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Detection of oxidants in uremic plasma by electron spin resonance spectroscopy

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Detection of oxidants in uremic plasma by electron spin resonance spectroscopy. Depletion of antioxidants and the presence of products of free radical damage in plasma suggest that oxidative stress is increased in uremia. We have developed an application of electron spin resonance spectroscopy, and used this method to show that a stable oxidizing component or components of plasma accumulate in uremia. No oxidizing activity was detectable in plasma from subjects with normal renal function. The oxidant was detected by its capacity to oxidize the spin trap 3,5-dibromo-4-nitrosobenzene sulphonate (DBNBS). The oxidant was dialyzable from plasma, had an upper molecular weight limit of about 3,000 Daltons and was stable over many months. Physiological plasma concentrations of vitamin C, a water soluble congener of vitamin E and reduced glutathione were unable to inhibit the oxidizing capacity of uremic plasma. Thus, uremia is associated with accumulation of an endogenous oxidizing activity at much higher concentrations than in subjects with normal renal function.

Oxidative damage to lipids and proteins has been proposed as an important event in the pathogenesis of atherosclerosis [1, 2], arthritis and inflammatory diseases [3]. Atherosclerotic vascular disease has been the leading cause of death in uremic subjects for more than 20 years [4]. While hypertension and dyslipidemia are major contributing factors to the pathogenesis of atherosclerosis, several lines of evidence suggest there may be an increased oxidant stress in uremic plasma contributing to the high prevalence of atherosclerosis associated with renal disease.

Evidence for increased oxidant stress in uremia includes low plasma concentrations of the antioxidant vitamin C [5], although normal concentrations have been measured [6, 7] using the same analytical method [8]. Concentrations of vitamin E may be low in uremic plasma [9] but normal in the low density lipoprotein (LDL) fraction [10]. Selenium, a component of the antioxidant enzyme glutathione peroxidase, is found in reduced concentrations in uremic plasma and leukocytes [11, 12]. Concentrations of superoxide dismutase, which catalyses the dismutation of superoxide radicals to hydrogen peroxide are also reduced in erythrocytes [13]. Concentrations of lipid peroxidation by-products are raised in red cell membranes [14, 15] and may be further increased by dialysis [12, 16], providing evidence of on-going lipid peroxidation in uremia. Recently, autoantibodies to oxidized LDL have been detected in uremic plasma, suggesting enhanced LDL oxidation *in vivo* [17].

The source of the oxidant stress in uremic subjects undergoing dialysis has been suggested to be dialysis itself, with reactive oxygen species being generated on the surface of dialysis membranes by activation of neutrophils to produce substances detectable by assays of by-products of lipid peroxidation or chemiluminescence [18–24]. However, endogenous oxidants peculiar to the uremic state might also play a role.

In this study, oxidizing activity of uremic plasma was detected by monitoring the one electron oxidation of 3,5-dibromo-4-nitrosobenzene sulphonate (DBNBS). Oxidizing activity was freely dialyzed, was present in patients with chronic uremia prior to receiving any treatment, and accumulated rapidly in patients with acute renal failure. Oxidizing activity was not inhibited by physiological plasma concentrations of vitamin C, Trolox (a water soluble congener of vitamin E) or glutathione, but was inhibited by glutathione at concentrations found intracellularly. Thus, uremia results in accumulation of endogenous oxidants in plasma.

Methods

Subjects

Thirteen female and 20 male subjects, age 51 ± 13 years (mean ± SD), with chronic renal failure treated by hemodialysis at the Royal London Hospital were studied. Causes of renal failure were (European Dialysis and Transplantation Association registry classification; number of patients): biopsy-proven chronic glomerulonephritis (10; 12), non-biopsied chronic glomerulonephritis (19; 3), adult polycystic kidney disease (41; 4), insulin-dependent diabetes mellitus (80;3), nephrolithiasis (25; 2), reflux nephropathy (24; 1), acquired obstruction (23; 1), congenital obstruction (22; 1), neurogenic bladder (21; 1), amyloidosis (83; 1), non-insulin dependent diabetes mellitus (81; 1), unknown (0; 3). Duration of dialysis was 3.2 ± 3.8 years, range 0.1 to 10.9 years. All patients were dialyzed using cuprophane membranes and acetate or bicarbonate buffers. In addition, 13 patients with end-stage renal disease had plasma oxidizing activity determined prior to any treatment. Causes of renal failure in this group were: unknown (0; 4), renal artery disease (70; 2), chronic glomerulonephritis (19; 1), insulin-dependent diabetes mellitus (80; 1),

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pyelonephritis (20; 1), focal segmental glomerulonephritis (11; 1), adult polycystic kidneys (41; 1), membranous nephropathy (14; 1), and Henoch Schönlein purpura (85; 1). Five laboratory personnel with normal renal function provided non-uremic plasma. Samples were also collected from patients with acute renal failure, receiving cadaveric renal allografts, or with nephrotic syndrome secondary to minimal change nephropathy but with a normal serum creatinine.

Blood samples

Venous blood (5 ml) was drawn into ethylene diamine tetraacetic acid (EDTA) at the end of an interdialytic period, either 48 or 72 hours after the last dialysis. Plasma was separated by centrifugation at 10°C. Creatinine and urea were determined by autoanalyzer (Technicon, DAX). Plasma for determination of oxidizing activity was either used immediately or stored at −70°C until use.

For determination of the relationship between oxidizing activity and neutrophil count, two patients on maintenance hemodialysis were studied. Blood access was via a forearm Cimino fistula. Blood (3.5 ml) was drawn into EDTA for determination of white cell and neutrophil count and for determination of the oxidizing activity. Hemodialysis was commenced, using a bicarbonate dialysate and a cuprophane dialysis membrane (Organon Teknika Nephross[TM]). Blood samples were taken from the arterial side of the fistula (exiting the patient) at regular intervals and neutrophil count and oxidizing activity determined on each sample. Samples for the measurement of oxidizing activity were stored on ice until analysis.

To investigate the relationship between acute pathophysiological changes in renal function and changes in oxidizing activity, venous blood was drawn from patients undergoing renal transplantation, or who were treated for acute renal failure.

Measurement of oxidizing activity

DBNBS was synthesized from 3,5-dibromosulphanilic acid (Aldrich Chemical Company) as described by Kaur, Leung and Perkins [25]. Measurements were made using a JEOL RE1X electron spin resonance spectrometer equiped with an X-band microwave and integral chart recorder with a four-minute sweep time (JEOL UK Ltd, Welwyn Garden City, Herts., UK). Spectrometer settings were: center field 335.0 milliTesla (mT), field width ± 5 mT, field modulation width 0.32 mT, time constant 1 second, microwave power 10 mW, frequency 9.44 GHz, receiver gain 1 to 10×10^3. To prevent auto-oxidation, DBNBS was stored as the solid at 4°C and protected from light until required. Working aliquots of 100 mM DBNBS in phosphate buffered saline (0.05 M NaH_2PO_4/Na_2HPO_4, pH 7.4 containing 0.15 M NaCl; PBS), were stored in the dark at −20°C until use. The oxidizing activity of plasma samples were determined by mixing an aliquot of plasma and DBNBS buffered with PBS solution at zero time. The mixture was aspirated into a quartz capillary tube, capped and placed in the spectrometer. Oxidation of DBNBS resulted in the appearance of a three line spectrum. Experiments were performed to determine the change in amplitude of the three line spectrum with time after mixing with DBNBS, plasma dilution, DBNBS concentration, change in pH and storage of plasma samples. Results from these preliminary studies allowed conditions to be set for which the amplitude of the three line spectrum generated by a given plasma sample was linearly related to the oxidizing activity of that sample. This consisted of adding 190 μl PBS to 300 μl plasma and 10 μl DBNBS, to give a final concentration of DBNBS of 2 mM. Under these conditions, the amplitude (signal height) of the center line of the spectrum measured in arbitrary units and normalized to the same receiver gain, was used as a measure of oxidizing activity.

Determination of clearance of oxidizing activity from plasma by dialysis

Clearance of oxidizing activity was measured in 18 patients. The dialysis circuit was modified to contain a length of sterile connecting tubing of known volume, with access ports at each end in series with the dialysis circuit between the membrane unit and the bubble trap. Blood flow was calculated by timing the passage of an air bubble through the tubing. The procedure was repeated three times and the average blood flow in mls per minute determined. Immediately after recording blood flow, samples of blood were taken into heparin from the arterial and venous sides of the dialysis filter. Plasma was separated and the paired samples were either analyzed the same day (for urea and creatinine by autoanalyzer) or stored at −40°C until determination of oxidizing activity within 24 hours.

Preliminary characterization of oxidizing activity

To determine some of the characteristics of the oxidizing activity in uremic plasma, oxidizing activity was measured before and after uremic plasma was subjected to acid hydrolysis (12 M hydrochloric acid), to trypsin or pepsin digestion (Sigma Chemicals), after addition of sodium azide (100 mM; Sigma Chemicals), or after extraction with hexane. Oxidizing activity was also measured after prolonged storage of plasma at −70°C. Because of the pH dependence of the reaction with DBNBS, all samples were brought to pH 7.4 for measurement of oxidizing activity.

Effect of vitamin C, glutathione and vitamin E on oxidizing activity

Vitamin C (Sigma Chemical Co., Poole, Dorset, UK) was prepared in a nitrogen saturated solution (50 mM) in PBS. Aliquots were added in 190 μl PBS to 300 μl uremic plasma to give concentrations of 0 to 2.5 mM vitamin C in a final volume of 500 μl. After five minutes, 10 μl DBNBS was added (final concentration 2 mM), and the amplitude of the three line spectrum recorded after 30 minutes. Experiments were repeated four times with aliquots of the same plasma sample.

Reduced glutathione (Sigma) was dissolved in PBS to give a 100 mM solution. Aliquots were added to 300 μl plasma as for vitamin C, but giving final concentrations of 0 to 20 mM. DBNBS was added as above and spectral amplitude recorded at 30 minutes.

Since DBNBS is water soluble, detection of oxidizing activity by DBNBS implies that the oxidant is water soluble. To investigate a possible direct interaction between oxidizing activity in uremic plasma and vitamin E, a water soluble congener of vitamin E, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, was used (Trolox, Sigma). The ability of Trolox to inhibit the capacity of uremic plasma to oxidize DBNBS was performed by incubating uremic plasma (300 μl) with PBS (190 μl) containing Trolox to a final concentration of 5 mM and DBNBS (10 μl), final concentration 2 mM.

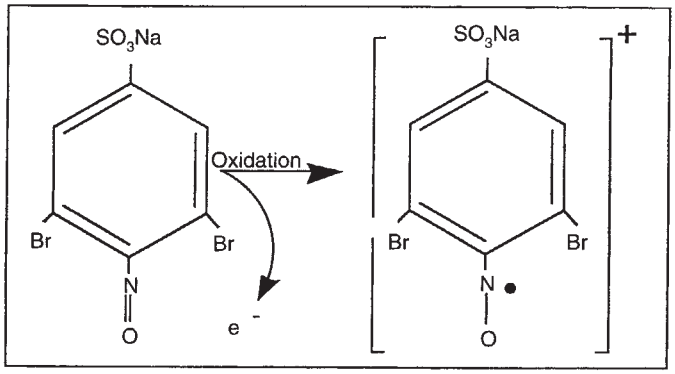

Fig. 1. *Oxidation of DBNBS leads to formation of a nitroso cation radical which is resonance stabilized by the benzene ring.*

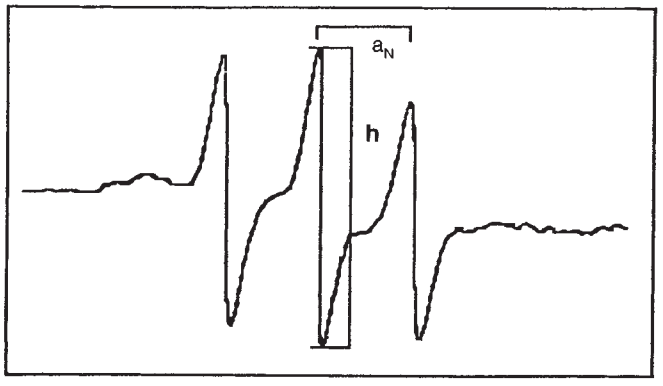

Fig. 2. *Measurement of oxidizing activity.* The amplitude of the center line of the three line spectrum (h) was measured in arbitrary units and corrected to a receiver gain of 7.9×10^3. This was termed the signal height and was directly related to the concentration of the oxidant, determined by dilutions of plasma samples at constant pH and temperature. The splitting (a_N) is representative of both the type of radical detected and its chemical environment, with the unpaired electron in proximity of the nitrogen atom.

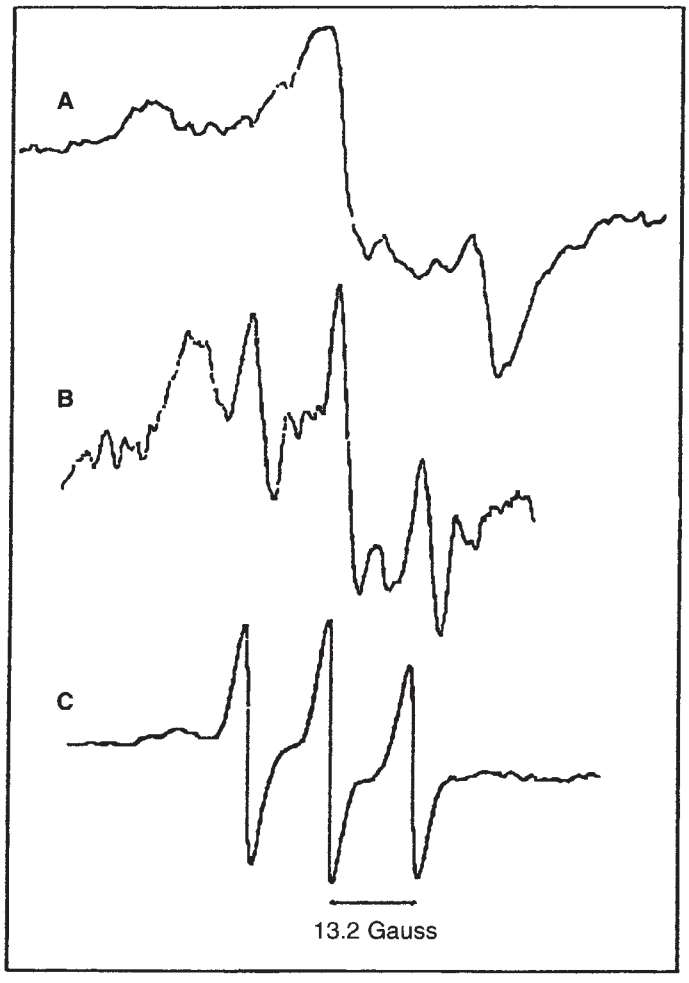

Fig. 3. A. *Spectrum generated when plasma from an individual with normal renal function was mixed with DBNBS (2 mM) at 20°C and pH 7.4.* **B.** Spectrum obtained with plasma from a patient with renal failure, taken prior to dialysis. **C.** Ultrafiltrate of uremic plasma from B, molecular weight cutoff at 3,000 Daltons. The ultrafiltration removed the broad background signal leaving the three line signal characteristic of the nitroso-cation radical of DBNBS.

Statistical analysis

The relationship between plasma creatinine and oxidizing activity was determined using Spearmans rank correlation coefficient using SigmaStat for Windows (Jandel Scientific, San Rafael, CA, USA), after testing the data for the appropriateness of using this test. The coefficient of variation of the electron spin resonance assay was 8%, determined by repeated measures ($N = 20$).

Results

Detection of oxidizing activity

DBNBS undergoes oxidation to form a stable nitroso-cation radical (Fig. 1). In an external magnetic field the odd electron interacts with the nuclear spin of nitrogen, leading to a characteristic three line spectrum, the amplitude of which (h, in Fig. 2) reflects the concentration of the radical. The concentration of the radical in turn relates to the concentration of the substances initiating oxidation within uremic plasma.

There was a clear difference between spectra obtained from normal and uremic plasma incubated with DBNBS. Plasma from subjects with normal renal function produced a broad background signal and no three line spectrum (Fig. 3A). Plasma obtained from uremic patients prior to dialysis caused DBNBS oxidation and a 3 line spectrum, characteristic of a nitroso cation radical, with hyperfine splitting of 1.32 mT, suggesting that the DBNBS radical was free and not covalently coupled to the oxidant (Fig. 3B). The three line spectrum was superimposed upon a broad background signal. DBNBS reacted with whole plasma passed through a 3,000 Dalton cut-off filter (Amicon) gave a clean three line spectrum without the background signal (Fig. 3C). Neither plasma from uremic or normal subjects, or DBNBS incubated alone had any detectable spectrum.

Characterization of assay of oxidizing activity

Because the amplitude of the spectrum increased with increasing pH, samples were buffered to pH 7.4 to allow comparison of different samples (Fig. 4A). Kinetic studies showed a rise in amplitude of the 3 line signal with time which plateaued by 30 minutes for all samples analyzed, so that comparisons of oxidizing

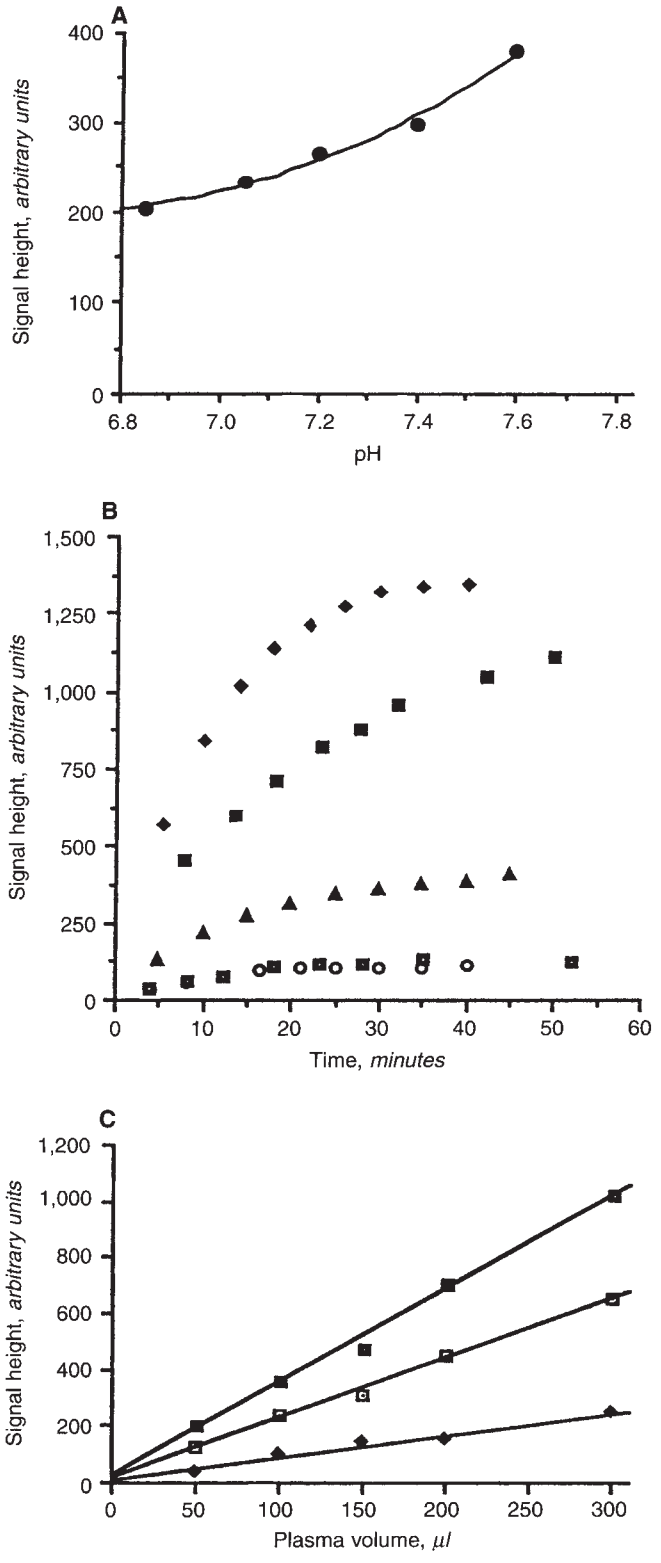

Fig. 4. A. *Oxidizing activity was measured on five aliquots of the same plasma sample from a patient with uremia, with each sample adjusted to a pH between 6.8 and 7.6 using an appropriate phosphate buffer.* Final volumes were plasma 300 µl, phosphate buffer 190 µl, DBNBS (2 mM final concentration) 10 µl. **B.** Data are shown for five individual patients. After incubation of plasma with DBNBS and PBS at zero time, spectra were recorded at the time intervals shown. There was a large variation in oxidizing activity, but a plateau was reached after 30 minutes. For comparison of oxidizing activity between plasma samples, the 30 minute measurement was recorded. **C.** Plasma samples were diluted with PBS and the 30 minute amplitude of the spectrum was recorded. Dilution led to a linear decrease in the amplitude of the spectrum recorded, indicating that the assay was linear for the range of oxidizing activity encountered and conditions of the assay. Lines represent plasma samples from different subjects.

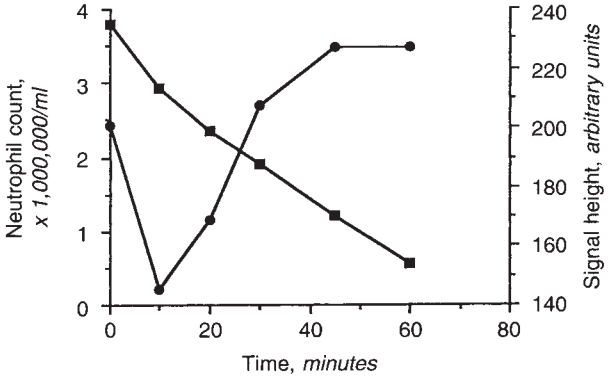

Fig. 5. *Neutrophil count (●) and oxidizing activity (■) determined during the first hour of hemodialysis.* Each data point representing oxidizing activity is the mean of duplicate determinations. Samples were collected as described in **Methods**.

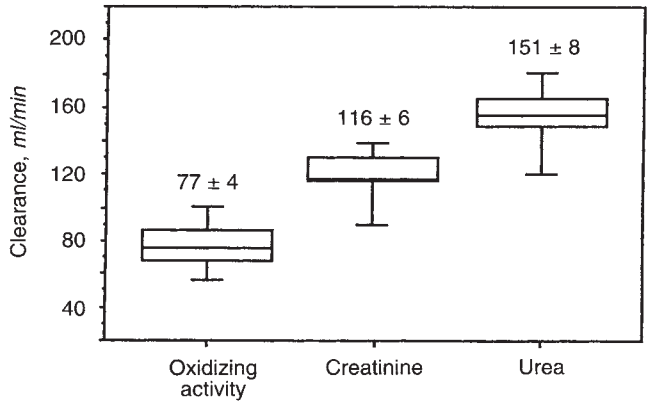

Fig. 6. *Box plot showing centiles (10th, 25th, 50th, 75th and 90th) of the clearance of oxidizing activity, creatinine and urea in 18 patients undergoing hemodialysis.* The numbers are the means ± SEM.

activity between samples were made at this time. Five representative samples are shown in Figure 4B. Serial dilutions of plasma samples with a fixed concentration of DBNBS and a range of oxidizing activities showed that there was a linear relationship between the amplitude of the signal and the concentration of plasma oxidizing activity (Fig. 4C).

Relationship between peripheral blood neutrophil count and oxidizing activity of plasma during dialysis

Figure 5 shows the relationship between whole blood neutrophil count and oxidizing activity in one of two patients studied

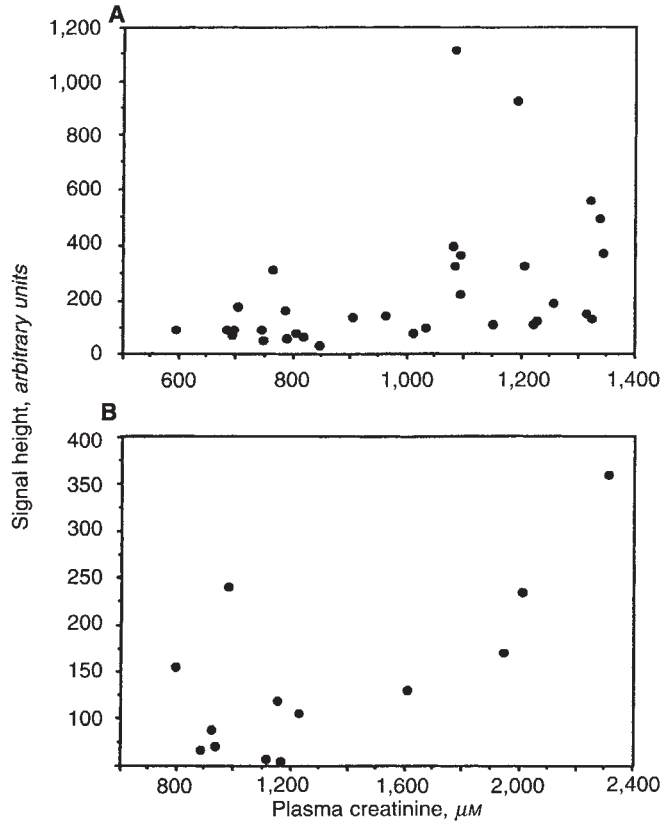

Fig. 7. A. *Association of plasma creatinine concentration with oxidizing activity in 33 subjects on maintenance hemodialysis, prior to a hemodialysis session (P < 0.001).* Individual points are single determinations of oxidizing activity. The c.v. of the assay was 8%. **B.** Oxidizing activity determined in 13 patients with end-stage renal failure, prior to their first hemodialysis sesssion ($P = 0.11$). Association was calculated using Spearman's rank correlation coefficient, as data points are skewed.

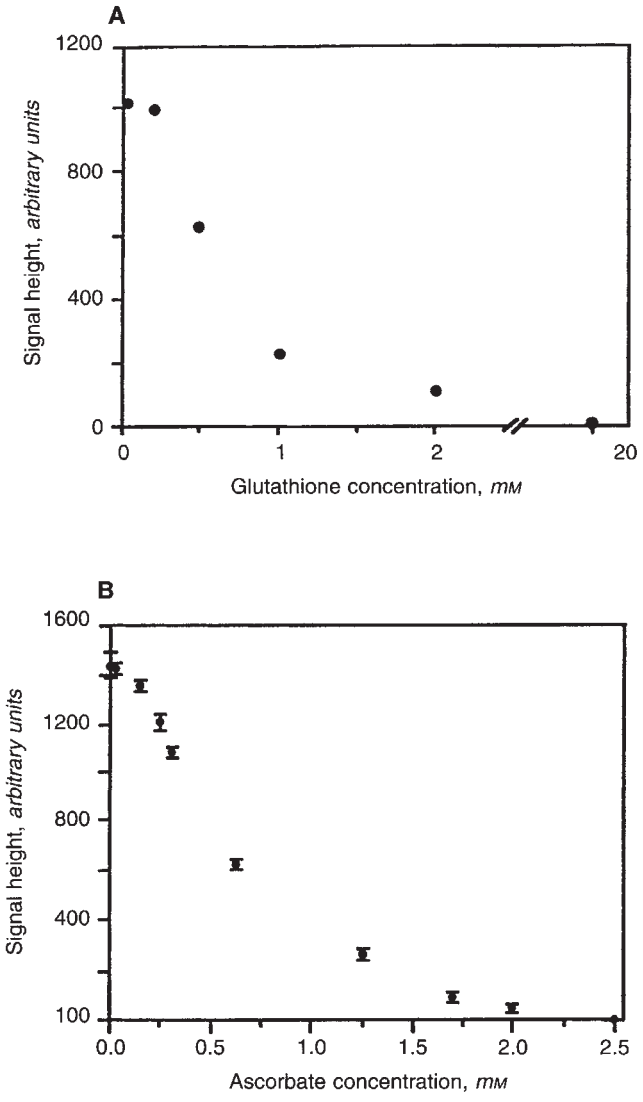

Fig. 8. A. *Glutathione caused a concentration dependent decrease in oxidizing activity, but only within the intracellularly physiological range of glutathione concentrations.* Points are the mean of duplicate determinations with the same plasma sample. **B.** Ascorbate failed to reduce oxidizing activity within the physiological range of plasma concentrations (40 to 80 μM), but reduced activity at a concentration in excess of that achievable by supplementation. Points represent the mean ($N = 4$). Error bars represent the SEM.

during the first hour of hemodialysis. The neutrophil count fell initially, reaching a nadir at around 20 minutes, but rising again and slightly exceeding pre-dialysis levels within the hour. The amplitude of the ESR signal, reflecting the capacity of the plasma sample to oxidize DBNBS, gradually decreased over the same time period.

Clearance of oxidizing activity from plasma by dialysis

Figure 6 shows that the clearance of oxidizing activity from plasma by dialysis was less than that of urea or creatinine. These data are consistent with that of the filtration data reported above, in regard to the approximate molecular mass of the oxidizing activity.

Relationship between oxidizing activity and creatinine pre-dialysis

There was a wide variation in the oxidizing activity of plasma from subjects on maintenance hemodialysis (Fig. 7A). However, there was a strong association between plasma creatinine concentration and oxidizing activity, determined using Spearman's rank correlation coefficient ($N = 33$, $P < 0.001$). Oxidizing activity was determined in 13 patients presenting with end stage renal failure, prior to any treatment (Fig. 7B). Oxidizing activity was clearly increased in this patient population.

Effect of acid hydrolysis, proteolytic digestion, azide or organic extraction on oxidizing activity

Oxidizing activity was reduced to 10% of starting activity by acid hydrolysis ($N = 3$), but was unaffected by either proteolytic digestion with pepsin or trypsin, or treatment with the metabolic inhibitor sodium azide. Activity was not extractable into hexane. Storage of plasma over 72 hours at 20°C, or for over 15 months at −70°C had no effect on the capacity of the sample to oxidize DBNBS.

Effect of antioxidants on oxidizing activity in uremic plasma

Both glutathione and vitamin C inhibited oxidation of DBNBS by uremic plasma. Only high concentrations of vitamin C were

able to inhibit oxidation of DBNBS, and no effect was seen at concentrations achievable physiologically in plasma or tissues, even with dietary supplementation. (Fig. 8). Trolox at a concentration of 5 mM had no effect on the ability of uremic plasma to oxidize DBNBS. Glutathione inhibited oxidation only at concentrations higher than were present in plasma, although at concentrations that were present intracellularly.

Association of oxidizing activity with pathophysiological alterations in renal function. Measurement of oxidizing activity in patients undergoing renal transplantation, or sustaining acute reversible renal failure, demonstrated a close pathophysiological relationship between progressively impaired renal function and accumulation of oxidizing activity in plasma (Fig. 9). Successful renal transplantation was associated with rapid clearance of oxidizing activity from plasma, followed by a slower decline in creatinine concentration (Fig. 9A). Conversely, oxidizing activity in plasma remained at pre-transplant concentrations with primary non-function of the allograft (Fig. 9B). Figure 9 C and D document the plasma creatinine and oxidizing activity profiles of two patients who developed acute renal failure while hospitalized. In both cases, oxidizing activity closely mirrored changes in renal function as reflected by the plasma creatinine. Nephrotic range proteinuria with a normal serum creatinine, occurring in three patients with minimal change nephrotic syndrome, was not associated with detectable oxidizing activity in plasma.

Discussion

Our results indicate that an oxidizing species, monitored by DBNBS oxidation is present in the plasma of subjects with severe renal disease. The oxidant apparently has a molecular weight below 3,000 Daltons, based on the preservation of oxidizing activity in plasma ultrafiltrates and clearance of oxidizing activity during dialysis. Neutrophil activation was demonstrated during dialysis by a transient fall in the peripheral neutrophil count, confirming previous reports [19]. However, it is unlikely that oxidizing activity was generated by neutrophil activation because dialysis was associated with a fall in oxidizing activity. Furthermore, the presence of oxidizing activity in subjects with renal failure prior to any treatment, the inability to detect similar oxidizing activity in subjects with normal renal function, and the strong positive association with plasma creatinine concentration suggests that accumulation of oxidizing activity in plasma is a consequence of falling glomerular filtration rate. The close relationship between oxidizing activity and changes in glomerular filtration rate is also supported by the observations made after transplantation and during episodes of acute renal failure.

The broad background signal generated by incubating DBNBS with normal plasma (Fig. 3A) is indicative of some oxidation of the DBNBS, being detected in a motionally restricted environment. Plasma or DBNBS alone generated no signal. The background signal was also present in uremic plasma but could be filtered out using a 3,000 Dalton filter, indicating that components of plasma causing both low levels of oxidation and motional restriction had a molecular weight above 3,000 Daltons. The background activity appeared of similar magnitude in all samples. It may be indicative of an oxidant separate from that measured by the three line spectrum, an issue that could be studied by fractionation of plasma. In addition, oxidants would be detected in the present system only if able to oxidize DBNBS. Therefore, although the data demonstrate the presence of oxidizing activity in uremic plasma, other potentially important biologically-active oxidants could exist but be undetectable.

Previous studies of pro-oxidants in uremia have focused on methods for determining lipid peroxidation by-products, such as the thiobarbituric acid assay or chemiluminescence to detect reactive oxygen species [18–24]. The thiobarbituric acid assay measures several products of lipid peroxidation as well as other compounds, so the results of this assay are difficult to interpret [26]. The use of DBNBS to characterize oxidizing activity in human plasma has not been reported. It was suggested previously that DBNBS was oxidized by superoxide with which it formed a stable adduct [27], but this conclusion was not substantiated by other studies [28, 29]. Inflamed human synovial tissue was shown to contain activity capable of oxidizing DBNBS, attributed to the presence of superoxide radicals [30]. Later work using pulse radiolysis showed DBNBS could act as a donor substrate to compound I intermediates of peroxidases. Compound I intermediates of peroxidases are formed in the presence of hydrogen peroxide, which is generated by dismutation of superoxide radicals either spontaneously, or catalyzed by superoxide dismutase. Thus superoxide would only be detectable indirectly, and only in systems containing peroxidases [29]. Thus, while the assay used here may be more specific than the thiobarbituric acic assay, it is not possible to characterize the chemical nature of oxidizing activity in uremic plasma from this study. It seems unlikely that the oxidizing activity is dependent on enzymatic activity, on the basis of its probable upper molecular mass limit, its stability and resistance to azide, and this and previous work would suggest that it is not due to superoxide radicals. Fractionation of ultrafiltrates of uremic plasma by HPLC may provide further information as to the chemical nature of the oxidant.

The presence of oxidizing activity in plasma of subjects with impaired renal function could be interpreted as evidence of impaired antioxidant defence mechanisms. This could occur through interaction of oxidizing compounds with endogenous antioxidants. Antioxidant defences in plasma include vitamin C, which scavenges water soluble radicals and also regenerates vitamin E, which is in turn a lipid soluble chain breaking antioxidant [31]. Oxidizing activity was not inhibited by physiologically relevent concentrations of either vitamin *in vitro*, or by glutathione. Glutathione concentrations are in the millimolar range intracellularly, so only if the oxidant species entered cells would there be a sufficiently high concentration of glutathione to be protective. Thiols such as glutathione may act as antioxidants, one mechanism being through their role as electron donors for the enzyme glutathione peroxidase. However, they may also act as pro-oxidants, particularly in the presence of free transition metal ions [32, 33], when superoxide radicals, thiyl radicals and reduced metal ions are formed. In the present system, oxidizing activity was detected in a system containing plasma anticoagulated with EDTA, making the presence of free transition metal ions unlikely. This may indicate that the thiol groups on reduced glutathione are important in inhibiting the oxidizing activity, possibly through an enzyme independent mechanism. Within the limits of this study, it is not possible to give further mechanistic interpretation of these observations.

Although there is a clear relationship between alterations in renal function and changes in oxidizing activity in a clinical setting, direct oxidation of LDL, or cytotoxic effects related to presence of oxidizing activity have not been demonstrated. Provision of such information will require isolation of the oxidizing

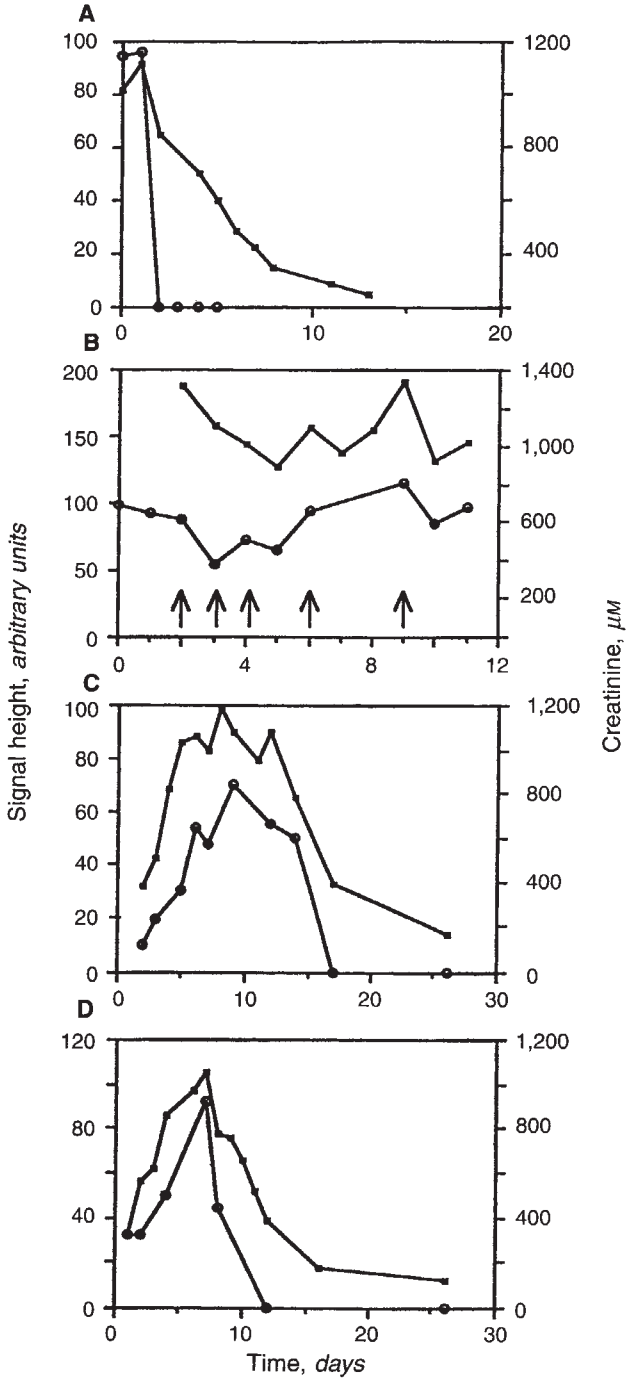

Fig. 9. A *Fall in oxidizing activity in plasma after successful renal transplantation in a 33 yr old male maintained on hemodialysis.* Immunosuppression was attained using cyclosporine A, azathioprine and prednisolone. Transplantation (day 0) was followed by a rapid decline in oxidizing activity which paralleled the swift decline in serum creatinine. **B.** Primary non-function of a renal allograft in a 36-year-old male was associated with retention of oxidizing activity in plasma. The arrows indicate hemodialysis sessions. **C.** Onset and resolution of acute oliguric renal failure in a 48-year-old male secondary to intravascular hemolysis of undetermined cause. Renal failure developed while the patient was hospitalized and serial measurements showed that oxidizing activity and serum creatinine paralleled each other. Hemodialysis was required on days 6, 8 and 11. **D.** Acute non-oliguric renal failure developing after an eclamptic seizure in a 23-year-old female. Resolution occurred without recourse to hemodialysis. The lack of availability of blood samples prior to the onset of renal failure did not allow the exclusion of oxidizing activity in plasma related to the pre-eclamptic phase of her illness.

components plasma. However, Maggi et al have recently shown that susceptibility of low density lipoprotein (LDL) to copper-induced oxidation was greater in uremic patients than controls [10], suggesting that unidentified factors promote oxidation in uremia. There was no difference in vitamin E content of LDL between patients with normal renal function, and those patients with uremia treated either conservatively, or by hemodialysis or peritoneal dialysis. Such findings are consistent with our finding of an endogenous oxidant in uremic plasma.

Supplementation with antioxidants to redress the accumulation of oxidizing activity may not be appropriate, because although resistance of LDL to *in vitro* oxidation has been equated with resistance to changes that promote atherogenicity, recent evidence suggests that these effects may be dissociated [34]. Furthermore, there is little consistent evidence in controlled animal studies that antioxidants at physiological concentrations protect against development of atherosclerosis [35].

In summary, uremia is associated with accumulation of oxidizing activity in plasma, resistant to the antioxidant actions of vitamin C, vitamin E and glutathione at physiological plasma concentrations. It is freely dialyzable and is not the product of dialysis. Such activity might contribute to the changes in the oxidation status in uremic patients noted by other investigators, and may be one factor contributing to the high prevalence of vascular disease in the uremic population.

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