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Effect of 3,4-dihydroxyphenylalanine on Cu^{2+}-induced Inactivation of HDL-associated Paraoxonase1 and Oxidation of HDL; Inactivation of Paraoxonase1 Activity Independent of HDL Lipid Oxidation

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Paraoxonase1 (PON1), one of HDL-asssociated antioxidant proteins, is known to be sensitive to oxidative stress. Here, the effect of endogenous reducing compounds on Cu^{2+}-mediated inactivation of PON1 was examined. Cu^{2+}-mediated inactivation of PON1 was enhanced remarkably by catecholamines, but not by uric acid or homocysteine. Furthermore, catecholamines such as 3,4-dihydroxyphenylalanine (DOPA), dopamine or norepinephrine were more effective than caffeic acid or pyrocatechol in promoting Cu^{2+}-mediated inactivation of PON1, suggesting the importance of dihydroxybenzene group as well as amino group. DOPA at relatively low concentrations showed a concentration-dependent inactivation of PON1 in a concert with Cu^{2+}, but not Fe^{2+}. The DOPA/Cu^{2+}-induced inactivation of PON1 was prevented by catalase, but not hydroxyl radical scavengers, consistent with Cu^{2+}-catalyzed oxidation. A similar result was also observed when HDL-associated PON1 (HDL-PON1) was exposed to DOPA/Cu^{2+}. Separately, it was found that DOPA at low concentrations (1–6 μM) acted as a pro-oxidant by enhancing Cu^{2+}-induced oxidation of HDL, while it exhibited an antioxidant action at ≥10 μM. In addition, Cu^{2+}-oxidized HDL lost the antioxidant action against LDL oxidation. Meanwhile, the role of DOPA/Cu^{2+}-oxidized HDL differed according to DOPA concentration; HDL oxidized with Cu^{2+} in the presence of DOPA (60 or 120 μM) maintained antioxidant activity of native HDL, in contrast to an adverse effect of DOPA at 3 or 6 μM. These data indicate that DOPA at micromolar level may act as a pro-oxidant in Cu^{2+}-induced inactivation of PON1 as well as oxidation of HDL. Also, it is proposed that the oxidative inactivation of HDL-PON1 is independent of HDL oxidation.

Keywords: PON1; Oxidative inactivation; Cu^{2+}; Hydroxyl radicals; DOPA; HDL oxidation

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; PON1, paraoxonase1; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; DMSO, dimethylsulfoxide; DOPA, 3,4-dihydroxyphenylalanine; HDL-PON1, HDL-associated paraoxonase1

INTRODUCTION

Paraoxonase1 (PON1), associated with HDL, is known to be an antioxidant protein capable of preventing LDL from oxidation.[1–3] A support for this contention is provided by animal model, in which a lower serum level of PON1, exclusively localized to HDL, is associated with a greater susceptibility of LDL to oxidation and an increased risk of atherosclerosis.[4] Moreover, there have been reports supporting the intimate association between the low activity of plasma PON1 and cardiovascular diseases.[5,6] Therefore, the reduction of HDL-associated PON1 (HDL-PON1) activity was supposed to lead to the decrease in the anti-atherogenicity of HDL.

Concerning the loss of HDL-PON1 activity under oxidative conditions, it was suggested that HDL-associated PON1 might undergo the oxidative inactivation during lipid peroxidation of HDL.[3] Related studies[3,7,8] demonstrated that PON1 was susceptible to hydrogen peroxide, HOCl, or hydroxyl radicals-generating systems such as

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activated macrophage, gamma irradiation, or ascorbate/Cu^{2+} system. Especially, PON1 was highly susceptible to Cu^{2+}-catalyzed oxidation employing ascorbate/Cu^{2+} system, whereas no remarkable inactivation of PON1 was demonstrated by thiol/Cu^{2+} system.[8] Additional system for Cu^{2+}-catalyzed oxidation would be catecholamine/Cu^{2+} couple, where catecholamine might act as a pro-oxidant to generate H_2O_2 and hydroxyl radicals.[9] Nonetheless, there has been no attempt to test catecholamine as a pro-oxidant in Cu^{2+}-catalyzed inactivation of PON1 as well as oxidation of HDL lipid. Instead, there is a report that DOPA acts as an antioxidant in Cu^{2+}-induced oxidation of LDL, where apolipoprotein B is implicated in the oxidation.[10] Thus, it seems that the effect of DOPA on the oxidation of lipoproteins may differ according to the target, protein or lipid, for the initiation of oxidation. In the meantime, there have been reports[11,12] that the administration of DOPA can elevate the plasma level of homocysteine. Since homocysteine was reported to promote the LDL oxidase activity of ceruloplasmin containing Cu^{2+},[13] it is conceivable that the oxidation of lipoproteins could be enhanced in DOPA-induced hypercysteinemia. In turn, the oxidative modification of lipoproteins could affect the function of lipoproteins, anti-atherogenicity of HDL and atherogenicity of LDL.

The aim of our study, therefore, is to determine how DOPA at low physiological concentrations, in combination with copper ion, affects the HDL-PON1 activity as well as the oxidation of HDL lipid. In addition, we also investigated whether the oxidative inactivation of HDL-PON1 occurs as a result of HDL oxidation.

MATERIALS AND METHODS

Chemicals and Reagents

Compounds including various catecholamines were obtained from Sigma Chemical Co. (St Louis. MO, USA). Metal ions including cupric sulfate or ferrous sulfate were of analytical grade.

Assay and Preparation of Purified Paraoxonase1 (PON1)

PON1 activity was determined by monitoring the formation of phenol at 270 nm in 0.5 ml of Tris buffer (50 mM, pH 7.4) containing 1 mM $CaCl_2$ and 10 mM phenyl acetate as described.[3,8,14] One unit of enzyme activity is expressed as one micromole of phenol produced per minute. PON1 was purified from human plasma through sequential chromatographies according to the published procedures[3,8,14], and the phenotype was characterized as reported previously.[15]

Preparation of HDL-PON1 or LDL

Serum LDL and HDL were isolated from fasted normolipidemic human volunteers by ultracentrifugation at a density of 1.019–1.063 g/ml as described.[8,16] LDL or HDL was dialyzed at 4°C against 10 mM PBS buffer (pH 7.4) and the same buffer containing 1 mM Ca^{2+}, respectively. The HDL preparation was used as HDL-PON1.

Effect of Various Oxidants on the Activity of Purified PON1 or HDL-PON1

Purified PON1 (0.5 unit) was preincubated with each oxidant system at 38°C in 0.1 ml of 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+}, and an aliquot (20 μl) was taken for the assay of remaining activity. In further study, purified PON1 (0.5 unit) was incubated with DOPA (1–100 μM) in the presence of 3 μM Cu^{2+} in the same buffer at 38°C for 30 min. Additionally, PON1 was incubated with DOPA (20 μM) in the presence of various concentrations of Cu^{2+} or Fe^{2+}. Separately, to see the inactivation of HDL-PON1 by DOPA/Cu^{2+}, HDL-PON1 (0.5 unit) was incubated with 20 μM DOPA in the presence of Cu^{2+} (5 μM) in the same buffer for various times. Additionally, HDL-PON1 was incubated with DOPA (1–200 μM) in the presence of 5 μM Cu^{2+} in the same buffer for 30 min. In the protection study, PON1 (0.5 unit) was incubated with 20 μM DOPA and 3 μM Cu^{2+} in the presence of each candidate compound in the same buffer as described above.

Concentration-dependent Effect of DOPA on Cu^{2+}-induced Oxidation of HDL and Inactivation of HDL-PON1

HDL (0.2 mg/ml) was incubated with 5 μM Cu^{2+} in 0.2 ml of 10 mM PBS buffer (pH 7.4) at 38°C for 60 min in the presence or absence of DOPA (1–30 μM), and the reaction was terminated by adding 500 μM butylated hydroxyltoluene and 1 mM EDTA. The TBARS value and PON1 activity were determined as described previously.[3,16] Unless otherwise described, values are expressed as means ± S.D. of triplicate experiments.

Effect of Native HDL or Oxidized HDL on Cu^{2+}-induced Oxidation of LDL

Oxidized HDL was prepared from the preincubation of HDL (1 mg protein/ml) with Cu^{2+} (5 μM) for 1 h in the presence or absence of DOPA (0–120 μM), followed by extensive dialysis in 10 mM PBS buffer (pH 7.4) at 4°C. Native HDL or oxidized

HDL (0.1 mg protein/ml) was incubated with LDL (0.1 mg protein/ml) in the presence of Cu^{2+} (0.5 μM) at 37°C, and 3 h later the lipid oxidation was assessed by the measurement of the peroxide value as described previously.[17,18] The inhibition of LDL oxidation by HDL under these conditions was calculated according to the formula:

$$\text{Inhibition of LDL oxidation (\%)} = [(A_{HDL} + A_{LDL}) - A_{HDL+LDL}] \times 100/(A_{HDL} + A_{LDL})$$

where $(A_{HDL} + A_{LDL})$ is the sum of the absorbances observed for HDL and LDL incubated separately with Cu^{2+} for 3 h, and $(A_{HDL+LDL})$ is the absorbance observed during co-incubation of LDL with HDL and Cu^{2+} for 3 h. Analysis of variance (ANOVA) and Duncan's multiple range test were performed using Statistical Analysis System software (SAS, Cary, NC, USA) to determine a significance of difference of values at $P < 0.01$ ($n = 5$) where indicated.

RESULTS

Susceptibility of Purified PON1 or HDL-PON1 to the Combination of Cu^{2+} and Endogenous Reductants

When purified PON1 was exposed to Cu^{2+} (3 μM) in the presence of each reducing compound in 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+} at 38°C for 30 min, it was observed (Table I) that Cu^{2+}-mediated inactivation of purified PON1 was enhanced prominently by catecholamines (20 μM), in contrast to caffeic acid or pyrocatechol showing a low enhancing effect. In addition, reducing compounds such as homocysteine or uric acid failed to display any substantial effect on Cu^{2+}-mediated inactivation. A similar result was also obtained (Table I) when HDL-PON1 was exposed to catecholamines (20 μM) and Cu^{2+}, although a higher concentration of Cu^{2+} (5 μM) was required for the same degree of inactivation as observed with purified PON1. Thus, catecholamines, in combination with copper ion, were found to be effective in inactivating PON1 activity, suggesting that dihydroxybenzene moiety and amino group in the structure of catecholamines are crucial in promoting Cu^{2+}-mediated inactivation of PON1. In the subsequent experiment, where the time course for the oxidative inactivation of purified PON1 by Cu^{2+} (3 μM), coupled with DOPA (20 μM), was monitored, PON1 was found to be inactivated in a time-dependent manner (Fig. 1A); after 60 min, the remaining activity was around 25% of control value. Separately, when PON1 was incubated with DOPA of various concentrations (3–100 μM) in the presence of Cu^{2+} (3 μM), DOPA promoted Cu^{2+}-catalyzed inactivation of PON1 in a dose-dependent manner (Fig. 1B); in combination with Cu^{2+}, DOPA at 10 and 30 μM showed 43 and 74% inactivation, respectively. To see the selectivity of metal ion in inactivating PON1, Cu^{2+} or Fe^{2+} of various concentrations was incubated with PON1 in the presence of DOPA (20 μM). As exhibited in Fig. 2, Cu^{2+} (1–10 μM), in combination with DOPA (20 μM), demonstrated a concentration-dependent inactivation of PON1 with a minimal effective concentration of Cu^{2+} being as low as 1 μM. In contrast, no apparent inactivation was achieved by Fe^{2+} up to 10 μM, in combination with DOPA. Next, in an attempt to define the mechanism by which DOPA, in combination with Cu^{2+}, inactivated the PON1, it was observed (Table II) that catalase expressed a remarkable (>90%) protection. Meanwhile, hydroxyl radical scavengers[19,20] such as mannitol, DMSO or quercetin failed to show any significant protection against the DOPA/Cu^{2+}-induced inactivation of PON1. In addition, quinone compounds such as 1,4-benzoquinone had no effect on PON1 activity. Taken together, these data may support the view[19,20] that Cu^{2+}-bound hydroxyl radicals may be responsible for the inactivation of PON1 by DOPA/Cu^{2+}. Generally, metal-catalyzed oxidative inactivation of some enzymes containing metal-binding sites at active center is known to be prevented by their substrates.[20] However, phenylacetate and paraoxon, substrates of PON1, exhibited no remarkable protection (<15%) at concentrations used (Table II), suggesting that the active center of PON1 may not be a major target for Cu^{2+} oxidation. Instead, the DOPA/Cu^{2+}-mediated inactivation of PON1 was partly (approximately 40%) prevented by oleic acid, similar to the previous observation with ascorbate/Cu^{2+}-induced inactivation.[21] Thus, the Cu^{2+} binding site responsible for

TABLE I Effect of various oxidants on the activity of purified paraoxonase1 (PON1)

Treatment	Concentration (μM)	Enzyme activity (%) Purified PON1	HDL-PON1
Cu^{2+}	3	92.5 ± 5.1	97.4 ± 6.4
	5		86.5 ± 5.1
+DOPA	20	38.8 ± 5.1	38.7 ± 4.6*
+Dopamine	20	46.4 ± 3.9	39.2 ± 3.6*
+Noradrenaline	20	50.2 ± 5.2	43.6 ± 2.2*
+Tyrosine	20	99.5 ± 3.5	83.5 ± 5.1*
+Pyrocatecol	20	91.4 ± 4.2	–
+Homocysteine	50	98.5 ± 5.7	–
+Uric acid	100	95.5 ± 4.8	–
+Caffeic acid	20	91.6 ± 2.6	92.6 ± 3.1*

PON1 or HDL-PON1 (5 units/ml) was incubated with each oxidant system at 38°C in 0.1 ml of 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+}, and 30 min later an aliquot (20 μl) was taken for the assay of remaining activity. Data are the mean ± SD of triplicate assays, presented as a percentage of control activity. –, Not determined. *, 5 μM Cu^{2+} was included.

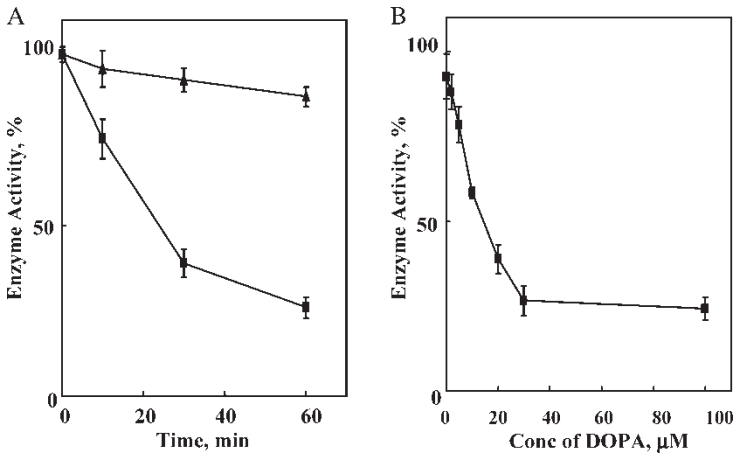

FIGURE 1 Oxidative inactivation of PON1 by DOPA/Cu^{2+}. (A) PON1 (5 units/ml) was incubated with 3 μM Cu^{2+} in the presence or absence of DOPA (20 μM) at 38°C in 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+} for 10, 30, or 60 min. ▲, Cu^{2+} alone; ■, DOPA/Cu^{2+}. (B) PON1 (5 units/ml) was incubated with DOPA (1–100 μM) in the presence of 3 μM Cu^{2+} at 38°C in 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+}. Data are expressed as a mean ± SD (bar) value of triplicate assays, presented as a percentage of control activity.

DOPA/Cu^{2+}-induced inactivation of PON1 is supposed to be the same as that for ascorbate/Cu^{2+}-mediated one.[21]

Oxidative Inactivation of HDL-PON1 by DOPA/Cu^{2+}

In an attempt to see the possible inactivation of PON1 by DOPA in a concert with Cu^{2+} *in vivo* system, we examined the oxidative inactivation of HDL-associated PON1 activity. First, when HDL-PON1 (5 units/ml) was incubated with DOPA (20 μM)/Cu^{2+} (5 μM) at 38°C, PON1 activity decreased in a time-dependent pattern; the activity decreased to approximately 20% of control value after 60 min (Fig. 3A), in contrast to 70% activity remaining after the exposure to Cu^{2+} (5 μM) alone. In related study, when HDL-PON1 was incubated with DOPA (20 μM) and Cu^{2+} (3 μM), the remaining activity after 30 min and 60 min was approximately 72 and 64% of control (data not shown). Thus, 3 μM Cu^{2+}, in combination with DOPA (20 μM), was not sufficient to achieve more than 50% inactivation of HDL-PON1. Thus, compared to purified PON1 (Fig. 1), HDL-PON1 was less sensitive to DOPA/Cu^{2+}-catalyzed oxidation. Separately (Fig. 3B), when the concentration-dependent effect of DOPA on Cu^{2+} (5 μM)-induced inactivation of HDL-PON1 was examined for 30 min, it was found that DOPA at concentrations as low as 3 μM enhanced Cu^{2+}-mediated inactivation of HDL-PON1 significantly. But, the increase of DOPA concentration beyond 30 μM did not further enhance

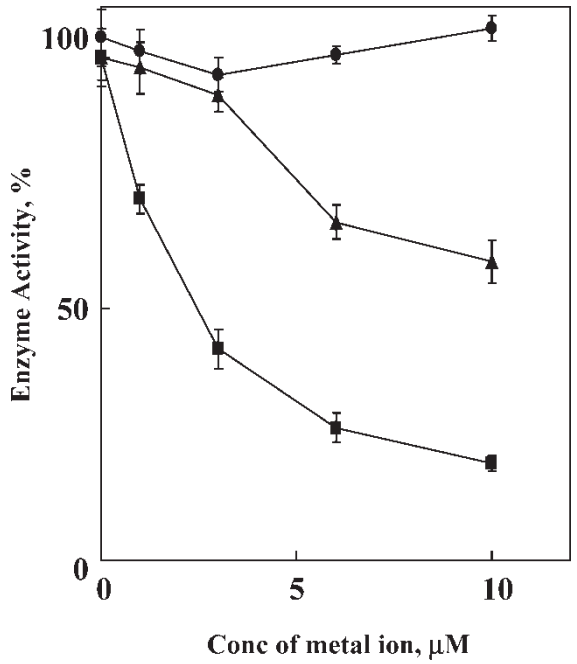

FIGURE 2 Concentration-dependent effect of divalent metal ion on the inactivation of PON1 by DOPA/metal ion. PON1 (5 units/ml) was incubated with DOPA (20 μM) in the presence or absence of various concentrations (1–10 μM) of Cu^{2+} or Fe^{2+} in 0.1 ml of 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+} at 38°C for 30 min; ▲, Cu^{2+} only; ■, DOPA/Cu^{2+}; •, DOPA/Fe^{2+}. Data are expressed as a mean ± SD (bar) value of triplicate sets.

TABLE II Prevention against the inactivation of purified PON1 by DOPA/Cu^{2+}

Treatment	Concentration	Enzyme activity (%)
Cu^{2+}/DOPA	3 μM/20 μM	38.8 ± 5.1
+Catalase	300 unit/ml	92.5 ± 10.2
+Ethanol	0.2 M	53.9 ± 2.3
+Mannitol	0.2 M	43.4 ± 2.8
+DMSO	0.2 M	46.8 ± 2.3
+Oleic acid	10 μM	64.3 ± 6.6
+Paraoxon	1 mM	53.1 ± 5.5
+Phenylacetate	1 mM	52.6 ± 4.0
+Quercetin	10 μM	43.5 ± 4.8
+Ascorbate	30 μM	37.3 ± 5.4

Purified PON1 (5 units/ml) was incubated with DOPA (20 μM) and Cu^{2+} (3 μM) in the presence of each protectant in 0.1 ml of 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+} as described in Table I.

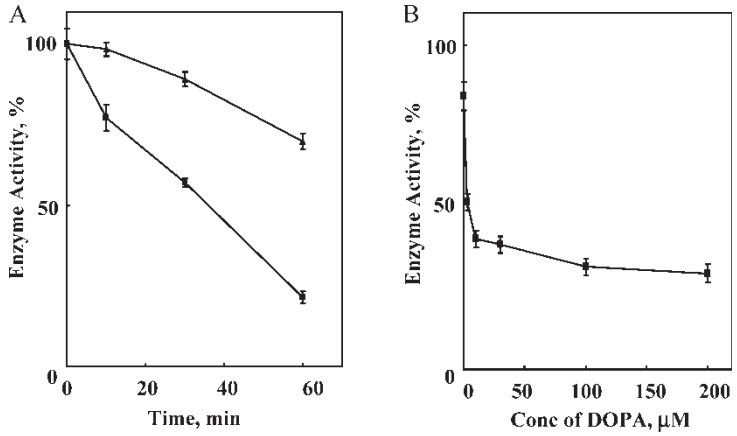

FIGURE 3 Oxidative inactivation of HDL-PON1 by DOPA/Cu^{+2}. (A) HDL-PON1 (5 units/ml) was incubated with 5 μM Cu^{2+} in the presence or absence of DOPA (20 μM) at 38°C in PBS buffer (pH 7.4) containing 1 mM Ca^{2+} for 10, 30, or 60 min. ▲, Cu^{2+} alone; ■, DOPA/Cu^{2+}. (B) HDL-PON1 (5 units/ml) was incubated with DOPA (1–200 μM) in the presence of 5 μM Cu^{2+} in the same buffer (pH 7.4) containing 1 mM Ca^{2+} at 38°C for 30 min. Data are expressed as a mean ± SD (bar) value of triplicate assays.

the inactivation degree. In addition, it was found that DOPA/Cu^{2+}-mediated inactivation of HDL-PON1 was prevented almost fully (> 90%) by catalase, but not hydroxyl radical scavengers.

Relationship between HDL Oxidation and PON1 Inactivation

To see whether the Cu^{2+} -induced oxidation of HDL affects the activity of HDL-associated PON1, HDL was exposed to 5 μM Cu^{2+} in the presence of DOPA (1–30 μM), and then the aliquot was taken for the determination of TBARS value and PON1 activity. Figure 4 indicates that the inclusion of DOPA at low concentrations (1–6 μM) increased both

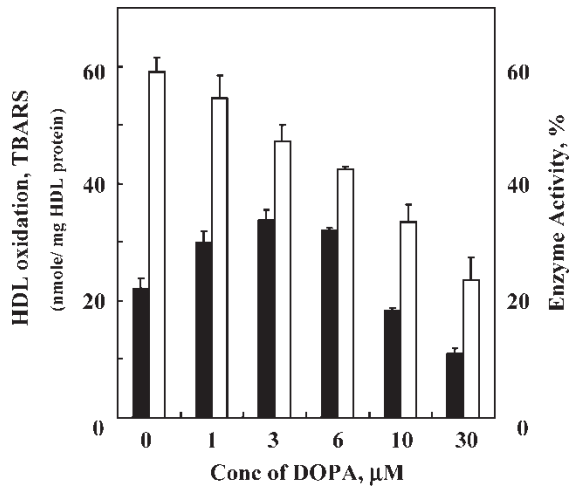

FIGURE 4 Effect of DOPA on Cu^{2+}-induced oxidation of HDL and inactivation of HDL-PON1 activity. HDL (0.2 mg protein/ml) was incubated with 5 μM Cu^{2+} in 0.2 ml of 10 mM PBS buffer (pH 7.4) containing 1 mM Ca^{2+} at 38°C for 60 min in the presence or absence of DOPA (1–30 μM), and the reaction was terminated by adding 500 μM butylated hydroxyltoluene and 1 mM EDTA. The TBARS value (■) and PON1 activity (□) were determined as described in "Materials and methods". Data are expressed as a mean ± SD (bar) value of triplicate assays.

Cu^{2+}-induced oxidation of HDL and Cu^{2+}-mediated PON1 inactivation, consistent with a pro-oxidant role of DOPA. This appears to support a positive relationship between HDL oxidation and PON1 inactivation. However, higher concentrations of DOPA (≥10 μM), which continued to enhance Cu^{2+}-mediated inactivation of HDL-PON1, tended to reduce Cu^{2+}-mediated oxidation of HDL lipid, complying with its antioxidant action. Therefore, it is supposed that the oxidation of HDL is not necessarily related to the loss of PON1 activity. In further support of this, Cu^{2+}-induced oxidation of HDL was prevented almost completely (> 90%) by quercetin (10 μM), which had no protective effect on Cu^{2+}-induced inactivation of HDL-PON1 (data not shown).

Effect of Native HDL or Oxidized HDL on LDL Oxidation

To assess the physiological relevance of the effect of DOPA on Cu^{2+}-mediated oxidation of HDL, the ability of native HDL or oxidized HDL (HDL-0, -3, -6, -20, -60 and -120) to protect LDL from Cu^{2+} (0.5 μM)-mediated oxidation was evaluated by the measurement of the peroxide value. As shown in Fig. 5, the oxidized HDL (HDL-0), which was prepared from the exposure of HDL to Cu^{2+} (5 μM) in the absence of DOPA, expressed only a slight protection (26%), while native HDL was highly effective (67% protection) in protecting LDL from the oxidation. Meanwhile, the inclusion of DOPA/Cu^{2+}-oxidized HDL preparations (HDL-20, -60 or -120) decreased lipid oxidation of LDL progressively with increasing concentrations of DOPA (20, 60 or 120 μM). However, HDL-3 or -6, which was prepared with DOPA of low concentrations (3 or 6 μM), was less efficient in preventing LDL oxidation, compared to HDL-0 ($P < 0.01$). Thus, it is suggested that

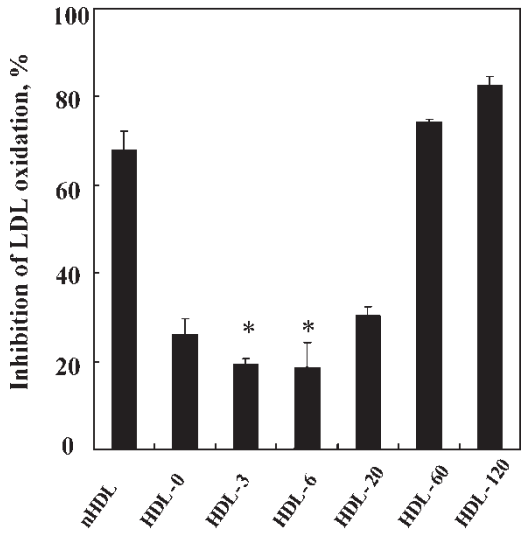

FIGURE 5 Inhibitory effect of native HDL or oxidized HDL on Cu^{2+}-induced oxidation of LDL. Native HDL or each oxidized HDL preparation (0.1 mg protein/ml) was incubated with LDL (0.1 mg protein/ml) in the presence of Cu^{2+} (0.5 μM) in 10 mM PBS (pH 7.4) at 38°C, and 3 h later an aliquot was taken for the assay of lipid peroxidation, based on the measurement of the peroxide value. HDL-0, -3, -6, -20, -60 and -120 indicate the HDL oxidized with Cu^{2+} (5 μM) in the presence of DOPA at 0, 3, 6, 20, 60, and 120 μM, respectively, with corresponding PON1 activity of 59, 47, 42, 33, 22, and 21%, compared to native HDL-PON1 activity (5 units/mg protein). Data are expressed as a mean ± SD ($n = 5$), *, $P < 0.01$ vs. HDL-0.

the ability of DOPA/Cu^{2+}-oxidized HDL in preventing LDL oxidation may be related to the oxidation state of HDL, but not PON1 activity (Fig. 4).

DISCUSSION

The present study indicates that PON1 is among the enzymes susceptible to DOPA/Cu^{2+}-mediated oxidation;[9] the concentration of DOPA, required for effective inactivation of purified PON1, is relatively low, and close to the physiological level.[22,23] The protective effect of catalase, but not hydroxyl radical scavengers, on DOPA/Cu^{2+}-mediated inactivation of PON1 is consistent with the notion that an inactivating intermediate may be Cu^{2+}-bound hydroxyl radical as well established for Cu^{2+}-catalyzed oxidation.[19,20] Although DOPA/Cu^{2+}-induced inactivation of PON1 is similar to ascorbate/Cu^{2+}-induced inactivation,[21] the DOPA system may differ from the ascorbate system in that DOPA/Fe^{2+} exhibited no inactivation of PON1, in contrast to some inactivation of PON1 by ascorbate/Fe^{2+}.[8] Thus, compared to ascorbate, DOPA seems to interact more selectively with copper ion, which was proposed to associate with PON1.[21] This might be supported by the structure-activity relationship for the effect of dihydroxyphenyl compounds; both ortho-dihydroxybenzene moiety and amino group of dihydroxyphenyl compounds are crucial for the enhancement of Cu^{2+}-catalyzed inactivation of PON1 whereas only dihydroxybenzene moiety is required for the Cu^{2+}-dependent oxidation of catechols.[24] Therefore, it is supposed that the DOPA/Cu^{2+}-induced inactivation of PON1 may occur at a specific site of PON1, which promotes the coordination of DOPA with copper ions. In turn, hydroxyl radicals generated from the redox cycle between Cu^{2+} and DOPA may modifiy amino acid residues close to Cu^{2+}-binding site. The binding site of Cu^{2+} in DOPA/Cu^{2+}-induced inactivation of PON1 might be the same as that proposed for Cu^{2+}-catalyzed inactivation of PON1.[8,21] A support for this may come from the common protection by oleic acid against DOPA/Cu^{2+}-mediated inactivation of PON1 and Cu^{2+} -induced inactivation of PON1.[21]

Although there is a controversy about the bioavailability of free copper ion in blood, it is possible that ceruloplasmin may participate in the supply of copper ions to HDL, which shows an affinity toward copper ions,[25] as demonstrated from the oxidase activity of ceruloplasmin-associated copper ion in the LDL oxidation.[13] In this regard, the interaction between ceruloplasmin and HDL in some pathological conditions, where the release of Cu^{2+} is facilitated, could enhance the local concentration of copper ions. Further, the possible implication of copper ions in the atherosclerosis was suggested from evidences of metal ion-catalyzed oxidation in atherosclerotic lesions.[26,27] A very recent report[28] provides an evidence for the elevation of copper level in diseased intima samples. Separately, there have been reports that the DOPA administration can elevate the plasma level of homocysteine, an independent risk factor for vascular diseases.[11] Since the serum level of ceruloplasmin is known to be frequently associated with hyperhomocysteinemia,[12,29] the DOPA/Cu^{2+}-mediated inactivation of HDL-PON1 could be greater in DOPA-induced hyperhomocysteinemia of some Parkinson's disease patients.[29]

Previous data have suggested that the oxidation of HDL might lead to the loss of HDL-associated PON1 activity.[3,7,16] A recent study shows that the formation of protein hydroperoxide is coupled tightly to lipid oxidation during Cu^{2+}-mediated LDL oxidation.[30] Nonetheless, there is no positive evidence for the idea that the oxidation of HDL lipid may lead to the inactivation of HDL-PON1. Although HOCl was also observed to inactivate HDL-associated PON1, the PON1 inactivation was independent of the oxidation of HDL lipid.[8] In addition, there was no significant loss of PON1 activity associated with HDL membrane which was oxidized with peroxynitrite or lipoxygenase (data not shown). Although the products from Cu^{2+}-induced LDL peroxidation had been reported to cause a partial loss of purified PON1 activity,[16]

HDL-associated PON1 activity was resistant to these lipid products.[8,21] Thus, the loss of HDL-PON1 activity was supposed to be independent of HDL oxidation. Furthermore, Cu^{2+}-induced inactivation of HDL-PON1 was enhanced by ascorbate,[8,21] while it prevented against Cu^{2+}-induced oxidation of HDL.[31] Likewise, present findings showed that Cu^{2+}-induced inactivation of HDL-PON1 was enhanced by DOPA ($\geq 10\,\mu M$), which exerted an antioxidant action in Cu^{2+}-mediated oxidation of HDL. Noteworthy, the effect of DOPA on Cu^{2+}-mediated oxidation of HDL differed according to the DOPA concentration; DOPA played a pro-oxidant role at lower concentrations ($1-6\,\mu M$), and anti-oxidant one at higher concentrations. Therefore, considering the micromolar DOPA concentration in some physiological conditions,[22,23] DOPA would more likely play as pro-oxidant rather than anti-oxidant in Cu^{2+}-mediated oxidation of HDL *in vivo* system. Nonetheless, the pro-oxidant effect of DOPA at low concentrations in Cu^{2+}-mediated oxidation of HDL is in contrast to the antioxidant action of DOPA against Cu^{2+}-induced oxidation of LDL.[10] Although this discrepancy was not further elucidated, it may be explained by the notion that the effect, pro-oxidant or antioxidant, of DOPA may differ according to the mechanism for the initiation of Cu^{2+}-mediated lipid oxidation; LDL oxidation is known to be initiated at apolipoprotein B100, while the oxidation of HDL proceeds through lipid peroxidation.[10,17,25]

In addition to a role in reverse cholesterol transport, HDL has been recognized to have an antioxidant action to protect LDL from oxidative modification.[32] Concerning the antioxidant action of HDL, there have been reports[1–3,5,6,16] that PON1 activity in HDL may be responsible for its antioxidant action. In present study, however, the antioxidant function of oxidized HDL preparation seems to be governed by lipid oxidation state, but not remaining PON1 activity. One explanation for this might be that the activiy of PON1 is not necessarily related to its antioxidant action; the oxidative destruction of histidine residue, responsible for the catalysis, after the exposure of PON1 to Cu^{2+} oxidation was not sufficient to account for the loss of antioxidant action of PON1, and the cysteine residue, resistant to Cu^{2+} oxidation, was also responsible for the antioxidant action.[8,16] Then, the mass, rather than the activity, of PON1 may be more important for antioxidant action of HDL-associated PON1. Such a view had been proposed by earlier study using EDTA-treated PON1.[33] Alternatively, the ability of HDL to protect LDL from Cu^{2+} oxidation may be linked at least partially to other proteins such as apolipoprotein A1[34], which is known to participate in the prevention against LDL oxidation. This might be supported by present finding that the oxidized HDL, prepared from the exposure of HDL to Cu^{2+} in combination with DOPA ($\geq 10\,\mu M$), exerted an antioxidant action against LDL oxidation, independent of PON1 inactivation. Nevertheless, the possibility is not ruled out that the protective action of the oxidized HDL may be due to the antioxidant function of not only apolipoprotein A1 but also cysteine residue in PON1 molecule. In addition, protein-bound DOPA, generated during HDL oxidation, might also participate in the inhibition of LDL oxidation as had been observed with peptide-bound DOPA.[10] However, protein-bound DOPA might act as an pro-oxidant in the presence of Cu^{2+} as demonstrated in oxidative cleavage of DNA.[35] Thus, the role of protein-bound DOPA may differ according to the environmental conditions such as oxidation target, Cu^{2+} concentration or amount of bound DOPA. In this regard, the role of protein-bound DOPA present in oxidized LDL[36] or atherosclerotic plaques[26] is to be examined under physiological conditions.

In summary, our results demonstrate that DOPA at physiological concentrations, in combination with copper ions, may augment the loss of the HDL-PON1 activity as well as the oxidation of HDL lipid, and additionally, suggest that the oxidative inactivation of HDL-PON1 by DOPA/Cu^{2+} may be independent of HDL oxidation. The practical effect of DOPA on HDL components in blood containing a higher level of Cu^{2+} remains to be elucidated.

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