Advanced oxidation protein products as a novel molecular basis of oxidative stress in uraemia

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Oxidative stress in uraemia syndrome

Considerable evidence has accumulated that chronic uraemia is associated with a multifactorial immunoinflammatory syndrome, which occurs early in the course of renal failure, is accentuated with the progression of uraemia and culminates in maintenance dialysis therapy [1,2]. Besides the dysregulation in the balance between pro-inflammatory cytokines and their inhibitors which has been described in uraemic patients [3,4], a disturbance in the balance between oxidants and antioxidants has also been pointed out. The haemodialysis setting could be considered as a human model of oxidative stress since blood–dialyser interaction triggers circulating neutrophils to produce a large amount of reactive oxygen species, including superoxide anion, hydrogen peroxide, hydroxyl radical, and hypochlorous acid [5,6] which are partially scavenged by plasma components. However, due to a profound deficiency in antioxidant systems, this scavenging potential is likely to be overwhelmed and chronic oxidative stress will thus take place [7,8]. Moreover, one of the features of uraemia is the presence of signs of oxidative stress before haemodialysis, thus emphasizing the importance of evaluating the physiopathological role of oxidative stress with respect to the uraemia-related immune dysregulation and inflammatory processes.

Description of advanced oxidation protein products (AOPPs)

Measurement of oxidative stress is not straightforward and standardized methods are still lacking. In our search for oxidative stress markers relevant to uraemia, we investigated the possibility that oxidants could induce oxidative damage to plasma proteins [9,10]. Although proteins are elective targets of oxidants, no clinical studies have been performed to quantify oxidant-induced protein damage in relation to clinical status in chronic renal failure. Indeed, we described for the first time, the presence in the plasma of haemodialysed patients high levels of oxidized proteins that we designated AOPPs [11]. Since oxidative damage to proteins modifies the spectroscopic characteristics of proteins, for example through the oxidation of aromatic amino acid residues, we studied spectral characteristics in plasma fractionated by size exclusion chromatography. We pointed out two UV-visible peaks of absorbance at 340 nm in plasma from haemodialysed patients which were absent in controls. These two peaks, corresponding to a molecular mass of 60 and 600 kDa, were called low molecular weight (LMW) and high molecular weight (HMW) AOPPs, respectively.

Interestingly, formation of AOPPs could be induced in control plasma by chlorinated oxidants such as chloramines or hypochlorous acid. Of note, the in vitro formation of AOPPs was much lower when proteins were submitted to H₂O₂ compared to identical concentrations of chlorinated oxidants. Moreover, the formation of AOPPs using purified human serum albumin was clearly correlated to the concentration of chlorinated oxidant added, thus demonstrating that AOPP resulted from the interaction between such oxidants and plasma proteins.

The HMW-AOPPs generated in plasma from haemodialysed patients suggested that they were cross-linking products. Since glycation-modified proteins also induced protein cross-linking and are elevated in uraemic patients [12,13], we studied the relationships between AOPPs and advanced glycation end products (AGEs) pentosidine, considered as a marker of protein glycation, and showed that AOPPs and AGEs were highly correlated. Moreover, AOPPs were highly correlated with dityrosine, a marker of protein oxidation [14]. Interestingly, recent work has demonstrated that biochemical reactions of protein glycation involve oxidative pathways, which is of great relevance in the uraemic syndrome [15].

Biological activity of AOPPs

Because of the structural analogy between AOPPs and AGEs, we hypothesized that AOPPs might exert bio-
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Gical activities by themselves as has been described for AGES. AGE induce proinflammatory activities such as upregulation of adhesion molecules [16] or cytokine induction [17] via specific receptors [18]. We showed that chlorinated oxidant-modified human serum albumin (AOPP-HSA) was able to trigger the respiratory burst in purified human monocytes as compared to native HSA. Interestingly, the intensity of the oxidant generation was greater than that obtained with AGE-HSA [19].

Clinical relevance of AOPPs

In vivo, we evaluated the possible relationships between plasma AOPPs and markers of monocyte activation in a cohort of non-dialysed uraemic patients (n = 162) at different stages of chronic renal failure. We demonstrated that AOPPs were highly correlated with creatinine clearance. AOPPs correlated with those of neopterin, and of inflammatory cytokines such as TNF-α and its soluble receptors, thus suggesting that, in vivo, AOPP formation is clearly related to or contributes to monocyte activation.

It is important to point out the crucial importance of chlorinated oxidants in the formation of AOPPs. In vivo, the generation of chlorinated oxidants is a feature of phagocytic cells which possess myeloperoxidase, the only enzyme able to generate chlorinated oxidants. Recent works have emphasized the potential importance of myeloperoxidase-derived chlorinated oxidants in the pathophysiological mechanisms of atherosclerosis by demonstrating the presence of myeloperoxidase in the atherosclerotic lesions as well as chlorinated oxidant-induced lipoprotein byproducts [20,21].

Interestingly, in uraemic patients, we found that the oxidative activity of circulating neutrophils, measured within whole blood by chemiluminescence, was correlated with plasma AOPP. These data strongly suggest that neutrophils, which constitute the most important source of chlorinated oxidants due to their high content in myeloperoxidase, might be involved in plasma AOPP formation [22].

Conclusion

Taken together, our data shows that, both in vitro and in vivo, AOPPs appear as markers of the oxidative stress associated with uraemia as well as true inflammatory mediators able to amplify monocyte activation (Figure 1).

With regard to the mechanisms of generation for AOPPs, we highlight the importance of the chlorinated oxidants, previously considered solely as microbicidal agents, in the generation of AOPPs. In fact, the AOPPs, which arise from the reaction between chlorinated oxidants and plasma proteins, constitute a new molecular basis for the deleterious activity of oxidants and as such might be considered as true mediators of the pro-inflammatory effects of oxidative stress [23].

References


Fig. 1. AOPP as novel mediators between neutrophils and monocytes and oxidative stress-associated dialysis complications.


