Nitric oxide modulation of blood vessel tone identified by arterial waveform analysis

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ABSTRACT
Traditionally, nitric oxide-mediated alteration in blood vessel tone has been inferred from changes in flow in response to physical and pharmacological interventions using plethysmographic or ultrasonic techniques. We hypothesized that alteration in pulsatile arterial function may represent a more sensitive measure to detect and monitor nitric oxide-mediated modulation of arterial smooth muscle tone. Healthy male volunteers (n=15) had radial artery pressure pulse waveforms recorded using a calibrated tonometer device. A computer-based assessment of the diastolic pressure decay was employed to quantify changes in arterial waveform morphology in terms of altered pulsatile (arterial compliance) and steady-state (peripheral resistance) haemodynamics. \(\text{N}^\text{G}\)-nitro-L-arginine methyl ester (L-NAME), a stereospecific inhibitor of nitric oxide synthesis, was infused intravenously in incrementally increasing doses of 0.25, 0.5 and 0.75 mg/kg for 8 min each. Subjects then received either L-arginine or D-arginine (200 mg/kg over 15 min) intravenously in a blinded fashion. On a separate day, subjects had radial artery pressure pulse waveforms recorded before and after the sublingual administration of glyceryl trinitrate, an exogenous donor of nitric oxide. Cardiac output and heart rate decreased and mean arterial blood pressure increased significantly (P < 0.01 for all) in response to the incremental intravenous infusion of L-NAME. Small artery compliance decreased, whereas systemic vascular resistance increased in response to nitric oxide synthesis inhibition (P < 0.01 for both). The intravenous infusion of L-arginine restored the pulsatile and steady-state haemodynamic parameters to pre-treatment values, whereas D-arginine had no effect. Sublingual glyceryl trinitrate decreased systemic vascular resistance by 11%, whereas large artery- and small artery-compliance increased by 25% and 44% respectively. Pressure pulse contour analysis represents a sensitive and convenient technique capable of tracking changes in the pulsatile function of arteries accompanying nitric oxide-mediated alteration in arterial smooth muscle tone.

INTRODUCTION
Endothelium-derived nitric oxide plays a pivotal role in controlling the structure and tone of arterial blood vessels [1]. Impaired nitric oxide-mediated vasodilation has been found in patients with risk factors for and disease states associated with an increase in cardiovascular events [2]. As endothelium-derived nitric oxide is viewed as vaso-protective, restoration of nitric oxide synthesis and activity is increasingly viewed as a therapeutic target for pharmacological interventions [2,3]. Clinical investigators most commonly employ venous occlusion plethysmography or ultrasound measurements of diameter changes in conduit arteries to monitor nitric oxide-mediated alteration in blood vessel tone.
Impaired pulsatile arterial function is now recognized as an independent predictor of cardiovascular risk [8,9]. Impaired pulsatile function of arteries and defective nitric oxide-mediated control of arterial tone are common accompaniments in disease states associated with an increase in cardiovascular events [10,11]. We have previously reported that endothelial dysfunction and impaired pulsatile arterial function are present in patients with diabetes mellitus [12,13], and that these measures can be favourably influenced by therapeutic interventions, without significant alteration in steady-state haemodynamic variables [14,15]. Furthermore, the recognition that the acute administration of glyceryl trinitrate, an exogenous donor of nitric oxide, can improve the compliance characteristics of arteries suggests a physiological link between nitric oxide-mediated control of smooth muscle tone and the pulsatile function of arteries [16].

Measures of pulsatile arterial function can be derived from quantitative analysis of the arterial pressure pulse contour, that can now be recorded non-invasively and reproducibly by applanation tonometry [11]. Using this technique we studied the relationship between nitric oxide modulation of arterial tone and pulsatile arterial function.

**METHODS**

**Participants**

Healthy male volunteers (n = 15) aged 19–48 years were recruited for study. All subjects underwent a full history and examination that included an electrocardiogram. No subjects were taking any drugs prior to or at the time of the study. All subjects gave written informed consent for all procedures. The studies were approved by the local Ethical Committee of the Queen’s University of Belfast.

**Procedures**

All studies were performed in a quiet laboratory in the Department of Therapeutics, with the subjects resting supine. The subjects refrained from consuming alcohol, tobacco or caffeine for 12 h prior to the study. An intravenous cannula was inserted into the left antecubital vein for infusion of drugs.

Radial artery pressure pulse waveforms were recorded by an acoustic transducer using the HDI/pulsewave™ CR-2000 research cardiovascular system (Hypertension Diagnostic Inc., Egan, MN, U.S.A.). A wrist stabilizer was positioned on the right wrist by two hooks and loop straps. This was designed to gently immobilize the wrist and stabilize the radial artery, making it readily accessible for placement of the arterial pulse pressure sensor. The pressure sensor, fabricated from medical-grade stainless steel, was positioned over the radial artery and held in place using a holding and positioning device on a manually adjustable shaft. Optimal pressure pulse waveforms were recorded by carefully placing the device and the attached sensor and adjusting the hold-down pressure by rotating the knob on top of the device. The recorded waveforms were calibrated by the oscillometric method, with the cuff on the opposite arm and a calibration system internal to the device. Once optimal waveforms and a stable baseline were achieved, no further manipulation of the device was required in any of the studies. Cardiac output was estimated from an algorithm which incorporated a multivariate function of age and body surface area in addition to heart rate and ejection time, determined from the arterial pressure waveforms, as previously described [17].

**Beat marking, waveform and data analysis**

Non-invasive radial artery waveforms were recorded for 30 sec for each subject in the supine position. Blood pressure waveforms were digitized at 200 samples/s, and stored on a personal computer. The data were automatically displayed on the computer screen for visual analysis to confirm that the recorded waveforms were uniform and without artifact. Individual beats, demarcated with the upstroke beat mark as a fiduciary time point, were cross-correlated. Those with a correlation coefficient of < 0.95 were discarded. To obtain a measure of arterial compliance, a model was used that divides the total systemic arterial compliance into large artery or capacitive and small artery or oscillatory compliances. The model describes diastolic pressure contours by the following equation:

\[
P_i(t) = A_{1i} - A_{2i} + A_{3i} - A_{4i} \cos (A_{5i} t + A_{6i})
\]

where \(P_i(t)\) is the diastolic pressure at time \(t\) relative to aortic value closure. A parameter-estimating algorithm was applied for determination of the best set of \(A_i\) values for matching the diastolic portion of the measured beat to this equation. These \(A_i\) parameters, together with an estimate of systemic vascular resistance, determine the 2 compliances [11]. The compliance values for each beat were weighted inversely with respect to an estimate of error (to minimize potential error estimates in the \(A_i\) parameters) and then averaged. The estimate of error was the predicted variance in the compliance divided by a measure of goodness-of-fit of the model to the data. This approach ensures that individual compliance values with high estimated variance will contribute proportionally less to the overall compliance value. Additionally, end-diastolic distortions were eliminated by defining
Table 1  Effect of nitric oxide modulation on pulsatile and steady-state haemodynamics

Data are the means and 95% confidence intervals (* P < 0.05 versus control, † P < 0.01 versus control, ** P < 0.05 d-arginine versus l-arginine, ‡ P < 0.01 d-arginine versus l-arginine). 1 dyn = 10⁻⁵ N.

<table>
<thead>
<tr>
<th>Haemodynamic parameters</th>
<th>Dose of l-NAME (mg/kg, n = 15)</th>
<th>l-Arginine (200 mg/kg, n = 9)</th>
<th>D-Arginine (200 mg/kg, n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (l/min)</td>
<td>Baseline</td>
<td>5.3 (5.1, 5.5)</td>
<td>5.1 (4.8, 5.4)</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>5.2 (5.0, 5.4)</td>
<td>4.8† (4.5, 5.0)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5.0† (4.8, 5.3)</td>
<td>5.0† (4.5, 5.0)</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>4.8† (4.5, 5.0)</td>
<td>4.8** (4.2, 5.3)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>60 (55, 66)</td>
<td>56 (51, 61)</td>
<td>56 (48, 65)</td>
</tr>
<tr>
<td></td>
<td>64 (51, 61)</td>
<td>52† (27, 57)</td>
<td>50† (45, 54)</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>Baseline</td>
<td>80 (77, 0.38)</td>
<td>83 (76, 90)</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>0.25</td>
<td>84* (79, 88)</td>
<td>90‡ (88, 100)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>89† (84, 93)</td>
<td>92† (87, 97)</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>92† (87, 97)</td>
<td>92† (87, 97)</td>
</tr>
<tr>
<td>Systemic vascular</td>
<td>Baseline</td>
<td>1174 (1105, 1243)</td>
<td>1293 (1271, 1367)</td>
</tr>
<tr>
<td>resistance (dyn·cm·s⁻¹)</td>
<td>1292† (1211, 1373)</td>
<td>1416† (1319, 1513)</td>
<td>1550† (1460, 1641)</td>
</tr>
<tr>
<td>Large artery compliance</td>
<td>Baseline</td>
<td>18.9 (16.8, 21.0)</td>
<td>19.9 (17.4, 22.3)</td>
</tr>
<tr>
<td>(ml/mmHg × 10)</td>
<td>10.9</td>
<td>18.9 (16.7, 21.2)</td>
<td>19.9 (17.4, 22.3)</td>
</tr>
<tr>
<td>Small artery compliance</td>
<td>Baseline</td>
<td>10.2 (8.9, 11.6)</td>
<td>10.4 (8.5, 12.3)</td>
</tr>
<tr>
<td>(ml/mmHg × 100)</td>
<td>9.3*</td>
<td>7.7† (6.3, 9.1)</td>
<td>6.9† (5.5, 8.3)</td>
</tr>
</tbody>
</table>

end-diastole as the point where diastolic pressure is no longer monotonically decreasing.

Protocol

L-NAME (N⁵-nitro-L-arginine methyl ester) HCl, L-arginine HCl and D-arginine HCl were purchased from Clinalfa, Läufelfingen, Switzerland. Intravenous L-NAME (0.25 mg/kg, 0.5 mg/kg and 0.75 mg/kg) was infused at each dosage for 8 min at 1 ml/min via a Braun Perfusor VI infusion pump. Haemodynamic and pulse contour parameters were measured over a 20 min control period, during which the stability of baseline measurements were confirmed, and during the last 2 min of each infusion of L-NAME. Subjects then received either L-arginine (n = 9) or D-arginine (n = 6) infused intravenously in a blinded fashion as a 10% solution over 15 min to a total dose of 200 mg/kg, as previously described [18]. Haemodynamic and pulse contour parameters were again estimated on completion of the L-arginine and D-arginine infusions respectively.

On another day each subject returned to the laboratory to study the effect of 300 μg of sublingual glyceryl trinitrate on haemodynamic and pulse contour parameters. On attaining optimal and stable radial artery waveforms, haemodynamic and pulse contour parameters were recorded before and at 3, 6 and 9 min after the administration of glyceryl trinitrate.

Statistical analysis

Data was statistically analysed using two-factor repeated measures ANOVA (SPSS for Windows, Version 6.1.3). If P < 0.05 for overall effect, the significance of difference between the mean at each dose level (× 1) and at baseline (X₀) was determined from the ‘r’ statistic calculated as (X₀ – X₁)/square root (residual mean square °2/n). The significance of difference between responses to D- and L-arginine was determined using the difference data from control and from the maximum cumulative dose of L-NAME; these difference data were analysed by one-factor ANOVA for group [D-arginine (n = 6), L-arginine (n = 9)]. Data was expressed as mean (95% confidence intervals), with data being regarded as statistically significant at P < 0.05.

RESULTS

Table 1 shows the changes in steady-state and pulsatile haemodynamics in response to the intravenous infusion of L-NAME. Cardiac output and heart rate decreased progressively from baseline values, with increasing doses of L-NAME 5.3 (5.1, 5.5) to 4.8 (4.5, 5.0) litres/min and 60 (55, 66) at baseline to 50 (45, 54) beats/min respectively (P < 0.01 for both) after L-NAME. Mean arterial pressure increased progressively from 80 (77, 84) at baseline to 92 (87, 97) mmHg (P < 0.01) after the L-NAME infusion. The infusion of L-arginine restored these variables to pre-treatment levels. The normalization of haemodynamic responses observed with the L-arginine infusion were significantly different to those recorded with D-arginine, which exhibited no significant haemodynamic effect (P < 0.01 for each variable).

Figure 1 illustrates the effect of endothelium-derived nitric oxide modulation on the intravenous infusion of L-NAME, L-arginine and D-arginine on systemic vascular resistance, large artery compliance and small artery compliance. Systemic vascular resistance increased pro-
The effect of nitric oxide modulation on (a) systemic vascular resistance, (b) large artery compliance and (c) small artery compliance.

Data are means and 95% confidence intervals (*P < 0.05 versus control; †P < 0.01 versus control and ‡P < 0.01 D-arginine versus L-arginine).

Figure 1 The effect of nitric oxide modulation on (a) systemic vascular resistance, (b) large artery compliance and (c) small artery compliance.

Progressively from a baseline value of 1174 (1105, 1243) to 1550 (1460, 1641) dyn.cm.s⁻¹, while small artery compliance decreased from 10.2 (8.9, 11.6) to 6.9 (5.5, 8.3) ml/mmHg <100 in response to the intravenous infusion of L-NAME (P < 0.05 for both). In contrast, no change was detected in the large artery compliance estimate to the infusion of L-NAME. The intravenous infusion of L-arginine largely reversed the changes in small artery compliance and systemic vascular resistance, and these responses were significantly different from that recorded with the infusion of D-arginine (P < 0.01 for both). Figure 2 depicts the characteristic changes in pressure pulse contour morphology observed in response to nitric oxide-modulation of smooth muscle tone.

The effects of sublingual glyceryl trinitrate on the haemodynamic parameters were not different after 3, 6 and 9 min. After 3 min, heart rate increased from 63 (57,68) to 66 (57,72) beats/min (P < 0.05), mean arterial pressure decreased from 81 (76,85) to 79 (75 to 85) mmHg (P > 0.05) and cardiac output remained unchanged 5.5 (5.3,5.7) to 5.7 (5.4 to 6.0) litres/min (P > 0.05). Systemic vascular resistance decreased from 1157 (1053,1265) to 1021 (933,1109) dyn.cm.s⁻¹ (P < 0.01), while large artery compliance increased from 17.0 (14.9,17.2) to 21.3 (18.1 to 24.6) ml/mmHg ×10 (P < 0.05), and small artery compliance increased from 9.6 (7.9,11.2) to 13.8 (10.8, 16.8) ml/mmHg ×10 (P < 0.01) respectively. Figure 3 depicts typical changes in pressure pulse contour morphology observed in response to the sublingual administration of glyceryl trinitrate.

**DISCUSSION**

Characteristic and consistent changes in the arterial pressure pulse waveform morphology were found in response to nitric oxide modulation of arterial blood vessel tone. Pronounced changes in the arterial waveshape and pulsatile parameters could be detected with minimal alteration in systemic haemodynamics, suggesting that estimates of pulsatile arterial function represent sensitive measures for identifying altered tone in the arterial vasculature.

Loss of the oscillatory waveform that distorts the proximal part of the diastolic pressure decay from a pure exponential has been consistently identified as a sensitive marker for altered structure or tone in the vasculature with ageing and disease states associated with an increase in cardiovascular events [11,12,19,20]. This morphological feature arises from wave reflection and a dampened resonance occurring in the arterial tree, with the major site of reflected waves originating in the smaller arteries and arterioles [21]. Loss of the oscillatory diastolic waveform is recognized as an early feature of impaired pulsatile arterial function, since it can be found in patients at increased cardiovascular risk without alteration in steady-state haemodynamics [12,19,22,23]. Inhibition of endothelial nitric oxide synthesis in response to increasing doses of L-NAME was associated with progressive diminution in the amplitude and frequency of the oscillatory diastolic waveform, identified as a decrease in small artery compliance in the model analysis. The change in waveform morphology during the diastolic interval was accompanied by the appearance or increase in amplitude of a secondary waveform in late systole. In every case, the infusion of L-arginine, in a dose previously shown to have no effect on baseline blood pressure [18], restored blood pressure almost to pre-treatment levels and reversed the altered waveform morphology observed in response to L-NAME. D-Arginine had no effect on these parameters, thus confirming the nitric oxide dependence of these changes. Arterial compliance can be
Influenced by a number of haemodynamic factors. With increasing doses of L-NAME changes in haemodynamic variables confound interpretation of the effect of nitric oxide modulation of smooth muscle tone and estimates of arterial compliance.

Sublingual glyceryl trinitrate provides an exogenous source of nitric oxide that directly relaxes vascular smooth muscle tone. Characteristic changes in arterial waveform morphology accompany the administration of nitrovasodilators, and have been recognized for more than a century as a sensitive means to detect the haemodynamic action of these compounds [24,25]. Alteration in pulsatile arterial function, identified by changes in the pressure pulse contour in response to
G. E. McVeigh and others

Alterations in pulsatile haemodynamics, identified by pressure pulse contour analysis, occurred consistently and predictably in response to nitric oxide modulation of arterial tone. It is possible that alteration in the pressure pulse contour, particularly loss of the oscillatory diastolic waveform, which has been repeatedly identified in association with cardiovascular risk factors, may have its origins in nitric oxide-mediated alteration in blood vessel structure and tone. Support for this hypothesis will require prospective outcome studies that demonstrate an association between nitric oxide synthesis in relation to changes in the pressure pulse contour in response to therapeutic interventions. With the advent of non-invasive techniques capable of accurately and reproducibly tracking changes in the arterial pulse contour over time, this goal can now become a reality.

REFERENCES

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