〈Original Article〉

Paraoxonase 1 associated with high-density lipoprotein transfers to oxidized low-density lipoprotein depending on the degree of oxidation

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Summary Paraoxonase 1 (PON1) is an important anti-atherosclerotic enzyme that is predominantly associated with high-density lipoprotein (HDL). PON1 protects low-density lipoprotein (LDL) from oxidation because it is able to hydrolyze lipid hydroperoxides (LOOHs). Although several studies have indicated that PON1 transfers to chylomicron and very low-density lipoprotein, it has not been elucidated whether a similar phenomenon occurs in LDL. We investigated the transferability of PON1 from HDL to LDL and oxidized LDL (oxLDL). After LDL or oxLDL had been incubated with HDL, the components of the mixture were re-isolated by gel filtration and ultracentrifugation, and PON1 activity in each fraction was measured. Following incubation with HDL, gel filtration analysis revealed PON1 activity in the LDL fraction of oxLDL sample, but not LDL sample. Ultracentrifugation analysis revealed that PON1 activity in the LDL fraction was apparently proportional to the degree of oxLDL oxidation ($r = 0.90$, $p = 0.003$). Moreover, the relative electrophoretic mobility of oxLDL was increased by oxidation and reduced by incubation with HDL. In conclusion, HDL-associated PON1 transfers to oxLDL depending on the extent of oxidation of LDL. This important antioxidant mechanism contributes to the anti-atherosclerotic effects of HDL.

Key words: Paraoxonase 1, Oxidized low-density lipoprotein, High-density lipoprotein, Atherosclerosis, Antioxidant

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1. Introduction

The oxidation of low-density lipoprotein (LDL) is a major factor in the development of atherosclerosis[1]. Free radicals and non-radical oxidants transform LDL into oxidized LDL (oxLDL)[2]. Various types of modified substances, including lipid hydroperoxides (LOOHs) and negatively charged apolipoprotein B-100, are produced in oxLDL; they are recognized by scavenger receptors on macrophages and induce foam cell formation, a crucial cause of atherosclerosis initiation[3, 4]. In addition, oxLDL induces several cellular responses that are involved in the progression of atherosclerosis: chemotaxis and proliferation of monocytes/macrophages; recruitment and proliferation of smooth muscle cells (SMCs); and apoptosis of endothelial cells, SMCs, and macrophages with ensuing necrotic core development[5].

However, antioxidant systems exist in human blood that are supposed to prevent harmful oxidation. Paraoxonase 1 (PON1), a glycoprotein enzyme, is a key player in these systems. PON1 is produced in the liver and secreted into the blood where it becomes associated with high-density lipoprotein (HDL). When HDL extracts LOOHs from LDL via the action of cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP), the PON1 in HDL hydrolyzes the LOOHs, thereby preventing the accumulation of LOOHs in LDL[6-8]. In addition to this antioxidant activity, recombinant PON1 specifically binds to macrophage binding sites[9], and reduces the secretion of pro-inflammatory cytokines from the macrophage[10]. Therefore, PON1 is considered an important anti-atherosclerotic enzyme. In fact, PON1-knockout mice are more susceptible to atherosclerosis[11], and PON1-overexpressed mice with a combined trait of metabolic syndrome are more resistant to atherosclerosis[12].

PON1 may exert these effects in other lipoproteins besides HDL. Several studies have shown that PON1 transfers from HDL to very low-density lipoprotein (VLDL) and chylomicrons[13, 14], and inhibits LDL oxidation[15]. Recently, it has been reported that PON1 also transfers to small dense LDL (sdLDL), which is highly susceptible to oxidation[16]. However, the results of that study were obtained by the incubation of serum, and there is no obvious evidence that PON1 transfers to LDL. Therefore, we investigated the transferability of PON1 from HDL to LDL and oxLDL.

2. Materials and methods

1. Chemicals and blood samples

Unless otherwise stated, all chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Blood samples were collected from healthy volunteers after obtaining their written informed consent, and the study was approved by the ethics committee of Tokyo Medical and Dental University (number 1441).

2. Preparation of lipoproteins

LDL (density (d) = 1.006–1.063 g/mL) and HDL (d = 1.063–1.210 g/mL) were isolated from the sera of healthy volunteers by ultracentrifugation, as described previously[17]. LDL and HDL were dialyzed against phosphate-buffered saline (PBS) and 1 mmol/L $CaCl_2$-containing PBS, respectively, then filtered through a 0.22-μm polyether sulfone (PES) Syringe Filter (Starlab Scientific, Shaanxi, China). The lipoproteins were kept at 4°C and used within 2 weeks. Before use, the protein concentrations of the lipoprotein fractions were assayed using the Folin–Lowry method[18].

3. PON1 activity assay

A serum sample obtained from a healthy volunteer was used as the standard for the PON1 activity assay. The PON1 activity of the standard serum was determined by the method described by Clement et al.[19] with some modifications. Briefly, 15 μL of the standard serum or PBS as a control was mixed with 385 μL of the assay buffer (1.32 mmol/L $CaCl_2$, 2.63 mol/L NaCl, 132 mmol/L Tris-HCl, pH 8.5). After pre-incubation at 37°C, the assay was initiated by addition of 100 μL of the substrate solution prepared

by 20-fold dilution of the substrate stock solution (120 mmol/L paraoxon in acetone) with 50 mmol/L Tris-HCl (pH 8.5 when used). A 40-μL aliquot was added to 100 μL of the stop reagent (100 mmol/L Tris-HCl, pH 8.5 containing 3 mmol/L ethylenediaminetetraacetic acid (EDTA)) at 2-min intervals. The absorbance of each mixture was measured at 405 nm using an Ultrospec 3300 Pro UV/visible spectrophotometer (Amersham Biosicences, Piscataway, NJ, USA). The PON1 activity (U/L) of the standard serum, expressed as the micromole value of paraoxon hydrolyzed per minute, was calculated using the change in absorbance per minute and the molar extinction coefficient of the product (*p*-nitrophenol, 18,050 $M^{-1} \cdot cm^{-1}$). The PON1 activities of the lipoprotein samples were measured using the standard serum. Samples (40 μL) and the standard serum diluted 20-fold with PBS were mixed with 30 μL of the assay buffer and 30 μL of the substrate solution. The absorbance of the mixture was monitored at 405 nm using a SUNRISE Rainbow microplate reader (Wako, Osaka, Japan) for 40 min. The PON1 activity of the sample was calculated from the change in absorbance per minute (ΔABS/min) compared with the ΔABS/min of the standard serum.

4. Quantification of total cholesterol and phospholipids

Total cholesterol (TC) and phospholipids were quantified using commercial enzymatic assay kits: Determinar L TC II and Determinar L PL (Kyowa Medex, Tokyo, Japan), respectively.

5. Distribution of PON1 in lipoprotein fractions

After ultracentrifugation of 3 mL of the serum and adjustment of its density to 1.063 g/mL using KBr, the top fraction (including VLDL and LDL), the middle fraction (the intermediate colorless fraction), and the bottom fraction (including HDL and plasma proteins) were separately recovered. The absolute PON1 activity in each fraction was calculated from the actual volume and the PON1 activity (U/L). The bottom fraction was further re-ultracentrifuged at a density of 1.210 g/mL. The top (including HDL), middle, and bottom (including serum proteins) fractions were obtained, and their absolute PON1 activities were determined, as described above.

6. Preparation of oxidized LDL

Equal volumes of LDL (2 mg protein/mL) and $CuSO_4$ (20 μmol/L in PBS) were mixed and incubated at 37°C every 30 min up to 4 h (0.5, 1, 1.5, 2, 2.5, 3, 3.5, or 4 h), followed by addition of 1/3 volume of EDTA (300 μmol/L in PBS) to stop oxidation. To evaluate the degree of oxidation, each sample was diluted with PBS and adjusted to 0.050 mg protein/mL; the levels of conjugated diene and LOOH were determined by measuring the absorbance at 234 nm, which is the wavelength of its maximum absorption[20], and by using the FOX-2 assay[21], respectively. In addition, electrophoresis of the lipoproteins was carried out on agarose gel (Helena Laboratories, Saitama, Japan) followed by visualization by Fat Red 7B staining. The relative electrophoretic mobility (REM) of the native LDL was defined as 1.

7. Gel filtration analysis

LDL, oxLDL (4 h oxidation) (final 0.5 mg protein/mL) with and without HDL (final 2.5 mg protein/mL), or HDL alone (final 2.5 mg protein/mL) were incubated at 37°C for 90 min. Each sample (2 mL) was introduced to a CL-6B Sepharose (GE Healthcare, Tokyo, Japan) column (1.5 × 95 cm) equilibrated with PBS containing 0.33 mmol/L $CaCl_2$ and 50 μmol/L EDTA. The levels of PON1 activity and TC in each fraction were measured as described above. The peak fractions corresponding to LDL and HDL were concentrated using Amicon Ultra-0.5 mL Centrifugal Filters (Merck Millipore, Billerica, MA, USA) for western blotting.

8. Western blotting

Western blotting was carried out as described previously[22]. After gel filtration, the concentrated peak fractions corresponding to LDL were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12.5% gel) in the same

amounts as the proteins. The amount of HDL-only fraction protein applied to the gel was adjusted to produce the same degree of PON1 activity as the peak fraction of LDL obtained from oxLDL pre-incubated with HDL. After electrophoresis, the proteins were transferred to an Immobilon-P, polyvinylidene fluoride membrane (Merck Millipore, Billerica, MA, USA). The membrane was then incubated with anti-paraoxonase 1 antibody (Sigma-Aldrich Japan, Tokyo, Japan) or anti-apolipoprotein A-I antibody (Academy Bio-Medical Company, Houston, TX, USA) followed by incubation with the corresponding secondary antibody. The PON1 and apolipoprotein A-I (apoA-I) bands were visualized using ECL Prime Western Blotting Detection Reagent (GE Healthcare Japan, Tokyo, Japan) and 3,3'-diaminobenzidine tetrahydrochloride/hydrogen peroxide, respectively.

9. Ultracentrifugation analysis

LDL, oxLDL (1-, 2-, and 4-h oxidation) (0.5 mg protein/mL) with and without HDL (2.5 mg protein/mL), or HDL alone (2.5 mg protein/mL) were incubated at 37°C for 90 min. LDL and HDL were re-isolated by ultracentrifugation (d = 1.063 g/mL) at 4°C for 19 h, and divided into three fractions (top, middle, and bottom), as described above. PON1 activity and the quantities of TC and phospholipids in each fraction were measured. The LDL in each top fraction (2 μL) was investigated by agarose gel electrophoresis to compare REMs.

10. Statistical analysis

We used one-way analysis of variance (ANOVA) followed by the paired t-test to compare multiple groups. The correlation was analyzed using the Spearman's rank correlation coefficient test. The results are expressed as mean ± SD. A p-value of less than 0.05 was considered statistically significant.

3. Results

1. Distribution of PON1 in lipoprotein fractions

PON1 is predominantly associated with HDL; however, it has been reported that PON1 can be released during ultracentrifugation[23]. Therefore, we first investigated the levels of PON1 activity, TC, and phospholipids after fractionation of HDL by ultracentrifugation. The levels of PON1 activity, phospholipids, and TC in the sera obtained from four subjects were 280 ± 92 U/L, 227 ± 44 mg/dL, and 178 ± 31 mg/dL (mean ± SD), respectively. The distribution of these factors in each fraction was expressed as the percentage of the absolute value in each serum. After the first ultracentrifugation (d = 1.063 g/mL), PON1 activities of 0.4 ± 0.1%, 0.2 ± 0.1%, and 78.8 ± 7.1% were observed in the top, middle, and bottom fractions, respectively (Fig. 1A). After the second ultracentrifugation (d = 1.210 g/mL), PON1 activities of 12.9 ± 7.3% and 36.1 ± 3.9% were detected in the top and middle fractions, respectively. However, the mean recoveries of phospholipids and TC were higher in the top fraction (32.2% and 28.3%, respectively) than in the middle fraction (2.9% and 2.5%, respectively) (Fig. 1B). The percentages of PON1 activity, phospholipids, and TC in the bottom fraction were 7.0 ± 2.2%, 5.8 ± 1.6%, and 1.0 ± 0.2%, respectively (Fig. 1B).

2. Evaluation of oxLDL oxidation degree

To evaluate the degree of oxidation in LDL induced by copper sulfate, we determined the REM and the levels of conjugated diene and LOOH, which are the markers of oxidation, in each sample. The REM gradually increased to 1.61 ± 0.30 and the levels of conjugated diene and LOOH gradually increased to 0.704 ± 0.029 and 416 ± 26 nmol/mg LDL protein, respectively, depending on the oxidation time (Fig. 2).

3. Gel filtration analysis

After gel filtration, the fractions of LDL and HDL were confirmed by measuring the concentration of TC (Fig. 3A and 3B). LDL and oxLDL (4 h) incubated without HDL indicated one peak with no PON1 activity. However, the peak for oxLDL (fraction number 59) was observed in a later fraction than LDL (fraction number 58) (Fig. 3A). In contrast, it was expected that PON1 activity would be detected

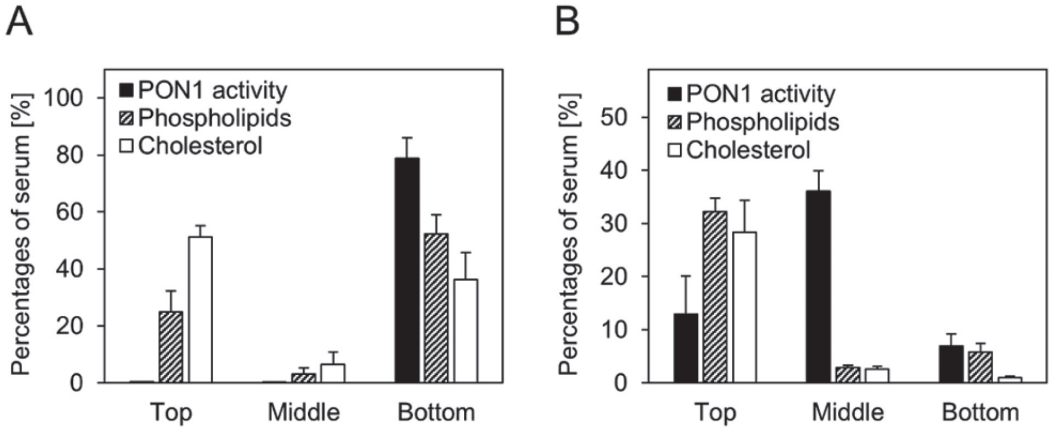

Fig. 1 Distribution of paraoxonase 1 (PON1) activity in each lipoprotein fraction. Serum, with density adjusted to 1.063 g/mL, was ultracentrifuged at 4°C for 24 h, and divided into three fractions: top, middle, and bottom fractions (A). Subsequently, the bottom fraction, with density adjusted to 1.210 g/mL, was ultracentrifuged again and divided into three fractions, as described above (B). The absolute values of PON1 activity, phospholipids, and cholesterol in each fraction were measured and expressed as a percentage of those in the serum. Results are given as mean ± SD (n = 4).

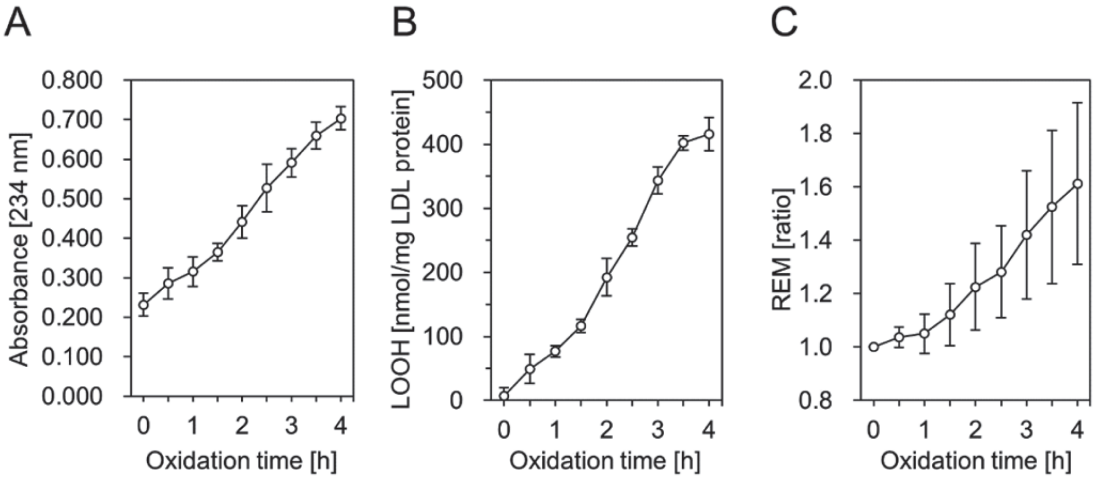

Fig. 2 Evaluation of degree of oxidation of oxidized low-density lipoprotein (oxLDL). LDL (1 mg/mL) was oxidized with copper sulfate (10 μmol/L) at 37°C for 0.5–4 h followed by addition of EDTA to stop oxidation. The levels of dienes (A), lipid hydroperoxides (LOOHs) (B), and relative electrophoretic mobility (REM) (C) in oxLDL were determined. The REM of native LDL was defined as 1. Results are given as mean ± SD (n = 3).

in the HDL fraction of the profile for HDL only; however, the actual peak of PON1 activity (fraction number 74) was observed just before that of cholesterol (fraction number 76) (Fig. 3A). When LDL was pre-incubated with HDL, extremely low PON1 activity (5.4%) was observed in the LDL fraction (fraction numbers 51–67), and the greatest PON1 activity (94.6%) was observed in the HDL fraction (fraction numbers 68–87) (Fig. 3B). However, PON1 activity in the LDL fraction was dramatically increased (13.7%) (fraction numbers 51–67) when oxLDL (4-h) was pre-incubated with HDL, although the greatest activity (86.3%) was still found in the HDL fraction (fraction numbers 68–87) (Fig. 3B).

Next, we confirmed that the increased PON1 activity in the LDL fraction was due to a transfer of

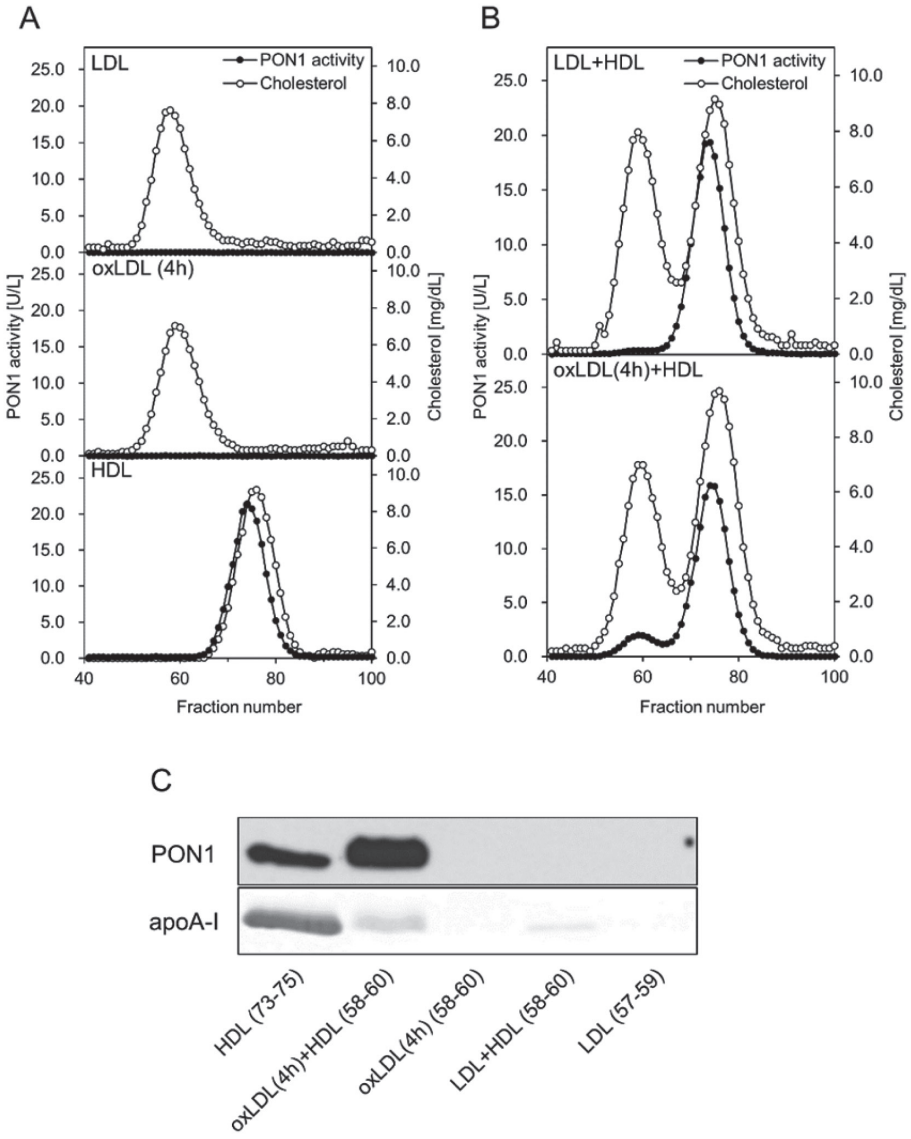

Fig. 3 Gel filtration analysis. LDL, oxLDL (4-h oxidation) (final 0.5 mg protein/mL) with and without HDL (final 2.5 mg protein/mL), or HDL alone (final 2.5 mg protein/mL) were incubated at 37°C for 90 min. After gel filtration, the PON1 activity and the total cholesterol in each fraction were measured (A, B). HDL peak fractions (73–75) of the HDL-only profile and LDL peak fractions (numbers in parentheses) of each profile were concentrated, and the apparent amounts of PON1 and apoA-I were compared by western blotting (C). Equal amounts of protein from each LDL fraction were applied to a gel. The amount of HDL-only fraction protein applied to the gel was adjusted to produce the same degree of PON1 activity as the peak fraction of LDL obtained from oxLDL pre-incubated with HDL.

PON1 from HDL to oxLDL and not contamination of HDL. The quantities of PON1 and apoA-I proteins in the HDL fraction separated from HDL alone were compared with those in the LDL fraction separated from oxLDL pre-incubated with HDL using SDS-PAGE followed by western blotting, adjusted to load the same degree of PON1 activity. A markedly greater amount of PON1 and less apoA-I was observed in the LDL fraction obtained from the mixture of oxLDL and HDL compared with the HDL fraction obtained from HDL alone (Fig. 3C). Moreover, the other three LDL fractions

separated from LDL and oxLDL alone, and LDL pre-incubated with HDL were investigated by western blotting by loading the same amount of proteins as the LDL fraction separated from oxLDL pre-incubated with HDL. No PON1 band was observed in any of those three LDL fractions, whereas a faint band due to apoA-I was observed in the LDL fractions separated from LDL pre-incubated with HDL (Fig. 3C).

4. Ultracentrifugation analysis

Because the possible transfer of PON1 from HDL to oxLDL was examined by gel filtration separation based on particle size, the transferability of PON1 was further investigated by ultracentrifugation separation based on density. LDL or oxLDL (1, 2, or 4 h) were incubated in the presence and absence of HDL, and the mixtures were divided into three fractions (top, middle, and bottom) by ultracentrifugation (d = 1.063 g/mL). The quantities of TC and phospholipid in the top fraction obtained from the mixture of oxLDL (4 h) pre-incubated with HDL were significantly reduced ($p < 0.05$ and $p < 0.01$, respectively) compared with those in LDL pre-incubated with HDL (Fig. 4A). In contrast, they were significantly increased ($p < 0.01$ and $p < 0.01$, respectively) in the middle fraction. However, these patterns of TC and phospholipid levels in fractions were not significantly different between with and without HDL treatment. On the other hand, the degree of PON1 activity in the top fraction increased depending on the time of oxidation of LDL pre-incubated with HDL, but not without HDL. In addition, a significant positive correlation was observed between the PON1 activities in the top fraction and diene levels of LDL just after the oxidation ($r = 0.90$, $p = 0.003$) (Fig. 4B). Moreover, higher PON1 activity was observed in the middle fraction (3.4 ± 1.1 mU) than in the top fraction (1.9 ± 0.3 mU) separated from oxLDL (4 h) pre-incubated with HDL (Fig. 4A). However, the levels of TC and phospholipids in the middle fraction were lower than in the top fraction. Among the bottom fractions including HDL, there was no significant difference in the amounts of PON1, TC, and phospholipids, regardless of the extent of LDL oxidation. To investigate the change in negative surface charge of oxLDL due to pre-incubation with HDL, the top fractions separated from the mixtures were subjected to agarose gel electrophoresis. The REM of LDL increased depending on the extent of oxidation and tended to be suppressed by pre-incubation with HDL (Fig. 4C).

4. Discussion

Our study demonstrates that PON1, which is associated with HDL, transfers to oxLDL. Based on the evidence provided by many studies, PON1 is essential for preventing the oxidation of LDL, which is an important initial event in atherosclerosis. This multitasking enzyme is potentially transferred from HDL to other lipoproteins. Considering that LDL can undergo oxidation and initiate atherogenesis, it is plausible that the transfer of PON1 from HDL to LDL could effectively remove the underlying cause of atherogenesis. The elucidation of this important interaction between HDL and LDL would help us understand the mechanism behind the development of atherosclerosis.

First, we confirmed the recovery rate of PON1 activity in HDL prepared by ultracentrifugation, because ultracentrifugation is thought to disrupt the lipoprotein structure resulting in the release of PON1 from HDL[23]. PON1 activity in the HDL fraction was only 12.9% of that in the serum (Fig. 1), suggesting that HDL loses most of its PON1 during ultracentrifugation. In contrast, 36.1% PON1 activity was observed in the middle fraction, which is supposed to be denser HDL such as very high-density lipoprotein (VHDL) (Fig. 1B). However, in the present study, we did not use the middle fraction as the HDL fraction to avoid contamination by other serum proteins. Considering the recovery of PON1 in HDL (12.9%), we decided to use five times more HDL protein than LDL as a mixing condition; however, the mixing ratio was not dramatically different from that under physiological conditions. Second, we confirmed the degree of oxidation in LDL treated with copper sulfate by determining the REM and the

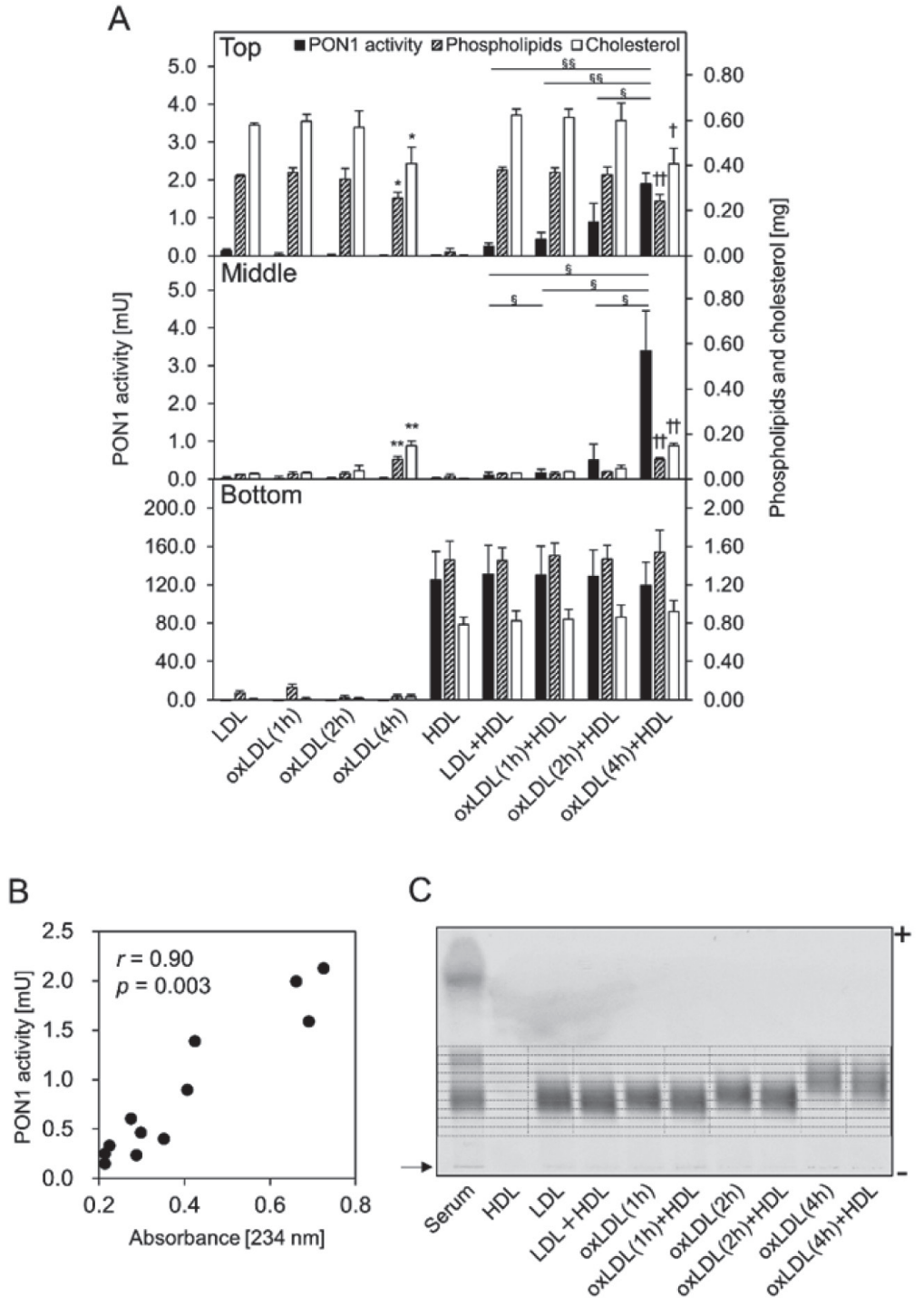

Fig. 4 Ultracentrifugation analysis. LDL, oxLDL (1, 2, or 4 h) (final 0.5 mg protein/mL) with and without HDL (final 2.5 mg protein/mL), or HDL alone (final 2.5 mg protein/mL) were incubated at 37°C for 90 min. After re-isolation by ultracentrifugation at a density of 1.063 g/mL, each sample was divided into top, middle, and bottom fractions. The PON1 activity and the amounts of phospholipids and cholesterol were measured (A). Correlation between the PON1 activities in top fractions, separated from LDL and oxLDL (1, 2 or 4 h) pre-incubated with HDL, and the diene levels in those LDL samples prior to incubation with HDL was analyzed by Spearman's rank correlation coefficient (B). Each top fraction (2 μL) was subjected to agarose gel electrophoresis and LDL was visualized by Fat Red 7B staining (C). The arrow shows the position of origin. Results are given as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. LDL, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. LDL+HDL, $^{\S}p < 0.05$, $^{\S\S}p < 0.01$.

levels of diene and LOOH; those values increased in a time-dependent manner. The REM of oxLDL (2 h) in Figure 2C (1.23) is similar to the REM of oxLDL obtained from coronary artery stenosis patients (1.3)[24]. Taken together, our experiments using these lipoprotein fractions reflect (patho)physiological conditions. The transferability of PON1 to LDL was then investigated using two re-isolation methods: gel filtration and ultracentrifugation.

Using gel filtration analysis, PON1 activity was obvious in the LDL fraction after the incubation of oxLDL (4 h) with HDL, although only slight PON1 activity was identified in the LDL faction after the incubation of native LDL with HDL (Fig. 3B). These results suggest that PON1 can transfer from HDL to LDL, and the oxidation of LDL emphasizes the transfer of PON1. PON1 transfer from HDL to normal LDL might mean that the normal LDL has been slightly oxidized during preparation and incubation and/or that PON1 only moves to sdLDL, as described previously[16]. Further, a few fractions at the LDL peak were concentrated and investigated by western blotting. Although the absolute PON1 activities of the HDL and the concentrated LDL fraction obtained from the mixture of oxLDL and HDL loaded on the gel were the same, the intensity of the PON1 band for the latter was markedly stronger than for the former (Fig. 3C), suggesting that a large part of the PON1 activity transferred to oxLDL was inactivated by oxidized lipids[25]. In contrast, only a weak apoA-I band was observed in the LDL fraction obtained from the mixture of oxLDL and HDL compared with the HDL sample (Fig. 3C). This implies that the increased level of PON1 in the LDL fraction obtained from the mixture of oxLDL and HDL was derived from the transfer from HDL. Because if the PON1 activity was due to the contamination by HDL or enlarged HDL, the rate of apoA-I amount to PON1 would be higher than that in LDL fraction from the mixture of oxLDL and HDL like as shown in HDL alone fraction (Fig. 3C). Whereas the transferability of apoA-I to oxLDL cannot be denied. The extremely small contamination can be ignored because a faint apoA-I band was also observed in the highly concentrated (approximately 50-fold) LDL fraction obtained from the mixture of native LDL and HDL. The peak of PON1 activity in the HDL fraction was earlier than its cholesterol peak regardless of pre-incubation with LDL or oxLDL (4 h), suggesting that PON1 is associated with larger HDL particles, which is consistent with a previous report[26].

The results from ultracentrifugation analysis were consistent with those from gel filtration analysis; PON1 activity in oxLDL, which was increased by pre-incubation with HDL depending on the oxidation time of LDL, indicated a significant correlation with the diene level, reflecting the degree of oxidation, in LDL just after the oxidation (Fig. 4A and 4B). The levels of TC and phospholipids in the middle fraction increased in oxLDL (4 h), regardless of incubation with HDL. The aggregation of LDL can be induced by excessive oxidation (72 h) using copper sulfate (5 μmol/L)[27]. A denser part of oxLDL, therefore, might move to the middle fraction. PON1 activity in oxLDL incubated with HDL was higher in the middle fraction than in the top fraction, suggesting that PON1 has a higher affinity for the denser, excessively oxidized LDL. In the bottom fraction, supposed to comprise HDL as well as serum, a wide range of PON1 activity was observed. PON1 has two genetic polymorphisms at positions 55 and 192, which are major determinants of its activity[28]. Although these polymorphisms were not examined in this study, the range of values in the bottom fraction could be due to genetic differences among volunteers. Interestingly, the negative surface charge in LDL was increased by oxidation using copper sulfate and might be slightly reduced by pre-incubation with HDL, regardless of the extent of oxidation in LDL (Fig. 4C). A previous study has shown that treatment of oxLDL with PON1 reduces the ability of oxLDL to induce monocyte–endothelial cell interactions[29]. Moreover, in the co-culture model of endothelial cells and smooth muscle cells, LDL from PON1-knockout mice induced more monocyte transmigration than LDL from wild-type mice[11]. Taken together, these facts imply the possibility that PON1 in HDL transfers to LDL and exerts an anti-oxidation effect. However, it is not clear

whether the addition of HDL prevents further oxidation of LDL during 90 min incubation and/or reduces the extent of oxLDL oxidation.

PON1 is known to bind to the surface phospholipids of HDL by anchoring its N-terminal helix structure[30], and a recent study has identified the specific PON1 residues (Leu-9, Tyr-185, and Tyr-293) that covalently crosslink with the phospholipids[31]. However, changes to the character of HDL could strongly affect the binding affinity and stability of PON1[32, 33]. In addition, our study also indicates that PON1 is easily released from HDL. When LDL is oxidized, propagation of lipid peroxidants and accumulation of LOOH occur in LDL. Then, the level of lysophosphatidylcholine (LPC), which is formed by the hydrolysis of phosphatidylcholine, increases and induces a truncation and modification of apolipoprotein B-100, ultimately leading to the acquisition of negative charge in LDL. Considering these characteristics of oxLDL, including a higher level of LPC and a more negative charge, oxLDL could approximate HDL in terms of physical properties. Although further experiments are required to confirm the suggestion, oxLDL could acquire a higher affinity for PON1 as a result of these changes in character.

In conclusion, this study indicates that HDL-associated PON1 transfers to LDL depending on the extent of oxidation, and suggests that the transferred PON1 could play an important role in anti-atherosclerotic function through its antioxidant effects.

Conflicts of interest

None.

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