In vivo fate and scavenger receptor recognition of oxidized lipoprotein[a] isoforms in rats

Yolanda B. de Rijke,[1,*] Günther Jürgens,[†] Esther M. A. J. Hessels,[*] Anita Hermann,[†] and Theo J. C. van Berkel[*]

Division of Biopharmaceutics, Center for Bio-Pharmaceutical Sciences,[*] Sylvius Laboratory, University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands, and Institute of Medical Biochemistry,[†] Karl-Franzens University Graz, Harrachgasse 21/III, A-8010 Graz, Austria

Abstract High levels of Lp[a] in blood form an independent risk factor for atherosclerosis. Oxidative modification of Lp[a] may be involved in the suggested atherogenic action of Lp[a]. After Cu^{2+}-mediated oxidative modification of the 440 kDa and 610 kDa apo[a] isoforms of lipoprotein[a] (Ox-Lp[a]), the in vivo fate was investigated in rats. Ox-Lp[a], when injected into rats, was rapidly removed from the blood circulation by the liver, in which the intrahepatic fate is dependent on the degree of oxidation of the isoforms. Upon oxidation to a slightly increased negative charge of Lp[a], the high molecular weight form of Lp[a] is recognized more efficiently by the Kupffer cells than by the endothelial cells. When the liver uptake of Ox-Lp[a] is blocked by preinjection of polyinosinic acid (poly I), the association of Ox-Lp[a] with the rat heart is increased 20-fold. In vitro studies show that the association and degradation of ^{125}I-labeled Ox-Lp[a] with liver endothelial and Kupffer cells was inhibited by oxidized LDL (Ox-LDL), poly I, or Ox-Lp[a] itself by 60–90%, while only a partial competition was found with acetylated-LDL (up to 25%). In conclusion, after oxidative modification of Lp[a], there is recognition of Ox-Lp[a] by specific oxidized-lipoprotein receptors on liver endothelial and Kupffer cells; the relative importance at low degrees of oxidation of Lp[a] is dependent on the molecular weight of the apo[a] isoforms. Under conditions in which liver uptake is not adequate, the deposition of Ox-Lp[a] in the heart may be of potential pathological importance.—**de Rijke, Y. B., G. Jürgens, E. M. A. J. Hessels, A. Hermann, and T. J. C. van Berkel.** In vivo fate and scavenger receptor recognition of oxidized lipoprotein[a] isoforms in rats. *J. Lipid Res.* 1992. **33:** 1315–1325.

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High lipoprotein[a] (Lp[a]) levels form an independent risk factor for atherosclerosis (1–3). Lp[a] resembles low density lipoprotein (LDL) in lipid composition (4) and also contains apolipoprotein B-100. However, Lp[a] can be distinguished from LDL by the presence of apo[a], a glycoprotein that shows remarkable size polymorphism. Due to the number and types of repetitive kringle IV units, the molecular mass of apo[a] ranges from 400 kDa to 838 kDa (5–8). It was reported that the higher molecular weight forms are specifically correlated with a greater risk for coronary heart disease (9). It was suggested that high Lp[a] levels (above 30 mg/ml) are a better predictor for coronary atherosclerosis than LDL levels (10). Additionally, Lp[a] serum levels correlated best among all lipid parameters with number and size of atherosclerotic plaques in patients with cervical atherosclerosis (3). The reported structural relationship of apo[a] to plasminogen (5, 11) led to a renewed interest in the mechanism by which Lp[a] levels can lead to atherosclerosis.

The major site of synthesis of plasma apo[a] appears to be the liver (12). The removal pathway for Lp[a], however, is under discussion. In vitro studies have revealed that Lp[a] interacts with the LDL receptor (13), although for an optimal affinity the thiol groups that link apo[a] with apoB-100 have to be reduced (14). In vivo studies (15) revealed that upon estrogen treatment of rats, which leads to a high increase of LDL-receptors on liver parenchymal cells, the blood decay and liver-association of Lp[a] are increased to a much lower extent than that of LDL. From this it was concluded that Lp[a] does not interact efficiently with the LDL receptor in vivo (16). These data are in accord with the ineffectiveness of HMG-CoA reductase inhibitors to reduce Lp[a] plasma level during treatment of familial hypercholesterolemic heterozygotes (10, 16). Recently it was reported for recombinant apo[a] that the interaction with the scavenger receptor might explain the relation between Lp[a] and atherosclerosis (17).

Abbreviations: Lp[a], lipoprotein[a]; Ox-Lp[a], oxidized Lp[a]; LDL, low density lipoproteins; apo[a], apolipoprotein[a]; BSA, bovine serum albumin; PBS, phosphate-buffered saline; EDTA, ethylene diaminetetraacetic acid; PAGE, polyacrylamide gel electrophoresis; REM, relative electrophoretic mobility; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; poly I, polyinosinic acid; TC, tyramine cellobiose.

[1]To whom correspondence should be addressed.

However, in vivo studies of Hofmann et al. (18) did lead to the conclusion that native Lp[a] does not show any affinity for the scavenger receptor.

It is generally accepted now that the atherogeneity of LDL may be related to oxidative modification (19). Besides LDL (20), Lp[a] was found to be highly susceptible to Cu^{2+}-mediated oxidation (21, 22). Previous studies indicate that oxidation of lipoproteins (23–26) leads to an interaction with scavenger receptors, present on liver endothelial cells, liver macrophages, and mouse peritoneal macrophages. The present study takes into consideration the possible change in fate of Lp[a] upon oxidation. The in vivo and in vitro data of both 440 kDa and 610 kDa forms of Lp[a], exposed to different oxidative conditions, were compared in order to determine whether the difference in atherogenic character might be explained by a variation of properties of oxidized Lp[a] isoforms.

MATERIALS AND METHODS

Chemicals

Type I collagenase and BSA, Fraction V were from Sigma Company, St. Louis, MO; B-grade pronase was obtained from Calbiochem-Behring Corporation, La Jolla, CA; Nycodenz was from Nyegaard and Company A/S, Oslo, Norway; Dulbecco's Modified Eagle's Medium was from Gibco-Europe, Hoofddorp, The Netherlands; and ^{125}I in NaOH was from Amersham, Buckinghamshire, England.

LDL isolation and modification

LDL (d 1.019–1.063 g/ml) was isolated by two repetitive centrifugations from plasma (in 1 mM EDTA) of normolipemic fasting donors as reported by Redgrave et al. (27). Acetylation of LDL was done according to Basu et al. (28). Before oxidation, LDL was dialyzed for 2 days against four changes of 500 ml phosphate-buffered saline (PBS) pH 7.4, containing 10 μM EDTA. Oxidation of LDL (1 mg/ml) was performed by incubation with 10 μM $CuSO_4$ in PBS for 20 h at 37°C (29). The degree of acetylation and oxidation of LDL was tested by the electrophoretic mobility on 0.75% agarose gels. As reported earlier (26), the relative (to native LDL) electrophoretic mobility (REM) of Ac-LDL was 2.50 ± 0.05 (R_f value 0.52 ± 0.01, n = 6 ± SE) and for Ox-LDL was 2.58 ± 0.03 (R_f value 0.54 ± 0.01, n = 6 ± SE, native LDL R_f value 0.21 ± 0.01 (23)).

Lp[a] isolation, oxidation, and characterization

Two different Lp[a] isoforms were isolated from the plasma of two male donors at density 1.050–1.110 g/ml by repetitive ultracentrifugation. Separation from contaminating LDL was achieved by gel filtration on Biogel A-15 as described previously (30). Purification of Lp[a] was done according to Gaubatz et al. (4) in the presence of 100 units kallikrein inactivator/ml, 10 μM phenylmethanesulfonyl-fluoride, and 50 mg/l chloramphenicol. Lp[a] preparations did not show any cryoscopic behavior and aggregation, regardless which isoform of Lp[a] we used. The purity of the intact Lp[a] was checked by polyacrylamide gel electrophoresis (PAGE) on 3.75% nondenaturing gels. The Lp[a] preparations were free of albumin and other lipoproteins. The apolipoproteins present in Lp[a] were apoB-100 and apo[a] as determined on PAGE in the presence of 2.5% β-mercaptoethanol. According to the nomenclature of Utermann et al. (7), the 440 kDa and 610 kDa apo[a] isoforms were identified by Western blot. Both native and oxidized Lp[a] were radioiodinated according to the procedure of McFarlane (31) as modified for lipoproteins by Bilheimer, Eisenberg, and Levy (32). Unbound iodine was removed by dialysis against 0.024 M NaBr, 0.01 M Tris-HCl, 1 mM EDTA, pH 8.0, and two times against PBS, 1 mM EDTA, pH 7.4. More than 95% of the radioactivity in the labeled Lp[a] preparations was precipitated by 35% TCA. The specific activity of the iodinated Lp[a] preparations was 66–307 cpm/ng of protein (recovery yield of the protein: 86 ± 14%). In native Lp[a], both apoB-100 and the apo[a] protein were radioiodinated to approximately a similar extent (range 20–140 cpm/ng protein).

Oxidation of both the low molecular mass (440 kDa) Lp[a] and the high molecular mass (610 kDa) Lp[a] was performed by exposure of 1 mg Lp[a]/ml to 10 μM or 50 μM Cu^{2+} at 37°C for 24 h in a closed flask containing at least 20 times more air than liquid (v/v). The oxidation was terminated by the addition of EDTA (final concentration 1 mM) and lipoproteins were subsequently dialyzed against 0.01 M sodium phosphate buffer (pH 7.4) containing 50 mg/l chloramphenicol and 1 mM EDTA at 4°C. The recovery of Lp[a] protein after oxidation was >90%, regardless of the molecular weight of the isoform.

During the oxidation of Lp[a], conjugated dienes were continuously recorded by the measurement of the increase of the 234 nm absorption (33). In addition, lipid peroxides were measured with a colorimetric assay at 365 nm (34). The generation of conjugated dienes and lipid peroxides of 1 mg low molecular mass (440 kDa) Lp[a]/ml upon oxidation with 50 μM Cu^{2+} at 37°C is shown in **Fig. 1**. The kinetics of Cu^{2+}-mediated oxidation of Lp[a] is similar to that described for LDL (35). The size of the Lp[a] particles was analyzed with laser-light scattering (submicron particle analyzer, Malvern Instruments Inc.) before and after oxidation. We did not find evidence for the presence of particles larger than 40 nm.

In vivo uptake studies

Throughout this study, 12-week-old male Wistar rats were used. Rats were anesthetized by intraperitoneal in-

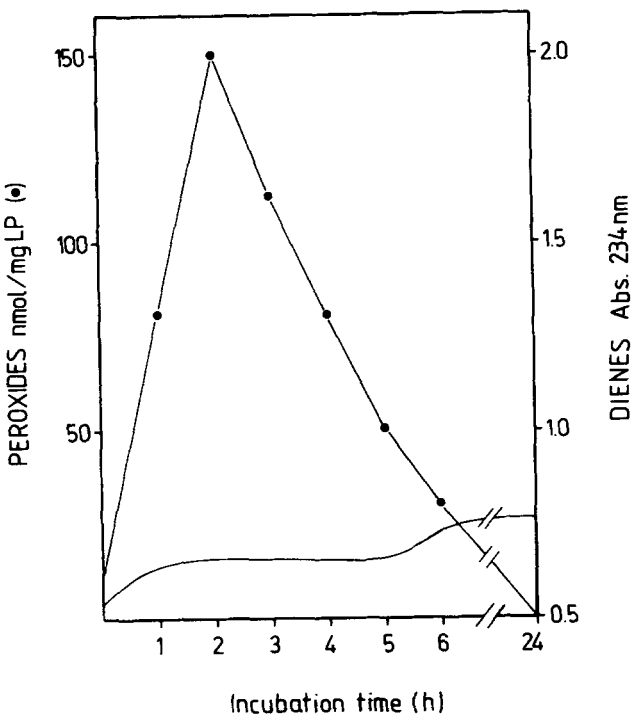

Fig. 1. Kinetics of Cu^{2+}-stimulated oxidation of Lp[a]. Oxidation of the 440 kDa form of Lp[a] was performed by exposure of 1 mg/ml in 0.01 M sodium-phosphate buffer, pH 7.4, to 50 μM $CuCl_2$ at 37°C (REM at 6 h was 1.56; REM at 24 h was 2.08). During the oxidation, conjugated dienes (—) were continuously monitored by absorption at 234 nm (31). Lipid peroxides (–●–) were measured by a spectrophotometric assay at 365 nm (30).

jection of 80 mg/kg Nembutal. The abdomen was opened and radiolabeled lipoproteins (600×10^3–1200×10^3 cpm) were injected into the vena penis. When indicated, 1 min prior to the radiolabeled compound, 5 mg polyinosinic acid (poly I) or PBS (control) was injected into the inferior vena cava. Blood clearance and liver uptake were determined as reported in previous studies (23). At indicated times, 0.2 ml blood was taken from the inferior vena cava and the radioactivity was counted. Liver lobules were tied off and excised at indicated times. Liver uptake was calculated by weighing liver lobules and counting the radioactivity. The radioactivity in the liver lobules was corrected for the amount of radioiodinated lipoproteins entrapped in the blood (36).

Isolation of rat liver cells

To determine the contribution of the various liver cell types to the total liver uptake of Lp[a] and Ox-Lp[a], the following collagenase/pronase procedure was performed (37). The radiolabeled lipoproteins were injected into the vena penis and after 10 min of circulation the vena porta was cannulated. A liver perfusion was started (flow: 14 ml/ml) with Hanks' buffer, containing 1.6 g HEPES/l, pH 7.4, at 8°C. After 8 min perfusion a lobule was tied off for the determination of the total liver uptake. Subsequently, the liver was perfused with Hanks' buffer plus 0.05% collagenase (w/v) at 8°C for 15 min. Thereafter the liver was excised and cut into pieces with a pair of scissors in ice-cold medium (Hanks' buffer supplemented with 0.3% BSA). The suspension was filtered through nylon gauze (mesh width 88 μm) and the filtrate was centrifuged for 30 sec at 50 *g*, after which the supernatants were transferred to 50-ml Falcon tubes. The pellets containing parenchymal cells were resuspended and the procedure was repeated three times. The combined supernatants of the first and second 50 *g* centrigations were then centrifuged for 10 min at 500 *g*. In a total volume of 25 ml, the remainder of the liver was further digested by stirring with 0.25% pronase (w/v) for 20 min at 4°C. Subsequently this suspension was filtered and the filtrate was centrifuged for 10 min at 500 *g*, after which the pellet was washed twice with medium. All pellets of the 500 *g* centrifugation were pooled and resuspended in 5 ml of buffer, and mixed with 7.3 ml of 29% Nycodenz. One ml of medium was layered on top of the mixture and tubes were centrifuged for 15 min at 1500 *g*. The cells that had floated into the interphase were collected and injected into the mixing chamber of the elutriation rotor. At a rotor speed of 3250 rpm, four fractions were collected at flows of 12.5, 25, 39, and 70 ml/min, respectively. At each flow 150 ml was collected. Fractions of endothelial and Kupffer cells were obtained at a flow of 25 and 70 ml/min, respectively. Purity of endothelial and Kupffer cell preparations was monitored by peroxidase staining and contained >90% of the indicated cell type.

For the in vitro competition studies, liver endothelial and Kupffer cells were isolated by a comparable procedure, except that this procedure was performed with collagenase (type I, 0.05%) at 37°C (37). Purity of endothelial and Kupffer cell preparations was monitored by peroxidase staining and contained >90% of the indicated cell type. The viability of the cells used for in vitro experiments was ≥90% as judged by 0.2% trypan blue exclusion.

Cell association and degradation of lipoproteins in vitro

To determine the cell-associated radioactivity and degradation of lipoproteins, incubations of freshly isolated Kupffer and endothelial cells with the indicated amounts of lipoproteins were performed in Dulbecco's Modified Eagle's Medium (DMEM), containing 2% (w/v) bovine serum albumin (BSA) at 37°C in a total volume of 0.5 ml (38). A circulating lab shaker (Adolf Kühner AG, Switzerland) at 150 rpm was used. After 2 h of incubation, samples were withdrawn and the cells were centrifuged for 2 min at 500 *g*. The pellets were washed two times in 1 ml of medium containing 50 mM

Tris-HCl, pH 7.4, 0.15 M NaCl, 2.5 mM $CaCl_2$, and 0.2% BSA, and the third time in medium without BSA. The cell-associated radioactivity was counted in a Packard ultrogamma counter. To determine the degradation of the lipoproteins, 0.5 ml of the first supernatant was incubated with 0.2 ml trichloroacetic acid for 15 min at 37°C. Subsequently, the mixture was centrifuged for 2 min at 14,000 rpm. To 0.5 ml of the supernatant, 10 μl of 20% KI and 25 μl of H_2O_2 were added and the mixture was shaken for 5 min. After centrifugation for 2 min at 14,000 rpm, 0.4 ml of the aqueous phase was counted; the degradation values shown in Fig. 4 represent this phase. The radioactivity in cell-free control incubations was routinely determined and subtracted from that obtained in the presence of cells.

Labeling of Ox-Lp[a] with ^{125}I-labeled tyramine cellobiose

Labeling of Ox-Lp[a] with ^{125}I-labeled tyramine cellobiose (TC) was done according to Pittman et al. (39). The reaction of tyramine cellobiose (20 nmol) with $Na^{125}I$ (0.5 mCi) was performed for 30 min at room temperature. Two hundred μg of lipoprotein was allowed to react with activated radioiodinated TC for 30 min at room temperature. The specific activities of these iodinated Lp[a] preparations varied between 208 and 385 cpm/ng of protein (recovery yield of the protein: 90 ± 5%). More than 95% of ^{125}I-labeled TC-Ox-Lp[a] was precipitable in 35% (w/v) trichloroacetic acid. Derivatization of the lipoproteins with the TC ligand did not alter their blood decay and liver uptake.

Total body distribution of oxidized-^{125}I-labeled TC-Lp[a]

Rats were anesthetized and injected with the radiolabeled compound. One minute prior to tissue isolation, a 0.2-ml blood sample was taken to measure the amount of radioactivity present in the blood. The radioactivity present in the organs and tissues was corrected for the amount of radiolabeled lipoproteins present in the entrapped blood (36). The recovered percentage of radioactivity was 97 ± 4 (n = 12 ± SEM). Values were expressed as percentage of the injected dose (ID) and % ID/mg of wet tissue weight.

RESULTS

Oxidation of lipoprotein[a]

Two Lp[a] fractions (440 and 610 kDa forms) were used for oxidative modification at two different Cu^{2+} concentrations. The degree of oxidation expressed as REM and TBARS is shown in **Table 1**. With SDS-PAGE, we found that exposure of Lp[a] isoforms (1 mg/ml) to Cu^{2+} ions for 20 h at 37°C led to fragmentation of both apoB-100 and apo[a]. For apoB-100, the extent of fragmentation was similar as reported earlier for LDL (29).

In vivo studies

The decays of the native 440 and 610 kDa forms of Lp[a] in rats were slow (**Fig. 2**) and at 10 min after injection only 2 ± 1% and 4 ± 1% of the injected dose were recovered in liver, respectively. Upon exposure to different oxidative conditions, the removal of both the 440 kDa and 610 kDa forms of Lp[a] was greatly enhanced as compared to native Lp[a]. Up to 50–60% of the injected dose became associated with the liver within 10 min after injection. It was observed that the blood decay and liver association of both isoforms of Lp[a], oxidized with either a low or a high [Cu^{2+}], were similar. In Fig. 2 only the blood decay and liver uptake are given for the two Lp[a] isoforms, oxidized with a low [Cu^{2+}]. After preinjection with poly I (1 min prior to injection of the radiolabeled lipoproteins), the clearance and the liver association of both the low and high molecular weight forms of oxidized

TABLE 1. Effect of Cu^{2+}-mediated oxidation of Lp[a] on the electrophoretic mobility and the generation of TBARS

Form of Lp[a]	R_f[a]	REM[b]	TBARS
			nmol MDA/mg apoLp[a]
440 kDa			
Control	0.26 ± 0.01		<1
Oxidized with 10 μM Cu^{2+}	0.46	1.75	29
Oxidized with 50 μM Cu^{2+}	0.52 ± 0.03	1.98 ± 0.10	35
610 kDa			
Control	0.27 ± 0.02		<1
Oxidized with 10 μM Cu^{2+}	0.43 ± 0.02	1.61 ± 0.01	30
Oxidized with 50 μM Cu^{2+}	0.56	2.06	36

The concentration of Lp[a] was 1 mg/ml. Values represent the mean of two or three experiments ± SD. TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.
[a]Electrophoretic mobility on agarose gel.
[b]Relative (to native Lp[a]) electrophoretic mobility.

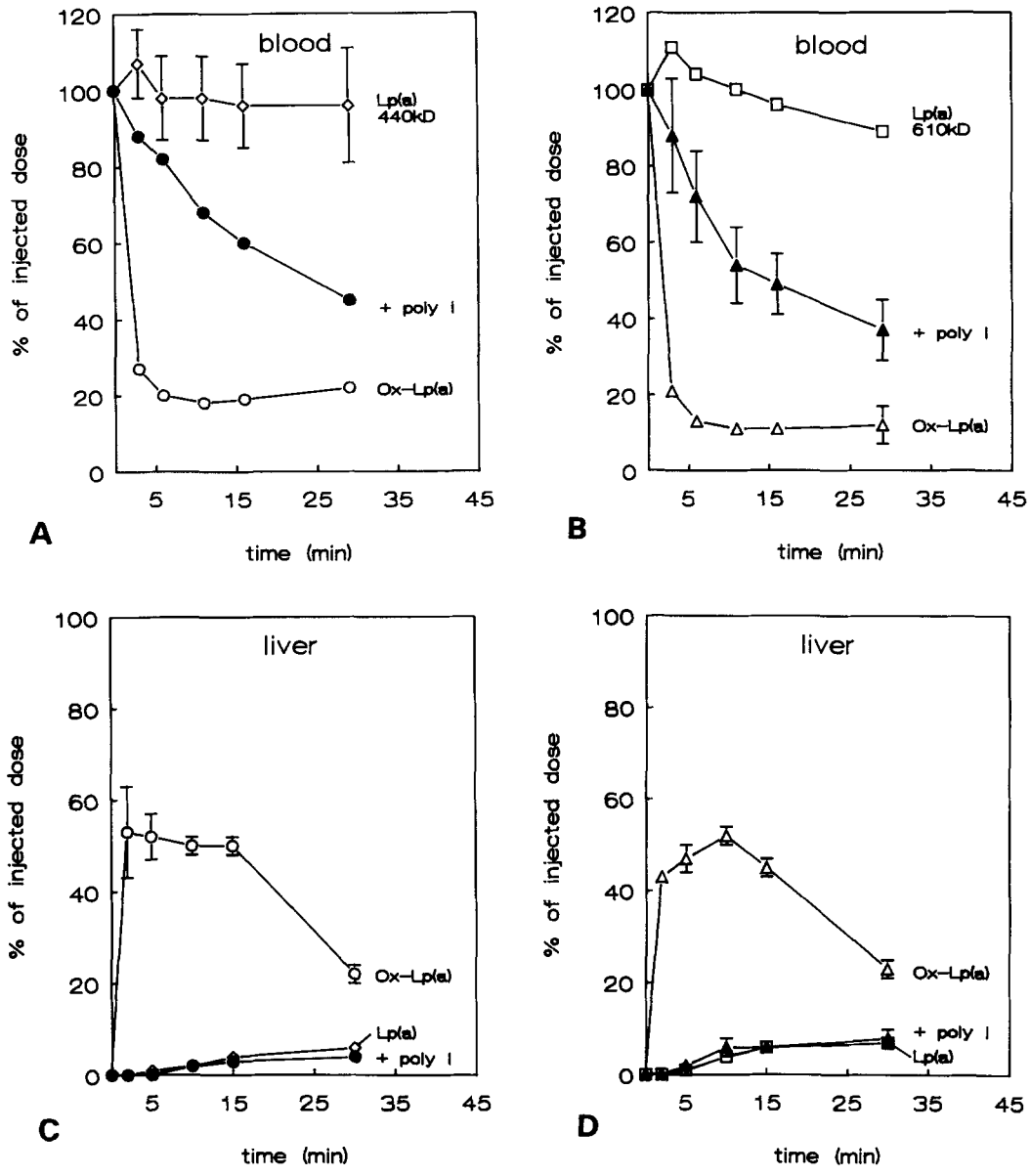

Fig. 2. The effect of Cu^{2+}-oxidation of the 440 kDa and 610 kDa forms of ^{125}I-labeled Ox-Lp[a] on blood decay and liver uptake in rats. One minute prior to the injection of 440 kDa (panels A and C) and 610 kDa (panels B and D) forms of ^{125}I-labeled Lp[a] or ^{125}I-labeled Ox-Lp[a], 0.5 ml PBS (open symbols) or 0.5 ml poly I (10 mg/ml) (closed symbols) was preinjected. Liver values were corrected for the amount of radioiodinated lipoproteins entrapped in blood (36). Values are expressed as percentage radioactivity of the injected dose (n = 2-3 ± SEM). The electrophoretic mobility (R_f) of the 440 kDa and the 610 kDa forms of Lp[a] on agarose gels was 0.26 ± 0.01 (n = 3) and 0.27 ± 0.02 (n = 3), respectively. For the 440 kDa and 610 kDa forms of Ox-Lp[a], the R_f values were 0.46 (n = 2) and 0.43 ± 0.02 (n = 3), respectively.

Lp[a] were effectively inhibited. Although poly I greatly blocks the liver uptake of oxidized Lp[a], it is clear that blockade of liver uptake is not paralleled by a complete blockade of the blood removal pathway.

Cellular distribution

The influence of the degree of oxidation and the molecular weight of the apo[a] isoform of Lp[a] on the in vivo association to the parenchymal, Kupffer, and endothelial cells at 10 min after injection is shown in **Fig. 3**. The total liver uptake was not influenced by the varying degrees of oxidation of the 440 kDa and 610 kDa forms of Lp[a]. However, it appears that the intrahepatic fate of the oxidized 440 kDa and 610 kDa forms of Lp[a] with lower R_f values are different (Fig. 3A). It is remarkable that the 440 kDa form of Lp[a] is taken up by liver endo-

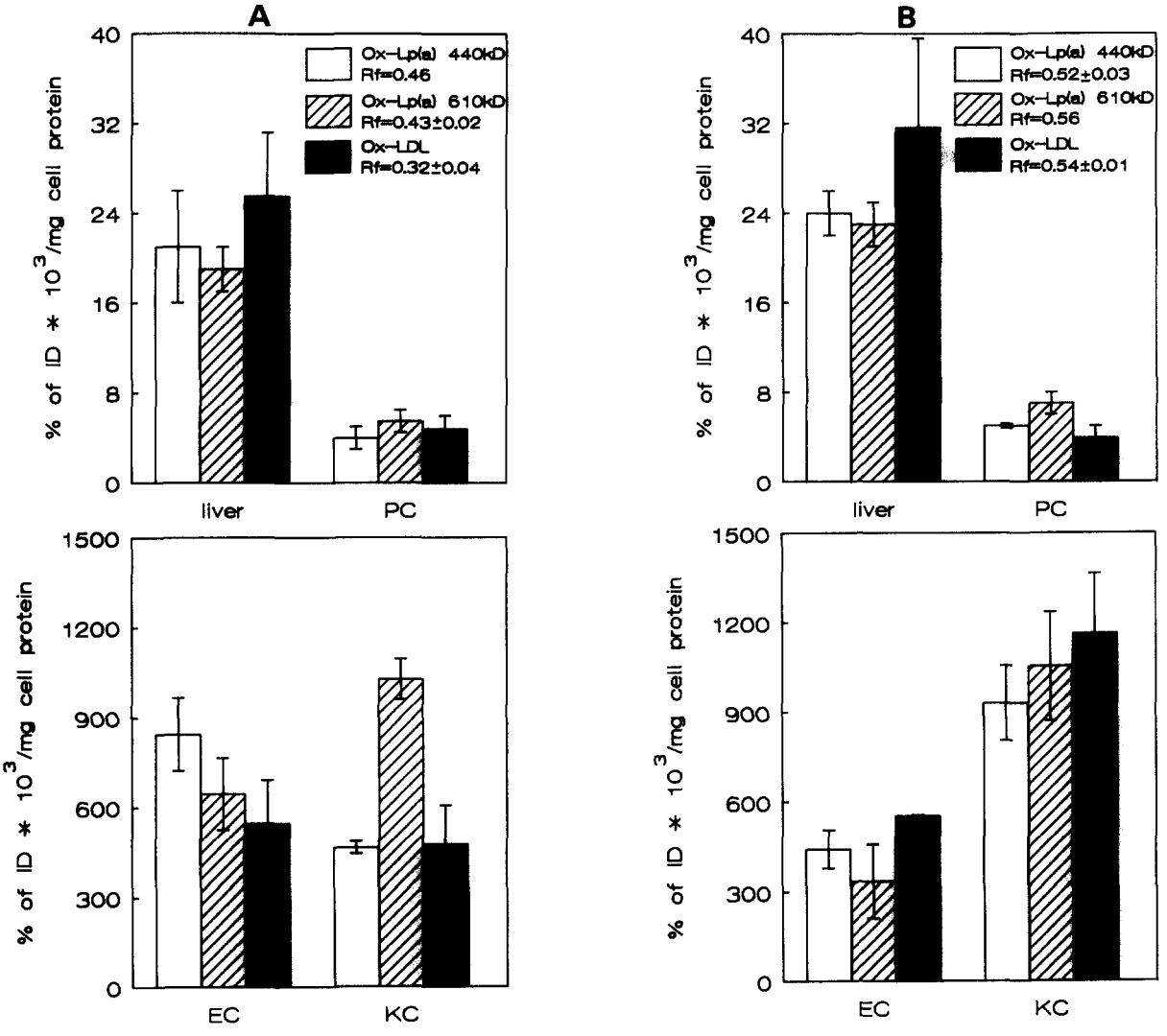

Fig. 3. The effect of Cu^{2+}-oxidation of ^{125}I-labeled LDL and the 440 kDa and 610 kDa forms of ^{125}I-labeled Lp[a] on the cell-association with parenchymal (PC), Kupffer (KC), and endothelial (EC) cells. A: Both LDL (from ref. 23) and the ^{125}I-labeled Lp[a] isoforms (440 kDa and 610 kDa) were oxidized to a low R_f value. B: Both LDL and the Lp[a] isoforms were oxidized to a high R_f value. These values are expressed as % injected dose/mg cell protein and are the mean of three experiments ± SEM.

thelial cells in particular (59 ± 1% of total liver uptake), while for the 610 kDa form of oxidized Lp[a] with a low R_f value, the Kupffer cell uptake was 2-fold higher compared with the 440 kDa form, leading to the Kupffer cell as the main site of liver uptake (49 ± 8%) (**Table 2**). The contribution of the endothelial and the Kupffer cells to the total liver uptake of 6-h-oxidized LDL is more similar to the 440 kDa form (Fig. 3A).

Oxidation of both the 440 and 610 kDa forms of Lp[a] to a higher degree, however, paralleled by an increased REM on agarose as compared to the lower oxidation degree (Table 1), resulted in a comparable cellular distribution within the liver at 10 min. The association values of the highly oxidized Lp[a] isoforms with the liver are very similar to the values obtained for highly oxidized LDL (22). The Kupffer cell now appears to be the main liver site for the uptake of both Lp[a] isoforms, oxidized to a high degree (52 ± 7% of the total liver uptake). For the three cell types, preinjection of poly I does inhibit the uptake of both the 440 kDa and 610 kDa forms of Lp[a], either oxidized to a low or a high degree (data not shown).

In vitro characterization of recognition sites for oxidized Lp[a] on liver endothelial and Kupffer cells

Competition experiments were performed in order to obtain information about the specificity of the recognition site for the highly oxidized Lp[a] isoforms on liver endothelial and Kupffer cells (**Fig. 4**). ^{125}I-labeled Ox-Lp[a] was incubated with liver endothelial and Kupffer cells in the presence or absence of poly I, Ox-LDL, Ac-LDL, or

TABLE 2. Relative contribution of the different cell types to the total uptake of Ox-Lp[a] by rat liver

Form of Lp[a]	PC	EC	KC
		%	
440 kDa			
Control	40 ± 2	11 ± 1	49 ± 1
Oxidized with 10 μM Cu^{2+}	16 ± 1	59 ± 1	25 ± 2
Oxidized with 50 μM Cu^{2+}	21 ± 2	27 ± 5	52 ± 7
610 kDa			
Control	53 ± 2	13 ± 2	33 ± 9
Oxidized with 10 μM Cu^{2+}	18 ± 5	32 ± 11	49 ± 8
Oxidized with 50 μM Cu^{2+}	26 ± 3	22 ± 6	52 ± 7

At 10 min circulation of ^{125}I-labeled Ox-Lp[a] after intravenous injection, liver perfusion at 8°C was started. Liver parenchymal cells (PC), endothelial cells (EC), and Kupffer cells (KC) were separated by a collagenase/pronase method (37). When the amount of protein that each cell type contributes to total liver protein is taken into account (23), the relative contribution of the three cell types to the total liver uptake of ^{125}I-labeled Ox-Lp[a] by rat liver can be calculated. PC contribute 92.5% of total liver volume; EC 3.3%; and Kupffer cells 2.5%.

Ox-Lp[a] itself. The association and degradation of ^{125}I-labeled Ox-Lp[a] (440 kDa isoform) by endothelial and Kupffer cells was inhibited by oxidized Lp[a] itself for 70–90% and 60–70%, respectively. Similar inhibition percentages were obtained with Ox-LDL and poly I (Fig. 4). For the competitor Ac-LDL, only a maximal inhibitory percentage of 25% was found. The cell-association and degradation of the 610 kDa form of highly oxidized ^{125}I-labeled Lp[a] by liver endothelial and Kupffer cells was competed for in a similar way by the various competitors as with the 440 kDa form (not shown).

Extrahepatic deposition of ^{125}I-labeled TC-Ox-Lp[a]

Upon in vivo injection of the 440 kDa and 610 kDa forms of ^{125}I-labeled Ox-Lp[a], the blood decay could not be completely explained by a quantitative recovery of the radioactivity in the liver. A determination of the possible extrahepatic uptake sites of ^{125}I-labeled tyramine cellobiose-labeled Ox-Lp[a] indicates that, in addition to the liver, spleen and bone marrow show a relatively high uptake/mg wet weight (**Fig. 5**). By preinjection of Poly I, the liver uptake of Ox-Lp[a] was greatly blocked. Under this condition it appears that the association of Ox-Lp[a] with the heart was 20-fold increased. This effect appears to be comparable with both the 440 kDa and the 610 kDa forms of Ox-Lp[a]. Of the injected dose of Ox-Lp[a], 75 ± 2% was recovered in the blood, liver, heart, spleen, and bone marrow (Fig. 5). The recovery percentage was not influenced by preinjection with poly I. In addition, 20% and 5% of the injected dose of ^{125}I-TC-Ox-Lp[a] was recovered in bone and skin, respectively. By preinjection of Poly I, the association of Ox-Lp[a] with bone was 8-fold decreased, while the association with skin was increased 2- to 3-fold. This association appears to be independent of either the degree of oxidation or the tested molecular weight of the apo[a] isoforms (data not shown).

DISCUSSION

Although the liver plays an important role in the elimination of various atherogenic lipoproteins from the blood (23, 26, 40), Lp[a] cannot be efficiently removed (15), leading to a prolonged circulation time. Lp[a] may also bind to intercellular matrices forming complexes with apoB-containing lipoproteins (41). This prolonged residence time might allow oxidative modification, which can be one of the mechanisms for the atherogenicity of Lp[a]. In this study we investigated the in vivo fate of the 440 kDa and 610 kDa forms of Lp[a] upon oxidation. With the tested variation of oxidation degree of the 440 kDa and 610 kDa forms of Lp[a], an indication for the increased risk for coronary heart disease for the higher molecular weight form compared to the lower molecular weight form of Lp[a] (9) was sought. Oxidation of both the 440 kDa and 610 kDa forms of Lp[a] results in a rapid blood removal caused by a 14-fold enhanced liver uptake as compared to native Lp[a]. The present data indicate that with the tested variation in oxidation degree of Lp[a] the blood decay and liver association are comparable. However, the intrahepatic fate of the 440 kDa and 610 kDa forms of Lp[a] with low R_f values is clearly different. Upon oxidation to a slightly increased negative charge (low R_f value) of Lp[a], the high molecular weight form of Lp[a] is recognized more efficiently by the Kupffer cells than the endothelial cells as compared to the lower molecular weight form of Lp[a]. As reported earlier for oxidized LDL (23), for highly oxidized Lp[a] the Kupffer cell is the main liver site for uptake of both the 440 and 610 kDa forms. Different receptor systems, responsible for the recognition of modified lipoproteins, were recently reported to be present on mouse peritoneal macrophages (24, 25) and liver macrophages (23, 26). Besides the Ac-LDL receptor, which recognizes both Ac-LDL and Ox-LDL, an additional oxidized lipoprotein receptor was found that recognizes oxidatively modified LDL but not Ac-LDL.

The involvement of scavenger receptors in the in vivo uptake of oxidized Lp[a] is indicated by the blockade of the liver uptake by preinjection of polyinosinic acid. In vitro competition studies indicate that the specific receptor for Ox-Lp[a] on liver endothelial and Kupffer cells is different from the recognition site for Ac-LDL. On both endothelial and Kupffer cells the recognition of the 440 kDa and 610 kDa forms of Ox-Lp[a] was only slightly competed for by Ac-LDL, while an efficient inhibition was noticed with poly I, Ox-LDL, and Ox-Lp[a] itself. These data indicate that Ox-Lp[a] is apparently avidly bound by the recently described so-called specific Ox-LDL receptor (23, 26).

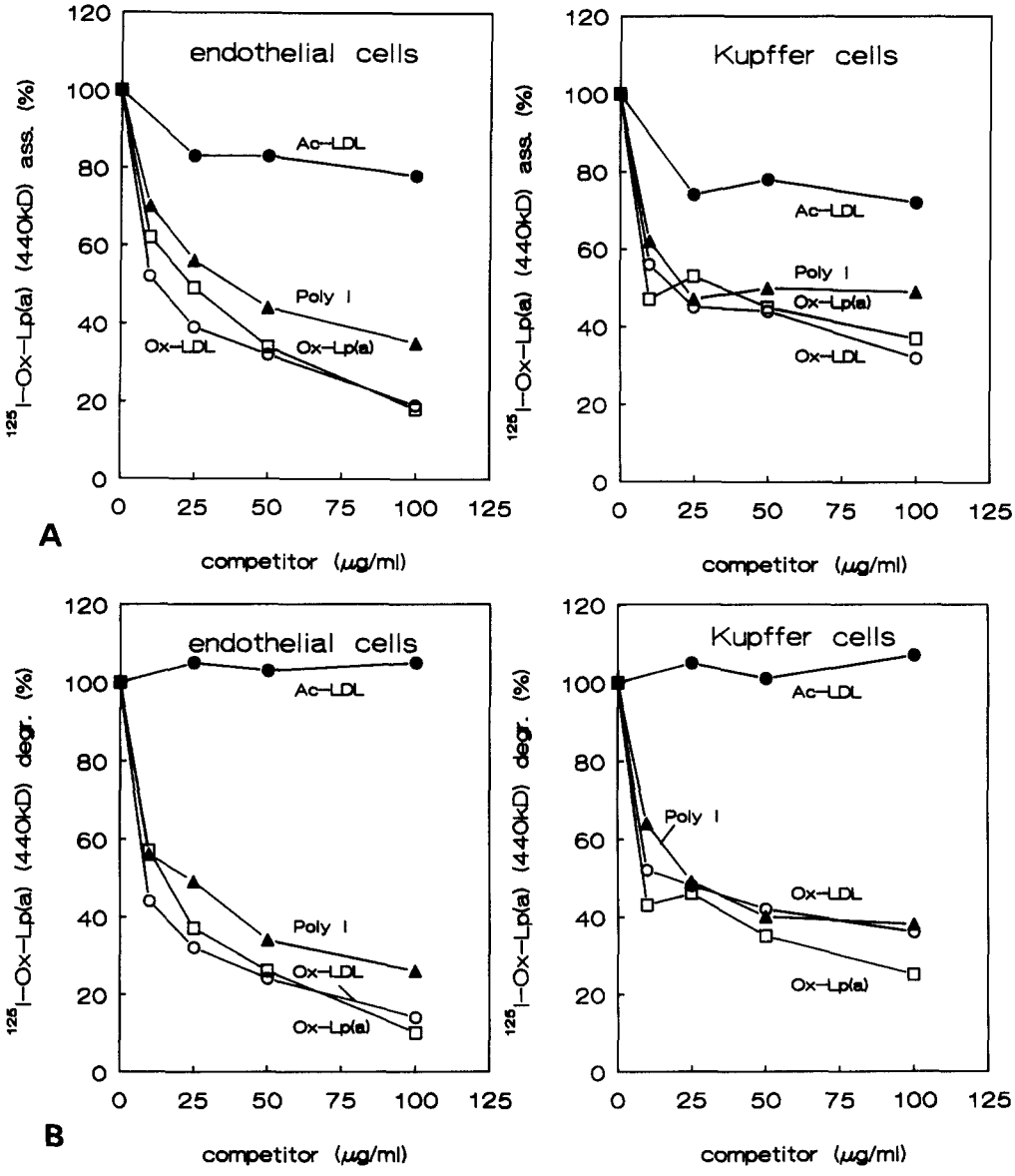

Fig. 4. Comparison of the ability of unlabeled lipoproteins or polyinosinic acid to compete for the cell-association (A) or degradation (B) of the 440 kDa form of highly oxidized ^{125}I-labeled Lp[a] by endothelial and Kupffer cells. Freshly isolated endothelial and Kupffer cells were incubated at 37°C for 2 h in DMEM medium (2% BSA) with 5 μg/ml ^{125}I-labeled Ox-Lp[a] and the indicated concentrations of unlabeled Ox-Lp[a] (□), Ox-LDL (○), Ac-LDL (●), or poly I (▲). Cell-association and degradation were determined. Values are given as percentage of the control incubations in the absence of competitors. The values for 100% association and degradation of ^{125}I-labeled Ox-Lp[a] by Kupffer cells were 1574 ± 285 and 1363 ± 323 ng/mg cell protein, respectively. For endothelial cells, these values were 3239 ± 598 and 2360 ± 651 ng/mg cell protein, respectively.

Although the liver is quantitatively most important for the clearance of Ox-Lp[a], 20% still became associated with bone. Preinjection of poly I did lead to an inhibition of the bone-associated Ox-Lp[a], indicating that the uptake by bone may also be mediated by scavenger receptors. Surprisingly, the heart appears to be an important organ in the deposition of Ox-Lp[a] when a prolonged blood circulation is induced by poly I blockade of liver uptake. Ox-Lp[a] is probably not actively internalized in the heart, because the association is already maximal within 10 min after injection and no further accumulation is found. The extrahepatic distribution of the 440 kDa and 610 kDa forms of Ox-Lp[a] is comparable and appears to be independent of the degree of oxidation. The association of Ox-Lp[a] with spleen and bone marrow may be explained by the recognition of oxidized Lp[a] by the

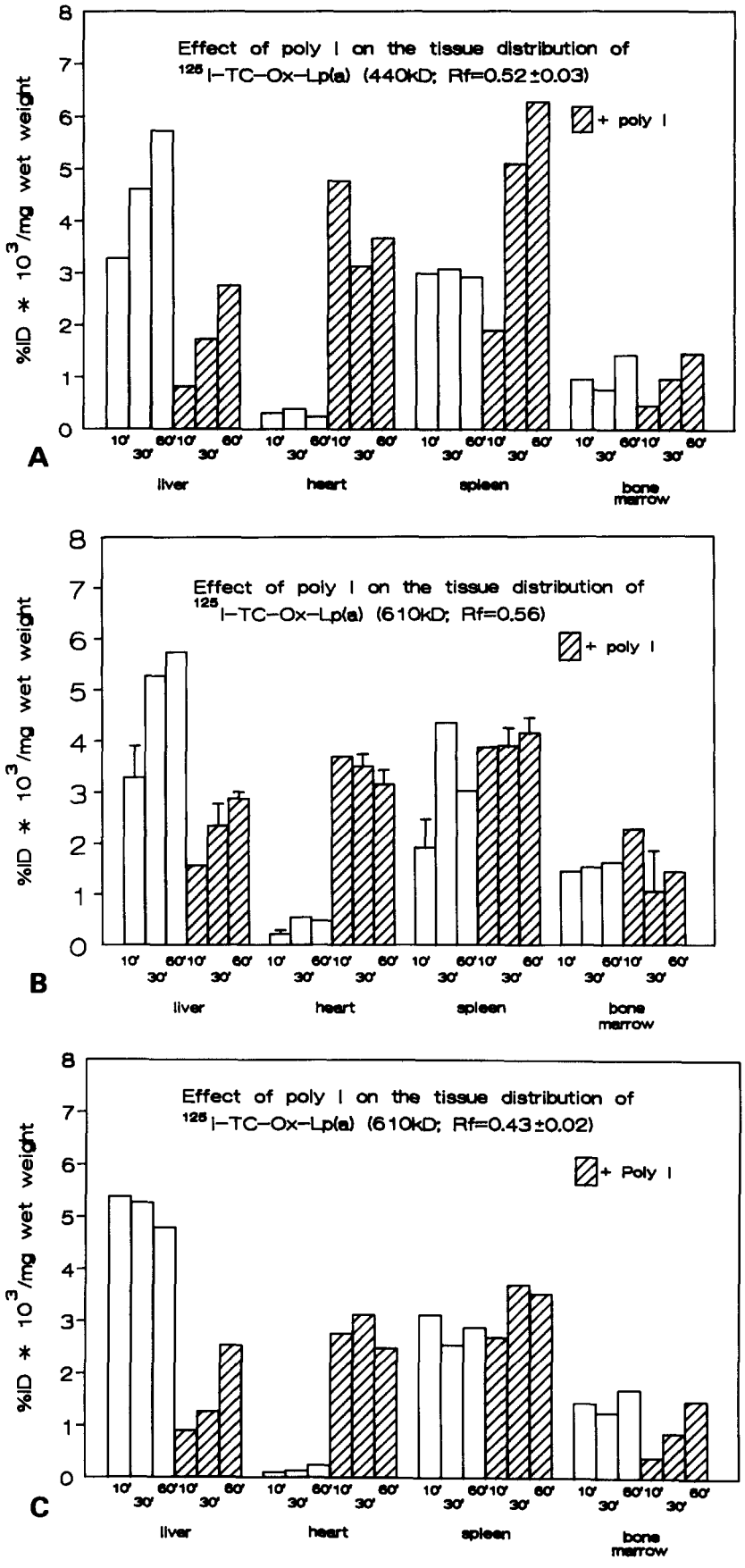

Fig. 5. The effect of polyinosinic acid on the uptake of the 440 kDa and 610 kDa forms of oxidized Lp[a], coupled to ^{125}I-labeled tyramine cellobiose, by liver, heart, spleen, and bone marrow. One minute prior to the injection of ^{125}I-labeled TC-Ox-Lp[a], 0.5 ml PBS (open bars) or 0.5 ml poly I (10 mg/ml) (hatched bars) was injected. At 10, 30, and 60 min after intravenous injection of ^{125}I-labeled TC-Ox-Lp[a], rats were killed and the ^{125}I content in the tissues was examined. Values are expressed as percentage of the injected dose × 10^3/mg wet weight, and are corrected for the amount of radiolabeled lipoproteins entrapped in the blood present in the tissues. A: The tissue distribution of the 440 kDa form of ^{125}I-labeled TC-Ox-Lp[a] with a high R_f value (R_f = 0.52 ± 0.03). B: The tissue distribution of the 610 kDa form of ^{125}I-labeled TC-Ox-Lp[a] with a high R_f value (R_f = 0.56). Bars represent extreme values (n = 2). C: The tissue distribution of the 610 kDa form of ^{125}I-labeled TC-Ox-Lp[a] with a low R_f value (R_f = 0.43 ± 0.02).

mononuclear phagocytic cells of the reticuloendothelial system, as shown previously for negatively charged modified LDL (42). In recent years the hypothesis concerning the occurrence of oxidative modification of lipoproteins in vivo was strengthened by the detection of epitopes (formed upon oxidation) on lipoproteins extracted from atherosclerotic lesions of Watanabe rabbits and from human aorta by means of antibodies (21, 43, 44). The positive reaction of the extracted lipoproteins from human aorta with polyclonal antibodies against MDA- or 4-hydroxynonenal-modified LDL (21) may be also explained by the presence of oxidatively modified Lp[a] (45). The oxidative damage of apoB-100 versus apo[a] can be estimated immunochemically by using monospecific antibodies against apoB and apo[a]. Initial data suggest that the oxidative degradation of apoB-100 is more extensive than that of apo[a]. However, a further characterization is necessary before more definite conclusions can be drawn.

In conclusion, oxidative modification of the high molecular weight form of Lp[a] converts Lp[a] into a ligand that is readily recognized by the specific oxidized-lipoprotein receptor on Kupffer cells. These data form the first indication for differential recognition of Lp[a] isoforms by scavenger receptors. Under conditions that the liver uptake of Ox-Lp[a] is not adequately exerted, accumulation of Ox-Lp[a] in the heart is apparent. Its potential pathological importance and the mechanism of uptake remain to be established.

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