A critical assessment of the effects of aminoguanidine and ascorbate on the oxidative modification of LDL: evidence for interference with some assays of lipoprotein oxidation by aminoguanidine[1]

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Abstract Several lines of evidence support a role for oxidized low density lipoprotein (LDL) in the genesis of the atherosclerotic lesion. Hence, the effect of compounds with antioxidant properties on LDL oxidation assumes great significance. Ascorbate, a potent water-soluble chain-breaking antioxidant, has been shown to inhibit LDL oxidation. Aminoguanidine (AMG) is a pharmacological inhibitor of advanced non-enzymatic glycosylation. Recently it has been suggested that aminoguanidine might have an inhibitory effect on LDL oxidation, but total lipid peroxidation assayed by conjugated diene formation was not inhibited. Thus, in this study, we compared the effect of aminoguanidine with ascorbate to obtain a better appreciation of the effect of AMG on Cu^{2+}-catalyzed LDL oxidation. Oxidative modification of LDL was monitored by assaying intermediates and end products of lipid peroxidation, conjugated dienes (CD), lipid peroxides (LPO), and relative electrophoretic mobility (REM). Apolipoprotein B-100 modification (increased fluorescence, fragmentation on SDS-PAGE, and ^{125}I-labeled LDL degradation by human macrophages) was also measured. Ascorbate (100 μM) inhibited LDL oxidation by >95%, as evidenced by all of the selected indices. Aminoguanidine (20 mM) substantially decreased thiobarbituric acid-reactive substances (TBARS) activity and lipid peroxide formation, but only partially prevented the increase of REM (−55%), apoB fluorescence (−39%), and degradation by macrophages (−54%). Unlike ascorbate, AMG failed to preserve α-tocopherol in LDL, prevent apoB-100 fragmentation, or inhibit conjugated diene formation during LDL oxidation. Furthermore, incubation of AMG with already oxidized LDL resulted in a significant decrease in TBARS activity and LPO, and 26.9% decrease in the REM of LDL. Separation of AMG from LDL by gel chromatography largely restored the lipid peroxides and REM of oxidized LDL. Thus AMG interferes with these assays of lipid peroxidation. In comparison with ascorbate, which inhibits LDL oxidation at physiologic levels, the significance of a partial inhibition of some indices of LDL oxidation by high levels of aminoguanidine (20 mM) remains to be established.—**Scaccini, C., G. Chiesa, and I. Jialal.** A critical assessment of the effects of aminoguanidine and ascorbate on the oxidative modification of LDL: evidence for interference with some assays of lipoprotein oxidation by aminoguanidine. *J. Lipid Res.* 1994. **35:** 1085–1092.

Supplementary key words LDL oxidation • apoB • ascorbate • aminoguanidine

Data continue to accumulate supporting a pro-atherogenic role for oxidatively modified low density lipoprotein (LDL) (1). Furthermore, the in vivo existence of oxidatively modified LDL is supported by several lines of evidence (2, 3). Hence compounds with antioxidant properties assume great significance in the protection of LDL against oxidative modification with the potential to prevent atherosclerosis.

Aminoguanidine (AMG), a nucleophilic hydrazine compound, is a pharmacological inhibitor of the advanced glycosylation pathway (4–6). Aminoguanidine prevents the formation of fluorescent advanced non-enzymatic glycosylation products and of glucose-derived collagen cross-links in vitro (4). It appears that the primary mechanism by which aminoguanidine inhibits the formation of advanced glycosylation end products (AGE) is by reacting with Amadori-derived fragmentation products such as 3-deoxyglucosone (5). Picard et al. (7) postulated that AMG might also have an inhibitory effect on LDL oxidation by competitively binding to reactive aldehydes formed during lipid peroxidation and preventing their subsequent conjugation to apoB. However, they did not clearly establish an antioxidant effect of AMG as total lipid peroxidation, assayed by conjugated diene formation, was not inhibited (7).

Ascorbate, the most effective water-soluble chain-breaking antioxidant in human plasma (8, 9) has a significant and substantial inhibitory effect on copper-

Abbreviations: LDL, low density lipoprotein; OX-LDL, oxidized LDL; AMG, aminoguanidine; TBARS, thiobarbituric acid-reactive substance; LPO, lipid peroxides; PBS, phosphate-buffered saline; REM, relative electrophoretic mobility; AGE, advanced glycosylation end products; CD, conjugated dienes.

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catalyzed and cell-mediated LDL oxidative modification (10, 11). Ascorbate is also known to react with amino groups of amino acids and proteins and to compete with glucose for binding protein, thereby decreasing glycosylation (12).

The aim of the present study was to compare the antioxidant capacity of aminoguanidine with that of an established water-soluble antioxidant, ascorbate, to obtain a better appreciation of the antioxidant effect of AMG on the oxidative modification of LDL. Modification of LDL was tested by measuring intermediates and products of lipid peroxidation (conjugated dienes, lipid hydroperoxides, thiobarbituric acid-reactive substances) as well as increase in negative charge, and apoB fluorescence and fragmentation. The effect on the processing of LDL by human monocyte-derived macrophages was also determined.

MATERIALS AND METHODS

Reagents

All reagents were obtained from Sigma Chemical Co. (St. Louis, Mo), except RPMI (Gibco, Grand Island, NY). Aminoguanidine was kindly supplied by Michael Yamin, Alteon, Northvale, NJ.

LDL preparation

Plasma was obtained from normolipidemic (LDL cholesterol < 130 mg/dl and plasma triglycerides < 200 mg/dl) volunteers (n = 12). None of these subjects were on any antioxidant therapy. Fasting plasma for LDL isolation was collected from normal human volunteers in tubes containing EDTA (1 mg/ml). LDL (d 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation, as previously described (10). The isolated LDL was dialyzed against 150 mM NaCl/1 mm EDTA (pH 7.4), filtered and stored at 4°C under nitrogen, and used within 10 days. Protein was measured by the method of Lowry et al. (13) using bovine serum albumin as standard. Aliquots of LDL were labeled with carrier-free ^{125}I by a modification of the iodine monochloride method (14).

Oxidation of LDL

LDL was dialyzed overnight against 1 liter phosphate-buffered saline (PBS, pH 7.4) at 4°C in the dark. LDL (200 μg protein/ml) was then oxidized in a cell-free system in the presence of 2.5 μM Cu^{2+} in PBS at 37°C for 6 h (11). The length of incubation corresponded to the steady state of the maximum LDL oxidative modification, as determined in preliminary time-course experiments (11). Oxidation was arrested by refrigeration and addition of 200 μM EDTA and 40 μM butylated hydroxytoluene (BHT). The oxidation of LDL was performed in the presence and absence of ascorbate (100 μM) and AMG (5–20 mM), added before the addition of Cu^{2+}. The concentration of ascorbate used was that which resulted in maximum inhibition of LDL oxidative modification, as previously reported (10).

The formation of conjugated dienes was measured by monitoring the absorbance of LDL at 234 nm and the results are expressed as either ΔA234 or nmol/mg protein (Fig. 1) using a molar extinction coefficient for conjugated dienes of E_{234} = 29,500 1 $mol^{-1}cm^{-1}$ (15).

Thiobarbituric acid-reactive substance (TBARS) was measured by a modification of the assay of Buege and Aust (16), as described previously (17). TBARS activity was expressed as malondialdehyde (MDA) equivalents (nmol MDA/mg protein) using freshly diluted 1,1,3,3-tetramethoxypropane as the standard.

Lipid peroxides (LPO) were estimated spectrophotometrically at 365 nm based on the oxidation of iodide to iodine by lipid hydroperoxides as described by El-Saadani et al. (18).

For fluorescence measurement, the LDL was diluted with PBS to a final concentration of 20 μg protein/ml and the emission was measured at 430 nm with excitation at 360 nm (LS-5, Perkin-Elmer), with emission and excitation slit widths at 5 nm (19).

LDL electrophoresis was performed at pH 8.6 in 0.05 M barbital buffer on 0.5% agarose gels (20). The gels were stained with Sudan B black. The increased electrophoretic mobility of LDL was expressed relative to the mobility (REM) of native LDL which was assigned a mobility of 1.0.

To separate AMG from LDL after oxidation, the reaction mixture was chromatographed on a Sephadex G-25 column (PD-10, Pharmacia) and the protein peak was determined by monitoring absorbance at 280 nm. The column was equilibrated with PBS.

For SDS-polyacrylamide gel electrophoresis, 2 μg of LDL protein (in 10 μl) was added to 30 μl sample buffer (containing 0.15% SDS, 0.25% glycerol, 0.2 M Tris-HCl, pH 6.8, and 6% β-mercaptoethanol), mixed and incubated in boiling water for 5 min. Vertical gel electrophoresis was performed according to Laemmi (21). For each sample, 15 μl was loaded onto a 5% SDS-PAGE minigel in a Mini-Protean II System (Bio-Rad) and electrophoresis was carried out at a constant current of 200 V for ~45 min at 4°C. The proteins were fixed in 10% trichloroacetic acid and stained with Coomassie-Blue R-250. Proteins of known molecular weights were used as standards.

The effect of ascorbate and aminoguanidine on the α-tocopherol content of LDL during oxidative modification was also determined. After extraction, α-tocopherol was measured by high-pressure liquid chromatography with fluorescence detection (22).

Macrophage preparation

Human monocytes were isolated from heparinized blood derived from apparently healthy fasting donors by Ficoll-Hypaque centrifugation as described previously (11). The mixed mononuclear cell band was removed by aspiration and the cells were washed twice in RPMI 1640 culture medium containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. The cells were plated at 2 × 10^6 mononuclear cells per 16-mm dish (Primaria brand, Falcon Labware, Becton, Dickson & Co., Oxnard, CA) in the same medium. After 2 h incubation at 37°C in 5% CO_2/95% air, nonadherent cells were removed by three washes with serum-free medium. The cells were then plated in fresh medium containing 20% autologous serum and were fed twice weekly with the same medium. Monocyte-derived macrophages were used within 10–14 days of plating.

Degradation assay

After a 6-h incubation of ^{125}I-labeled LDL with 2.5 μM Cu^{2+} in the presence and absence of ascorbate and aminoguanidine, an aliquot was added to cultured macrophages to yield a final concentration of 10 μg/ml in RPMI-1640 medium (23). The uptake and degradation of ^{125}I-labeled LDL by human monocyte-derived macrophages were measured as appearance of trichloracetic acid-soluble radioactivity (noniodide) formed by the cells and excreted in the medium. Degradation rates were corrected for cell-free controls incubated in parallel. The macrophages remaining in the wells after aspiration of the media were washed thrice with cold PBS, dissolved in 0.1 N NaOH and their protein content was determined. Results were expressed as μg of protein degraded per milligram of cell protein.

Statistical analysis

Data are presented as mean ± standard error. Statistical analysis was performed using a one-factor analysis of variance and Scheffe's method for multiple comparisons.

RESULTS

The dose-response effect of AMG in inhibiting Cu^{2+}-catalyzed LDL modification was tested by measuring conjugated dienes, TBARS, LPO, and REM after a 6-h incubation at 37°C (**Table 1**). AMG was very efficient in decreasing TBARS and LPO at low concentration (5 mM), while only 20 mM AMG was able to significantly ($P < 0.01$) decrease the change in electrophoretic mobility (REM decrease 54.5%). Hence, in all further experiments 20 mM AMG was used. Conjugated diene formation was not decreased by aminoguanidine at all concentrations tested. However, in agreement with the findings of Picard et al. (7), a 5-h time-course experiment revealed that AMG (20 mM) prolonged the lag phase of oxidation.

Fig. 1 summarizes the comparative effects of ascorbate and AMG on Cu^{2+}-catalyzed LDL modification. Ascorbate (100 μM) was able to almost completely inhibit LDL oxidative modification, detected both as lipid peroxidation products (conjugated dienes, LPO, and TBARS), increase in the negative charge (REM), and in apoB fluorescence. AMG (20 mM) totally decreased TBARS and LPO formation, but only partially decreased the increase of REM (−55%) and apoB fluorescence (−39%). Once again in these experiments, conjugated dienes were not decreased by aminoguanidine. As it has previously been shown that aminoguanidine can react with proteins (24), we tested the effect of co-incubation of AMG with native LDL for 6 h to see whether this explained the partial inhibition in fluorescence and REM. Co-incubation of LDL with AMG did not alter the electrophoretic mobility of LDL and only slightly increased the absorbance at 234 nm (4.1%) and apoB fluorescence (7.7%).

To exclude the possibility that AMG could generate material with 234 nm absorbance independently from the presence of oxidizable substrates, we incubated AMG in PBS with and without Cu^{2+} (data not shown). In both situations (AMG with and without Cu^{2+}) we did not find

TABLE 1. Dose-response effect of aminoguanidine on copper-catalyzed oxidation of low density lipoprotein

	ΔA234	Lipid Peroxides	Thiobarbituric Acid-Reactive Substances	Relative Electrophoretic Mobility
		nmol/mg protein	*nmol MDA/mg protein*	
Native LDL		6.1 ± 0.1	2.3 ± 0.3	1
OX-LDL	3.0 ± 0.56	610 ± 45	55.6 ± 5.5	3.2 ± 0.2
+ 5 mM AMG	2.47 ± 0.11	64 ± 1.0[a]	12.2 ± 2.7[a]	2.7 ± 0.6
+ 10 mM AMG	3.15 ± 0.2	17 ± 1.2[a]	8.0 ± 1.3[a]	2.4 ± 0.3
+ 20 mM AMG	3.87 ± 0.59	7.2 ± 1.0[a]	6.7 ± 1.1[a]	2.0 ± 0.2[b]

LDL (200 μg protein/ml) was incubated at 37°C for 6 h with 2.5 μM Cu^{2+} in the presence of increasing concentrations of AMG (0–20 mM). Values represent the mean of three experiments done in triplicate.
[a] $P < 0.001$ by one-factor ANOVA.
[b] $P < 0.01$ by one-factor ANOVA.

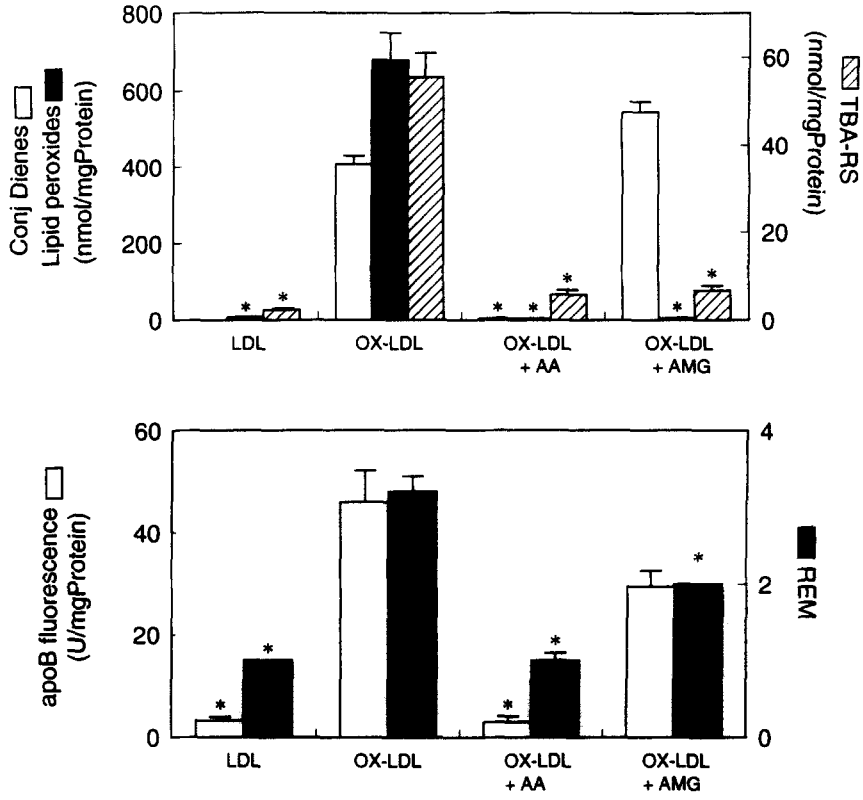

Fig. 1. Effect of ascorbic acid (AA) and aminoguanidine (AMG) co-incubation on copper-catalyzed LDL oxidation. LDL was incubated at 37°C for 6 h with 2.5 μM Cu^{2+} in the absence and presence of ascorbic acid (100 μM) and aminoguanidine (20 mM). Results represent mean ± SE of 9 experiments in triplicate. REM denotes electrophoretic mobility compared to control LDL. *, $P < 0.001$ from OX-LDL by ANOVA, Scheffe's test.

any increase in the absorbance at 234 nm after addition of AMG as compared to PBS alone. Moreover, the addition of AMG to LDL already oxidized by Cu^{2+} did not change the absorbance at 234 nm (**Table 2**).

These results indicate that AMG inhibited the assays used to monitor LDL lipid peroxidation perhaps by reacting with aldehydes and other functional groups. Moreover, high concentrations (20 mM) of AMG were only partially able to protect apoB from oxidative modification, as evidenced by the changes in electrophoretic mobility and apoB fluorescence of oxidized LDL.

Reactive aldehydes, final products of lipid peroxidation, bind apoB to generate a form of LDL that is recognized by the scavenger receptors of macrophages (1). **Fig. 2** shows the degradation by human monocyte-derived macrophages of ^{125}I-labeled LDL after oxidation in the presence and absence of ascorbate and AMG. The results are expressed as percentage of degradation of oxidized

TABLE 2. Effect of aminoguanidine on indices of oxidation after copper-catalyzed oxidation of low density lipoproteins

	Oxidized LDL	
Indices	+ Aminoguanidine	− Aminoguanidine
ΔA234	2.8 ± 0.01	2.9 ± 0.04
TBARS (nmol MDA/mg protein)	55.9 ± 1.2	3.6 ± 14
Lipid peroxides (nmol/mg protein)	675.6 ± 26.5	4.7 ± 0.2
ApoB fluorescence (U/mg protein)	33 ± 1.5	32 ± 1.0
Relative electrophoretic mobility	3.6	2.9

LDL (200 μg protein/ml) was incubated at 37°C for 6 h with 2.6 μm Cu^{2+}. The reaction was stopped and AMG was added to triplicate tubes and PBS was added to the remaining tubes to yield a final concentration of AMG of 20 mM. Values represent the mean of three experiments done in triplicate.

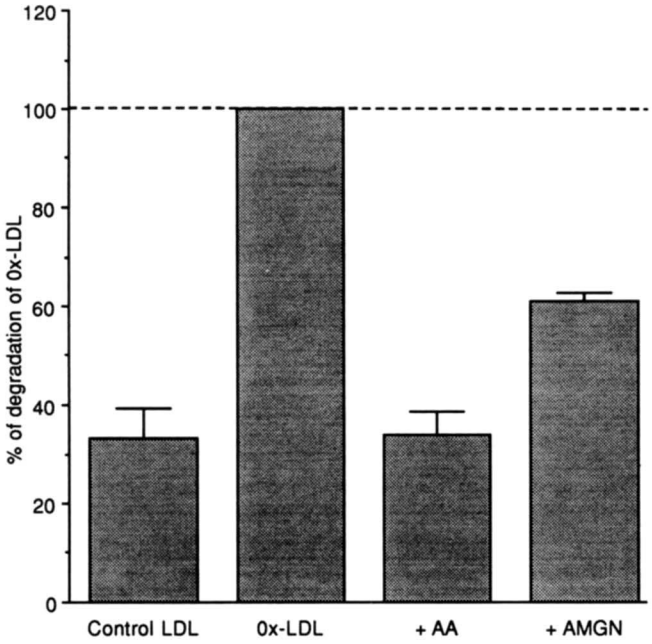

Fig. 2. Effect of ascorbate and aminoguanidine on the degradation of ^{125}I-labeled LDL by human macrophages. ^{125}I-labeled LDL (200 μg/ml) was incubated at 37°C for 6 h with 2.5 μM Cu^{2+} in the presence of AA (100 μM) or AMG (20 mM). After incubation, an aliquot was added to human monocyte-derived macrophages (16-mm dishes) to yield a final concentration of LDL of 10 μg protein/ml in RPMI media. After a 6-h incubation period of 37°C, trichloracetic acid-soluble radioactivity corrected for cell-free controls was determined. Results are expressed as % of degradation of OX-LDL (100%) and represent mean ± SE of 4 experiments in triplicate. OX-LDL degradation was 2.8 μg of LDL protein/mg of cell protein. Anova, Scheffe's test: control vs. OX-LDL and AMG, $P < 0.001$; OX-LDL vs. AA and AMG, $P < 0.001$; AMG vs. AA, $P < 0.01$.

LDL (OX-LDL). The degradation of ^{125}I-labeled LDL in the presence of ascorbate was not different from that of native LDL after a 6-h incubation. The degradation by macrophages of LDL oxidized by Cu^{2+} in the presence of AMG was significantly different from native LDL ($P < 0.001$) and from LDL oxidized in the presence of ascorbate ($P < 0.01$); AMG inhibited only 54% the macrophage degradation obtained with LDL oxidized in the absence of inhibitors, while ascorbate inhibited ^{125}I-labeled LDL degradation by 97%. Aminoguanidine added to LDL that had already been oxidized had no effect on the subsequent degradation of OX-LDL by macrophages.

Oxidative modification of LDL is also accompanied by extensive non-proteolytic scission of apoB-100, due to the oxidative attack on the polypeptide chain, directly or secondary to the peroxidation of the lipids (25). **Fig. 3** shows the effect of ascorbate and aminoguanidine on the breakdown of apoB-100 by Cu^{2+}-catalyzed oxidation. There was substantial preservation of intact apoB-100 when LDL was oxidized in the presence of 100 μM ascorbate (lane 3). However, co-incubation of LDL with Cu^{2+} and aminoguanidine resulted in a complete loss of intact apoB (lane 4), similar to the apoB fragmentation of oxidized LDL (lane 2).

As another measure to study the effect of ascorbate and aminoguanidine on copper-catalyzed LDL oxidative modification, α-tocopherol, the major antioxidant in LDL, was determined after a 6-h period of Cu^{2+}-catalyzed LDL oxidation. Co-incubation of LDL with ascorbate during oxidative modification resulted in a preservation of α-tocopherol compared to the native LDL (6.7 ± 1.3 and 6.9 ± 0.9 nmol/mg protein respectively). However, co-incubation with aminoguanidine failed to preserve α-tocopherol in LDL (0.6 ± 0.2 nmol/mg protein) resulting in a 91% loss with a level similar to that of oxidized LDL (0.5 ± 0.1 nmol/mg protein).

As AMG did not prevent apoB-100 fragmentation, preservation of α-tocopherol in LDL, or significantly inhibit formation of conjugated dienes, the question arises whether AMG interferes with the detection of lipid hydroperoxides. To determine whether AMG interfered with the methods used to monitor LDL oxidation, LDL was subjected to copper-catalyzed LDL oxidation for 6 h. At the end of the incubation, the effect of AMG on the

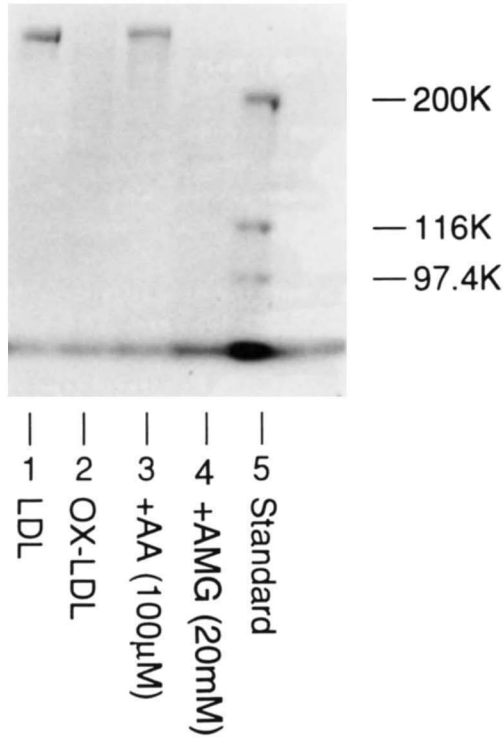

Fig. 3. Effect of ascorbate (AA) and aminoguanidine (AMG) on the oxidative breakdown of apoB during Cu^{2+}-catalyzed LDL oxidation. LDL (200 μg/ml) was incubated at 37°C for 6 h with 2.5 μM Cu^{2+} in the presence of AA (100 μM) or AMG (20 mM). After incubation, samples were analyzed by SDS-PAGE as described in Methods. Lane 1, native LDL; lane 2, OX-LDL; lane 3, AA-treated LDL; lane 4, AMG-treated LDL; lane 5, molecular weight standards.

assays used to monitor LDL oxidation was tested (Table 2). While AMG did not alter the increased absorbance at 234 nm or the increased fluorescence of oxidized LDL, AMG substantially decreased the amount of lipid peroxides and TBARS activity; also AMG decreased the REM of LDL by 26.9%. As AMG binds aldehydes, its effect on the TBARS assay is predictable and is in agreement with Picard et al. (7). However, the interference in the LPO assay and electrophoretic mobility was unexpected. To obtain a better appreciation of the effect of AMG on the LPO assay and REM, LDL was oxidized in the absence and presence of AMG and thereafter both oxidized LDL fractions were subjected to chromatography (Sephadex G-25) to reisolate the LDL and separate it from AMG. The reisolated LDL fractions were then assayed for lipid peroxides and subjected to electrophoresis. It is evident from **Fig. 4** that co-incubation with AMG both decreased the lipid peroxide levels and retarded the electrophoretic mobility of oxidized LDL. However, after separation of LDL from AMG by column chromatography, the reisolated LDL had 82.5% the lipid peroxide content of reisolated OX-LDL. Furthermore, the increased electrophoretic mobility of LDL was partly restored with AMG, resulting in lesser inhibition in the electrophoretic mobility (35.4%).

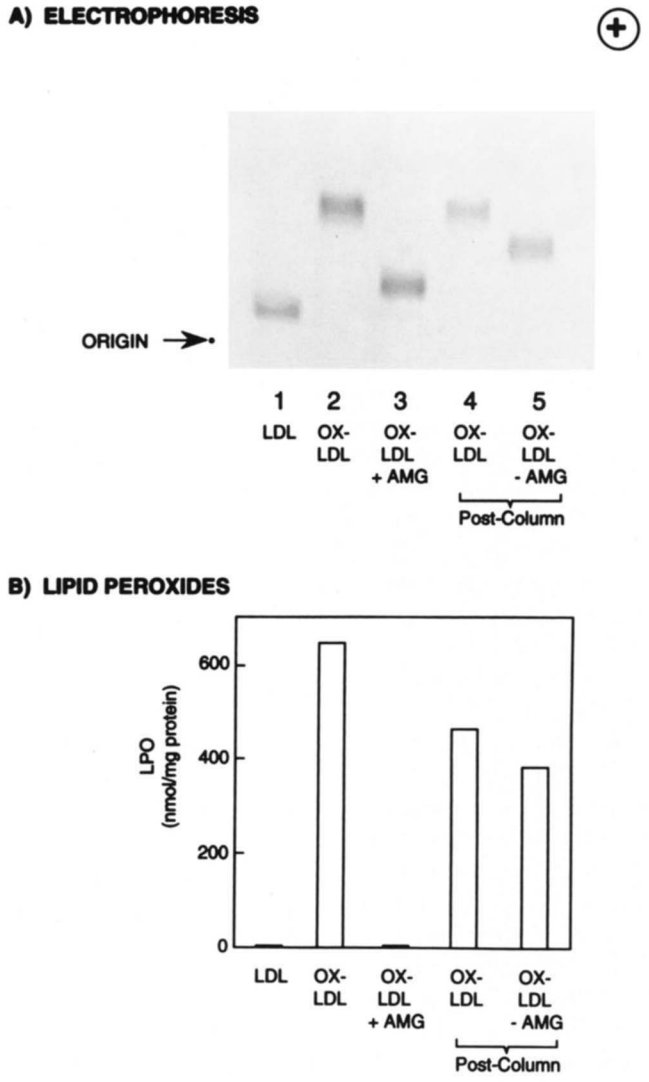

Fig. 4. Effect of aminoguanidine on copper-catalyzed oxidation of LDL. LDL (200 μg protein/ml) was incubated at 37°C for 6 h with 2.5 μm Cu^{2+} in the absence and presence of AMG (20 mM). The reaction was stopped and an aliquot (1.0 ml) of the pooled triplicate tubes of LDL oxidized in the absence and presence of AMG was subjected to chromatography on a Sephadex G-25 column. The major protein peak determined by monitoring the column fractions at 280 nm was collected. All experimental points, including the co-incubation experiment and the reisolated LDLs, were assayed for lipid peroxides. They were also subjected to electrophoresis. +AMG denotes co-incubation of LDL with Cu^{2+} and AMG (lane 3) while −AMG denotes separation of the AMG from OX-LDL by column chromatography prior to electrophoresis and lipid peroxide determination of the LDL (lane 5).

DISCUSSION

Although several lines of evidence support the in vivo existence of OX-LDL, the most persuasive data on the role of OX-LDL in atherogenesis derives from studies showing that antioxidants prevent atherosclerosis in animal models (26–28).

Aminoguanidine, a nucleophilic hydrazine compound that inhibits AGE formation, has been shown to prevent or ameliorate diabetic retinopathy, nephropathy, and neuropathy in animal models (4–6). In a recent report, Picard et al. (7) suggest that aminoguanidine could inhibit LDL oxidation, but they did not clearly establish an antioxidant effect. The goal of the present study was to compare the effect of these two water-soluble compounds on LDL oxidation to obtain a better appreciation of the antioxidant effect of aminoguanidine by comparing it with an established antioxidant.

In the present report we show that AMG is much less potent than ascorbate in inhibiting LDL oxidation. Unlike ascorbate, aminoguanidine failed to prevent apoB-100 fragmentation, preservation of α-tocopherol, or significantly inhibit conjugated diene formation. Furthermore, aminoguanidine, unlike ascorbate, only had a partial effect on preventing the increased fluorescence, electrophoretic mobility, and ^{125}I-labeled LDL degradation by macrophages of LDL subjected to oxidation. However, aminoguanidine had a substantial effect on decreasing the TBARS activity and lipid peroxides in LDL. This effect of aminoguanidine on TBARS activity and lipid peroxides was also evident when it was added to LDL that had already been oxidized. The decrease in TBARS activity is not surprising as AMG can react with aldehydes such as malondialdehyde and prevent their detection. This finding accords with that of Picard et al. (7). However, the decrease in lipid peroxides assayed by iodometry during

both co-incubation and post-oxidation experiments with AMG suggest that AMG interfered with the detection of lipid peroxides. To confirm this, LDL that was oxidized in the presence of AMG was subjected to chromatography to separate the oxidized LDL from AMG and then the lipid peroxides were determined. The lipid peroxides in OX-LDL after separation from AMG were restored to 82.5% of that of OX-LDL. These experiments confirm that AMG interferes with the detection of lipid peroxides by iodometry. The electrophoretic mobility of LDL was also monitored during the post-oxidation incubations with AMG and the chromatographic studies. It appears that AMG can retard the electrophoretic mobility of OX-LDL; during the post-oxidation experiment, AMG decreased the mobility of OX-LDL partially (26.9%). After the co-incubation experiment with AMG and separation of AMG from LDL by column chromatography, the increased mobility of OX-LDL was partially restored.

The only other study that has specifically tested the effect of AMG on the oxidative modification of LDL is the report of Picard et al. (7) in which they showed that AMG inhibited the oxidation of LDL as evidenced by the REM, ^{125}I-labeled LDL degradation, and apoB-100 fluorescence. These studies are not strictly comparable quantitatively because of the different incubation conditions; Picard et al. (7) oxidized LDL with 5 μM Cu^{2+} in Hams F-10 media which contains (Cu^{2+}, iron, and thiols) while in the present study LDL was oxidized in PBS with 2.5 μM Cu^{2+}. Although both studies showed that AMG did not inhibit the maximum amount of conjugated dienes formed, it appears that Picard et al. (7) obtained a greater inhibition of the REM, apoB fluorescence, and ^{125}I-labeled LDL degradation. The present report indicated that higher concentrations of AMG only partially inhibit these indices of apoB modification. In support of our findings is the observation that, unlike ascorbate, AMG failed to protect apoB-100 from fragmentation during oxidative conditions. Ascorbate, in contrast, protected the apoB-100 in LDL and prevented the increased apoB-100 fluorescence and uptake by the scavenger receptors. In addition, the major endogenous antioxidant in LDL, α-tocopherol, was preserved by ascorbate but not by AMG.

With respect to AMG, it appears that its ability to react with aldehydes to form Schiff bases does not prevent the formation of conjugated dienes (first step of the lipid peroxidation chain), suggesting that its action is not that of a general antioxidant such as ascorbate and α-tocopherol. The inability of aminoguanidine to prevent increased conjugated diene formation was also recently reported in the sciatic nerve of streptozotocin-induced experimental diabetic neuropathy (29). In a recent report, the authors suggest that when LDL is incubated in a high glucose medium, in addition to AGE modification of LDL, there is increased oxidation and both appear to be inhibited by aminoguanidine (30). However, as the authors only used the TBARS assay to quantitate oxidation, it is difficult to interpret their finding as a direct antioxidant effect of aminoguanidine because it can react with aldehydes. Furthermore, the concentration of aminoguanidine used (300 mM) to show an effect was far in excess of that reported by Picard et al. (7) and the present study. In this regard, the relevance to the in vivo situation of a partial inhibitory effect of 20 mM AMG on LDL oxidation needs comment. Oral feeding to animals has resulted in plasma levels of about 13 mM (31). It thus appears that the concentrations required to achieve an inhibition of LDL oxidation are greater. In fact, these levels clearly exceed the concentrations of other amino groups in plasma (arginine 88 μM and lysine 157 μM) (32). Thus, in comparison with ascorbate, physiologic concentrations of which inhibit LDL oxidation, the significance of a partial inhibition of some indices of LDL oxidation by aminoguanidine brings into question its antioxidant effect with respect to LDL oxidation. In this regard, it should be pointed out that aminoguanidine was recently shown to inhibit catalase and generate hydrogen peroxide in vitro (33).

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