Increased low density lipoprotein degradation in aorta of irradiated mice is inhibited by preenrichment of low density lipoprotein with α-tocopherol

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Abstract **We previously reported that upper thoracic exposure to ionizing radiation (IR) accelerates fatty streak formation in C57BL/6 mice and that such effects are inhibited by overexpression of the antioxidant enzyme CuZn-superoxide dismutase (SOD). Notably, IR-accelerated lesion formation is strictly dependent on a high fat diet (i.e., atherogenic lipoproteins) but does not involve alterations in circulating lipid or lipoprotein levels. We thus proposed that IR promotes changes in the artery wall that enhance the deposition of lipoprotein lipids. To address this hypothesis, we examined the effects of IR on aortic accumulation and degradation of low density lipoproteins (LDL). Ten-week-old C57BL/6 mice were exposed to a single (8-Gy) dose of ^{60}Co radiation to the upper thoracic area or were sham irradiated (controls) and were then placed on the high fat diet. Five days postexposure, the mice received either ^{125}I-labeled LDL (^{125}I-LDL) (which was used to measure intact LDL) or ^{125}I-labeled tyramine cellobiose (^{125}I-TC)-LDL (which was used to measure both intact and cell-degraded LDL) via tail vein injection. On the basis of trichloroacetic acid (TCA)-precipitable counts in retroorbital blood samples, ≥95% of donor LDL was cleared within 24 h and there were no differences in time-averaged plasma concentrations of the two forms of LDL among irradiated and control mice. Aortic values increased markedly within the first hour and thereafter exhibited a slow increase up to 24 h. There were no differences between irradiated and control mice at 1 h, when values primarily reflected LDL entry, but a divergence was observed thereafter. At 24 h, ^{125}I-TC-associated counts were 1.8-fold higher in irradiated mice ($P = 0.10$). In contrast, ^{125}I-LDL-associated counts were 30% lower in irradiated mice ($P < 0.05$), suggesting that most of the retained ^{125}I-TC was associated with LDL degradation products. Consistent with the proposed involvement of oxidative or redox-regulated events, IR-induced LDL degradation was lower in SOD-transgenic than wild-type mice ($P < 0.05$). The importance of LDL oxidation was suggested by observations that IR-induced LDL degradation was significantly reduced by preenriching LDL with α-tocopherol. On the basis of these results, we propose that IR elicits SOD-inhibitable changes in the artery wall that enhance LDL oxidation and degradation leading to the deposition of LDL-borne lipids. These studies provide additional support for the role of oxidation in lipoprotein lipid deposition and atherogenesis and suggest that IR promotes an arterial environment that stimulates this process in vivo.**—Tribble, D. L., R. M. Krauss, B. M. Chu, E. L. Gong, B. R. Kullgren, J. O. Nagy, and M. La Belle. **Increased low density lipoprotein degradation in aorta of irradiated mice is inhibited by preenrichment of low density lipoprotein with α-tocopherol.** ***J. Lipid Res.*** **2000.** 41: **1666–1672.**

Supplementary key words ionizing radiation • lipoproteins • oxidation • antioxidants • atherosclerosis

Early fatty streaks, the first histologically defined lesions in the development of atherosclerosis, are characterized by the presence of lipid-engorged foam cells in the arterial intima (1). These foam cells originate primarily from macrophages that engulf and degrade arterially retained lipoproteins. Most plasma lipoprotein classes are subject to uptake by macrophages, but low density lipoproteins (LDL) appear to be the primary contributors to foam cell formation in humans. This is attributed both to LDL particle attributes and relative concentrations.

Over the past several decades, trapped ligand methods have been used to investigate arterial LDL retention and degradation in vivo. As initially demonstrated by Carew et al. (2), ^{125}I-labeled LDL (^{125}I-LDL) can be used to monitor intact LDL whereas LDL that is covalently coupled with ^{125}I-labeled tyramine cellobiose (^{125}I-TC), a residualizing agent, can be used to monitor both intact LDL and intracellular LDL degradation products (2–7). Studies employing this methodology in hyperlipidemic rabbits and White Carneau pigeons have shown that LDL particles preferentially accumulate within atherosclerosis-susceptible sites of the vasculature and that this occurs

Abbreviations: IR, ionizing radiation; LDL, low density lipoprotein; PBS, phosphate-buffered saline; SOD, superoxide dismutase; TC, tyramine cellobiose; TCA, trichloroacetic acid.

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prior to the development of focal lesions (3–5). Susceptible sites often show a greater permeability to plasma LDL than resistant sites (4), but evidence suggests that the enhanced LDL accumulation is due largely to reduced arterial LDL efflux (5). In the early stages of lesion development initiated by a high fat atherogenic diet, most of the accumulated LDL remains intact and extracellular. Over time, the relative extent of arterial LDL degradation increases, presumably as macrophages become an active part of the process (6). Consistent with this scenario, Carew, Schwenke, and Steinberg (7) observed that trapped LDL degradation products are present primarily within macrophages in lesioned but not nonlesioned areas of the artery wall.

Current models of atherogenesis propose a key role for LDL modifications, particularly oxidative modifications, in promoting LDL-macrophage interactions (8, 9). This is attributed to oxidation-associated changes in apolipoprotein B epitope exposure that enhance recognition and uptake by macrophage scavenger receptors (10, 11). The extent of LDL degradation in the artery wall is thus proposed to be determined not only by LDL availability and the presence of competent macrophages, but also by the balance of conditions that determine the potential for and extent of LDL oxidation (or analogous modifications that enhance LDL-macrophage interactions). Evidence that the lipophilic antioxidant probucol inhibits the uptake and degradation of LDL by arterial macrophages supports the importance of this process in influencing LDL-macrophage interactions in vivo (6).

In the present studies, we examined the arterial accumulation and degradation of LDL in mice after thoracic-targeted exposures to ionizing radiation (IR). Our interest in this model was based on observations that IR markedly accelerates fatty streak formation in fat-fed C57BL/6 mice without altering circulating lipid levels (12). Importantly, the atherogenic effects of IR are inhibited by overexpression of the antioxidant enzyme CuZn-superoxide dismutase (SOD) (12). We thus proposed that IR promotes changes in the artery wall that lead to increased lipoprotein lipid deposition and, moreover, that these events are linked, either directly or indirectly, to the SOD inhibitory effects. Using the trapped ligand methodology, we show that LDL is degraded more readily in the irradiated aorta and that the enhanced degradation is affected by antioxidants.

MATERIALS AND METHODS

Materials

Tyramine cellobiose was synthesized and purified according to published procedures (13). The structure was confirmed by ^{1}H nuclear magnetic resonance spectroscopy; the spectrum was taken at 400 MHz in deuterated methanol. Iodobeads were obtained from Pierce (Rockford, IL). All other reagents were obtained from commercial sources and were of the highest grade available.

Mice

Studies were performed with atherosclerosis-susceptible C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME). To investigate the effects of CuZn-SOD overexpression, we used the transgenic strain C57BL/6-TgN(SOD1)10cje. These mice contain a 14-kilobase segment encompassing the entire human CuZn-SOD gene (in eight tandem copies) and all necessary regulatory sequences (14). Nontransgenic littermates were used as experimental controls. The transgenic mice were identified by polyacrylamide gel electrophoresis separation of red blood cell extracts, with staining for SOD activity as previously described (14).

Radiation exposures and dietary conditions

Anesthetized mice between the ages of 10 and 14 weeks were exposed to a single dose (2, 4, or 8 Gy) of IR, using a ^{60}Co source. Exposures were confined to the thorax by placing lead shielding on the exterior of the animal housing compartment. Immediately after irradiation, animals were switched from standard chow to a high fat atherogenic diet comprising 1.5% cholesterol, 15% saturated fat, and 0.5% sodium cholate (15). Control mice were placed on an identical dietary regimen.

Lipoprotein isolation and radioiodination

Donor LDL was prepared from human plasma by sequential ultracentrifugation, using standard procedures (16). For preparation of ^{125}I-LDL, the d = 1.019–1.063 g/ml fraction was dialyzed overnight at 4°C against 0.1 M sodium phosphate, pH 6.5, and then was incubated for 12 min at room temperature with ^{125}I and Iodobeads (the oxidizing agents) according to the manufacturer instructions. Iodinated TC was prepared identically except that the incubation with Iodobeads was carried out for 15 min. For preparation of ^{125}I-TC-LDL, the d = 1.019–1.063 g/ml plasma fraction was dialyzed at 4°C overnight against 0.1 M sodium phosphate, pH 9.5, and then was covalently modified with ^{125}I-TC according to the method of Pittman et al. (17). To remove unincorporated label, the radiolabeled LDL preparations were dialyzed against 0.1 M ammonium bicarbonate overnight, followed by phosphate-buffered saline (PBS), pH 7.4, overnight and then 0.15 M saline, pH 7.4. Dialyzed samples were analyzed for protein content by a modification of the method of Lowry (18). Radiolabel incorporation into the protein and lipid components was determined after 10% trichloroacetic acid (TCA) precipitation and chloroform–methanol 2:1 (v/v) extraction. Virtually all counts (>95%) were recovered within the TCA-precipitable (protein) fraction. Specific activities of ^{125}I and ^{125}I-TC ranged from 167 to 1,150 cpm/ng LDL protein.

Lipoprotein α-tocopherol enrichment and oxidative susceptibility measurements

In some experiments, aliquots of LDL were enriched with α-tocopherol prior to radioiodination. This was accomplished by a modification of the method of Esterbauer et al. (18) that involved incubating the d >1.019 g/ml plasma fraction with α-tocopherol [300 nM in dimethyl sulfoxide (DMSO)] followed by centrifugation at d = 1.063 g/ml to isolate the LDL fraction. The isolated LDL samples were extensively dialyzed against 0.15 M PBS, pH 7.4, to remove any unincorporated α-tocopherol. Control LDL samples were exposed to similar conditions but without α-tocopherol. Concentrations of α-tocopherol were measured in lipid extracts of enriched and nonenriched samples by high-performance liquid chromatography with UV detection as previously described (19); quantification was accomplished by comparison with standards of known amount.

LDL oxidative susceptibility was evaluated by monitoring conjugated diene formation, as an index of lipid peroxidation, after

addition of 5 μM Cu^{2+}. This was accomplished by monitoring the change in absorbance at 234 nm in a Shimadzu (Kyoto, Japan) model UV2101 spectrophotometer equipped with a temperature-controlled, six-position automatic sample changer. Initial absorbance was set at zero and measurements were taken at 2-min intervals for up to 8 h at 37°C. The relative oxidative susceptibility of LDL was assessed on the basis of the lag time, which was defined as the time interval between initiation and the intercept of the slope of the absorbance curve.

Lipoprotein injections and blood collections

Donor LDL preparations (10 μg of LDL protein) were injected into recipient mice within 5 days of preparation. All samples were mixed with an equal volume of mouse plasma and were administered to anesthetized animals via the tail vein (50 μl per injection). Plasma concentrations of donor LDL were monitored by collecting serial retroorbital blood samples (at times ranging from 2 min to 24 h) and determining TCA-precipitable (LDL-associated) counts.

Isolation of aortic tissue and determination of aortic lipoprotein levels

At selected intervals, animals were anesthetized with metaphane and were exsanguinated by perfusion with 15 ml of 0.15 M PBS, pH 7.4., with 1 mM ethylenediaminetetraacetic acid followed by 5 ml of 10% buffered formalin. Flow entered the left ventricle and exited the vena cava, which was severed below the renal artery. The thoracic aorta was gently stripped of excess adventitia and removed as described elsewhere (20). Aortic samples were placed in buffered formaldehyde for 4 h to allow non-protein-bound radioactivity to diffuse away. Prior to measurement, samples were transferred to fresh fixative in gamma counting tubes. Total ^{125}I radioactivity was determined by counting in a Packard (Downers Grove, IL) 800 auto gamma counter with autocorrection for background counts. Plasma samples were counted for 1–10 min (with a statistical counting error of 2%) and aortic samples were counted for 10 min (with a statistical counting error of 3–4%).

Calculations and statistical analyses

^{125}I-LDL was used to measure aortic levels of intact donor LDL whereas LDL covalently coupled with ^{125}I-TC was used to measure both intact LDL and LDL degradation products and thus provided an estimate of the total amount of donor LDL that had accumulated in the aorta (2–7). Estimates of donor LDL degradation were obtained by subtracting values obtained for ^{125}I-LDL from those obtained for ^{125}I-TC. Areas under the plasma concentration curves were determined by integrating a monoexponential equation fit to data of the time-dependent decline in the concentration of radiolabeled LDL. In contrast to previous studies using the trapped ligand methodology, aortic values at later times were not normalized according to time-averaged plasma concentrations of donor LDL because there were no significant differences in concentrations among the groups being compared (as shown in Results). We also chose not to express LDL accumulation and degradation in terms of absolute plasma LDL equivalents because mice, unlike hyperlipidemic rabbits and pigeons used in previous studies, have low plasma LDL concentrations even when placed on high fat diets.

A total of seven LDL preparations were used for these experiments. For each LDL preparation, two to five mice were used per experimental condition. Results for individual mice were averaged to obtain a single value per condition for each LDL preparation. These values were then averaged to obtain the group means presented in this report. Differences between irradiated and control mice and between ^{125}I-LDL and ^{125}I-TC-LDL were evaluated using analysis of variance with $P < 0.05$ considered as significant in two-tailed tests.

RESULTS

Time-dependent changes in plasma and aortic concentrations of injected ^{125}I-labeled LDL and ^{125}I-labeled C-LDL

Experiments were performed 5 days after irradiation and initiation of the high fat diet. This time was selected on the basis of previous studies suggesting that IR-induced changes in the artery wall are particularly enhanced during the first weeks postexposure (12). Because the atherosclerosis-promoting effects of IR are strictly dependent on the high fat diets, all experiments were performed in fat-fed mice.

As shown in **Fig. 1**, more than 90% of the donor LDL preparations were cleared from the plasma within 24 h (1440 min). There were no significant differences in plasma clearance or areas under the plasma concentration curves between ^{125}I-LDL and ^{125}I-TC-LDL or between irradiated and control mice, indicating that the donor LDL pools available for aortic sequestration were similar for the four groups. These results were not unexpected because identical quantities were injected and previous studies showed that upper thoracic irradiation did not affect plasma lipid and lipoprotein (TG-rich and HDL) concentrations (12).

Aortic concentrations of ^{125}I-TC were determined at selected times from 30 min up to 24 h. As previously reported for other animal models, aortic values increased rapidly within the first hour and thereafter exhibited a slow increase up to 24 h (data not shown). At this time, ~0.03% of the injected LDL could be accounted for in the aorta. In some experiments, we also monitored ^{125}I-TC accumulation in the liver of recipient mice. Up to 35% of injected

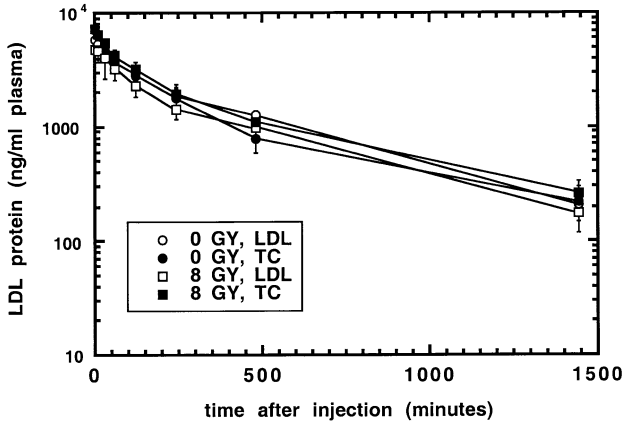

Fig. 1. Plasma decay curves for ^{125}I-LDL and ^{125}I-TC-LDL in control and 8 Gy-irradiated mice. Plasma decay curves for ^{125}I-LDL and ^{125}I-TC-LDL were determined by monitoring TCA-precipitable (protein-bound) radioactivity in serial retroorbital blood samples. Plasma values are expressed as nanomoles of LDL protein per milliliter of plasma and represent means (±SEM) from 7 LDL preparations with values from a total of 18–24 mice contributing to each curve.

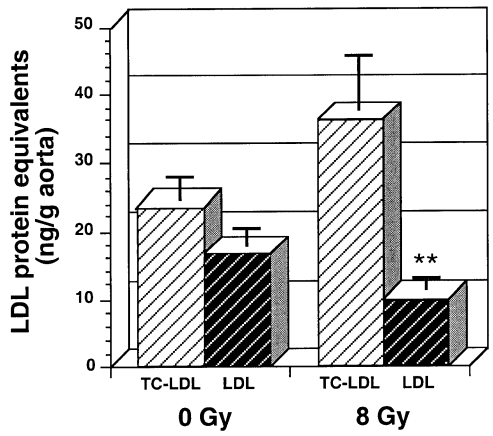

Fig. 2. ^{125}I-LDL and ^{125}I-TC-LDL concentrations in the thoracic aorta at 24 h. Aortic values are expressed as nanograms of LDL protein equivalents per gram of aortic tissue (wet weight) and represent the means (±SEM) from 7 LDL preparations and a total of 18–24 mice per condition. Values for ^{125}I-TC are represented by light columns and values for ^{125}I-LDL are represented by dark columns. Significant differences (control vs. irradiated mice) are marked with asterisks.

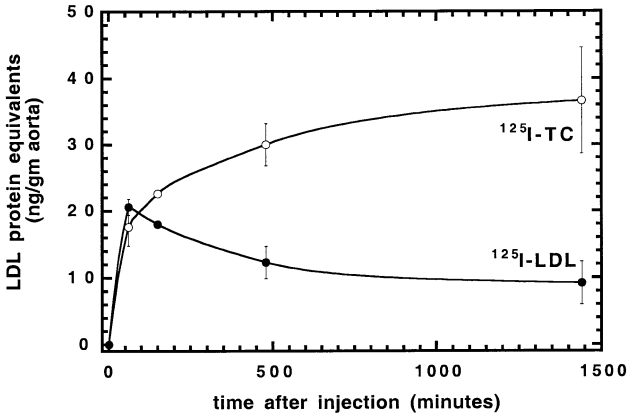

Fig. 4. Time-dependent changes in ^{125}I-LDL and ^{125}I-TC-LDL concentrations in the irradiated aorta. Values are expressed as nanograms of LDL protein equivalents per gram of aortic tissue (wet weight) and were obtained with three LDL preparations and nine or more mice (with the exception of the 2-h value, which was determined with one LDL preparation and three mice).

LDL was recovered at 24 h. When expressed on an identical weight basis (per gram of tissue), 24-h values were >10-fold higher in the liver than in the aortic segment under study.

Previous studies in other animal models have shown that the extent of aortic LDL accumulation at early times (<1 h) is determined primarily by LDL entry, whereas values at later times also reflect loss resulting from a return of LDL to circulation or LDL degradation (4). We did not observe any effect of IR on LDL accumulation at 1 h (data not shown). However, at 24 h, ^{125}I-TC-associated counts were 1.8-fold higher in irradiated mice ($P = 0.10$), suggesting that more LDL or LDL degradation products had accumulated in the irradiated aorta (see **Fig. 2**). Values for ^{125}I-LDL were 30% lower in irradiated than control mice ($P < 0.05$), indicating that more LDL had been degraded (79 vs. 30% in control mice; see **Fig. 3**).

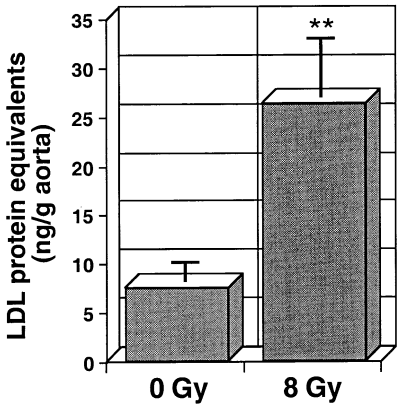

Fig. 3. Estimates of aortic LDL degradation at 24 h in control and irradiated mice. Values were derived from the data represented in Fig. 2 as follows: ^{125}I-TC (nanograms of LDL protein equivalent per gram of aorta) – ^{125}I-LDL (nanograms of LDL per gram of aorta). The asterisks indicate values that are significantly greater for irradiated (8 Gy) than control mice at $P < 0.05$.

The course of LDL degradation in the irradiated aorta is illustrated in **Fig. 4**, which compares aortic counts associated with ^{125}I-LDL versus those associated with ^{125}I-TC at selected times after injection (from 1 to 24 h). Values for the two forms of LDL were almost identical at 1 h, but began to diverge soon thereafter, ultimately leading to the large difference at 24 h in irradiated mice.

Extent of LDL degradation in irradiated aorta from SOD-TG mice

In previous studies, we observed that IR-induced atherosclerosis is reduced in mice overexpressing CuZn-SOD, suggesting the importance of the superoxide anion (O_2^-) and/or its reactive by-products. To test whether CuZn-SOD overexpression impacts LDL-vascular interactions, we examined aortic accumulation of LDL-associated counts and degradation of LDL in SOD-transgenic mice. As above, experiments were carried out 5 days after the IR exposure. **Figure 5** shows that LDL degradation (estimated from independent observations involving ^{125}I-LDL and ^{125}I-TC-LDL) was almost 2-fold lower in SOD-transgenic than wild-type mice at 24 h. These results suggest that the atherosclerosis inhibitory effects of SOD may operate, at least in part, through effects on aortic LDL degradation.

Inhibition of aortic LDL degradation by LDL α-tocopherol enrichment

To test whether LDL oxidation might be involved in promoting LDL degradation in the irradiated aorta, we performed experiments using LDL enriched in vitro with α-tocopherol. By the procedures described in Materials and Methods, we were able to increase the LDL α-tocopherol content 4.6 ± 1.5-fold (n = 3 LDL preparations). Enrichment of LDL with α-tocopherol led to a 3- to 4-fold decrease in ex vivo LDL oxidative susceptibility (based on conjugated diene lag times; data not included), suggesting that the

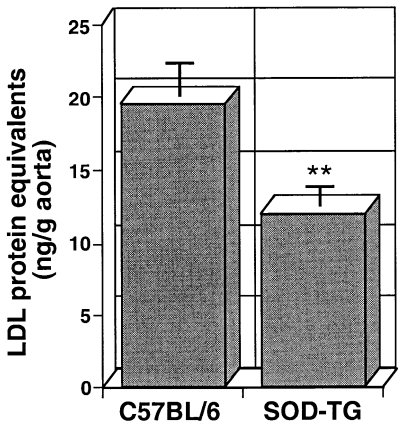

Fig. 5. Estimates of aortic LDL degradation in irradiated C57BL/6 and SOD-TG mice. Values were derived from independent observations of ^{125}I-TC-LDL and ^{125}I-LDL as follows: ^{125}I-TC (nanograms of LDL protein equivalent per gram of aorta) – ^{125}I-LDL (nanograms of LDL per gram of aorta). Each of the four conditions used to calculate these values was evaluated with three or more LDL preparations and nine or more mice. The asterisks indicate that values are significantly lower for SOD-TG than wild-type mice at $P < 0.05$.

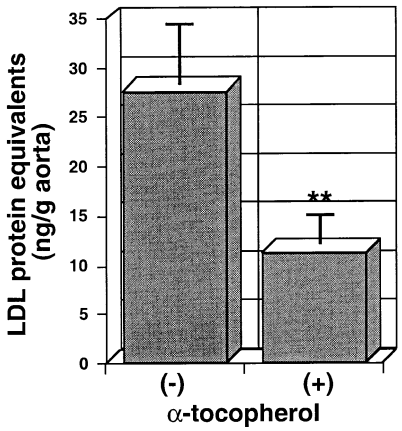

Fig. 7. Effect of α-tocopherol enrichment on LDL degradation in the irradiated aorta. Values were derived from the data represented in Fig. 6 as follows: ^{125}I-TC (nanograms of LDL protein equivalent per gram of aorta) – ^{125}I-LDL (nanograms of LDL per gram of aorta). The asterisks indicate that the values for LDL pre-enriched with α-tocopherol were significantly greater than those for LDL without α-tocopherol at $P < 0.05$.

α-tocopherol had been incorporated in a manner that allowed antioxidant protection of the LDL particle.

Enrichment of LDL with α-tocopherol did not affect plasma clearance rates in either the irradiated or nonirradiated condition (data not shown). In contrast, as illustrated in **Figs. 6** and **7**, this treatment did alter the extent of LDL degradation in the irradiated aorta. When enriched LDL samples were used, values for ^{125}I-TC were ~22% lower (albeit not significantly) and values for ^{125}I-LDL were almost 2-fold higher in the irradiated condition ($P < 0.05$ vs. nonenriched LDL). Thus, less of the retained LDL had been degraded. These results suggest that oxidation may contribute to the enhanced degradation of LDL in the irradiated aorta. No effect of α-tocopherol enrichment was observed in the nonirradiated condition (data not included), likely because of the low extent of LDL degradation.

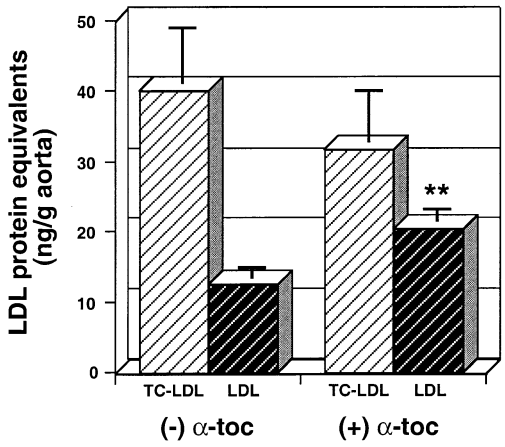

Fig. 6. Effect of α-tocopherol enrichment on concentrations of ^{125}I-TC and ^{125}I-LDL in the irradiated aorta. Values are expressed as nanograms of LDL protein equivalents per gram of aortic tissue (wet weight) and were obtained with 3 LDL preparations and 6–12 mice per condition.

DISCUSSION

We previously reported that IR accelerates fatty streak formation in fat-fed mice via SOD-inhibitable processes, leading us to propose a key role for oxidative- or redox-regulated events (12). In the present studies, we tested the hypothesis that IR promotes changes in the artery wall that lead to increased LDL lipid deposition through oxidative mechanisms. Using ^{125}I-LDL and ^{125}I-TC-LDL to monitor both intact LDL and LDL degradation products, we demonstrated a greater accumulation of LDL constituents and a greater extent of LDL degradation in aorta from irradiated mice. Consistent with a role for LDL oxidation in stimulating LDL degradation, this effect was inhibited by antioxidants.

The extent of accumulation of lipoproteins and lipoprotein constituents in the artery wall is determined by a complex set of factors that influence LDL entry and loss including that due to degradation (4). Our results suggest that LDL degradation was of major importance in the IR model because a large fraction of the retained ^{125}I-TC was in the form of LDL degradation products at 24 h. This finding distinguishes IR from other experimental conditions used to stimulate LDL-vascular interactions, including short-term cholesterol feeding and balloon injury. With short-term cholesterol feeding (16 d), arterial LDL accumulation is increased but this primarily involves increased retention of intact LDL (4). Fractional rates of degradation are in fact lower under these conditions possibly due to saturation and down-regulation of LDL receptors in arterial cells. Likewise, after vascular injury a persis-

tent increase in levels of intact LDL is seen in the neointima (21, 22).

With the exception of arterial localization and age of onset, IR-associated atherosclerosis is similar to idiopathic atherosclerosis as evidenced by accumulations of macrophage-derived foam cells, intimal thickening, fibrosis, elastic degeneration, and calcification (23, 24). It is presently unclear why the early effects of IR on LDL accumulation/degradation differ from those of other models of accelerated atherosclerosis. Presumably, there are a number of ways to initiate and/or accelerate the disease. All of these should involve enhanced lipoprotein lipid accumulation, but the specific factors driving that accumulation could differ, at least in the early stages.

To test whether LDL oxidation might be involved in stimulating aortic LDL degradation in irradiated mice, we examined the effects of preenriching LDL with the antioxidant α-tocopherol. As hypothesized, α-tocopherol-enriched LDL particles were degraded less readily in the irradiated aorta. Because α-tocopherol was delivered within the LDL particle, it is assumed that the protection was specific for LDL and involved an inhibition of oxidative changes that alter LDL receptor recognition (8–11). However, α-tocopherol also has been shown to directly affect properties of the artery wall and these effects cannot be discounted.

Whether antioxidant protection was most relevant during the in vitro processing of LDL or in vivo cannot be established at this time. Radioiodination is known to promote oxidative changes in LDL (25). However, because oxidized LDL is removed more readily from the plasma than nonoxidized LDL (26), plasma decay curves presumably would have been different for enriched and nonenriched samples if the relevant oxidative changes/antioxidant protection had occurred in vitro. This was not observed. We cannot rule out the in vitro occurrence of mild oxidative changes in LDL that do not affect plasma clearance. However, such changes do not stimulate cellular uptake and would therefore be insufficient to explain the increased arterial LDL degradation. Considering all of these issues, the observation that IR-induced LDL degradation is reduced by preenrichment of LDL with α-tocopherol is interpreted as an indication that IR elicits changes in artery wall that promote α-tocopherol-inhibitable LDL oxidation. Consistent with this hypothesis are previous studies in irradiated rats showing that local protein oxidation is markedly enhanced for days following irradiation (27).

We further hypothesize that the enhanced oxidation and degradation of LDL in the IR model reflects the ability of IR to stimulate inflammation. Leukocyte infiltration is a recognized early response of cells/tissues to IR, and is linked to the induction of leukocyte adhesion molecules. Hallahan and colleagues (28, 29) have demonstrated a rapid and persistent induction of E-selectin and intercellular adhesion molecule-1 after irradiation in both isolated cells and in vivo. A pronounced upregulation of platelet endothelial cell adhesion molecule 1 also has been demonstrated in isolated endothelial cells (30). These effects were observed after exposure to doses similar to those used in the present studies and previously shown to promote atherosclerosis in C57BL/6 mice (12). Inflammatory cells would be expected to participate directly in LDL degradation as well as to promote localized LDL oxidation, which would increase the potential for cellular recognition and uptake of these particles via the scavenger receptor pathway. In support of this possibility, we have observed that IR-induced LDL degradation is reduced in ICAM-deficient mice (data not included). Nonetheless, the identify of the cells participating in LDL degradation remains to be determined by methods directly addressing this issue.

The inhibitory effects of SOD overexpression on aortic LDL degradation could reflect increased neutralization of LDL-oxidizing species such as those generated by activated inflammatory cells. However, SOD also is expected to intercept oxidizing species generated directly in response to IR and thus could limit the initial impact of such species on the artery wall. Whether the effects of SOD involve direct inhibition of lipoprotein oxidation or inhibition of other events stimulated by the initial IR exposure cannot be determined at this time. Regardless of the mechanism of SOD inhibition, the inhibitory effects on LDL degradation are proposed to contribute to the inhibitory effects of SOD on IR-induced atherosclerosis (12).

In summary, we adapted the trapped ligand methodology to investigate whether the atherogenic effects of IR in C57BL/6 mice involve increased lipoprotein retention and degradation in the artery wall. Our results show that LDL degradation products accumulate in the irradiated aorta. This effect is inhibited by SOD overexpression and preenrichment of LDL with α-tocopherol, both of which have been shown by others to reduce the potential for LDL oxidation. On the basis of these results, we propose that IR is useful for stimulating an arterial environment that promotes LDL oxidation and degradation and for identifying aortic and lipoprotein-related factors, such as antioxidants that influence these processes.

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REFERENCES

1. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
2. Carew, T. E., R. C. Pittman, E. R. Marchand, and D. Steinberg. 1984. Measurement in vivo of irreversible degradation of low density lipoprotein in the rabbit aorta. Predominance of intimal degradation. *Arteriosclerosis.* **4:** 214–224.
3. Schwenke, D. C., and T. E. Carew. 1989. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. *Arteriosclerosis.* **9:** 895–907.
4. Schwenke, D. C., and T. E. Carew. 1989. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of

LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis.* **9:** 908–918.
5. Schwenke, D. C., and R. W. St Clair. Influx, efflux, and accumulation of LDL in normal arterial areas and atherosclerotic lesions of White Carneau pigeons with naturally occurring and cholesterol-aggravated atherosclerosis. *Arterioscler. Thromb.* **93:** 1368–1381.
6. Schwenke, D. C., and T. E. Carew. 1988. Quantification in vivo of increased LDL content and rate of degradation in normal rabbit aorta occurring at sites susceptible to early atherosclerotic lesions. *Circ. Res.* **62:** 699–710.
7. Carew, T. E., D. C. Schwenke, and D. Steinberg. 1987. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc. Natl. Acad. Sci. USA.* **84:** 7725–7729.
8. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum. 1989. Beyond cholesterol. Modifications of low-density lipoprotein cholesterol that increase its atherogenicity. *N. Engl. J. Med.* **320:** 915–924.
9. Steinberg, D. 1997. Low density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.* **272:** 20963–20966.
10. Steinbrecher, U. P. 1987. Oxidation of human low density lipoprotein results in derivitization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. *J. Biol. Chem.* **262:** 3603–3608.
11. Steinbrecher, U. P., J. L. Witztum, S. Parthasarathy, and D. Steinberg. 1987. Decrease in active amino groups during oxidation or endothelial cell modification of LDL: correlation with changes in receptor-mediated catabolism. *Arteriosclerosis.* **1:** 135–143.
12. Tribble, D. L., M. H. Barcellos-Hoff, B. M. Chu, and E. L. Gong. 1999. Ionizing radiation accelerates aortic lesion formation in fat-fed mice via SOD-inhibitable processes. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1387–1392.
13. Pittman, R. C., and C. A. Taylor. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–628.
14. Epstein, C. J., K. B. Avraham, M. Lovett, S. Smith, O. Elroy-Stein, G. Rotman, C. Bry, and Y. Groner. 1987. Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome. *Proc. Natl. Acad. Sci. USA.* **84:** 8044–8048.
15. Paigen, B., A. Morrow, P. A. Holmes, D. Mitchell, and R. A. Williams. 1987. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis.* **68:** 231–240.
16. Lingren, F. T., L. C. Jensen, R. D. Wills, and N. K. Freeman. 1969. Flotation rates, molecular weights, and hydrated densities of the low density lipoproteins. *Lipids.* **4:** 337–345.
17. Pittman, R. C., T. E. Carew, C. K. Glass, S. R. Green, C. A. Taylor, and A. D. Attie. 1983. A radioiodinated, intracellularly trapped ligand for determining the sites of plasma protein degradation in vivo. *Biochem. J.* **212:** 791–800.
18. Esterbauer, H., M. Dieber-Rothenender, G. Striegl, and G. Waeg. 1991. Role of vitamin E in preventing the oxidation of low-density lipoprotein. *Am. J. Clin. Nutr.* **53:** 314S–321S.
19. Tribble, D. L., P. M. Thiel, J. J. van den Berg, and R. M. Krauss. 1995. Differing alpha-tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL. *Arterioscler. Thromb. Vasc. Biol.* **15:** 2025–2031.
20. Tangirala, R. K., E. M. Rubin, and W. Palinski. 1995. Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. *J. Lipid Res.* **36:** 2320–2328.
21. Alavi, M., and S. Moore. 1984. Kinetics of low density lipoprotein interactions with rabbit aortic wall following balloon catheter deendothelialization. *Arteriosclerosis.* **4:** 395–402.
22. Chang, M. Y., A. M. Lees, and R. S. Lees. 1992. Time course of ^{125}I-labeled LDL accumulation in the healing, balloon-deendothelialized rabbit aorta. *Arterioscler. Thromb.* **12:** 1088–1098.
23. Rotman, M., B. Seidenberg, I. Rubin, C. Botstein, and M. Bosniak 1969. Aortic arch syndrome secondary to radiation in childhood. *Arch. Intern. Med.* **124:** 87–90.
24. Glick, B. 1972. Bilateral carotid occlusive disease. *Arch. Pathol. Lab. Med.* **93:** 352–355.
25. Khouw, A. S., S. Parthasarathy, and J. L. Witztum. 1993. Radioiodination of low density lipoprotein initiates lipid peroxidation: protection by use of antioxidants. *J. Lipid Res.* **34:** 1483–1496.
26. Juul, K., L. B. Nielsen, K. Munkholm, S. Stender, and B. G. Nordestgaard. 1996. Oxidation of plasma low-density lipoprotein accelerates its accumulation and degradation in the arterial wall in vivo. *Circulation.* **94:** 1698–1704.
27. Fliss, H. M. 1994. Rapid neutrophil accumulation and protein oxidation in irradiated rat lungs. *J. Appl. Physiol.* **77:** 2727–2733.
28. Hallahan, D., E. T. Clark, J. Kuchibhotla, B. L. Gewertz, and T. Collins. 1995. E-selectin gene induction by ionizing radiation is independent of cytokine induction. *Biochem. Biophys. Res. Commun.* **217:** 784–795.
29. Hallahan, D. E., and S. Virudachalam. 1997. Ionizing radiation mediates expression of cell adhesion molecules in distinct histological patterns within the lung. *Cancer Res.* **57:** 2096–2099.
30. Quarmby, S., P. Kumar, J. Wang, J. A. Macro, J. J. Hutchinson, R. D. Hunter, and S. Kumar. 1999. Irradiation induces upregulation of CD31 in human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **19:** 588–597.