Augmented endothelial uptake of oxidized low-density lipoprotein in response to endothelin-1

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ABSTRACT

Endothelin-1 (ET-1) may be involved in the development and progression of atherosclerosis. Furthermore, endothelin receptor blockade was shown to reduce the formation of atherosclerotic lesions in experimental studies. Another potent pro-atherosclerotic risk factor is oxidized low-density lipoprotein (oxLDL). Endothelial cells mediate the uptake of oxLDL by the recently identified lectin-like oxLDL receptor-1 (LOX-1), which accumulates in atherosclerotic lesions. In the present study, we analysed the effects of ET-1 on oxLDL uptake and LOX-1 expression in primary cultures of human umbilical vein endothelial cells (HUVEC). ET-1 stimulated uptake of oxLDL in HUVEC, which reached a maximum after 1 h. In further studies, we found a similar induction of LOX-1 mRNA and protein expression in response to ET-1. The augmented oxLDL uptake and the increased LOX-1 expression in response to ET-1 are mediated by the endothelin receptor B. Our data support a new pathophysiological mechanism by which locally and systemically increased ET-1 levels, e.g. in hypertensive patients, could promote LOX-1-mediated oxLDL uptake in human endothelial cells. This mechanism could promote the development and progression of endothelial dysfunction and atherosclerosis. In addition, endothelin receptor blockade could be considered as a new anti-atherosclerotic therapeutic principle.

INTRODUCTION

Hypertension and hypercholesterolaemia are well known risk factors in the pathogenesis of atherosclerosis [1]. The plasma level of the potent vasoconstrictor endothelin-1 (ET-1) is increased in patients with coronary artery disease [2]. In addition, augmented local ET-1 concentrations have been found in atherosclerotic plaques. Therefore ET-1 has the potential to be involved in the pathogenesis of cardiovascular diseases [3]. Further evidence for the involvement of ET-1 in the pathogenesis of atherosclerosis is the reduced development of atherosclerotic lesions in apolipoprotein E-deficient mice treated with endothelin receptor blockers [4].

The pro-atherosclerotic risk factor oxidatively modified low-density lipoprotein (oxLDL) has been shown to impair endothelial relaxation [5] via a decrease in endothelial NO synthase expression and induction of the vascular formation of reactive oxygen species [6,7]. Other pro-atherosclerotic effects of oxLDL in endothelial cells are augmented expression of adhesion molecules [8] and increased apoptosis [9].

Endothelial cells mediate the uptake of oxLDL by the recently cloned lectin-like oxLDL receptor-1 (LOX-1)
while macrophages internalize oxLDL via scavenger receptors, leading to foam cell formation. LOX-1 accumulates in atherosclerotic lesions [11]. Another interesting function of LOX-1 is the recently described phagocytosis of aged and apoptotic endothelial cells [12]. The expression of LOX-1 can be stimulated by tumour necrosis factor-α, PMA, shear stress and transforming growth factor β [13–15]. LOX-1 has been suggested to provide a link between hypertension and atherosclerosis [16]. This is supported by the induction of LOX-1 in response to angiotensin II in human endothelial cells [17,18]. The effects of the potent vasoconstrictor ET-1 on the endothelial uptake of oxLDL and the expression of LOX-1 have not been studied so far. Therefore we measured the effects of ET-1 on oxLDL uptake and LOX-1 expression in human umbilical vein endothelial cells (HUVEC).

METHODS

Cell culture
All cell culture reagents and chemicals were purchased from Sigma Chemical Co, unless indicated otherwise. Primary cultures of HUVEC were isolated with collagenase IV and cultured in M199 medium (Life Technologies) supplemented with 20% (v/v) calf serum, as described previously [19]. Confluent cell cultures were incubated with medium containing 0.5% (v/v) calf serum for 24 h, and were subsequently treated with ET-1 (1–100 nM) or with ET-1 (100 nM) and the ET$_B$ receptor antagonist BQ-788 (1 μM; Alexis Corp.).

Uptake of oxLDL in human endothelial cells
LDL was isolated as described previously [20] and labelled with 1,1'–dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes). Confluent primary cultures of HUVEC were incubated with medium containing 0.5% (v/v) serum for 24 h, subsequently stimulated with ET-1 (100 nM) for the indicated times, and then incubated with DiI-labelled oxLDL (100 μg/ml) for 3 h. The uptake of oxLDL was determined by fluorescence and normalized to the protein concentration of each sample, as described previously [18].

Quantification of LOX-1 mRNA and protein expression
Total RNA from endothelial cells was isolated by guanidinium thiocyanate/caesium chloride centrifugation. Quantification of LOX-1 mRNA expression by standard calibrated competitive reverse transcriptase–PCR using an internal-deleted and in vitro-transcribed LOX-1 standard, and Western blot analysis using a primary anti-LOX-1 antibody, were done as described previously [18].

Data analysis
Data are given in relative units compared with control, and are means ± S.E.M. Statistical analysis was performed with Student’s t test or ANOVA followed by Bonferroni’s method (multiple comparison) (SigmaStat software; Jandel Corp.). A value of P < 0.05 was taken as the level of statistical significance.

RESULTS

Augmented uptake of oxLDL in response to ET-1 in HUVEC
HUVEC were stimulated with ET-1 and subsequently the uptake of DiI-labelled oxLDL was quantified. oxLDL uptake in HUVEC was augmented in response to ET-1 (Figure 1A). Maximal uptake (2.2 ± 0.3-fold increase compared with control) was found after a 1 h
ET-1 increases the expression of LOX-1

In order to find an endothelial receptor mediating the increased oxLDL uptake, HUVEC were stimulated with ET-1 in a time- and dose-dependent manner. The mRNA expression of the endothelial oxLDL receptor LOX-1 was quantified by standard-calibrated competitive reverse transcriptase–PCR. ET-1 induced LOX-1 mRNA expression in HUVEC (Figure 1B). Maximum induction was found using 100 nM ET-1 after 1 h, which resulted in a 1.6 ± 0.1-fold increase compared with the control (P < 0.05). This induction of LOX-1 mRNA expression was dose-dependent (maximal at 100 nM). As illustrated in Figure 1(B), the induction of LOX-1 mRNA by ET-1 (100 nM) was inhibited by the ET<sub>B</sub> receptor antagonist BQ-788 (1 μM).

The impact of ET-1 stimulation on LOX-1 protein expression was determined by Western blot analysis. In agreement with the findings at the mRNA level, stimulation of HUVEC with ET-1 (100 nM) augmented LOX-1 protein expression after 1 h by 1.7 ± 0.2-fold compared with the control (P < 0.05). This induction of oxLDL uptake and LOX-1 mRNA and protein expression was determined by Western blot analysis. In order to find an endothelial receptor mediating the increased oxLDL uptake, HUVEC were stimulated with ET-1 in a time- and dose-dependent manner. The mRNA expression of the endothelial oxLDL receptor LOX-1 was quantified by standard-calibrated competitive reverse transcriptase–PCR. ET-1 induced LOX-1 mRNA expression in HUVEC (Figure 1B). Maximum induction was found using 100 nM ET-1 after 1 h, which resulted in a 1.6 ± 0.1-fold increase compared with the control (P < 0.05). This induction of LOX-1 mRNA expression was dose-dependent (maximal at 100 nM). As illustrated in Figure 1(B), the induction of LOX-1 mRNA by ET-1 (100 nM) was inhibited by the ET<sub>B</sub> receptor antagonist BQ-788 (1 μM).

DISCUSSION

The principal finding of the present study is that ET-1 induces LOX-1 mRNA and protein expression after 1 h by 1.7 ± 0.2-fold compared with the control (P < 0.05). In summary, ET-1 induces oxLDL uptake and LOX-1 mRNA and protein expression in a similar manner.

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