

# Are advanced oxidation protein products potential uremic toxins?

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**Are advanced oxidation protein products potential uremic toxins?** Oxidative stress, defined as a disruption of the equilibrium between the generation of oxidants and the activity of anti-oxidant systems, plays a significant role in the development of the inflammatory syndrome associated with chronic renal failure and hemodialysis. In our recent work, the aim of which was to better characterize oxidative stress in dialysis patients, we described the presence of oxidized protein products, which we have termed advanced oxidation protein products (AOPP), in the plasma of dialysis patients and we proposed AOPP as new markers of oxidative stress and potential inflammatory mediators. AOPP represent an exquisite marker of phagocyte-derived oxidative stress, and their role in the pathophysiology of chronic renal failure and dialysis-related complications might be of great importance. Regarding the mechanisms of generation of AOPP, we pointed out the importance of myeloperoxidase and the subsequent generation of chlorinated oxidants, previously considered solely as microbicidal agents, in the formation of AOPP. Indeed, AOPP appear to act as true inflammatory mediators since they are able to trigger the oxidative burst and the synthesis of inflammatory cytokines in neutrophils, as well as in monocytes. Thus, it could be hypothesized that the AOPP, which arise from the reaction between chlorinated oxidants and plasma proteins, constitute new uremic toxins with pro-inflammatory effects.

The presence of a chronic inflammatory state, which is, to a large extent, induced by an oxidative stress subsequent to disruption of the natural balance between the generation of oxidants and the activity of anti-oxidant systems, has been widely documented in end-stage renal disease patients on maintenance hemodialysis (HD) [1]. The oxidative stress is commonly attributed to the recurrent activation of polymorphonuclear neutrophils (PMN) and monocytes (MN) during blood passage through dialysis circuits, and subsequent generation of activated complement components following contact with bioincompatible membranes and/or possible transfer of endotoxins from the dialysate. Activated PMN generate the cascade of highly reactive oxygen species (ROS) (e.g.,  $O_2^-$ ,  $H_2O_2$ , and  $OH^-$ ) which results from NADPH oxidase

complex [2]. Likewise, activated MN also release these compounds, although at much lower rates than PMN, and are mainly endowed with the production of the potent pro-inflammatory cytokines IL1, TNF- $\alpha$ , and IL-6.

In addition to the formation of NADPH oxidase-derived ROS, phagocytic cells have the capacity to produce chlorinated oxidants via the myeloperoxidase (MPO) system, which, in the presence of chloride ions, converts  $H_2O_2$  into hypochlorous acid ( $HOCl^-$ ). In humans, MPO is the sole enzyme capable of generating such microbically-chlorinated oxidants [3]. However, more recent work on MPO has clearly shown that MPO is implicated in the inflammatory process associated with atherosclerosis since MPO is detected in aortic atherosclerotic plaques, but not in normal aortae [4, 5].

In the dialysis patient, the pro-inflammatory effects of these oxidants, proteases, and cytokines are reinforced by the uremia-associated defect in anti-oxidant systems [6], anti-proteases [7], and cytokine inhibitors [8]. Recent studies have clearly demonstrated that inflammation, together with malnutrition, largely contributes to the accelerated atherosclerosis process of uremia, which still represents the leading cause of mortality in HD patients [9].

## DESCRIPTION OF ADVANCED OXIDATION PROTEIN PRODUCTS IN UREMIA

Until recently, biological evidence of oxidative stress in the dialysis patient in vivo relied almost entirely on the measurement of lipid peroxidation by-products, such as malondialdehyde and thiobarbituric acid reactive substances (TBARS), which, in general, poorly reflect the intensity of oxidative stress. The exquisite vulnerability of proteins to ROS is now well documented [10]. Oxidation of amino acid residues such as tyrosine, which leads to the formation of dityrosine, protein aggregation, cross-linking, and fragmentation are but a few examples of ROS-mediated protein damage in vitro. In contrast, evidence for the presence of such oxidatively-damaged proteins in vivo and their possible clinical significance has been lacking until recently. In the search whether

**Key words:** inflammation, phagocyte, myeloperoxidase.

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such protein oxidative damage could reflect the dialysis-associated oxidative stress, we were able to isolate and characterize dityrosine-containing protein cross-linking products in the plasma of dialysis patients; these we designated as advanced oxidation protein products (AOPP) in reference to the well-established advanced glycation end products (AGEs), to which AOPP appeared to be closely related [11]. The formation of AGEs has been widely documented during diabetes and aging, and is thought to be responsible for tissue degradation. The presence of increased plasma levels of AGEs has been observed in dialysis patients, independent of diabetes [12]. Interestingly, the hypothesis that oxidative stress is implicated in AGEs formation might also be relevant to the uremic toxicity syndrome. We reported that the well-characterized AGEs pentosidine accumulates with progression of chronic renal failure in close relationship with neopterin, a well-characterized monocyte activation marker.

### **ARE AOPP UREMIC TOXINS?**

The contribution of uremia to chronic inflammatory state has been suggested and numerous studies have shown that both monocyte activation and defect in antioxidant systems occur early in the course of chronic renal failure and gradually increase with its progression to end-stage renal disease. Interest has focused on the role of uremic toxins generated during the course of chronic renal failure, some of which have known effects on neutrophil and monocyte functions. Among these, AGEs have been the focus of numerous studies. In the search whether AOPP could act as a uremic toxin, we have spent some effort during the last 10 years to determine if AOPP could meet the criteria that characterizes a uremic toxin, according to Vanholder et al [13].

#### **The compound should be chemically identified, and accurate quantitative analysis in biological fluids should be possible**

Size exclusion chromatography of uremic plasma allowed us to isolate high-molecular-weight AOPP (600 kD), which are protein aggregates, and low-molecular-weight compounds (around 80 kD), with albumin as a main component. Isolation of the two main plasma fractions containing the two distinct AOPP peaks [11] has been reproduced by other groups [14]. Biochemical characteristics allow us to consider AOPP as final cross-linking products of protein oxidation. Compared with controls, AOPP were found at very high concentrations in the plasma of dialysis patients and were closely related to dityrosine, a marker of protein oxidation. In vitro, exposure of control plasma or purified plasma albumin to chlorinated oxidants triggered the formation of AOPP in an oxidant concentration-dependent manner. Antioxidants such as ascorbic acid or glutathione did not

decrease AOPP levels, thus revealing that they are devoid of oxidant activity. However, the precise chemical structure of plasma AOPP has still not been elucidated. Our recent data indicate that in vivo plasma AOPP might not be identical to in vitro HOCl-treated plasma, and more complicated reactions might occur in vivo. Like AGEs, AOPP represent a generic name for oxidatively-modified proteins, especially via the myeloperoxidase system. The methodology to determine plasma AOPP is well characterized and several groups have now confirmed the high AOPP levels in HD patients [15] and in other oxidative stress-related pathologies [16]. This methodology relies on their spectrophotometric properties, which are the absorbance at 340 nm in acidic conditions, as previously described.

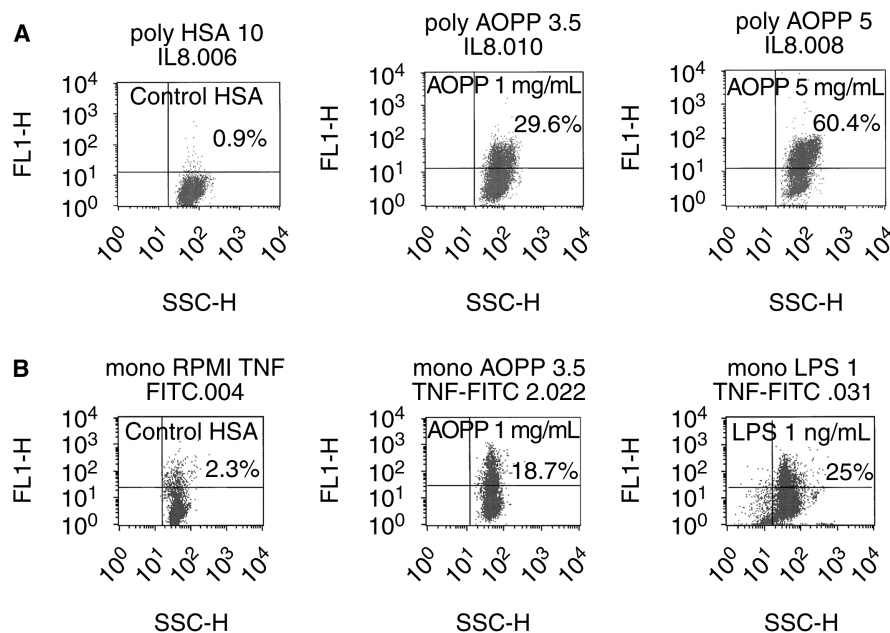
#### **The plasma concentration of the toxin should be higher in uremic than in nonuremic patients**

We showed that increased levels of AOPP are already observed at an early stage of chronic renal failure (CRF), and rise with the progression of uremia. Our previous data have clearly shown that plasma levels of AOPP were significantly higher in predialysis CRF patients than in control groups and culminated in HD patients. Among predialysis patients, an inverse relationship between plasma AOPP and creatinine clearance (Ccr) levels was found. Interestingly, AOPP levels are closely related to plasma levels of monocyte activation markers such as neopterin, TNF- $\alpha$ , and its soluble receptors. There was no correlation with IL-6 and no correlation with markers of activated T cells (soluble IL-2 receptors) or B cells (soluble CD23) [17].

#### **The compound must be a chemical or biological agent capable of interacting with biological systems and produce a deleterious biological response**

The study of the relationship between AOPP and neutrophil oxidative metabolism showed that neutrophil basal NADPH oxidase activity (as measured by lucigenin-amplified chemiluminescence [CL]) was within the range of controls, regardless of the stage of CRF in predialysis patients, but was significantly increased in HD patients. Likewise, neutrophil MPO activity (as measured by luminol-amplified CL) remained within the normal range in predialysis patients, regardless of the degree of CRF, but was markedly higher in HD patients. These data strongly suggest that neutrophil oxidative potential is involved in plasma AOPP formation.

In vitro studies showed that AOPP could be generated by exposing proteins such as human serum albumin (HSA) to oxidants, among which HOCl<sup>-</sup> was found to be the most powerful. We have previously shown that HSA-AOPP has the ability to trigger an oxidative burst in neutrophils as well as in monocytes. Interestingly, the intensity of the respiratory burst is dependent on the



**Fig. 1. Intracellular detection of IL-8 in PMN and TNF- $\alpha$  in MN stimulated with AOPP.** PMN and MN were isolated from blood of healthy donors. Cells were stimulated with appropriate stimuli to induce cytokine production and intracellular IL-8 or TNF- $\alpha$  were measured by flow cytometry after cell permeabilization as indicated by the manufacturer (Becton Dickinson). (A) PMN (106 cells/ml) were stimulated either with purified human serum albumin (HSA at 10 mg/ml) or with different concentrations of AOPP for 15 hours. (B) MN ( $0.5 \times 10^6$  cells/ml) were stimulated overnight either with purified human serum albumin (HSA at 10 mg/ml) or with AOPP or with LPS.

level of protein oxidation. The respiratory burst was triggered by different preparations of AOPP obtained with various ratios of HSA/HOCl [17]. More recently, we have shown that AOPP purified from uremic plasma also trigger the respiratory burst of normal neutrophils.

Recently, we observed that AOPP were potent stimuli in triggering interleukin-8 synthesis in neutrophils, as shown in Fig. 1A. We were able to show by flow cytometry analysis that marked IL-8 synthesis occurs 12 hours following AOPP stimulation, and that it is potentiated by GM-CSF. Interestingly, HSA-AOPP was also able to trigger the synthesis of inflammatory cytokines in monocytes, such as TNF- $\alpha$  (Fig. 1B).

#### High concentrations should be related to specific uremic syndroms that decrease or disappear when the concentration is reduced

So far, no study has been performed to see whether pharmacologic modulation of AOPP could improve the inflammatory state associated with uremia. However, we have recently described that, *in vitro*, N-acetylcysteine could selectively decrease AOPP-induced respiratory burst without any effect on zymosan-induced respiratory burst in purified neutrophils. This was observed both in neutrophils from control groups and from hemodialyzed patients [18]. This potential pathway for modulating an AOPP-induced pro-inflammatory effect has yet to be explored *in vivo*.

AOPP belong to the compounds that could play a role in the development of cardiovascular complications. In a recent study, we observed a significant association of plasma AOPP with common carotid artery intima-media

thickness (CCA-IMT) and CCA wall-to-lumen ratio in uremic patients. AOPP levels were also positively correlated with ferritin levels and the dose of intravenous iron administered. Interestingly, there was no correlation between other oxidative stress markers, such as malondialdehyde (MDA), and with cardiovascular parameters. These findings support the concept of a role of phagocyte-derived oxidative stress in the accelerated atherosclerosis of uremic patients, which may be enhanced by usual recommended doses of intravenous iron [19]. This study points out the relevance of AOPP follow-up in the management of uremia patients in their assessment for cardiovascular risk factors.

#### CONCLUSION

In conclusion, we propose that AOPP generated via the MPO system could not only be exquisite markers of the oxidative stress, but also active mediators of the inflammation associated with the uremic state. AOPP appear to meet the criteria of a novel uremic toxin. Neutrophils and monocytes, which could themselves amplify the inflammatory process by increasing the neutrophil recruitment via enhanced IL-8 synthesis and oxidant generation, appear to be elective targets of AOPP. These observations lead us to propose that AOPP are key molecules in the cross-talk between these two types of phagocytes. It is plausible that they make a large contribution to the immune dysregulation and inflammatory process in uremia.

#### ACKNOWLEDGMENT

This work was supported by the Extramural Grant Program, Baxter Company.

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## REFERENCES

1. DESCAMPS-LATSCHA B, DRUEKE T, WITKO-SARSAT V: Dialysis-induced oxidative stress: Biological aspects, clinical consequences, and therapy. *Semin Dial* 14:193–199, 2001
2. WITKO-SARSAT V, RIEU P, DESCAMPS-LATSCHA B, et al: Neutrophils: Molecules, functions and pathophysiological aspects. *Lab Invest* 80:617–653, 2000
3. KLEBANOFF SJ: Myeloperoxidase. *Proc Assoc Am Physicians* 111:383–389, 1999
4. HEINECKE JW: Atherosclerosis: Cell biology and lipoproteins. *Curr Opin Lipidol* 7:U131–U136, 1996
5. HAZELL LJ, ARNOLD L, FLOWERS D, et al: Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *J Clin Invest* 97:1535–1544, 1996
6. CANAUD B, CRISTOL J, MORENA M, et al: Imbalance of oxidants and antioxidants in haemodialysis patients. *Blood Purif* 17:99–106, 1999
7. HORL WH: Neutrophil function in renal failure. *Adv Nephrol Necker Hosp* 31:173–192, 2001
8. DESCAMPS-LATSCHA B, HERBELIN A, NGUYEN AT, et al: Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. *J Immunol* 154:882–892, 1995
9. STENVINKEL P, HEIMBURGER O, PAULTRE F, et al: Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 55:1899–1911, 1999
10. DAVIES KJ: Protein damage and degradation by oxygen radicals. I. General aspects. *J Biol Chem* 262:9895–9901, 1987
11. WITKO-SARSAT V, FRIEDLANDER M, CAPELLERE-BLANDIN C, et al: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 49:1304–1313, 1996
12. VLASSARA H: Serum advanced glycosylation end products: A new class of uremic toxins? *Blood Purif* 12:54–59, 1994
13. VANHOLDER R, ARGILES A, BAURMEISTER U, et al: Uremic toxicity: Present state of the art. *Int J Artif Organs* 24:695–725, 2001
14. KANEDA H, TAGUCHI J, OGASAWARA K, et al: Increased level of advanced oxidation protein products in patients with coronary heart disease. *Atherosclerosis* 162:221–225, 2002
15. WRATTEN ML, SERENI L, TETTA C: Hemolipodialysis attenuates oxidative stress and removes hydrophobic toxins. *Artif Organs* 24:685–690, 2000
16. BUONOCORE G, PERRONE S, LONGINI M, et al: Oxidative stress in preterm neonates at birth and on the seventh day of life. *Pediatr Res* 52:46–49, 2002
17. WITKO-SARSAT V, FRIEDLANDER M, NGUYEN KHOA T, et al: Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 161:2524–2532, 1998
18. WITKO-SARSAT V, GAUSSON V, NGUYEN AT, et al: AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: a potential target for N-acetylcysteine treatment in dialysis patients. *Kidney Int* 63, In press.
19. DRUEKE T, WITKO-SARSAT V, MASSY ZA, et al: Iron therapy, advanced oxidation protein products and carotid intima media thickness in end-stage renal disease. *Circulation* 106:2212–2217, 2002