Glucose-enriched medium enhances cell-mediated low density lipoprotein peroxidation

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Abstract A 1 week preculture of endothelial or smooth muscle cells in glucose-enriched (11.2 to 44.8 mM) media resulted in a marked enhancement of the subsequent ability of cells to oxidize low density lipoprotein, as assessed by the lipid peroxidation end product and conjugated diene content of the particle, its relative electrophoretic mobility and its degradation by macrophages. This phenomenon is correlated to a marked stimulation of superoxide anion secretion by cells. Such an effect of elevated glucose concentration on cell-induced LDL oxidative modification could be involved in the increased occurrence of atherosclerotic lesions in diabetes mellitus.

Key words: LDL peroxidation; Glucose; Endothelial cell; Smooth muscle cell; Diabetes mellitus; Atherosclerosis

1. Introduction

Low density lipoprotein (LDL) peroxidation by endothelial cells, smooth muscle cells or monocytes is currently believed to be one of the main factors involved in the development of atherosclerotic lesions [1–4]. Oxidized LDL are less catabolized by the normal apo B/E receptor pathway, but are taken up by macrophages inducing cholesteryl ester accumulation and appearance of foam cells, which contribute to the formation of atherosclerotic lesions [1–4].

During the last few years, an increasing number of studies have pointed out a possible role of LDL peroxidation in the occurrence of atherogenic lesions, which are one of the most frequent complications of diabetes mellitus [5–7]. The involvement of oxidative stress in diabetes is supported by several arguments such as increased concentration of lipid peroxidation end products (thiobarbituric acid reactive substances: TBARS) and decreased content of antioxidants in the plasma of patients [8–10]. Recent studies have suggested that elevated glucose concentration in plasma could promote LDL oxidation by leading to glycated forms of the particle which are more sensitive to transition metal-dependent auto-oxidation [11]. Glucose by itself seems to enhance the in vitro LDL oxidative modification induced by copper ions [12,13]. However, there are at the present time no studies on a possible effect of elevated glucose concentration on the capacity of cultured cells to oxidize LDL, leading to modified forms being taken up by macrophages. In the present work, we demonstrate that culture of endothelial or smooth muscle cells in glucose-enriched media results in increased ability of these cells to subsequently oxidize LDL, and that this phenomenon is accompanied by enhanced secretion of superoxide anion.

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Abbreviations: LDL, low density lipoprotein; TBARS, thiobarbituric acid reactive substance (lipid peroxidation end products).

2. Materials and methods

2.1. Materials

Cell culture media and foetal calf serum were from Gibco, Grand Island, NY, USA. J774 murine macrophage-like cells were from The American Type Culture Collection, Rockville, MD, USA. The UNA endothelial cells and A7r5 smooth muscle cells, both of murine origin, were generous gifts from Prof. J.D. Chapman (Fox Chase Cancer Institute, Philadelphia, USA) and Dr. O. Colard (Faculté de Médecine Saint-Antoine, Paris, France), respectively. Na^{125}I (13–17 Ci/mg) was purchased from Amersham, Buckinghamshire, UK.

2.2. LDL preparation and labelling

LDL (d = 1.024–1.050) was prepared from normal human serum by sequential ultracentrifugation according to Havel et al. [14], and dialyzed against 0.005 M Tris, 0.05 M NaCl, 0.02% EDTA, pH 7.4, for conservation. Prior to oxidation, EDTA was removed by dialysis for 24 h, and LDL stored at 4°C before oxidation by cultured cells within 2 days. ^{125}I labelling of LDL was performed by the method described by Bilheimer et al. [15]. The specific activity was about 250 cpm/ng.

2.3. Cell culture

UNA endothelial cells were cultured in Ham's F10 medium, and A7r5 smooth muscle cells in Dulbecco's modified minimum essential medium, both supplemented with 10% foetal calf serum. The J774 macrophage cell line was maintained in suspension in RPMI 1640 medium supplemented with 10% heat inactivated foetal calf serum. For LDL degradation determination, the cells were seeded in 3.5 cm Petri dishes (2×10^6 cells/dish). All experiments were performed on subconfluent cultures.

2.4. LDL oxidation

Cells were precultured for 1 week in serum supplemented medium in the presence of increasing (5.6 to 44.8 mM) concentrations of glucose. LDL (0.1 mg/ml LDL protein) were then incubated for 24 h with cells in standard Ham's F10 medium without further addition of glucose. The TBARS content of LDL was checked by the fluorometric assay of Yagi [16]. Results are expressed in nmol equivalent malondialdehyde/mg LDL protein, using malondialdehyde from tetramethoxypropane as a standard, and calculated as % of control. Conjugated dienes were measured at 234 nm as previously described [17]. The modification of the negative net charge of LDL was assessed by agarose gel-electrophoresis at pH 8.6 in a Ciba Corning system.

2.5. LDL degradation by J774 macrophage-like cells

Oxidation of [^{125}I]LDL by cells was first carried out with 0.02 μg/ml LDL as previously described [18]. The medium was then transferred to J774 cells. LDL degradation was determined after 6 h as the trichloroacetic acid-soluble non-iodide radioactivity, and expressed in ng LDL degraded/mg cellular protein. Blank values from parallel incubations without cells were also determined.

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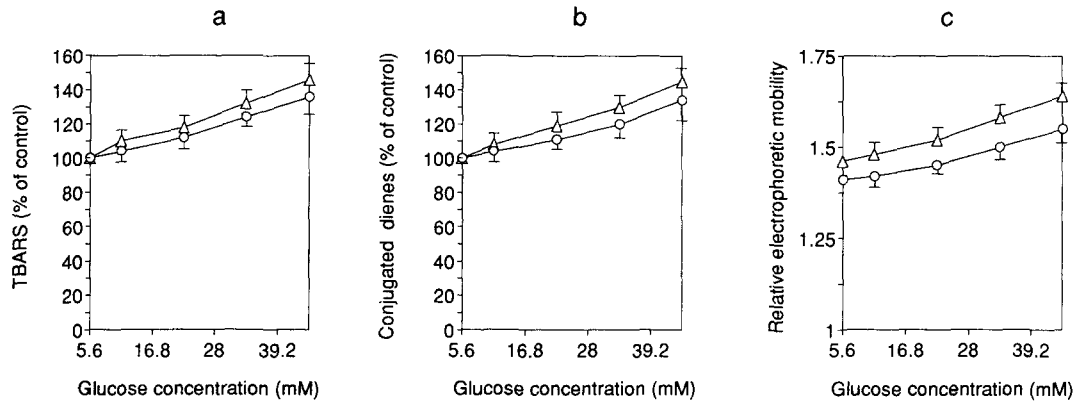

Fig. 1. Effect of glucose on LDL oxidative modification by endothelial or smooth muscle cells. Cells were precultured for 1 week in medium containing increasing (5.6 to 44.8 mM) concentrations of glucose, washed, and then incubated for 24 h with 0.1 mg/ml LDL in standard Ham's F10 medium. The degree of LDL modification was assessed by the TBARS (a) or conjugated dienes (b) content of the particle, and by measurement of its relative electrophoretic mobility (c). Triangles, UNA endothelial cells; circles, A7r5 smooth muscle cells. 100% for the LDL TBARS contents were 44 ± 6 and 36 ± 4 nmol/mg LDL protein for UNA and A7r5 cells, respectively. 100% for conjugated dienes, expressed as the differential OD at 234 nm were 0.25 ± 0.03 and 0.20 ± 0.02 for UNA and A7r5 cells, respectively. Means of 6 experimental values ± S.D.

2.6. Secretion of superoxide anion by cells

Cells were cultured for 1 week in medium supplemented with different concentrations of glucose. After washing, the cells were then incubated for 1 h in Dulbecco's modified minimum essential medium devoid of Phenol red, in the presence of $2 \cdot 10^{-5}$ M horse heart cytochrome *c* [19]. Superoxide anion release was calculated from the difference in OD at 550 nm in the absence and presence of superoxide dismutase (100 μg/ml), using a molar extinction coefficient of 21/mM/cm.

Each experiment was performed at least 3 times in duplicate. Statistical analysis was performed by Student's *t*-test.

3. Results

Fig. 1 displays the effect of a 1 week preculture in medium containing increasing glucose concentrations, on the ability of endothelial or smooth muscle cells to subsequently oxidize LDL. It can be clearly observed that the 3 parameters tested for checking LDL oxidation (TBARS content, conjugated diene formation, and relative electrophoretic mobility of the particle) were increased in a dose-dependent manner with the glucose concentration in the culture medium. A 35–45% increase in TBARS content and diene formation was observed at the highest tested glucose concentration (44.8 mM); under the same culture conditions, the relative electrophoretic mobility of the particle was increased by about 30% as compared to LDL oxidized by cells cultured in control (5.6 mM) medium.

Since oxidized LDL is rapidly taken up by the scavenger pathway of macrophages, we also studied the degradation of the labelled particle by J774 monocyte macrophages. Table I shows that LDL degradation was augmented after modification by cells cultured in glucose-enriched medium, with an about 65 and 50% increase for endothelial and smooth muscle cells exposed to 44.8 mM glucose, respectively.

As active oxygen species have been suggested to be involved in LDL oxidative modification [20,21], we then measured the influence of glucose on superoxide anion secretion by UNA or A7r5 cells. Table 2 shows that superoxide production was markedly and dose-dependently stimulated by a 1 week preculture in glucose-enriched medium, with an about 1.5 or 2 fold increase for glucose concentrations of 22.4 or 44.8 mM, respectively.

4. Discussion

The above results clearly show that preculture of endothelial or smooth muscle cells in glucose-enriched medium induced a dose-dependent increase in LDL oxidative modification and recognition by macrophages, the latter process being currently believed to be one of the important steps involved in the initiation and progression of atherogenic lesions.

It is now believed that LDL modification is involved in the pathogenesis of atherosclerotic lesions, which occur with increased frequency in patients with diabetes mellitus. Although a lot of studies have been concerned with the role of non-enzymatic LDL glycosylation in the atherosclerotic complications of diabetes [22–24], only a few have focused attention on

Table 1
Degradation by J774 monocyte macrophages of LDL modified by endothelial or smooth muscle cells precultured in glucose-enriched medium

	[^{125}I]LDL degradation (ng/mg protein)	
	Endothelial cells	Smooth muscle cells
LDL without cells, 125 ± 18		
LDL modified by glucose concentration (mM)		
5.6 (control)	437 ± 49	345 ± 39
11.2	552 ± 67*	380 ± 46
22.4	628 ± 55**	448 ± 52*
33.6	715 ± 82***	490 ± 62**
44.8	790 ± 95***	527 ± 75***

UNA endothelial cells or A7r5 smooth muscle cells were precultured for 1 week in medium supplemented with various glucose concentrations, then washed 3 times and incubated for 24 h with 0.02 mg/ml [^{125}I]LDL in standard Ham's F10 medium. [^{125}I]LDL degradation was then studied as previously described. Results are expressed as ng [^{125}I]LDL degraded/mg cell protein. Means of 6 experimental values ± S.D. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ by Student's *t*-test.

Table 2
Effect of glucose on superoxide anion release by endothelial and smooth muscle cells

Glucose (g/l)	Superoxide release (nmol/h/mg protein)	
	Endothelial cells	Smooth muscle cells
5.6 (control)	134 ± 12	102 ± 18
11.2	172 ± 21*	124 ± 15
22.4	215 ± 38**	145 ± 18*
33.6	256 ± 33***	163 ± 22**
44.8	270 ± 42***	176 ± 19***

UNA endothelial cells or A7r5 smooth muscle cells were precultured for 1 week in medium containing 5.6 to 44.8 mM glucose before determination of the superoxide anion release. Means of 6 experimental values ± S.D. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ by Student's t-test.

a possible involvement of LDL lipid peroxidation in this process. In particular, it has recently been shown that high glucose concentrations enhance in vitro LDL auto-oxidation induced by copper ions [12,13]. Glycated LDL also seems to be more sensitive to copper-induced oxidative modification [11]. In all these studies, attention has mainly been paid to the direct influence of high glucose concentrations or of LDL glycation on the oxidisability of the particle in in vitro models (LDL auto-oxidation). However, it might also be supposed that elevated glucose concentrations in the blood of patients with diabetes mellitus results in an alteration in the metabolism of the cells involved in LDL oxidative modification, such as endothelial and smooth muscle cells. To our knowledge, the present work is the first demonstration of a stimulatory effect of glucose on cell-mediated oxidative modification of LDL. Similar results were obtained with U937 monocyte-like cells (data not shown), which are also widely used as an experimental model for studying LDL modification.

It must be stressed that in our experimental system, LDL modification by cells was conducted in the absence of added glucose (standard Ham's F10 medium containing 5.6 mM glucose), which eliminated the possibility of a direct effect of glucose on the oxidative process. It is also noteworthy that a significant enhancement of cell-mediated LDL modification was already observed at 22.4 mM of glucose, a concentration which is much lower than that used in most of the studies dealing with LDL glycation [11,22]. Moreover, only a 1 week culture of cells in glucose-enriched medium is sufficient to observe a significant effect. A longer pretreatment period (2–4 weeks) did not led to a proportional increase in the ability of cells to modify LDL (data not shown). Our studies also demonstrate for the first time that the production (or/and secretion) of superoxide anion by endothelial or smooth muscle cells is increased by a preculture in glucose-enriched media. It is noteworthy that under experimental conditions, up to 44.8 mM, no significant effect of glucose was noted on cell proliferation as assessed by cell counting or by [^{3}H]thymidine incorporation into DNA. Regardless of the mechanisms, which remain to be specified, the observation that elevated glucose concentrations in the culture medium enhances superoxide production and the subsequent ability of endothelial and smooth muscle cells to oxidatively modify LDL brings a new insight to the pathogenesis of atherosclerotic lesions in diabetes mellitus.

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