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Doxazosin Treatment and Peroxidation of Low-Density Lipoprotein among Male Hypertensive Subjects: *In Vitro* and *Ex Vivo* Studies

*Ingeborg R. Brude, Christian A. Drevon, Kjersti Viken, Jon E. Arnstad, Kolbjørn N. Valnes and Marit S. Nenseter**

INSTITUTE FOR NUTRITION RESEARCH, UNIVERSITY OF OSLO, P.O. BOX 1046, BLINDERN, 0316 OSLO, NORWAY

ABSTRACT. Doxazosin is an antihypertensive drug that gives rise to 6- and 7-hydroxydoxazosin during hepatic metabolism. The structures of the hydroxymetabolites suggest that they may possess antioxidative properties. The aim of the present study was to examine whether doxazosin and 6- and 7-hydroxydoxazosin were able to scavenge free radicals and whether these compounds might protect low-density lipoprotein (LDL) against *in vitro* and *ex vivo* oxidation. Both 6- and 7-hydroxydoxazosin showed radical scavenging capacity as assessed by measuring scavenging of 1,1-diphenyl-2-picrylhydrazyl radicals. *In vitro* incubation with 10 μM 6- and 7-hydroxydoxazosin significantly reduced human mononuclear cell-mediated oxidation of LDL, measured as the formation of lipid peroxides and the relative electrophoretic mobility of LDL (to 10 and 6% of the control, respectively). Furthermore, formation of conjugated dienes in LDL during Cu^{2+}-induced oxidation was significantly reduced in the presence of 5 μM 6- and 7-hydroxydoxazosin (to 28% of t_{max} [time to maximum] of control). However, treatment of hypertensive patients with increasing doses of doxazosin (from 1 to 8 mg/day) for 8 weeks altered neither Cu^{2+}-catalyzed, 2,2′azobis-(2-amidinopropane hydrochloride)-initiated, nor cell-mediated oxidation of patient LDL *ex vivo*. Furthermore, the total antioxidative capacity of plasma was unaffected by treatment. In conclusion, the present study shows that 6- and 7-hydroxydoxazosin have radical scavenging properties and protect LDL against *in vitro* oxidation. However, treatment of hypertensive male subjects with increasing doses of doxazosin for 8 weeks did not affect *ex vivo* oxidation of LDL. BIOCHEM PHARMACOL **58**;1:183–191, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. doxazosin; human mononuclear cells; hypertensive; LDL; oxidation; lipid peroxidation

Hypertension constitutes a major risk factor for stroke and cardiovascular disease in addition to several other risk factors such as smoking and hypercholesterolemia [1]. Thus, LDL† from hypertensive patients has been suggested to be more easily oxidized than LDL isolated from healthy subjects [2]. The ideal cardioprotective and antihypertensive drug should therefore, in addition to lowering elevated blood pressure, prevent or retard the development of atherosclerosis. Antihypertensive drugs such as calcium antagonists [3] and lipophilic β-blockers [4] may protect LDL against *in vitro* oxidation. Thus, the possible antioxidative capacity of antihypertensive agents may contribute to prevent cardiovascular diseases via this mechanism.

Doxazosin is a selective α_1-adrenergic antagonist which reduces blood pressure through relaxation of peripheral blood vessels [5]. In addition, doxazosin has been reported to favorably affect serum lipids in humans [5] as well as in animals [6, 7] by increasing plasma concentrations of high-density lipoprotein cholesterol and by lowering concentrations of LDL cholesterol and triacylglycerol. Interestingly, doxazosin may inhibit development of atherosclerotic plaques in hypercholesterolemic hamsters [7] and in cholesterol-fed rabbits [8]. However, the mechanism by which doxazosin exerts its antiatherogenic effect is not fully understood. Reduced accumulation of cholesterol and reduced formation of atherosclerotic plaques in cholesterol-fed rabbits was not associated with changes in serum lipids [8]. Moreover, Foxall *et al.* found that a 20-mg/kg dose of doxazosin decreased the lesion area in hamsters as compared to a 10-mg/kg dose, without further effect on plasma lipid concentration [7]. These findings suggest possible additional effects of doxazosin on lesion formation, occurring independently of, or in concert with, a lowering of lipoprotein cholesterol.

Doxazosin (Fig. 1) undergoes extensive hepatic metabolism in part by hydroxylation, giving rise to 6- and 7-hydroxydoxazosin. The structure of these substances may suggest that they possess antioxidative properties, potentially protecting LDL against oxidative modification *in vivo*. Chait *et al.* reported reduced Cu^{2+}-induced oxidation of

* Corresponding author: Dr. Marit S. Nenseter, Lipidklinikken, Cardiovascular Research Center, University Hospital, Rikshospitalet, 0027 Oslo, Norway. Tel. +47-22867010, ext 6851; FAX +47-22868458.

† *Abbreviations:* AAPH, 2,2′-azobis-(2-amidinopropane hydrochloride); ABTS, 2,2′-azino-di-(3-ethylbenzhiazoline sulphonate); DPPH, 1,1-diphenyl-2-picrylhydrazyl; Ham's F-10, Ham's nutrient mixture F-10; LDL, low-density lipoprotein; and PMA, phorbol 12-myristate 13-acetate.

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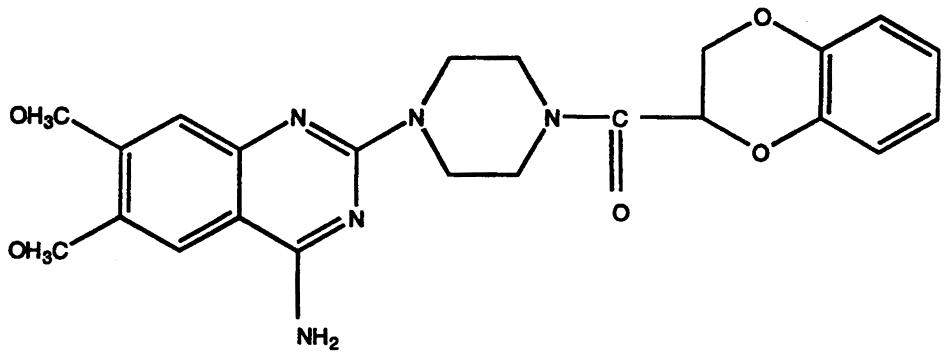

FIG. 1. Molecular structure of doxazosin. A hydroxyl group is attached to a phenol ring in doxazosin, giving 6- and 7-hydroxydoxazosin, respectively.

LDL *in vitro* exposed to 6- and 7-hydroxydoxazosin [9]. The authors suggested that 6- and 7-hydroxydoxazosin might exert their antioxidative effect in the aqueous milieu of the lipoprotein, similar to vitamin C, and might be important for the prevention of atherosclerosis in hypertensive individuals.

In the present study, the radical scavenging capacity of the metabolites and their effect on cell-mediated as well as Cu^{2+}-catalyzed oxidation of LDL were studied during *in vitro* incubation with doxazosin and its 6- and 7-hydroxymetabolites. Furthermore, the effect of treatment with doxazosin on the total antioxidative capacity of plasma and *ex vivo* oxidation of LDL was studied in male hypertensive subjects.

MATERIALS AND METHODS

Materials

Carduran capsules, doxazosin, and its 6- and 7-hydroxymetabolites were provided by Pfizer Research Laboratories. Ham's nutrient mixture Ham's F-10 and gentamicin were obtained from BioWhittaker. PMA and butylated hydroxytoluene were purchased from Sigma Chemical Co. Dynabeads M-450 antiglycophorin A were purchased from Dynal AS, and AAPH from Polysciences Inc. Polymorphprep was delivered by Nycomed Pharma AS, and tissue culture dishes were supplied by Costar. Bicinchoninic acid protein assay was obtained from Pierce Laboratories Inc. Kits for determination of total cholesterol, triacylglycerol, and phospholipids in LDL were purchased from Bio Merieux, a kit for determination of lipid peroxides was from Kamiya Biochemical, and a kit for detection of total antioxidative capacity of plasma was delivered from Randox Laboratories Ltd. Paragon lipoprotein electrophoresis agarose gels were supplied by Beckman Instruments, Inc. Vacutainer® tubes for blood collection were delivered by Becton Dickinson.

In Vitro *Incubation with Doxazosin and 6- and 7-Hydroxydoxazosin*

SCAVENGING OF DPPH RADICAL. DPPH was dissolved in methanol to obtain a concentration of approximately 45 μg/mL, corresponding to an absorbance of approximately 1.3 at 517 nm. Pyrogallol, a potent radical scavenger, was dissolved in methanol to a concentration of 2% (w/v) and used as a positive control for scavenging. The decline in radical concentration in the presence of 6- or 7-hydroxydoxazosin was determined by adding 100 μL test substance, dissolved to indicated concentrations in methanol, to 100 μL DPPH solution, and the absorbance at 517 nm was continuously monitored for 15 min in a Beckman DU 640 spectrophotometer.

ISOLATION OF LDL. LDL was isolated from freshly prepared EDTA plasma obtained from healthy volunteers by sequential ultracentrifugation [10, 11]. LDL was stored in the presence of EDTA (2 mmol/L) under N_2 at 4° and used within 1 to 2 weeks. Before use, LDL was dialyzed extensively against PBS (0.15 mol/L NaCl, 20 mmol/L sodium phosphate, pH 7.4) to remove EDTA, at 4°. Freshly dialyzed LDL was immediately subjected to oxidation by mononuclear cells or Cu^{2+} ions. Protein concentrations were determined by the bicinchoninic acid protein assay (see Materials). The intra-assay coefficient of variation for protein determination was 2.6% (N = 10).

ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS. Cells were isolated from citrated, freshly collected blood from healthy volunteers as previously described [12].

CELL-MEDIATED OXIDATION OF LDL. The freshly isolated cells were seeded in triplicate 24-well tissue culture plates at a density of 2×10^6 cells/mL. All experiments were performed in Ham's F-10 medium with gentamicin (250 μg/mL) in a total volume of 250 μL/well. Before addition of LDL, the cells were preincubated for 30 min at 37° (95% air and 5% CO_2 atmosphere), in the absence (control) or presence of 10 μmol/L of doxazosin or its 6- or 7-hydroxymetabolites. Oxidation was carried out at 37° (95% air and 5% CO_2 atmosphere) in the presence of LDL (100 μg/mL), PMA (100 ng/mL), and $CuSO_4$ (5 μmol/L), and stopped by chilling on ice with immediate addition of EDTA (200 μmol/L) and butylated hydroxytoluene (40 μmol/L). Microscopic examination of the cells was performed at the end of the incubation.

KINETICS OF CU^{2+}-INDUCED OXIDATION OF LDL. This was monitored as the absorbance at 234 nm [13] using a Beckman DU 640 spectrophotometer with a 12-position micro multicell. LDL (25 μg/mL) was incubated at 37° in the presence of 1.67 μmol/L $CuSO_4$ and the absence (control) or presence of 5 μmol/L doxazosin or its 6- or 7-hydroxymetabolites. The absorbance was measured every 5 min for 200 min. From these analyses the lag time (min), the formation rate of conjugated dienes (nmol/mg LDL protein/min), and the maximum amount of conjugated dienes formed (nmol/mg LDL protein) were calculated [14].

Treatment with Doxazosin of Male Hypertensive Subjects

SUBJECTS. Seven male subjects with mild to moderate essential hypertension were recruited from investigator centers in the Oslo area. To be included in the study, the patient had to be 40–65 years of age and have essential hypertension: diastolic blood pressure ≥ 95 mmHg ≤ 115 mmHg in the sitting position (average of three readings) at baseline. Previously treated patients discontinued their antihypertensive therapy at least 6 weeks before entering the study. Patients with a history of myocardial infarction, stroke or malignant diseases, and smokers, alcoholics or drug abusers were not included.

STUDY DESIGN. The study was an open non-comparative pilot trial of 8 weeks' duration. Patients were administered an initial dose of doxazosin of 1 mg/day at baseline, and the dose was doubled after 1, 3, and 5 weeks of treatment until a blood pressure of ≤85 mmHg was reached. Doxazosin was administered as commercial Carduran tablets (2, 4, and 8 mg). Blood pressure and heart rate were measured every week after 5 min in the sitting position (average of three readings). Venous blood samples were drawn from the patients after overnight fasting at week 0, 4, and 8. Assessment of dietary intake was performed by a self-administered quantitative food frequency questionnaire at the start and at the end of the study [15], and the patients were informed not to use any vitamin or cod-liver oil supplementation during the trial. Informed consent was obtained from all participants, and the study protocol was assessed by the Regional Committee of Medical Ethics and by the Norwegian Medicine Control Authority according to local regulations.

ISOLATION OF LDL. LDL was isolated from freshly prepared heparin plasma collected from the patients at week 0, 4, and 8 by sequential ultracentrifugation [12]. Freshly isolated LDL was dialyzed extensively against PBS at 4°, pH 7.4. Prior to cryopreservation [12, 16] LDL was diluted with PBS to obtain a final concentration of 1.5 mg LDL protein/mL. LDL remained frozen for 8 months. LDL samples isolated at week 0, 4, and 8 were thawed (by immersion in a 37° water bath) and dialyzed (against PBS at 4° to remove sucrose) simultaneously, and immediately subjected to cell-mediated, copper-induced, or AAPH-initiated oxidation. The concentration of cholesterol, triacylglycerol, and phospholipids in the LDL fractions were determined enzymatically by kit methods (see Materials). Intra-assay coefficients of variation were 3.9%, 3.8%, and 6.1% for cholesterol, triacylglycerol, and phospholipid determinations, respectively (N = 10).

CELL-MEDIATED OXIDATION OF LDL. Patient LDL fractions (100 μg/mL) isolated at week 0, 4, and 8 were oxidized simultaneously in the presence of PMA (100 ng/mL) and $CuSO_4$ (2.5 μmol/L), as described above.

THE KINETICS OF CU^{2+}- AND AAPH-INDUCED OXIDATION OF LDL. This was monitored as the absorbance at 234 nm, as described above. LDL (25 μg/mL) was incubated at 37° in the presence of 5 μmol/L $CuSO_4$ or 1 mmol/L AAPH [12]. The intra-assay coefficients of variation were 9.4%, 5.4%, and 1.1% for lag time, formation rate, and maximum amount of conjugated dienes formed, respectively, for Cu^{2+}-catalyzed oxidation (N = 12), and 4.8%, 6.5%, and 4.5% for lag time, formation rate, and maximum amount of conjugated dienes formed, respectively, for AAPH-induced oxidation (N = 10).

CU^{2+}-INDUCED OXIDATION OF LDL. Patient LDL (100 μg/mL) was incubated at 37° for 0, 1, and 3 hr in the presence of freshly prepared 5 μmol/L $CuSO_4$. The intra-assay coefficient of variation of Cu^{2+}-catalyzed oxidized LDL (N = 8) was 4.6% when measured as the amount of lipid peroxides formed, and 1% when measured as changes in relative electrophoretic mobility.

AAPH-INITIATED OXIDATION OF LDL. Patient LDL (100 μg/mL) was incubated at 37° for 0, 2, and 4 hr in the presence of 4 mmol/L AAPH, dissolved in PBS [12, 17, 18]. The intra-assay coefficient of variation of AAPH-induced oxidation of LDL (N = 8) was 6% when measured as the amount of lipid peroxides formed, and 10% when measured as changes in relative electrophoretic mobility.

DETERMINATION OF DOXAZOSIN AND 6- AND 7-HYDROXY-DOXAZOSIN. Plasma concentrations of doxazosin and 6- and 7-hydroxydoxazosin were measured by HPLC, with minor modifications according to the method validated by Hazleton Wisconsin Inc. [19].

TOTAL ANTIOXIDANT STATUS. The total antioxidative capacity of plasma was determined by a kit method (Randox) using a Cobas Mira, where ABTS and metmyoglobin, reacting with hydrogen peroxide, produced a radical cation ($ABTS^+$) with a stable blue–green color that was detected at 600 nm. In the presence of heparin plasma, the absorbance of this radical cation was quenched to an extent related to the antioxidant capacity of plasma, using Trolox as a standard [20].

Extent of LDL Oxidation

LIPID PEROXIDES. The amount of lipid peroxides in Cu^{2+}-catalyzed, AAPH-induced, and cell-mediated oxidized LDL was determined by a colorimetric end point kit [21]. The intra-assay coefficient of variation was less than 2% (N = 10).

ELECTROPHORETIC MOBILITY. Changes in the net negative surface charge of Cu^{2+}-catalyzed, AAPH-induced, and cell-mediated oxidized LDL were determined using 0.5% agarose gel electrophoresis (Paragon) [22]. The intra-assay coefficient of variation was less than 1% (N = 10).

Statistical Methods

Median values as well as minimum and maximum values are presented. The non-parametric Mann–Whitney U test and Wilcoxon rank sum test were chosen because most of the variables were not distributed normally and the number of observations was low [23]. Level of significance was set at 5%. Data analyses were performed using the Stat View 4.01 statistical package.

RESULTS

In Vitro *Incubation with Doxazosin and 6- and 7-Hydroxydoxazosin*

SCAVENGING OF DPPH RADICAL. The radical scavenging capacity of 6- and 7-hydroxydoxazosin was studied by the DPPH test. Radical scavenging was shown at concentrations ranging from 25–250 μmol/L and 50–500 μmol/L for 6- and 7-hydroxydoxazosin, respectively. After 15-min incubation with 50 μmol/L metabolites, 43% (N = 2) and 23% (N = 5) scavenging was obtained for 6- and 7-hydroxydoxazosin, respectively, as compared to DPPH alone (no scavenging). At concentrations below 10 μmol/L, no scavenging of DPPH radicals was observed (data not shown).

CELL-MEDIATED OXIDATION OF LDL. To examine the effect of doxazosin and its hydroxymetabolites on cell-mediated oxidation of LDL, human mononuclear cells were preincubated for 30 min in the absence (control) or presence of doxazosin or 6- or 7-hydroxydoxazosin before incubation with LDL was begun. The formation of lipid peroxides (Fig. 2A) and the relative electrophoretic mobility of LDL (Fig. 2B) were measured. By incubating mononuclear cells with 6- and 7-hydroxydoxazosin, the amount of lipid peroxides formed after 6 hr of LDL oxidation was reduced by 90% and 94%, respectively, as compared to control (N = 5). Similarly, the relative electrophoretic mobility of LDL was reduced by 47% for both 6- and 7-hydroxydoxazosin, as compared to control (N = 6). In contrast to the hydroxymetabolites, no effect of preincubation was observed with doxazosin (Fig. 2A and B).

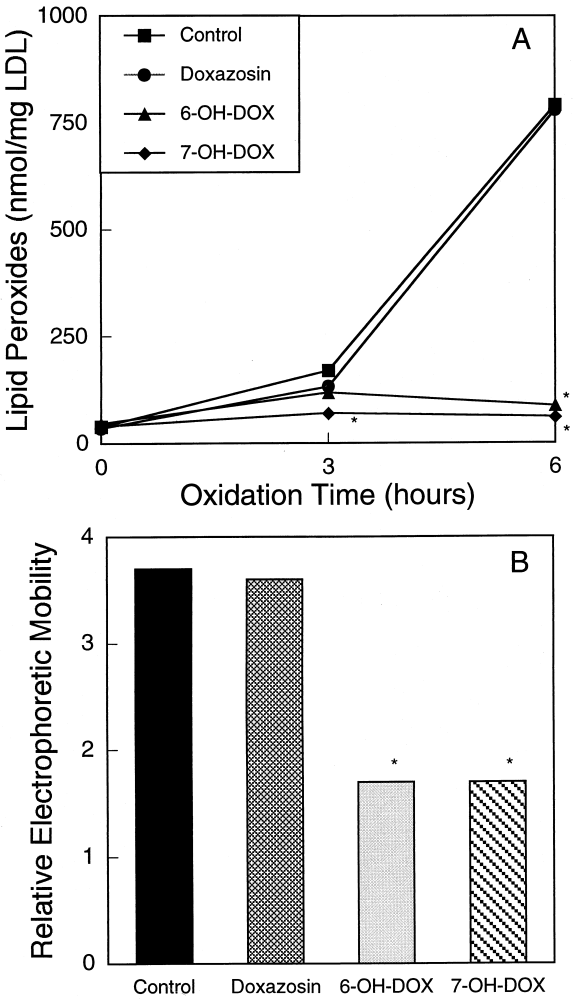

FIG. 2. Effect of *in vitro* incubation with doxazosin and its 6- and 7-hydroxymetabolites on formation of lipid peroxides (panel A) and the relative electrophoretic mobility (panel B) of LDL during cell-mediated oxidation. Peripheral blood mononuclear cells from healthy volunteers were preincubated for 30 min in the absence (control) or presence of 10 μmol/L doxazosin or 6- and 7-hydroxydoxazosin (OH-DOX) prior to addition of LDL. Oxidation of LDL (100 μg/mL) was performed in Ham's F10 medium with 5 μmol/L $CuSO_4$ and 100 ng/mL PMA at 37°. Formation of lipid peroxides in LDL was measured after 0, 3, and 6 hr oxidation (panel A), whereas the relative electrophoretic mobility of LDL was calculated after 6 hr oxidation only (panel B). Each point/block represents the median of five/six separate experiments performed in triplicate. Differences versus control were tested using the Mann–Whitney U test. *$P < 0.01$ versus control.

KINETICS OF CU^{2+}-INDUCED OXIDATION. Oxidation of LDL in the presence of 6- and 7-hydroxydoxazosin caused striking differences in the formation of conjugated dienes as compared to control conditions (Fig. 3). There was no defined lag before onset of formation, and the amount of conjugated dienes formed increased slowly and continuously. In control LDL, the maximum amount of conjugated dienes formed was 577 (484, 599) nmol/mg LDL and time of maximum (t_{max}) was 95 (95, 130) min (N = 5). Oxidation of LDL in the presence of 5 μmol/L 6- and

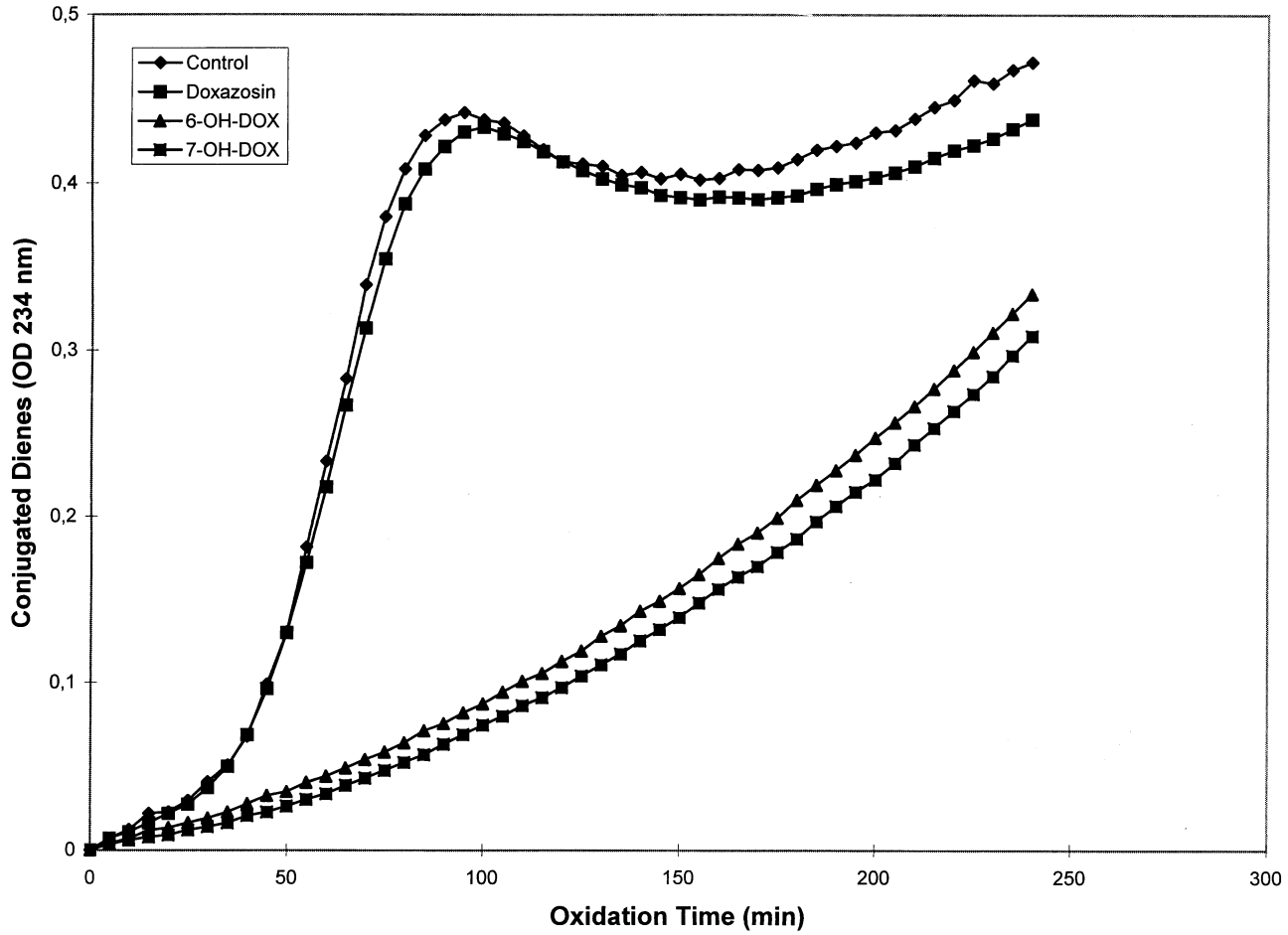

FIG. 3. Effect of *in vitro* incubation with doxazosin and its 6- and 7-hydroxymetabolites on formation of conjugated dienes in LDL during Cu^{2+}-induced oxidation. LDL (25 μg/mL) was incubated at 37° in PBS containing 1.67 μmol/L $CuSO_4$ in the absence (control) or presence of 5 μmol/L doxazosin or 6- and 7-hydroxydoxazosin (OH-DOX). The data show results from one of five representative experiments. OD, optical density.

7-hydroxydoxazosin significantly reduced the amount of conjugated dienes formed at t_{max} for control LDL to 149 (111, 387) nmol/mg LDL and 169 (87, 367) nmol/mg LDL, respectively, $P < 0.01$, N = 5). The presence of doxazosin did not significantly alter the formation of conjugated dienes in LDL subjected to Cu^{2+}-induced oxidation, as compared to control.

These data raise the question as to whether pharmacological doses of doxazosin might have antioxidative properties *in vivo* possibly by protecting LDL against oxidative modification or by increasing the total antioxidative capacity of plasma. Therefore, an intervention study was conducted.

Treatment with Doxazosin of Male Hypertensive Subjects

CHARACTERIZATION OF PATIENTS. All participants completed the study without major problems. Laboratory safety parameters (Table 1) and dietary nutrient intake of the patients, as assessed by a quantitative food frequency questionnaire (data not shown), remained unchanged during the trial. All but two patients raised the daily doxazosin dose as stated in the protocol. These two patients followed the protocol until week 5, but kept the maximum dose at 4 mg due to adequate lowering of blood pressure. Serum concentration of doxazosin increased significantly from baseline to week 4 and from week 4 to week 8 (Table 2),

TABLE 1. Baseline characteristics of participants

Characteristics	Data
Age (year)	48 (47, 62)
Height (cm)	175 (174, 186)
Weight (kg)	84 (76, 93)
Hemoglobin (g/100 mL)	15.2 (14.8, 16.5)
Erythrocytes ($\times 10^{12}$/L)	5.1 (4.7, 5.4)
Leucocytes ($\times 10^{9}$/L)	4.9 (4.3, 8.4)
Creatinine (μmol/L)	98 (89, 141)
Creatine kinase (U/L)	101 (68, 132)
Potassium (mmol/L)	4.3 (3.4, 4.9)
Alanine aminotransferase (U/L)	25 (18, 72)
Uric acid (μmol/L)	387 (308, 452)

Data presented as median (minimum, maximum) values, N = 7.

TABLE 2. Effects of treatment with doxazosin

	Week 0	Week 4	Week 8
Doxazosin (ng/mL)	ND —	7.4* (1.3, 23.0)	18.9*† (8.2, 52.0)
6-OH-DOX (ng/mL)	ND —	ND (ND, 1,06)	0.83*† (0.73, 1.45)
7-OH-DOX (ng/mL)	ND —	ND —	ND —
Systolic blood pressure (mmHg)	151 (139, 161)	140* (120, 150)	139* (121, 141)
Diastolic blood pressure (mmHg)	100 (97, 105)	91* (83, 96)	89* (82, 89)
Total cholesterol (mmol/L)	6.1 (4.3, 8.5)	6.3 (4.3, 8.0)	6.0 (4.2, 9.2)
HDL-cholesterol (mmol/L)	1.4 (0.9, 1.8)	1.3 (0.9, 1.9)	1.4 (0.9, 1.8)
Triacylglycerol (mmol/L)	1.5 (0.6, 3.2)	1.1 (0.5, 3.2)	0.9 (0.7, 6.0)
LDL Composition			
Cholesterol (%)	49 (43, 52)	49 (46, 51)	49 (41, 52)
Triacylglycerol (%)	5.3 (3.5, 10.9)	5.2 (3.7, 8.5)	6.0 (3.5, 13.2)
Phospholipids (%)	24 (22, 25)	24 (23, 25)	24 (23, 24)
Protein (%)	21 (19, 23)	23 (21, 23)	21 (21, 23)
Antioxidative capacity (mmol/L)	1.31 (1.27, 1.31)	1.29 (1.22, 1.38)	1.30 (1.15, 1.43)

Data presented as median (minimum, maximum) values; OH-DOX, hydroxydoxazosin; ND, not detectable (≤ 0.4 ng/mL). Blood pressure was measured in the sitting position. Differences between week 0, 4, and 8 were tested using Wilcoxon signed rank test, N = 7.

* $P < 0.03$ versus week 0.

† $P < 0.03$ versus week 4.

indicating that overall compliance was satisfactory. Moreover, serum concentration of 6-hydroxydoxazosin was significantly increased at week 8 as compared to baseline and 4 weeks, and correlated positively to serum doxazosin ($r = 0.89$, $P < 0.03$, N = 7). The 7-hydroxydoxazosin was not detectable in serum during the 8-week treatment (Table 2). Systolic and diastolic blood pressure was significantly lowered by 13% and 14%, respectively, at week 8 as compared to baseline (N = 7; Table 2). Serum total cholesterol and high-density lipoprotein cholesterol remained unchanged during the study (Table 2). LDL particle composition, measured as % distribution of cholesterol, triacylglycerol, phospholipids, and protein, was not altered by intervention with doxazosin, and the total antioxidative capacity of plasma remained unchanged during the intervention period (Table 2).

Cu^{2+}-CATALYZED AND AAPH-INITIATED OXIDATION. Intervention with doxazosin altered neither the Cu^{2+}-catalyzed nor the AAPH-induced oxidation of LDL *ex vivo* as assessed by measuring lag time, rate, or maximum conjugated dienes formed (Table 3). The formation of lipid peroxides and the relative electrophoretic mobility of LDL subjected to Cu^{2+}-catalyzed or AAPH-induced oxidation were also similar at all time points (Fig. 4).

CELL-MEDIATED OXIDATION. To examine the effect of doxazosin treatment in a more physiological system, patient LDL isolated at week 0, 4, and 8 was subjected to oxidation by human mononuclear cells isolated from a healthy volunteer. The formation of lipid peroxides was similar for LDL particles isolated at the different time points (192 [172, 866] nmol/mg LDL vs 167 [86, 521] nmol/mg LDL and 149 [86, 239] nmol/mg LDL, for week 0, 4, and 8, respectively, N = 7).

DISCUSSION

The present study showed that 6- and 7-hydroxydoxazosin had radical scavenging capacity and were able to protect LDL against cell-mediated as well as Cu^{2+}-induced oxidation *in vitro*. However, treatment of male hypertensive subjects with increasing doses of doxazosin (from 1 to 8 mg/day) for 8 weeks did not change the susceptibility of

TABLE 3. Effect of intervention with doxazosin on formation of conjugated dienes in LDL

	Week 0	Week 4	Week 8
Cu^{2+}-catalyzed oxidation			
Lag time (min)	64 (50, 75)	58 (48, 74)	56 (49, 65)
Rate (nmol/mg/min)	20 (17, 26)	21 (16, 23)	21 (16, 24)
Max CD (nmol/mg)	865 (734, 956)	818 (722, 921)	822 (717, 972)
AAPH-induced oxidation			
Lag time (min)	115 (67, 125)	112 (100, 123)	116 (108, 129)
Rate (nmol/mg/min)	7.8 (7.2, 8.9)	8.5 (5.3, 11.3)	8.8 (4.3, 11.1)
Max CD (nmol/mg)	1115 (966, 1273)	991 (575, 1410)	1092 (644, 1296)

Data presented as median (minimum, maximum) values; CD, conjugated dienes. LDL (25 μg/mL) isolated from patients at week 0, 4, and 8 was incubated in PBS at 37° in the presence of either 5 μM Cu^{2+} or 1 mM AAPH. Differences between week 0, 4, and 8 were tested using Wilcoxon signed rank test (no significant differences, N = 7).

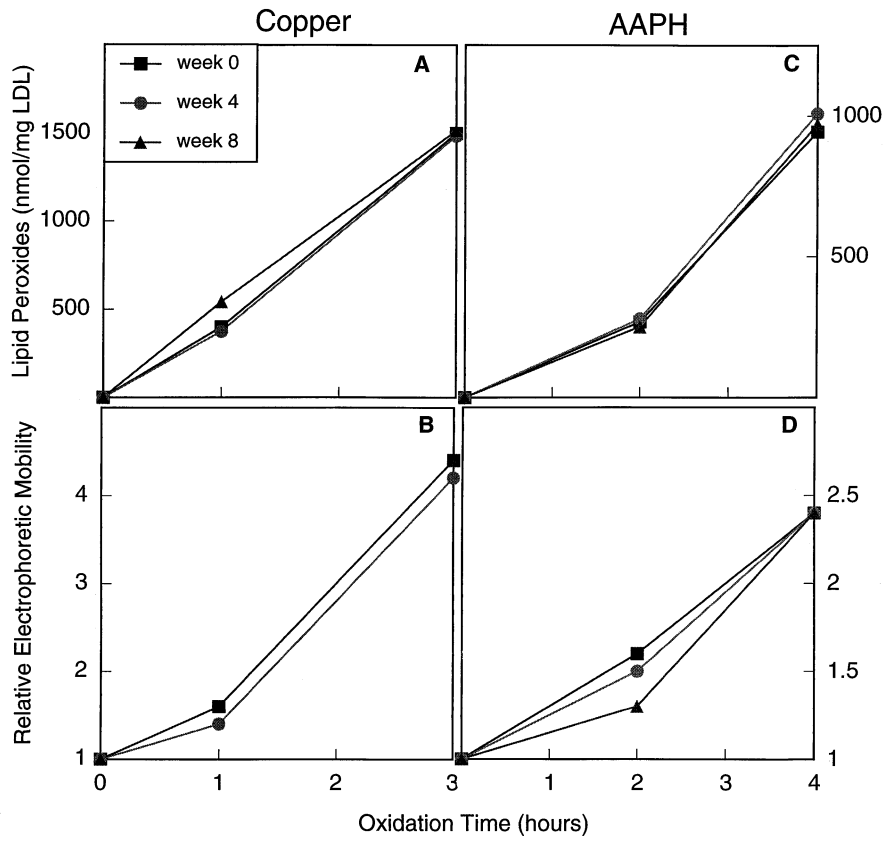

FIG. 4. Effect of treatment with doxazosin on Cu^{2+}-catalyzed (panels A and B) or AAPH-induced (panels C and D) oxidation of LDL. LDL (100 μg/mL) isolated from the patients at week 0, 4, and 8 was incubated at 37° in PBS in the presence of either 5 μM $CuSO_4$ for 0, 1, and 3 hr or 4 mM AAPH for 0, 2, and 4 hr. Extent of oxidation was measured as formation of lipid peroxides (panels A and C) and the relative electrophoretic mobility of LDL (panels B and D). Each point represents median values from 7 patients. Differences between week 0, 4, and 8 were calculated using the Wilcoxon signed rank test; no significant differences were observed.

LDL to undergo oxidative modification *ex vivo*, initiated by either Cu^{2+} ions, AAPH compound, or human mononuclear cells. Furthermore, the total antioxidative capacity of plasma remained unchanged during the treatment period with doxazosin.

The water soluble 6- and 7-hydroxydoxazosin are not incorporated into the LDL particle [9], but may act as aqueous phase antioxidants, similar to vitamin C. Vitamin C is able to regenerate endogenous antioxidants such as vitamin E [24] and thereby potentially protect LDL against oxidation. Previous studies have shown that physiologic concentrations of ascorbate can reduce LDL oxidation *in vitro* and prevent its subsequent uptake by macrophages [25, 26] and preserve the endogenous antioxidants in LDL [27]. Furthermore, supplementation with ascorbate to healthy smokers increased the lag phase and decreased the oxidation rate of LDL as compared to baseline [28]. The authors suggested that ascorbate supplementation reduced the inherent propensity of LDL to oxidation prior to its isolation from plasma. In the present study, cell-mediated as well as Cu^{2+}-catalyzed oxidation of LDL were strongly reduced when *in vitro* oxidation was performed in the presence of 6- and 7-hydroxydoxazosin. These data are consistent with the findings of Chait *et al.* [9], who reported reduced Cu^{2+}-induced oxidation of LDL in the presence of 6- and 7-hydroxydoxazosin. The antioxidative effect was lost after re-isolation of LDL incubated with the metabolites. However, these findings [9] do not exclude the possibility that 6- and 7-hydroxydoxazosin may regenerate endogenous antioxidants *in vivo*, and thereby protect LDL against oxidation, similar to what is reported for vitamin C [28].

Treating male hypertensive subjects with increasing doses of doxazosin for 8 weeks did not alter the susceptibility of LDL to undergo *ex vivo* oxidation in our present study. The low doses of metabolites present in serum from the patients may explain why the oxidizability of LDL remained unchanged during the trial. Five out of seven subjects reached a maximum dose of 8 mg doxazosin per day, and serum doxazosin and 6-hydroxydoxazosin concentrations measured after overnight fast increased significantly for all participants, ranging from 8.2 to 52 ng/mL and from 0.73 to 1.45 ng/mL, respectively (Table 2), whereas the 7-hydroxymetabolite was not detectable (<0.4 ng/mL). Unpublished data from Pfizer AS (Norway)* show that plasma doxazosin concentrations 24 hr after administration of 8 mg doxazosin were about 20 ng/mL, corresponding to the median concentration measured in our patients (18.9 ng/mL at week 8, Table 2). It might be argued that 8 weeks' treatment with increasing doses of doxazosin is too short to detect any effects on LDL oxidizability. However, according to Elliott *et al.* [29], measurement of plasma doxazosin concentrations on 2 occasions 3 months apart in hyperten-

* Vashi V, Chung M, Puente J and Sweeney M, unpublished observations.

sive patients showed no evidence of time-dependent changes in the disposition of the drug. Furthermore, with doses ranging from 1 to 16 mg, plasma concentrations and bioavailability were directly proportional to the dose of doxazosin. Thus, administration of increasing doses of doxazosin is necessary to obtain higher plasma concentrations of doxazosin and its metabolites.

To further investigate the antioxidative properties of doxazosin and its metabolites, the total antioxidative capacity of plasma was measured. Since 6- and 7-hydroxydoxazosin act as radical scavengers *in vitro*, increasing serum concentrations of the metabolites might increase the total antioxidative capacity in plasma. However, the total antioxidative capacity of plasma, measured as quenching of the $ABTS^{\cdot+}$-radical, remained unchanged during the trial.

In conclusion, the present study showed that 6- and 7-hydroxydoxazosin possessed free radical scavenging properties, and that the metabolites were able to protect LDL against lipid peroxidation *in vitro*. However, the protective effect of the doxazosin metabolites was not detectable after treatment of hypertensive subjects for 8 weeks with increasing doses of doxazosin.

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