AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: A potential target for N-acetylcysteine treatment in dialysis patients

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AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: A potential target for N-acetylcysteine treatment in dialysis patients.

Background. Oxidative stress largely contributes to hemodialysis-associated lethal complications, thus explaining the urgent need of antioxidant-based therapeutic strategies in hemodialysis patients. We previously identified advanced oxidation protein products (AOPP) in the uremic plasma as exquisite markers of oxidative stress and potent mediators of monocyte activation. The present study was aimed at searching whether (1) AOPP can also trigger activation of polymorphonuclear neutrophils (PMN), and (2) whether AOPP-induced activation could be inhibited by N-acetylcysteine (NAC), a widely used compound which has been shown to prevent oxidative injury to kidney.

Methods. Both human serum albumin (HAS) AOPP (i.e., HOCl-modified HAS in vitro preparations and AOPP extracted from plasma of hemodialysis patients) were tested for their capacity to trigger phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase (MPO)-dependent activities as measured by lucigenin- and luminol-amplified chemiluminescence (CL), respectively, as compared to receptor-dependent [opsonized zymosan or receptor-independent phorbol myristate acetate (PMA)]. The effect of PMN priming by platelet-activating factor (PAF), and the effect of NAC on normal monocyte and on normal or hemodialysis patient’s (N = 16) PMN oxidative responses were compared.

Results. HAS-AOPP triggered in a HOCl dose-dependent manner both NADPH-oxidase- and MPO-dependent CL of PMN. This latter was further enhanced by PAF priming. Plasma-derived AOPP obtained from hemodialysis patients also triggered PMN respiratory burst. NAC significantly reduced HAS-AOPP-mediated responses of normal monocyte and of normal and uremic PMN but had no significant effect on opsonized zymosan- or PMA-induced CL responses.

Conclusion. This dual potential of NAC to inhibit phagocyte oxidative responses induced by HAS-AOPP without affecting those mediated by compounds mimicking pathogens supports the proposal of a therapeutic trial with NAC aimed at reducing oxidative stress–related inflammation in hemodialysis patients.

Oxidative stress, resulting from a disruption of the natural balance between pro- and anti-oxidant systems in favor of the former [1, 2] has long been reported in patients on maintenance hemodialysis [3]. In these patients recurrent blood interaction with bioincompatible dialysis membranes triggers polymorphonuclear neutrophil (PMN) and monocyte activation and their subsequent generation of highly reactive oxygen species (ROS) including O2− and its derivatives (H2O2, OH− and ‘O2·) via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and OCI− via myeloperoxidase (MPO)-dependent reaction between chloride and H2O2 [4]. Moreover, the profound impairment in antioxidant systems as for instance glutathione peroxidase activity [5–8], together with regular peridialytic loss of antioxidant molecules (such as glutathione), favors oxidant-mediated damage [9].

Evidence for oxidative stress in hemodialysis patients has long relied solely on elevated circulating levels of lipid oxidation markers [10–13]. More recently, increased levels of carbonyl compounds in relation to the formation of advanced glycation end products (AGE) have also been reported in these patients [14, 15].

In the search for specific markers of protein oxidation [16] in the plasma of hemodialysis patients, we recently described a novel family of oxidized protein compounds, which we designated advanced oxidation protein products (AOPP) [17] in keeping with their close relationship with AGE compounds such as pentosidine. The generation of AOPP could also be obtained in vitro by exposing proteins such as human serum albumin (HSA) to various oxidants, among which HOCl was the most powerful com-
A close correlation was found between AOPP and carbonyls or dityrosine levels. This latter observation was in keeping with the well-documented capacity of HOCl to induce the formation of tyrosine products [18]. In a following study [19] we showed that (I) in vivo AOPP levels are in fact elevated early in the course of chronic renal failure (CRF), increase with the progression of CRF and are closely related to monocyte activation state, and (2) in vitro, HOCl-modified HSA (referred herein as HSA-AOPP) also retain the capacity to trigger monocyte respiratory burst, and this was not induced by dityrosine alone. Taken together, these findings led us to propose AOPP as both an ultrasensitive gauge of oxidative stress and a novel class of mediators of inflammation in CRF [20]. More recently, Himmelfarb and McMonagle [21] also concluded that albumin was the major plasma protein target of oxidative stress in uremia and that its oxidation contributes to the development of cardiovascular risk.

The role of oxidative stress in the major dialysis-associated complications such as amyloidosis and accelerated atherosclerosis has been well documented [22]. However, antioxidant therapeutic strategies have not yet been extensively used in hemodialysis patients, with the exception of the recent development of vitamin E–coated dialysis membranes. Among potential antioxidant drugs, N-acetylcysteine (NAC), an aminothiol and synthetic precursor of cysteine and glutathione, might be a good candidate worth testing [23]. Indeed, NAC has already been used therapeutically in several disorders related to oxidative stress such as chronic bronchitis and acetaminophen poisoning, and it has also recently been shown to protect renal function in conditions of acute [24] and CRF [25, 26]. Specifically, NAC is capable of directly scavenging ROS and HOCl [27], and after deacetylation, it releases cysteine, which in turn increases the formation of glutathione, within the intracellular pool of antioxidant molecules [28].

The present study conducted in vitro was aimed at (I) characterizing the effects of AOPP on monocyte and PMN oxygenation activities, and (2) searching whether these effects could be counteracted by NAC both in normal and uremic (hemodialysis patients) phagocytes.

METHODS

If not otherwise indicated, chemicals were purchased from Sigma-Aldrich Chemical Co. (Saint-Quentin Fallavier, France).

Preparation of AOPP

HSA (Calbiochem, San Diego, CA, USA) was exposed to HOCl (Fluka, Buchs, Switzerland) to produce AOPP [17]. Briefly, HOCl stock solution was freshly prepared in phosphate-buffered saline (PBS) and concentration was measured by spectrophotometry using a molar extinction coefficient of 350 mol⁻¹/cm⁻¹ at 290 nm at pH 12. HSA (100 mg/mL) was exposed to HOCl (100 mmol/L) for 30 minutes at room temperature and then dialyzed overnight against PBS.

AOPP concentrations were measured by a spectrophotometric assay, as described previously [17]. Briefly, 200 μL of AOPP-HSA or HSA preparation in PBS were placed in a 96-well plate (Nunc-immuno plate, Roskilde, Denmark) and mixed with 20 μL acetic acid. AOPP concentrations were measured in a microplate reader MR5000 (Dynatech, Paris, France) at 340 nm and calibrated versus standard reference wells containing 200 μL of chloramine-T solution (0 to 100 μmol/L), 10 μL of 1.16 mol/L potassium iodide, and 20 μL of acetic acid. The content of AOPP in the stock HSA-AOPP preparation used for all experiments in this study was ≤300 μmol/L versus 23 μmol/L in native HSA. The concentrations of dityrosine were 9.6 μmol/L versus 0.8 μmol/L, respectively, and those of carbonyls of 20,940 μmol/L versus 100 μmol/L, respectively.

In an attempt to concentrate high-molecular-weight AOPP fraction formed in vivo [17], 1 mL of hemodialysis plasma (or control plasma) was first diluted 1:4 in PBS and further submitted to differential centrifugation (1000g × 20 minutes at 4°C) using a 100 kD cutoff centrificon (Amicon, Beverly, MA, USA).

Isolation of PMN and monocyte

PMN and monocytes were isolated from ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood of healthy controls (N = 11) recruited among volunteer blood donors of the Etablissement du Sang (Paris) and hemodialysis patients (N = 16) treated three times a week (4- to 5-hour sessions) with dialyzers equipped with cellulosic (N = 5), or synthetic (N = 11) membranes. The dialysate was of standard ionic composition with bicarbonate buffer.

Mononuclear cells were obtained by density gradient centrifugation over Ficoll-Hypaque 400 (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient as described previously [29]. Isolation of monocytes from the mononuclear cells, harvested at the interface of the gradient, was performed by depletion of nonmonocyte using the magnetic cell sorting (MACS) cell isolation system (Miltenyi Biotec, Gladbach, Germany). Briefly, T cells, natural killer (NK) cells, B cells, dendritic cells, and basophils were labeled using a cocktail of hapten-conjugated CD3, CD7, CD19, CD45RA, CD56 and anti-immunoglobulin E (IgE) antibodies. After washing, labeled cells were depleted by retaining them on a MACS column placed in the magnetic field of the VarioMACS, yielding to a highly purified monocyte suspension (90% to 95%).
Monocytes were adjusted at a concentration of 2.5 × 10^6 cells/mL in phenol-red free Hank’s balanced salt solution (HBSS) (Eurobio, Paris, France).

PMN and erythrocytes sedimented through the Ficoll gradient were resuspended in HBSS to the original volume of blood layered on the gradient, mixed in a 2:3 ratio with a 5% solution of Dextran 500 (Pharmacia) in saline and maintained 45 minutes at 4°C. The PMN containing supernatant was then gently aspirated and centrifuged (200g at 4°C for 10 minutes). After lysis of residual erythrocytes by 1.6% NaCl, PMN (>95%) were resuspended (5 × 10^6 /mL) in HBSS.

NADPH oxidase and myeloperoxidase-dependent oxygenation activities

Monocyte and PMN NADPH oxidase and myeloperoxidase (MPO)-dependent oxygenation activities were measured by chemiluminescence (CL) using a single-photon luminometer (AutoLumat LB953, Berthold Co., Wildbad, Germany), as described previously [30]. Briefly, two distinct activity-specific lipopolysaccharide (LPS) free chemilumogenic substrates obtained from Lumino-stix (San Antonio TX, USA) were used: lucigenin (10, 10-dimethyl-9,9-biacridium dinitrate), which selectively measures NADPH oxidase–dependent extracellular superoxide anion formation, and the cyclic hydrazide luminal (5-amino-2,3-dihydro–1,4-phthalazinedione), which measures intracellular MPO-dependent formation of H_2O_2 and HOCl [31]. One hundred microliters of PMN suspension (5 × 10^6 cells) were distributed into polystyrene tubes containing 100 μL of a lumigenic substrate and 50 μL of either HBSS (resting CL) or agents to be tested, including HSA or HSA-AOPP [final concentration (fc) of 2 mg/mL]; 4-phorbol,12-miristate,13-acetate (PMA) (fc, 16 μmol/L), serum AB opsonized zymosan (2 × 10^10 particles/mL, fc, 2 mg/mL). Luminescence activities were measured in duplicate over 40 minutes and expressed as integrated total counts.

Study of the effect of PMN priming by platelet-activating factor (PAF) on AOPP-induced activation of NADPH oxidase and MPO oxygenation activities was performed by using PAF (1 μg)-coated tubes as described [32] and following the same steps as above.

Effect of NAC and thiol compounds on PMN and monocyte oxygenation activities

The effect of NAC on PMN and monocyte oxidative responses was tested according to the technical conditions described in [33]. Briefly, 5 × 10^6 cells were set to incubate (30 minutes at 37°C under constant agitation) with NAC (fc, 0.01 mg/mL up to 1 mg/mL) or HBSS as controls. It was verified in preliminary experiments that (1) 30 minutes was an optimal time of incubation; (2) cell viability was not affected up to a 2 mg/mL of NAC; and (3) washing after cell treatment had no influence on the effect of NAC. To quantify the action of NAC, results were expressed as % control CL response = 100 × CL (cells + NAC) − CL (cells + HBSS)/CL (cells + HBSS).

The effect of glutathione and dithiothreitol (fc, 5 mmol/L for both, previously reported as optimal in [34]) was tested in the same conditions as those used for NAC.

Statistical analysis

Results were expressed as mean ± SEM and compared using analysis or variance (ANOVA) or Mann-Whitney or Wilcoxon tests for unpaired and paired data, respectively. Differences were considered significant when the P value was 0.05 or less.

RESULTS

AOPP triggers oxidative responses of PMN in a dose-dependent manner

Both NADPH oxidase, measured by lucigenin-amplified CL, and MPO-dependent activity, measured by luminol-amplified CL, of normal PMN were significantly increased in a dose-dependent manner with increasing AOPP concentrations (Fig. 1). Most important, there was a linear relationship between HSA-AOPP concentrations and lucigenin or luminol CL intensity (r correlation coefficient of 0.98 and 0.95, respectively).

The possibility that LPS contamination contributes to the observed effect of HSA-AOPP on PMN oxygenation activities could be ruled out as follows: (1) LPS concentration as measured by Limulus amoebocyte lysate (LAL) test was similar in HSA-AOPP (0.53 EU/mL) and in HSA (0.58 EU/mL), which itself did not trigger any CL response as opposed to HSA-AOPP and as compared to HBSS (Table 1); (2) LPS (from 0.5 EU/mL up to 50 EU/mL) had no enhancing effect on lucigenin or luminol CL production by PMN as compared to HBSS alone; and (3) polymyxin B did not significantly inhibit HSA-AOPP–induced PMN lucigenin CL [572,850 ± 8582 counts/40 minutes versus 458,100 ± 6380 counts/40 minutes, respectively (N = 4, NS)] or PMN luminol CL response [608,500 counts/40 minutes versus 569,700 ± 8138 counts/40 minutes (N = 4, NS)].

Priming by PAF enhances PMN response capacity to HSA-AOPP

As previously described [32], PAF-elicited priming of PMN selectively increased luminol-amplified, but not lucigenin-amplified, CL responses to opsonized zymosan (CR1/CR3 receptor-mediated). In contrast, PAF enhanced HSA-AOPP–induced PMN responses via both NADPH oxidase and MPO-dependent pathways, although the increase was less pronounced for the former than for the latter (Fig. 2).
Comparative study of PMN and monocyte responses to HSA-AOPP and to receptor-dependent or receptor-independent stimuli

**NADPH oxidase oxygenation activity.** Regardless of the compound tested, lucigenin-amplified CL production was higher in PMN than in monocytes. However, the difference was statistically significant only for stimulated CL (Table 1). For monocytes, HSA-AOPP–induced responses did not significantly differ from those induced by opsonized zymosan or PMA. For PMN, HSA-AOPP–induced responses were also of the same order of magnitude as those induced by PMA but were significantly higher than those induced by opsonized zymosan ($P < 0.01$).

**MPO-dependent oxygenation activity.** Likewise, HSA-AOPP triggered significantly higher luminol-amplified CL responses than native HSA in both monocyte and PMN or medium alone (Table 1). However, HSA-AOPP–induced responses of both monocyte and PMN remained significantly lower than those induced by opsonized zymosan or PMA ($P < 0.001$, for both).

**NAC inhibits HSA-AOPP–induced oxidative responses of both PMN and monocytes**

Preincubation of monocytes or PMN with NAC (1 mg/mL) significantly reduced their capacity to elicit HSA-AOPP–induced responses via either NADPH oxidase or MPO-dependent pathways (Fig. 4). At a lower concentration of 0.1 mg/mL, NAC still inhibited both types of oxygenation activities of monocyte but not those of PMN. At a NAC concentration of 0.01 mg/mL, a 30% increase of both CL responses was observed in PMN but not in monocytes. Thus, the dose of 1 mg/mL of NAC was used in the following experiments.

Figure 5 compares the effect of NAC (1 mg/mL) on HSA-AOPP–mediated CL responses to that on other stimulating agents. Whereas NAC inhibited PMA-induced MPO-dependent oxygenation activities of both PMN and monocytes, it had no effect on PMA-induced NADPH oxidase activity of monocyte and an enhancing effect on that of PMN. In contrast, regardless of the cell type, NAC exerted an enhancing effect on opsonized zymosan-induced NADPH oxidase and MPO-dependent oxygenation activities. Such an effect was still observed at a higher NAC concentration (up to 2 mg/mL) (data not shown).

In an attempt to investigate the mechanisms by which NAC may inhibit AOPP-mediated phagocyte activation, we tested the effect of thiol-related compounds (e.g., glutathione and dithiothreitol) (both at 5 mmol/L) on HSA-AOPP–induced responses of PMN. As shown in Figure 6, both compounds induced a significant inhibition of CL response. Interestingly, the inhibition induced by glutathione was of the same order of magnitude as that induced by NAC (5 mmol/L), and this was true...
Table 1. Comparative study of monocyte and neutrophil oxygenation activities in healthy subjects

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent oxygenation activity

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<th>HBSS</th>
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<th>HSA-AOPP</th>
<th>Zymosan</th>
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<td><strong>Monocytes</strong></td>
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<td>Lucigenin-amplified chemiluminescence (CL) production (mean ± SEM, counts/40 minutes)</td>
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<td>11,091 ± 5471</td>
<td>9729 ± 9094</td>
<td>34,528 ± 14,033</td>
<td>54,688 ± 12,311</td>
<td>42,747 ± 15,557</td>
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<td><strong>Neutrophils</strong></td>
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<td>21,541 ± 5535</td>
<td>35,301 ± 10,432</td>
<td>271,650 ± 79,868</td>
<td>134,412 ± 31,655</td>
<td>227,787 ± 64,504</td>
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Myeloperoxidase (MPO)-dependent oxygenation activity

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<th>HBSS</th>
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<th>HSA-AOPP</th>
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<td><strong>Monocytes</strong></td>
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<td>Luminol-amplified CL production (mean ± SEM, counts/40 minutes)</td>
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<td>19,224 ± 8533</td>
<td>13,606 ± 7388</td>
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<td>244,999 ± 92,075</td>
<td>107,409 ± 36,970</td>
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<td>P = 0.005</td>
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<td><strong>Neutrophils</strong></td>
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<td></td>
<td>64,917 ± 14,214</td>
<td>62,756 ± 28,773</td>
<td>159,403 ± 44,681</td>
<td>1,959,833 ± 307,264</td>
<td>1,176,958 ± 243,746</td>
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Abbreviations are: HBSS, Hank’s balanced salt solution; HSA, human serum albumin; HSA-AOPP, human serum albumin–advanced oxidation protein products; PMA, phorbol myristate acetate. Statistical comparison of differences between monocyte and neutrophil oxygenation activities using Mann Whitney test.

Fig. 2. Effect of platelet-activating factor (PAF) priming on human serum albumin-advanced oxidation protein products (HSA-AOPP)–induced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase (MPO)-dependent oxidative responses of normal neutrophils. Lucigenin (A, left panel) and luminol (B, left panel) amplified chemiluminescence (CL) production in normal polymorphonuclear neutrophils (PMN) previously treated (30 minutes at 22°C) with PAF or Hank’s balanced salt solution (HBSS) and tested in presence of the indicated agents. On the left, CL response kinetics (in a representative case). On the right, results of integrated CL production over 40 minutes (mean ± SEM of six experiments).
Witko-Sarsat et al: Effect of N-acetylcysteine on AOPP-induced monocyte and neutrophil activation

AOPP formed in vitro also behaved as potent mediators of monocyte activation, triggering both their respiratory burst and tumor necrosis factor-α (TNF-α) synthesis.

In the present study, we show that HSA-AOPP obtained by exposure of HSA to HOCl trigger in a HOCl dose-dependent manner oxidative responses not only of monocytes but also of PMN and via both NADPH oxidase and MPO-dependent pathways. In PMN, NADPH oxidase oxygenation activity triggered by HSA-AOPP was significantly higher than those induced by complement opsonized zymosan (which acts via CR1 and CR3 ligation). Like HSA-AOPP, uremic plasma fractions enriched in AOPP obtained by fast protein liquid chromatography (FPLC) also retained the capacity to activate monocytes. However, this in vitro data should not be misinterpreted in the context of uremia. It is highly probable that plasma-derived AOPP also triggered significant phagocyte activation in vivo, but that inhibitory mechanisms might also counter regulate such proinflammatory reaction.

In contrast to lucigenin, the CL probe luminol used to measure MPO-dependent activity penetrates inside the cells and thus mainly measures intracellular oxidant formation [31, 36]. Surprisingly, intracellular MPO-derived activity induced by AOPP was much lower than that obtained with opsonized zymosan or PMA, regardless of the cell type. Taking into account the fact that PMN could be activated by LPS, it was verified that AOPP-induced respiratory burst was not due to LPS contamination, thus definitively ruling out an artifact. These observations support our working hypothesis that AOPP behave as mediators of inflammation, which, however, still need to be precisely defined. Indeed, like AGE, which under their name comprise a wide variety of heterogeneous compounds [37], AOPP might also be considered not as single species but rather as a family of compounds. Among these, both dityrosine and carbonyls, which are produced following exposure of albumin to HOCl [15, 18, 21] were found to be present in our HOCl-modified HSA preparation, and in our in vivo study of a cohort of uremic patients were closely correlated to AOPP levels [19]. Interestingly, in these previous studies we also observed that these protein oxidation derivatives were not, when tested alone, capable to trigger monocyte respiratory burst, as was the whole AOPP preparation. This observation does not rule out the possibility that other HOCl-mediated albumin oxidation products compounds (e.g., oxidized fatty acids or reactive aldehydes attached to HSA, and protein nitration-derived products) could as single exert such an effect and their proper contribution to AOPP biochemical composition and mediated biologic effects remains to be determined.

Our results showing that AOPP generated in vivo and
extracted from uremic plasma are capable to trigger neutrophil respiratory burst in a similar manner as does HOCl-treated control plasma are in support of their physiopathologic relevance for the phagocyte activation associated with uremia. Since AOPP are preferentially formed by chlorinated oxidants [17, 19] and MPO is the sole enzyme capable of producing such oxidants in vivo, this AOPP-induced activation of neutrophil MPO-dependent activity is also in favor of our working hypothesis that these products as numerous mediators may by themselves amplify their own formation.

We also showed that PMN priming by PAF enhances their potential to mount oxidative responses to AOPP. PAF has been found to trigger up-regulation of CR1, CR3, and FcγRII, the receptors ligated upon opsonized zymosan stimulation [32, 38, 39]. Since the ligation of

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**Fig. 4.** Dose-dependent effect of *N*-acetylcysteine (NAC) on human serum albumin-advanced oxidation protein products (HSA-AOPP)-induced monocyte and neutrophil NADPH-oxidase and MPO-dependent oxidative responses. HSA-AOPP-induced monocyte (A) and neutrophil (B) nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [lucigenin-amplified chemiluminescence (CL) responses, top panels] and myeloperoxidase (MPO)-dependent oxygenation activities (luminol-amplified CL responses, bottom panels) were measured after cell incubation (30 minutes at 37°C) with Hank’s balanced salt solution (HBSS) or NAC at the indicated concentrations. Results (mean ± SEM of six experiments) were expressed as % control CL response 100 × CL (cells + NAC)/CL (cells + HBSS).

**Fig. 5.** Comparative effect of *N*-acetylcysteine (NAC) (1 mg/mL), opsonized zymosan, and phorbol myristate acetate (PMA) on human serum albumin-advanced oxidation protein products (HSA-AOPP)-induced monocyte and neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase (MPO)-dependent oxidative responses. Monocyte (A) and neutrophil (B) NADPH oxidase [lucigenin-amplified chemiluminescence (CL) responses, top panels] and MPO-dependent oxygenation activities (luminol-amplified CL responses, bottom panels) triggered by HSA-AOPP, opsonized zymosan, or PMA were measured after cell incubation (30 minutes at 37°C) with Hank’s balanced salt solution (HBSS) or NAC (1 mg/mL). For each stimulus, results (mean ± SEM of six experiments) were expressed as % control CL response 100 × CL (cells + NAC)/CL (cells + HBSS).
opsonized zymosan with opsonin receptors induces rapid receptor internalization, up-regulation of opsonin receptors by PAF results in a selective increase in intracellular oxidants, as evidenced by enhanced MPO-derived activities, without increasing extracellular superoxide production. In contrast, PAF did not increase PMA-induced respiratory burst because it is receptor independent. Interestingly, PAF significantly increased AOPP-induced extracellular superoxide anion release and intracellular MPO activities, thus suggesting that AOPP triggers respiratory burst via the ligation of a specific receptor, which is distinct from opsonin receptor. This receptor might be stored within intracellular pools, but would be easily mobilizable toward the plasma membrane We therefore hypothesized that AOPP-induced respiratory burst could be a pertinent parameter to be modulated via appropriate therapeutic strategies aimed at reducing oxidative stress in hemodialysis patients. In this context,
NAC was chosen as a candidate for antioxidant therapy. Regardless of cell type, NAC at a dose of 0.5 to 1 mg/mL inhibited AOPP-induced oxygenation activities via both NADPH oxidase and MPO-dependent pathways. With regard to other stimulating agents we also found that, while NAC did not inhibit NADPH oxidase activation induced by PMA, it reduced MPO-dependent activity of both PMN and monocytes. In contrast, NAC had no inhibitory effect and even exerted an enhancing effect, on both opsonized zymosan-induced types of oxygenation activities. As opposed to PMA, which activates phagocyte oxidative metabolism via a receptor-independent pathway, opsonized zymosan triggers phagocyte oxidative responses via CR1 and CR3 receptor-dependent pathways [31]. Interestingly, an enhancing effect of NAC on the phagocytic activity of neutrophils has been observed [40].

The inhibitory effect of NAC on AOPP- and PMA-induced but not on opsonized zymosan-induced respiratory burst strongly suggests that NAC acts via the modulation of intracellular signaling, and not via a nonspecific intracellular oxidant scavenging effect. Of note, both glutathione and dithiothreitol, which, like NAC, also act mainly intracellularly and upon signal transduction pathways [34], exerted a similar inhibitory effect on AOPP-induced PMN oxygenation activities. Of interest is the consideration that signaling involved in AOPP and in PMA-induced respiratory burst might share some common steps since they are both inhibited by NAC, whereas opsonized zymosan-induced respiratory burst is not. Although NAC did not inhibit opsonized zymosan-induced respiratory burst, we cannot rule out that NAC might interfere, in vivo, with some mechanisms involved in host defense.

The last part of our study, which was conducted with PMN of a homogeneous series of dialysis patients, showed that basal luminol-amplified CL is much higher than those of controls. This suggests that PMN of hemodialysis patients are in a primed state. As to the effect of PAF on uremic PMN, we observed an unexpected phenomenon namely a significant decrease in basal CL, which did not occur with control PMN and remains to be elucidated. We herein provide evidence that uremic PMN retain a normal capacity to mount MPO-dependent oxidative responses to AOPP and that these responses are selectively enhanced by PAF priming. Interestingly, the respiratory burst induced by AOPP in uremic PMN was, as observed with normal PMN, selectively inhibited following pretreatment by NAC. It must be mentioned that the abnormal decrease of plasma glutathione in uremia may further aggravate the deleterious effect of potential AOPP-mediated ROS production.

There is an increasing body of evidence of the involvement of ROS in the pathogenesis of renal disease, and its most harmful complication (i.e., accelerated atherosclerosis). Interestingly, recent reports have stressed on AOPP as a most relevant index of phagocyte-derived oxidative stress and associated cardiovascular complications, not only in uremic patients [41] but also in nonuremic patients with coronary disease [42]. Interestingly, in our own study, AOPP but not another oxidative stress marker such as malondialdehyde (MDA) were found to be an independent risk factor for atherosclerosis and AOPP were highly correlated with ferritin.

Thus, antioxidant administration seems a promising approach [43] to prevent oxidative stress-related complications in end-stage renal disease (ESRD) patients [9, 14, 22]. Among the numerous antioxidants tested, there is a great deal of recent evidence to suggest that NAC administration may protect renal function [24–26].

AOPP-induced respiratory burst appears to be a promising target for modulating the inflammatory state in CRF. More important, the fact that the effect of NAC differs according to the stimulating agent and the intracellular signaling is of special relevance in the context of therapeutic intervention. Indeed, our results are in keeping with the hypothesis that, in vivo, NAC will selectively inhibit oxygenation activities triggered by AOPP, a circulating inflammatory mediator, without decreasing intracellular oxidants, which are induced via the ligation of opsonin receptors, and required for host defense. Clinical trials should be developed in order to verify whether this new indication of an old drug is relevant and whether NAC can be useful in the treatment of uremic patients.

NOTE ADDED IN PROOF

During the publication process of this paper, Tepel et al [44] reported that the oral administration of NAC to chronic hemodialysis patients over 14.5 months was associated with a significant reduction of cardiovascular events, in keeping with the probable clinical relevance of our study.

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