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Antioxidant treatment of diabetic rats inhibits lipoprotein oxidation and cytotoxicity

Diane W. Morel[1] **and Guy M. Chisolm**[2]

Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195

Abstract Increased lipid peroxidation products were detected in a lipoprotein fraction containing very low density lipoprotein (VLDL) and low density lipoprotein (LDL) obtained from rats made diabetic by streptozotocin injection. The enhanced oxidation in the diabetic VLDL plus LDL fraction correlated with the *in vitro* toxicity of this lipoprotein fraction to proliferating fibroblasts. In contrast, high density lipoprotein (HDL) was not cytotoxic. That the increased oxidation and development of cytotoxic activity in the diabetic VLDL + LDL was related to the diabetes was shown by the fact that insulin treatment of diabetic animals inhibited both oxidation and cytotoxicity of VLDL + LDL. In contrast, treatment of diabetic rats with the antioxidants vitamin E or probucol after diabetes was established also inhibited both the in vivo oxidation and in vitro cytotoxicity of diabetic VLDL + LDL, but without altering hyperglycemia. Vitamin E or probucol treatment thus allowed separation of the oxidation process from the hyperglycemia occurring in experimental diabetes. The mechanisms by which diabetes in humans or experimental animals leads to the various manifestations of tissue damage are unknown; however, these studies demonstrate for the first time that a relationship exists between the in vivo oxidation of lipoproteins in diabetes and the potential for tissue damage as monitored by in vitro cytotoxicity. Furthermore, these results suggest that the mechanism for certain aspects of tissue damage accompanying experimental diabetes may be mediated by lipid peroxidation products. —**Morel, D. W., and G. M. Chisolm.** Antioxidant treatment of diabetic rats inhibits lipoprotein oxidation and cytotoxicity. *J. Lipid Res.* 1989. **30:** 1827–1834.

Supplementary key words streptozotocin • fibroblasts • VLDL • LDL • HDL • vitamin E • probucol

Tissue damage in arteries and in organs such as the eyes and kidneys is characteristic of diabetes mellitus both in humans and experimental models (1–3), making diabetics more prone to atherosclerosis, blindness, and renal failure. The mechanisms by which tissue damage occurs in diabetes are not well understood.

Lipid peroxidation has been implicated in adverse tissue changes in aging as well as in certain deseases (4–6). Plasma lipid peroxides have also been reported to be elevated in humans with diabetes (7,8), particularly those with poorly controlled plasma glucose or angiopathy (7). Increased levels of plasma lipid peroxidation products have been reported in streptozotocin (SZ)-induced diabetes in rats (9, 10) and can be reduced by dietary vitamin E supplementation (10).

Our previous studies demonstrated that very low density lipoprotein (VLDL) and low density lipoprotein (LDL), when oxidized, become toxic to cells in culture. We found that the oxidation of LDL renders it cytotoxic by a free radical process (11, 12) that can be mediated in culture by human monocyte-macrophages (13) as well as by certain endothelial cells and smooth muscle cells (14). In these studies, antioxidants such as vitamin E inhibited both the oxidation and the formation of cytotoxic LDL (12). Probucol has been shown in studies by others to inhibit effectively the oxidative modification of LDL (15). The cytotoxicity of oxidized lipoproteins in vitro suggests at least the potential for lipoprotein-mediated tissue damage in vivo. In particular, oxidized lipoproteins, when present in vivo in a disease such as diabetes, might be responsible for some of the tissue damage characteristic of diabetes.

In the present studies we have explored the possibility that lipoprotein oxidation in vivo is responsible for the reported (16) in vitro cytotoxicity of diabetic rat sera and lipoprotein fractions. Since we found previously that both LDL and VLDL become cytotoxic when oxidized (11, 12) and since rats have relatively small amounts of LDL (17), we chose to investigate the correlation between in vivo oxidation and in vitro cytotoxicity of one lipoprotein fraction containing both LDL and VLDL and a second lipoprotein fraction containing HDL. By examining the effect of antioxidant treatment of the diabetic animals (using both vitamin E and probucol) we were able to separate the

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Abbreviations: SZ, streptozotocin; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TBA, thiobarbituric acid; LPDS, lipoprotein-deficient serum; MDA, malondialdehyde; TBARS, thiobarbituric acid reacting substances.

[1]Present address: Department of Physiology and Biochemistry, The Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129.

[2]To whom correspondence should be addressed at: Research Institute FF4, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195.

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effects of oxidation from those directly related to hyperglycemia. The results indicate, first, that lipoprotein oxidation, in particular in the VLDL + LDL fraction, occurs as a result of hyperglycemia in experimental diabetes and is related to the in vitro cytotoxicity of that fraction, and secondly, that interference with the in vivo oxidation via antioxidant treatment markedly decreases in vitro cytotoxicity without affecting hyperglycemia.

MATERIALS AND METHODS

Animals and treatments

Diabetes was induced in male Sprague-Dawley rats (175-225 g, Hilltop) by a single intraperitoneal injection of streptozotocin (SZ, 75 mg/kg, Sigma) in 0.2 ml citrate buffer (0.05 M, pH 4.5). Control animals were injected with buffer (0.2 ml). Beginning after hyperglycemia was established (4-6 days after SZ injection), as detected by glucosuria (Labstix®, Miles Laboratory), rats were treated with the antioxidants vitamin E or probucol as described in the legends of Figs. 1-3. That these antioxidants affected the diabetic rats was indicated by decreased lipid peroxidation in their lipoprotein fractions and, in the case of probucol, by lowered serum cholesterol. Some of the diabetic rats were treated with daily subcutaneous injections of insulin (protamine zinc iletin, 6 U/day).

Blood collection and lipoprotein isolation

After a 12-h fast, rats were exsanguinated (5-10 ml blood/rat) under ether anesthesia into syringes containing 0.2 ml of 0.2 M ethylenediaminetetraacetic acid (EDTA). The plasma was removed by centrifugation at 1000 g for 20 min, then clotted by addition of calcium ($CaCl_2$-$6H_2O$) to yield a calcium concentration 5 mM in excess of the equivalent molarity of EDTA. The clot was removed by centrifugation at 23,500 g for 30 min and samples of the plasma-derived serum were removed for glucose, cholesterol, and triglyceride measurements. Results were corrected for the initial dilution with the EDTA and calcium solutions. Plasma-derived sera of rats from the same treatment group were pooled in certain cases (see Statistical considerations) in order to obtain sufficient quantities of serum for the described assays and lipoprotein isolation. Prior to testing sera for cytotoxicity, samples were dialyzed overnight against 0.15 M NaCl with 0.5 mM EDTA, then against tissue culture medium for 4-6 h at 4°C.

In order to isolate lipoprotein fractions, the solvent density of the plasma-derived serum was adjusted to 1.050 g/ml with a high density salt solution (containing NaCl, KBr, and EDTA). After centrifugation for 18-20 h at 50,000 rpm, 10°C in a Beckman L5-75 ultracentrifuge using a 50.3 Ti rotor, the supernatant was collected as a fraction containing VLDL and LDL. The infranatant was adjusted to solvent density 1.21 g/ml with solid KBr, centrifuged as before, and a high density lipoprotein (HDL) fraction was collected. The lipoprotein fractions were dialyzed under sterile conditions at 4°C for 48-66 h against four changes of at least 50 volumes of 0.15 M NaCl with 0.5 mM EDTA, adjusted to pH 8.0-9.0, then against tissue culture medium for 4-6 h at 4°C. In all of these experiments, it was particularly important to inhibit oxidation of lipoproteins subsequent to the removal of blood from the animals. Our previous experiments (12, 14) indicated that the use of EDTA is sufficient for this purpose. The lipoprotein fractions were filter-sterilized, assayed for cholesterol, and aliquoted for the thiobarbituric acid (TBA) assay immediately before addition to the cytotoxicity assay.

Lipoprotein-deficient serum (LPDS) was prepared from pooled human serum as previously described (14).

Plasma-derived serum was assayed for final glucose (Sigma kit), cholesterol (Boehringer Mannheim kit), and triglyceride (Sigma kit); lipoprotein fractions and LPDS were assayed for cholesterol (Boehringer Mannheim kit) and protein (18).

Measure of oxidation

Lipid peroxides were measured in 400-μl samples of lipoproteins diluted with tissue culture medium to 300 μg chol/ml (the concentration used in the cytotoxicity assay) using the TBA assay as previously described (12) except that fluorescence at 515 nm excitation, 553 nm emission was recorded. Lipoprotein lipid peroxides are expressed in terms of malondialdehyde (MDA) equivalents (12). Oxidation was not monitored in the plasma-derived serum because uncontrolled serum components interfere with the TBA assay (D. W. Morel and G. M. Chisolm, unpublished observations).

Because of the potential for glycosylation products to interfere in the TBA assay (19), normal plasma-derived rat serum was incubated at 37°C for 14 days with or without exogenous glucose added to elevate glucose concentration an additional 1800 mg/dl above the serum content. EDTA (0.5 mM) was present and nitrogen was introduced prior to closing the container to inhibit additional oxidation. At the end of this period HDL and VLDL + LDL fractions were isolated as above and the TBA and cytotoxicity assays were performed.

Cell culture

Human dermal fibroblasts were obtained from neonatal foreskins as previously described (11). Explants were placed in a DVF-12 media (1:1 mixture of Dulbecco-Vogt modified Eagle's medium with high glucose and Ham's F-12, supplemented with 0.24% sodium bicarbonate, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and 4

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mM L-glutamate) with 15% fetal bovine serum (MA Bio-products) and incubated in a humidified 5% CO_2 in air environment for 3 to 4 weeks, then subcultured. Cytotoxicity assays were performed with cells in passages 2-9.

Cytotoxicity assay

To test cytotoxicity of sera and lipoprotein fractions, fibroblast cell survival was monitored at the end of a 66-h incubation. Fibroblasts were plated in 96-well microtiter plates 24 h prior to the start of the assay. Cells were rinsed twice with medium prior to addition of test medium consisting of DVF-12 plus 20% volume displacement of plasma-derived serum or 300 μg cholesterol/ml of the lipoprotein fraction and a 10% volume displacement of LPDS (final concentration 4 mg protein/ml). After 66 h, cells were rinsed twice with phosphate-buffered saline to remove nonadherent cells and cell survival was assessed by determining the hexosaminidase activity of the cells remaining attached; this assay has been shown to correlate well with other indices of cell survival (20). Cell survival was determined by comparing the absorbance in experimental wells to that in wells that were exposed to LPDS but no lipoprotein and is expressed as a percentage of this control.

Statistical considerations

Data presented are means ± standard error. Statistical analysis was performed using one-way analysis of variance and Scheffe's method of multiple comparisons (21). Differences with $P<0.05$ were considered statistically significant.

Final plasma samples from animals in the experiments presented in this report were pooled where necessary to obtain sufficient quantities of samples for lipoprotein separation and the various assays. Animals that had not become hyperglycemic by 5 days after SZ injection as assessed by measurement of urine glucose (Labstix®, Miles Laboratory) were removed from the study before any treatment began. In the experiment shown in Figs. 1 and 2, the four experimental groups had the following numbers of animals at termination: nondiabetic control, 12; SZ + vehicle, 5; SZ + probucol, 7; and SZ + vitamin E, 5. These values of n apply to Fig. 1. Measurements of serum glucose, cholesterol, triglyceride, and body weight were made on the final plasma samples from individual animals. Assessments of serum toxicity and measurements made on lipoprotein fractions were performed on samples from either individual animals or pools of plasmas from pairs of animals for which plasma volume was insufficient. From pooling, the group sizes were reduced to n = 7, 3, 4, and 4 for the four groups, respectively; these values of n apply to Fig. 2.

In the experimental results displayed in Fig. 3, a second pooling scheme applied. Nonresponders to SZ had been removed as above prior to treatment with antioxidants or insulin, pooling of plasma samples in pairs was done randomly within an experimental group prior to all measurements, including glucose, cholesterol, and triglyceride. The numbers of animals used in each group were: nondiabetic control, 5; SZ, 6; SZ + vehicle, 8; SZ + probucol, 7; SZ + vitamin E, 6; and SZ + insulin, 6. These numbers were reduced by paired pooling where necessary to n = 3,4,4,4,4, and 3, respectively. The latter values of n apply to Fig. 3.

Because of the variable and occasionally low values of n used in these studies, it is pertinent to point out that we performed nine experiments on the oxidation and toxicity of lipoproteins in SZ-treated rats. We examined and observed in all nine experiments the correlation between VLDL + LDL oxidation in vivo and cytotoxicity in vitro of the sera and/or VLDL + LDL fraction. In seven of the nine experiments antioxidants were administered; in three, an insulin-treated group was included and in three, HDL was isolated and its toxicity and oxidation were assessed. The results shown from the experiments in Figs. 1-3 are representative of the findings.

RESULTS

Rats injected with streptozotocin (SZ) exhibited serum glucose levels elevated above those of controls injected with buffer alone. The increase was observed within 1 week after SZ injection (data not shown) and the glucose levels remained elevated throughout the course of each experiment. As shown in **Fig. 1a**, serum glucose levels 6.5 weeks after injection with SZ were five times that in control animals injected with citrate ($P<0.01$). Neither vitamin E nor probucol treatment for 6-8 weeks had any effect on hyperglycemia.

Serum cholesterol was modestly elevated in the SZ rats but was unaffected by vitamin E treatment (Fig. 1b). Probucol treatment decreased serum cholesterol slightly. This decrease was not statistically significant in the experiment shown in Fig. 1, but the analogous decrease in cholesterol by probucol for the experiment depicted in Fig. 3 was significant ($P\leq 0.05$). Serum triglycerides were markedly elevated in SZ rats (Fig. 1c) but probucol and vitamin E treatment had no effect on serum triglyceride in these experiments despite a previous report that vitamin E treatment lowered diabetes-induced hypertriglyceridemia (22).

As shown in Fig. 1d, diabetic animals gained little or no weight during the course of an experiment, whereas nondiabetic control animals almost doubled in weight; antioxidant treatment had no effect on the body weight of the diabetic rats.

As shown in **Fig. 2**, a lipoprotein fraction containing VLDL + LDL from SZ rats exhibited increased oxidation as monitored by thiobarbituric acid reactivity (TBARS) compared to that from control rats ($P<0.01$). The

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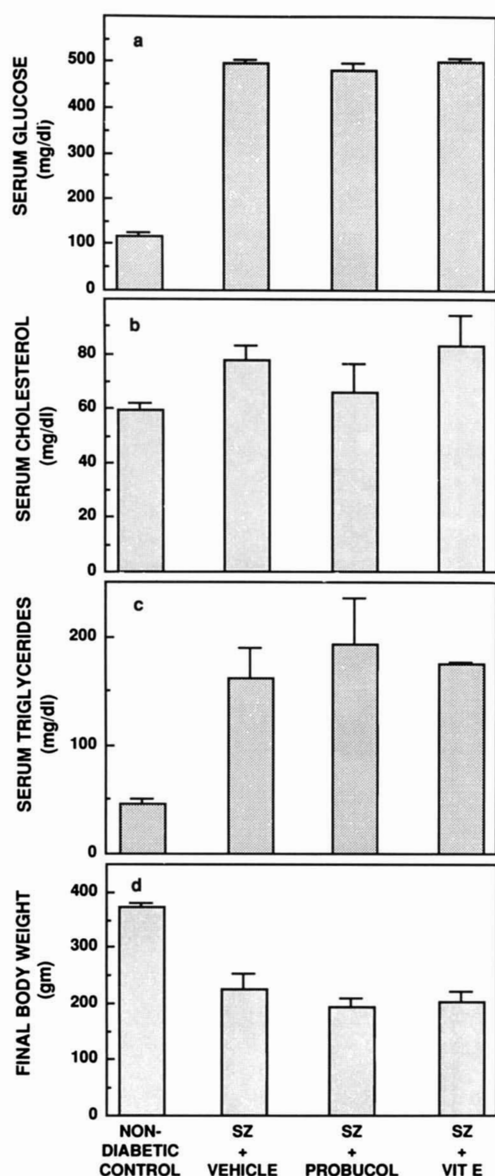

Fig. 1. Final levels of (a) serum glucose, (b) serum cholesterol, (c) serum triglycerides, and (d) body weight of rats made diabetic by streptozotocin: effect of antioxidant treatment. Treatment was accomplished by feeding rats 1 g cream cheese (vehicle) with or without probucol or vitamin E, 5 days/wk for 5 weeks and 7 days/wk for a final week. Probucol-treated rats received 200 mg probucol/day for weeks 1-3 and 400 mg/day for weeks 4-6. Vitamin E-treated rats received 400 mg vitamin E acetate/day for all 6 weeks. All data are expressed as mean ± SE.

VLDL + LDL fraction from SZ rats in all experiments was cytotoxic compared to the VLDL + LDL fraction from control rats ($P<0.01$). Vitamin E or probucol treatment of SZ rats reduced the level of oxidation in the VLDL + LDL fraction compared to SZ + vehicle ($P<0.025$). In addition, the toxicity of the VLDL + LDL fraction from vitamin E-treated SZ rats or probucol-treated SZ rats was less than that observed with the VLDL + LDL fraction from SZ + vehicle rats ($P<0.025$ for probucol and $P<0.05$ for vitamin E).

In contrast to our findings for the VLDL + LDL fraction of diabetic rats, the TBA reactivity of the HDL fraction of SZ rats was altered variably and only slightly from the controls. (In two of three experiments HDL-associated TBARS were elevated approximately 2 nmol MDA/mg chol and in the third they were decreased by approximately the same amount.) Neither vitamin E treatment nor probucol treatment had a significant effect on the level of HDL-associated TBARS. None of the HDL fractions was cytotoxic compared to HDL from nondiabetic animals.

The VLDL + LDL fraction from SZ rats was sufficiently cytotoxic that, as shown in Fig. 2c, when diabetic serum was added to fibroblast cultures at a 20% volume displacement, cell survival was markedly reduced com-

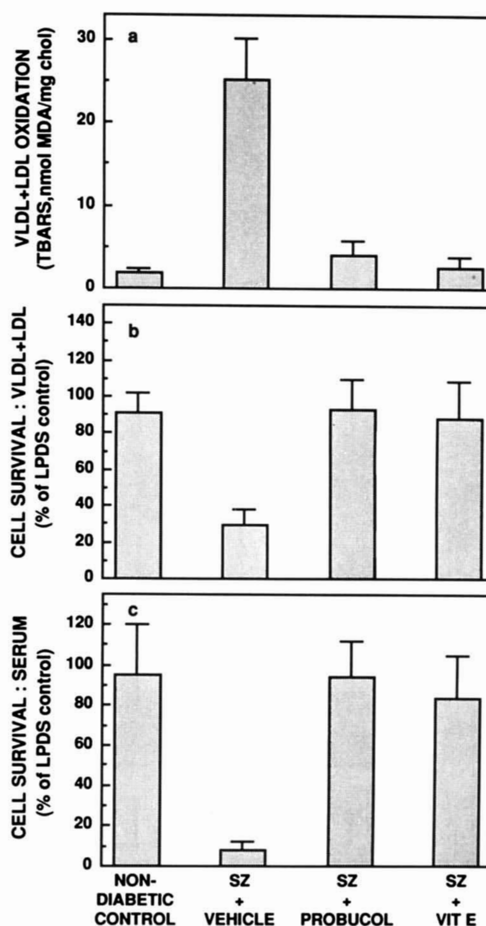

Fig. 2. Effect of antioxidant treatment of diabetic rats on (a) in vivo oxidation of diabetic VLDL + LDL , (b) the in vitro toxicity to fibroblasts of diabetic VLDL + LDL , and (c) the cytotoxicity of diabetic serum. Treatments were as described in the legend to Fig. 1. Cytotoxicity was monitored in fibroblasts after a 66-h exposure to either 300 μg cholesterol/ml of the lipoprotein fraction plus 10% LPDS or to 20% plasma-derived serum. Cytotoxicity is expressed as percent cell survival compared to a LPDS control. All data are expressed as mean ± SE.

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pared to that in cultures exposed to sera from nondiabetic rats ($P<0.025$). The cytotoxicity of the diabetic sera was not directly due to SZ since *a*) the half-life of SZ is such that it would be removed or inactivated within hours of injection (3) and *b*) serum fractions from rats exsanguinated 6 h after SZ injection did not exhibit TBA reactivity or cytotoxicity (data not shown). The survival of cells exposed to sera from antioxidant-treated SZ rats was significantly greater than that of the vehicle-treated SZ groups ($P<0.025$ for probucol and $P<0.05$ for vitamin E) despite the fact that neither vitamin E treatment nor probucol treatment had any effect on hyperglycemia or on hypertriglyceridemia. The enhancement of cell survival in the vitamin E group appeared to be due to the antioxidant effect of vitamin E in vivo since probucol had the same effect and, in a separate experiment, the addition of vitamin E (25 μM) to cultures exposed to diabetic serum did not increase fibroblast survival (data not shown).

In the experiment shown in **Fig. 3** we explored the role of hyperglycemia in the oxidation of the VLDL + LDL fraction and in rendering it cytotoxic in vitro. Insulin treatment of diabetic rats normalized serum glucose levels to those of the nondiabetic controls. Insulin treatment also decreased the hypertriglyceridemia observed in untreated diabetic rats; serum triglycerides were 63 ± 12, 254 ± 65 and 94 ± 23 in nondiabetic controls, untreated diabetics, and insulin-treated diabetics, respectively. Insulin and probucol reduced serum cholesterol levels to those of nondiabetic controls, whereas vitamin E treatment had no effect on either serum triglycerides or serum cholesterol. Insulin treatment of diabetic animals also abrogated the cytotoxicity of serum (Fig. 3c) and of the VLDL + LDL fraction (data not shown) as did treatment with probucol or vitamin E. Thus, in vivo oxidation of diabetic VLDL + LDL and its in vitro cytotoxicity as well as the toxicity of diabetic serum can be inhibited by reversing hyperglycemia with insulin treatment or by antioxidant treatment which does not affect hyperglycemia.

In a separate experiment, control rat plasma-derived serum was incubated with or without added glucose to a final concentration of 1800 mg/dl under nitrogen for 2 weeks at 37°C in order to explore the possibility that glucosylation products might contribute to the readings of TBARS (19) or to the cytotoxicity. The lipoprotein fractions were subsequently isolated and tested for TBARS and for cytotoxicity. The TBARS of the VLDL + LDL fraction from the high glucose incubation was 0.7 nmol MDA/mg cholesterol higher than the low glucose control, and that for HDL was 2.2 nmol MDA/mg cholesterol higher, indicating a minor, if any, contribution of glucosylation products to the TBARS readings shown for diabetic animals. None of the lipoprotein fractions glucosylated in vitro was cytotoxic. Thus, glucosylation is not responsible for the elevated TBARS or the toxic action of the diabetic VLDL + LDL fraction.

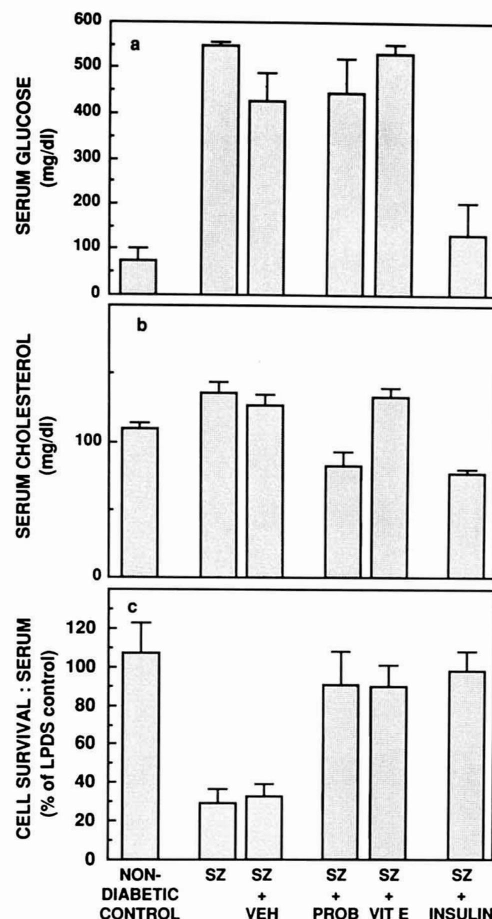

Fig. 3. Effect of insulin or antioxidant treatments of diabetic rats on final (a) serum glucose, (b) serum cholesterol, and (c) in vitro serum cytotoxicity. Insulin treatment was for 8 weeks with daily subcutaneous injections of insulin (protamine zinc iletin, 6 U/day). Antioxidant treatment was for 8 weeks using twice weekly intraperitoneal injections of vitamin E acetate (200 mg/kg) or probucol (200 mg/kg) in Captex 300 oil (0.2 ml) or Captex 300 oil alone (0.2 ml, vehicle). Cytotoxicity (decreased cell survival) was monitored in fibroblast cultures after a 66-h exposure to 20% plasma-derived serum. All data represent mean ± SE.

In other experiments (data not shown), we found that a serum fraction from diabetic rats that contained HDL plus nonlipoprotein components of serum (d > 1.050 g/ml) was also not toxic in vitro; thus, the cytotoxic activity of diabetic sera resides only in the d < 1.050 g/ml; VLDL + LDL fraction.

DISCUSSION

The studies described in this report demonstrate a correlation between increased lipoprotein oxidation in vivo in experimental diabetes and lipoprotein cytotoxicity in vitro. This relationship holds for a VLDL + LDL fraction, not for the HDL fraction which was only slightly and variably TBA-reactive and was not cytotoxic. Insulin

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treatment of diabetic rats reversed the hyperglycemia and inhibited both the oxidation of VLDL + LDL and the development of cytotoxic activity. Antioxidant treatment of diabetic rats, begun after hyperglycemia was established, had no effect on the hyperglycemia yet inhibited the oxidation of the lower density lipoproteins as well as the toxicity to cultured cells of both this lipoprotein fraction and the whole plasma-derived serum. The results shown are representative of experiments we have performed of varying durations of diabetes and varying doses, routes of administration, and duration (1-8 weeks) of antioxidant treatment. We consistently found that antioxidant treatment of diabetic rats inhibited the in vitro toxicity of diabetic serum and reduced the TBA reactivity and cytotoxicity of diabetic VLDL + LDL.

These studies represent an in vivo counterpart to our previous studies in vitro which showed that LDL or VLDL, when allowed to oxidize, became toxic to cells in culture (11-14). We chose to look at oxidation and cytotoxicity in a combined VLDL + LDL fraction from diabetic rats in order to optimize our ability to detect differences and because rats have very low levels of LDL (17). Vitamin E and probucol were used as antioxidants because both are lipophilic and neither is toxic at relatively high doses. In addition, vitamin E, in our hands, inhibited the in vitro oxidation and subsequent cytotoxicity of LDL (12-14) and probucol treatment in humans has been shown by others to inhibit the oxidative modification of LDL in vitro (15). Since neither vitamin E nor probucol had any effect on hyperglycemia, their use in parallel with insulin allowed us to distinguish the effects of in vivo oxidation from those directly related to hyperglycemia.

It was recently reported that vitamin E supplementation had little effect on plasma vitamin E levels, although it increased liver vitamin E content (22). We chose doses and routes of administration of vitamin E which have been shown to be effective in inhibiting lipid peroxidation processes in vivo (23-25). The doses of probucol were based on approximate equivalency to the vitamin E doses and on the bioavailability of probucol (26). That probucol and vitamin E were effective at these doses was evidenced by decreased oxidation (TBARS) in the VLDL + LDL fraction and in the case of probucol, by decreased serum cholesterol. Vitamin E and probucol levels in plasma were not monitored since in these experiments we were not seeking the minimum effective dose.

These studies are distinct from those of Slonim et al. (27) and Sandler and Andersson (28) showing that pretreatment of animals with vitamin E or dimethyl urea (a hydroxyl radical scavenger) prevented or inhibited the diabetogenic action of SZ. In our studies, antioxidant treatment was not initiated until after the diabetes was established and it had no effect on the subsequent course of the diabetes as monitored by serum glucose. It was important to ascertain that the enhanced lipoprotein oxidation we observed was a result of diabetes rather than the free radical process believed to take place in SZ-induced beta cell destruction. Since the half-life of active SZ is only 1-2 h (3), our finding that the sera from rats killed 6 h after SZ injection was not cytotoxic indicates that oxidation results from the diabetes, not SZ directly. This is further supported by our observation that insulin treatment as well as antioxidant administration, begun several days after the SZ injection, inhibited both the enhanced oxidation and cytotoxicity of diabetic VLDL + LDL.

The identity of the cytotoxic moiety of oxidized lipoproteins is as yet undetermined. The toxic agent of oxidized LDL appears to be a lipid, as it can be extracted with organic solvents (11). It has been demonstrated that the majority, but not all, of TBARS produced when LDL becomes oxidized are water-soluble and do not remain associated with the lipoprotein (29); yet we found that all of the cytotoxic activity remains with the lipoprotein (14). Thus, although we use the TBARS measurement as an index of oxidation, we actually measure only a portion of the TBARS produced during lipoprotein oxidation (since water-soluble TBARS are removed by dialysis). It is as yet unknown whether the toxic moiety itself is TBA-reactive.

Glucosylated proteins have been shown to occur in diabetes (30, 31) and to exhibit crosslinking and formation of fluorescent pigments (30) which could possibly interfere with the TBA assay (19). A distinction, therefore, needed to be made between TBARS from lipid peroxidation and TBARS from glucosylation of LDL, since LDL, like other plasma proteins, can be readily glucosylated (31). The elevated TBARS detected in diabetic sera was not due to the elevated glucose itself since adding glucose to control sera did not enhance TBARS values. Furthermore, since incubation for 14 days of control serum with high glucose failed to enhance TBARS markedly and failed to render the lipoproteins toxic, it is unlikely that glucosylation of the lipoprotein is responsible for our results. The reduced TBARS observed in the sera of vitamin E and probucol-treated rats probably reflects decreased lipid peroxidation since neither treatment had any effect on serum glucose and presumably no effect on the extent of plasma protein glucosylation.

Arbogast, Lee, and Raymond (16) first reported that diabetic rat sera and VLDL are toxic to cultured endothelial cells. Although their studies included experiments designed to rule out the occurrence of lipoprotein oxidation during lipoprotein isolation from the rat plasma, the possibility of lipoprotein oxidation in vivo as a result of the diabetes was not considered and oxidation products were not measured. Thus, their results and those of the present study are not contradictory in this respect. Our studies and those of Arbogast et al. (16), Chi et al. (32), and Arbogast, Berry, and Newell (33) differ in that in the latter studies the diabetes was accompanied by a marked hypertriglyceridemia (up to 500 mg/dl compared to about

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250 mg/dl in our studies) and the degree of toxicity appeared to correlate with the degree of hypertriglyceridemia (33). This is consistent with the findings of Gianturco et al. (34) that hypertriacylglycerolemic human VLDL was toxic to bovine endothelial cells in culture. In contrast, we have found that antioxidant treatment of diabetic animals inhibits oxidation and cytotoxicity without affecting the hypertriglyceridemia that was induced.

Our decision to use human foreskin fibroblasts as a target cell in these studies was derived from previous studies by us and others examining the characteristics of oxidized lipoprotein or diabetic lipoprotein toxicity. The toxicity of oxidized VLDL or LDL does not appear to be specific for a certain cell type. Hessler et al. (11, 35) and Henriksen, Evenson, and Carlander (36) demonstrated potent toxicity of these oxidatively modified lipoproteins to vascular smooth muscle cells and endothelial cells as well as fibroblasts. We have previously used fibroblasts as a convenient model to evaluate various aspects of the oxidized lipoprotein toxicity, such as the cell cycle dependency (37). That the toxicity of lipoproteins isolated from SZ-treated rats is similarly nonspecific is indicated by the corroboration of our results of toxicity of VLDL + LDL to fibroblasts and those of Arbogast et al. (16, 32, 33) showing VLDL toxicity to endothelial cells.

The concept that lipoprotein oxidation might be important in diabetes was introduced by the studies of Sato et al. (7), showing that lipid peroxides were enhanced in the plasma of diabetic patients, and Nishigaki et al. (8), showing that the lipid peroxides in diabetic sera resided in a lipoprotein fraction, primarily HDL. Higuchi (9) reported that lipid peroxides were elevated in the sera and liver of rats with SZ-induced diabetes but lipoprotein fractionation was not performed. It is unclear why the enhanced lipid peroxides we find in SZ-induced diabetic rats are primarily in the lower density lipoproteins while Nishigaki et al. (8) found the enhanced lipid peroxides in the HDL fraction of human diabetics. This difference may be related to the expression of significant lipid transfer protein activity in human plasma but not rat plasma (38); however, further investigations will be required to understand this species difference.

The mechanism by which lipoprotein oxidation increases in experimental diabetes is not known. Kitahara et al. (39) have reported that monocytes from poorly controlled diabetic humans exhibit an enhanced respiratory burst upon stimulation with zymosan; their results suggest the effect is related to increased plasma glucose. Since our previous studies showed that monocytes, when stimulated, can oxidize LDL and make it cytotoxic (13), it is possible that increased cell-mediated oxidation, in response to an unknown stimulus, produces the observed elevation in oxidation of diabetic lipoproteins. It has been reported that antioxidants in certain tissues of diabetic rats are decreased (40-42), which may reflect the increased occurrence of oxidation and a decrease in capability to protect against oxidation.

Others have hypothesized that increased lipid peroxides might be related to tissue damage in aging and various diseases (4-6). The relationship between tissue damage and lipid or lipoprotein oxidation is currently unknown for experimental diabetes or other human diseases. Our studies indicate that the potential for tissue damage, as measured by in vitro cytotoxicity, is increased by the elevated lipoprotein-associated lipid peroxides in diabetic rats. Longer term studies are needed to determine whether a correlation exists between in vivo lipoprotein oxidation and the many and various forms of tissue damage occurring in experimental diabetes.

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