Hypochlorous acid and low serum paraoxonase activity in haemodialysis patients: an in vitro study

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Abstract

Background. Serum paraoxonase 1 (PON1) is an oxidant-sensitive enzyme associated with high-density lipoprotein (HDL) that inhibits the atherogenic oxidation of low-density lipoprotein (LDL). In haemodialysis patients, production of reactive oxygen species, such as hypochlorous acid (HOCl) and hydrogen peroxide, is increased and serum PON1 arylesterase is abnormally low. We have examined the effect of HOCl and the uraemic milieu on serum PON1 arylesterase activity and the ability of HDL to inhibit LDL oxidation in vitro.

Methods. Serum was incubated with HOCl, hydrogen peroxide and products of HOCl reaction with excess cysteine, lysine and taurine and then serum PON1 arylesterase and serum protein tryptophan fluorescence were measured. The ability of plasma HDL fractions isolated by a dextran-sulphate method, to protect LDL from mild oxidation in air, was determined by a fluorimetric method using oxidation of 2,7-dichlorofluorescein (DCFH).

Results. Incubation of healthy serum with HOCl in the range 6.5–32.9 mmol/l resulted in a linear decrease in serum PON1 arylesterase activity to 40% of that without HOCl and a parallel decrease in protein tryptophan fluorescence were measured. The ability of plasma HDL fractions isolated by a dextran-sulphate method, to protect LDL from mild oxidation in air, was determined by a fluorimetric method using oxidation of 2,7-dichlorofluorescein (DCFH).

Conclusions. These results suggest that high concentrations of HOCl that severely oxidize serum proteins and tryptophan residues in the active site of PON1 are required to decrease PON1 arylesterase activity in serum. In haemodialysis patients, overproduction of HOCl that leads to high concentrations of severely oxidized proteins and increased oxidants in plasma might also contribute to low serum PON1 arylesterase activity, but does not appear to impair the ability of an HDL molecule to protect LDL from mild oxidation.

Keywords: haemodialysis; hypochlorous acid; low-density lipoprotein oxidation; paraoxonase
cardiovascular disease is a major cause of death. However, the ability of HDL from haemodialysis patients to protect LDL against copper ion oxidation in vitro is controversial and may be impaired [6] or unchanged [4]. Low serum HDL concentration [5], but not PON1 genotype [7], is thought to contribute to the abnormally low serum PON1 activity in haemodialysis patients. There is evidence that other factors may also contribute to low serum PON1 activity in these patients. The ratio PON1 activity/HDL cholesterol is abnormally low in haemodialysis patients [5]. Since PON1 is sensitive to oxidants and is inactivated by oxidized lipids [8], it can be postulated that increased oxidative stress may decrease serum PON1 activity and impair the antioxidant activity of HDL in haemodialysis patients.

Increased output of reactive oxygen species, including hydrogen peroxide, superoxide and hypochlorous acid (HOCl), by activated neutrophils enhances oxidative stress in haemodialysis patients. HOCl oxidizes proteins leading to a decrease in thiol groups and then to an increase in protein carbonyl groups [9,10]. The active site of PON1 contains a cysteine thiol group that is essential for PON1-mediated protection of LDL against atherogenic oxidative damage [11] and tryptophan residues that are essential for PON1 arylesterase activity [12]. Both cysteine and tryptophan are susceptible to oxidation by HOCl. In haemodialysis patients, plasma concentrations of protein thiols are low and protein carbonyls and advanced oxidation protein products (AOPP) are high [9,13], similar to the pattern when serum is treated with relatively high concentrations of HOCl in vitro [10]. Also, there is evidence that serum from haemodialysis patients contains increased concentrations of low molecular weight oxidants [9] and compounds that generate advanced glycation end-products (AGE) [14]. Increased glycation of PON-1 decreases its activity [15]. Whether increased protein oxidation and glycation contribute to lower serum PON1 arylesterase activity and impair the ability of HDL to protect LDL against oxidation in haemodialysis patients is unclear. The aims of the present study were (i) to determine the effect of exposing serum to the oxidants HOCl and hydrogen peroxide on PON-1 activity and the ability of HDL to protect isolated LDL against oxidation in vitro, (ii) to test the effect of components of serum from haemodialysis patients on PON-1 activity and (iii) to assess the capacity of HDL from these patients to inhibit mild oxidation of LDL.

Subjects

Nineteen haemodialysis patients (15 men and four women) aged 30–79 years (mean 48 ± 13 years) were recruited from the Otago Regional Dialysis Service to compare their serum PON-1/HDL cholesterol ratios with those of 19 healthy volunteers (15 men and four women) aged 23–75 years (mean 41 ± 13 years) who were recruited from the local community and staff of the University of Otago. The haemodialysis patients were receiving dialysis with cellulose acetate membranes and the duration of chronic dialysis was 4–270 months (median 36 months). A further nine patients (eight men and one woman) aged 33–80 years (mean 55 ± 16 years) receiving dialysis with a cellulose acetate membrane (median duration: 12 months; range 2–48 months) and 10 of the healthy subjects (six men and four women) aged 35–75 years (mean 47 ± 12 years) provided serum for the other in vitro studies.

Venous blood from patients with chronic renal failure receiving maintenance haemodialysis and from healthy subjects was collected in plain tubes and in tubes containing lithium heparin. Blood was taken in the morning and, in haemodialysis patients, immediately prior to a haemodialysis session. In most patients, this pre-dialysis blood sample was taken after the long interval between dialysis sessions. Serum was obtained by centrifuging the clotted blood at 1000 g for 20 min at 4 °C. Plasma was obtained by centrifuging blood in the lithium heparin tube under similar conditions. Informed consent was obtained from the subjects and the study was approved by the Otago Ethics Committee.

Preparation of HOCl

A solution of HOCl was prepared by adding 7.5 ml sodium hypochlorite solution (Merck) to 10 ml potassium phosphate solution (100 mmol/l). The concentration of HOCl in the solution was determined from the absorbance at 292 nm (molar absorption coefficient ε = 350 M⁻¹ cm⁻¹) after dilution (1/501) into pH 12 buffer. The solution of HOCl was diluted with phosphate-buffered saline (PBS) solution (pH 7.4) containing disodium hydrogen phosphate (13.5 mmol/l) and sodium chloride (150 mmol/l), to give working solutions of lower HOCl concentration.

Incubation of serum with HOCl and hydrogen peroxide

The HOCl solution (50 μl) and PBS (50 μl) were added to 400 μl serum from healthy individuals to give concentrations of 6.5–32.9 mmol/l and the mixtures were incubated for 30 min at room temperature (21 °C). Unless stated otherwise, all incubations of serum or plasma with HOCl were for 30 min at room temperature. Serum (450 μl) from healthy individuals was also incubated with aliquots (50 μl) of hydrogen peroxide solution (104 mmol/l) or water (control) at room temperature for 30 min. The concentration of hydrogen peroxide was determined from absorption at 240 nm and using the molar absorption coefficient 39.4 M⁻¹ cm⁻¹.

PON1 assay

An aliquot of the serum reaction mixture was diluted 40-fold in 20 mmol/l Tris buffer (pH 7.4) and 60 μl was assayed for PON1 arylesterase activity as described previously [16].

Subjects and methods

Materials

Sodium hypochlorite solution (~1N in 0.1 M sodium hydroxide) and hydrogen peroxide solution (30% w/v) were obtained from Merck Chemicals Ltd (Poole, UK). 2,7-Dichlorofluorescein diacetate and all other chemicals were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA).
The intra-assay coefficient of variation for the PON1 assay was 5%.

**Protein tryptophan fluorescence and fluorescent AGE**

Protein tryptophan fluorescence was measured at excitation 282 nm and emission 331 nm in an aliquot of the serum/HOCl incubation mixture that had been delipidated. Serum (0.1 ml) was delipidated by adding ethanol/diethyl ether (3/1 v/v), vortexing the mixture and allowing it to stand for 15 min before centrifugation for 15 min at 1500 g and 4°C. The supernatant was decanted and the pellet was washed briefly with ethanol/diethyl ether (3/1) and then redissolved in deionized water (2 ml). Fluorescent AGE in redissolved, delipidated serum protein solutions were measured at excitation 350 nm and emission 460 nm and were expressed in arbitrary units relative to quinine sulphate (0.1 μg/ml in 0.1 M sulphuric acid) [17].

**HDL protection of LDL from oxidation**

The ability of HDL to inhibit mild oxidation of LDL *in vitro* was tested essentially by the method of Navab et al. [18], which used HDL isolated by dextran-sulphate precipitation. LDL was isolated from EDTA-plasma by density gradient ultracentrifugation and EDTA was removed by gel-filtration of the LDL solution through a PD-10 column (Bio-Rad, Hercules, CA, USA) equilibrated with Chelex (Bio-Rad, Hercules, CA, USA) treated PBS. Heparin plasma (1 ml) was incubated for 30 min at room temperature with 20 μl HOCl solution, to give a final concentration of 0.95 mmol/l, and 20 μl distilled water (control). Thiol concentration was measured in this incubation mixture after removal of HOCl by gel-filtration through a PD-10 column. HDL was prepared from the HOCl-treated heparin plasma by precipitation with dextran sulphate and magnesium ions [19]. The HDL fraction was passed through a PD-10 gel-filtration column equilibrated with PBS, to remove small molecules. In the experiment that compared the ability of HDL from haemodialysis patients and healthy subjects to prevent LDL oxidation, the HDL fractions were not gel-filtered. The LDL oxidation assay was performed by adding LDL (45 nmol cholesterol) with or without HDL (25 nmol cholesterol) to triplicate polystyrene tubes containing 20 μg of 2,7-dichlorofluorescein (DCFH). After adding PBS to a final volume of 1.075 ml, the tubes were gently vortexed and then incubated at room temperature in the dark for 6 h. Lipid oxidation products formed during lipoprotein oxidation by oxygen in air converts DCFH to a fluorescent form (DCF) that was measured at excitation 485 nm and emission 530 nm. A blank reading that was obtained by incubating PBS and DCFH under similar conditions was subtracted from all readings.

**Other laboratory methods**

Cholesterol and triglycerides in plasma and lipoprotein fractions were measured enzymatically using commercial kits (Roche Diagnostics, Mannheim, Germany). Protein thiol concentration was measured colorimetrically [20].

**Statistical analysis**

Values are means ± SD unless stated otherwise. The paired *t*-test and repeated measures of analysis of variance (ANOVA) with Scheffe’s *F*-test were used to analyse paired data. Student’s *t*-test was used to compare mean values between unpaired samples. Two-tailed significance tests were used and a *P*-value of <0.05 was considered to be statistically significant.

**Results**

Serum PON1 activity (haemodialysis: 56 ± 18 μmol/ml/min; control: 86 ± 17 μmol/ml/min; *P < 0.0001*) and the ratio serum PON1 activity/HDL cholesterol (haemodialysis: 55.3 ± 20.6; control: 70.2 ± 17.9; *P = 0.02*) were significantly lower in the 19 haemodialysis patients compared with the 19 healthy controls. As expected, mean plasma triglyceride (TG) concentration was significantly higher (*P = 0.02*), HDL cholesterol concentration was lower, albeit non-significantly (*P = 0.18*), and total cholesterol (TC) was not significantly different in the haemodialysis patients compared with the healthy controls (haemodialysis: TG 1.89 ± 0.61 mmol/l, TC 4.93 ± 1.03 mmol/l, HDL 1.10 ± 0.47 mmol/l; healthy controls: TG 1.44 ± 0.57 mmol/l, TC 5.43 ± 1.10 mmol/l, HDL 1.27 ± 0.27 mmol/l). Mean age was not significantly different (*P = 0.1*) between these groups of subjects.

PON1 activity decreased linearly in healthy serum incubated at room temperature with increasing HOCl concentration (Figure 1A). A decrease of 40% in serum PON1 activity was achieved at the highest concentration of HOCl (32.9 mmol/l). There was an essentially parallel decrease in serum PON1 activity in serum incubated at 37°C with increasing HOCl concentration, indicating that HOCl has a similar effect on PON1 activity at physiological temperature. Tryptophan fluorescence (excitation: 282 nm; emission: 331 nm) in delipidated serum protein (Figure 1B) also paralleled the decrease in PON1 activity in serum incubated with increasing concentrations of HOCl. Incubation of serum from healthy subjects with hydrogen peroxide (10 mmol/l) did not significantly alter serum PON1 arylesterase activity (hydrogen peroxide: 76 ± 21 μmol/ml/min; distilled water: 76 ± 24 μmol/ml/min; *n = 3*).

To determine the effect of HOCl on PON1 activity in serum from haemodialysis patients, serum from six patients receiving maintenance haemodialysis (mean PON1 activity: 56 ± 18 μmol/ml/min) and eight healthy subjects (mean PON1 activity: 91 ± 16 μmol/ml/min) was incubated with HOCl (32.9 mmol/l). The magnitude of the decrease in serum PON1 arylesterase activity in haemodialysis serum was not significantly different from the magnitude of the corresponding decrease in serum from control healthy subjects (haemodialysis: −35 ± 11 μmol/ml/min, *n = 6*; control: −37 ± 10, *n = 8*; *P = 0.73*).

The effect of product(s) from the reaction of HOCl with an excess of cysteine, lysine and taurine on the
PON1 activity of serum *in vitro* is shown in Figure 2. Compared with controls (PBS with no amino acid and amino acid plus NaOH), serum PON1 activity decreased significantly (*P* = 0.006) following incubation of serum with taurine plus HOCl reaction products and did not change significantly in serum treated with reaction products of cysteine and lysine with HOCl. Measurement of taurine N-chloramine formation at 252 nm and using a molar absorption coefficient of 429 M$^{-1}$cm$^{-1}$ [21] indicated a concentration of 13.86 mmol/l taurine N-chloramine in the final incubation mixture with serum. The amount of HOCl reacted with taurine is calculated to give a final concentration of 21 mmol/l in the serum incubation mixture. The absorption at 292 nm was low, indicating minimal (if any) remaining HOCl and was not significantly different in 1/501 dilutions of stock solutions of lysine (0.026 ± 0.005, *n* = 3) and taurine (0.028 ± 0.004, *n* = 3) that had been reacted with HOCl. Relative fluorescent intensity of protein tryptophan decreased significantly (*P* = 0.008) in healthy serum (*n* = 3) following incubation with the reaction product(s) of taurine plus HOCl (control: 36.0 ± 0.9 AU; HOCl plus taurine: 32.4 ± 0.9 AU).

To determine the effect of uraemic oxidants and glycating precursors in haemodialysis serum on PON1 activity *in vitro*, serum from haemodialysis patients and healthy controls was incubated for a week at 37°C and PON1 activity and fluorescent AGE were measured. The data in Table 1 indicate that serum PON1 activity decreased significantly (*P* < 0.001) by 65–70% and fluorescent AGE increased significantly (*P* < 0.001) during the incubation period in both groups of subjects. The magnitude of the decrease in serum PON1 activity was significantly less while the increase in fluorescent AGE was significantly larger in incubated serum from haemodialysis patients compared with healthy subjects. When serum from healthy subjects was incubated for a week at 37°C, serum PON1 activity did not decrease during the first day and then decreased in an essentially linear fashion during the remaining 6 days (Figure 3).

To determine the ability of a plasma HDL fraction isolated from haemodialysis patients to inhibit the mild oxidation of LDL by air *in vitro*, plasma HDL fractions from seven healthy subjects and seven haemodialysis patients were incubated with LDL from a healthy subject in the presence of DCFH and the formation of
DCF fluorescence induced by oxidized LDL was monitored. Figure 4 indicates that the proportion of LDL control fluorescence remaining after incubation of HDL from haemodialysis patients with LDL was significantly higher compared with the corresponding proportion for healthy subjects. The 95% confidence intervals of these proportions (haemodialysis: 0.23–0.47; control: 0.52–0.66) indicate that they are significantly different from the control LDL value of 1.

The proportion of control fluorescence present when HDL alone was incubated with DCFH was also significantly higher with HDL from haemodialysis patients compared with healthy subjects. The mean percentage decrease in fluorescence due to addition of HDL to LDL calculated after subtraction of fluorescence due to HDL alone was 100 ± 6% for haemodialysis patients and this value was not significantly different (P = 0.14) from the corresponding mean value of 95 ± 6% for healthy subjects.

To examine the effect of HOCl on the ability of plasma HDL to inhibit isolated LDL oxidation by air, plasma from healthy subjects was incubated with HOCl (0.95 mmol/l) and the HDL fraction was isolated, gel-filtered to remove small molecules and then incubated with LDL plus DCFH. This concentration of HOCl oxidizes nearly all plasma protein thiols and may include the cysteine thiol in PON1 that is required for HDL to protect LDL against oxidation. Figure 5 shows that the proportion of oxidized LDL-generated DCF fluorescence remaining after incubation of HOCl-treated HDL was significantly (P = 0.008) higher compared with the corresponding proportion after incubation with control HDL. This increase occurred in all four separate experiments and the magnitude of the increases varied substantially (5-fold). Data were log-transformed to minimize the impact of this variance in statistical analysis using the paired t-test. The amount of fluorescent DCF that was formed and expressed as a proportion of oxidized LDL-induced fluorescence was significantly (P = 0.0009) higher when HDL from HOCl-treated plasma compared with control HDL was incubated alone with DCFH. The mean ± SD percentage decrease in fluorescence due to addition of HOCl-treated HDL to LDL calculated after subtraction of fluorescence of HDL alone was 95 ± 4% (n = 4) and this value was not significantly different at P = 0.004 in log-transformed data; significantly different at P = 0.002 in log-transformed data.

### Table 1. Effect of prolonged incubation of serum from healthy subjects and haemodialysis patients on PON1 arylesterase activity and the concentration of protein fluorescent AGE

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n = 10)</th>
<th>Haemodialysis patients (n = 9)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>PON1 arylesterase activity (μmol/ml/min)</td>
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<tr>
<td>Baseline</td>
<td>88 ± 16</td>
<td>56 ± 16</td>
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<tr>
<td>1 Week</td>
<td>31 ± 15</td>
<td>17 ± 7</td>
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<tr>
<td>Change</td>
<td>−57 ± 18</td>
<td>−39 ± 18</td>
<td>0.04</td>
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<tr>
<td>Fluorescent AGE (AU/ml)</td>
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</tr>
<tr>
<td>Baseline</td>
<td>9.06 ± 1.82</td>
<td>35.43 ± 15.45</td>
<td></td>
</tr>
<tr>
<td>1 Week</td>
<td>10.96 ± 1.64</td>
<td>39.24 ± 15.55</td>
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<tr>
<td>Change</td>
<td>1.90 ± 0.56</td>
<td>3.81 ± 1.67</td>
<td>0.01</td>
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</table>

Values are means ± SD. Serum was incubated in sealed tubes for 1 week at 37°C. *Significantly different compared with baseline at P < 0.001.
different from the corresponding value for control HDL (96 ± 5%, n = 4). The concentration of protein thiol groups was 231 μmol/l in control plasma and 15 μmol/l in plasma treated with 0.95 mmol/l HOCl then gel-filtered to remove small molecules. The mean (± SD) relative fluorescent intensity of DCF generated by LDL oxidation alone was 3.36 ± 1.2 AU. Blank values with DCFH alone and with HDL or LDL alone were low and in the range 0.03–0.09 relative fluorescent units. Values are means ± SEM of four experiments with separate serum samples done in triplicate. *Significantly different at P = 0.008 in log-transformed data; †significantly different at P = 0.0009 in log-transformed data.

The impact of increasing the concentration of HOCl on the oxidation of DCFH by HDL isolated from plasma incubated with HOCl was investigated. The relative fluorescent intensity increased significantly (P = 0.001) when a fraction rich in HDL and other high molecular weight compounds from plasma that had been treated with increasing concentrations of HOCl was incubated with DCFH (Figure 6).

**Discussion**

These data indicate that incubation of serum from both healthy subjects and haemodialysis patients with high concentrations of HOCl substantially decreases serum PON1 arylesterase activity. This HOCl-induced decrease in serum PON1 activity is totally inhibited by excess cysteine and lysine, but not taurine. The data also indicate that high concentrations of endogenous oxidants, uraemic toxins and glycating precursors do not accelerate the decrease in PON1 activity during prolonged incubation of serum from haemodialysis patients in air. In addition, the ability of HDL to protect isolated LDL against mild oxidation is not abnormally impaired in haemodialysis patients. It is possible that oxidation of PON1 by HOCl may contribute to the abnormally low serum PON1 activity and the low ratio PON1 activity/HDL in haemodialysis patients that we confirmed in the present study. The decrease in PON1 arylesterase activity during incubation of serum with HOCl might be due to oxidation of the PON1 tryptophan residue 280. Tryptophan residues in proteins are readily oxidized by HOCl and the tryptophan 280 residue in PON1 appears to be in the active site and vital for enzyme activity [12]. In the present study, there was a decrease in serum protein tryptophan fluorescence that paralleled the corresponding decrease in serum PON1 activity during incubation of serum with increasing concentrations of HOCl. The high concentration of HOCl that was required to inhibit serum PON1 activity might partly reflect the potent antioxidant capacity of serum albumin thiol groups and might also suggest that severe protein oxidation is needed to impair PON1 arylesterase activity in serum. In human serum, free thiol groups are quantitatively the most important scavengers of HOCl and are mainly located on albumin [10]. It is possible that protein oxidation of sufficient severity to inhibit PON1 activity may occur in haemodialysis patients. Concentrations of protein...
carboxyls [9] and AOPP are markedly elevated in haemodialysis patients and incubation of human serum with high concentrations of HOCl is required to generate these compounds [13]. Concentrations of HOCl > 10 mmol/l are needed to generate appreciable quantities of AOPP in human serum in vitro [13]. Activated neutrophils can generate ~100 μmol/l HOCl in vitro [22]. However, it has been suggested that the localized concentration of HOCl near activated neutrophils in vivo may be much higher [10]. Thus, severe oxidation of circulating proteins by high concentrations of HOCl that are generated by activated leukocytes may potentially contribute to the low serum PON1 activity in haemodialysis patients.

Taurine is present in high concentrations in neutrophils and is thought to act physiologically as a trap for HOCl. α-Amino acids also react readily with HOCl and form reactive aldehydes [23] that can modify proteins and enzymes. The present findings suggest that α-amino acids, but not taurine, may protect serum PON1 against inactivation by HOCl. Reaction with a nearly 3-fold excess of cysteine and lysine completely abolished the ability of HOCl to inactivate serum PON1 activity. In contrast, a similar excess of taurine did not prevent a HOCl-induced decrease in serum PON1 activity. Excess taurine also did not prevent a decrease in serum protein tryptophan fluorescence, indicating a residual capacity of taurine/HOCl reaction products to oxidize serum proteins. Taurine N-chloramine is the stable reaction product of HOCl with taurine and may oxidatively inactivate serum PON1 activity. A previous study has reported that taurine N-chloramine has oxidant activity [21]. It has been suggested that taurine N-chloramine might propagate at a reduced concentration the damaging effects of HOCl on biological macromolecules [21] and this may include PON1. The present findings are in keeping with this concept.

Serum from haemodialysis patients contains low concentrations of antioxidants, including ascorbate, protein thiols and albumin, that scavenge HOCl [10]. However, in the present study the uraemic milieu did not influence appreciably the effect of added HOCl on serum PON1 arylesterase activity. The absolute decrease in PON1 activity caused by incubation with HOCl was similar in serum from haemodialysis patients and healthy subjects. Thus, protection against some forms of HOCl-induced damage does not appear to be compromised in haemodialysis serum.

Serum from haemodialysis patients also contains increased concentrations of low molecular weight compounds that increase protein glycation during prolonged incubation with the high molecular weight fraction of serum [14] and increased concentrations of low molecular weight oxidants [10]. Both increased glycation [15] and oxidation of HDL and PON1 can lead to a decrease in PON1 activity. In the present study, PON1 arylesterase activity decreased markedly in serum incubated for 1 week and the absolute magnitude of this decrease was less, despite a larger increase in fluorescent AGE on serum proteins, in haemodialysis serum compared with serum from healthy subjects. This finding suggests that glycating precursors and low molecular weight oxidants in serum from haemodialysis patients might not damage PON1 sufficiently to decrease its arylesterase activity in vitro. The mechanism responsible for the decrease in serum PON1 activity during prolonged incubation of serum in air remains to be determined.

A recent study has reported an assay for detecting HDL that is dysfunctional in preventing mild oxidation of isolated LDL [18]. In this assay, a HDL fraction from normal plasma inhibits the fluorescent signal that is generated as oxidized lipids that are formed during incubation of LDL in air oxidize DCFH to the fluorescent DCF. In the present study, we have tested the ability of HDL fractions from haemodialysis patients and from serum that has been severely depleted of protein thiols by a relatively low concentration of HOCl (0.95 mmol/l) to inhibit this fluorescent signal. Although the HDL fraction from haemodialysis patients inhibited the fluorescent signal in the presence of LDL less than did the corresponding fraction from healthy subjects, this does not indicate that haemodialysis HDL is dysfunctional in preventing the formation and/or inactivation of oxidized lipids in LDL. The HDL fraction from haemodialysis patients alone increased DCF fluorescence more than did healthy HDL. It is possible that this increase in DCF fluorescence may be due to oxidants in plasma from haemodialysis patients. The HDL-rich fraction contained all plasma components, excepting apolipoprotein B-lipoproteins. Our data tend to support the previous finding that the ability of HDL from haemodialysis patients to inhibit LDL oxidation is not abnormal [4]. In contrast, Morena et al. [6] have reported that the ability of HDL from haemodialysis patients to inhibit LDL oxidation by copper ions is impaired. Differences in methodology and characteristics of the haemodialysis patients might potentially contribute to these discrepant findings.

The present data indicate that reaction of plasma with a comparatively low concentration of HOCl that oxidizes almost all free thiol groups does not affect the ability of isolated HDL to protect isolated LDL from mild oxidation. It appears that HOCl-induced oxidation of the free thiol group on cysteine-283 of PON1 that is essential for HDL-mediated protection of LDL against oxidation [11] (but not for PON1 activity) does not impair the protective effect of HDL. This resistance of HDL antioxidant activity to the effects of HOCl may partly underlie the unimpaired ability of HDL from haemodialysis patients to protect LDL from in vitro oxidation.

Our findings suggest that the reaction of plasma with HOCl might produce high molecular weight oxidants. The HDL fraction from plasma that had been incubated with a relatively low concentration of HOCl and then depleted of low molecular weight compounds, oxidized DCFH to DCF. This fraction contained all high molecular weight components of plasma, excepting apoB-containing lipoproteins. It is possible that high molecular weight oxidants generated by HOCl...
might be uraemic toxins and that they might contribute to the higher oxidant activity of the HDL fraction from haemodialysis patients compared with healthy subjects.

This study has limitations. The number of subjects in the in vivo studies was relatively small. Thus, care should be exercised in extrapolating the findings to the general population of haemodialysis patients.

In conclusion, the results of this study indicate that serum PON1 arylerase activity is reduced by relatively high concentrations of HOCl that severely oxidize serum proteins and decrease concentrations of protein tryptophan, which is vital for PON1 arylerase activity. Serum from haemodialysis patients contains high concentrations of severely oxidized proteins. Thus, conditions including overproduction of HOCl, which lead to elevated concentrations of severely oxidized serum proteins, might be expected to contribute to low serum PON1 arylerase activity in these patients. A normal decrease in PON1 activity during prolonged incubation of serum suggests that conditions capable of enhancing the loss of PON1 activity do not persist in serum from haemodialysis patients. Less severe serum protein oxidation by lower concentrations of HOCl does not influence PON1 activity or the ability of HDL to protect LDL from mild oxidation in vitro. This protective effect of HDL appears to be normal in haemodialysis patients, suggesting that it may not be directly affected by uraemic oxidants in vivo. However, lower HDL concentrations may reduce HDL-associated antioxidant protection and coupled with increased concentrations of oxidants derived from HOCl this may increase the risk of oxidative modification of LDL in the arterial intima in haemodialysis patients. α-Amino acids, but not the β-amino acid taurine, nullify the inhibitory effect of HOCl on PON1 arylerase in vitro. Whether administration of α-amino acids as part of intradialytic parenteral nutrition increases serum PON1 arylerase activity in haemodialysis patients remains to be determined.

Conflict of interest statement. None declared.

References


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