Assessment of endothelial function: comparison of the pulse wave response to β_2 -adrenoceptor stimulation with flow mediated dilatation

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AIMS

To assess the reproducibility of the digital pulse wave response to β_2 -adrenoceptor stimulation and to determine if an attenuated response to β_2 -adrenoceptor stimulation is associated with impaired flow mediated dilatation (FMD).

METHODS

Subjects ($n = 20$) with endothelial dysfunction (ED), were compared with healthy control subjects ($n = 20$). Change in reflection index (ΔRI) of the digital volume pulse in response to salbutamol (SALB, $5 \mu\text{g min}^{-1}$ i.v.) and to nitroglycerin (NTG, $5 \mu\text{g min}^{-1}$ i.v.) was used to assess endothelium-dependent (ΔRI_{SALB}) and endothelium-independent (ΔRI_{NTG}) pressure wave reflection. ΔRI_{SALB} was assessed on two occasions to examine reproducibility. High resolution ultrasound of the brachial artery was used to measure FMD and also dilation to NTG (NTGD).

RESULTS

The mean difference in ΔRI_{SALB} between two visits was -0.2% , with SD of the difference 4.9% . Both ΔRI_{SALB} and FMD were impaired in subjects with ED compared with values in control subjects (5.0 ± 0.7 vs. $11.3 \pm 1.2\%$, mean values $\pm$ SEM, $P < 0.01$ and 4.2 ± 0.6 vs. $7.5 \pm 0.8\%$, $P < 0.02$ for ΔRI_{SALB} and FMD, respectively), whereas ΔRI_{NTG} and NTGD were similar in the two groups. ΔRI_{SALB} was correlated with FMD ($r = 0.44$, $P < 0.01$) and had 88% sensitivity and 79% specificity to detect abnormal (FMD $< 4\%$).

CONCLUSIONS

The pulse wave response to a β_2 -adrenoceptor agonist correlates with FMD and has high sensitivity and specificity in detecting abnormal endothelial function as defined by FMD. However, FMD is the preferred test to detect effects of interventions on endothelial function.

Introduction

Endothelial function *in vivo* in man is usually assessed by examining the vasodilator response to an endothelium-dependent stimulus, such as intra-arterial administration of acetylcholine (ACh) [1, 2] or an increase in blood flow and hence in shear stress. Shear stress stimulates the endothelium to release nitric oxide (NO) with subsequent vasodilation that can be imaged and quantified as an index of vasomotor function. Dilatation of the brachial artery to

an increase in flow (flow mediated dilatation, FMD) during reactive hyperaemia following restoration of blood flow after a period of ischaemia in the hand is the current 'gold standard' for noninvasive assessment of endothelial function [3]. This technique, however, requires specialist imaging equipment and great attention to detail in order to obtain reproducible results [4].

We and others have suggested an alternative approach to assessing endothelial function, measuring the vasodilator response to β_2 -adrenoceptor agonists [5–7]. Vasodilator

Table 1

Subject characteristics

	Endothelial dysfunction (n = 20)	Control subjects (n = 20)
Age (years)	46.7 (9.8)*	41.1 (8.6)
Sex (M/F)	11/9	11/9
Height (m)	1.66 (0.11)	1.71 (0.11)
Weight (kg)	80.9 (13.5)*	71.7 (11.8)
BMI (kg m ⁻²)	29.2 (5.2)*	24.2 (2.6)
Smoker/nonsmoker	2/20	3/20
Systolic BP (mmHg)	146.5 (21.3)**	118.1 (9.1)
Diastolic BP (mmHg)	92.5 (11.7)**	72.5 (7.2)
Total-cholesterol (mmol l ⁻¹)	5.0 (0.9)	4.7 (0.9)
HDL-cholesterol (mmol l ⁻¹)	1.6 (0.5)	1.8 (0.6)
LDL-cholesterol (mmol l ⁻¹)	2.8 (0.8)	2.4 (0.8)
Triglycerides (mmol l ⁻¹)	1.1 (0.7)	1.1 (0.6)
Glucose (mmol/l)	4.1 (0.9)	4.5 (0.8)
Diuretics (%)	7 (35)	
α-adrenoceptor blockers (%)	5 (25)	

Values are means (SD) or numbers (%). *P < 0.05, **P < 0.01 compared with control subjects.

responses to β_2 -adrenoceptor agonists are mediated in part by endothelium-derived NO [7,8]. Low doses of salbutamol (SALB), a selective β_2 -adrenoceptor agonist, have minimal effects on blood pressure but influence the arterial pulse waveform [5]. SALB reduces reflection index (RI) [5] of the digital volume pulse and augmentation index of the radial pressure pulse [6] as a result of vasodilation of muscular arteries proximal to resistance vessels but distal to conduit vessels. Using this technique we have demonstrated reduced vasodilator responsiveness to β_2 -adrenoceptor stimulation in association with essential hypertension/metabolic syndrome and ethnic differences in vascular responsiveness between Afro-Caribbeans and Caucasians [9, 10]. However, because the pulse wave response to β_2 -adrenoceptor stimulation involves a different mechanism in activating NO mediated vasodilation from that for FMD and is assessed in a different vascular bed (systemic arteries vs. forearm conduit arteries) it is unknown whether an abnormal pulse wave response to β_2 -adrenoceptor stimulation is associated with impaired FMD.

The purpose of this present study was 1) to assess the reproducibility of the pulse wave response to β_2 -adrenoceptor stimulation and 2) to determine if an attenuated response to β_2 -adrenoceptor stimulation is associated with impaired FMD. We studied a group of subjects with risk factors in whom our previous work had identified impaired β_2 -adrenoceptor responses (endothelial dysfunction, ED group) and a group of healthy control subjects.

Methods

Subjects

Subjects with risk factors and hence assumed to have ED were recruited from the Vascular Risk Clinic at King's

College Hospital, London. The majority of subjects were overweight/obese hypertensives and included treated hypertensives. Subjects treated with β -adrenoceptor blocking agents and drugs known to modify endothelial function: aspirin [11], statins [12], angiotensin converting enzyme inhibitors [13], angiotensin II receptor antagonists [14] and calcium channel antagonists [15] were excluded. Healthy normotensive volunteers were recruited by advertisement within the local community. The study was approved by King's College Hospital Research Ethics Committee and informed consent was obtained from each participant. Subject characteristics are summarized in Table 1.

DVP response to SALB and nitroglycerin and reproducibility of response to SALB

The digital volume pulse (DVP) was recorded using an infrared photoplethysmograph (Micro Medical, Gillingham, UK) placed on the index finger of the left hand. This was used to assess the response to SALB and nitroglycerin (NTG, an endothelium-independent NO donor) [5]. In order to avoid confounding effects due to variable absorption or first pass metabolism, SALB and NTG were administered by the intravenous route as previously described [10]. The response to SALB was repeated after 1 week in order to determine its reproducibility. All studies were performed in a temperature controlled ($24 \pm 1^\circ\text{C}$) vascular laboratory in the morning with subjects having fasted overnight and having avoided caffeine and alcohol containing beverages from the evening before. Following venous cannulation, subjects rested supine for 30 min. Baseline measurements of blood pressure (using an Omron 705CP oscillometric device, Omron, Japan) and DVP were obtained at 5 min intervals for 15 min. SALB ($5 \mu\text{g min}^{-1}$, Allen & Hanbury, UK) was then infused for 30 min during which haemodynamic

measurements were recorded at 3 min intervals. After a washout period of 60 min during which 0.9% sodium chloride was infused, all parameters returned to baseline. NTG ($5 \mu\text{g min}^{-1}$, Faulding, UK) was then infused for 30 min with haemodynamic measurements recorded at 3 min intervals. The response to SALB was assessed in an identical manner on a second occasion, 1 week after the first pulse wave study.

Flow mediated dilatation (FMD)

FMD was measured in the morning after an overnight fast within 1 week of pulse wave studies and under identical conditions according to current guidelines [4]. A high resolution ultrasound (Accuson 128XP/10) system with 7 MHz linear array transducer, positioned by a stereotactic manipulator) was used to scan the brachial artery in a longitudinal section 2–15 cm above the elbow. After optimal positioning of the transducer a baseline scan was recorded. Increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the arterial segment being scanned) to a pressure of 250 mmHg for 5 min, followed by release. A second scan commenced 10 s before release of the cuff and continued for 3 min after cuff deflation. After 10 min to allow vessel recovery, another resting scan was taken. Sublingual GTN ($500 \mu\text{g}$) was then administered, and a final scan performed 3–4 min later. Images were coded and recorded on VHS videotape, then digitized for subsequent blinded analysis using automated edge detection software (Brachial Analyser, Medical Imaging Applications, LCC, Iowa, USA). FMD was expressed as the percentage increase in brachial artery diameter from baseline to maximal dilation which occurred 30–90 s after release of the cuff. Dilation to NTG (NTGD) was expressed as the percentage increase in brachial artery diameter from baseline to maximal dilation after NTG.

Statistical analysis

Subject characteristics are expressed as means (SD) and results as means $\pm$ SEM. Change in RI in response to SALB ($\Delta\text{RI}_{\text{SALB}}$), was quantified by measuring the change from baseline (mean of values recorded at 15, 10, 5 and 0 min before the start of the infusion of SALB), during the period 12–21 min after the start of infusion [10], expressed as a percentage of the baseline value. $\Delta\text{RI}_{\text{SALB}}$ was compared in the two groups by Student's *t*-test (two-tailed), $P < 0.05$ was considered significant. Reproducibility of $\Delta\text{RI}_{\text{SALB}}$ was investigated using a Bland-Altman plot [16] and expressed in terms of the mean difference of $\Delta\text{RI}_{\text{SALB}}$ for the two visits and SD of this difference. The correlation between $\Delta\text{RI}_{\text{SALB}}$ (mean value for both visits) and FMD was examined by linear regression analysis using Pearson's test of correlation. Previous studies using FMD have shown that in healthy people FMD is 7–10% of the baseline diameter but in patients with cardiovascular disease, FMD is impaired or absent with FMD $< 5\%$ [17]. We used cut off values of

FMD $> 8\%$ as indicative of normal endothelial function and FMD $< 4\%$ as abnormal endothelial function [18]. The sensitivity and specificity of ΔRI to detect normal and abnormal endothelial function according to this definition was determined using a receiver operating characteristic (ROC) plot.

Results

Subject characteristics are summarized in Table 1. Subjects with ED had higher blood pressure, weight and higher BMI than control subjects. Values of RI at baseline were similar in subjects with ED compared with control subjects (77.0 ± 1.5 vs. $76.0 \pm 3.4\%$, $P = \text{NS}$), a finding consistent with that in other studies [10]. There was no significant difference in baseline brachial artery diameter between the groups (4.1 ± 0.9 in controls vs. 3.9 ± 0.6 mm in ED subjects, $P = \text{NS}$).

Reproducibility of DVP response to SALB

Baseline values and haemodynamic changes in response to SALB on the 2 study days were similar. The Bland-Altman plot for $\Delta\text{RI}_{\text{SALB}}$ determined on two occasions separated by 1 week is shown in Figure 1. There was no relation between the difference in paired measurements and the size of $\Delta\text{RI}_{\text{SALB}}$ (no trend for within subject variability to be dependent on the mean value of $\Delta\text{RI}_{\text{SALB}}$). The mean difference in $\Delta\text{RI}_{\text{SALB}}$ between the two visits was -0.2% with SD of this difference 4.9% .

Comparison of pulse wave response and FMD in ED and control subjects

Haemodynamic changes in response to SALB and NTG are summarized in Table 2. In ED subjects, both $\Delta\text{RI}_{\text{SALB}}$ and FMD were impaired compared with values in control subjects (5.0 ± 0.7 vs. $11.3 \pm 1.2\%$, $P < 0.01$ and 4.2 ± 0.6 vs. $7.5 \pm 0.8\%$, $P < 0.02$ for $\Delta\text{RI}_{\text{SALB}}$ and FMD, respectively, Figure 2). $\Delta\text{RI}_{\text{NTG}}$ and NTGD were preserved in ED compared to control subjects (12.6 ± 0.7 vs. $15.1 \pm 1.1\%$, $P = 0.25$ and 16.4 ± 0.8 vs. $15.7 \pm 0.8\%$, $P = 0.45$ for $\Delta\text{RI}_{\text{NTG}}$ and NTGD, respectively). In all subjects there was a significant correlation between $\Delta\text{RI}_{\text{SALB}}$ and FMD ($r = 0.44$, $P < 0.01$). $\Delta\text{RI}_{\text{NTG}}$ and NTGD were not significantly correlated. Using a cut-off value of 8% for $\Delta\text{RI}_{\text{SALB}}$, the DVP response to SALB had 88% sensitivity and 79% specificity to detect abnormal endothelial function (as defined by FMD $< 4\%$). The ROC plot is shown in Figure 3.

Discussion

The pulse wave response to SALB is a simple test which does not require specialized equipment or training and can be used in studies with more than one centre [10]. In the present study we assessed the correlation of $\Delta\text{RI}_{\text{SALB}}$

Figure 1

Bland-Altman plot showing the difference in response of the reflection index of the digital volume pulse to salbutamol (ΔRI_{SALB}) measured on two occasions vs. the mean value for the two occasions

Table 2

Haemodynamic and digital pulse parameters at baseline and during intravenous infusion of salbutamol (SALB) and nitroglycerin (NTG)

	Control subjects			Endothelial dysfunction		
	Baseline	SALB (5 $\mu\text{g min}^{-1}$)	NTG (5 $\mu\text{g min}^{-1}$)	Baseline	SALB (5 $\mu\text{g min}^{-1}$)	NTG (5 $\mu\text{g min}^{-1}$)
Heart rate (beats min^{-1})	63 $\pm$ 1	70 $\pm$ 3	69 $\pm$ 2	70 $\pm$ 2	76 $\pm$ 3	78 $\pm$ 3
SBP (mmHg)	117 $\pm$ 2	119 $\pm$ 3	119 $\pm$ 3	148 $\pm$ 4	148 $\pm$ 3	143 $\pm$ 4
DBP (mmHg)	74 $\pm$ 2	69 $\pm$ 3	70 $\pm$ 2	93 $\pm$ 3	86 $\pm$ 3	86 $\pm$ 3
RI (%)	77.3 $\pm$ 1.5	66.0 $\pm$ 3.1	62.2 $\pm$ 3.3	76.4 $\pm$ 3.4	71.4 $\pm$ 3.4	63.8 $\pm$ 2.2
ΔRI (%)		11.3 $\pm$ 1.2	15.1 $\pm$ 1.1		5.0 $\pm$ 0.7	12.6 $\pm$ 0.7

Values are means $\pm$ SEM; BP: blood pressure; RI: reflection index of the digital volume pulse.

with FMD and the reproducibility of ΔRI_{SALB} . The correlation between ΔRI_{SALB} and FMD was significant but relatively low. This may be because the measurements are in different vascular beds and activated by different mechanisms. It is notable that the correlation of forearm blood flow methods for measuring endothelial function with FMD has also been reported to be weak [19].

The within-subject standard deviation of ΔRI_{SALB} for successive visits is determined by the physiological variability in vascular reactivity and by the intrinsic error in the test. The within subject SD for ΔRI_{SALB} was 4.9%, slightly less than the difference in ΔRI_{SALB} between the ED and control groups in this study. This degree of variability means that a relatively large sample size would be required to detect a modest change in ΔRI_{SALB} (i.e. a change substantially less than the difference in ΔRI_{SALB} between the ED and control groups in this study). The ratio of the within subject SD to the difference between the ED and control subjects for ΔRI_{SALB} in this study was 0.8 which compares with a ratio of approximately 0.5 for FMD (assuming a within subject SD for FMD of approximately 1.5%). Thus the pulse wave technique is substantially less powerful than FMD in detecting

a within-subject change in endothelial function. A similar conclusion was reached by Donald *et al.* [20] when comparing the reproducibility of the salbutamol response assessed by pulse wave analysis (using both the digital pulse and radial tonometry) in children and adults.

The power of a test to discriminate between two groups with differing endothelial function depends upon the SD of the test in each group and the size of the difference between the groups. Variation in vascular function between subjects is likely to play a more important role in determining within group SD, and thus the intrinsic error of the test may be of less importance when comparing differences between groups. In the present study the ratios of the within group SD to the between group difference were 1.3 and 1.0 for ΔRI_{SALB} and FMD respectively, so the difference in power of the two tests to detect differences in endothelial function between groups was less marked than that to detect a within-subject change but still favored FMD. The ROC analysis we performed suggests that the pulse wave response to SALB has adequate sensitivity and specificity in determining the presence of endothelial dysfunction as determined by FMD. A limitation of

Figure 2

(a) Response of the reflection index (ΔRI) of the digital volume pulse to salbutamol (SALB) and nitroglycerin (NTG) in subjects with endothelial dysfunction and control subjects. (b) Dilation of the brachial artery to hyperaemic flow (FMD) and to NTG in the same subject groups. Endothelial dysfunction, (▨); Control subjects, (□)

Figure 3

ROC curve for the pulse wave response to salbutamol (ΔRI_{SALB}) as a predictor of abnormal FMD (<4%). Sensitivity equals the number of subjects with abnormal FMD and abnormal ΔRI_{SALB} divided by the total number of subjects with abnormal FMD. Specificity equals the number of subjects with normal FMD and normal ΔRI_{SALB} divided by total number of subjects with normal FMD. The area under the curve (AUC: 0.92, 95% CI 0.83, 1.00) is close to 1 and suggests that the discriminatory value of ΔRI_{SALB} in the detection of abnormal endothelial function as defined by FMD is satisfactory

the present study is that the relationship between ΔRI , NTGD and FMD may depend in part on the doses of salbutamol and NTG used for the two tests. The doses used for the pulse wave response were chosen to produce a change in RI with minimum systemic effects but we cannot exclude the possibility that the sensitivity and specificity might be improved by altering these doses.

In conclusion, the digital pulse wave response to SALB correlates with FMD and is broadly similar in identifying subjects with endothelial dysfunction. However, the within subject variation of the pulse wave response is greater than FMD and thus, where available, FMD is the preferred test to detect effects of interventions on endothelial function.

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