Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6876–6880, August 1992
Medical Sciences

Aminoguanidine inhibits oxidative modification of low density lipoprotein protein and the subsequent increase in uptake by macrophage scavenger receptors

SYLVIE PICARD, SAMPATH PARTHASARATHY, JOACHIM FRUEBIS, AND JOSEPH L. WITZTUM*

Division of Endocrinology and Metabolism, Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0613

Communicated by Daniel Steinberg, May 1, 1992

ABSTRACT **Aminoguanidine decreases the formation of advanced glycosylation end products that occurs during chronic hyperglycemia. Presumably this occurs because early glycosylation products preferentially bind to aminoguanidine rather than to lysine groups of adjacent proteins. Because oxidative modification of low density lipoprotein (LDL) also involves derivatization of lysine residues of apolipoprotein (apo) B by reactive aldehydes formed during the decomposition of oxidized fatty acids, we postulated that aminoguanidine might also inhibit the oxidatively induced modification of LDL protein. To test this hypothesis we oxidized LDL by incubation with Cu^{2+} or with endothelial cells in the absence or presence of aminoguanidine. Aminoguanidine prevented apo B lysine modification, as measured by fluorescence spectroscopy, and inhibited in a dose-dependent manner the oxidatively induced increase in subsequent macrophage uptake. At concentrations that inhibited apo B modification (5–10 mM), aminoguanidine increased the lag time in diene conjugation but did not affect the plateau value reached. These data indicate that aminoguanidine inhibits oxidative modification of LDL protein in large part by binding reactive aldehydes formed during lipid peroxidation and preventing their subsequent conjugation to apo B. Thus, aminoguanidine (and related compounds) may be of dual benefit in inhibiting atherosclerosis, both by inhibiting formation of advanced glycosylation end products and by inhibiting the modification of LDL apo B that makes it a ligand for scavenger receptors.**

Although the etiology of atherosclerosis is multifactorial, there is increasing evidence that oxidative modification of low density lipoprotein (LDL) is an important, if not integral, step in the pathogenesis of the atherosclerotic lesion (1). We have reviewed elsewhere the evidence that oxidized LDL occurs *in vivo* and the many ways in which it may contribute to atherogenesis (2, 3). This suggests that antioxidants could prove useful in prevention of the early atherosclerotic lesion. Probucol, a lipophilic antioxidant, has been shown to inhibit atherosclerosis in hypercholesterolemic rabbits (4, 5), but its ability to alter lipoprotein concentrations, as well as to affect levels of cholesteryl ester transfer protein and inhibit interleukin 1 production by macrophages, makes less certain its exact mode of action (6, 7). Several other natural antioxidants, such as vitamin C, vitamin E, and β-carotene, are now being tested for their ability to protect LDL from oxidation.

Many of these antioxidants presumably protect LDL against the initial lipid peroxidation and the subsequent formation of lipid peroxidation breakdown products, which include highly reactive aldehydes, such as malondialdehyde (MDA). Once these reactive aldehydes form, they can derivatize lysine residues of apolipoprotein B (apo B) (8, 9). Consequently, LDL loses its ability to be recognized by cellular LDL receptors but instead becomes recognized by alternative receptors ("scavenger" receptors) on macrophages, such as the acetyl-LDL receptor (10) and possibly others (11). MDA can also induce crosslinking of proteins (reviewed in ref. 8), and such aldehydes may be involved in mediating the damage of the oxidative reactions (8). These mechanisms are analogous to the modifications of lysine residues that occur with glucose to form Schiff bases and Amadori products, and the more extensive crosslinking of proteins leading to generation of advanced glycosylation end products (AGE) that are formed at a greater rate in patients with chronic hyperglycemia (12). It has been suggested that such glucose-modified proteins and AGE may be responsible in part for many of the long-term complications of hyperglycemia in diabetic subjects (12), including the accelerated and diffuse atherosclerosis.

Aminoguanidine is a hydrazine compound that can prevent the formation of AGE by competitive inhibition. Because the early (Amadori-type) glycosylation products have a stronger affinity for aminoguanidine than for lysine ε-amino groups of proteins, they bind to aminoguanidine, inducing formation of unreactive substitutes that are unable to crosslink to other similar proteins or to trap plasma proteins with which they may be in contact (13). Aminoguanidine is currently being tested for its ability to prevent chronic complications of diabetes.

Since oxidative modification of LDL also involves the modification of lysine ε-amino groups of apo B by MDA and other similarly reactive aldehydes, we postulated that aminoguanidine might be able to react with such aldehyde products and in that way prevent their binding to apo B. Consequently, the formation of the epitopes on modified apo B that leads to its recognition by macrophage scavenger receptors should be prevented.

Here we show that aminoguanidine can decrease the alterations in LDL apo B that occur as a consequence of lipid peroxidation, induced either by endothelial cells (EC) or by Cu^{2+}, and consequently can decrease the rate of conversion of LDL to the oxidized form, which is more readily degraded by mouse peritoneal macrophages. We present evidence that aminoguanidine accomplishes this in large part by binding with high affinity to reactive aldehydes produced during the oxidative process, such as MDA, and thus preventing their conjugation to LDL apo B.

METHODS

Aminoguanidine. Aminoguanidine (kindly supplied by Michael Yamin, Geritech, Northvale, NJ) was diluted in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AGE, advanced glycosylation end product(s); apo, apolipoprotein; EC, endothelial cell(s); LDL, low density lipoprotein; MDA, malondialdehyde; TBARS, thiobarbituric acid-reactive substances.

*To whom reprint requests should be addressed.

sterile Ham's F10 medium to obtain a 0.5 M solution with pH of 7.3–7.8.

Lipoproteins. Fresh plasma from healthy human donors was pooled, and LDL was isolated by sequential ultracentrifugation (11). LDL was radioiodinated with carrier-free $Na^{125}I$ (11). Unlabeled LDL and ^{125}I-LDL were dialyzed against phosphate-buffered saline (PBS) with 0.01% EDTA and then sterile-filtered. The protein content was measured with the Lowry method (14).

Cells. Rabbit aortic EC (provided by V. Buonassissi, Alton Jones Cell Science Center, Lake Placid, NY) were grown in Ham's F10 medium containing 10% fetal bovine serum (FBS) (Irvine Scientific) (11, 15).

Mouse peritoneal macrophages were harvested from 1-month-old female Swiss Webster mice (25–30 g) (Simonsen Laboratories, Gilroy, CA) by peritoneal lavage with PBS and cultured overnight in 24-well dishes (Costar) at 1.5×10^6 cells per well in RPMI 1640 medium containing 10% (vol/vol) heat-inactivated FBS (15).

LDL Oxidation. Unlabeled LDL or ^{125}I-LDL (100 μg/ml) was diluted in Ham's F10 medium and incubated in 60-mm plastic dishes (Nunc) with confluent EC cultures or with 5 μM $CuSO_4$ in the absence of cells. In some experiments oxidation was performed in the presence of 5, 10, or 25 mM aminoguanidine. Native LDL was diluted in Dulbecco's modified Eagle's medium (DMEM), to prevent oxidation. After a 20-hr incubation at 37°C (4°C for the native LDL), the medium was harvested and the LDL was tested for measures of lipid peroxidation and/or for rate of degradation by macrophages in a subsequent incubation as described below.

Lipid Peroxidation. Lipid peroxidation was determined by quantification of thiobarbituric acid-reactive substances (TBARS) (11). Tetramethoxypropane was used as a standard, and results are expressed as nmol equivalents of MDA. Conjugated-diene formation was also measured by determining the absorbance at 234 nm of a solution of LDL (100 μg/ml) in PBS incubated with 5 μM $CuSO_4$ in the absence or presence of various concentrations of aminoguanidine. The absorbance was measured every 15 min for 16 hr in a Uvikon 810 spectrophotometer (Kontron, Basel) (16). Results are expressed as absolute absorbance at 234 nm.

Macrophage Degradation of LDL. Prior to use, plated mouse peritoneal macrophages were washed twice with 1 ml of DMEM and then incubated with the oxidized LDL diluted (1:10, vol/vol) in 0.5 ml of DMEM in the absence or presence of aminoguanidine (0.5–2.5 mM). Control dishes were incubated under the same conditions in the absence of macrophages. After 5 hr at 37°C, trichloroacetic acid-soluble radioactivity in the medium and cell-associated radioactivity were determined (17, 18).

Modification of LDL apo B by Exogenous MDA in Absence or Presence of Aminoguanidine. MDA-LDL was prepared by a modification of previously described methods (19). Two milligrams of unlabeled LDL was incubated at 37°C for 30 min in 1 ml of 0.475 mM tetramethoxypropane to yield an LDL preparation with ≈40% of its lysine residues modified by MDA. In some experiments various concentrations of aminoguanidine were added to the LDL before incubation with MDA. The samples were then dialyzed against PBS containing 0.5 mM ascorbic acid to prevent further oxidation. After determination of the protein concentration, the percentage of lysines modified by MDA was estimated using trinitrobenzenesulfonic acid (20). An aliquot of each sample was also tested for its electrophoretic mobility in agarose gel (CIBA/Corning). The extent of lysine modification was also monitored by determining the fluorescence of native or modified LDL samples, using an excitation wavelength of 390 nm and monitoring emission fluorescence with a spectrofluorometer (model LS 50; Perkin–Elmer) (8).

Ability of Aminoguanidine to Act as an Antioxidant. We tested the ability of aminoguanidine to inhibit oxidation in a defined system—xanthine with xanthine oxidase. The reduction of cytochrome *c* by superoxide anion generated in the xanthine/xanthine oxidase reaction was estimated by measuring continuously for 105 sec the increase in the absorbance at 550 nm (21).

RESULTS

Effects of Aminoguanidine on Macrophage Uptake of LDL Exposed to Oxidative Conditions. When LDL unsaturated fatty acids undergo oxidation, reactive aldehydes are formed that bind to apo B, generating novel epitopes that are recognized by macrophage scavenger receptors. We postulated that aminoguanidine would bind to such reactive aldehydes and, consequently, block the modifications of apo B. To directly test this hypothesis we oxidized LDL by incubation with EC or Cu^{2+}, in the absence or presence of aminoguanidine (Fig. 1 and Table 1). Aminoguanidine inhibited LDL modification in a dose-dependent manner, and even at concentrations as low as 5 mM, the subsequent macrophage degradation of LDL was inhibited by up to 60% (Fig. 1). In these experiments, the aminoguanidine present in the oxidative incubation mixtures was transferred to the macrophages along with the modified LDL. To demonstrate that the aminoguanidine carried over did not directly affect the ability of the macrophages to take up and degrade oxidized LDL, aminoguanidine was also added directly to macrophage cultures at the same concentrations. Aminoguanidine added directly to the macrophages had little effect on the rate of degradation of previously oxidized LDL (Fig. 1). To rule out the possibility that aminoguanidine might, like NH_4Cl, alter lysosomal processing, we also measured cell-associated radioactivity and saw no alteration in the presence of aminoguanidine (data not shown).

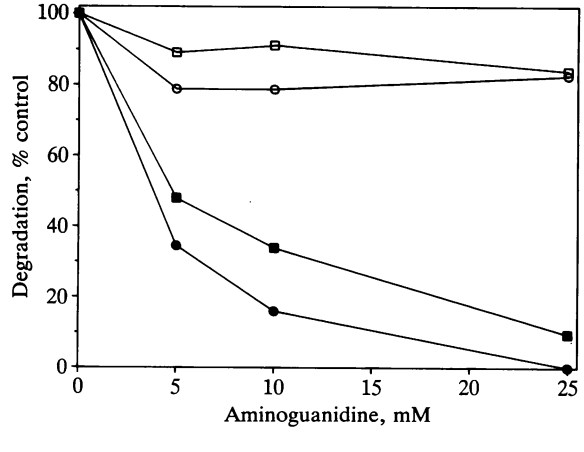

FIG. 1. Aminoguanidine inhibits oxidative modification of LDL. ^{125}I-LDL (100 μg/ml) was incubated in Ham's F10 with EC (○, ●) or with 5 μM Cu^{2+} (□, ■) for 20 hr at 37°C in the absence (○, □) or presence (●, ■) of the indicated concentrations of aminoguanidine. Media were then diluted 1:10 with DMEM and added to macrophage cultures, and degradation of ^{125}I-LDL was determined. To demonstrate that the aminoguanidine transferred from the oxidation reactions did not directly alter macrophage function, 0.5, 1, or 2.5 mM aminoguanidine was added directly to macrophage cultures of LDL samples originally oxidized in the absence of aminoguanidine (i.e., to achieve concentrations of aminoguanidine equal to those in macrophage cultures for samples incubated with aminoguanidine) (○, □). Results are expressed as the percentage of the value obtained for the LDL sample oxidized in the absence of aminoguanidine and after subtraction of the amount of native LDL degraded. Results are the mean of two experiments, each of which was performed in duplicate.

Table 1. Effects of aminoguanidine (AMGN) on LDL oxidation

	AMGN, mM	TBARS, nM MDA/mg of LDL	Relative electrophoretic mobility	Macrophage degradation μg/mg	
EC	0	42.47 ± 4.27	1.79 ± 0.14	5.67	(6.47, 4.88)
	5	9.51 ± 1.01	1.57 ± 0.12	3.12	(3.49, 2.75)
	10	6.13 ± 3.29	1.41 ± 0.11	2.35	(2.40, 2.31)
	25	8.65 ± 3.01	1.26 ± 0.08	1.63	(1.95, 1.31)
Cu^{2+}	0	46.33 ± 3.66	1.78 ± 0.14	5.44	(5.52, 5.37)
	5	10.91 ± 1.92	1.49 ± 0.15	3.53	(4.11, 2.95)
	10	8.60 ± 1.46	1.35 ± 0.15	3.00	(3.33, 2.68)
	25	10.17 ± 2.03	1.17 ± 0.15	2.09	(2.28, 1.91)
Nat	0	2.17 ± 1.04	1.00	1.74	(1.69, 1.79)

^{125}I-LDL (100 μg/ml in Ham's F10) was oxidized by EC or by 5 μM Cu^{2+} for 20 hr at 37°C in the absence or presence of aminoguanidine. Native LDL (Nat) was incubated in DMEM at 4°C. An aliquot of the harvested medium was tested for TBARS and for mobility on agarose gel electrophoresis. The migration distance of LDL was divided for each sample by the migration distance of the native LDL. Another aliquot of the medium was used for the determination of macrophage degradation of LDL, expressed as (μg of LDL protein taken up and degraded during a 5-hr incubation)/(mg of cell protein). Each experiment was performed in duplicate. Data for TBARS and agarose gels are the mean ± SD of five experiments and data for macrophage degradation are from two experiments.

As LDL undergoes progressive oxidative modification, its apo B becomes progressively negatively charged and its migration during agarose electrophoresis is progressively enhanced. Inclusion of aminoguanidine in the medium when LDL was subjected to oxidative conditions, whether by incubation with EC or by Cu^{2+}, progressively inhibited oxidative modification as assessed by enhanced electrophoretic migration of LDL (Table 1). As another measure to indicate that aminoguanidine had inhibited protein modification of apo B, we recorded the fluorescence spectrum of LDL subjected to oxidative conditions in the absence or presence of 25 mM aminoguanidine (Fig. 2). Oxidized LDL showed the fluorescence properties attributed to the formation of Schiff base products, with excitation and emission maxima at 350 and 420 nm, respectively (8). Presumably, this represents adduct formation between reactive aldehydes and lysine residues of apo B. LDL subjected to the same oxidant stimulus, but in the presence of aminoguanidine, failed to show any increase in fluorescence. Aminoguanidine itself did not quench the fluorescence when added to an LDL preparation that was already oxidatively modified (Fig. 2).

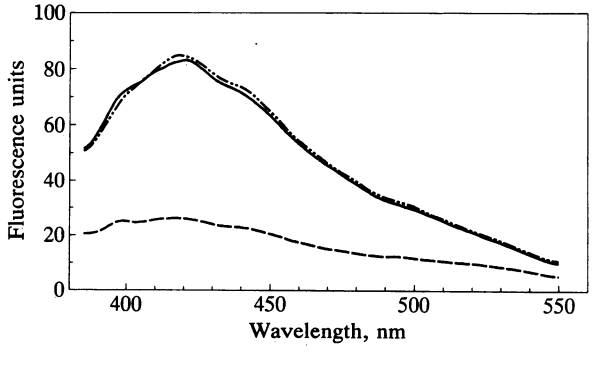

FIG. 2. Fluorescence of LDL (100 μg/ml) incubated with 5 μM Cu^{2+} in the absence or presence of 25 mM aminoguanidine. After 20 hr, the emission fluorescence spectrum was determined for each sample. The fluorescence peak at 420 nm is characteristic of aldehyde-modified lysines in oxidized LDL. Excitation wavelength was 350 nm. ——, LDL oxidized by Cu^{2+} in the absence of aminoguanidine; – – –, LDL oxidized in the presence of aminoguanidine; –·–, a previously Cu^{2+}-oxidized LDL sample to which 25 mM aminoguanidine was added 1 hr prior to fluorescence measurement. Native LDL did not show fluorescence at these wavelengths.

To directly demonstrate that aminoguanidine could inhibit aldehyde incorporation into apo B, we added MDA to LDL in the presence of aminoguanidine. In the absence of aminoguanidine, under the conditions used, 37% of apo B lysine residues were derivatized with MDA (as determined by trinitrobenzenesulfonate assay). The presence of 25, 50, and 100 mM aminoguanidine resulted in progressive inhibition of MDA incorporation, so that only 24%, 19%, and 17%, respectively, of lysine residues were modified. The increase in electrophoretic mobility of these MDA-modified LDL preparations was also progressively reduced by aminoguanidine (data not shown). Treatment of LDL with MDA leads to the formation of Schiff base adducts with lysine groups of apo B. The resulting MDA-modified LDL shows a strong fluorescence with excitation/emission at 390/460 nm. The presence of increasing concentrations of aminoguanidine progressively eliminated this peak (Fig. 3), consistent with the ability of aminoguanidine to prevent MDA modification of lysine.

Effects of Aminoguanidine on Lipid Peroxidation. From its structure, we would not predict that aminoguanidine would act directly as a traditional antioxidant. To test this, we oxidized LDL in the presence of aminoguanidine and measured the formation of TBARS (Table 1). There was a marked decrease in apparent TBARS formation. However, this result could have occurred because the aminoguanidine was reacting directly with the MDA (or other aldehydes) produced during the lipid peroxidation, thus preventing them from reacting with thiobarbituric acid to form a colored product. To demonstrate this point, aminoguanidine (0.001–7 mM) was added to solutions of thiobarbituric acid in PBS containing 2.8 μM MDA (Fig. 4). The addition of aminoguanidine to the thiobarbituric acid solution in the absence of MDA did not change the TBARS value with concentrations of aminoguanidine up to 7 mM. In the presence of MDA, aminoguanidine inhibited TBARS formation in a dose-dependent fashion. At 0.083 mM aminoguanidine, TBARS formation was inhibited 50%. Under these conditions, thiobarbituric acid (25.8 mM) was present at a 10^4 molar excess relative to MDA, whereas aminoguanidine was present at only a 30-fold molar excess. This suggests that the affinity of MDA for aminoguanidine is much higher than its affinity for thiobarbituric acid.

To further assess the impact of aminoguanidine on lipid peroxidation, we evaluated the effects of aminoguanidine on the formation of conjugated dienes. Since conjugated-diene formation is an early step of lipid peroxidation and would precede fatty acid breakdown and consequent aldehyde

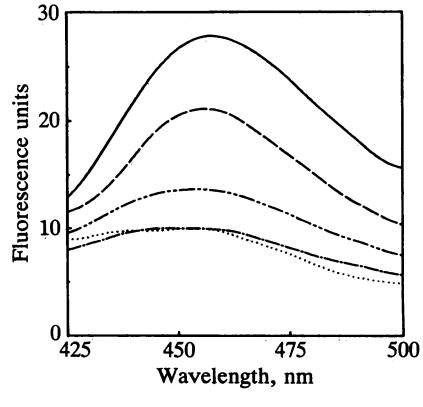

FIG. 3. Fluorescence spectra of LDL modified by MDA in the absence (——) or presence of 25 mM (– – –), 50 mM (–·–), or 100 mM (—) aminoguanidine. This resulted in approximately 37%, 24%, 19%, and 17%, respectively, of lysine residues derivatized as measured with trinitrobenzenesulfonate. Excitation wavelength was 390 nm. Spectrum of native LDL (·····) is shown for comparison.

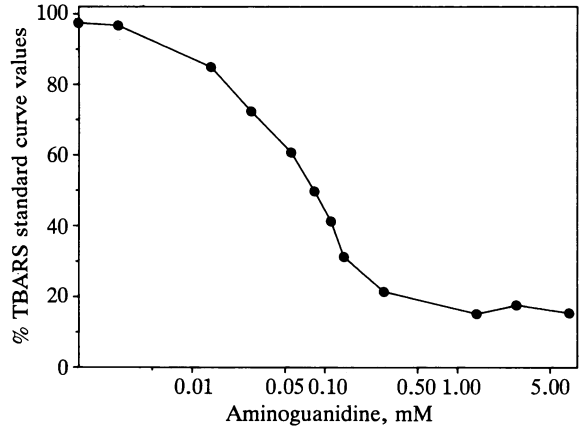

FIG. 4. Aminoguanidine inhibits MDA binding to thiobarbituric acid. Various amounts of aminoguanidine were added to solutions of thiobarbituric acid containing 0–2.8 μM MDA (tetramethoxypropane). The formation of TBARS was measured as absorbance at 532 nm. Results are expressed as the percentage of the value obtained in the absence of added aminoguanidine. Shown is a representative curve obtained when the MDA concentration was 2.8 μM, but identical curves were obtained in seven other experiments in which the MDA concentration varied from 0.28 to 2.8 μM. The addition of aminoguanidine to the thiobarbituric acid solution in the absence of MDA did not change TBARS values when the reaction was conducted in PBS. When the reaction was conducted in Ham's F-10 at >0.44 mM aminoguanidine there was an increase in color formation even in the absence of MDA.

formation, the aldehyde-complexing effects of aminoguanidine should be independent of such an antioxidant effect. At 1 mM, aminoguanidine actually shortened the lag time of the curve (Fig. 5). At 2.5 mM, there was no apparent effect. However, at 5 mM there was a definite prolongation of the lag phase and at 10 mM a marked prolongation. This suggests that at high concentrations, aminoguanidine acts directly as an antioxidant. To directly address the possibility that it could trap superoxide anion, for example, we assessed the ability of aminoguanidine to inhibit cytochrome *c* reduction mediated by superoxide anion generated by xanthine and xanthine oxidase. Under the conditions of assay, there was

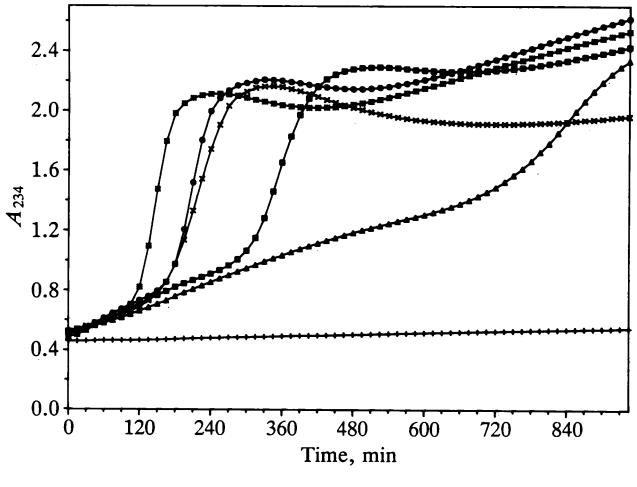

FIG. 5. Conjugated-diene formation of LDL exposed to Cu^{2+} in the absence or presence of aminoguanidine. LDL (100 μg/ml) was incubated with or without 5 μM Cu^{2+} for the indicated times in the absence or presence of aminoguanidine. Conjugated dienes were monitored at 15-min intervals at 234 nm and absolute values of absorbance are plotted. +, LDL incubated without Cu^{2+}; x, LDL plus Cu^{2+}; *, LDL plus Cu^{2+} plus 1 mM aminoguanidine; ●, LDL plus Cu^{2+} plus 2.5 mM aminoguanidine; ■, LDL plus Cu^{2+} plus 5 mM aminoguanidine; ▲, LDL plus Cu^{2+} plus 10 mM aminoguanidine.

an excess of aminoguanidine relative to the amount of superoxide anion generated. The free radical-generating system induced an increase in absorbance at 550 nm of 0.046 unit/min in the absence of aminoguanidine. The value observed under the same conditions, but in the presence of 1–30 mM aminoguanidine, was unchanged [0.043 ± 0.007 unit/min (mean ± SD, n = 7)]. The alternative interpretation is that, at higher concentrations, aminoguanidine reacts directly with the oxidants themselves, such as fatty acid peroxy radicals, thus slowing diene conjugation. Note, however, that the *overall* increase in conjugated dienes over 16 hr was not reduced, even by 10 mM aminoguanidine. Under these conditions, a maximum OD of 3 units is obtainable, and thus the level of conjugated-diene formation achieved in the experiment was below the maximal level that could be measured, and was not limited by the capacity of the instrument.

DISCUSSION

To date, inhibition of the susceptibility of LDL to oxidation has been achieved by supplementing LDL with hydrophobic antioxidants, such as probucol or vitamin E (4, 15, 22), or by decreasing the LDL content of polyunsaturated fatty acids (23). Alternatively, increasing the content of ascorbate in the aqueous medium containing LDL also affords protection, presumably by helping to maintain the endogenous vitamin E in the reduced state (24). Here we present another approach to the inhibition of oxidative modification—namely, trapping of reactive aldehydes formed from the breakdown of the oxidized fatty acids, thus preventing them from forming adducts with lysine residues of apo B. Presumably, it is these lysine adducts that form the epitopes on oxidized LDL that are recognized by the scavenger receptors on macrophages (9, 17, 25). It is possible that aldehyde adducts with other amino acids are involved as well (26).

Aminoguanidine is a nucleophilic hydrazine compound that has been used to inhibit diamine oxidase activity (27) and, more recently, to inhibit the formation of AGE that occurs from nonenzymatic glycosylation secondary to hyperglycemia (12). Aminoguanidine has been postulated to inhibit AGE formation by selectively reacting with carbonyl groups of early glycosylation products and thus preventing the subsequent electrophilic attack of these intermediates on lysine residues of adjacent proteins. Consequently, aminoguanidine can inhibit AGE-mediated modification of proteins, including crosslinking. In animal models of diabetes, aminoguanidine has resulted in decreased AGE formation in tissues such as aorta, lens, and kidney (28–30). Aminoguanidine is relatively nontoxic and clinical studies in humans are currently underway.

In our *in vitro* experiments, significant inhibition of the oxidative modification of LDL occurred with as little as 5 mM aminoguanidine. There are few data as to the tissue levels of aminoguanidine that can be produced *in vivo* with oral feeding, but the LD_{50} in rodents is very high (1800 mg/kg) and plasma levels of about 13 mM have been reported after oral feeding to animals (31). In our experiments, aminoguanidine, even though it is a relatively hydrophilic compound, was effective in preventing the formation of the ligand on LDL that causes oxidized LDL to be recognized by macrophage scavenger receptors. More-hydrophobic derivatives of aminoguanidine may achieve better penetration of the hydrophobic core of LDL and be even more effective at lower concentrations.

Aminoguanidine prevented lysine modification of apo B when LDL was subjected to oxidative stress. Concentrations of aminoguanidine that did not inhibit total lipid peroxidation over extended periods of time (e.g., 5–10 mM) nevertheless inhibited the generation of epitopes on apo B that cause

oxidized LDL to be an effective ligand for macrophage scavenger receptors, presumably by binding to reactive aldehydes formed. For example, during a 20-hr incubation period, 10 mM aminoguanidine inhibited macrophage uptake and degradation of LDL exposed to oxidizing conditions by up to 80% (Fig. 1). Although the *rate* of lipid peroxidation was slowed at this concentration of aminoguanidine, after a 16-hr period, *total* lipid peroxidation (measured as conjugated-diene formation) was not inhibited (Fig. 5). The ability to strongly inhibit apo B lysine modification without inhibiting the ultimate extent of lipid peroxidation might prove useful in separating out which components of oxidized LDL are responsible for the various biological effects it appears to have (3, 32, 33). For example, LDL oxidized for 20 hr in the presence of 5–10 mM aminoguanidine does not develop the changes in apo B that lead to enhanced uptake via macrophage scavenger receptors, yet at this time, total conjugated-diene formation is unchanged. Therefore, it seems likely that oxidized sterols, which may mediate the cytotoxicity of oxidized LDL (34), and decomposition products of oxidized fatty acids are being generated at a normal rate. It should be emphasized again, however, that even at 5 mM, aminoguanidine inhibited the rate of conjugated-diene formation and thus appears to act as an antioxidant by inhibiting propagation. Possibly it does this by acting as an amine donor directly reacting with peroxy radicals. This latter effect could account for the ability of aminoguanidine at higher concentrations to delay (but not completely prevent) the increase in conjugated dienes (Fig. 5). Thus, it is possible that aminoguanidine inhibits oxidative modification of LDL by several mechanisms.

Atherosclerosis is widespread and accelerated in the diabetic state. In part, this may be attributable to AGE formation of arterial wall proteins such as the extracellular matrix, which in turn could lead to disruption of matrix structure as well as trapping of serum proteins via the process of nonenzymatic glycosylation and AGE formation (12). For example, nonenzymatically glycosylated matrix proteins may trap LDL (13), prolonging its residence time in the intima and consequently increasing its potential to be oxidatively modified. Recently, evidence has accumulated that the process of AGE formation may involve oxidative and free radical-based mechanisms (35, 36) that in turn can potentiate and accelerate lipid peroxidation (37). In addition, lipid peroxidation products may potentiate AGE formation as well (38). Thus, one might anticipate enhanced formation of AGE in atherosclerotic tissue even in the absence of frank hyperglycemia. Indeed, we have recently used immunocytochemical techniques to demonstrate AGE in aortic lesions of euglycemic Watanabe heritable hyperlipidemic rabbits (unpublished data). Thus, aminoguanidine may be of dual benefit in inhibiting atherosclerosis, both by inhibiting AGE formation and by directly inhibiting the oxidative modification of LDL. It will be important to determine whether aminoguanidine can inhibit atherosclerosis in nondiabetic animal models of atherosclerosis, as well as prevent diabetic complications and atherosclerosis in diabetic animals.

We thank Dr. Daniel Steinberg for his advice and encouragement and Dr. Michael Yamin for supplying aminoguanidine. This research was supported in part by National Institutes of Health Grant HL14197 (Specialized Center of Research on Arteriosclerosis). S. Picard was supported by an International Lilly Fellowship, a Lavoisier Fellowship (French Government), and a fellowship of the International Institute Lipha for Medical Research.

1. Steinberg, D. & Witztum, J. L. (1990) *J. Am. Med. Assoc.* **264,** 3047–3052.
2. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witztum, J. L. (1989) *N. Engl. J. Med.* **320,** 915–924.
3. Witztum, J. L. & Steinberg, D. (1991) *J. Clin. Invest.* **88,** 1785–1792.
4. Carew, T. E., Schwenke, D. C. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7725–7729.
5. Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H. & Kawai, C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5928–5931.
6. McPherson, R., Hogue, M., Milne, R. W., Tall, A. R. & Marcel, Y. L. (1991) *Arterioscler. Thromb.* **11,** 476–481.
7. Akeson, A. L., Woods, C. W., Mosher, L. B., Thomas, C. E. & Jackson, R. L. (1991) *Atherosclerosis (Dallas)* **86,** 261–270.
8. Esterbauer, H., Schaur, R. J. & Zollner, H. (1991) *Free Radical Biol. Med.* **11,** 81–128.
9. Steinbrecher, U. P. (1987) *J. Biol. Chem.* **262,** 3603–3608.
10. Kodama, T., Freeman, R., Rohrer, L., Zabrecky, J., Matsudaira, P. & Krieger, M. (1990) *Nature (London)* **343,** 531–535.
11. Sparrow, C. P., Parthasarathy, S. & Steinberg, D. (1989) *J. Biol. Chem.* **264,** 2599–2604.
12. Brownlee, M., Cerami, A. & Vlassara, H. (1988) *N. Engl. J. Med.* **318,** 1315–1321.
13. Brownlee, M., Vlassara, H. & Cerami, A. (1985) *Diabetes* **34,** 938–941.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
15. Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L. & Steinberg, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3883–3887.
16. Esterbauer, H., Striegl, G., Puhl, H. & Rotheneder, M. (1989) *Free Radical Res. Commun.* **6,** 67–75.
17. Steinbrecher, U. P., Witztum, J. L., Parthasarathy, S. & Steinberg, D. (1987) *Arteriosclerosis (Dallas)* **1,** 135–143.
18. Henriksen, T., Mahoney, E. & Steinberg, D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6499–6503.
19. Palinski, W., Rosenfeld, M. E., Ylä-Herttuala, S., Gurtner, G. C., Socher, S. S., Butler, S., Parthasarathy, S., Carew, T., Steinberg, D. & Witztum, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1372–1376.
20. Steinbrecher, U. P., Witztum, J. L., Parthasarathy, S. & Steinberg, D. (1987) *Arteriosclerosis (Dallas)* **7,** 135–143.
21. McCord, J. M. & Fridovich, I. (1969) *J. Biol. Chem.* **244,** 6049–6055.
22. Esterbauer, H., Dieber-Rotheneder, M., Striegl, G. & Waeg, G. (1991) *Am. J. Clin. Nutr.* **53,** 314S–321S.
23. Reaven, P., Parthasarathy, S., Grasse, B. J., Miller, E., Almazan, F., Mattson, F. H., Khoo, J. C., Steinberg, D. & Witztum, J. L. (1991) *Am. J. Clin. Nutr.* **54,** 701–706.
24. Jialal, I. & Grundy, S. M. (1991) *J. Clin. Invest.* **87,** 597–601.
25. Parthasarathy, S., Fong, L. G., Otero, D. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 537–540.
26. Jürgens, G., Lang, J. & Esterbauer, H. (1986) *Biochim. Biophys. Acta* **875,** 103–114.
27. Rokkas, T., Vaja, S., Murphy, G. M. & Dowling, R. H. (1990) *Digestion* **46** (Suppl. 2), 447–457.
28. Brownlee, M., Vlassara, H., Kooney, A., Ulrich, P. & Cerami, A. (1986) *Science* **232,** 1629–1632.
29. Lewis, B. S. & Harding, J. J. (1990) *Exp. Eye Res.* **50,** 463–467.
30. Soulis-Liparota, T., Cooper, M., Papazoglou, D., Clarke, B. & Jerums, G. (1991) *Diabetes* **40,** 1328–1334.
31. Baylin, S., Horakova, Z. & Beaven, M. A. (1975) *Experentia* **31,** 562–564.
32. Rosenfeld, M. E. (1991) *Circulation* **83,** 2137–2140.
33. Liao, F., Berliner, J. A., Mehrabian, M., Navab, M., Demer, L. L., Lusis, A. J. & Fogelman, A. M. (1991) *J. Clin. Invest.* **87,** 2253–2257.
34. Hessler, J. R., Morel, D. W., Lewis, L. J. & Chisolm, G. M. (1983) *Arteriosclerosis (Dallas)* **3,** 215–222.
35. Hunt, J. V., Smith, C. C. T. & Wolff, S. P. (1990) *Diabetes* **39,** 1420–1424.
36. Baynes, J. W. (1991) *Diabetes* **40,** 405–412.
37. Mullarkey, C. J., Edelstein, D. & Brownlee, M. (1990) *Biochem. Biophys. Res. Commun.* **173,** 932–939.
38. Hicks, M., Delbridge, L., Yue, D. K. & Reeve, T. S. (1989) *Arch. Biochem. Biophys.* **268,** 249–254.