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Clinical Science

Ageing is associated with impairment of nitric oxide and prostanoid dilator pathways in the human forearm

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ABSTRACT

Ageing is associated with endothelial dysfunction and increased cardiovascular risk. We assessed the activity of nitric oxide (NO) and prostaglandin pathways in older subjects. Bilateral venous occlusion plethysmography was used to measure forearm blood flow during intra-arterial infusion of the NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA; 1, 2 and 4 μmol/min), the cyclo-oxygenase inhibitor, aspirin (3, 9 and 30 μmol/min), and the smooth muscle constrictor, noradrenaline (60, 120 and 240 pmol/min); each dose infused for 5 min. Eighteen young and 15 healthy older subjects (mean age $\pm$ S.E.M., 32 ± 1 and 65 ± 1 years respectively) were studied. Effects of treatment were calculated from the ratio of blood flow in the infused to control arm, expressed as a percentage. Dose-response curves were compared by analysis of the area under the curve (AUC) using independent samples *t* test. All agents caused dose-dependent decreases in basal forearm blood flow. AUC values for noradrenaline, aspirin and L-NMMA in younger and older subjects were 162 ± 24, 173 ± 24 and 170 ± 17, and 138 ± 22, 70 ± 22 and 89 ± 22 respectively. Effects of aspirin and L-NMMA, but not noradrenaline, were reduced in older subjects ($P = 0.004$, 0.007 and 0.461 respectively). Our findings suggest a generalized abnormality of basal endothelial function in older people, with similar impairment of NO and prostanoid dilator pathways. Defects in both pathways could contribute to the development of age-related cardiovascular disease.

INTRODUCTION

The vascular endothelium has vasodilator, anti-thrombotic and athero-protective properties through the production of vasoactive mediators, such as nitric oxide (NO) and prostacyclin [1]. NO is generated from the amino acid L-arginine by the enzyme NO synthase (NOS) [2]. Prostacyclin is produced from arachidonic acid by a series of enzymes including cyclo-oxygenase (COX) and prostaglandin polymerase [3]. In healthy humans, local inhibition of NOS or COX causes forearm vasoconstriction [4,5], which is consistent with a role for these mediators in the regulation of basal vascular tone.

Ageing is an independent risk factor for the development of atherosclerosis and is associated with a progressive decline in endothelium-dependent vasodilatation in resistance and conduit vessels [6,7]. Endothelial dysfunction might have a pathogenic role in the development of atherosclerosis and its complications in the elderly. Studies to date [8] implicate reduced NO bioactivity in the vasculopathy associated with ageing, but it is unclear to what extent other endothelial dilator

Key words: elderly, endothelium.

Abbreviations: AUC, area under the curve; COX, cyclo-oxygenase; L-NMMA, N^G-monomethyl-L-arginine; NOS, NO synthase.

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pathways are altered in healthy elderly subjects. We hypothesized that ageing-related endothelial dysfunction was not confined to the NO pathway. The aim of the present study was to test this hypothesis by assessing the vasoconstrictor effects of NOS and COX inhibition in the forearm of healthy young and elderly subjects.

METHODS

Subjects

Fifteen healthy older subjects (7 males; mean age 65 ± 1 years) and 18 younger subjects (12 males; mean age 32 ± 1 years) were recruited from the local population. All subjects gave written informed consent to the study protocol, which was approved by the local ethics committee and conforms with the principles outlined in the Declaration of Helsinki.

Subjects were screened by medical history, physical examination, electrocardiograph and laboratory analysis. All subjects were fit and active, but none were trained athletes. They were excluded if any of the following were present: cardiovascular disease, diabetes mellitus, transient ischaemic attack or cerebrovascular accident, current smokers (or had smoked within the previous 12 months), treatment for hyperlipidaemia, on antihypertensive or other vasoactive medication, treatment with antioxidants, vitamin supplements or aspirin and other non-steroidal anti-inflammatory drugs. Sitting arterial blood pressure was measured on two separate occasions using the automated oscillometric device (Omron HEM 705CP; Omron Cooperation Healthcare Division, Tokyo, Japan) [9]. Three readings were taken on each occasion after 10 min of rest. The mean of the readings from the second visit were used. Subjects' heights and weights were measured, and blood samples were taken for biochemical analysis (renal and liver function, fasting glucose and fasting lipid profile).

Forearm plethysmography

Mercury-in-rubber strain gauge plethysmography [10] was used to measure bilateral forearm blood flow. Alcohol and caffeine were withheld for 12 h before the studies, which were performed in a temperature-controlled laboratory (26–28 °C) in the post-absorptive state. Drugs or physiological saline [0.9% (w/v) NaCl] were infused continuously at 0.5 ml/min into the brachial artery of the non-dominant arm through a 27 SWG needle introduced under local anaesthetic (1% lignocaine). During the recording period, the hands were occluded from the circulation by inflating a cuff placed around each wrist to supra-systolic pressure. Blood flow was derived by measuring the rate of increase in forearm volume caused by inflating upper arm congesting cuffs to 40 mmHg for 10 s in every 15-s cycle. After a baseline period of 15 min following brachial artery cannulation, cumulative dose–response curves to noradrenaline (to assess smooth muscle reactivity; 60, 120 and 240 pmol/min; each dose for 5 min), aspirin (COX inhibitor; 3, 9 and 30 μmol/min; each dose for 5 min), and L-NMMA (NOS inhibitor; 1, 2 and 4 μmol/min; each dose for 5 min) were constructed. Drug infusions were separated by 15 min (after noradrenaline) and 25 min (after aspirin), which allowed forearm blood flow to return to resting control values.

Drugs

Noradrenaline (Abbott Laboratories, Queenborough, Kent, U.K.) and L-NMMA (Calbiochem–Novabiochem, Nottingham, U.K.) were obtained from commercially available sources. Aspirin (Aspisol) was supplied generously by Bayer (Leverkusen, Germany). Drugs were diluted to the desired concentration using normal saline. Fresh solutions were made for each study.

Data analysis

Forearm blood flow, expressed as $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ of forearm volume, was calculated according to the method of Whitney [10]. The ratio of blood flow in the infused to control arm was calculated for each measurement period. Constriction in response to the infused drugs was expressed as the percentage decrease in this blood flow ratio during control (saline) infusion. Responses were compared with analysis of the area under the dose–response curve (AUC) and expressed in arbitrary units. All results are expressed as mean $\pm$ S.E.M. and compared using the independent samples t test, where $P < 0.05$ is considered significant.

RESULTS

Clinical and biochemical characteristics

The baseline clinical and biochemical characteristics of the study population are shown in Table 1. The two groups were well matched for gender, body mass index, systolic blood pressure, diastolic blood pressure and fasting blood glucose levels. Fasting total cholesterol and low-density lipoprotein cholesterol levels were higher in the older age group ($P < 0.02$) but the ratio of total cholesterol to high-density lipoprotein cholesterol was similar ($P = 0.835$). Basal forearm blood flow was $3.80 \pm 0.48\ ml \cdot min^{-1} \cdot 100\ ml^{-1}$ of forearm volume in the older and $3.13 \pm 0.25\ ml \cdot min^{-1} \cdot 100\ ml^{-1}$ of forearm volume in the younger group ($P = 0.226$).

Relationship between age and endothelial function

All three agents caused dose-dependent decreases in basal forearm blood flow (Figure 1). At the highest doses of noradrenaline, aspirin and L-NMMA, the mean reduction in basal blood flow in younger subjects was 36.9 ± 5.2%,

Table 1 Clinical characteristics of study populations

Values are expressed as the mean ± S.E.M. $^*P < 0.05$ compared with younger subjects. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

	Value	
Characteristic	Younger subjects ($n = 18$)	Older subjects ($n = 15$)
Age (years)	32 ± 1 (range 22–44)	65 ± 1 (range 57–71)*
Sex (male/female)	12/6	7/8
Smoking history	15 never smoked	8 never smoked
	3 ex-smokers (6 ± 2 years)	7 ex-smokers (20 ± 6 years)
Body mass index (kg/m^2)	25.0 ± 0.8	25.1 ± 0.7
Blood pressure (mmHg)	115/79 ± 2/10	123/75 ± 5/3
Total cholesterol (mmol/l)	4.6 ± 0.2	5.7 ± 0.3*
LDL cholesterol (mmol/l)	2.8 ± 0.2	3.5 ± 0.2*
HDL cholesterol (mmol/l)	1.4 ± 0.1	1.6 ± 0.1
Triacylglycerols (mmol/l)	0.89 ± 0.07	1.25 ± 0.20
Total cholesterol/HDL ratio	3.6 ± 0.3	3.7 ± 0.2
Glucose (mmol/l)	5.1 ± 0.1	5.0 ± 0.2

34.9 ± 4.9% and 34.5 ± 4.0% respectively, and in older subjects it was 30.7 ± 6.5%, 25.0 ± 5.1% and 25.0 ± 4.0% respectively. The constrictor effects of aspirin and L-NMMA were blunted in the elderly compared with the younger group. The AUC values for aspirin in the younger and older group were 172.9 ± 24.2 and 70.2 ± 21.6 respectively ($P = 0.004$), and for L-NMMA were 170.1 ± 16.8 and 88.5 ± 22.4 respectively ($P = 0.007$). The response to noradrenaline was similar in the younger and older groups (AUC values, 162.3 ± 23.8 and 137.9 ± 22.5 respectively; $P = 0.461$).

DISCUSSION

The results of the present study demonstrate that the responses to endothelium-dependent constrictors (L-NMMA and aspirin) in the human forearm diminish with increasing age. L-NMMA and aspirin reduce forearm blood flow through the inhibition of endothelial production of NO and dilator prostanoids respectively. A reduction in the constrictor response to these inhibitors is consistent with diminished basal endothelial function. The response to noradrenaline (a directly acting smooth muscle constrictor) was not significantly reduced by increasing age, indicating that smooth muscle reactivity was largely preserved. Reduced activity of both the NO and prostacyclin pathways with increasing age is consistent with a generalized abnormality of basal endothelial function in elderly subjects.

Animal models indicate that ageing causes arterial endothelial dysfunction throughout the arterial vascular bed [11,12]. Similarly, impaired endothelial function has been described in elderly humans in conduit [13,14] and resistance arteries [7,15–17] and is independent of other known risk factors for endothelial dysfunction [7,13–17]. A consistent finding in these previous studies is that the direct response of the vascular smooth muscle to dilators (including glyceryl trinitrate) is normal, which is consistent with preserved smooth muscle function in the elderly. However, the mechanism of the response to endothelium-dependent dilators may include stimulation of NO, endothelium-derived hyperpolarizing factor [4] and prostaglandins [18], and it is unclear which of these mediators is affected by ageing.

In order to specifically assess background NO-mediated dilatation, we infused L-NMMA into the forearm, and the resulting reduction in blood flow was taken as a measure of NO-mediated endothelial function. L-NMMA caused a significant reduction in forearm blood flow in both age groups, which was diminished with increasing age. However, our results also indicate that the endothelial defect is not confined to the NO pathway. The present study is the first to investigate the relationship between prostaglandin-mediated dilatation and age. Dilator prostaglandins contribute to the regulation of vascular tone in a variety of animal species in the coronary and peripheral circulations [19–23]. In humans, a number of studies have also determined a role for dilator prostanoids in the regulation of the brachial [5,18,24] and coronary [25–27] vascular beds. In these studies, indomethacin or aspirin was infused locally to reduce regional blood flow, most likely through reduction of the production of prostacyclin, which is the principal vascular prostanoid produced in the human vasculature [28]. In the present study, we observed that the constrictor effect of aspirin was reduced in elderly subjects compared with the younger subjects, results that mirror the findings with L-NMMA. These observations suggest that the role played by prostaglandins in the

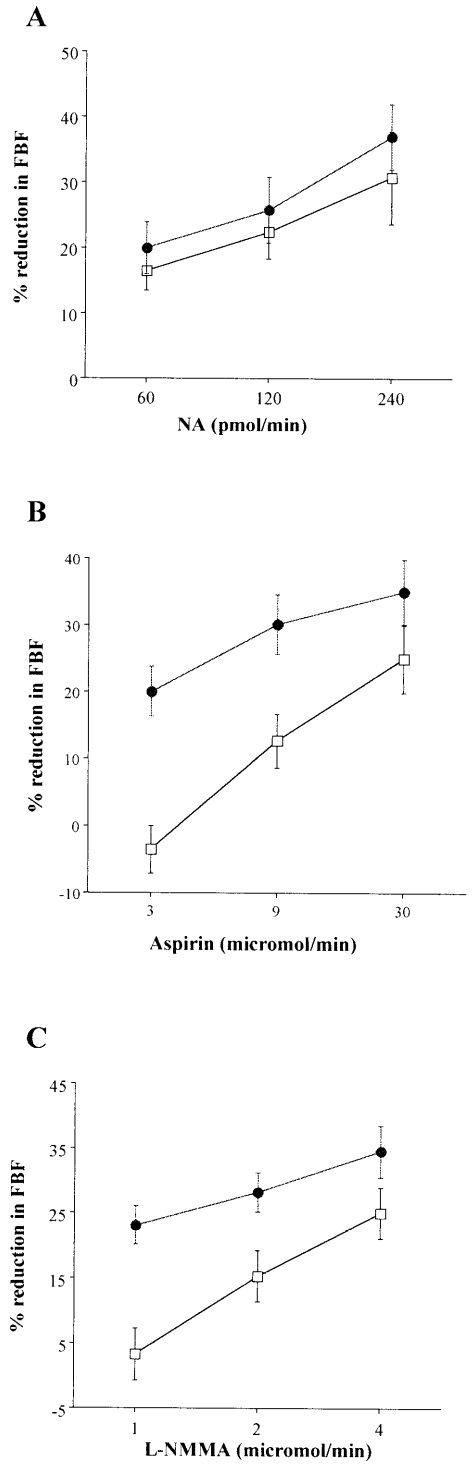

Figure 1 Constrictor response of the forearm resistance vasculature to noradrenaline (NA; A), aspirin (B) and L-NMMA (C) in older (□) and younger subjects (●)

Results are expressed as the percentage reduction in forearm blood flow (FBF; percentage reduction in the ratio of blood flow in the infused to control arm). There was no significant difference in the constrictor response to noradrenaline between the younger and older groups ($P = 0.461$). There was a significant difference in the constrictor response to aspirin and to L-NMMA between the younger and older groups ($P = 0.004$ and $P = 0.007$ respectively).

regulation of vascular tone, like that of NO, diminishes with age.

The high concentrations of aspirin used in the present study raise the possibility that non-specific actions (including reduced blood pH or increased noradrenaline release) might contribute to the constriction seen. However, at the doses infused, blood pH and noradrenaline release are unchanged [5]. Moreover, at these doses there is a close correlation between the concentration of prostacyclin metabolites and forearm blood flow [5]. Given that anti-inflammatory systemic doses of aspirin do not alter forearm blood flow or systemic blood pressure, these results suggest that near-maximal inhibition of COX is required before basal prostanoid-mediated vasodilatation is blocked.

To assess smooth muscle responsiveness, we measured the constrictor response to noradrenaline [29], which was similar in both groups. At the doses we used, noradrenaline predominantly acts on smooth muscle α-1 adrenoceptors to reduce blood flow [30,31]. Taken together, these findings suggest an impairment of basal endothelial function in older people, with largely preserved smooth muscle function.

The two groups that we studied were well matched for many known confounders of endothelial function. The younger group had an increased male:female ratio (although this was not statistically significant; $P = 0.264$, χ-squared test), but we did not observe any difference in endothelial or smooth muscle function between the males and females in our study group. Although the total cholesterol was higher in the older group, so too was high-density lipoprotein cholesterol, and adjusting for the cholesterol profile did not alter the analysis. We did not assess plasma homocysteine, which increases with age and might account for a proportion of the differences we observed in the two groups. Whether structural differences between young and older subjects (e.g. altered forearm fat to muscle ratios with ageing) might also influence vascular function remains to be determined.

The mechanisms for age-associated impairment of basal endothelial function have not been fully elucidated. Free radical oxidant stress increases with ageing and may impair many of the processes required for normal bioactivity of endothelium-dependent relaxing factors. There may also be reduced activity of endogenous antioxidant defences [32,33]. Free radicals (particularly superoxide) inactivate NO [34], or cause direct endothelial damage. Other possible mechanisms for age-related endothelial dysfunction include reduced synthesis [35] and impaired effector pathways for NO and other endothelium-derived relaxant factors [36–38].

In summary, our findings suggest that NO and prostanoid pathways exhibit reduced activity with increasing age. Reduced basal endothelium-dependent dilatation might contribute to age-related increases in peripheral vascular resistance and systemic hypertension.

Moreover, these endothelial mediators reduce leucocyte and platelet adhesiveness, smooth muscle proliferation and endothelial permeability, and reduction of these effects might contribute to the development of age-related atherosclerosis and its complications. Restoration of basal endothelial function in the elderly will require future therapies to target at least the NO and prostanoid pathways.

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