Hypochlorite modification of high density lipoprotein: effects on cholesterol efflux from J774 macrophages

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Received 17 March 1999; received in revised form 4 May 1999

Abstract The present study was aimed at investigating effects of hypochlorite (HOCl) modification of high density lipoproteins subclass 3 (HDL_3) on their ability for cellular cholesterol removal from permanent J774 macrophages. Our findings indicate that HOCl (added as reagent or generated enzymatically by the myeloperoxidase/H_2O_2/Cl^- system) damages apolipoprotein A-I, the major protein component of HDL_3. Fatty acid analysis of native and HOCl-modified HDL_3 revealed that unsaturated fatty acids in both major lipid subclasses (phospholipids and cholesteryl esters) are targets for HOCl attack. HOCl modification resulted in impaired HDL_3-mediated cholesterol efflux from J774 cells, regardless of whether reagent or enzymatically generated HOCl was used to modify the lipoprotein. Decreased cholesterol efflux was also observed after HOCl modification of reconstituted HDL particles. Impairment of cholesterol efflux from macrophages was noticed at low and physiologically occurring HOCl concentrations.

Key words: Reverse cholesterol transport; High density lipoprotein modification; Myeloperoxidase; Hypochlorite

1. Introduction

In contrast to low density lipoproteins (LDL), high plasma concentrations of high density lipoproteins (HDL) are associated with a decreased risk for the development of coronary artery disease, an effect commonly attributed to their central role in reverse cholesterol transport [1]. During this process HDL promotes cholesterol efflux from peripheral tissues and the accepted cholesterol is partially re-esterified by the action of lecithin-cholesterol acyltransferase (LCAT) [2]. Cholesteryl esters (CEs) formed by the LCAT reaction are then transferred from HDL to other lipoproteins by the cholesterol ester transfer protein (CETP) or are delivered to the liver for biliary secretion or reutilization during lipoprotein assembling [3]. Hepatic (selective) uptake of HDL-associated CEs is mediated via scavenger receptor BI (SR-BI) in rodents [4]. SR-BI participates in cellular lipid/cholesterol efflux [5] and phospholipids may have a major role in mediating this process [6]. However, not only phospholipids, but also apolipoprotein A-I (apoA-I) plays a crucial role during removal of cellular cholesterol [7,8] and some distinct regions within apoA-I are involved this process [9–13]. Oxidation/modification of HDL was demonstrated to result in diminished cholesterol efflux, findings attributed to alterations of the apoprotein domain [14–16]. Within these modifications oxidative tyrosylation appears to be an exception since tyrosyl radical modification of HDL enhanced cellular cholesterol efflux and mobilization [17,18].

Among the numerous in vitro oxidation systems the myeloperoxidase (MPO)/H_2O_2/halide system has emerged as an oxidant being of potential in vivo relevance. Hypochlorite (OCl^-), formed in vivo via the MPO/H_2O_2/Cl^- system from activated neutrophils and/or monocytes, may react with a wide range of biological target molecules, including lipids, antioxidants and proteins [19]. The importance of MPO as a potential in vivo oxidant is further underlined by the presence of enzymatically active MPO in human atherosclerotic lesions [20], the presence of HOCl-modified (lipo)proteins in advanced human atherosclerotic lesions [21] and inflammatory kidney tissues rich in MPO [22]. In vitro, the MPO/H_2O_2/halide system transforms LDL [23] and HDL [24] into high uptake forms for macrophages and it was shown that HDL_3 modified by polymorphonuclear cells (containing high concentrations of MPO) had a markedly reduced ability to remove cholesterol from cells [25].

Therefore, the present study was aimed at investigating the consequences of HOCl added as reagent or generated by the MPO/H_2O_2/halide system on HDL_3-mediated cholesterol efflux from J774 macrophages. In order to differentiate between effects mediated by modified lipids and/or apoprotein modification we used human HDL_3 and reconstituted HDL particles (rHDL), the latter composed of saturated fatty acids which are not susceptible to OCl^- modification.

2. Materials and methods

2.1. Preparation of human, apo E-free HDL_3

Human apo E-free HDL_3 was prepared by density gradient ultracentrifugation of plasma obtained from human normolipidemic volunteers in a TL120 Beckman tabletop ultracentrifuge (Beckman, Austria) as described [26]. SDS-PAGE on 15% gels and subsequent Coomassie staining revealed the presence of apoA-I as the major apolipoprotein.

2.2. Isolation of apoA-I

HDL was isolated from plasma obtained from human normolipidemic volunteers by sequential ultracentrifugation and delipidated as described [27]. The delipidated apoproteins were redissolved in 50 mM glycine, 4 mM NaOH, 0.5 M NaCl and 6 M urea (pH 8.8). HDL apoproteins were then separated by size exclusion chromatography on a Sephacryl S-200 column (3×150 cm) as described [27]. The fractions

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Abbreviations: Apo, apolipoprotein; BSA, bovine serum albumin; CE, cholesteryl ester; DMEM, Dulbecco's modified Eagle's medium; FAME, fatty acid methyl ester; FCS, fetal calf serum; GC, gas chromatography; HDL_3, high density lipoprotein subclass 3; LPDS, lipoprotein-deficient serum; MPO, myeloperoxidase; PBS, phosphate buffered saline; PL, phospholipid; rHDL, reconstituted HDL; TBS, Tris-buffered saline

0014-5793/99/$20.00
PII: S0014-5793(99)00677-8

containing the major peak were pooled and dialyzed against NH_4HCO_3. The product was homogeneous as assessed by SDS-PAGE, reversed phase HPLC and amino acid analysis.

2.3. Preparation of rHDL

rHDL was prepared by the sodium cholate dialysis method using dipalmitoyl phosphatidylcholine (DPPC), apoA-I and Na-cholate in molar ratios of 120:1:100 as described [28]. Briefly, 14.2 mg DPPC was dissolved in 1 ml buffer (10 mM Tris, pH 8.0, 0.15 M NaCl, 0.005% EDTA and 1 mM NaN_3) containing 6.2 mg Na-cholate. The mixture was stirred at 40°C for 90 min. After the addition of apoA-I (4 mg/1.4 ml) and stirring at 40°C (20 min) the mixture was incubated at 37°C for 16 h. Dialysis with exhaustive buffer changes was performed at 20°C over 3 days. Samples were kept under N_2 and protected from light. rHDL was reisolated by gradient ultracentrifugation using a TLA 100.4 rotor (Beckman, Austria) as described [26]. The chemical composition of rHDL was obtained by protein determination using the Lowry procedure [29], the phospholipid (PL) content was measured enzymatically (BioMerieux, France). The content of apoA-I molecules/rHDL was obtained by dimethylsuberimidate (DMS) crosslinking as described by Swaney [30].

2.4. NaOCl modification of apoA-I

One mg apoA-I/ml PBS (10 mM, pH 6) was incubated with NaOCl (Sigma, Austria) solution at the indicated molar ratio of NaOCl: apoA-I as described earlier [24]. The concentration of NaOCl was determined using a molar absorption coefficient for NaOCl of 350 cm^{-1} at 292 nm. NaOCl was added once, the mixture gently vortexed, then kept on ice for 30 min and 90 min at 37°C. Modified apoA-I was dialyzed against water at 4°C.

2.5. MPO modification of apoA-I

To 1 mg apoA-I/ml PBS (50 mM, pH 7.4) 15 additions of 20 μM H_2O_2 were made at 10 min intervals at 37°C [24]. 13 nM MPO (25 U/ml; Alexis Co., Switzerland) was added at the start and subsequently at every second addition of H_2O_2. At alternate additions of H_2O_2, 2 μM ascorbate was added. The reaction mixture was incubated for 1 h at 37°C and dialyzed against water at 4°C.

2.6. SDS-PAGE and Western blotting

ApoA-I samples were run for 90 min at 150 V in a Bio-Rad mini-protean chamber (Bio-Rad, Austria). Proteins were electrophoretically transferred to nitrocellulose membranes (150 mA, 4°C, 90 min). For the detection of native apoA-I a polyclonal rabbit anti-human apoA-I antibody was used [24], followed by a HRP-conjugated goat anti-rabbit IgG (Sigma, Austria). Immunochemical detection of NaOCl-modified apolipoproteins was performed with a monoclonal antibody 2D10G9 [31] followed by peroxidase-conjugated goat anti-mouse IgG (Sigma). Detection was performed by the ECL method (Amersham, Austria). Amino acid analysis of native and NaOCl-modified HDL_3 was performed as described previously on 6 N HCl hydrolysates (24 h, 105°C) [24].

2.7. Fatty acid analysis of lipid subclasses and total HDL_3 lipids

FA analysis of total HDL_3 lipids and HDL_3 lipid subclasses was performed as described [32]. Briefly, total HDL_3 lipids were converted to the corresponding FA methyl esters (FAMEs) in toluene (0.5 ml) and methanol/BF_3 complex (Sigma; 20%, v/v; 1 ml) with heptadecanoic acid as the internal standard (100 μg). The FA composition of the PL and CE fractions was analyzed after in situ transesterification of the corresponding TLC spots exactly as described [32]. Separation was performed on a FFAP-CB column (25 m, 0.35 mm i.d.; Chrompack, Austria) using a HP 5890 gas chromatograph equipped with a flame ionization detector and a split/splitless injector (Hewlett-Packard Co., Austria). After an initial isothermal period of 5 min at 150°C the temperature was programmed to 190°C at 4°/min and then to 215°C at 2°/min, with a hold at 215°C for 15 min. Concentrations of the individual FAs were calculated by peak area comparison with the internal standard.

2.8. Cholesterol efflux studies

J774 mouse macrophages (ATCC, Rockville, MD, USA) were plated on 6 well trays in RPMI 1640 medium containing 10% (v/v) fetal calf serum (FCS) as described [33]. One day prior to the experiments at approximately 80% confluence the cells were preincubated in RPMI 1640 containing lipoprotein-depleted serum (LPDS, 10%, v/v) and the indicated activities of [^{3}H]cholesterol (added as an ethanolic solution; final volume $< 0.1\%$ of total volume) as described [34]. This procedure was shown to result in predominant labeling of the plasma membrane-located cholesterol pool. Before initiating the efflux experiments the [^{3}H]cholesterol-containing medium was aspirated then the cells were washed twice in TBS (containing 5% (w/v) BSA) and twice in TBS. Efflux experiments were then initiated by the addition of RPMI 1640 containing LPDS (10%, v/v) and native and modified HDL_3 or rHDL at the concentrations indicated. At the indicated time points the medium was collected and counted on a β-counter (Packard-Canberra, Austria). The cells were lysed in 0.3 N NaOH to determine the cellular protein content [29]. Efflux of radioactive label to the medium was calculated as the percentage of radioactivity present in the cells prior to the addition of the indicated cholesterol acceptors.

3. Results

The FA composition of total HDL_3 lipids and the FAs contained in the PL and CE fractions of HDL_3 is shown in Table 1. The PL fraction contains about one third more unsaturated FAs as compared to the CE fraction (306 vs. 212 nmol/mg HDL_3). With respect to FA composition of the two lipid subclasses, unsaturated FAs constitute 85% of the CE and 47% of the PL fraction (Table 1).

It is well established that during HDL_3-mediated cholesterol efflux PLs have an important contribution to the particle's efflux abilities [35,36]. To identify whether one of the major HDL_3 lipid subclasses represents a preferred target for NaOCl attack, HDL_3 was modified with increasing molar ratios of NaOCl, the lipids were extracted, separated by TLC, transmethylated and analyzed by gas chromatography (GC) as described in Section 2. Modification of HDL_3 with NaOCl:HDL_3 molar ratio $\geq 100:1$ led to pronounced consumption of FAs in the PL (Fig. 1A) and CE fractions (Fig. 1B). At all NaOCl ratios studied, the degree of FA consumption was related to the degree of unsaturation, i.e. modification occurred in the order C22:6 > 20:4 > 18:3 > 18:2 > 18:1. For more clarity only FAs contributing $\geq 6\%$ of the total FA content are shown. From the data shown in Fig. 1 it appears that PL-located FAs are slightly more sensitive to HOCl damage as compared to polyunsaturated FAs in the CE fraction.

The effects of reagent and enzymatically generated OCl^- on

Table 1
Fatty acid composition of HDL_3 phospholipids, cholesteryl esters and total HDL_3 lipids

Fatty acids	nmol/mg total HDL_3 mass		
	Total lipids	Phospholipids	Cholesteryl esters
14:0	12.1 ± 0.5	9.2 ± 1.1	1.2 ± 1.6
16:0	307.8 ± 9.8	234.1 ± 7.6	33.9 ± 4.1
16:1	28.4 ± 1.4	16.5 ± 4.2	1.1 ± 0.9
18:0	102.6 ± 5.4	99.7 ± 2.7	2.9 ± 0.8
18:1 (n-9)	201.9 ± 4.2	115.1 ± 5.9	67.3 ± 3.1
18:1 (n-7)	12.0 ± 1.1	n.d.	6.4 ± 4.6
18:2	264.0 ± 13.5	140.0 ± 3.5	105.6 ± 4.7
18:3	1.1 ± 0.9	1.5 ± 0.7	n.d.
20:4	59.8 ± 3.7	31.0 ± 4.9	29.9 ± 2.1
22:6	7.2 ± 2.6	4.7 ± 2.5	1.2 ± 0.2
TOTAL	996.9 ± 43.1	651.8	249.5

Total HDL_3 lipids were either directly transmethylated with methanol-containing BF_3 (20%) or preseparated by TLC to analyze the FAs present in the PL and CE fractions. The FA content was quantitated by peak area comparison with heptadecanoic acid used as internal standard. Results shown represent mean ± S.D. from triplicate analyses and are given in nmol FA/mg total HDL_3 mass.

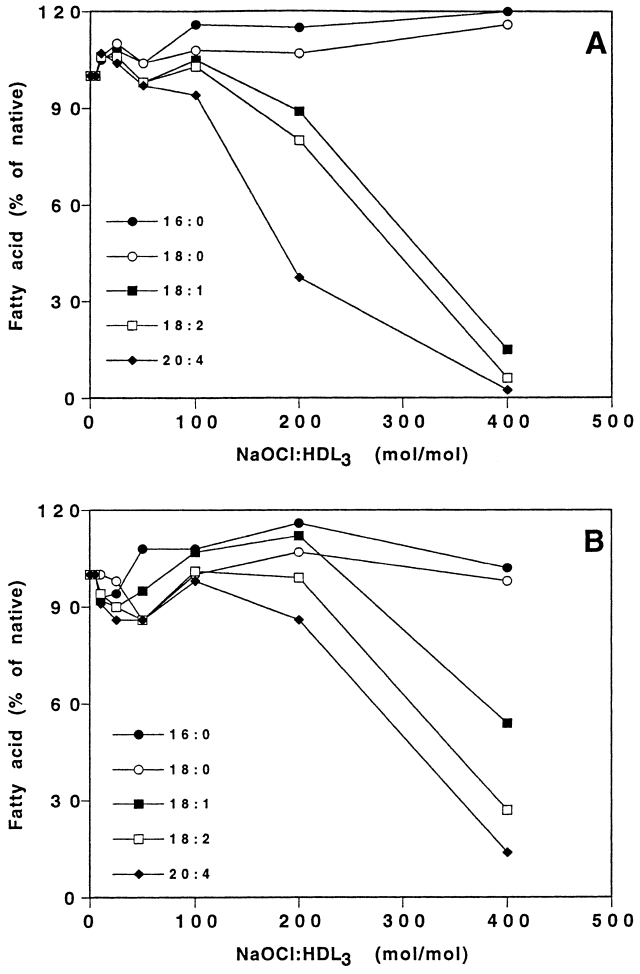

Fig. 1. FA composition of HDL_3 phospholipids (A) and cholesteryl esters (B) prior to and after NaOCl modification. HDL_3 (1 mg/ml) was modified in the presence of the indicated molar excess of NaOCl as described in Section 2. After modification the lipids were extracted and the main lipid subclasses were separated on silica gel 60 plates. The bands corresponding to the PL and CE fractions were scraped off the plate and analyzed as described in Section 2. Results shown are mean values from two independent experiments. The absolute FA concentrations of the corresponding fraction in native HDL_3 are shown in Table 1.

the apoprotein domain of HDL_3 is shown in Fig. 2. HDL_3 or rHDL was modified in the presence of a 100-fold molar excess of NaOCl or in the presence of the MPO/H_2O_2/Cl^- system. The apoproteins were separated by SDS-PAGE (5–25% gels), transferred to nitrocellulose and analyzed by immunoblotting using either a rabbit human anti-apoA-I antiserum or a monoclonal antibody (clone 2D10G9) specifically recognizing HOCl-modified epitopes. ApoA-I in native HDL_3 or rHDL was detected as a single, homogeneous band with an apparent molecular mass of 28 kDa (lanes 1 and 5) and was free of any preformed epitopes recognized by 2D10G9 (lanes 2 and 6). However, NaOCl modification of HDL_3 and rHDL resulted in extensive apoprotein modification and generation of high molecular weight apoA-I aggregates readily recognized by 2D10G9 (lanes 3 and 7). Most importantly enzymatic modification with the MPO/H_2O_2/Cl^- system resulted in similar OCl^--modified apoA-I aggregates in HDL_3 (lane 4) and rHDL (lane 8). To identify preferential amino acid targets,

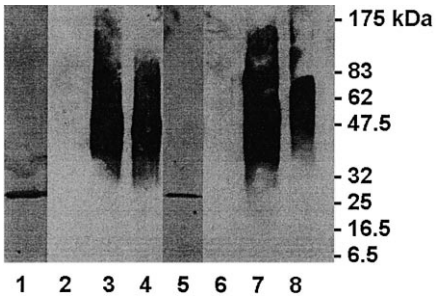

Fig. 2. Western blot of native, NaOCl- and MPO-modified HDL_3 and rHDL. Prior to SDS-PAGE, HDL_3 or rHDL was modified with NaOCl (100-fold molar excess) or in the presence of the MPO/H_2O_2/Cl^- system. Samples containing 100 ng apoA-I were separated on 5–25% gels and transferred to nitrocellulose. Native apoA-I of HDL_3 (lane 1) or rHDL (lane 5) was detected with rabbit anti-human apoA-I antiserum and was undetectable with monoclonal 2D10G9 (lanes 2 and 9, respectively). NaOCl- and MPO-modified apoA-I of HDL_3 (lanes 3 and 4) or the corresponding rHDL samples (lanes 7 and 8) were detected with monoclonal antibody, clone 2D10G9 specifically recognizing OCl^--modified epitopes. Visualization was performed with peroxidase-conjugated goat anti-rabbit or goat anti-mouse IgG using the ECL method. The molecular mass of the marker proteins is indicated.

the amino acid composition of native and NaOCl-modified (25- and 100-fold molar excess) apoA-I was analyzed. From the data shown in Table 2 it is evident that methionine residues are early targets and are destroyed at low oxidant:protein ratios (25:1). At a molar oxidant:apoA-I ratio of 100:1 Lys and Arg were partially (16 and 75%, respectively) modified, while Tyr, Phe, and His were quantitatively lost.

Next we have investigated the effect of increasing NaOCl modification on the ability of HDL_3 to promote cellular cholesterol efflux from J774 macrophages. It is evident that at all time points analyzed (3, 6 and 24 h; Fig. 3A) the degree of cholesterol efflux was dependent on the degree of HDL_3 modification (ranging from 25 to 800:1). The cholesterol acceptor properties of HDL_3 modified with a $\geq$100-fold molar excess of NaOCl were significantly lower than observed with native HDL_3 and HDL_3 modified with lower NaOCl ratios. The percentage of cholesterol efflux to the acceptors was dependent on the modification rate and decreased from 29 ± 0.3 to 11 ± 1.1 (3 h), 38 ± 0.5 to 20 ± 0.9 (6 h) and 62 ± 2.9 to 40 ± 0.5% (24 h) (native vs. HDL_3 modified at a molar ratio of 400:1). Modification of HDL_3 with an 800-fold molar excess of NaOCl led to only a small further decrease in choles-

Table 2
Amino acid analysis of NaOCl-modified apoA-I

Amino acid[a]	NaOCl:apoA-I (mol/mol)		
	0	25	100
Met	2	n.d.	n.d.
Tyr	6	6	n.d.
Phe	6	5	n.d.
Lys	19	18	16
His	6	6	n.d.
Arg	12	11	3

Native apoA-I (500 μg) in PBS (1 ml) was modified with NaOCl (25- and 100-fold oxidant excess). Amino acid analysis was performed on hydrolysates prepared with 6 N HCl (105°C, 24 h).
n.d. = not detectable.
[a]Values are given in mol amino acid/mol apoA-I and represent mean values from two independent experiments. Only amino acids which are modified by NaOCl treatment are shown.

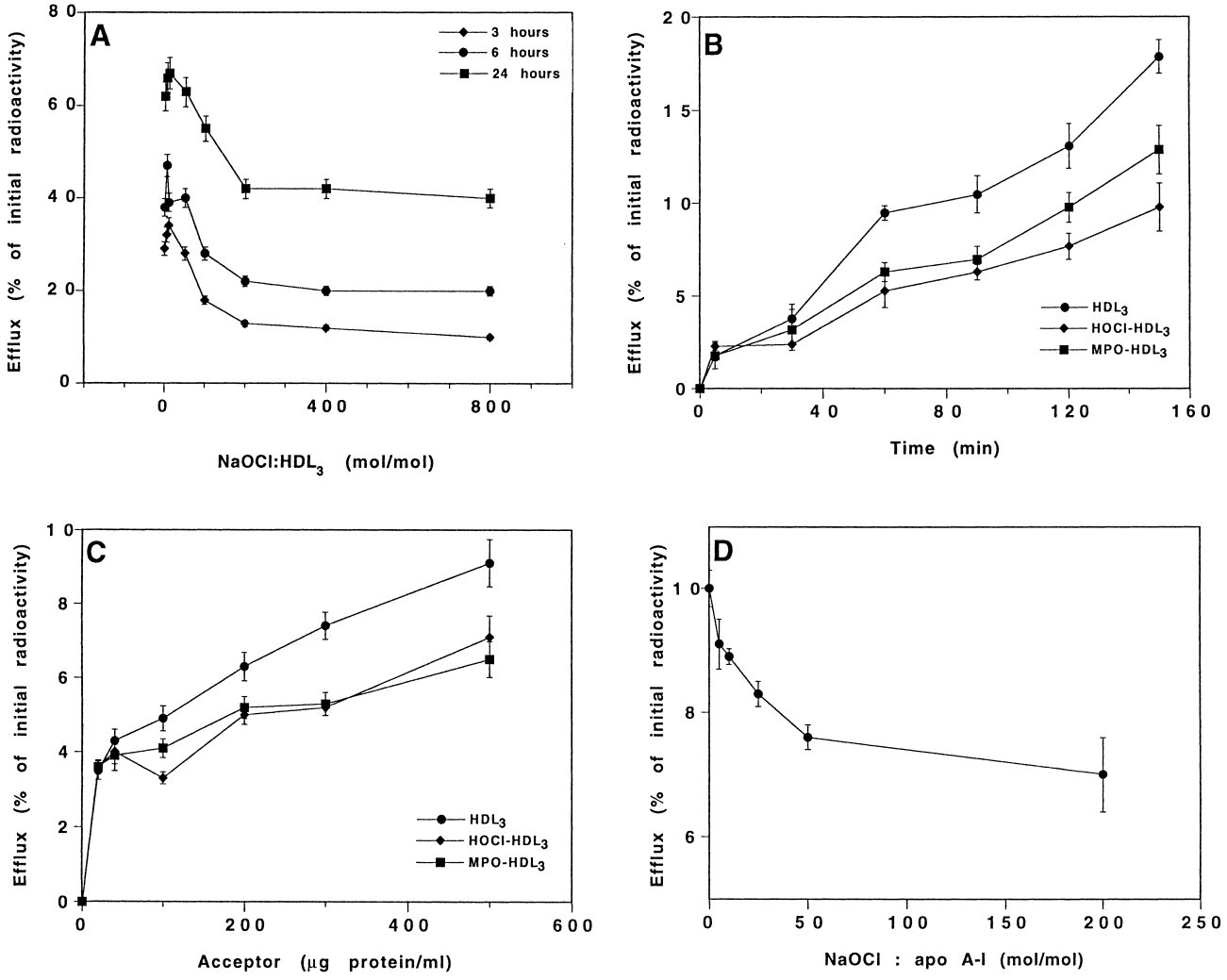

Fig. 3. NaOCl or MPO modification of HDL_3 or rHDL results in decreased cellular cholesterol efflux. J774 macrophages were cultivated in RPMI 1640 medium until ~80% confluence as described in Section 2. The cells were labeled in the presence of [^{3}H]cholesterol (0.1 or 1 μCi/well/ml; 24 h) and washed as described in Section 2. A: 500 μg of native or modified HDL_3 (molar NaOCl:apoA-I ratio = 25:1–800:1) was added to the cells in RPMI 1640 medium. The medium was removed at 3, 6 and 24 h to determine the radioactivity. At time zero the cells acquired 9918 ± 911 cpm/well ($n = 6$). B: Cells were labeled with [^{3}H]cholesterol (1 μCi/well), washed and received fresh medium containing 225 μg of native, NaOCl- (200:1) or MPO-modified HDL_3 protein. At the indicated time the medium was removed to determine the radioactivity. At time zero the cells acquired 131 807 ± 9711 cpm/well ($n = 3$). C: Cells were labeled with [^{3}H]cholesterol (1 μCi/well), washed and received the indicated concentrations of native, NaOCl- (200:1) or MPO-modified HDL_3 protein in fresh medium. After 90 min the medium was removed to determine the radioactivity. At time zero the cells acquired 131 807 ± 9711 cpm/well ($n = 3$). D: Cells were labeled with [^{3}H]cholesterol (1 μCi/well) and received the indicated concentrations of native and rHDL (containing 100 μg apoA-I) modified with a 5–200-fold NaOCl excess. After an incubation period of 240 min the medium was removed to determine the radioactivity. At time zero the cells have acquired 267 668 ± 16 232 cpm/well. Cellular cholesterol efflux is expressed as percentage medium radioactivity in terms of the radioactivity present in the cells prior to the addition of the indicated acceptor particles (A–D). All results shown represent mean ± S.D. of triplicate dishes.

terol efflux. From the data shown in Fig. 3 we have calculated (by non-linear regression analysis) the time necessary to mediate 50% cholesterol efflux ($t/2$). The $t/2$ values for native and the various HDL_3 modifications are summarized in Table 3. These calculated values show that the time necessary to remove half of [^{3}H]cholesterol progressively increased from ~15 h (native) to ≥ 30 h with increasing modification rates.

To compare the effects of reagent OCl^- and enzymatically generated OCl^- on cholesterol efflux properties of HDL_3 efflux experiments were performed in a time- (Fig. 3B) and concentration-dependent (Fig. 3C) manner and were conducted for shorter incubation times (90–150 min). With native HDL_3 cholesterol efflux occurred in a nearly linear manner and resulted in a removal of ~6% of the initially cell-associated radioactivity per hour (Fig. 3B). The corresponding values for NaOCl- and MPO-modified HDL_3 were 3.6 and 4.8%/h. From the data shown in Fig. 3B it is evident that HDL_3 modification with either reagent or the $MPO/H_2O_2/Cl^-$ system resulted in a comparable impairment in cholesterol acceptor properties. This was also obvious in concentration-dependent experiments where cholesterol efflux mediated by NaOCl- and MPO-modified HDL_3 was 10–30% lower than observed in the presence of native HDL_3 (Fig. 3C).

Modification of HDL_3 with NaOCl results in alterations of the apoprotein and the lipid domain (Figs. 1 and 2). To be able to differentiate between effects of lipid and apoprotein modifications on cholesterol acceptor properties of HDL_3 we prepared rHDL particles with DPPC as the PL species. As

Table 3
Calculated $\tau/2$ values

NaOCl:HDL_3 (mol/mol)	$\tau/2$ (h)
0	15 ± 0.9
5	9 ± 1.2
10	13 ± 0.8
50	14 ± 1.3
100	21 ± 0.8
200	32 ± 1.2
400	34 ± 1.1
800	35 ± 0.7

Data shown in Fig. 3A were subjected to non-linear regression analysis to calculate the times necessary to remove half of cell-associated [^{3}H]cholesterol ($\tau/2$).

can be seen from Fig. 1A, palmitic acid in the PL fraction is not affected by OCl^- treatment even at the highest OCl^-:apoA-I molar ratios used (400:1). The rHDL preparation reisolated by ultracentrifugation contained three rHDL species with Stokes radii of 18, 15 and 11 nm (as analyzed by non-denaturing gradient gel electrophoresis) with the 11 nm population contributing about 70%. The stoichiometry of apoA-I in rHDL was determined by chemical crosslinking using DMS [30]. In line with other reports we have observed the presence of 2, 3 and 4 apoA-I molecules per rHDL particle [37]. The different rHDL subpopulations were not further separated prior to the efflux experiments. rHDL was modified with an increasing molar excess of NaOCl (5–200 mol/mol apoA-I) and used as cholesterol acceptor vesicles during efflux experiments. J774 cells were labeled with 1 μCi [^{3}H]cholesterol and acquired 267 668 ± 16 232 cpm/well, corresponding to approximately 25% of the initially added radioactivity. Cells were then washed and incubated in the presence of 100 μg rHDL protein. When rHDL was modified at increasing NaOCl:apoA-I molar ratios the efficacy to promote cholesterol efflux from J774 macrophages was significantly diminished (Fig. 3D). The radioactivity recovered in the medium gradually decreased with increasingly modified rHDL preparations (26 260 ± 624 cpm, native rHDL vs. 18 327 ± 296 cpm for rHDL modified at 200:1). Most importantly, a small but significant decrease in cholesterol efflux became apparent at the lowest modification (5:1).

4. Discussion

The functional integrity of apoA-I and the PL composition of HDL are important determinants affecting the particle's capability to promote cellular cholesterol efflux [6–8,38]. In the present study we have observed impaired cholesterol efflux from J774 macrophages to (reagent or enzymatically generated) OCl^--modified HDL. NaOCl modification of HDL_3 resulted in the dose-dependent loss of unsaturated FAs in the two major HDL lipid subclasses (PLs and CEs), a fact most probably related to FA chlorohydrin formation [24,39,40]. From the data shown in Fig. 1 it appears that polyunsaturated fatty acids (PUFAs) present in the outer PL shell are slightly more susceptible to OCl^- modification than PUFAs located in the hydrophobic CE core, in line with earlier observations during copper-mediated oxidation of HDL [32]. It is well documented that the quantitative PL content of HDL [35,36] as well as the PL subspecies composition of rHDL [41] modulate the efficacy for cellular cholesterol removal of a given acceptor particle. At present we have no reasonable explanation inasmuch chlorohydrin formation in the PL moiety contributes to the observed impairment in cellular cholesterol efflux by NaOCl-modified HDL_3. We have, however, obtained two lines of evidence that apoprotein (rather than PL) modifications are responsible for decreased cholesterol efflux to OCl^--modified acceptor particles: (i) MPO modification does not affect the lipid moiety of HDL_3 [24] yet results in a comparable decrease of cholesterol efflux as compared to NaOCl-modified HDL_3 (Fig. 3B,C) and (ii) NaOCl modification of discoidal apoA-I/dipalmitoyl phosphatidylcholine particles results in diminished cholesterol removal (Fig. 3D) although palmitic acid is not modified by NaOCl (Fig. 1).

In contrast to the lipid domain (see above), the apoprotein domain of HDL undergoes comparable changes upon modification with either reagent or enzymatically generated HOCl (Fig. 2). One of the amino acids present in apoA-I that are highly sensitive to chemical and/or enzymatic oxidation is methionine. Methionine oxidation of human apoA-I was observed in vitro and in vivo [42,43]. ApoA-I can directly reduce lipid hydroperoxides in vitro, a redox process accompanied by oxidation of Met^{112} and Met^{148} to the corresponding methionine sulfoxides [44,45]. Whether this reaction also occurs in vivo (resulting in the formation of oxidized apoA-I in the circulation) awaits clarification. H_2O_2 oxidation of Met^{112} and Met^{148} in apoA-I resulted in a 30% impairment of cholesterol efflux capacity from human skin fibroblasts as compared to HDL containing unoxidized apoA-I [46]. In line with these findings we have observed Met modification at low molar oxidant:apoA-I ratios (25:1; Table 2) and found a small but significant reduction in cellular cholesterol efflux to rHDL modified with a 5-fold OCl^- excess (Fig. 3D). Whether the cholesterol acceptor properties of methionine sulfoxides containing apoA-I could be restored by treatment with peptide methionine sulfoxide reductase as suggested recently [47] remains to be elucidated.

In addition to methionine residues, other amino acids located in distinct regions of the apoA-I molecule are also of importance for HDL-mediated cellular cholesterol removal. The formation of aggregated apoA-I could be a result of either inter- or intra-particle crosslinks via chloramine and subsequent Schiff base [48] or dityrosine formation [49], affecting amino acids contributing to the functional properties of HDL. Banka and colleagues [9] have identified two distinct regions within apoA-I (amino acids 74–105 and 96–111) apparently involved in cholesterol efflux from monocytes to apoA-I proteoliposomes. Inhibition experiments with monoclonal antibodies directed against continuous epitopes of apoA-I have revealed that regions within, or adjacent to, residues 149–186 [10] and 140–150 [11] determine the ability of apoA-I to promote cellular cholesterol efflux. Another amino acid stretch important for cellular cholesterol removal was identified in pre-β-HDL [13]. All of these epitopes which are involved in the mobilization of cellular cholesterol contain amino acids that are susceptible to NaOCl modification (Table 2). We are currently attempting to identify distinct regions in the apoA-I molecule that are affected by HOCl treatment and could be linked to impaired cholesterol efflux abilities of apoA-I. Whether the structural integrity of the above mentioned apoA-I sequences is a requirement for SR-BI-dependent cholesterol efflux [5,6] is at present not clear. However, regardless of the precise mechanisms it is noteworthy that

modification at low and physiologically occurring HOCl:apoA-I molar ratios (5:1–50:1) during the present study led to impaired cholesterol acceptor properties of rHDL. The HOCl concentrations at sites of acute inflammation were calculated to be about 340 μM [50] and HDL_3 plasma concentrations are between 6 and 12 μM, resulting in an approximate HOCl:apo A:I molar ratio of 30:1 to 50:1, supporting the in vivo relevance of our study.

Acknowledgements: This work was supported by Grants P12000 MED (W.S.) and P11276 MED (E.M.) from the Austrian Research Foundation (FWF). We are grateful for financial support by the Franz-Lanyar Stiftung and the Austrian National-Bank (Projects 6232 to W.S. and 7434 to E.M.).

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