Glycation amplifies lipoprotein(a)-induced alterations in the generation of fibrinolytic regulators from human vascular endothelial cells

Jianying Zhang, Song Ren, Garry X. Shen *

Departments of Internal Medicine and Physiology, The University of Manitoba, BS440 730 William Ave, Winnipeg, Manitoba, Canada R3E 3J7

Received 26 January 1999; received in revised form 23 August 1999; accepted 3 September 1999

Abstract

Increased lipoprotein(a) [Lp(a)] in plasma is an independent risk factor for premature cardiovascular diseases. The levels of glycated Lp(a) are elevated in diabetic patients. The present study demonstrated that glycation enhanced Lp(a)-induced production of plasminogen activator inhibitor-1 (PAI-1), and further decreased the generation of tissue-type plasminogen activator (t-PA) from human umbilical vein endothelial cells (HUVEC) and human coronary artery EC. The levels of PAI-1 mRNA and its antigen in the media of HUVEC were significantly increased following treatments with 5 μg/ml of glycated Lp(a) compared to equal amounts of native Lp(a). The secretion and de novo synthesis of t-PA, but not its mRNA level, in EC were reduced by glycated Lp(a) compared to native Lp(a). Treatment with aminoguanidine, an inhibitor for the formation of advanced glycation end products (AGEs), during glycation normalized the generation of PAI-1 and t-PA induced by glycated Lp(a). Butylated hydroxytoluene, a potent antioxidant, inhibited native and glycated Lp(a)-induced changes in PAI-1 and t-PA generation in EC. The results indicate that glycation amplifies Lp(a)-induced changes in the generation of PAI-1 and t-PA from venous and arterial EC. This may attenuate fibrinolytic activity in blood circulation and potentially contributes to the increased incidence of cardiovascular complications in diabetic patients with hyperlipoprotein(a). EC-mediated oxidative modification and the formation of AGEs may be implicated in glycated Lp(a)-induced alterations in the generation of fibrinolytic regulators from vascular EC. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Glycated lipoprotein(a); Vascular endothelial cells; Plasminogen activator inhibitor-1; Tissue-type plasminogen activator; Antioxidants; Aminoguanidine

1. Introduction

Numerous epidemiological studies suggest that increased plasma lipoprotein(a) [Lp(a)] is a strong and independent risk factor for premature cardiovascular diseases [1,2]. Lp(a) particles have been detected in the atherosclerotic vascular wall [3,4]. The risk of developing cardiovascular disease is greatly increased in diabetic individuals [5]. Circulating Lp(a) levels were increased in Type 2 diabetic patients and positively correlated with blood concentrations of hemoglobin A1c, an index of glucose control [6]. The impact of Lp(a) on the development of cardiovascular complications in diabetes had not been known.

The Lp(a) particle is composed of low density lipoprotein (LDL) and apolipoprotein(a) [apo(a)]. Apo(a) has more than 30 sizes of isoforms or phenotypes. The sizes of apo(a) depend on the number of kringle repeats in the polypeptide. The kringle of apo(a) are to a great extent a homologue of the kringle IV of plasminogen. Lp(a) competes with plasminogen in binding to fibrin and plasminogen receptors on the surfaces of monocytes and vascular endothelial cells (EC), which may attenuate the generation of plasmin and reduces fibrinolytic activity in blood circulation [7,8]. The capacity for the generation of fibrinolytic activity responding to vascular stress is reduced in diabetic patients [9]. In Type 2 diabetes, plasma levels of plasmi-
nogen activator inhibitor-1 (PAI-1) were significantly elevated [10] and that was correlated with body mass index, plasma insulin and triglycerides [11]. Increased levels of PAI-1 were found in Type 1 diabetic patients with a high risk for developing atherosclerotic complications [12]. PAI-1 is the major physiological inhibitor for plasminogen activators. The dominant plasminogen activator in blood circulation is tissue-type plasminogen activator (t-PA), which modulates the formation of plasmin.

Vascular EC synthesize both PAI-1 and t-PA. The generation of fibrinolytic regulators in EC is modulated by a variety of biological agonists, including proinsulin [13], glucose [14,15] and several types of plasma lipoproteins [16–22]. Very low density lipoprotein from hypertriglyceridemia, native and oxidized LDL stimulated the release of PAI-1 from EC or hepatocytes [16–23]. Treatments with Lp(a), LDL or high density lipoprotein reduced the secretion of t-PA from EC [24]. Lp(a) also elevated the production of PAI-1 in EC [25]. Previous studies in our laboratory demonstrated that oxidation enhanced the production of PAI-1 in EC induced by Lp(a) [26]. Glycation promotes the peroxidation of LDL [27]. Our recent studies indicate that glycated LDL stimulated PAI-1 but reduced t-PA generation from the human umbilical vein EC (HUVEC) [28]. The present study examined the effect of glycated Lp(a) on the generation of fibrinolytic regulators in HUVEC and human coronary arterial EC (HCAEC). The involvement of EC-mediated oxidative modification and advanced glycation end products (AGEs) in glycated Lp(a)-induced generation of fibrinolytic regulators was investigated.

2. Methods

2.1. Isolation of lipoproteins

Density < 1.21 fraction was separated from fresh human plasma by density ultracentrifugation in the presence of 1 mM EDTA [29]. The lipoprotein-rich fraction was applied on a lysine Sepharose-CL 4B affinity column. Lp(a)-free LDL (1.019–1.063) was isolated previously described [31]. The levels of endotoxin in lipoproteins were monitored by Limulus amebocyte lysate test using a commercially available kit (E-TOXATE, Sigma, St. Louis, MO). The lowest limit for detecting endotoxin using this system is 0.05 ng/ml. No detectable amount of endotoxin was found in lipoprotein preparations.

2.2. Modification of lipoproteins

Lp(a) and Lp(a)-free LDL were glycated by 25, 50 or 200 mM glucose for 1–3 weeks in the presence of equimolar sodium cyanoborohydride in 0.1 M phosphate buffer (pH 7.4) containing 0.01% EDTA and 0.01% sodium azide in sealed tubes under N2 at 37°C. At the end of glycation, lipoproteins were dialyzed to remove free glucose. Native lipoproteins used in this study were proceeded in parallel without exposure to glucose. Glycated and native lipoproteins were analyzed on 1% non-denatured agarose gel electrophoresis (Corning, Oneonta, NY) at 90 V for 50 mins in 75 mM barbital buffer (pH 8.6) to assess the electrophoretic mobility of lipoproteins. The extent of glycation of lipoproteins was estimated by measuring the abundance of glucitollysine using reverse phase HPLC as described previously [28]. Integrity of apo(a) in glycated Lp(a) was examined using the aforementioned Western blotting analysis [31]. As outlined earlier, no fragmentation of apo(a) in glycated Lp(a) was found in comparison to its unmodified form [6], and lipid peroxidation of lipoproteins was evaluated by measuring thiobarbituric acid reaction substances (TBARS) [26]. No significant increase in TBARS was detected in native and glycated Lp(a) and LDL.

2.3. Cell culture and stimulation

HUVEC were obtained by collagenase digestion and verified as previously outlined [26]. HUVEC were grown in M-199 medium supplemented with 10% fetal bovine serum, 30 μg/ml of EC growth stimulator, 100 μg/ml of heparin, 0.1 mM nonessential amino acids, 200 U/ml of penicillin and 200 μg/ml of streptomycin in a humidified incubator under 95% air and 5% CO2 at 37°C. Confluent HUVEC were stimulated in heparin-free M-199 medium for the evaluation of t-PA and PAI-1. Seed HCAEC and Endothelial Growth Medium-MV Bulletkits were purchased from Clonetics (San Diego, CA). HCAEC were grown and stimulated in the same medium and used within passage eight. Cytotoxicity of lipoproteins was determined by incubating cells with 5 × 106 dpm/well of 3H-leucine (54 Ci/mM, ICN Radiochemical, Irvine, CA) in leucine-free medium for 2 h after treatments with lipoproteins [32]. The incorporation of 3H-leucine in EC was not significantly altered by treatment with up to 10 μg/ml of Lp(a), 100 μg/ml of LDL or their glycated forms for ≤ 48 h.
2.4. Measurement of PAI-1 and t-PA antigen

The medium was collected from cultured cells at the end of incubations. Cells were harvested in phosphate buffered saline (PBS, pH 7.4) containing 0.1% SDS and 0.5% Triton X-100. Total amounts of PAI-1 and t-PA antigen in free or complex form in the medium from replicated cultures were estimated by using IMUBIND PAI-1 or t-PA ELISA kits with monoclonal antibodies against human PAI-1 or t-PA (American Diagnostica Inc., Greenwich, CT). The levels of PAI-1 and t-PA antigen were measured on a microplate reader at an absorbency of 490 nm and values were corrected with total cellular proteins from each well [28].

2.5. Northern blotting analysis

The total cellular RNA cultured in 150 mm dishes was extracted from cells at the end of incubation by guanidine isothiocyanate-cesium chloride method [33]. RNA was denatured and subject to electrophoresis on a 1% agarose-formaldehyde gel, then transferred to Zeta-probe GT blotting membranes (BioRad, Hercules, CA). Plasmids containing cDNA fragments of encoded human PAI-1, t-PA or β-actin were labelled with [32P]-dCTP (> 111 TBq/mM, Dupont NEN) using random primer labelling kits. Blots were prehybridized in 0.25 M Na2HPO4 (pH 7.2) and 7% SDS for 10 mins, then hybridized with denatured radioactive probe for 16 h at 25°C [34]. After hybridization, blots were washed, then subjected to autoradiography. The levels of PAI-1 and t-PA mRNA were quantified from autoradiogram by density scanning, and subsequently corrected with the levels of β-actin mRNA in corresponding lanes.

2.6. Metabolic labelling and immunoprecipitation

Confluent cells in 60 mm dishes were treated with 100 μCi/ml of Trans35S-label (85% methionine/15% cystine, 38 TBq/mM, ICN) in methionine- and cystine-free medium supplemented with 2 mM glutamine and 10% serum, with and without lipoproteins [28]. Cellular lysates were diluted with buffer containing 50 mM Tris, 0.5 M NaCl, 0.1% NP-40, 1 mM EDTA, 2.5% gelatine and 0.5% bovine serum albumin (NET buffer). Media and cellular lysates were incubated with 30 mg/ml of rabbit IgG at 25°C for 1 h, then treated with 40 μl of a 50% slurry of protein A Sepharose for 30 mins. Beads bound with non-specific IgG were removed by centrifugation. Resultant supernatant was incubated at 25°C for 2 h with goat anti-human uterine t-PA IgG (American Diagnostica Inc.). The immune complexes of t-PA were collected using protein A Sepharose and recovered from beads using 125 mM Tris buffer (pH 6.8) containing 20% glycerol and 4.6% SDS. The mixture was analyzed on 12% SDS-PAGE. Radioactive t-PA on dried gels was visualized using autoradiography, and quantified by density scanning.

2.7. Measurement of protein concentrations in lipoproteins and cells

Protein concentrations in lipoproteins were determined using a modified Lowry method [34]. For measuring the total cellular proteins, cells were lysated in PBS containing 0.5% Triton X-100 and 0.1% SDS.

2.8. Statistical analysis

Values were presented as mean ± SD from quadruplicate or triplicate cultures. Student’s t-test was used for the comparison of probability of values between two groups. Comparisons among multiple groups were achieved using one-way ANOVA followed by Duncan’s test analysis. The level of significance was defined as P < 0.05. Statistical analyses were performed using Number Cruncher Statistical System software.

3. Results

3.1. Effects of glycated Lp(a) on PAI-1 and t-PA secretion

The levels of PAI-1 and t-PA antigen were determined in post-cultural medium of HUVEC treated with up to 1–10 μg protein/ml of native or glycated Lp(a) (modified by 50 mM glucose). Incubation with above 5 μg/ml of native or glycated Lp(a) for 48 h significantly increased PAI-1 release from HUVEC compared to controls. Glycated Lp(a) ≥ 5 μg/ml increased PAI-1 release compared to identical concentrations of native Lp(a) (P < 0.05, Fig. 1, upper). In contrast, the secretion of t-PA from HUVEC treated with various amounts of native or glycated Lp(a) for 24 h was dose-dependently decreased. The reductions in t-PA generation reached significant levels following treatments with ≥ 2.5 mg/ml of native or glycated Lp(a) (Fig. 1, bottom). Glycated Lp(a)-induced inhibition of t-PA secretion was significantly greater than native Lp(a). The levels of t-PA antigen in the media of HUVEC treated with 5 μg/ml of native Lp(a) were reduced by 36.6% in comparison to no addition controls. Glycated Lp(a) at the same concentration decreased t-PA antigen level in the medium by 52.3% compared to controls or by 25% compared to native Lp(a) (P < 0.001).

PAI-1 and t-PA levels were time-dependently increased in the media of HUVEC treated with 5 μg/ml of native or glycated Lp(a). Native Lp(a) moderately but significantly stimulated PAI-1 release compared to controls, following a 48 h treatment (P < 0.01). Glycated Lp(a) modified by 50 mM glucose accelerated and
enhanced PAI-1 secretion from EC compared to native Lp(a) (*P < 0.01 or 0.001, Fig. 2, upper). Incorporation of \(^3\)H-leucine was significantly reduced in cells treated with native or glycated Lp(a) for either 56 or 72 h but not ≥ 48 h. The results generated from cultures with ≥ 56 h of Lp(a)-treatments were not included in the present study. Accumulation of t-PA in media was time-dependent in cultures treated with Lp(a) or medium without addition. Significant decreases in t-PA generation were detected in EC treated with 5 μg/ml of native Lp(a) for ≥ 24 h compared to appropriate controls. The inhibitory effect of Lp(a) on t-PA generation in EC was aggravated by treatment with glycation of Lp(a). The levels of t-PA in the medium of HUVEC treated with glycated Lp(a) for 24 or 48 h were significantly lower than that in native Lp(a)-treated cells (*P < 0.05 or 0.01, Fig. 2, bottom).

The influence of glucose concentration and exposure time on glycated Lp(a)-induced PAI-1 and t-PA generation from EC was examined. Treatments with glycated Lp(a) were modified by 25 mM glucose for 3 weeks, 50 mM glucose for 2 weeks, or 200 mM glucose for 1 week which altered the generation of PAI-1 and t-PA to similar extents (Fig. 3). The extents of glycation evaluated by glucitollysine/lysine ratio in those lipoproteins were comparable: 1.12 for glycated Lp(a) modified by 25 mM glucose for 3 weeks; 1.09 for that modified by 50 mM glucose for 2 weeks; 1.14 for that by 200 mM glucose for 1 week, compared to 0.19 for native Lp(a) (average of two separate preparations with variations ≤ 15%). Glycated Lp(a) (10 μg/ml) modified by 25 mM glucose for 3 weeks (glucitollysine/lysine ratio 0.34) did not significantly affect PAI-1 or t-PA release from EC compared to controls. The same amount of glycated Lp(a) modified by 50 mM glucose for 1 week (glucitollysine/lysine ratio 0.62) moderately altered PAI-1 (+18.9 ± 2.1%) and t-PA release (−23.2 ± 3.7%) from EC compared to controls (mean ± SD,

---

Fig. 1. Dose-response of native and glycated Lp(a) on the generation of PAI-1 and t-PA antigen from cultured HUVEC. Cells were incubated with 1–10 μg/ml of native Lp(a) [n-Lp(a)] or glycated Lp(a) [gly-Lp(a), modified by 50 mM glucose] for 48 h (PAI-1, upper) or 24 h (t-PA, bottom). The levels of PAI-1 and t-PA antigen in the medium were determined by ELISA as described in Section 2. Values were expressed as μg/mg of cellular proteins (mean ± SD) from quadruplicate cultures. * , ** : * P < 0.05, 0.01 or 0.001 versus cultures without addition; + , ++ : P < 0.05 or 0.01 versus n-Lp(a).

Fig. 2. Time-dependence of glycated Lp(a) on the generation of PAI-1 and t-PA antigen from cultured HUVEC. Cells were treated with medium without addition (control), with 5 μg/ml of native Lp(a) [n-Lp(a)] or glycated Lp(a) [gly-Lp(a), modified by 50 mM glucose] for 8–48 h. The levels of PAI-1 (upper) and t-PA (bottom) antigen in the media were analysed by ELISA. Values were expressed as μg/mg of cellular proteins (mean ± SD) from quadruplicate cultures, * * * * * : * P < 0.05; 0.01 or 0.001 versus controls following matching period of incubation; + , ++ : P < 0.05 or 0.01 versus n-Lp(a).
Our findings suggest that the effects of glycated Lp(a) on PAI-1 and t-PA generation are dose-dependent to glucose used in glycation. Lp(a) modified by 25 or 50 mM glucose for longer periods (2–3 weeks) may alter the generation of fibrinolytic regulators from EC to a similar extent as that modified by 200 mM glucose for a shorter period.

The effects of native and glycated Lp(a) on the generation of PAI-1 and t-PA were also examined in HCAEC. Due to required growth condition and the cost of specific medium/ supplements, limited experiments were performed in HCAEC. The basal level of PAI-1 released from HCAEC was 2.7-fold of that for HUVEC. The level of t-PA released from HCAEC was 18% lower than that from HUVEC without addition (Fig. 4). Treatment with 10 μg/ml of native Lp(a) induced comparable increases in PAI-1 generation from HCAEC (+40%) and HUVEC (+33%). PAI-1 release was significantly increased by 50 mM glucose-modified glycated Lp(a) in HCAEC (+109%) and HUVEC (+68%) (Fig. 4, upper). Inhibition of t-PA generation from HCAEC by native and glycated Lp(a) (−18 and −32%) was considerably less than that from HUVEC (−42 and −56%) (Fig. 4, bottom).

### 3.2. Effect of glycated Lp(a) on PAI-1 mRNA

PAI-1 mRNA in HUVEC was presented as two bands, approximately 3.4 and 2.4 kb, as previously described [26]. Density of the 2.4 kb PAI-1 mRNA was obviously enhanced by treatments with 5 μg/ml of glycated Lp(a) for 48 h compared to an equal amount of native Lp(a) or controls. The levels of 3.4 kb PAI-1 mRNA were slightly reduced in HUVEC by glycated Lp(a) compared to native Lp(a)-treated cells. No substantial change in the levels of β-actin mRNA was found in cells treated with native or glycated Lp(a) (Fig. 5). The steady state levels of 2.4 kb PAI-1 mRNA in HUVEC treated with 5 μg/ml of native and glycated Lp(a) for 48 h were quantified by density scanning of Northern blots. Native Lp(a) increased 2.4 kb PAI-1 mRNA levels more than twofold (2.23 ± 0.25-fold of controls, mean ± SD from triplicate cultures). Significant increases in the abundance of 2.4 kb PAI-1 mRNA were detected in HUVEC treated with glycated Lp(a) (3.52 ± 0.21-fold) compared to native Lp(a) (P < 0.01).
3.3. Effect of glycated Lp(a) on mRNA level and synthesis of t-PA

No obvious change in the density of t-PA mRNA was detected in HUVEC treated with 5 μg/ml of native or glycated Lp(a) for 24 h compared to controls (Fig. 6). In order to determine which step in the process of t-PA generation in EC was affected by Lp(a) or its glycated form, HUVEC were metabolically labelled with 35S-methionine-cystine (70%/30%) in the presence of 5 μg/ml of native or glycated Lp(a) for 48 h. Radioactivity of t-PA was mainly detected in extracellular fluid but not in cell-associated pool. Synthesis of t-PA in EC treated with native Lp(a) was 79.1 ± 3.4% (mean ± SD from triplicated cultures) of control group (P < 0.01). In EC with 5 μg/ml of glycated Lp(a) (modified by 50 mM glucose), the synthesis of t-PA was 62.3 ± 5.6% of controls (P < 0.001), and that was significantly lower than the sample in EC that was treated with the same amount of native Lp(a) (P < 0.05).

3.4. Comparison of the effects of glycated Lp(a) and LDL on PAI-1 and t-PA generation

Lp(a) and Lp(a)-free LDL isolated from a healthy donor, which apo(a) phenotype is B/S2 (double-band phenotype with B type as the major isoform and S2 as the minor), were applied in the experiments described in Fig. 7. Since the size of apo(a) B is approximately equal to apoB-100, the protein content in Lp(a) of this individual is close to 2-fold of that in equimolar amount of LDL. LDL and Lp(a) from the individual was glycated with 50 mM glucose for 2 weeks. Ratios of glucitolysine:lysine in glycated Lp(a) and LDL were 0.97 and 1.04, respectively. Native and glycated Lp(a) at 5 μg/ml significantly affected the generation of PAI-1 and t-PA from EC. Treatment with 2.5 μg/ml of native or glycated LDL modified by the same condition, which contains similar amount of apoB-100 as in 5 μg/ml of Lp(a) B isoform, did not significantly alter PAI-1 or t-PA generation. Native LDL at the level of 100 μg/ml significantly increased PAI-1 generation and reduced t-PA release compared to controls (P < 0.001). Glycated LDL (100 μg/ml) induced significantly greater increase in PAI-1 production and further decreased t-PA generation from EC compared to equimolar amount of native LDL (P < 0.05) (Fig. 7). Those findings indicate that Lp(a) in either native or glycated form is more potent than LDL in the generation of PAI-1 and t-PA from EC.
3.5. Influence of antioxidants and AGEs inhibitor on glycated Lp(a)-induced PAI-1 and t-PA in EC

To determine the role of EC-mediated oxidative modification in native and glycated Lp(a)-induced changes in PAI-1 and t-PA production, HUVEC were treated with 80 mM butylated hydroxytoluene (BHT), a potent antioxidant, with and without the presence of 5 μg/ml of native or glycated Lp(a). BHT treatment completely prevented native Lp(a)-induced changes in PAI-1 and t-PA generation. The same concentration of BHT inhibited 80% of PAI-1 overproduction and 49% of the decrease in t-PA generation induced by glycated Lp(a) (Table 1). Our previous studies demonstrated that the presence of 25 mM aminoguanidine during the glycation prevented glycated LDL-induced alterations in the generation of fibrinolytic regulators from HUVEC [28]. In the present study, Lp(a) was glycated by 50 mM glucose in the presence of 25 mM aminoguanidine for 2 weeks at 37°C. No detectable glucolysine was found in aminoguanidine-treated glycated Lp(a) as it was found in glycated Lp(a). The levels of PAI-1 and t-PA released from EC incubated with aminoguanidine-treated glycated Lp(a) for 48 h were significantly different from glycated Lp(a)-treated cells (P < 0.05 or 0.01), and similar to that induced by native Lp(a) (Table 1).

4. Discussion

Peripheral levels of Lp(a) in diabetes have been examined by a number of studies. Increased Lp(a) levels were found in some studies in Type 1 diabetes [35–40] but not in most others [41–50]. In comparison, the majority of studies in Type 2 diabetes found increased levels of Lp(a) in plasma [36,38,40,51–58] with a few exceptions [44,59–63]. Correlation between Lp(a) levels and macrovascular diseases was found in many studies in Type 2 diabetes [41,45,55–59] but only in a few in Type 1 diabetes [39,40]. In diabetic patients, the glycation of plasma proteins, including Lp(a), is expected to be increased. Elevated levels of glycated Lp(a) were detected in diabetic subjects and the proportion of glycated Lp(a) correlated to glucose control [6]. The results of the present study demonstrated that glycation amplified Lp(a)-induced production of PAI-1 and further reduced t-PA generation from vascular EC. This suggests that increased levels of glycated Lp(a) potentially attenuate EC-derived fibrinolytic activity. The effects of Lp(a) isolated from diabetic patients on EC-derived fibrinolytic regulators will be determined in a future study.

Oxidized LDL stimulates PAI-1 secretion and reduced t-PA release from coronary artery EC [64]. The present study demonstrates that Lp(a) and its glycated form altered the generation of PAI-1 and t-PA from HCAEC. HCAEC produced 2- to 3-fold more of PAI-1 and moderately less amounts of t-PA compared to HUVEC at the basal condition. The variations in PAI-1 generation between HCAEC and HUVEC may also result from differences in the types of media and/or passage numbers. The responses of HCAEC and HUVEC to native or glycated Lp(a) in the generation of PAI-1 and t-PA were comparable. Increased levels in Lp(a) or its glycated form in blood may attenuate EC-derived fibrinolytic activity in arterial as well as venous systems.

Our results demonstrated that LDL in either native or glycated form was substantially weaker than Lp(a) for the alteration in PAI-1 and t-PA generation. The presence of apo(a) may amplify the susceptibility of Lp(a) to cell-dependent and -independent oxidation. Glycation may further increase the oxidative stress of Lp(a) as it found in LDL [27]. Native Lp(a)-induced changes in PAI-1 and t-PA generation likely resulted from cell-mediated oxidative modification, since treatment of EC with BHT almost completely inhibited the effects of native Lp(a). Antioxidant treatment partially inhibited glycated Lp(a)-induced changes in EC-derived fibrinolytic regulators, which suggests that cell-mediated oxidative modification also plays an important role in glycated Lp(a)-induced PAI-1 and t-PA generation. TBARS assay may not be sensitive enough to reveal lipid peroxidation in glycated lipoproteins. Our previous study demonstrated that the formation of conjugated dienes but not TBARS was detected in glycated LDL [28]. We speculated that glycated Lp(a) has a similar pattern of lipid peroxidation as that in glycated LDL. The detection of conjugated dienes in Lp(a) was limited by the amounts of Lp(a) (milligrams) required for the assay.
Extended exposure of Lp(a) to high levels of glucose promotes the formation of conjugated dienes. The cross-link between AGEs and proteins in the vascular wall may promote atherogenesis. Increased levels of AGEs were detected in the tissues of diabetes, Alzheimer’s disease, end stage kidney diseases and normal ageing [65]. In addition, AGEs increase the oxidative stress of glycated products [66]. The present study demonstrates that treatment of Lp(a) with aminoguanidine, an inhibitor of AGEs formation, during glycation prevented glycated Lp(a)-induced over-production of PAI-1 and reduced t-PA generation from EC. The finding suggests that AGEs formation may also play an important role in glycated Lp(a)-induced generation of fibrinolytic regulators in EC.

In summary, glycation enhances the production of PAI-1 and attenuates the synthesis of t-PA induced by Lp(a) in arterial and venous EC. The formation of AGEs and EC-mediated oxidative modification may contribute to the alterations of the generation of PAI-1 and t-PA induced by glycated Lp(a). The combination of hyperglycaemia and hyperlipoprotein(a) may reduce EC-derived fibrinolytic activity, which may promote the development of thrombosis and atherosclerosis in diabetes. Management of hyperglycaemia and hyperbetalipoproteinemia, including hyperlipoprotein(a), potentially improves fibrinolytic activity and reduces the incidence of cardiovascular complications in diabetes.

Acknowledgements

The authors thank Dr Paul E. DiCorleto (Cleveland Clinic Foundation, Cleveland, OH) for providing cDNA probes and Dr Angelo M. Scanu (University of Chicago, Chicago, IL) for the advice on Lp(a) purification. This study is supported by the Canadian Diabetes Association in the memory of late Archibald Mitchell to G.X.S.

References


