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Methylglyoxal: a stimulus to neutrophil oxygen radical production in chronic renal failure?

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Abstract

Background. Chronic renal failure is characterized by oxidant stress, resulting in part from increased reactive oxygen species production by neutrophils. Plasma concentrations of methylglyoxal are increased in uraemia. Methylglyoxal activates p38 mitogen-activated protein kinase (MAPK) in endothelial cells. Activation of p38 MAPK in neutrophils enhances reactive oxygen species production through exocytosis of intracellular storage granules. We tested the hypothesis that methylglyoxal enhances reactive oxygen species production by activating p38 MAPK in neutrophils.

Methods. Neutrophils were exposed to methylglyoxal *in vitro*. Activation of p38 MAPK was determined by immunoblot analysis. Exocytosis was determined by measuring plasma membrane expression of CD35 and CD66b, specific markers of secretory vesicles and specific granules, respectively. Reactive oxygen species production was determined by measuring H_2O_2 and O_2^- production.

Results. Methylglyoxal activated p38 MAPK and caused dose-dependent increases in CD35 and CD66b expression, which were blocked by the methylglyoxal scavenger, aminoguanidine, or the p38 MAPK inhibitor, SB203580. Methylglyoxal caused dose-dependent increases in basal and *Staphylococcus aureus*-stimulated H_2O_2 production and basal and formyl-methionyl-leucyl-phenylalanine-stimulated O_2^- production. Enhancement of reactive oxygen species production was blocked by aminoguanidine and SB203580.

Conclusions. Methylglyoxal enhances reactive oxygen species production in neutrophils through a process involving p38 MAPK-dependent exocytosis of intracellular storage granules. These findings, together with the observation that methylglyoxal concentrations are increased in renal failure, suggest a possible role for methylglyoxal as a uraemic toxin that contributes to the oxidant stress associated with renal failure.

Keywords: methylglyoxal; neutrophil; oxidant stress; reactive oxygen species; uraemia

Introduction

Damage to cells and proteins contributes to the long-term morbidity associated with chronic renal failure. Processes that cause this damage include oxidant stress, carbonyl stress and the formation of advanced glycation end products (AGEs). These processes interact. For example, oxidant stress can promote carbonyl stress by increasing production of reactive carbonyl compounds, which can then non-enzymatically modify proteins to form AGEs [1]. In turn, AGEs may contribute to oxidant stress by enhancing production of reactive oxygen species [2]. The extent of these interactions, and the molecular species involved, remains incompletely understood.

Methylglyoxal is a reactive carbonyl compound formed primarily from triose phosphates [3]. Concentrations of methylglyoxal and its protein adducts are increased in end-stage renal disease [4,5]. Methylglyoxal is biologically active. It activates p38 mitogen-activated protein kinase (MAPK) in endothelial [6] and mesangial cells [7], and inhibiting p38 MAPK blocks activation by methylglyoxal of the pro-apoptotic enzyme, caspase 3 [6]. Methylglyoxal also primes reactive oxygen species production and apoptosis in U266 cells [8]. We have shown that neutrophil reactive oxygen species production is enhanced by a factor(s) retained in plasma as renal function decreases [9]. We have also shown that p38 MAPK is involved in enhancing neutrophil reactive oxygen species

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production [10,11]. These observations suggested the hypothesis that methylglyoxal primes neutrophil reactive oxygen species production through activation of p38 MAPK. Demonstrating such an action of methylglyoxal would establish a further link between carbonyl stress and oxidant stress in uraemia.

Subjects and methods

Experimental design

The ability of methylglyoxal to activate p38 MAPK and stimulate exocytosis and respiratory burst activity was determined by incubating neutrophils in Krebs-Ringer phosphate buffer (KRPB) or ACD-anticoagulated normal human plasma at 37°C with different concentrations of methylglyoxal for times up to 60 min. Incubation times were based on previous studies of the time course of p38 MAPK activation by agents that prime neutrophil reactive oxygen species production [10]. Activation of p38 MAPK was assessed by SDS–PAGE of cell lysates, followed by immunoblot analysis for phosphorylated p38 MAPK. Exocytosis was assessed by measuring plasma membrane expression of CD35 and CD66b, respectively, specific markers of secretory vesicles and specific granules, which are intracellular storage sites of the NADPH oxidase component, cytochrome b_{558}. Respiratory burst activity was assessed by measuring basal and stimulated H_2O_2 and O_2^- production. Experiments were performed with or without 5 mM of the methylglyoxal scavenger, aminoguanidine, to confirm that exocytosis and changes in respiratory burst activity were induced by methylglyoxal. To confirm a role for p38 MAPK in methylglyoxal-induced exocytosis and changes in respiratory burst activity, experiments were performed after pre-incubation of neutrophils for 60 min with or without 3 μM of the p38 MAPK inhibitor, SB203580.

Materials

Endotoxin-free reagents and plastics were used in all experiments. Methylglyoxal and aminoguanidine were from Sigma Chemical Company (St Louis, MO). The p38 MAPK inhibitor, SB203580, was from Calbiochem (La Jolla, CA). Polyclonal antibodies specific for dual phosphorylated (Thr 180/Tyr 182) p38 MAPK and total p38 MAPK were from Cell Signaling Technology (Beverly, MA). FITC-labelled anti-CD35 was from Pharmingen (San Diego, CA) and FITC-labelled CD66b was from Accurate Chemical and Scientific (Westbury, NY).

Neutrophils

Blood was obtained from healthy donors using a protocol approved by the University of Louisville Human Studies Committees. Neutrophils were isolated using a plasma-Percoll gradient as described previously [11] and washed in endotoxin-free KRPB (pH 7.2) containing 0.2% dextrose. More than 97% of the cells were neutrophils by microscopic evaluation and viability was >95% by Trypan Blue exclusion.

Respiratory burst activity

Respiratory burst activity was determined by measuring H_2O_2 production stimulated by phagocytosis of *Staphylococcus aureus* and by measuring O_2^- production stimulated by formyl-methionyl-leucyl-phenylalanine (fMLP) as described previously [9]. H_2O_2 production was measured using a flow cytometric assay based on the stoichiometric conversion of non-fluorescing 2′,7′-dichlorofluoresin to fluorescent dichlorofluorescein by H_2O_2. Neutrophils (4×10^6/ml), suspended in ACD-anticoagulated normal human plasma, were incubated with 2′,7′-dichlorofluoresin diacetate (50 μM) for 10 min at 37°C. Fifty microliters of cell suspension was sampled before, and 10 min after, addition of 50 μl of opsonized, propidium iodide-labelled *S.aureus* (final concentration $\sim 10^8$ bacteria/ml). Sampled cells were washed in KRPB and fixed in 1% paraformaldehyde before analysis for phagocytosis and H_2O_2 production by flow cytometry (Epics XL, Beckman-Coulter, Hialeah, FL). The mean channel number of fluorescence (mcf) was used to quantify phagocytosis and H_2O_2 production. The flow cytometer was calibrated before analysis of each set of samples using Flow Check and Flow Set beads (Coulter). O_2^- production was determined by measuring the reduction of ferricytochrome C. Neutrophils (4.5×10^6/ml) were suspended in KRPB containing calcium and magnesium and 1 mg/ml ferricytochrome C. O_2^- production was stimulated by adding 3×10^{-7} M fMLP for 10 min at 37°C. The reaction was stopped by placing the tubes on ice and pelleting the cells by centrifugation at 4°C. The amount of O_2^- produced was quantified using the change in absorbance of the supernatant at 550 nm and was expressed as nanomoles of O_2^- per 10^6 cells using an extinction coefficient of $2.1 \times 10^4\,M^{-1}\,cm^{-1}$. All assays of respiratory burst activity were performed in duplicate.

Expression of plasma membrane proteins

Plasma membrane expression of CD35 and CD66b was determined by flow cytometry. Neutrophils were suspended in ice-cold KRPB and incubated at 4°C for 30 min with FITC-conjugated monoclonal anti-CD35 or anti-CD66b. FITC-conjugated mouse IgG_1 was used as an isotype control. Labelled cells were washed in KRPB and analysed for fluorescence intensity by flow cytometry.

p38 MAPK activity

p38 MAPK activation was determined using an antibody specific for p38 MAPK phosphorylated at Thr 180 and Tyr 182. After incubation with methylglyoxal or KRPB, 20×10^6 neutrophils were pelleted by centrifugation. The cells were lysed with 100 μl of ice-cold Laemmli's SDS-sample buffer, sonicated and boiled for 5 min. Samples were microfuged for 10 min then subjected to 10% SDS–PAGE. Proteins were electrotransferred to nitrocellulose and the membranes washed with PBS and blocking buffer. The blot was probed for total and phospho-p38 MAPK following the manufacturer's protocol. Immunoreactivity was localized by horseradish peroxidase-conjugated secondary antibodies and visualized by ECL enhanced chemiluminescence (Lumiglo, Cell Signaling). For each condition, the ratio of phospho-p38 MAPK to total p38 MAPK was calculated from the quantified intensities of the bands (ImageQuaNT, Molecular Dynamics, Sunnyvale, CA).

Data analysis

Differences in H_2O_2 and O_2^- production, plasma membrane expression of CD35 and CD66b, and p38 MAPK activation in the presence or absence of methylglyoxal and inhibitors were evaluated by analysis of variance. Where significant differences were detected in the primary analysis, differences between methylglyoxal and control and between individual inhibitors were examined using Bonferroni's adjustment for multiple comparisons or Tukey's test, as appropriate. Statistical analyses were performed using SPSS for Windows (version 11, SPSS Inc., Chicago, IL). Differences were considered significant at $P < 0.05$. Data are presented as mean ± SEM for n observations.

Results

Methylglyoxal activates p38 MAPK in neutrophils

Essentially no phosphorylated p38 MAPK was detected in control cells (Figure 1). In contrast, cells incubated with methylglyoxal showed marked phosphorylation of p38 MAPK after 15 min of incubation, demonstrating that methylglyoxal activates p38 MAPK in neutrophils.

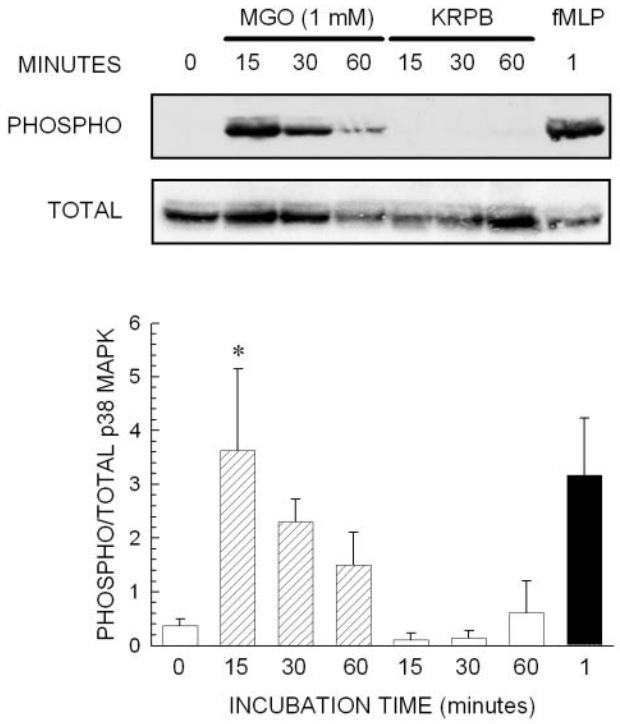

Fig. 1. Methylglyoxal induces time-dependent phosphorylation of p38 MAPK in neutrophils. Neutrophils were incubated with or without 1 mM methylglyoxal for 15–60 min. The cells were lysed and subjected to immunoblot analysis with anti-phospho-p38 MAPK antibody. The membranes were stripped and reprobed with anti-p38 MAPK antibody. fMLP (3×10^{-7} M for 1 min) was included as a positive control. Representative immunoblots for phospho-p38 MAPK and total p38 MAPK are shown in the upper part of the figure. The lower part of the figure shows the ratio of phospho-p38 MAPK to total p38 MAPK ($n = 4$). The ratio of phospho-p-38 MAPK to total p38 MAPK differed significantly between cells incubated in methylglyoxal and cells incubated in KRPB ($P = 0.001$). * Indicates different from zero time.

Methylglyoxal induces p38 MAPK-dependent neutrophil exocytosis

Neutrophils incubated with 1 mM methylglyoxal showed increased plasma membrane expression of CD35 and CD66b, indicating exocytosis of secretory vesicles and specific granules. The increase in CD35 and CD66b expression was significant after 45 min of incubation with methylglyoxal (Figure 2). The ability of methylglyoxal to induce exocytosis was dose-dependent, as shown by a significant increase in plasma membrane expression of CD35 and CD66b at concentrations of 0.5 mM, and above, when neutrophils were incubated with methylglyoxal for 60 min (Figure 3). The data in Table 1 confirm that exocytosis was induced by methylglyoxal by showing that pre-incubation for 5 min with 5 mM of the methylglyoxal scavenger, aminoguanidine, blocked the increase in CD35 and CD66b observed when the cells were incubated for 60 min with 1 mM methylglyoxal. Pre-incubation of neutrophils with 3 μM of the p38 MAPK inhibitor, SB203580, for 60 min also blocked the exocytosis induced by 1 mM methylglyoxal (Table 1), demonstrating that the ability of methylglyoxal to induce exocytosis depended on the p38 MAPK pathway. The data in Figure 3 also show that methylglyoxal induced exocytosis whether the incubation took place in plasma or protein-free buffer, demonstrating that the effect of methylglyoxal is directly on the cell and not through modification of a plasma protein. Taken together, these data demonstrate that methylglyoxal acts directly on human neutrophils to induce exocytosis of secretory vesicles and specific granules through a p38 MAPK-dependent mechanism.

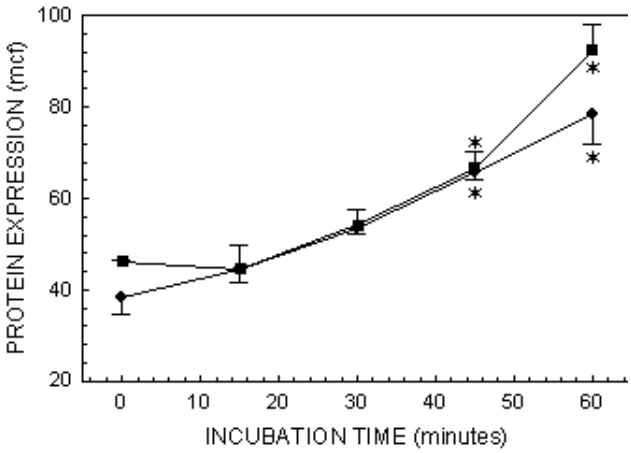

Fig. 2. Time course of methylglyoxal-induced exocytosis in human neutrophils. Neutrophils in buffer were incubated for varying times with 1 mM methylglyoxal at 37°C. Exocytosis of secretory vesicles and specific granules was determined by measuring plasma membrane expression of CD35 (circles) and CD66b (squares), respectively. Data are presented as mean ± SEM for $n = 3$. * Indicates different from zero time.

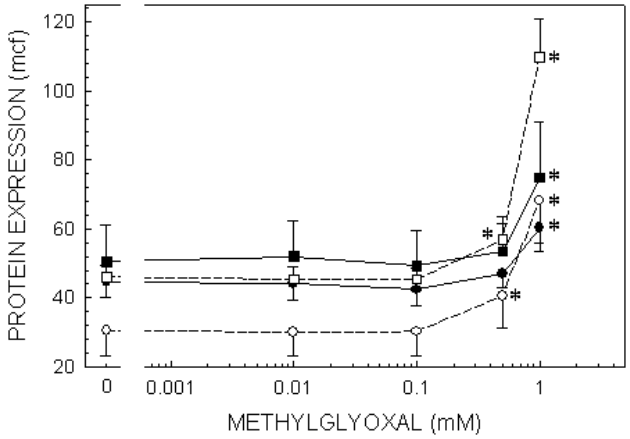

Fig. 3. Methylglyoxal induces a dose-dependent exocytosis of secretory vesicles and specific granules in neutrophils. Neutrophils were incubated with varying concentrations of methylglyoxal (0–1 mM) for 60 min at 37°C. Exocytosis of secretory vesicles and specific granules was determined by measuring plasma membrane expression of CD35 (circles) and CD66b (squares), respectively. Methylglyoxal at doses of 0.5 mM, and above, induced significant exocytosis both in protein-free buffer (open symbols, $n=3$) and plasma (closed symbols, $n=6$). Data are presented as mean ± SEM. * Indicates different from control.

Table 1. Both scavenging of carbonyl compounds and inhibition of p38 MAPK prevent methylglyoxal-induced exocytosis

	Protein expression (mcf)	
	CD35	CD66b
Control	27.8 ± 1.9	38.5 ± 2.7
Methylglyoxal	60.9 ± 3.2*	60.2 ± 4.9*
Control + 5 mM aminoguanidine	26.0 ± 2.7	37.6 ± 3.3
1 mM Methylglyoxal + 5 mM aminoguanidine	27.5 ± 2.5	39.4 ± 2.8
Control + 3 μM SB203580	25.2 ± 3.2	33.5 ± 1.6
1 mM Methylglyoxal + 3 μM SB203580	30.5 ± 2.8	28.8 ± 1.5

Neutrophils (4×10^6/ml in buffer) were pre-incubated with 5 mM aminoguanidine for 5 min or 3 μM SB203580 for 60 min before being stimulated with 1 mM methylglyoxal for 60 min. CD35 and CD66b expression was measured by flow cytometry. Data are presented as mean ± SEM for four experiments. * Indicates a significant difference from control.

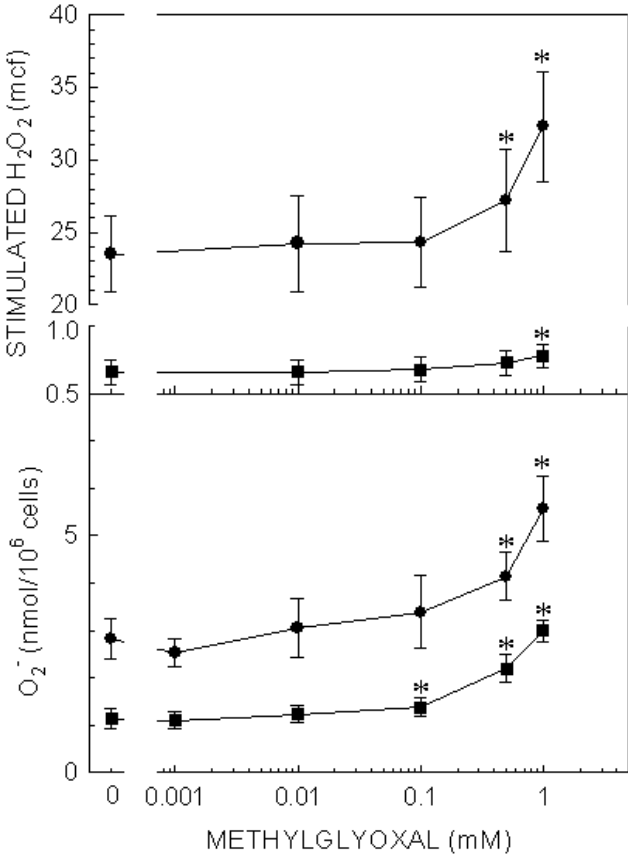

Fig. 4. Methylglyoxal primes neutrophil respiratory burst activity in a dose-dependent manner. The upper panel shows baseline (squares) and *S.aureus*-stimulated (circles) H_2O_2 production by neutrophils incubated in plasma for 60 min at 37°C with varying concentrations of methylglyoxal (0–1 mM). Methylglyoxal caused a significant dose-dependent increase in both baseline and *S.aureus*-stimulated H_2O_2 production, and the increase in H_2O_2 production induced by *S.aureus* depended significantly on the concentration of methylglyoxal. The lower panel shows fMLP-stimulated O_2^- production by neutrophils incubated in protein-free buffer for 60 min at 37°C with varying concentrations of methylglyoxal (0–1 mM). fMLP caused a significant increase in both baseline and fMLP-stimulated O_2^- production, and the increase in O_2^- production induced by fMLP depended significantly on the concentration of methylglyoxal present in the reaction mixture. Data are presented as mean ± SEM for $n=7$ (upper panel) and $n=8$ (lower panel). * Indicates different from control.

Methylglyoxal increases neutrophil respiratory burst activity

Incubation for 60 min with methylglyoxal produced small, but significant, dose-dependent increases in basal H_2O_2 and O_2^-, as well as priming both *S.aureus*-stimulated H_2O_2 production and fMLP-stimulated O_2^- production (Figure 4). As with exocytosis, the effect of methylglyoxal became significant at concentrations of 0.5 mM, and above (Figure 4), and followed a similar time course to that observed for exocytosis (data not shown). Similar to what was observed for exocytosis, the increase in *S.aureus*-stimulated H_2O_2 production produced by 1 mM methylglyoxal was blocked by pre-incubation with aminoguanidine or SB203580 (Table 2). Moreover, the ability of methylglyoxal to increase basal respiratory burst activity and prime the response to *S.aureus* and fMLP was independent of whether or not plasma proteins were present (Figure 4), demonstrating that these actions of methylglyoxal also occur through a direct action on the cell and not through modification of a plasma protein. These findings demonstrate that methylglyoxal acts directly on human neutrophils to enhance reactive oxygen species production through a process that involves p38 MAPK-dependent exocytosis of granules that act as intracellular stores of the NADPH oxidase component, cytochrome b_{558}.

Table 2. Both scavenging of carbonyl compounds and inhibition of p38 MAPK prevent methylglyoxal-induced priming of respiratory burst activity

	Stimulated H_2O_2 production
Control	28.3 ± 3.5
Control + 5 mM aminoguanidine	24.7 ± 2.2
1 mM Methylglyoxal	43.2 ± 3.0*
1 mM Methylglyoxal + 5 mM aminoguanidine	28.3 ± 3.3
Control	21.8 ± 1.4
Control + 3 μM SB203580	17.2 ± 1.1
1 mM Methylglyoxal	28.6 ± 1.3*
1 mM Methylglyoxal + 3 μM SB203580	20.7 ± 1.2

Neutrophils (4×10^6/ml in plasma) were pre-incubated with 5 mM aminoguanidine for 5 min or 3 μM SB203580 for 60 min before being incubated with 1 mM methylglyoxal for 60 min. *Staphylococcus aureus*-stimulated H_2O_2 production was measured by flow cytometry. Data are presented as mean ± SEM for seven (aminoguanidine) or 12 (SB203580) experiments. * Indicates a significant difference from control.

Discussion

Neutrophils kill bacteria by releasing proteolytic enzymes and generating reactive oxygen species. To prevent unwanted tissue damage, peripheral blood neutrophils normally respond poorly to pro-inflammatory stimuli. To effectively kill bacteria, these relatively benign cells convert to a primed state, wherein they are capable of responding to stimuli, and then to an activated state in which they release proteolytic enzymes and reactive oxygen species. Priming of neutrophils by inflammatory mediators involves exocytosis of intracellular granules, leading to a progressive change in the complement of proteins expressed in the plasma membrane. This process depends on activation of p38 MAPK [11]. The present study shows for the first time that the reactive carbonyl compound, methylglyoxal, can induce exocytosis and enhance respiratory burst activity in human neutrophils, and that these actions depend on activation of p38 MAPK. The time courses of p38 MAPK activation and neutrophil functional response observed for methylglyoxal are similar to those observed with other priming agents, such as LPS and TNFα; that is, initial activation of p38 MAPK followed after a time lag by exocytosis and enhancement of respiratory burst activity [10,11].

Renal failure is associated with increased neutrophil respiratory burst activity and a primed response to soluble and particulate stimuli [9,12]. This priming is caused by a substance(s) retained in uraemia [13,14]. Neutrophil reactive oxygen species production may be increased by a number of agents that can be present in uraemic plasma, including complement fragment C5a [15], AGEs [2] and advanced oxidation protein products (AOPP) [16]. Several pieces of evidence suggest, however, that these factors are not solely responsible for the increased respiratory burst activity observed in uraemia. We have demonstrated that respiratory burst activity decreases from pre- to post-dialysis in patients treated with high-flux membranes [12]. This observation, together with the findings of Rhee *et al*. [13] and Daniels *et al*. [14], suggests that at least one factor responsible for priming the respiratory burst in uraemia is of lower molecular weight than most AGEs and AOPPs. Complement fragment C5a has been implicated in a transient increase in reactive oxygen species production during haemodialysis, particularly when cellulose membranes are used [17]. However, priming of the respiratory burst is evident in chronic kidney disease before initiation of dialysis [9], and chronic kidney disease generally is not associated with complement activation. Also, plasma C5a concentrations are normal pre-dialysis, a time when significant priming of reactive oxygen species production is evident [12,17]. The demonstration that methylglyoxal can increase basal respiratory burst activity in neutrophils and prime their response to other stimuli, together with the observation that plasma methylglyoxal concentrations are increased in uraemia [4], suggests methylglyoxal as an additional candidate priming factor, one which creates a possible link between carbonyl stress and oxidant stress that may be relevant to long-term morbidity in renal failure.

Our results indicate that methylglyoxal is able to activate p38 MAPK in human neutrophils, as well as in human endothelial [6] and rat mesangial cells [7]. The mechanism by which methylglyoxal activates p38 MAPK in neutrophils is not known. Methylglyoxal could act on the cell directly or indirectly, through modification of a plasma protein. Recently, Fan *et al*. reported that methylglyoxal-modified albumin activated p38 MAPK and stimulated reactive oxygen species production in RAW 264.7 cells [18]. Methylglyoxal reacts reversibly with arginine, cysteine and lysine residues of proteins to form structures that bind to AGE receptors [3] and Collison *et al*. have reported the presence of an AGE receptor on neutrophils [2]. Ligation of this receptor with AGE-modified albumin primed the respiratory burst stimulated by fMLP, although not phagocytosis of *S.aureus* [2]. However, our finding that methylglyoxal caused exocytosis and stimulated and primed respiratory burst activity when incubated with neutrophils in protein-free buffer argues against methylglyoxal acting through an effect on plasma proteins. Alternatively, methylglyoxal may enter the cell and act directly on components of the p38 MAPK signaling pathway. Methylglyoxal decreases intracellular reduced glutathione concentrations in endothelial cells [6], and changes the redox state of a cell by decreasing reduced glutathione concentrations activates p38 MAPK [19]. That priming of the respiratory burst in uraemia may be mediated by changes in redox state is supported by the observations that the ratio of oxidized to reduced glutathione in plasma is increased [20], and that total glutathione concentrations in neutrophils are decreased [21], in haemodialysis patients compared with normal subjects. Further study will be required to determine if this

mechanism is responsible for the exocytosis and enhanced respiratory burst response induced by methylglyoxal.

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Conflict of interest statement. None declared.

References

1. Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of 'carbonyl stress' in long-term uremic complications. *Kidney Int* 1999; 55: 389–399
2. Collison KS, Parhar RS, Saleh SS *et al*. RAGE-mediated neutrophil dysfunction is evoked by advanced glycation end products (AGEs). *J Leukoc Biol* 2002; 71: 433–444
3. Thornalley PJ. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. *Gen Pharmacol* 1996; 27: 565–573
4. Odani H, Shinzato T, Matsumoto Y *et al*. Increase in three α,β-dicarbonyl compound levels in human uremic plasma: specific *in vivo* determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun* 1999; 256: 89–93
5. Odani H, Shinzato T, Usami J *et al*. Imidazolium crosslinks derived from reaction of lysine with glyoxal and methylglyoxal are increased in serum proteins of uremic patients: evidence for increased oxidative stress in uremia. *FEBS Lett* 1998; 427: 381–385
6. Akhand AA, Hossain K, Mitsui H *et al*. Glyoxal and methylglyoxal trigger distinct signals for MAP family kinases and caspase activation in human endothelial cells. *Free Radic Biol Med* 2001; 31: 20–30
7. Liu B-F, Miyata S, Hirota S *et al*. Methylglyoxal induces apoptosis through activation of p38 mitogen-activated protein kinase in rat mesangial cells. *Kidney Int* 2003; 63: 947–957
8. Godbout JP, Pesavento J, Hartman ME *et al*. Methylglyoxal enhances cisplatin-induced cytotoxicity by activating protein kinase Cδ. *J Biol Chem* 2002; 277: 2554–2561
9. Ward RA, McLeish KR. Polymorphonuclear leukocyte oxidative burst is enhanced in patients with chronic renal insufficiency. *J Am Soc Nephrol* 1995; 5: 1697–1702
10. McLeish KR, Knall C, Ward RA *et al* Activation of mitogen-activated protein kinase cascades during priming of human neutrophils by TNFα and GM-CSF. *J Leukoc Biol* 1998; 64: 537–545
11. Ward RA, Nakamura M, McLeish KR. Priming of the neutrophil respiratory burst involves p38 mitogen-activated protein-kinase-dependent exocytosis of flavocytochrome b_{558}-containing granules. *J Biol Chem* 2000; 275: 36 713–36 719
12. Ward RA, Ouseph R, McLeish KR. Effects of high-flux hemodialysis on oxidant stress. *Kidney Int* 2003; 63: 353–359
13. Rhee MS, McGoldrick MD, Meuwissen HJ. Serum factor from patients with chronic renal failure enhances polymorphonuclear leukocyte oxidative metabolism. *Nephron* 1986; 42: 6–13
14. Daniels I, Lindsay M, Porter C *et al*. Effect of peritoneal dialysis effluent on superoxide anion production by polymorphonuclear neutrophils. *Nephron* 1993; 64: 382–387
15. Van Epps DE, Garcia ML. Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. *J Clin Invest* 1980; 66: 167–175
16. Witko-Sarsat V, Gausson V, Nguyen A-T *et al*. AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: a potential target for *N*-acetylcysteine treatment in dialysis patients. *Kidney Int* 2003; 64: 82–91
17. Ward RA, McLeish KR. Hemodialysis with cellulose membranes primes the neutrophil oxidative burst. *Artif Organs* 1995; 19: 801–807
18. Fan X, Subramaniam R, Weiss MF, Monnier VM. Methylglyoxal-bovine serum albumin stimulates tumor necrosis factor alpha secretion in RAW 264.7 cells through activation of mitogen-activating protein kinase, nuclear factor κB and intracellular reactive oxygen species formation. *Arch Biochem Biophys* 2003; 409: 274–286
19. Haddad JJ. The involvement of L-γ-glutamyl-L-cysteinyl-glycine (glutathione/GSH) in the mechanism of redox signaling mediating $MAPK^{p38}$-dependent regulation of pro-inflammatory cytokine production. *Biochem Pharmacol* 2002; 63: 305–320
20. Himmelfarb J, McMenamin E, McMonagle E. Plasma aminothiol oxidation in chronic hemodialysis patients. *Kidney Int* 2002; 61: 705–716
21. Schettler V, Wieland E, Methe H *et al*. Oxidative stress during dialysis: effect on free radical scavenging enzyme (FRSE) activities and glutathione (GSH) concentration in granulocytes. *Nephrol Dial Transplant* 1998; 13: 2588–2593

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