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Paraoxonase-1 concentrations in end-stage renal disease patients increase after hemodialysis: Correlation with low molecular AGE adduct clearance

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Abstract

Background: Hemorrhagic stroke and ischemic heart disease continue to be key problems in patients with end stage renal failure. Reduced serum paraoxonase (PON-1) activity has been described in these patients, which could contribute to the accelerated development of atherosclerosis. We hypothesized that retention of uremic toxins and or "middle molecules" including advanced glycation (AGE) free adducts and peptides could play a mechanistic role in decreasing PON-1 activity.
Methods: We enrolled 22 ESRD patients undergoing hemodialysis in whom paired pre- and post-dialysis samples were studied along with 30 age-matched control subjects.
Results: ESRD patients showed a 76% decrease in PON-1 activity. As expected, ESRD patients had an increase in lipoperoxides and advanced oxidation protein products (AOPP). Our patients had a 3-fold increase in serum AGEs and a striking 10-fold increase in low molecular weight (<10 kDa) AGEs. Post-dialysis samples in all patients displayed an increase in PON-1 activity, which ranged from 4 to 40% of the predialysis value. HDL-cholesterol, apoAI, free cholesterol (as a LCAT surrogate), HDL-subclasses and TG did not change significantly after dialysis. Changes in PON-1 activity display a good correlation ($r=0.66$, $p<0.001$) with rates in which creatinine and urea are cleared. Clearance of low molecular weight AGEs after hemodialysis explains 79% of the changes in PON-1 activity and are hence a much better predictor than creatinine changes ($r=0.89$, $p<0.00$). *In vitro* incubation of paraoxonase with serum ultrafiltrates show a time and concentration dependent inhibition of PON-1 by the ultrafiltrates, an inhibition that is up to 3 times higher (from 8 to 24%) when chronic renal failure patients are the source of the ultrafiltrate.
Conclusion: We showed that HD results in a significant, consistent increase in the activity of the antioxidant enzyme PON-1. The effect, correlates with the effectiveness of dialysis to clear creatinine and urea, and with the clearance of AGE adducts of low molecular weight. This effect was replicated *in vitro*, showing time and dose dependency. Our results suggest that another cause for the observed lower PON-1 concentrations in CRF are the retention of low-middle molecules and demonstrate a positive effect of hemodialysis in the delicate oxidant–antioxidant state of these patients, that should be weighted against other pro-oxidant effects that have also been shown to occur previously. If the hypothesis that AGEs are the main culprits is proved in further research, this opens a putative therapeutic avenue for AGE blockers in ESRD.

Keywords: Oxidative stress; HDL; Uremia; Maillard reaction; Macroangiopathy; Atherosclerosis; Chronic renal failure; Uremia

1. Introduction

The major cause of mortality in patients with end stage renal disease (ESRD) receiving renal replacement therapy is cardiovascular disease [1,2]. They have multiple metabolic abnormalities that may accelerate atherosclerosis, such as

Abbreviations: apoAI, apolipoprotein AI; AGE, advanced glycation endproducts; AOPP, advances oxidation protein products; CRF, chronic renal failure; ESRD, end stage renal disease; HD, hemodialysis; HDL, high density lipoprotein; IMA, ischemia modified albumin; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; PON-1, paraoxonase 1.

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hypertension, insulin resistance, and dyslipoproteinemia, along with other ESRD-related risk factors [1–4]. Chronic renal failure (CRF) patients frequently have lipoprotein abnormalities such as low high-density lipoprotein (HDL)-cholesterol concentrations and hypertriglyceridemia [3–5]. HDL-cholesterol concentrations are inversely correlated with atherogenic risk. HDL not only is a key player in reverse cholesterol transport but has the ability to protect low-density lipoprotein (LDL) against oxidation [6]. LDL oxidation is at present believed to be an early key event in the progress of atherosclerosis, leading to foam-cell formation. Oxidized-LDL is linked to other atherogenic properties, which include cytotoxicity and the stimulation of thrombotic and inflammatory events [6]. The essential mechanism by which HDL inhibits LDL oxidation is partly enzymatic. There is mounting evidence that paraoxonase (PON-1) could be implicated in this process [7–10]. Human PON-1 (aryldialkylphosphatase, EC 3.1.8.1) is an esterase associated with apolipoprotein AI (apoAI) and clusterin (apolipoprotein J) in HDL. PON-1 displays paraoxonase and arylesterase activities since it hydrolyzes organophosphate compounds such as paraoxon, and aromatic carboxylic acid esters such as phenylacetate [8,9]. On the other hand, PON-1 was shown to possess peroxidase-like activity that can contribute to its protective effect against lipoprotein oxidation [8–10]. It also possesses homocysteine-thiolactonase activity that may be a linked with its anti-atherogenic properties [10,11]. PON-1 protects lipids in lipoproteins, macrophages and erythrocytes from oxidation. Along with its antioxidative properties, PON-1 has added anti-atherogenic properties against macrophage foam cell formation: reduction of cholesterol and oxidized lipids influx, inhibition of macrophage cholesterol synthesis and stimulation of macrophage cholesterol efflux [12].

Another characteristic feature of chronic renal failure is a profound increase in plasma advanced glycation (AGE) free adducts [13–16]. AGE residues are formed on long and short-lived cellular and extracellular proteins. Cellular proteolysis forms AGE-peptide and AGE free adducts from these proteins, which are released into plasma for urinary excretion [13,14]. AGE free adducts are the chief molecular form by which AGEs are excreted in urine [15,16]. They display a high renal clearance, which distinctly declines in chronic renal failure patients, leading to accumulation of plasma AGE free adducts. This buildup is further augmented in end stage renal disease patients on hemodialysis (HD) by increased AGE formation [14,16]. The toxicity of AGEs resides not only on resistance of the extracellular matrix to proteolysis but mainly to AGE receptor-mediated responses. AGE free adducts may also participate in vascular disease in ESRD.

Paraoxonase activity can be affected by epigenetic factors such as diet, smoking and hormonal milieu [10]. Moreover, serum paraoxonase activity was found to be reduced in many conditions such as myocardial infarction, diabetes and hypercholesterolemia and renal failure [10,17–19]. Putative causes for reduced paraoxonase activity in chronic renal failure patients are reduced HDL concentrations, altered HDL subfraction distribution, reduced PON-1 concentration and different paraoxonase phenotype distributions [17]. The ultimate mechanism that determines the reduction in paraoxonase activity found in patients with renal failure remains uncertain.

We hypothesized that paraoxonase activity is inhibited in the uremic milieu and that contributes to the lower activity seen in ESRD patients, probably in a synergic fashion with the other several factors described above. If our hypothesis is correct, we reasoned that PON-1 activity should rise after a successful hemodialysis intervention, irrespective of changes in HDL concentration or subclass distribution. We further hypothesized that low molecular weight fractions of serum, containing AGE adducts that are cleared in part by dialysis, may be involved in this modification. Finally we tested the hypothesis that low molecular weight substances in ESRD patients serum directly inhibit PON-1 activity and therefore the results *in vivo* could be reproduced *in vitro*.

2. Materials and methods

2.1. Patients and specimen preparation

The renal failure patients were obtained from our outpatient ESRD population consulting the Department of Internal Medicine and Department of Laboratory Medicine and Central Clinical Laboratory, Showa University Northern Yokohama Hospital, Tsuzuki-ku, Yokohama City. A total of 22 patients (10 females and 12 males, 62±12 y) on long-term dialysis with a mean dialysis course of 6.1 y (range 1–19 y) were recruited. Patients had been on maintenance hemodialysis for at least 2 months and were dialyzed three times a week for 3–4 h on a non-reprocessed synthetic dialyzer using polysulfone membranes. Body mass index of the subjects was 21.03±2.9 kg/m2. Patients received no other medication except phosphate binders, ferrous sulfate, and angiotensin-converting enzyme inhibitors.

The underlying disease causing the renal failure were diabetes mellitus nephropathy (7 cases), chronic glomerulonephritis (8 cases), one case each for glomerulosclerosis, lupus nephritis, acute progressive glomerulonephritis, and 5 cases of CRF of unknown origin (no biopsy performed). The age and gender-matched control subjects in the study (n=30) were selected from a healthy population of hospital staff workers with no history of diabetes or renal disease. The research protocol was approved by the institutional review board of Showa University, and investigations were performed in accordance with the principles of the Helsinki declaration.

A fasting blood sample from both subsets was obtained by venipuncture and collected in evacuated tubes. Dry tubes were used for serum or for plasma sodium citrate (5 mmol/l final concentration) or sodium EDTA (5 mmol/l final concentration) were added as anticoagulants. Blood was centrifuged at 1600 ×g, at 4 °C for 7 min and separated serum or plasma was immediately analyzed or frozen at −80 °C until use.

2.2. Analytical measurements

All chemicals are analytical grade and were from Sigma (St. Louis, MO). Cholesterol and HDL-cholesterol as well as triglycerides were measured by enzymatic methods. As an indirect estimation of LCAT activity we measured free cholesterol by a cholesterol oxidase method (Wako, Richmond, VA). Albumin, urea, creatinine and other analytes were measured by standard routine clinical laboratory methods. Unless indicated otherwise, spectrophotometric measurements were made in a DU 800 Spectrophotometer (Beckman Coulter Inc, Fullerton, CA). Protein concentrations were measured by the Bradford method (BioRad, Hercules, CA).Serum lipid peroxides were measured by the method of El-Saadani et al. [20].Determination of advanced oxidation protein products (AOPP) is based on spectrophotometric detection according to Witko-Sarsat et al. [21]. We employ a VERSAmax (Tunable) Microplate Reader (Molecular Devices, Sunnyvale, CA). Concentration of AOPP is expressed in chloramine units (μmol/l). Determination of AGEs is based on the spectrofluorimetric detection. Fluorescence intensity is recorded at the emission maximum (440 nm) upon excitation at 350 nm and at 335/385 for pentosidine