested from patients undergoing myocardial revascularization surgery. Results: Diltiazem at 10 μM, which strongly inhibits L-type voltage-gated Ca²⁺ channels, induced partial relaxation (mean ± SEM, 44.6 ± 3.5%, n = 31) of phenylephrine-evoked contraction, but only 14.0 ± 4.1% (n = 10) and 12.2 ± 4.2% (n = 10) relaxation of U46619- (a thromboxane A₂ analogue) or endothelin-1-evoked contraction. Strikingly, nicorandil relaxed agonist-evoked contractions that were resistant to diltiazem or nicardipine. In the absence of a Ca²⁺ antagonist, nicorandil (30 μM) evoked 74.1 ± 5.6% (n = 24), 36.8 ± 9.3% (n = 10) and 64.5 ± 7.9% (n = 14) relaxation of phenylephrine-, U46619- and endothelin-1-evoked contractions. Conclusions: Nicorandil has a marked relaxant effect on contractions evoked by three different vasoconstrictor agonists, and relaxes vasospasm that is resistant to conventional Ca²⁺ antagonists. These in vitro data suggest that nicorandil might be a useful drug for the inhibition of radial artery vasospasm in myocardial revascularization surgery. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Grafting; Vasospasm; Radial artery; Nicorandil

1. Introduction

Having been abandoned in the 1970s due to post-operative spasm, the last few years have seen the return of radial artery as an important conduit for myocardial revascularization [1–5]. Although long term patency rates are not yet available, the short–mid term patency rates are similar to those of other arterial grafts [1–3,6,7]. The technical implications of radial artery harvesting are probably less demanding than for other arterial conduits, such as gastroepiploic and inferior epigastric arteries. However, it remains a concern that, when compared with other conduits, such as internal mammary and gastroepiploic arteries, the radial artery has a greater reactivity and propensity to spasm [8].

The L-type voltage-dependent Ca²⁺ channel is a well-recognized Ca²⁺-entry pathway which is blocked by clinically-used Ca²⁺-antagonists, such as diltiazem and nifedipine. Diltiazem, in particular, is one of the agents preferred by some surgeons to prevent spasm of the radial artery during and after coronary artery surgery [1–6]. Nevertheless, it has been reported that diltiazem, at a concentration of 200 ng/ml, has little effect on the human radial artery [9]. This justifies an interest in searching for more effective anti-spastic drugs, such as nicorandil, which has been shown to have an anti-spasmogogenic effect on coronary arteries [10].

In order to help inform decisions about the choice of vasodilator following radial artery revascularisation surgery, we have investigated the effectiveness of several Ca²⁺ antagonists (diltiazem, mibebradil and nicardipine) on human radial artery segments in vitro. On finding that substantial components of agonist-evoked contractions were resistant to Ca²⁺ antagonists, we went on to study whether nicorandil, a combined potassium channel opener and nitric oxide donor drug, might be able to prevent the residual vasospasm.

2. Materials and methods

Samples obtained from discarded segments of radial
artery from patients undergoing coronary artery bypass surgery were transported to the laboratory in Hanks solution. Arteries were used either on the same day of surgery, or the next day following over-night storage in Hanks solution at 4°C. Hanks solution contained: NaCl, 137 mM; KCl, 5.4 mM; CaCl₂, 0.01 mM; NaH₂PO₄, 0.34 mM; K₂HPO₄, 0.44 mM; D-glucose, 8; and Hepes, 5 mM (pH 7.4). Loose connective tissue was removed and 3-mm rings were prepared. The rings were mounted on two stainless steel hooks, one of which was fixed and the other connected to an isometric tension transducer in a glass organ bath containing 25 ml of Krebs solution bubbled with 95% O₂/5% CO₂ at 37°C. Krebs solution contained: NaCl, 118.3 mM; KCl, 4.6 mM; MgSO₄, 1.2 mM; NaHCO₃, 25 mM; KH₂PO₄, 1.2 mM; CaCl₂, 2.5 mM; and glucose, 11 mM. In preliminary experiments, it was observed that arterial rings stretched to a resting tension of 1 g and equilibrated for 90 min (during which time they were rinsed every 30 min) exhibited consistent responses to phenylephrine, and these conditions were used for all subsequent experiments.

Contractions evoked by phenylephrine faded spontaneously in the continuous presence of the agonist. In all experiments involving phenylephrine, a control response was obtained and then phenylephrine was washed out. A second application of phenylephrine then occurred and the vasodilator was applied. Two methods were used to analyze the resulting data: (1), the percentage relaxation in control conditions was compared with that in the presence of the vasodilator at the same time-point after phenylephrine application (Table 1); and (2), the difference between the tone level in the control and in the presence of the vasodilator was obtained at the same time-point after phenylephrine application, and then divided by the tone level in the control to give a percentage (Fig. 2D and data in the abstract). Contractions to U46619 and endothelin-1 did not fade. Vasodilator effects are expressed as a percentage of the initial (pre-vasodilator) tone to eliminate differences due to variations in the absolute level of tone in each vessel segment.

All statistical comparisons were made using paired, two-tailed Student’s t-tests, and differences were taken to be statistically significant at \( P < 0.05 \). Data within sets were approximately normal in distribution. Results are expressed as mean ± SEM. The value of \( n \) indicates the number of arterial segments. Concentration–response curves to nicorandil were constructed cumulatively, and thus \( n \) is the same for each concentration applied, except in cases where the highest concentration was not applied because full relaxation had already been obtained (see legend to Fig. 2D). The numbers of patients from whom samples were obtained were: fifteen for the phenylephrine/diltiazem experiments; seven for phenylephrine/nicorandil experiments; four for U46619 experiments; and four for endothelin-1 experiments. When phenylephrine was the agonist, diltiazem and nicorandil were tested in separate vessel segments. When U46619 or endothelin-1 were the agonists, nicorandil was tested first, and then nicorandil and the agonist were washed out and the agonist was reapplied before testing diltiazem. Nicorandil again produced vasodilation in the presence of diltiazem (data not shown), suggesting that the first applications of nicorandil had been effectively washed out before diltiazem was applied. All data presentation and mathematical fitting of functions to data using a least-squares method were performed by the program Origin (version 4.1; MicroCal, Inc, Northampton, MA). Concentration-effect data were fitted to the Hill equation (Eq. (1))

\[
y = \left(\frac{x^A}{x^B + EC_{50}}\right)
\]

where \( x \) is the slope and \( A \) is the maximum value of \( y \).

Diltiazem, nicardipine and Heps were from Sigma (Poole, Dorset, UK). General salts were from BDH (Merk Ltd.) (Poole, Dorset, UK). Nicorandil and mibefradil were gifts from Rhône-Poulenc Rorer (Kings Hill, Kent, UK) and Roche (Basel, Switzerland), respectively. Stock solutions of diltiazem, nicorandil and mibefradil were prepared in Milli-Q-grade water, while nicardipine was prepared in dimethylsulphoxide (DMSO). The final concentration of DMSO in the recording bath was 0.01%.

Ethics committee approval was obtained from the Research Ethics Committee, Leeds Health Authority/United Leeds Teaching Hospitals, Leeds, UK.

### 3. Results

Phenylephrine (3 \( \mu \)M), endothelin-1 (10 nM) or U46619 (30 nM) induced submaximal contractions in human radial artery segments. The contractile effect of phenylephrine usually faded in the continued presence of the agonist, whereas responses to endothelin-1 or U46619 were more slowly-developing and sustained (see below). Some arterial segments showed spontaneous oscillatory contractile activity or agonist-induced oscillations, notably in the presence of U46619 (data not shown).

The effect of diltiazem on phenylephrine-contracted segments was investigated by applying 10 \( \mu \)M diltiazem at the peak of the phenylephrine-induced contraction, and

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Relaxation (%)</th>
<th>( n )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem (10 ( \mu )M)</td>
<td>11.32 ± 0.51</td>
<td>55.68 ± 2.91</td>
<td>31</td>
</tr>
<tr>
<td>Control</td>
<td>11.32 ± 0.51</td>
<td>17.10 ± 3.99</td>
<td>31</td>
</tr>
<tr>
<td>Nicorandil (10 ( \mu )M)</td>
<td>9.83 ± 0.61</td>
<td>39.13 ± 4.94</td>
<td>24</td>
</tr>
<tr>
<td>Control</td>
<td>9.83 ± 0.61</td>
<td>4.54 ± 2.23</td>
<td>24</td>
</tr>
<tr>
<td>Nicorandil (30 ( \mu )M)</td>
<td>17.77 ± 0.61</td>
<td>77.16 ± 4.77</td>
<td>24</td>
</tr>
<tr>
<td>Control</td>
<td>17.77 ± 0.61</td>
<td>10.37 ± 2.61</td>
<td>24</td>
</tr>
<tr>
<td>Nicorandil (100 ( \mu )M)</td>
<td>26.07 ± 1.38</td>
<td>79.69 ± 4.93</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>26.07 ± 1.38</td>
<td>19.85 ± 3.08</td>
<td>13</td>
</tr>
</tbody>
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\( ^* \) P-values are for comparisons of the control and test, e.g. diltiazem, data sets. See section 2 for the analysis procedure.
then comparing the degree of fade of the contraction with a time-matched control response to phenylephrine. An example of a control response to phenylephrine, and then, subsequently in the same vessel segment, a response to phenylephrine with the addition of diltiazem are shown (Fig. 1A,B). As described in section 2, phenylephrine-induced contraction was measured under control conditions and in the presence of diltiazem in the same arterial segment. The time-point for measurement of the response varied depending on the speed of effect of diltiazem. Results of these experiments are given in Table 1. Diltiazem (10 μM) had a significant relaxant effect against phenylephrine-evoked contraction, but did not abolish contraction. Diltiazem was used at a concentration of 10 μM because this blocks Ca^{2+}-flux through smooth-muscle L-type, voltage-gated Ca^{2+} channels by at least 90% [11]. The effectiveness of diltiazem (10 μM) in blocking these Ca^{2+} channels was confirmed in experiments in which diltiazem abolished spontaneous or agonist-induced oscillatory contractions that occurred in some radial artery segments (data not shown). Mibefradil, an L- and T-type Ca^{2+} channel antagonist, had a small relaxant effect against phenylephrine-induced contraction, and 10 μM diltiazem had no additional effect in the presence of mibefradil (Fig. 1C). Therefore, there appeared to be a component of phenylephrine-evoked contraction that did not depend on L-type Ca^{2+} channel activity.

Diltiazem (10 μM) had a small effect or no effect on U46619-evoked contraction (Fig. 1D), on average evoking a 14.0 ± 4.1% relaxation (n = 10). Diltiazem (1 and 10 μM) had a small or no effect on endothelin-1-induced contraction (Fig. 2A). On average, 10 μM diltiazem induced a 12.2 ± 4.2% relaxation of endothelin-1-evoked contraction (n = 10).

In the presence of 1 μM nicardipine, which completely blocks L-type Ca^{2+} channel-current in arterial smooth muscle, phenylephrine evoked a contraction, confirming that there is a component of contraction which is resistant to conventional Ca^{2+} antagonists (Fig. 2B). Our aim was to determine if this resistant contraction might be susceptible to nicorandil. This did appear to be the case because a strong relaxation was induced by 10 μM nicorandil in the presence of diltiazem.
of 1 μM nicardipine (Fig. 2B). Furthermore, nicorandil relaxed endothelin-1-induced contraction, which we have already shown is resistant to 10 μM diltiazem (Fig. 2A).

In the absence of Ca²⁺ antagonists, nicorandil evoked concentration-dependent relaxation of contractions elicited by phenylephrine, endothelin-1 or U46619 (Fig. 2C, D). The data presented in Fig. 2D are corrected for the spontaneous fade observed in phenylephrine-evoked contraction (see section 2). Endothelin-1- and U46619-evoked contractions were sustained, and thus a correction was not made. Nicorandil elicited similar concentration-dependent relaxant effects against endothelin-1-induced contraction in the presence of 10 μM diltiazem (data not shown). Thus, nicorandil elicited relaxation of tension evoked by three different spasmogens, and relaxed tension that was resistant to classical Ca²⁺ antagonists.

4. Discussion

The radial artery is being increasingly used as a graft for myocardial revascularization because of its good short- and mid-term patency rates [1–3, 7]. Some surgeons suggest the routine use of bilateral radial arteries [4]. Better outcomes have, in fact, been reported with the use of the radial artery compared to the right internal thoracic artery [12]. Being a type III vessel [13], the radial artery is a muscular artery and therefore more prone to spasm peri-operatively, especially with the use of α-adrenoceptor agonists.

According to the surgical literature, mid-term patency rates of the radial artery vary from 93.5% at 9–10 months to 83% at 5 years [1, 3, 6, 7]. This would indicate that as many as 17% of patients develop occlusion of the radial artery graft, which may conceivably be due to the spastic charac-
teristics of the graft. With the current trend towards total arterial revascularization, such incidence of spastic occlusion of the graft has a potentially important impact on a large number of patients undergoing coronary artery grafting.

Varying pharmacological strategies have been used to prevent peri-operative spasm of the radial artery. A number of topical solutions have been used during harvesting of the grafts. Papaverine, a non-specific vasodilator, is commonly used [1,4,5,14]. However, He and colleagues showed that a topical solution of a Ca$^{2+}$-channel antagonist (verapamil) and a nitrate (nitro-glycerine) is as effective as papaverine in relaxing radial artery rings in vitro, but has a more rapid onset and more long lasting effect [15].

In addition to the topical application of anti-spasmodics, the use of systemic vasodilators is considered essential by some surgeons [8]. Some workers have advocated the use of an intravenous infusion of diltiazem (1 μg/kg per min) which is continued in the intensive care unit [1,3–6], whereas others prefer the use of intravenous phosphodies-terase inhibitors or nitro-glycerine [14,16]. Oral administration of Ca$^{2+}$-channel antagonists, such as diltiazem or amlodipine, is begun early after the operation and maintained for 6–12 months [1,3,5,7,14,16].

Despite the relative success of the radial artery as a graft for coronary revascularisation, a recent report questions the effectiveness of diltiazem in the prevention of radial artery spasm. In it, Cable and colleagues consider the clinical use of diltiazem for the prevention of graft spasm to be empirically based and sub-optimal. They also report that organic nitrates (nitric oxide donors), such as isosorbide dinitrate and nitro-glycerine, inhibited and reversed radial artery contractions effectively, and that these compounds represent a more reasonable choice for preventing radial artery graft spasm [9].

In this context, we decided to study the effectiveness of nicorandil in counteracting the vasospastic characteristics of the radial artery graft. No report has yet been published on the use of nicorandil on the radial artery in vitro. Its usefulness in the treatment of ischaemic heart disease has been widely accepted. Nicorandil (N-2-hydroxyethylnicotinamide nitrate) is a hybrid between a nitrate and an ATP-sensitive potassium channel opener [17,18]. It produces vasodilation by a dual-action mechanism, a nitrate-like action with an increase in intracellular cyclic GMP levels, and hyperpolarizing action via activation of ATP-sensitive potassium channels [19]. Nicorandil has been found to dilate human coronary and internal mammary arteries and small resistance arteries in vitro and in vivo [20,21]. Previous reports have suggested that, in vitro, nicorandil has a significantly lower negative inotropic effect than Ca$^{2+}$-channel antagonists, such as diltiazem and nifedipine, in the failing and non-failing ventricle [10].

We investigated the effectiveness of several clinically-available vasodilators to relax in vitro human radial artery segments obtained from patients undergoing myocardial revascularization surgery. Arterial segments were contracted by submaximally-effective concentrations of three receptor agonists: phenylephrine; endothelin-1; or U46619 (a stable thromboxane A$_2$ mimetic). These vasoconstrictors were chosen because they are likely to play an important role in producing radial artery-graft spasm in patients undergoing myocardial revascularization surgery. Plasma levels of all three vasoconstrictors are raised during and after conventional coronary bypass surgery [22–26]. They might all act synergistically in producing peri-operative vasospasm. It has been reported that endothelin may play an even more important role in diabetic patients undergoing myocardial revascularization surgery [27]. The effect of thromboxane A$_2$ antagonists on the human internal mammary artery and its implications on coronary artery surgery have been discussed elsewhere [28].

The Ca$^{2+}$-antagonist diltiazem did inhibit some elements of contraction observed in the radial artery, notably the spontaneous rhythmical activity and part of the phenylephrine-induced contraction. However, a large component of phenylephrine-induced contraction was resistant to diltiazem, and endothelin-1- and U46619-induced contractions were almost completely resistant. Two other, structurally unrelated, Ca$^{2+}$-antagonists (mibefradil and nicardipine) did not offer any advantage. In contrast, nicorandil induced strong relaxations and was more broadly effective. Therefore, nicorandil may help to minimize vasospasm of radial arteries used in coronary bypass surgery.

In light of these results, it seems that nicorandil may be a better alternative to diltiazem in preventing spasm of the radial artery graft following coronary artery bypass surgery. Caution must, however, be exercised when attempting to extrapolate these in vitro studies to the clinical setting. Determination of the relative effectiveness of nicorandil in clinical practice will, of course, only be shown by randomized, controlled trials involving the use of post-operative coronary angiography or another type of imaging.

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