

# Oxidant Stress in Hemodialysis Patients: What Are the Determining Factors?

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**Abstract:** Oxidant stress contributes to morbidity in hemodialysis patients. Three possible causes of oxidant stress have been suggested: the uremic state, the dialyzer membrane, and bacterial contaminants from the dialysate. Oxidant stress occurs in uremia before dialysis therapy is initiated, as evidenced by increased production of reactive oxygen species, increased levels of oxidized plasma proteins and lipids, and decreased antioxidant defenses. It has been proposed that increased production of reactive oxygen species during hemodialysis is also an important contributor to oxidant stress. Hemodialysis is associated with a transient increase in production of reactive oxygen species, particularly with cellulose membranes. In addition,

surveys have shown widespread contamination of dialysate by endotoxin, which may cross membranes and prime production of reactive oxygen species by phagocytic cells. Recent studies, however, show a decrease in protein oxidation from pre- to post-dialysis and a normalization of neutrophil reactive oxygen species production. Taken together, these data suggest that uremia, per se, is the most important cause of oxidant stress in hemodialysis patients. Dialysate quality may also contribute to oxidant stress, but evidence that the dialyzer membrane plays a role is weak. **Key Words:** Oxidant stress—Hemodialysis—Neutrophils—Uremia—Protein—Endotoxin.

Cardiovascular disease is the leading cause of mortality in hemodialysis patients (1). Both inflammation (2,3) and malnutrition (4) increase the risk of cardiovascular death in a synergistic process that Stenvinkel and colleagues have termed malnutrition, inflammation, and atherosclerosis (MIA) syndrome (5,6). Oxidative modification of proteins and lipids has been implicated in the etiology of numerous disorders and diseases (7,8) and such oxidant damage is believed to contribute to inflammation in hemodialysis patients (9).

The causes of oxidant stress in hemodialysis patients are poorly understood. Oxidant stress arises when the normal balance between production of reactive oxygen species and antioxidant activity is tilted in favor of the former. Protein and lipid oxidation may be a consequence of increased production

of reactive oxygen species, a deficiency in antioxidant systems, or both. Further, there are several possible stimuli for increased production of reactive oxygen species in hemodialysis patients, including the uremic state, the choice of dialyzer membrane, and bacterial contaminants derived from the dialysate. This review outlines the evidence for increased production of reactive oxygen species in hemodialysis patients and examines the potential role for each of these stimuli.

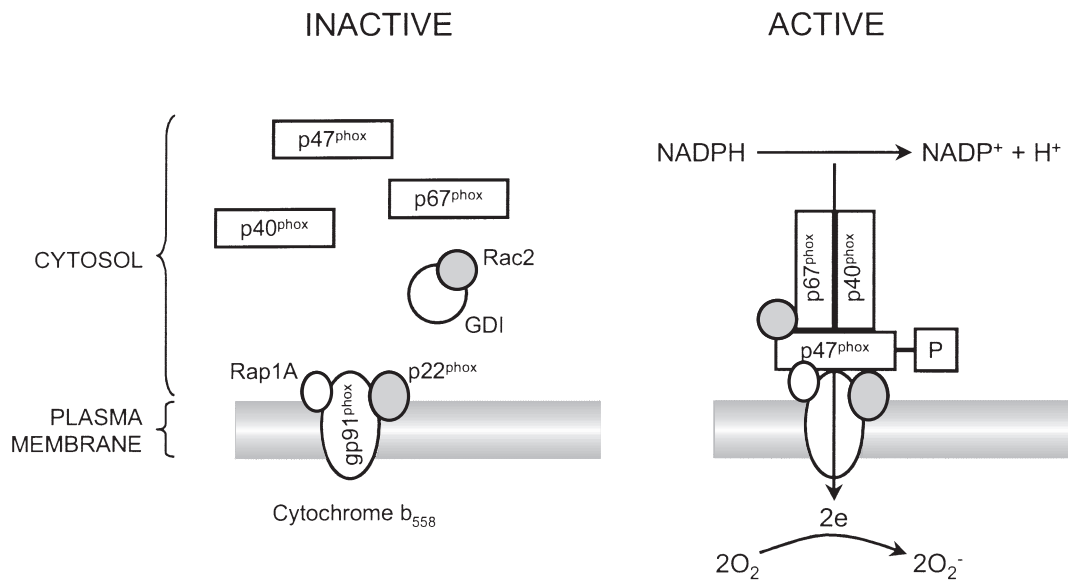
## Production of reactive oxygen species

Phagocytes, such as neutrophils and monocytes, produce reactive oxygen species as part of the host defense against invading bacteria. These cells generate reactive oxygen species using a multicomponent enzyme complex, the NADPH oxidase, in a process referred to as the respiratory burst. Under normal conditions, the NADPH oxidase complex is unassembled, with the individual components being distributed between the cytosol (p40<sup>phox</sup>, p47<sup>phox</sup>, p67<sup>phox</sup>, Rac-2) and the plasma membrane (the two components of cytochrome b<sub>558</sub>, gp91<sup>phox</sup> and p22<sup>phox</sup>, and Rap1A) (10) (Fig. 1). Following activation, the cytosolic components translocate to the plasma membrane, where they associate with cytochrome

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**FIG. 1.** This is a schematic representation of the NADPH oxidase enzyme complex responsible for the production of reactive oxygen species in phagocytic cells. Under normal conditions, the complex is unassembled with its components distributed between the cytosol and plasma membrane (left-hand panel). Following activation, the cytosolic components translocate to the plasma membrane and form the active oxidase (right-hand panel).

b<sub>558</sub> to form the active oxidase (10). In resting neutrophils, only 10–25% of cytochrome b<sub>558</sub> is located in the plasma membrane, with the remaining 75–90% distributed among secretory vesicles and gelatinase and specific granules (11,12). Sequestration of cytochrome b<sub>558</sub> in these storage granules limits the extracellular release of reactive oxygen species following inadvertent stimulation, thereby protecting healthy tissue from unregulated oxidant damage. To kill bacteria effectively, the normal level of neutrophil respiratory burst activity must be enhanced, a process referred to as priming. The neutrophil respiratory burst can be primed by a variety of agents released during the host response to invading bacteria, including cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (13), granulocyte-macrophage colony-stimulating factor (GM-CSF) (14), interleukin-8 (15), bacterial lipopolysaccharide (LPS) (16), and platelet activating factor (PAF) (17). Both TNF- $\alpha$  and LPS prime the respiratory burst by increasing plasma membrane expression of cytochrome b<sub>558</sub> through translocation of secretory vesicles and specific granules (12,18).

#### Neutrophil respiratory burst activity and protein and lipid oxidation in hemodialysis patients

Neutrophils obtained from hemodialysis patients immediately before a dialysis treatment demonstrate a higher spontaneous rate of production of reactive oxygen species than do neutrophils from healthy sub-

jects (19,20). Further, these neutrophils are primed for an enhanced respiratory burst following exposure to both particulate and soluble stimuli (19,21).

Consistent with an increased production of reactive oxygen species, proteins and lipids from hemodialysis patients exhibit higher levels of oxidation than do those from healthy subjects. Increased protein oxidation is evidenced by changes in the concentration of free sulfhydryl groups, carbonyl groups, and 3-chlorotyrosine on plasma proteins (22–25), and the appearance of advanced oxidation protein products (AOPP) (26). Early studies of lipid peroxidation produced ambiguous results, with levels of lipid peroxidation products being reported as normal (27,28) or increased (29,30). However, the discrepancies between these studies may reflect the short plasma half-lives of lipid peroxidation products and the relative non-specificity of the tests (31). More recently, measurement of plasma concentrations of esterified F2-isoprostanes, which are chemically stable products of arachidonic acid oxidation, by a sensitive and specific gas chromatography–mass spectrometry assay demonstrated significantly higher levels of esterified F2-isoprostanes in hemodialysis patients than in normal subjects (32).

#### Neutrophil respiratory burst activity and protein and lipid oxidation in uremia

Several lines of evidence support the argument that uremia, per se, is a major contributor to the

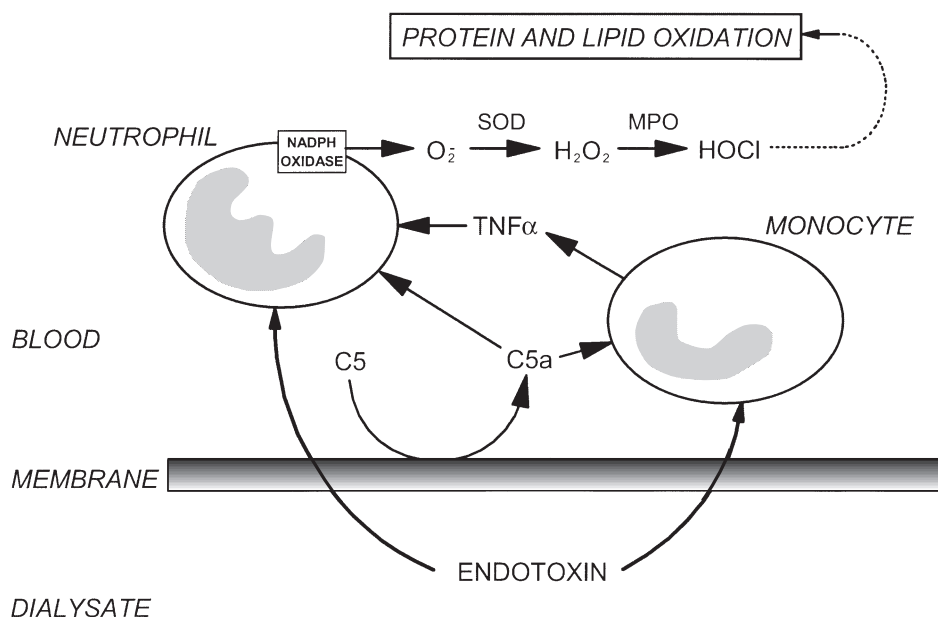
oxidant stress observed in hemodialysis patients. Neutrophils from patients with renal insufficiency are primed for an enhanced respiratory burst in response to both soluble and particulate stimuli well before the initiation of dialysis therapy (33–35). At least some of this priming appears to involve a factor(s) retained in plasma as renal function decreases, since incubation in plasma from patients with renal failure (19) or peritoneal dialysis effluent (36) primes and stimulates production of reactive oxygen species by normal neutrophils. In addition, while standard hemodialysis does not normalize neutrophil respiratory burst activity, there is partial correction with hemodiafiltration (37) and complete correction by transplantation (19).

Patients with renal insufficiency show increased levels of protein oxidation, as indicated by a decrease in plasma protein free sulfhydryl groups (23), increases in plasma protein carbonyl groups (23), advanced oxidation protein products (26,38), and increased leukocyte DNA oxidation (35). Increased lipid oxidation is also reported (29). Some of these changes occur as early as a creatinine clearance of 20 ml/min (34,38). Also, patients with renal failure exhibit comorbid conditions linked to oxidant damage before the initiation of dialysis therapy. For example, these patients are reported to have advanced atherosclerosis as evidenced by significant thickening of their carotid artery walls (39), and coronary artery stenosis (40).

### Effects of hemodialysis on neutrophil respiratory burst activity and protein and lipid oxidation

The hemodialysis procedure is associated with an increase in the production of reactive oxygen species, to a degree depending on the membrane material. Cellulose membranes are associated with a significant increase in resting and stimulated  $H_2O_2$  production during the first 30 minutes of dialysis (41–43). Lesser increases in the production of reactive oxygen species are observed with modified cellulose membranes and synthetic membranes, such as polymethylmethacrylate and polysulfone (41,44). There are two potential mechanisms by which hemodialysis could stimulate the production of reactive oxygen species. First, interactions between blood and the dialyzer membrane could lead to the generation of inflammatory mediators, such as the complement components C3a and C5a or platelet activating factor (PAF), which may then stimulate neutrophils. Second, bacterial products in the dialysate might cross the dialyzer membrane and directly or indirectly stimulate release of reactive oxygen species by neutrophils. These pathways to neutrophil activation, which may interact, are shown schematically in Fig. 2.

Hemodialysis membranes activate the alternative pathway of complement to a degree dependent on the membrane material (45–47). The complement fragment, C5a, which is produced by this activation stimulates (48), or at substimulatory concentrations



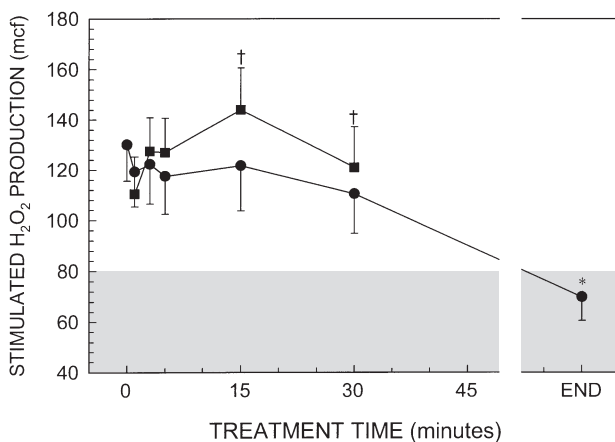
**FIG. 2.** Potential pathways to neutrophil activation during hemodialysis are illustrated.

primes (49), neutrophil respiratory burst activity. That complement activation is a stimulus to the production of reactive oxygen species during hemodialysis is supported by the observation that those dialyzer membranes that cause the most complement activation also show the highest level of reactive oxygen species production during hemodialysis (41,47,50). The two events occur with similar time courses (42,44), and infusion of soluble complement receptor 1, a potent inhibitor of complement activation, significantly reduces production of reactive oxygen species by neutrophils in an ex vivo model of hemodialysis (51). PAF is released in the first few minutes of dialysis, particularly when AN69 membranes are used (52). PAF is known to prime the neutrophil respiratory burst (17) and some investigators have suggested that PAF may also act as a stimulus to the production of reactive oxygen species by neutrophils during hemodialysis (43,53).

While the evidence that inflammatory mediators are released as a result of blood-membrane contact is convincing, the effect is transient. Indeed, a recent study has shown that the overall impact of dialysis with high-flux membranes is to reduce neutrophil production of reactive oxygen species, in spite of a small increase during the first 30 minutes of dialysis (Fig. 3) (44). Along with a reduction in the production of reactive oxygen species, dialysis is also associated with an improvement in some biomarkers of oxidant stress. The concentration of plasma protein free sulfhydryl groups increases from pre- to post-dialysis (23,44), indicating an improvement in oxidant

stress status. This improvement is limited to oxidation reactions that are reversible, such as sulfhydryl group oxidation, and does not occur for irreversible reactions, such as protein carbonyl or AOPP formation (23,44). Taken together, these data suggest that the overall effect of high-flux hemodialysis is to mitigate oxidant stress. That the dialyzer membrane is not a major contributor to oxidant stress is also supported by the finding that continuous ambulatory peritoneal dialysis (CAPD) is characterized by significant oxidant stress (19,26,35).

Bacterial contamination of dialysate to levels in excess of recommended quality standards is widespread (54–56); a situation made more remarkable by the considerable debate over whether or not these standards are sufficiently stringent (57). Under laboratory conditions, lipopolysaccharide (LPS) fragments and other bacterial products in the dialysate have been shown to stimulate mononuclear cells across both high-flux and low-flux dialysis membranes, leading to production of cytokines, such as interleukin-1 and TNF- $\alpha$  (58–60). TNF- $\alpha$  is a potent primer of neutrophil respiratory burst activity (13,18). LPS also primes neutrophil respiratory burst activity in a dose dependent manner by causing translocation of the NADPH oxidase component, cytochrome  $b_{558}$ , to the plasma membrane (12,18). Thus, the conditions exist whereby bacterial products derived from the dialysate may stimulate production of reactive oxygen species during hemodialysis. It is not clear, however, whether this mechanism contributes significantly to the generation of reactive oxygen species during hemodialysis. Clinical studies comparing pre- and post-dialysis serum cytokine concentrations during dialysis with standard dialysate or dialysate with undetectable levels of LPS have failed to find differences in cytokine levels that could be attributed to the presence of LPS in the dialysate (61,62). These negative results come with the caveats that the level of LPS in the standard dialysate was low (24 pg/ml) in one study (62) and the observation by Haeffner-Cavaillon et al. that the peak in cytokine production may not occur until some hours after the end of dialysis (63). Some of the apparent differences between in vitro and clinical studies may relate to the presence in plasma of the LPS binding proteins, bactericidal/permeability-increasing protein, and LPS-binding protein, which modulate endotoxin activity (64). So far there are no data to show whether the use of ultrapure dialysate reduces oxygen radical production or protein and lipid oxidation. However, bacterial contamination of the dialysate does not appear to be a prerequisite for increased production of reactive oxygen species during dialysis. Ex vivo



**FIG. 3.** The graph shows *S. aureus*-stimulated H<sub>2</sub>O<sub>2</sub> production by neutrophils obtained from the inlet (●) and outlet (■) of dialyzers containing high-flux polysulfone and cellulose triacetate membranes at various times during hemodialysis. (\*) This is significantly different from other time points. (†) It is significantly different from inlet to dialyzer ( $p < 0.002$ ). The shaded area represents the normal range (mean  $\pm$  2SD).

perfusion of modules containing cellulose membranes results in an increase in the production of reactive oxygen species similar to that observed during clinical dialysis even though the dialysate compartment is endotoxin-free (42,65).

### Antioxidant defenses

In addition to an increased production of reactive oxygen species, increased protein oxidation may also reflect compromised antioxidant defenses. Superoxide dismutase, catalase, and the glutathione system provide major protection against damage by oxygen radicals. The status of these enzymes in hemodialysis patients appears to be highly variable. Superoxide dismutase activity in erythrocytes is reported to be normal or decreased (24,66,67), glutathione peroxidase activity to be normal (24,66,67), and glutathione reductase activity to be normal or decreased (24,67). Whole blood total glutathione concentrations are reported to be decreased (67), whereas erythrocyte total glutathione levels are reported to be normal (68). Superoxide dismutase, catalase, and the glutathione system are primarily intracellular antioxidants—for example, the plasma concentration of glutathione is only 2  $\mu$ M (69)—and they are unlikely to play a significant role in protecting against oxidation of plasma proteins. Important antioxidants in plasma include vitamins C and E, uric acid, and albumin (70). Vitamin C levels have been reported to be low in some, but not all hemodialysis patients (24,71,72), possibly depending on whether or not the patients receive a supplement containing vitamin C. Vitamin E levels are reported to be normal in hemodialysis patients (24,71). Most free sulfhydryl groups in plasma are found on albumin (73), and a recent report by Himmelfarb and McMonagle showed that albumin is also a major target for protein carbonyl formation in plasma (74). These findings suggest that albumin may be an important defense against oxidant stress in hemodialysis patients. Such a role for albumin is supported by the finding of Soejima et al. that patients with hypoalbuminemia demonstrate a greater degree of erythrocyte membrane lipid peroxidation than do patients with normal serum albumin concentrations (75).

### CONCLUSION

Hemodialysis patients are in a state of oxidant stress, as indicated by significant oxidation of plasma proteins and lipids. One component of this oxidant stress is increased production of reactive oxygen species by neutrophils. The principal stimulus to reactive oxygen species production in hemodialysis

patients is uremia, per se. This effect of uremia appears to be manifest, at least in part, by retention of one or more substances in plasma as renal function decreases. Certain aspects of the dialysis procedure, including the membrane material and the presence of bacterial products in the dialysate, also have the potential to stimulate increased production of reactive oxygen species. However, the results of recent studies suggest that the dialyzer membrane is not a major stimulus to oxidant stress in hemodialysis patients. The importance of bacterial contaminants in the dialysate is less clear. While evidence for increased cytokine production during dialysis is inconclusive, the use of ultrapure water to prepare dialysate is associated with a reduction in inflammation (76) and improvements in morbidity thought to be related to inflammation (76,77). Further investigation will be needed to clarify this aspect of oxidant stress in hemodialysis. Whether or not depressed antioxidant defenses act in concert with the increased production of reactive oxygen species to facilitate protein and lipid oxidation also remains unclear.

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