RHEUMATOLOGY

Rheumatology 2014;53:1958–1965
doi:10.1093/rheumatology/keu218
Advance Access publication 4 June 2014

Original article

Myeloperoxidase and oxidation of uric acid in gout: implications for the clinical consequences of hyperuricaemia

Lisa K. Stamp[1], Rufus Turner[2], Irada S. Khalilova[2], Mei Zhang[3], Jill Drake[3], Louisa V. Forbes[2] and Anthony J. Kettle[2]

Abstract

Objectives. The aims of this study were to establish whether, in patients with gout, MPO is released from neutrophils and urate is oxidized to allantoin and if these effects are attenuated by allopurinol.

Methods. MPO, urate, allantoin and oxypurinol were measured in plasma from 54 patients with gout and 27 healthy controls. Twenty-three patients had acute gout, 13 of whom were receiving allopurinol, and 31 had intercritical gout, 20 of whom were receiving allopurinol. Ten additional gout patients had samples collected before and after 4 weeks of allopurinol.

Results. Plasma MPO and its specific activity were higher ($P < 0.05$) in patients with acute gout not receiving allopurinol compared with controls. MPO protein in patients' plasma was related to urate concentration ($r = 0.5$, $P < 0.001$). Plasma allantoin was higher ($P < 0.001$) in all patient groups compared with controls. In controls and patients not receiving allopurinol, allantoin was associated with plasma urate ($r = 0.62$, $P < 0.001$) and MPO activity ($r = 0.45$, $P < 0.002$). When 10 patients were treated with allopurinol, it lowered their plasma urate and allantoin ($P = 0.002$). In all patients receiving allopurinol, plasma allantoin was related to oxypurinol ($r = 0.65$, $P < 0.0001$). Oxypurinol was a substrate for purified MPO that enhanced the oxidation of urate.

Conclusion. Increased concentrations of urate in gout lead to the release of MPO from neutrophils and the oxidation of urate. Products of MPO and reactive metabolites of urate may contribute to the pathology of gout and hyperuricaemia. At low concentrations, oxypurinol should reduce inflammation, but high concentrations may contribute to oxidative stress.

Key words: gout, myeloperoxidase, cardiovascular disease, urate, allopurinol, oxidative stress.

CLINICAL SCIENCE

Introduction

Hyperuricaemia is critical for the development of gout and is also linked to numerous pathologies [1, 2]. Painful attacks of gout occur when crystals of monosodium urate precipitate within joints and promote an acute inflammatory response. Other inflammatory diseases associated with hyperuricaemia include diabetes, hypertension, pre-eclampsia, metabolic syndrome, renal failure and cardiovascular disease (CVD) [3, 4]. Whether urate is a causative factor in these diseases is controversial, but evidence linking hyperuricaemia to their pathology continues to mount.

The pernicious effects of urate are often attributed to its ability to activate the inflammasome [5]. It is also proposed to act as a pro-oxidant when oxidized to its radical [6]. Recently urate was demonstrated to be a physiological substrate for the neutrophil enzyme MPO [7]. This haem enzyme uses hydrogen peroxide (H_2O_2) to oxidize chloride to hypochlorous acid (HOCl) [8], but under physiological conditions will also convert urate to the urate radical. The urate radical is readily reduced by ascorbate [9], but it also gives rise to several electrophiles including dehydrourate, 5-hydroxyisourate and urate hydroperoxide [7]. These species will react with

[1]Department of Medicine, [2]Department of Pathology, Centre for Free Radical Research, University of Otago, and [3]Clinical Pharmacology Canterbury Health Laboratories, Christchurch, New Zealand.

Submitted 10 September 2013; revised version accepted 27 March 2014.

Correspondence to: Anthony J. Kettle, Department of Pathology, University of Otago, Christchurch, PO Box 4345, Christchurch 8140, New Zealand. E-mail: tony.kettle@otago.ac.nz

endogenous nucleophiles or break down to the stable biomarker allantoin.

In gout, MPO is likely to be discharged from neutrophils when they are recruited to affected joints and attempt to phagocytose urate crystals. Urate should compete with chloride and be oxidized by MPO. Whether MPO is present in the circulation of patients with gout and oxidizes urate to allantoin is unknown. This is clinically relevant because MPO contributes to cardiovascular risk [10] and both hyperuricaemia and gout are associated with an increased risk of CVD and death [11–14].

Successful long-term management of gout requires sustained reduction of plasma urate to $<360\,\mu M$ [15]. Allopurinol, which inhibits xanthine oxidoreductase, thereby preventing the production of urate [16], is the most commonly used urate-lowering therapy. Allopurinol is rapidly metabolized by aldehyde oxidase to oxypurinol and H_2O_2 [17]. By virtue of its longer half-life, oxypurinol exerts most of the inhibitory effects on xanthine oxidoreductase.

We hypothesize that during acute inflammation in gout, MPO will be released from neutrophils and oxidize urate to reactive intermediates that will contribute to the adverse effects of hyperuricaemia. The aims of this study were to determine in patients with gout whether MPO is released from neutrophils and oxidizes urate and if these effects are attenuated by allopurinol.

Patients and methods

Subjects

Patients with gout as defined by ARA preliminary classification criteria were recruited [18]. Acute gout was defined as the presence of a red, hot, swollen joint typical for gout and intercritical gout was defined as a patient with a confirmed diagnosis of gout with no current inflammatory joint symptoms. Healthy controls were randomly selected from the Christchurch electoral role. Patient demographic and clinical details were collected. Laboratory assessment included plasma urate and oxypurinol concentrations. Peripheral blood for MPO and allantoin was collected and processed as previously described [19]. Ethical approval was obtained from the Upper South A and B Regional Ethics Committees, New Zealand. Written informed consent was obtained from each patient.

Laboratory assays

Urate concentrations in plasma were measured by the Trinder reaction and using HPLC with ultraviolet (UV) light detection [20]. These methods gave comparable results. Plasma oxypurinol concentrations were measured as previously described [21]. There is currently no validated therapeutic range for plasma oxypurinol. In healthy volunteers, xanthine oxidase is maximally inhibited (90% inhibition) at a plasma oxypurinol concentration of $34\,\mu M$ [22].

Plasma MPO was determined by ELISA as described previously [23]. In this assay, MPO is initially captured with a monoclonal MPO antibody and its activity is determined using H_2O_2 and Amplex Red. Then the amount of MPO that was bound to the plate is determined using a polyclonal MPO antibody. Plasma allantoin was measured by stable isotope dilution mass spectrometry as described elsewhere [24].

The production of HOCl by purified MPO in an *in vitro* assay was determined by measuring the accumulation of taurine chloramine [10]. Reactions were started by adding $50\,\mu M$ H_2O_2 to 20 nM MPO in 10 mM phosphate buffer (pH 7.4) containing 140 mM sodium chloride and 5 mM taurine. The reactions were stopped after 4 min by adding $20\,\mu g/ml$ of catalase and accumulated taurine chloramine was determined. Tyrosine ($10\,\mu M$), which is a good substrate for compound II of MPO [8], was included with the purines to determine whether they acted by promoting formation of this redox intermediate, which is unable to oxidize chloride. None of the purines reacted directly with taurine chloramine. MPO was obtained from Planta (Vienna, Austria) and had a purity index of 0.82. Oxidation of urate ($300\,\mu M$) by purified MPO (25 nM) with various concentrations of oxypurinol in 50 mM phosphate buffer (pH 7.4) was started by adding H_2O_2 ($50\,\mu M$) and stopped after 30 min by adding catalase ($10\,\mu g/ml$). Production of allantoin from urate oxidation was measured using stable isotope dilution mass spectrometry as described elsewhere [24]. Xanthine, urate, allopurinol and oxypurinol were obtained from Sigma (St Louis, MO, USA). All other reagents were of the highest analytical grade available.

Statistical analysis

Data are expressed as the median and interquartile range (IQR). The statistical analysis of differences between group medians was carried out using either the Mann–Whitey rank sum test, analysis of variance (ANOVA) on ranks or the Wilcoxon signed rank test. Correlations were tested using Spearman's rank order correlation. In all cases significance was accepted as $P < 0.05$. Insufficient sample was available to analyse MPO in one patient with acute gout receiving allopurinol.

Results

Patient demographics

Fifty-four patients with gout and 27 healthy controls of a similar age range and gender mix were recruited. They had peripheral blood samples obtained on a single occasion. The patients were allocated into four groups: acute gout not receiving allopurinol ($n = 10$), acute gout receiving allopurinol ($n = 13$), intercritical gout not receiving allopurinol ($n = 11$) and intercritical gout receiving allopurinol ($n = 20$). An additional 10 patients with gout had peripheral blood samples taken prior to commencing treatment with allopurinol and again after 4 weeks of therapy. No patients were receiving other urate-lowering therapies such as probenecid or febuxostat. Demographic and clinical details are shown in Table 1.

The plasma urate concentrations of patients not on allopurinol with either acute gout [median $552\,\mu M$ (IQR 496–586), $n = 10$] or intercritical gout [median $530\,\mu M$

TABLE 1 Demographics and laboratory data of patients

	Acute gout ($n = 10$)	Acute gout + allopurinol ($n = 13$)	Intercritical gout ($n = 11$)	Intercritical gout + allopurinol ($n = 20$)	Gout patients starting allopurinol ($n = 10$)	Healthy controls ($n = 27$)
Age, mean (range), years	60.6 (40–91)	52.2 (30–83)	59.2 (34–82)	63.2 (42–82)	55.2 (40–78)	58.1 (39–79)
Male, %	80	69.2	72.7	95	80	74
NZ European, %	70	76.9	90.9	95	30	100
Plasma urate, mean (range), mmol/l	0.54 (0.43–0.61)	0.42 (0.28–0.64)	0.53 (0.45–0.64)	0.36 (0.22–0.65)	0.57 (0.51–0.63)	0.37 (0.23–0.52)
Creatinine, mean (range), mmol/l	0.11 (0.07–0.15)	0.10 (0.06–0.20)	0.10 (0.08–0.14)	0.10 (0.07–0.15)	0.10 (0.07–0.15)	nd
Receiving allopurinol, %	0	100	0	100	0	0
Allopurinol dose, mean (range), mg/day	—	269.2 (100–500)	—	280 (50–400)	—	—
Plasma oxypurinol, mean (range), μmol/l	—	84 (11–235)	—	92 (53–188)	—	—
NSAID, %	40	23	36	15	20	0
Prednisone, %	30	46	18	5	20	0
Colchicine, %	0	23	18	5	10	0
History of ischaemic heart disease, %	30	31	9	47	40	0

NZ: New Zealand; nd: not determined.

(IQR 460–570), $n = 11$] were significantly elevated ($P < 0.05$) compared with controls [median 359 μM (IQR 302–427), $n = 27$]. Plasma urate concentrations of patients receiving allopurinol were not significantly different from controls (data not shown).

Plasma MPO is increased in patients with acute gout

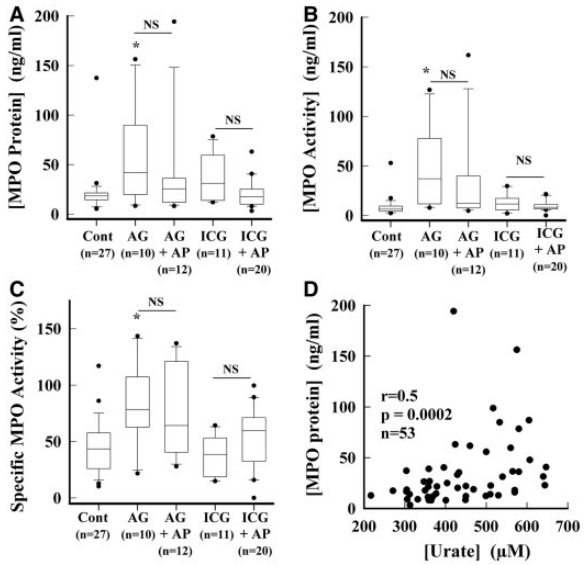
MPO protein and MPO enzyme activity were measured in the plasma of controls and the four patient groups, who were sampled on one occasion, to determine whether gout is associated with systemic increases in this neutrophil-derived protein. Both the activity and concentration of the enzyme were measured because MPO is susceptible to inactivation and is inhibited in plasma by ceruloplasmin [23]. Plasma MPO protein was significantly higher ($P < 0.05$) in patients with acute gout not receiving allopurinol [median 41.9 ng/ml (IQR 19.6–89.8), $n = 10$] compared with healthy controls [median 18.4 ng/ml (IQR 14.5–21.5), $n = 27$] (Fig. 1A), but not in patients with intercritical gout not receiving allopurinol [median 31.2 ng/ml (IQR 13.9–59.7), $n = 11$]. The enzyme activity of MPO in plasma was significantly higher ($P < 0.05$) in patients with acute gout not receiving allopurinol [median 37.0 ng/ml (IQR 11.8–77.8), $n = 10$] compared with healthy controls [median 6.3 ng/ml (IQR 4.3–9.5), $n = 27$] (Fig. 1B). MPO activity was not significantly elevated in patients with intercritical gout not receiving allopurinol [median 11.6 ng/ml (IQR 5.7–17.4), $n = 11$]. The specific activity of MPO (MPO activity/MPO protein) was significantly higher in patients with acute gout not on allopurinol compared with controls (Fig. 1C), but not for patients with intercritical gout not on allopurinol. Allopurinol had no significant effects on the levels of MPO protein, MPO activity or the enzyme's specific activity (Fig. 1A–C).

To determine whether plasma urate affects the release of MPO from neutrophils, we assessed its relationship with plasma MPO protein and activity in all acute and intercritical gout patients irrespective of whether they were receiving allopurinol (Fig. 1D). In these patients, plasma urate concentration was significantly associated with MPO protein ($r = 0.50$, $P = 0.0002$, $n = 53$) and MPO activity ($r = 0.29$, $P = 0.04$, $n = 53$). The effect of urate on MPO activity was more significant ($P = 0.005$) when patients were split into normouricaemic [median 8.1 ng/ml (IQR 6.6–11.0), $n = 23$] and hyperuricaemic individuals [median 12.9 ng/ml (IQR 7.9–33.6), $n = 30$]. Collectively these data suggest that acute gout leads to an increase in plasma MPO that is associated with the concentration of urate. Allopurinol did not appear to affect the concentrations of MPO.

Plasma allantoin is increased in patients with gout

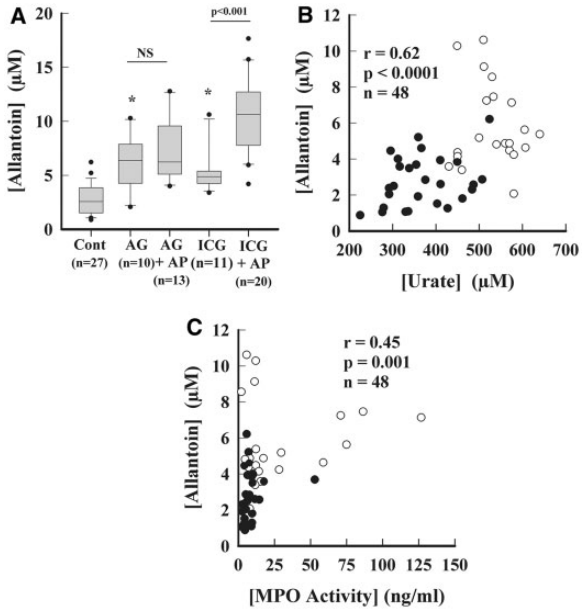
To assess whether urate is oxidized in patients with gout, we measured allantoin in plasma. The concentration of allantoin in plasma of both patients with acute gout not on allopurinol [median 6.4 μM (IQR 4.2–7.98), $n = 10$] and intercritical gout not on allopurinol [median 4.9 μM (IQR 4.2–5.4), $n = 11$] was significantly higher ($P < 0.05$) than that in healthy controls [median 2.6 μM (IQR 1.5–3.8),

Fig. 1 MPO in plasma of patients with gout



(**A**) MPO protein was measured in plasma from healthy controls (Cont), patients with acute gout off (AG) or on allopurinol (AG + AP) and patients with intercritical gout off (ICG) and on allopurinol (ICG + AP). (**B**) MPO activity was also measured in these groups and (**C**) the specific MPO activity was calculated from the ratio of MPO activity to MPO protein. Box plots show medians with 25th and 75th percentiles as boundaries. Whiskers are for the 10th and 90th percentiles. Symbols: closed circle indicates an outlier. Groups were compared using analysis of variance on ranks or the Mann–Whitney rank sum test. Asterisk indicates $P < 0.05$ compared with the control. NS: not significant. (**D**) The relationship between plasma urate and plasma MPO protein in all patients with gout. The association was determined using Spearman's rank order correlation.

Fig. 2 Allantoin in plasma of patients with gout



(**A**) Allantoin was measured in plasma from controls and patients with gout and analysed as described in Fig. 1. Closed circle indicates an outlier. Allantoin was compared with (**B**) plasma urate or (**C**) plasma MPO activity. Filled and empty dots are for controls and patients, respectively. Correlations were determined using Spearman's rank order correlation. NS: not significant.

$n = 27$] (Fig. 2A). The concentration of allantoin was similar in acute patients irrespective of whether they were receiving allopurinol, but in intercritical patients allantoin was significantly higher ($P < 0.001$) in those treated with allopurinol [median 10.6 μM (IQR 7.8–12.7), $n = 20$] compared with those not receiving allopurinol (Fig. 2A).

There was a significant correlation between the concentrations of allantoin and urate in the plasma of controls and gout patients not treated with allopurinol ($r = 0.62$, $P < 0.0001$, $n = 48$) (Fig. 2B). In this group there was also a significant correlation between the concentrations of allantoin and MPO activity in plasma ($r = 0.45$, $P < 0.002$, $n = 48$) (Fig. 2C). There was a weaker correlation of MPO protein with allantoin ($r = 0.31$, $P < 0.035$, $n = 48$). Patients treated with allopurinol were excluded from these analyses because of its positive effect on allantoin in the intercritical group. These data indicate that urate is oxidized in patients with gout and that oxidation is associated with higher concentrations of urate and MPO.

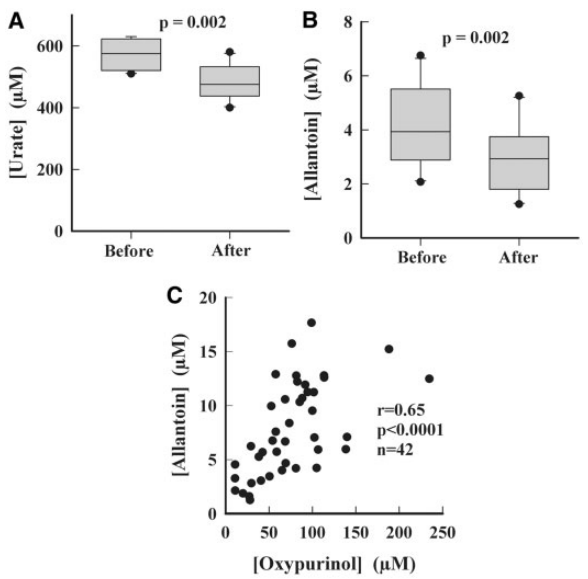
Effects of allopurinol on the oxidation of urate

A notable feature of the data on plasma allantoin was that even though allopurinol lowered urate concentrations in patients with gout, it did not lower the concentration of allantoin. In fact, the plasma concentration of allantoin was highest in patients with intercritical gout treated with allopurinol (Fig. 2A). To directly assess the effect of allopurinol on allantoin and MPO levels, we selected 10 additional patients with gout who were commenced on allopurinol and looked for changes in urate, allantoin and MPO after 4 weeks of therapy. In a paired analysis, allopurinol was found to lower circulating urate ($P = 0.002$) (Fig. 3A) as well as the plasma concentration of allantoin ($P = 0.002$) (Fig. 3B). It had no significant effect on concentrations of MPO protein or MPO activity (data not shown).

These data were in conflict with those in Fig. 2A for the effect of allopurinol in patients with intercritical gout. Therefore we assessed how the concentration of oxypurinol affected the plasma concentration of allantoin in all patients receiving allopurinol. As shown in Fig. 3C, there was a strong association between plasma oxypurinol concentrations and allantoin ($r = 0.65$, $P < 0.0001$, $n = 42$).

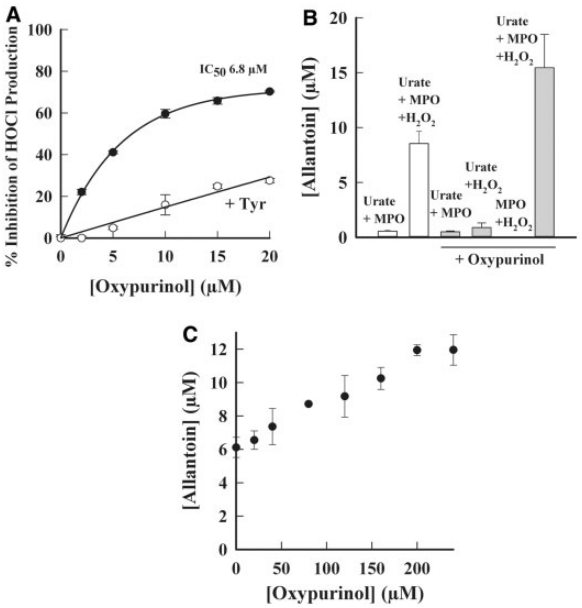
Allopurinol and oxypurinol are analogues of hypoxanthine and xanthine and are potential targets for oxidation by reactive oxygen species and peroxidases. Therefore, to understand how oxypurinol might affect the oxidation of

Fig. 3 The effects of allopurinol and oxypurinol on plasma concentrations of urate and allantoin



Plasma samples were collected from 10 patients presenting with gout and again 4 weeks after treatment with allopurinol. (**A**) Urate and (**B**) allantoin were measured before and after treatment. Statistically significant differences ($P < 0.05$) were determined using the Wilcoxon signed rank test; closed circle indicates an outlier. (**C**) Plasma oxypurinol was measured in all patients receiving allopurinol and compared with the concentration of allantoin. The correlation was determined using Spearman's rank order correlation.

Fig. 4 Oxypurinol as an inhibitor and substrate of MPO



(**A**) The effect of oxypurinol on the production of hypochlorous acid (HOCl) by purified MPO. Reactions were started by adding 50 μM hydrogen peroxide (H_2O_2) to 20 nM MPO in 10 mM phosphate buffer (pH 7.4) containing 140 mM chloride and 5 mM taurine with (open circle) or without (closed circle) 10 μM tyrosine. Reactions were stopped after 5 min by adding 20 μg/ml of catalase and the accumulated taurine chloramine was measured. (**B**) Oxidation of urate to allantoin by MPO in the absence and presence of 200 μM oxypurinol and (**C**) with increasing concentrations of oxypurinol. Reactions were started by adding 50 μM H_2O_2 to 50 mM phosphate buffer (pH 7.4) containing 25 nM MPO, 300 μM urate and varying concentrations of oxypurinol. After 30 min of production, allantoin was measured using mass spectrometry. Data are means and ranges of duplicates and are representative of at least three separate experiments. IC_{50}: half maximal inhibitory concentration.

urate, we determined the effects of these purines on the *in vitro* activity of purified MPO by assessing whether they interfere with its production of HOCl (Fig. 4A). Oxypurinol inhibited MPO by 50% (IC_{50}) at 6.8 μM (Fig. 4A), while xanthine had an IC_{50} of 2.8 μM (not shown). Allopurinol and hypoxanthine had no effect (data not shown). Inhibition by oxypurinol reached a maximum of only 75% and was less effective in the presence of tyrosine (Fig. 4A), which prevents accumulation of an inactive form of MPO [8]. These results suggest that oxypurinol is a substrate for the redox intermediates of MPO. To test this possibility we determined how oxypurinol affected the oxidation of urate by MPO. Adding oxypurinol to a system containing MPO, urate and H_2O_2 enhanced the production of allantoin (Fig. 4B). Importantly, allantoin was not formed in the absence of urate. Oxypurinol also enhanced the conversion of urate to allantoin by MPO in a dose-dependent manner (Fig. 4C). These results indicate that oxypurinol is a substrate for MPO that can promote the oxidation of urate.

Discussion

Neutrophils swarm into the joints of patients with gout to phagocytose MSU crystals [2] and in the process they generate numerous cytokines and release enzymes into the extracellular milieu [26]. MPO is the most abundant enzyme discharged by neutrophils [8]. Therefore it is surprising that until now there have been no reports on how MPO contributes to the pathology of gout. Our identification of elevated MPO in the circulation of patients with acute gout and its association with increased oxidation of urate strongly suggest that it will participate in the sequelae of gout, including the predisposition to CVD.

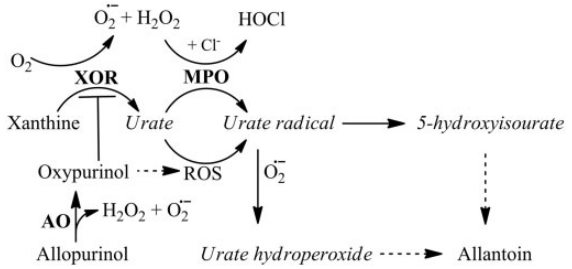
Our results indicate that during an attack of gout neutrophils are stimulated and release MPO. The significant correlation between the concentrations of urate and MPO in plasma is in accord with the established findings that MSU crystals activate the NACHT, LRR and PYD domains-containing protein 3 (NALP3) inflammasome, which promotes an influx of neutrophils to the site of

crystal deposition [27]. These crystals also stimulate neutrophils to produce superoxide and release proteins from their granules, including MPO [26, 28]. The higher specific activity of MPO in patients with acute gout suggests that neutrophils release fully active MPO during an attack of gout but over time it becomes inactivated either by its substrate H_2O_2 [29] or its product HOCl [30].

We found that allantoin, the oxidative metabolite of urate, is elevated in patients with gout not receiving allopurinol. This result is important because it demonstrates that urate is oxidized during episodes of gout so that oxidative stress is a feature of this inflammatory pathology. The strong association of allantoin with urate suggests that the inflammation induced by urate promotes oxidative stress. The correlation between allantoin and the activity of MPO also suggests that MPO actively produces oxidants during episodes of gout. Although significant, this correlation was not strong, which suggests that other oxidants also contribute to the oxidation of urate, and it may also reflect the fact that MPO sticks avidly to the endothelium so that only a minor fraction is detected in plasma [31]. During inflammation in gout, MPO would be expected to produce mainly HOCl, as occurs in numerous other inflammatory diseases [19, 32–34]. As illustrated in Fig. 5, our data suggest that MPO will also produce urate radicals during inflammation. Some of these radicals will break down to allantoin, which should be considered a stable biomarker of urate oxidation. However, urate radicals can also scavenge nitric oxide, combine with superoxide to form a reactive hydroperoxide or give rise to 5-hydroxyisourate, which is known to be cytotoxic [35]. Most animals use urate oxidase coupled with 5-hydroxyisourate hydrolase [36, 37] to degrade urate to allantoin. Urate oxidase produces 5-hydroxyisourate without releasing reactive intermediates while 5-hydroxyisourate hydrolase prevents accumulation of this toxic metabolite [35]. Both these enzymes are absent in humans. Thus oxidation of urate in humans is potentially dangerous because no enzymes are present to negate the harmful effects of its oxidation products. Consequently MPO-dependent oxidation of urate may contribute to the sequelae of inflammatory diseases, particularly gout.

From our results it is apparent that allopurinol has contrasting effects on oxidative stress depending on the oxypurinol concentration. Low concentrations of oxypurinol lowered allantoin. This effect was lost with increasing plasma concentrations of oxypurinol, to the point where it increased formation of allantoin. At low concentrations, oxypurinol should act by decreasing inflammation through its urate-lowering action. However, high concentrations of oxypurinol could promote oxidation of urate via two pathways (see Fig. 5). First, allopurinol is chiefly metabolized to oxypurinol by aldehyde oxidase and in the process produces superoxide and H_2O_2 [17, 38, 39]. These oxidants may evade endogenous scavengers and contribute to oxidation of urate by spawning several reactive oxidants. Secondly, our data show that oxypurinol is a good substrate for the redox intermediates of MPO and gives rise to a radical that is capable of oxidizing urate. Consequently,

Fig. 5 Oxidative metabolism of urate



Urate is produced by xanthine oxidoreductase (XOR) from xanthine and in the presence of oxygen can form hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) [16]. Myeloperoxidase (MPO) uses H_2O_2 to oxidize urate to the urate radical and to produce hypochlorous acid (HOCl) from chloride [7, 8]. Urate is also susceptible to one-electron oxidation by reactive oxygen species (ROS). The urate radical reacts rapidly with superoxide to form urate hydroperoxide or is converted to 5-hydroxyisourate. These electrophilic products can either react or decay to allantoin. Aldehyde oxidase (AO) oxidizes allopurinol to oxypurinol, which inactivates xanthine oxidoreductase. Oxypurinol is oxidized to a radical that promotes the oxidation of urate. Arrows with dashed lines indicate pathways with multiple stages between reactants and products. Urate is toxic because it can crystalize and activate the NACHT, LRR and PYD domains-containing protein 3 (NALP3) inflammasome or is oxidized to the reactive species shown in italics. Allantoin is a stable biomarker of oxidative stress.

when oxypurinol is metabolized by MPO, related peroxidases or one-electron oxidants, urate will be oxidized and there will be an increase in the production of allantoin.

Increased MPO and allantoin in patients with acute gout point to a potential mechanism for the increase in CVD observed in patients with gout and asymptomatic hyperuricaemia [40]. MPO is an emerging player in the promotion and progression of CVD [41, 42]. Once released from neutrophils, it sticks to the vascular endothelium, where it generates HOCl that damages endothelial cells. It also oxidizes low-density lipoprotein (LDL) to a form readily taken up by macrophages, as well as impairing the reverse cholesterol transport function of high-density lipoprotein (HDL) by selectively nitrating and chlorinating tyrosine residues [43]. In addition to these oxidative reactions, MPO also scavenges nitric oxide by producing free radicals, including the urate radical [7, 44]. Depleting nitric oxide, a potent vasodilator, may lead to hypertension and endothelial cell dysfunction [44]. Enhanced oxidation of urate in patients with gout will also result in formation of cytotoxic urate hydroperoxide and 5-hydroxyisourate [7]. These reactions of MPO-derived oxidants and metabolites of urate link MPO and urate to CVD. In support of this proposal, MPO is also elevated in RA [45] and promotes increased oxidation of HDL, especially in those patients

with CVD [45]. Further long-term clinical studies are required to determine the association between MPO and CVD in patients with gout.

Successful long-term management of gout requires a sustained reduction of plasma urate to $<360\,\mu M$ and potentially $<300\,\mu M$ if tophi are present. Achieving this target is associated with improved clinical outcomes [46–48]. It is plausible that achieving the target will also have a beneficial effect on cardiovascular outcomes in patients with gout by lowering inflammation and associated oxidative stress. However, the target concentration of urate is more likely to be obtained with a plasma oxypurinol concentration between 100 and $150\,\mu M$ [21]. These concentrations of oxypurinol tilt the antioxidant/oxidant balance to a pro-oxidant position by increasing the production of reactive oxygen species, as observed by the elevation in plasma allantoin. Whether this effect is clinically relevant will require long-term clinical trials to ascertain, but it raises the issue of how long patients need to maintain the target plasma urate concentration once attacks of gout and tophi have abated. A two-phase approach to treatment has been suggested whereby the lower urate target must be achieved in the first phase but once attacks of gout and tophi have resolved a higher target may be acceptable [49]. This may have benefits if lower doses of allopurinol, and hence lower plasma oxypurinol concentrations, could be used in the second phase to minimize oxidative stress.

In conclusion, we have found that elevated MPO and urate oxidation are associated with gout. In future work valuable mechanistic insights into the link between hyperuricaemia and inflammatory diseases may become apparent when probing the involvement of MPO-dependent oxidative stress, especially that related to oxidation products of urate.

Rheumatology key messages

- Circulating MPO is elevated during acute gout.
- High urate in gout is associated with increased oxidative stress.

Acknowledgements

We thank Professor Vicky Cameron for supplying plasma samples from healthy controls. All authors were involved in drafting the article or revising it critically for important intellectual content and all authors approved the final version to be published. L.K.S. and A.J.K. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Author contributions were as follows: L.K.S. and A.J.K., study conception and design; L.K.S., R.T., I.S.K., M.Z., J.D., L.V.F., acquisition of data; L.K.S. and A.J.K., analysis and interpretation of data.

Funding: This study was funded by the Health Research Council of New Zealand.

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med 2008;359:1811–21.

2 McQueen FM, Chhana A, Dalbeth N. Mechanisms of joint damage in gout: evidence from cellular and imaging studies. Nat Rev Rheumatol 2012;8:173–81.

3 Krishnan E. Inflammation, oxidative stress and lipids: the risk triad for atherosclerosis in gout. Rheumatology 2010; 49:1229–38.

4 Rosendorff C, Jogendra MR. Uric acid: where are we? J Clin Hypertens 2013;15:5–6.

5 Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006;440:237–41.

6 Bagnati M, Perugini C, Cau C *et al*. When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid. Biochem J 1999;340:143–52.

7 Meotti FC, Jameson GN, Turner R *et al*. Urate as a physiological substrate for myeloperoxidase: implications for hyperuricemia and inflammation. J Biol Chem 2011; 286:12901–11.

8 Kettle AJ, Winterbourn CC. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Rep 1997;3:3–15.

9 Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci USA 1988;85:9748–52.

10 Nicholls SJ, Hazen SL. Myeloperoxidase and cardiovascular disease. Arterioscler Thromb Vasc Biol 2005;25: 1102–11.

11 Krishnan E, Baker JF, Furst DE, Schumacher HR. Gout and the risk of acute myocardial infarction. Arthritis Rheum 2006;54:2688–96.

12 Krishnan E, Svendsen K, Neaton JD, Grandits G, Kuller LH. Long-term cardiovascular mortality among middle-aged men with gout. Arch Intern Med 2008;168: 1104–10.

13 Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. Circulation 2007; 116:894–900.

14 Edwards NL. The role of hyperuricemia in vascular disorders. Curr Opin Rheumatol 2009;2:132–7.

15 Zhang W, Doherty M, Bardin T *et al*. EULAR evidence based recommendations for gout. Part II: management. Report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 2006;65:1312–24.

16 Cantu-Medellin N, Kelley EE. Xanthine oxidoreductase-catalyzed reactive species generation: a process in critical need of reevaluation. Redox Biol 2013;1:353–8.

17 Day RO, Graham GG, Hicks M *et al*. Clinical pharmacokinetics and pharmacodynamics of allopurinol and oxypurinol. Clin Pharmacokinet 2007;46:623–44.

18 Wallace SL, Robinson H, Masi AT *et al*. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977;20:895–900.

19 Stamp LK, Khalilova I, Tarr JM *et al*. Myeloperoxidase and oxidative stress in rheumatoid arthritis. Rheumatology 2012;51:1796–803.

20 Jen JF, Hsiao SL, Liu KH. Simultaneous determination of uric acid and creatinine in urine by an eco-friendly solvent-free high performance liquid chromatographic method. Talanta 2002;58:711–7.

21 Stamp LK, Barclay ML, O'Donnell JL *et al*. Relationship between serum urate and plasma oxypurinol in the management of gout: determination of minimum plasma oxypurinol concentration to achieve a target serum urate level. Clin Pharmacol Ther 2011;90:392–8.

22 Graham S, Day RO, Wong H *et al*. Pharmacodynamics of oxypurinol after administration of allopurinol to healthy subjects. Br J Clin Pharmacol 1996;41:299–304.

23 Chapman AL, Mocatta TJ, Shiva S *et al*. Ceruloplasmin is an endogenous inhibitor of myeloperoxidase. J Biol Chem 2013;288:6465–77.

24 Turner R, Stamp LK, Kettle AJ. Detection of allantoin in clinical samples using hydrophilic liquid chromatography with stable isotope dilution negative ion tandem mass spectrometry. J Chromatogr B 2012;891–892:85–9.

25 Dypbukt JM, Bishop C, Brooks WM *et al*. A sensitive and selective assay for chloramine production by myeloperoxidase. Free Radic Biol Med 2005;39:1468–77.

26 Burt HM, Jackson JK. The priming action of tumour necrosis factor-alpha (TNF-alpha) and granulocyte-macrophage colony-stimulating factor (GM-CSF) on neutrophils activated by inflammatory microcrystals. Clin Exp Immunol 1997;108:432–7.

27 Martinon F. Mechanisms of uric acid crystal-mediated autoinflammation. Immunol Rev 2010;233:218–32.

28 Thomas MJ. Urate causes the human polymorphonuclear leukocyte to secrete superoxide. Free Radic Biol Med 1992;12:89–91.

29 Paumann-Page M, Furtmuller PG, Hofbauer S *et al*. Inactivation of human myeloperoxidase by hydrogen peroxide. Arch Biochem Biophys 2013;539:51–62.

30 Maitra D, Shaeib F, Abdulhamid I *et al*. Myeloperoxidase acts as a source of free iron during steady-state catalysis by a feedback inhibitory pathway. Free Radic Biol Med 2013;63:90–8.

31 Marshall CJ, Nallaratnam M, Mocatta T *et al*. Factors influencing local and systemic levels of plasma myeloperoxidase in ST segment elevation acute myocardial infarction. Am J Cardiol 2010;106:316–22.

32 Kettle AJ, Chan T, Osberg I *et al*. Myeloperoxidase and protein oxidation in the airways of young children with cystic fibrosis. Am J Respir Crit Care Med 2004;170:1317–23.

33 Hazell LJ, Arnold L, Flowers D *et al*. Presence of hypochlorite-modified proteins in human atherosclerotic lesions. J Clin Invest 1996;97:1535–44.

34 Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. J Clin Invest 1997;99:2075–81.

35 Stevenson WS, Hyland CD, Zhang JG *et al*. Deficiency of 5-hydroxyisourate hydrolase causes hepatomegaly and hepatocellular carcinoma in mice. Proc Natl Acad Sci USA 2010;107:16625–30.

36 Alvarez-Lario B, Macarron-Vicente J. Uric acid and evolution. Rheumatology 2010;49:2010–5.

37 Tipton PA. Urate to allantoin, specifically (S)-allantoin. Nat Chem Biol 2006;2:124–5.

38 Kundu TK, Hille R, Velayutham M, Zweier JL. Characterization of superoxide production from aldehyde oxidase: an important source of oxidants in biological tissues. Arch Biochem Biophys 2007;460:113–21.

39 Kundu TK, Velayutham M, Zweier JL. Aldehyde oxidase functions as a superoxide generating NADH oxidase: an important redox regulated pathway of cellular oxygen radical formation. Biochemistry 2012;51:2930–9.

40 Kuo CF, See LC, Luo SF *et al*. Gout: an independent risk factor for all-cause and cardiovascular mortality. Rheumatology 2010;49:141–6.

41 Nicholls SJ, Hazen SL. Myeloperoxidase, modified lipoproteins, and atherogenesis. J Lipid Res 2009;50(Suppl):S346–51.

42 Nussbaum C, Klinke A, Adam M, Baldus S, Sperandio M. Myeloperoxidase: a leukocyte-derived protagonist of inflammation and cardiovascular disease. Antioxid Redox Signal 2013;18:692–713.

43 Zheng L, Nukuna B, Brennan ML *et al*. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. J Clin Invest 2004;114:529–41.

44 Eiserich JP, Baldus S, Brennan ML *et al*. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. Science 2002;296:2391–4.

45 Vivekanandan-Giri A, Slocum JL, Byun J *et al*. High density lipoprotein is targeted for oxidation by myeloperoxidase in rheumatoid arthritis. Ann Rheum Dis 2013;72:1725–31.

46 Stamp LK, Zhu X, Dalbeth N *et al*. Serum urate as a soluble biomarker in chronic gout-evidence that serum urate fulfills the OMERACT validation criteria for soluble biomarkers. Semin Arthritis Rheum 2011;40:483–500.

47 Becker MA, Schumacher HR Jr, Wortmann RL *et al*. Febuxostat compared with allopurinol in patients with hyperuricemia and gout. N Engl J Med 2005;353:2450–61.

48 Becker MA, Schumacher HR, MacDonald PA, Lloyd E, Lademacher C. Clinical efficacy and safety of successful longterm urate lowering with febuxostat or allopurinol in subjects with gout. J Rheumatol 2009;36:1273–82.

49 Perez-Ruiz F, Herrero-Beites AM, Carmona L. A two-stage approach to the treatment of hyperuricemia in gout: the ''dirty dish'' hypothesis. Arthritis Rheum 2011;63:4002–6.