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Pyridoxine prevents dysfunction of endothelial cell nitric oxide production in response to low-density lipoprotein

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Abstract

Low-density lipoprotein (LDL) inhibits endothelium-dependent vasorelaxation. The aim of this study was to determine whether pyridoxine supplementation improves indices of LDL-induced endothelial dysfunction. Human umbilical vein endothelial cells (HUVEC) were incubated with native LDL (nLDL) from healthy subjects, oxidized LDL (oxLDL, formed by nLDL oxidation) or nLDL from type II diabetic patients (dLDL), in the absence or presence of pyridoxine; nitric oxide synthase (NOS) activity, cyclic GMP and expression of NOS isoforms were measured, as well as thiobarbituric acid reactive substances (TBARS) in HUVEC supernatants and amino acid concentrations in HUVEC lysates. All LDL species inhibited total NOS activity, whilst increasing the much smaller Ca^{2+}-independent component of NOS activity, the effects of oxLDL being greatest and those of nLDL smallest; in accordance with these findings, NOS type 3 expression decreased and NOS type 2 expression increased, with a resultant decrease in bioactive nitric oxide (NO), in HUVEC treated with each LDL species, with the same rank order of potency. LDL species also increased TBARS in HUVEC supernatants as well as homocysteine concentrations in HUVEC lysates, nLDL < dLDL < oxLDL. Pyridoxine largely prevented all LDL-induced changes in NOS activity and isoform expression, as well as in TBARS and homocysteine. The findings suggest that pyridoxine prevents LDL-induced dysfunction of endothelial cell NO generation, most likely through its antioxidant effects as well as through its effects on cellular homocysteine metabolism. This has important potential therapeutic implications for cardiovascular disease prevention.

Keywords: Endothelial cells; Low-density lipoproteins; Nitric oxide; Nitric oxide synthases; Pyridoxine

1. Introduction

Vascular endothelial dysfunction is found in a variety of cardiovascular disease states, such as type II diabetes and hypercholesterolemia as well as in established atherosclerosis, and studies suggest that the degree of endothelial dysfunction increases with the number of risk factors present in an individual [1,2]. This dysfunction is characterized by reduced bioactivity of endothelium-derived nitric oxide (NO); this may be due either to suppression of NO synthase (NOS) activity, or to increased NO scavenging attributable to enhanced $O_2^{\bullet -}$ production, since increased oxidative stress is a characteristic feature of these conditions [3]. Indeed, it has been suggested that endothelial dysfunction and impairment of NO availability may play a pathogenetic role in atherosclerosis [4,5].

Increased low-density lipoprotein (LDL) cholesterol is associated both with stimulation of atherogenesis and impairment of vascular endothelium-dependent relaxation [6–8]. This impairment can largely be attributed to a decrease in NO availability, principally through an increase in reactive oxygen species generation with a resultant increase in NO scavenging. Additionally, LDL can itself undergo oxidation

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in the presence of reactive oxygen species; oxidized (including minimally oxidized) LDL exerts a greater impairment of endothelium-dependent relaxation than does non-oxidized LDL [9,10], as does glyc-oxidized LDL, as found for example, in patients with type II diabetes [11]. Furthermore, LDL, and in particular oxidized LDL (oxLDL), can inhibit the high-affinity cationic amino acid transporter-1 (CAT-1), which is largely responsible for L-arginine uptake into endothelial cells, and this can be mimicked by its major lipid constituent, lysophosphatidylcholine [12]. Thus, qualitative as well as quantitative differences in LDL can be important as regards effects on vascular endothelial function.

We have recently reported that native LDL (nLDL) from healthy subjects, oxidized LDL (formed by nLDL oxidation) or nLDL from type II diabetic patients (dLDL) all inhibit NO generation by human umbilical vein endothelial cells (HUVEC), both through suppression of NOS activity and increased generation of reactive oxygen species, and that supplementation with either L-arginine or L-ascorbate or both together can ameliorate – but not completely normalize – NO production [13]. Therefore, although L-arginine and L-ascorbate are of potential clinical usefulness in cardiovascular disease prevention, by virtue of their ability to attenuate LDL-induced endothelial dysfunction, more effective therapies need to be sought. We have previously reported that pyridoxine (Vitamin B6) can improve endothelium-dependent relaxation in rabbit aortic rings exposed to nLDL, oxLDL or dLDL [14]. Pyridoxine acts as a cofactor in several enzymatic reactions but, additionally, has been reported to scavenge reactive oxygen species [15]. We therefore hypothesized that, in human vascular endothelial cells, pyridoxine will abrogate the impairment in NO generation caused by LDL. In the present study, therefore, we examined the effect of nLDL, oxLDL and dLDL on NO biosynthesis as well as expression of different NOS isoforms, by HUVEC, in the absence and presence of supplementation with pyridoxine.

2. Materials and methods

2.1. Materials and chemicals

The Glycacor kit was from BioGnosis Ltd (Hailsham, East Sussex, UK). Radiochemicals and the cyclic guanosine-3′,5′-monophosphate (cGMP) assay kit were from Amersham Life Science Ltd (Little Chalfont, Buckinghamshire, UK). Antibodies for immunostaining were obtained from BD Biosciences (San Diego, CA, USA). All other drugs and chemicals were from Sigma (Dorset, UK).

2.2. Subjects and LDL preparation

Seven healthy control subjects (5 male, 2 female) and 11 subjects with type II diabetes (8 male, 3 female) were recruited for these experiments. Controls and type II diabetic subjects were of similar age and sex distribution, and had comparable lipid profiles; however, both plasma glucose and HbA_{1C} were significantly higher in type II diabetics than in controls (Table 1). Native LDL from controls and type II diabetic subjects (nLDL and dLDL, respectively), were prepared from 100 mL venous blood, and nLDL and dLDL samples were pooled for the purpose of the experiments described here; oxLDL was derived from nLDL, as previously described [13]. All LDL isolates were adjusted to a protein concentration of 0.5 mg/mL, as measured using the bicinchoninic acid (BCA) method [16], and were stored at 4 °C and used within 1 month.

Fresh umbilical cords were obtained following delivery of healthy babies to healthy normotensive mothers, either by vaginal delivery or by elective Caesarean section. From these cords, HUVEC were isolated and cultured as previously described [17].

Informed consent was obtained from all subjects, and the study was approved by the institutional review board for human studies of Nanjing Medical University and by the King's College London Research Ethics Committee.

2.3. Oxidation and glycation status of isolated LDL

LDL oxidation status was determined by measuring the concentrations of thiobarbituric acid reactive substances (TBARS) in the different LDL samples, as previously described [13]. TBARS measurement revealed that dLDL was more oxidized than nLDL, and oxLDL even more so, as evidenced by an approximately 10- and 20-fold increase in malondialdehyde equivalents in dLDL and oxLDL, respectively, as compared with nLDL (nLDL: 2.7, dLDL: 28.1 and oxLDL: 58.5 nmol/mg protein). Storage of each of our LDL preparations for up to 1 month was not associated with an increase in measured TBARS (data not shown).

LDL glycation was quantified in our samples by competitive ELISA, using a proprietary assay kit (Glycacor), from which the percentage of total LDL in the glycated state was calculated. nLDL and oxLDL were glycated to only a small degree (14.3 and 15.0% of total LDL, respectively), whereas dLDL exhibited a much greater degree of glycation (49.0% of total LDL).

Table 1
Subject characteristics

	Controls	Type II diabetics
Number and sex	5 male, 2 female	8 male, 3 female
Age (years)	46.7 ± 2.9	48.2 ± 7.3
Total cholesterol (mmol/L)	5.5 ± 0.5	5.7 ± 0.9
HDL cholesterol (mmol/L)	1.4 ± 0.5	1.1 ± 0.2
LDL cholesterol (mmol/L)	3.2 ± 0.4	3.1 ± 0.8
Triglycerides (mmol/L)	1.7 ± 0.2	2.1 ± 0.5
Glucose (mmol/L)	4.9 ± 0.3	9.1 ± 1.6**
HbA1c (%)	4.9 ± 0.2	8.8 ± 1.8**

** $P < 0.01$ as compared with control subjects.