Impairment of forearm vasodilatation to acetylcholine in hypercholesterolemia is reversed by aspirin

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Abstract

Objective: Impaired cholinergic vasodilatation in the forearm in hypertension and hypercholesterolemia has been attributed to impaired nitric oxide bioavailability. However, inhibition of cyclooxygenase reverses the impaired cholinergic dilatation in hypertensive animals and patients. The aim of this study was to examine the effect of aspirin on cholinergic vasodilatation in hypercholesterolemic patients. Methods: We examined responses to brachial artery infusion of acetylcholine and the endothelium-independent vasodilator sodium nitroprusside in the presence or absence of aspirin in 10 hypercholesterolemic patients (7 men/3 women; aged 38–63 yr; systolic blood pressure 133 ± 5 mmHg; diastolic blood pressure 80 ± 3) compared with 10 matched controls (7 men/3 women; aged 38–63 yr; systolic blood pressure 126 ± 2; diastolic blood pressure 77 ± 2). Results: In hypercholesterolemics, forearm vasodilatation was impaired in response to acetylcholine (112 ± 20 vs. 346 ± 30% increase in blood flow in controls, at the highest dose [15 μg min −1]; P < 0.0001) but not in response to sodium nitroprusside. With the addition of aspirin, baseline forearm blood flow was unaltered. However, forearm vasodilatation to acetylcholine was partially restored in hypercholesterolemics from 112 ± 20 to 193 ± 30%; P < 0.001 though not affected in controls. Vasodilator responses to sodium nitroprusside were unaffected by aspirin in either group. Conclusions: In hypercholesterolemia, an altered balance between vasoconstrictor and dilator prostanoids, favouring constrictors, may contribute to endothelial dysfunction either directly or through an effect on nitric oxide synthesis. Restoration of this imbalance may be a component of the therapeutic benefit of aspirin in cardiovascular disease. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cholinergic vasodilatation is dependent on a functioning vascular endothelium [1] and may be mediated by generation of nitric oxide, dilator prostanoids, [2] or endothelium-derived hyperpolarising factor [3]. As in animal models, [4–6] patients with essential hypertension [7,8] and hypercholesterolemia [9,10] have impaired vasodilatation in forearm and coronary [11,12] vessels following intra-arterial infusion of acetylcholine. Responses to the endothelium-independent vasodilators, glyceryl trinitrate and sodium nitroprusside, are normal suggesting that vascular smooth muscle sensitivity to nitric oxide is not impaired. In the forearm, by the criterion of lesser vasoconstriction in response to the nitric oxide synthase inhibitor, L-N ω-monomethyl-arginine (L-NMMA), basal nitric oxide generation is impaired in essential hypertension [13] but not in hypercholesterolemia [14,15]. However, nitric oxide generation following acetylcholine stimulation is impaired in both conditions [13–16]. This ‘endothelial dysfunction’ could contribute to both vasoconstriction and platelet aggregation, and may be important in atherogenesis [17].

There is a contribution of prostanoids to both endothelium dependent vasodilatation and constriction, for example through prostacyclin and thromboxane A 2 respectively. In health, aspirin has no effect on basal forearm
blood flow or on cholinergic vasodilatation [8]. However, in patients with essential hypertension, indomethacin normalises forearm cholinergic dilatation [18]. This may occur because the balance between dilator and constrictor prostanoids is altered, consistent with work in hypertensive rats, [19,20] or because prostanoids may act indirectly to impair nitric oxide generation. In the present study, our aim was to establish whether cyclooxygenase inhibition would restore endothelial function in hypercholesterolemic patients.

2. Methods

2.1. Subjects

Ten hypercholesterolemic patients (total serum cholesterol > 7.0 mmol l⁻¹) and 10 healthy control subjects (total serum cholesterol < 5.3 mmol l⁻¹) were studied (Table 1). Subjects were not included if they were taking lipid-lowering or anti-hypertensive medication, or had received vasoactive drugs, aspirin, or non-steroidal anti-inflammatory agents during the 2 weeks before the study. All subjects abstained from alcohol for 24 h, and from food, caffeine-containing drinks and cigarettes for at least 4 h before each phase. Subjects rested recumbent during the measurements. Upper-arm congesting cuffs were inflated to 220 mmHg to exclude the hand circulation from the measurements. Upper-arm congesting cuffs were inflated to 40 mmHg to exclude the hand circulation from the measurements. Upper-arm congesting cuffs were inflated to 40 mmHg and blood flow was recorded for 10 s followed by a 5-s refilling period.

Multiple 10 s measurements were made over a three min period and the slopes of the final 5 recordings averaged to determine forearm blood flow. To correct for the effects of non-specific variations in blood flow during the protocol, changes in forearm blood flow during drug infusions are presented in the Figures as the ratio of flow in the infused arm to that in the control arm, expressed as percentage change in this ratio from baseline, as previously validated [22,23]. After each recording, blood pressure was measured in the non-infused arm using a Takeda UA-751 semi-automated oscillometric sphygmomanometer [24].

For drug infusions, a 27 standard wire gauge needle (Cooper’s Needle Works, Birmingham, UK), connected to a 16G epidual catheter (Portex, Hythe, Kent, UK), was introduced into the brachial artery of the non-dominant arm under local anaesthesia of 1% lidocaine (Astra Pharmaceuticals, Kings Langley, UK). Patency was maintained by infusion of physiologic saline (0.9%; Baxter Healthcare, Thetford, Norfolk, UK) or drug solutions (see below) at a rate of 1.0 ml min⁻¹.

2.3. Experimental protocol

Subjects were studied on two occasions in random order separated by a period of at least 14 days. On one occasion, aspirin was administered as an intravenous bolus (600 mg diluted in 5 ml 5% dextrose solution) (Aspegic, Laboratories Synthelabo, Paris, France). On the other occasion saline placebo was injected. After intra-arterial saline had been infused for 20 min, basal forearm blood flow was measured in both arms simultaneously. Then, acetylcholine (Miochol, IOLAB, Bracknell, UK) or sodium nitroprusside (Nipride, Roche Pharmaceutical Products, UK) was infused in physiologic saline at cumulative doses of 7.5 and 15 µg min⁻¹ and 3 and 10 µg min⁻¹ respectively for 6 min at each dose. Saline was then infused to provide a 12 min washout period, then a cumulative dose response was obtained with the other drug (sodium nitroprusside or acetylcholine). Forearm blood flow measurements were obtained during the final 3 min of each dose of drug or saline infusion. The order of administration of acetylcholine and sodium nitroprusside was randomised.

Table 1

Demographic data from hypercholesterolemic patients and age- and sex-matched control subjects

<table>
<thead>
<tr>
<th>Hypercholesterolemic patients</th>
<th>Control subjects</th>
<th>t-test patients vs. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range (yr)</td>
<td>38–63 (mean 52 ± 3)</td>
<td>38–63 (mean 49 ± 4)</td>
</tr>
<tr>
<td>Sample size (Male/Female)</td>
<td>7/3</td>
<td>7/3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133 ± 5</td>
<td>126 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 ± 3</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>98 ± 4 (95% CI 90–106)</td>
<td>93 ± 3 (95% CI 87–99)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>87 ± 4</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol l⁻¹)</td>
<td>7.9 ± 0.2 (range 7.2–9.2)</td>
<td>4.2 ± 0.2 (range 3.2–5.1)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.
Table 2
Absolute forearm blood flow results

<table>
<thead>
<tr>
<th></th>
<th>Hypercholesterolemic patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Aspirin</td>
</tr>
<tr>
<td><strong>Baseline forearm blood flow during intra-arterial saline (ml min⁻¹ 100 ml⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infused arm</td>
<td>2.56 ± 0.16 (p = 0.26 vs. controls)</td>
<td>2.33 ± 0.22 (p = 0.35 vs. controls)</td>
</tr>
<tr>
<td>Control arm</td>
<td>2.47 ± 0.21 (p = 0.13 vs. controls)</td>
<td>2.39 ± 0.24 (p = 0.19 vs. controls)</td>
</tr>
<tr>
<td><strong>Absolute increase in blood flow in the infused arm vs. baseline (ml min⁻¹ 100 ml⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine 15 μg min⁻¹</td>
<td>2.90 ± 0.65 (p &lt; 0.001 vs. controls)</td>
<td>5.83 ± 0.91 (p = 0.47 vs. controls)</td>
</tr>
<tr>
<td>Sodium nitroprusside 10 μg min⁻¹</td>
<td>7.03 ± 1.23 (p = 0.17 vs. controls)</td>
<td>9.26 ± 1.39 (p &lt; 0.01 vs. controls)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.
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Fig. 1. Forearm vasodilatation in response to intra-arterial infusion of acetylcholine (ACh) in hypercholesterolemic patients (○), expressed as percentage change from baseline, was significantly lower than the vasodilatation in control subjects (□) (P < 0.0002). Bolus intravenous injection of aspirin (600 mg) did not affect the response in control subjects (■), but potentiated the response in the patients (●) (P < 0.001).

2.4. Statistical analysis

Data were normally distributed within groups and are represented as mean ± SEM. Analysis of differences in forearm blood flow was by repeated measures analysis of variance followed by Student’s t tests where appropriate. Significance was accepted at P < 0.05.

3. Results

There was a trend toward higher blood pressure in hypercholesterolemic patients, but this did not reach statistical significance (Table 1). Basal forearm blood flows were not different and not affected by aspirin in either group (Table 2). Analyses of relative changes in blood flow showed that the increase in forearm blood flow following intra-arterial infusion of acetylcholine was significantly blunted in hypercholesterolemic patients compared with healthy controls (Fig. 1). Aspirin had no effect on acetylcholine mediated dilatation in controls, but restored the vasodilator response towards normal in hypercholesterolemic patients (Fig. 1). Forearm responses to sodium nitroprusside were not different between groups, and not affected by aspirin (Fig. 2). A similar pattern was confirmed by analysis of absolute changes in blood flow in the infused arm (Table 2), except that in this analysis aspirin had an additional effect which reduced the vasodilator response to both acetylcholine and sodium nitroprusside in controls.

4. Discussion

In keeping with other studies [9,10,14,15], the present findings confirm that cholinergic vasodilatation in the forearm vascular bed is impaired in hypercholesterolemic patients. There were no differences in forearm vasodilatation to sodium nitroprusside between the two groups, suggesting that sensitivity to nitric oxide in forearm resistance vessels was not different in hypercholesterolemia.

Also consistent with other work [8], there was no effect of aspirin on baseline forearm blood flow or on relative cholinergic vasodilatation in healthy subjects. Absolute vasodilatation to both aspirin and sodium nitroprusside was reduced by aspirin. By contrast, the defect in acetylcholine mediated vasodilatation was partially reversed by aspirin in hypercholesterolaemic patients, suggesting that the balance between vasodilator and constrictor prostanoids is altered in favour of vasoconstriction in this vascular bed.

In summary, in hypercholesterolemic patients impaired acetylcholine mediated vasodilatation in the forearm may
be due, at least in part, to altered generation of prostanoids, directly or indirectly favouring vasoconstriction. Restoration of this imbalance by aspirin may be an important component of its therapeutic benefit in cardiovascular disease.

Acknowledgements

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References