

Cholesterol lowering therapy inhibits the low-flow mediated vasoconstriction of the brachial artery in hypercholesterolaemic subjects

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- 1 We tested whether lipid lowering treatment with HMG CoA reductase inhibitor modified the flow mediated large artery reactivity in primary pure hypercholesterolaemia.
- 2 Abnormalities in arterial reactivity have been described in the presence of high blood cholesterol, in particular an enhanced constriction of the brachial artery in response to acute induction of a low flow state.
- 3 Using pulsed-Doppler, we measured brachial artery diameter and flow velocity at rest and their changes induced by wrist occlusion before and after 3 months of double-blind treatment by pravastatin (40 mg orally) in 13 subjects and placebo in 15 others.
- 4 The significant decrease ($P < 0.01$) in diameter induced by wrist occlusion before (0.34 ± 0.08 mm) placebo and pravastatin (0.39 ± 0.10 mm) persisted after placebo (0.26 ± 0.07 mm) but was abolished after pravastatin (0.07 ± 0.05 mm). The absolute change in diameter induced by wrist occlusion was lower after than before pravastatin ($P < 0.01$) and lower after pravastatin than after placebo ($P < 0.05$). Diameter during wrist occlusion was higher after pravastatin than after placebo (4.35 ± 0.16 vs 3.89 ± 0.09 mm); $P < 0.01$).
- 5 These findings indicate that the lipid changes induced by pravastatin and/or some unknown but direct mechanism of the drug itself inhibit low-flow-mediated vasoconstriction associated with hypercholesterolaemia. Such effects may have important implications for the treatment of vasospasm often seen in the presence of high blood cholesterol.

Keywords large artery diameter blood velocity pravastatin pulsed Doppler flow meter vasoconstriction.

Introduction

Abnormalities in the reactivity of arteries consisting of potentiated constriction and impaired relaxation have been reported in animals with hypercholesterolaemia even in the absence of atherosclerotic lesions [1–8]. These changes may be the result of several mechanisms, including functional alterations of the endothelium and vascular smooth muscle [8–13]. In hypercholesterolaemic man similar alterations in arterial reactivity have been observed by studying the response of coronary or peripheral arteries to intra-arterial infusion of vaso-

active substances, or by investigating the flow mediated changes in the calibre of peripheral arteries during ischaemia and/or hyperaemic manoeuvres [14–19]. Since arterial reactivity may contribute to the pathogenesis of vasospasm especially in the presence of atherosclerosis, it is important to determine whether alterations induced by hypercholesterolaemia can be reversed by lipid lowering treatment. Experimental studies have shown that HMG CoA reductase inhibitors change the arterial endothelium dependent reactivity [21] as well as the endothelium-mediated response in the coronary arteries of hypercholesterolaemic patients with athero-

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sclerosis [18, 19, 22–24]. Nevertheless the effect of cholesterol lowering therapy in subjects exempt from atherosclerosis has not been examined in the clinical setting. Therefore we investigated the effect of an HMG CoA reductase inhibitor (pravastatin) on the low flow mediated constriction of the brachial artery reported previously [14] in subjects with primary pure hypercholesterolaemia. The examination of the brachial artery generally free of atherosclerosis [25] allowed elimination of a confounding effect of atherosclerosis on vascular reactivity. We tested changes in brachial artery diameter in response to the induction of low flow state by wrist occlusion [14, 26, 27] before and after 3 months of double-blind administration of pravastatin and placebo.

Methods

Patients

Twenty-eight hypercholesterolaemic subjects (26 men and 2 women) 35–63 years of age were included in the study. A preliminary diagnosis of hypercholesterolaemia was established at the subjects' worksites by a group of occupational health physicians [28] (groupe de prevention cardiovasculaire en médecine du travail, or PCV METRA Group); the diagnosis was confirmed by lipid measurements in the biochemistry laboratory of the Hôpital Broussais in samples obtained after a 14 h fast [14, 28]. All subjects provided informed consent for the procedure, which was approved by the local ethics committee. Serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured by enzymatic methods after precipitation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) by phosphotungstic acid [14, 28, 29]. LDL-cholesterol (mmol l^{-1}) was calculated according to the following formula: $\text{LDL-cholesterol} = \text{Total cholesterol} - \text{HDL-cholesterol} - \text{triglycerides}/2.2$ [30].

The selection criteria for the study required that total blood cholesterol be above 6.2 mmol l^{-1} and that plasma triglycerides be below 2 mmol l^{-1} (type IIa hypercholesterolaemia). Primary hypercholesterolaemia was confirmed in all subjects by exclusion of diseases or factors that might cause secondary hypercholesterolaemia [30]. Subjects were required to have abstained from lipid-lowering drugs for at least 3 months before selection. Subjects with sustained blood pressure elevation greater than 160/95 mmHg on at least three outpatient visits [31], or with a history of treatment for hypertension, coronary heart disease, and clinical evidence of advanced atherosclerotic lesions of the abdominal aorta, the cervical arteries, or the lower-limb arteries were excluded from the study. The latter point was confirmed by ultrasonic examination of carotid, abdominal aortic, and femoral sites [28].

Study design

Subjects who fulfilled the selection criteria cited above were given placebo single-blind for 6 weeks and were

counselled on establishing a low-cholesterol diet ($< 300 \text{ mg}$ daily) with a caloric distribution of protein, carbohydrate, and fat of 15%, 55%, and 30%, respectively. At the end of this placebo lead-in period (baseline), 31 subjects whose total cholesterol remained above 5.2 mmol l^{-1} and whose triglycerides were below 2 mmol l^{-1} [28] were randomly assigned to 3 months of double-blind therapy with pravastatin 40 mg day^{-1} or matching placebo.

During the double-blind treatment period, two subjects were discontinued in the pravastatin group and one in the placebo group. One of these subjects left the study for personal reasons unrelated to any effects of study treatment, and the other two were discontinued because of technical difficulties in making arterial measurements due to uncontrolled arm motion during the examination.

Arterial measurements

The subjects were studied in the course of the morning in a quiet room at a controlled temperature of $21 \pm 1^\circ \text{C}$ while in the recumbent position, with the right arm supported at midthoracic level and the hand relaxed and open. After 10 min of rest, systemic blood pressure was determined in the left arm with a mercury sphygmomanometer, with mean blood pressure calculated as diastolic pressure plus a third of the pulse pressure. The internal arterial diameter and the lumen flow velocity of the right brachial artery were determined at rest by transcutaneous pulsed Doppler (Echovar Doppler, Alvar Electronic, Montreuil, France) [14, 26, 27, 32].

The system and method of operation have been described in detail previously [26, 27]. We will present here some pertinent characteristics. The system has two original features: a double transducer probe to adjust the incidence angle at $60 \pm 1^\circ$ between the ultrasonic beam and the arterial axis, and a range-gated time system of reception of emitted pulses. By electronically adjusting the delay and duration of reception, it was possible to focus the sample volume of the Doppler signals to 0.4 mm and to advance the sample in successive 0.4 mm steps across the artery. Synchronization with an electrocardiogram made it possible to automatically start the step advance at the QRS complex for every other cardiac pulse. The number (n) of pairs of peaks of the velocity profile allowed us to calculate arterial diameter (in cm) as: $n \times 0.4 \times 0.866$, where 0.866 is the sine of 60° (the angle between the beam direction and the arterial axis). Mean flow velocity was determined by increasing the sample volume to the value of arterial diameter and superimposing the former on the lumen of the artery.

To improve the reliability of the method, the Doppler probe was fixed throughout the investigation over the course of the artery by means of a stereotaxic device placed above the arm. Measured diameter was defined as the average of at least two consecutive measurements on each probe and was expressed in centimetres. Measured velocity was defined as the average of at least

10 measures (corresponding to 10 consecutive cardiac cycles) on each probe and was expressed in cm/s. The variability of measurements has been previously shown to be $7 \pm 2\%$ for diameter and $5 \pm 2\%$ for velocity [33].

Acute induction of the low-flow state in the brachial artery

Blood flow through the brachial artery was acutely decreased with an occluding cuff placed around the wrist [14, 26]. Wrist occlusion was obtained by inflating the cuff to suprasystolic levels (200 mmHg), and then the distal circulation to the hand was arrested and flow was reduced [14, 26]. Pulse-Doppler measurements of brachial artery diameter and velocity were performed after 5 min of wrist occlusion.

All the arterial measurements (at rest and during wrist occlusion) were performed twice in each subject: before treatment (at the end of the single-blind placebo period) and after 3 months of double-blind administration of pravastatin or placebo.

Statistical analysis

Data are expressed as mean \pm s.e.mean. Comparisons between groups (at baseline or after 3 months of treatment) and within groups (before and after treatment) were performed by analysis of variance (ANOVA) or by repeated-measures ANOVA when appropriate. Differences were considered significant for P values < 0.05 .

Results

Of the 31 patients randomized for the double-blind period treatment, 16 subjects were assigned to the placebo treatment and 15 subjects were assigned to the pravastatin treatment; as two subjects in the pravastatin group and one subject in the placebo group were withdrawn subsequently the comparisons between groups were done in 15 and 13 subjects. Before treatment, there were no significant differences between the two groups in age, sex ratio, body mass index, or blood pressure (Table 1), as well as in blood lipids (Table 2).

Compared with pretreatment values, placebo did not modify blood lipids significantly after 3 months of treatment, except for LDL-cholesterol, which decreased slightly ($P < 0.05$) (Table 2). After 3 months of treatment, pravastatin decreased total and LDL-cholesterol ($P < 0.001$) and triglycerides ($P < 0.05$) and increased HDL-cholesterol ($P < 0.05$). In addition, total and LDL-cholesterol were lower ($P < 0.001$) and triglycerides were lower ($P < 0.05$) in the pravastatin group than in the placebo group at the end of the 3 month double-blind treatment period.

Table 1 Demographic characteristics of the two treatment groups before treatment

Parameters	Placebo group (n = 15)	Pravastatin group (n = 13)
Age (years)	49 \pm 2	50 \pm 2
Sex ratio (Men/Women)	14/1	12/1
Body mass index (kg m ⁻²)	25 \pm 1	26 \pm 1
Blood pressure (mmHg)		
Systolic	124 \pm 3	120 \pm 3
Diastolic	84 \pm 3	80 \pm 2

n = number of subjects. Data shown are means \pm s.e. mean, except for the sex ratio.

Arterial parameters: pretreatment comparison

Values of diameter and velocity at rest and during wrist occlusion did not differ between placebo and pravastatin groups (Table 3).

The changes from resting values induced by wrist occlusion in diameter and blood velocity are shown in Table 3 and Figure 1: diameter decreased significantly in placebo group and in pravastatin group ($P < 0.01$) (Figure 1) and blood velocity decreased in both groups ($P < 0.001$) (Table 3). Wrist occlusion induced changes in diameter and velocity were not different between pravastatin and placebo groups (Table 3).

Arterial parameters: comparison between before and after treatment

Compared with pretreatment values placebo did not modify diameter and velocity at rest and during wrist occlusion (Table 3). After placebo the decrease in diameter induced by wrist occlusion was significant ($P < 0.01$) (Figure 1) as well as the decrease in velocity induced by wrist occlusion ($P < 0.001$, Table 3). Compared with pretreatment values placebo did not modify the wrist occlusion induced changes in diameter and velocity (Table 3).

Compared with pretreatment values, pravastatin did not modify diameter and velocity at rest and during wrist occlusion, except for the diameter during wrist occlusion which increased significantly ($P < 0.01$, Table 3). After pravastatin the change from resting value induced by wrist occlusion in diameter was not significant (Figure 1) while the decrease in blood velocity induced by wrist occlusion was significant ($P < 0.001$, Table 3). Compared with pretreatment values, pravastatin decreased the absolute change in diameter induced by wrist occlusion ($P < 0.01$, Table 3) but did not modify the wrist occlusion changes in velocity (Table 3).

Arterial parameters: post treatment comparison

At rest, diameter and velocity did not differ after the two treatments (Table 3). During wrist occlusion diameter was higher after pravastatin than after placebo ($P = 0.01$) while velocity did not differ after the two treatments

Table 2 Lipid parameters before and after 3 months of double-blind treatment with placebo or pravastatin

Parameters	Placebo group (n=15)		Pravastatin group (n=13)	
	Before treatment	After treatment	Before treatment	After treatment
Total cholesterol (mmol l ⁻¹)	7.88 ± 0.20	7.49 ± 0.24	7.48 ± 0.33	6.07 ± 0.29§ ^b
Triglycerides (mmol l ⁻¹)	1.42 ± 0.10	1.52 ± 0.15	1.32 ± 0.11	1.08 ± 0.09* ^a
HDL-cholesterol (mmol l ⁻¹)	1.34 ± 0.08	1.38 ± 0.08	1.30 ± 0.10	1.50 ± 0.12‡ ^a
LDL-cholesterol (mmol l ⁻¹)	5.90 ± 0.1	5.43 ± 0.22*	5.58 ± 0.33	4.08 ± 0.25§ ^b

n=number of subjects; HDL=high-density lipoprotein; LDL=low-density lipoprotein. Data shown are means ± s.e. mean. Comparisons are performed between values obtained before treatment and those obtained after 3 months of double-blind treatment. **P* < 0.05, within-group comparison. ‡*P* < 0.01, within-group comparison. §*P* < 0.001, within-group comparison. ^a*P* < 0.05, between-group comparison. ^b*P* < 0.001, between-group comparison.

Table 3 Arterial parameters before and after 3 months of double-blind treatment with placebo or pravastatin

Parameters	Placebo group (n=15)		Pravastatin group (n=13)	
	Before treatment	After treatment	Before treatment	After treatment
Resting values				
D (mm)	4.23 ± 0.11	4.15 ± 0.07	4.42 ± 0.13	4.42 ± 0.15
V (cm s ⁻¹)	4.13 ± 0.40	3.79 ± 0.36	4.01 ± 0.41	4.47 ± 0.45
WO values				
D (mm)	3.89 ± 0.14	3.89 ± 0.09	4.03 ± 0.10	4.35 ± 0.16‡ ^b
V (cm s ⁻¹)	2.24 ± 0.20	2.00 ± 0.14	2.25 ± 0.21	2.34 ± 0.12
WO Changes				
ΔD (mm)	-0.34 ± 0.08	-0.26 ± 0.07	-0.39 ± 0.10	-0.07 ± 0.05‡ ^a
ΔV (cm s ⁻¹)	-1.88 ± 0.40	-1.79 ± 0.38	-1.75 ± 0.36	-2.13 ± 0.41

n=number of subjects; D=arterial diameter; ΔD=change in diameter; V=blood velocity; ΔV=change in velocity; WO=wrists occlusion. Data shown are means ± s.e. mean. Comparisons are performed between values before treatment and those after treatment (within-group) and between groups at the same period (before or after treatment). ‡*P* < 0.01, within-group comparison. ^a*P* < 0.05, between-group comparison. ^b*P* < 0.01, between-group comparison.

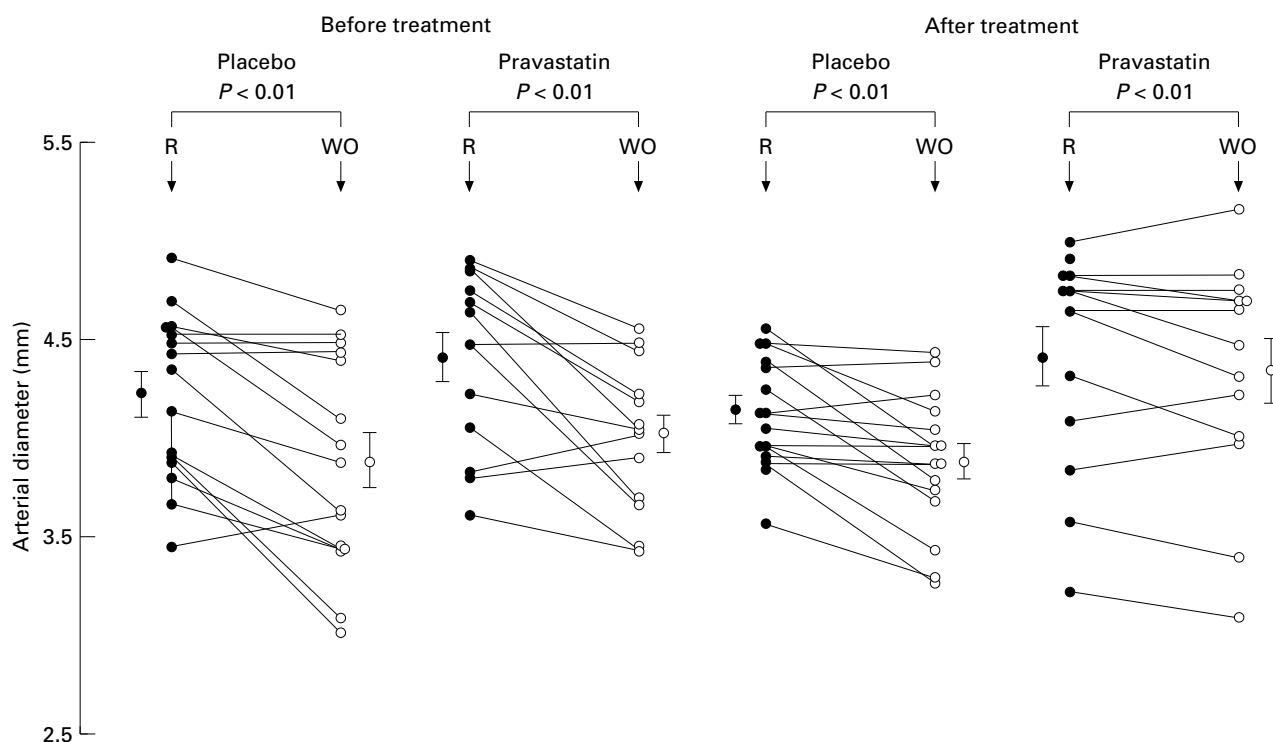


Figure 1 Plot of individual values of brachial artery diameter at rest (R) and during wrist occlusion (WO) before and after 3 months of double-blind treatment with placebo and pravastatin. Comparisons were made between WO and R. NS=not significant.

(Table 3). The absolute change from resting value induced by wrist occlusion in diameter was lower after pravastatin than after placebo ($P < 0.05$, Table 3) but the decrease in blood velocity induced by wrist occlusion was similar after the two treatments (Table 3).

Discussion

The main objective of the present study was to analyse the effects of lipid lowering therapy on the low flow mediated vasoconstriction of the brachial artery observed in hypercholesterolaemic subjects. We have used a non-invasive methodology based on a bidimensional pulsed Doppler system previously validated and used for the clinical investigation of peripheral arterial circulation [14, 26, 27]. This system allows concomitant and accurate determinations of arterial diameter and lumen flow velocity of the brachial artery at rest (the hand opened and relaxed) and during transient low flow state induced by wrist occlusion [27, 33]. At baseline we observed with this technique that the diameter of the brachial artery decreased concomitantly with blood velocity during wrist occlusion in the two hypercholesterolaemic pretreated groups. This phenomenon has been already described in a previous report in hypercholesterolaemic patients but not in normocholesterolaemic subjects and it was considered as reflecting an increased reactivity of the artery tone to the low flow state in the presence of high blood cholesterol [14]. Although the mechanisms have not been elucidated it can be speculated that the endothelium may play a role in this low flow mediated vasoconstriction [14]. The major finding of the present study was that the reduction in brachial artery diameter induced by wrist occlusion was lower after 3 months with pravastatin than after 3 months with placebo. Indeed the wrist occlusion induced decrease in diameter observed after pravastatin represented on average 1% of the resting diameter before wrist occlusion while that observed after placebo treatment was 6% of resting diameter. As a consequence of the pravastatin abolishment of this brachial artery vasoconstriction, the diameter of the brachial artery during wrist occlusion (the hand circulation being excluded) was higher after pravastatin than after placebo treatment suggesting a relative vasodilating effect of pravastatin in the condition of wrist occlusion.

The interpretation of the arterial effect of pravastatin raises several alternative hypotheses. An observation preliminary to the discussion is that the stimulus responsible for the changes in arterial diameter, i.e. the decreased flow velocity during wrist occlusion [14, 26, 27] was not modified by pravastatin. Therefore, the attenuation of vasoconstriction during wrist occlusion observed with pravastatin cannot be attributed to a lower reduction in flow velocity. The possibility remains that the drug may have modified endothelial function, the vascular smooth muscle, or the structure of the arterial wall [15]. The latter possibility is important to consider, because the presence of atherosclerotic structural changes can interfere with arterial reactivity [34,

35]. However, it is unlikely that atherosclerotic lesions affected the results in our hypercholesterolaemic subjects, because this process rarely affects the brachial artery [25], and our subjects were free of any symptoms or clinical signs of occlusive arterial disease. Thus it does not seem plausible that pravastatin may have changed the low flow-mediated brachial artery reactivity through an action on the atherosclerotic process of the arterial wall.

In contrast, an effect of pravastatin on endothelial function is supported by considerable evidence on the role of the endothelium in the flow-mediated changes in arterial caliber [27, 36–42] and on its dysfunction in hypercholesterolaemia [10, 14–16]. The latter point is suggested by the observation of an impairment of endothelium-derived relaxing factor release in vessels taken from hypercholesterolaemic animals that did not yet have overt plaque formation [10]. Moreover, an impaired endothelium-dependent vasodilation of forearm arteries has been described recently in men with primary hypercholesterolaemia who do not have clinical atherosclerosis [17]. Experimental studies show that inhibitors of HMG CoA reductase normalize endothelium-dependent relaxation in the thoracic aorta isolated from hypercholesterolaemic rabbits [21]. Finally, a recent clinical study has suggested that the impaired post-ischaeamic vasodilatory capacity of the forearm arterioles of hypercholesterolaemic patients is restored to normal by prolonged treatment with simvastatin, another HMG CoA reductase inhibitor [24].

Another important mechanism of pravastatin's effect on arterial reactivity could involve the vascular smooth muscle. It has been shown experimentally that a reduced response of the arterial smooth muscle to endothelial vasodilators and an increased sensitivity to vasoconstriction agents may be involved in the abnormal arterial reactivity seen in hypercholesterolaemia [11–13]. Furthermore, LDL-cholesterol, particularly if oxidatively modified, may directly potentiate contraction of the smooth muscle of perfused vessels *in vitro* [12–13].

Unfortunately, intriguing as the foregoing hypotheses are, they cannot be proven or disproven by the present work.

Limitation of the study

A possible limitation of our findings is relevant to the technical accuracy of the Doppler device for measuring brachial artery diameter. It depends on the precision of the location of the proximal and distal arterial walls, with a sample volume that has a 0.4 mm size and is displaced by 0.4 mm gradations [33]. This problem was previously tested *in vitro* in our laboratory by correlating the actual diameter of calibrated tubes and their calculated apparent echo-Doppler diameter [33]. The intercept of this correlation gives a quantitative estimation of the error of measurement of diameter, which was 0.35 mm, that is 7% of the brachial artery diameter [33]. This error is only two percentage points below the percent diameter change after occlusion in the hypercholesterolaemic group. However, the correlation

obtained from calibrated tubes shows that the error of diameter measurements is systematic and represents the over-estimation of diameter due to the sample volume size. When comparing the effects of occlusion on arterial diameter in the same patient, the systematic error is mathematically eliminated and does not affect the statistical evaluation of the difference in diameters before and during occlusion [14].

In conclusion, our findings suggest that the low flow mediated vasoconstriction of the brachial artery observed in hypercholesterolaemic humans in the absence of atherosclerosis may be pharmacologically reversed by the lipid changes induced by pravastatin and/or by some unknown but direct mechanism of the drug itself. Furthermore, the fact that the enhanced constriction of the large artery in response to a low-flow state may be attenuated by HMG CoA reductase inhibition may have important implications for the treatment of vasospasm [43, 45]. Indeed, vasospasm often occurs in the presence of high blood cholesterol levels in vessels without evidence of overt atherosclerosis, and is thought to contribute to the precipitation of cardiovascular complications [44].

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