Nitrosation of Uric Acid by Peroxynitrite

FORMATION OF A VASOACTIVE NITRIC OXIDE DONOR*

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Peroxynitrite (ONOO\(^{-}\)), formed by the reaction between nitric oxide (NO) and superoxide, has been implicated in the etiologies of numerous disease processes. Low molecular weight antioxidants, including uric acid, may minimize ONOO\(^{-}\)-mediated damage to tissues. The tissue-sparing effects of uric acid are typically attributed to oxidant scavenging; however, little attention has been paid to the biology of the reaction products. In this study, a previously unidentified uric acid derivative was detected in ONOO\(^{-}\)-treated human plasma. The product of the uric acid/ONOO\(^{-}\) reaction resulted in endothelium-independent vasorelaxation of rat thoracic aorta, with an EC\(_{50}\) value in the range of 0.03–0.3 \(\mu\)M. Oxyhemoglobin, a NO scavenger, completely attenuated detectable NO release and vascular relaxation. Uric acid plus decomposed ONOO\(^{-}\) neither released NO nor altered vascular reactivity. Electrochemical quantification of NO confirmed that the uric acid/ONOO\(^{-}\) reaction resulted in spontaneous (thiol-independent) and protracted (\(t_{\text{frac}} \sim 125 \text{ min}\)) release of NO. Mass spectroscopic analysis indicated that the product was a nitrated uric acid derivative. The uric acid nitration/nitrosation product may play a pivotal role in human pathophysiology by releasing NO, which could decrease vascular tone, increase tissue blood flow, and thereby constitute a role for uric acid not previously described.

Reactive oxygen and nitrogen species are continuously generated in vivo and play an integral role in numerous physiologic and pathologic processes (1–3). To minimize the consequences of oxidant damage to biologic molecules, human plasma is endowed with an integrated antioxidant system of enzymatic and expendable soluble antioxidants. Uric acid is reportedly one of these physiologically important plasma antioxidants (4). Reactive species can oxidize uric acid to relatively stable products that can serve as quantitative estimates of oxidant stress in vivo (5). Uric acid is of special relevance because of its role as the terminal oxidation product of purine metabolism due to the evolutionary loss of urate oxidase, which catalyzes uric acid to allantoin (6). Consequently, human plasma contains uric acid at concentrations approaching 500 \(\mu\)M (7). Endogenous antioxidants constitute the first line of defense against oxidant-induced tissue injury. However, in a variety of pathologic conditions, antioxidant defenses may become overwhelmed, thus allowing reactive oxygen and nitrogen species to react with target molecules and to impair essential biochemical processes (8–11).

When produced in proximity, superoxide (O\(_{2}\)\(^{\cdot}\)) and nitric oxide (NO) can react at an almost diffusion-limited rate (6.7 \(\times\) 10\(^{9}\) \(\text{M}^{-1} \text{s}^{-1}\)) to produce peroxynitrite (ONOO\(^{-}\)) (12). Recent evidence suggests that under certain conditions, nitric-oxide synthase can simultaneously generate both O\(_{2}\)\(^{\cdot}\) and NO (13). Peroxynitrite is an oxidizing and nitrating agent that reacts with a variety of biomolecules, including lipids, proteins, carbohydrates, and deoxyribonucleic acid (14–18). Potential pathophysiologic effects of ONOO\(^{-}\) include its action as a bactericidal agent (19), inactivation of mitochondrial manganese-superoxide dismutase (20) and glutamine synthetase (21), alteration of the lipid aggregatory properties of surfactant protein A (22, 23), inactivation of sodium transport (24), inactivation of \(\alpha_{1}\)-antiproteinase (17), and modification of tyrosine phosphorylation (25, 26). Plasma antioxidants also serve as significant biologic targets that can be decreased by ONOO\(^{-}\), leading to oxidative damage to tissues and compromised function (11, 14, 18, 27, 28).

Nitration and nitrosation reactions are increasingly recognized as important mediators of damage in biologic systems, although the precise mechanism of interaction of the reactive nitrogen species with endogenous biomolecules remains unclear. Peroxynitrite, and possibly other reactive nitrogen species, can react readily with phenolic compounds, such as tyrosine, to form nitrated (3-nitrotyrosine) and dimerized (dityrosine) products (29, 30). The stable 3-nitrotyrosine product has been detected in human atherosclerotic lesions (31), and tissues following acute injury (32, 33), inflammatory disorders (34), and liver ischemia/reperfusion and preservation/transplantation (35). It has also been reported that the reaction of ONOO\(^{-}\) with proteins, carbohydrates, and thiols can result in the formation of products, which can act as NO donors (36–38). Therefore, it was the purpose of this study to determine if the reaction between uric acid and ONOO\(^{-}\) could result in the formation of recognized oxidation products as well as form novel uric acid nitration/nitrosation derivatives that could release NO and induce vasorelaxation.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals and reagents, unless otherwise specified, were obtained from Sigma. Cyanuric acid, oxonic acid, uracil, urazole, and alloxan monohydrate were purchased from Aldrich. Methanol (Op-
tima grade) was ordered from Fisher. Water used in solution preparation was purified by passage through a Milli-Q water purification system (Millipore Corp., Milford, MA). Prior to use, the purified water was polished using a Sep-Pak C<sub>18</sub> column (Waters) and then filtered with a 0.22-μm membrane filter.

**Preparation of Peroxynitrite—ONOO<sup>-</sup>** was prepared by mixing NaNO<sub>2</sub>, NaOH, and acidified H<sub>2</sub>O<sub>2</sub>, as described by Beckman et al. (40). Any excess H<sub>2</sub>O<sub>2</sub> was removed by passage over a MnO<sub>2</sub> column. Typical ONOO<sup>-</sup> yields were 180–200 mM. Peroxynitrite was quantified spectrophotometrically (ε<sub>250</sub> = 1670 M<sup>−1</sup> cm<sup>−1</sup>) and stored in 1.2 N NaOH.

**Incubation of Purines and Purine Oxidation Products with ONOO<sup>-</sup>**—Hypoxanthine, xanthine, uric acid, allantoin, and parabanic acid (0.1–0.3 mM) were prepared in 100 mM potassium phosphate buffer containing 0.1 mM DTPA (pH 7.4) and 0.3 mM potassium phosphate (pH 3.0) containing 1.56 mM DTPA (pH 7.4). The mixture was shielded and then incubated at room temperature. Samples were then centrifuged (15 min, 15,000 g) and the supernatant was collected and stored in 1.2 N NaOH served as the vehicle control. Peroxynitrite reactivity is pH-dependent; therefore, the pH of all samples was determined and maintained at 7±0.8 under our experimental conditions.

For peroxynitrite experiments, ONOO<sup>-</sup> (10 mM) or decomposed ONOO<sup>-</sup> (0.25–0.1 mM) was added to the reaction chamber. The thiol and metal were added to the reaction chamber either prior to or after the addition of the purine/ONOO<sup>-</sup> mixture. As an additional control, in a subset of experiments, ONOO<sup>-</sup> was added to the reaction chamber either prior to or after following the addition of sample to the chamber. The mean half-life for each condition was calculated by fitting the data to a single exponential decay model (half-life = t<sub>1/2</sub> = A<sub>0</sub> e<sup>−t/τ</sup>, where τ = asymptotic value of the y variable, t<sub>0</sub> = initial time, t = time of decay, A<sub>0</sub> = y value when t<sub>0</sub> = 0, and τ = decay constant).

**Vessel Reactivity Studies—**Isometric tension was measured in isolated rat aortic ring segments. Animals were euthanized by an anesthetic overdose. The aorta was excised, cleansed of fat and adherent tissue, cut into ring segments (2–3 mm), and suspended from a force-displacement transducer in a tissue bath. Segments were bathed in a Krebs-Henseleit solution. A passive load of 2 g was applied and maintained throughout the experiment. Indomethacin (0.05 mM)-treated ring segments with KCl (70 mM) maximally maximized vessel contractile capacity. In subsequent experiments, phenylephrine (3×10<sup>−8</sup> M) was added to the indomethacin-treated ring segments to obtain a contraction equivalent to ~40% of the KCl response. When tension development reached a plateau, uric acid or xanthine reaction mixtures (obtained by treatment of purine with ONOO<sup>-</sup> or decomposed ONOO<sup>-</sup>) were added to achieve a cumulative dose-response curve. Vessels were then exposed to the nominal concentration of purine to which the tissue was exposed.

In some experiments, endothelium was removed by rubbing the luminal surface of the vessel with a serrated forceps. Endothelial denudation was confirmed by the absence of vessel relaxation in response to acetylcholine (0.001 mM), an endothelium-dependent vasodilator. To determine whether vessel relaxation was due to the release of NO from the purine products of ONOO<sup>-</sup> and peroxynitrite, oxyhemoglobin (0.001 mM) was added to the tissue bath in a subset of experiments. Data are reported as weighted means representing two or three observations per animal (n = eight animals). Real time data were acquired for all experiments using a computerized data acquisition system.

**RESULTS**

Both uric acid and xanthine were significantly (p < 0.001) oxidized by 0.25, 0.5, 0.75, and 1.0 mM ONOO<sup>-</sup> (Fig. 1). The extent of oxidation of uric acid was markedly greater than that of xanthine at all concentrations of peroxynitrite and ONOO<sup>-</sup> studied. Hypoxanthine was significantly (p < 0.05) oxidized only with 1.0 mM ONOO<sup>-</sup>. Parabanic acid and allantoin were not oxidized by any concentration of ONOO<sup>-</sup>.
significantly oxidized at any concentration of ONOO\(^-\). Concomitant with uric acid oxidation was the formation of allantoin, parabanic acid, urazole, oxonic acid, and a novel peak not observed following addition of decomposed ONOO\(^-\). Spectral characteristics and retention times of authentic oxaluric acid, cyanuric acid, alloxan monohydrate, and uracil excluded these purine oxidation products as this novel peak. Xanthine oxidation also produced allantoin, parabanic acid, and a novel product, but this product exhibited a different retention time and different spectra than the ONOO\(^-\)-treated nitrated uric acid product. The novel peak was not detected when xanthine was treated with decomposed ONOO\(^-\).

The hypochromic shift of the uric acid/ONOO\(^-\) product (Fig. 2) is consistent with the pH-dependent absorbance changes of nitrated aromatic compounds (42) and suggests that uric acid may be nitrated (or nitrosated) upon reaction with ONOO\(^-\). Addition of decomposed ONOO\(^-\) to uric acid resulted in an absorbance spectrum indistinguishable from uric acid alone and was pH-insensitive (data not shown).

In human plasma samples, the endogenous uric acid concentration was 0.20 \pm 0.01 mM (mean \pm S.E.) in pool donor plasma. Upon addition of ONOO\(^-\), plasma uric acid decreased concurrently with the appearance of oxidation products (allantoin and parabanic acid) and the nitrated uric acid product, similar to the effect observed in buffer. 9 \pm 2\% of the plasma uric acid was converted to the nitrated peak, whereas 13 \pm 4\% was converted following exposure to 1.0 mM ONOO\(^-\), again similar to the results with uric acid in buffer (Fig. 3). Treatment with neither decomposed ONOO\(^-\) nor vehicle resulted in significant oxidation of uric acid or formation of the nitrosation product.

Mass spectrophotometric analysis of the ONOO\(^-\)-treated uric acid revealed that the uric acid concentration \((m/z = 169, (uric acid + H)^+\), retention time = 5.4 min) was reduced 87\% by addition of 1.0 mM ONOO\(^-\) (Fig. 4A) compared with addition of an equivalent amount of decomposed ONOO\(^-\) (Fig. 4B). Concomitant with the reduction in uric acid was the formation of a novel peak at 6.5 min. This product was predicted to have a \(M_e\) of 146 based upon the mass-to-charge ratio \((m/z)\) of 147 (nitrated product + H\(^+\) ion) (Fig. 4C). Addition of decomposed ONOO\(^-\) (equivalent to 1.0 mM active ONOO\(^-\)) did not result in a significant reduction in the uric acid concentration (Fig. 4B) or formation of the novel nitrated product at the retention time of 6.5 min or molecular ion at \(m/z\) 147 (Fig. 4D).

The ONOO\(^-\)-treated uric acid product was scanned by electrospray ionization mass spectrum analysis. Scans acquired at 6.5 min (retention time of nitrated product) demonstrated that fragmentation of the nitrated product contained ions at 130, 104, 87, and 61 (Fig. 5A). The resulting spectrophotometry also
H) ity to directly quantify the nitrated product. The actual EC50 is decrease the uric acid concentration (m/z m/z in the range of 0.03–0.3 zture spontaneously liberated 2 Treatment of xanthine with ONOO 

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\text{FIG. 4. Chromatography of ONOO}^\text{-treated uric acid. The nitrated/nitrosated compound produced upon addition of ONOO}^\text{- to uric acid was separated by HPLC prior to passage through an electrospray ionization interface and injection into a triple quadrupole mass spectrometer. Addition of 0.1 mM ONOO}^\text{- reduced the uric acid concentration (m/z 169 (uric acid + H)\text{)), retention time = 5.4 min by 87\% (A). Concomitant with the reduction in uric acid was the formation of a novel peak at 6.5 min. This product was predicted to have a M_r of 146 based on the mass-to-charge ratio (m/z) of 147 (nitrated product + H)\text{+ ion)} (B). Addition of decomposed ONOO}^\text{- (equivalent to 1.0 mM active ONOO}^\text{-) did not significantly decrease the uric acid concentration (B) or result in the formation of the novel nitrated product at the retention time of 6.5 min or in the formation of the molecular ion at m/z 147 (D). Rel. Int., relative intensity.}
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showed molecular ions at m/z 164 ((uric acid + NH_4)\text{+}) and 169 ((uric acid + Na\text{+})). The data presented in Fig. 5B represent scans acquired at 5.41–5.43 min (retention time of uric acid). Liquid chromatography-mass spectrophotometric analysis confirmed the molecular weight of uric acid at 168 (uric acid + H\text{+) ion, m/z 169) as well as the anticipated associated molecular ions in the acetate mobile phase at 186 (uric acid + NH_4\text{+)\text{,} 191 (uric acid + Na})\text{+}), 337 (2(uric acid) + H\text{+}), and 505 (3(uric acid) + H\text{+}). Using mass spectrophotometric bombardment fragments as a guide, the nitrated uric acid product (M_r 146) has been identified as 2-nitro-4-amino-5-hydroxyimidazoline (Fig. 6). Cumulative administration of the uric acid/ONOO^-mixture induced a dose-dependent relaxation of rat aortic ring segments with EC_{50} = 0.27 ± 0.19 \text{\mu M (Fig. 7A). The calculated EC}_{50} represents an underestimate due to the 10-fold dilution of the uric acid/ONOO^-mixture in the tissue bath and the inability to directly quantify the nitrated product. The actual EC_{50} is in the range of 0.03–0.3 \text{\mu M. The relaxation response was completely reversed by treatment with oxyhemoglobin. In contrast, uric acid alone or uric acid exposed to decomposed ONOO^- had little effect on vessel tone, although a modest decrease in tension was observed at the highest cumulative concentration of uric acid treated with decomposed ONOO^-}. Treatment of xanthine with ONOO^- or decomposed ONOO^- did not yield reaction products with vasodilatory activity. Ring segments denuded of endothelium displayed similar dose-response characteristics for ONOO^-treated uric acid, as did intact vessels (Fig. 7B).

Direct electrochemical detection of NO, utilizing a selective microelectrode, demonstrated that the uric acid/ONOO^-mixture spontaneously liberated NO (Fig. 8A). A similar extent of NO release was seen when either 0.3 or 3.0 mM uric acid was treated with 1.0 mM ONOO^- . Therefore, in subsequent studies, 3.0 mM was used to ensure that excess substrate was present. Addition of oxyhemoglobin to the reaction chamber consumed the NO present and completely attenuated any further release of NO by subsequent additions of the uric acid/ONOO^-mixture.

The reaction of 3.0 mM uric acid with 1.0 mM ONOO^- resulted in the production of 13.7 ± 1.1 \text{\mu M NO. There was no detectable NO produced from the reaction of ONOO^- alone, uric acid alone, uric acid treated with decomposed ONOO^- , or xanthine treated with ONOO^- . The release of NO from the uric acid/ONOO^-mixture was spontaneous and was not enhanced by the addition of thiols (cysteine or glutathione) or metal (Cu\text{2+}). Addition of DTPA (0.1 mM), a metal chelator, did not alter NO release.

Finally, it was demonstrated that both temperature and light significantly (p < 0.01) alter the half-life of this NO-donating product. The half-life of NO release in the dark at 4 °C was 123 ± 20 min (Fig. 8B) and was significantly decreased at room temperature (20 ± 3 min) and further decreased in the presence of light (13 ± 1 min).

DISCUSSION

Peroxynitrite is both a nitrating and oxidizing agent that can compromise antioxidant defenses and that simultaneously results in oxidative damage to tissues (11). In this study, we demonstrated that uric acid is considerably more susceptible to oxidation by ONOO^- than other purines (hypoxanthine and xanthine) or purine oxidation products (allantoin and parabanic acid). The interaction of ONOO^- with both uric acid and xanthine resulted in the formation of putatively nitrated purine products. However, unlike the nitrated uric acid product, the xanthine product was not formed in our plasma experiments, nor did the reaction of xanthine with ONOO^- result in release of NO or vascular relaxation. Formation of the putatively nitrated uric acid product was dependent upon the concentration of ONOO^- in both plasma and buffer systems. Over 50% of the endogenous uric acid was oxidized in human plasma, which was remarkable in view of the multitude of other potential target molecules present that could react with ONOO^- . The pH-dependent hypochromic
absorbance shift of the uric acid product was suggestive of nitration, although a nitrosation product could not be dismissed solely on these data. A pathway for formation of the fragmentation products was developed from the liquid chromatography-mass spectrophotometric data. Electrospray ionization mass spectrometry indicated that uric acid ($M_r$ 168) was most likely oxidized to a product similar in structure to para-benzoic acid, a known uric acid oxidation product. This oxidation product could be subsequently nitrated or nitrosated to form a product(s) with a $M_r$ of 146. The structure of a nitrosated product would be consistent with a potential role as a $\cdot$NO donor, which was supported by ex vivo vessel relaxation data in this study. The $\cdot$NO donor produced by the reaction of uric acid with ONOO$^-$ resulted in a dose-dependent relaxation of rat aortic ring segments that was completely reversed by addition of oxyhemoglobin, a $\cdot$NO scavenger. Aortic ring segments denuded of endothelium displayed similar dose-response characteristics as intact vessels, indicating that the $\cdot$NO donating properties of the uric acid product were not mediated by the stimulation of endothelium-dependent $\cdot$NO production. The release of $\cdot$NO from the nitrated uric acid product was confirmed electrochemically and was not thiol-dependent, in marked contrast to organic nitrites that release $\cdot$NO (44). The treatment of uric acid with decomposed ONOO$^-$ did not result in production of a $\cdot$NO-donating metabolite. Based on these cumulative lines of evidence, we propose that the uric acid product is 2-nitrito-4-amino-5-hydroxyimidazoline (Fig. 6). Detection of the nitrosated uric acid product (in plasma) may also be a good index of $\cdot$NO-derived oxidant production and provide insight into the relative roles of reactive oxygen and nitrogen species in the etiology of oxidant-induced tissue injury.

Release of $\cdot$NO from the nitrosated uric acid derivative following insults such as ischemia/reperfusion could minimize tissue injury and thereby constitute a previously uncharacterized role for uric acid in physiology. Although $\cdot$NO-releasing drugs have been used clinically for >100 years in the treatment of cardiovascular dysfunction, the full potential for $\cdot$NO therapy is only now being recognized. Recently, it was demonstrated that $\cdot$NO could decrease lung injury following intestinal ischemia and that $\cdot$NO donors can prevent hydrogen peroxide-me-
dependent relaxation of rat aortic ring segments with an EC50 in the range of 0.03–0.3 μM (A). Addition of oxyhemoglobin (10 μM), a scavenger of ·NO, completely ablated this response. Uric acid exposed to decomposed ONOO• (○) had little effect on vessel tone, although a slight decrease in tension was observed at the highest cumulative concentrations. Incubation with uric acid (●), xanthine treated with ONOO• (△), or xanthine treated with decomposed ONOO• (△) did not exhibit significant vasodilatory activity. Ring segments denuded of endothelium (○) displayed similar dose-response characteristics for ONOO•-treated uric acid, as did intact vessels (●). *, p < 0.05 versus inactive control.

Vasodilator actions of organic nitrates and nitrates in vivo may be due to the formation of S-nitrosothiols. These stable sulphydryl-containing compounds liberate ·NO and induce relaxation via activation of vascular smooth muscle guanylate cyclase (50, 51). Conversion of numerous organic nitrates to their ·NO-donating forms has been attributed to the action of membrane-associated enzymes, whereas production of ·NO from organic nitrates appears to involve a distinct cytosolic enzyme (52, 53). The nominal potency of 2-nitroso-4-amino-5-hydroxyimidazoline as a ·NO donor (EC50 = 0.03–0.3 μM) is comparable to authentic ·NO (EC50 ~ 0.1 μM) and greater than ·NO donors used clinically, including glyceryl trinitrate (EC50 ~ 1.0 μM) and amyl nitrite (EC50 ~ 1.1 μM) (54, 55).

Nitric oxide is also released following the reaction of ONOO• with glucose, glycerol, and other biologic molecules that contain an alcohol functional group (37, 55) by a thiol- and Cu2+-dependent mechanism. This is a property shared with organic nitrates and nitrates (44). Although the precise mechanisms of ·NO release remain somewhat controversial, it has been suggested that an S-nitrosothiol intermediate is involved and that the decomposition of the nitrosothiol is accelerated by Cu2+ (56). In marked contrast, the release of ·NO from the nitrated uric acid product was 123 ± 20 min when fitted by a single-order exponential decay equation (solid line).

Fig. 7. Uric acid treated with ONOO• acts as a vasodilator. Administration of uric acid treated with ONOO• (●) induced a dose-dependent relaxation of rat aortic ring segments with an EC50 in the range of 0.03–0.3 μM (A). Addition of oxyhemoglobin (10 μM), a scavenger of ·NO, completely ablated this response. Uric acid exposed to decomposed ONOO• (○) had little effect on vessel tone, although a slight decrease in tension was observed at the highest cumulative concentrations. Incubation with uric acid (●), xanthine treated with ONOO• (△), or xanthine treated with decomposed ONOO• (△) did not exhibit significant vasodilatory activity. Ring segments denuded of endothelium (○) displayed similar dose-response characteristics for ONOO•-treated uric acid, as did intact vessels (●). *, p < 0.05 versus inactive control.

Fig. 8. Release of NO and stability of product formed by treatment of uric acid with ONOO•. Uric acid (UA; 3.0 mM) was reacted with ONOO• (1.0 mM). An aliquot of this reaction mixture was then added to a reaction chamber containing a NO microelectrode. It can be seen that with each repeated addition of the reaction mixture, ·NO was spontaneously released (A). Upon the addition of oxyhemoglobin to the reaction chamber, all of the ·NO was consumed, and further detection of ·NO by the reaction mixture was completely inhibited. Over a 5-h time period, aliquots of the uric acid (3.0 mM) and ONOO• (1.0 mM) mixture were added to the reaction chamber containing a NO microelectrode, and the ·NO released was detected. The reaction chamber was extensively rinsed with distilled water between the addition of aliquots of the uric acid/ONOO• mixture. In this manner, a curve was generated that depicts the stability of the nitrated uric acid product, as determined by the release of ·NO, with time (B). The reaction mixture was kept at 4 °C in the dark throughout each experiment. The half-life of the ·NO-donating uric acid product was 123 ± 20 min when fitted by a single-order exponential decay equation (solid line).
of p-hydroxymethyl fumaric acid (59), albumin (58), and tyrosine (58, 62). Uric acid effectively reduces ONOO−-induced nitration of albumin, whereas the addition of 0.2 mM uric acid decreases nitrotyrosine formation by ~40% (58). In this study, to ensure decomposition of ONOO− in the vessel relaxation studies, ONOO− was reacted with uric acid for 20 min in phosphate buffer (100 mM, pH 7.4) prior to addition to the vessel chamber containing Krebs-Henseleit buffer. The electrochemical NO detection experiments were also conducted in phosphate buffer at pH 7.4 prior to addition to the reaction vessel. It was unlikely that the adventitious carbonate associated with absorbed CO2 and contamination by the salts used in the buffer preparation was of sufficient concentration to catalyze nitration (59).

We have yet to demonstrate that this nitrated uric acid derivative is formed in vivo, and these studies are ongoing. If this derivative is formed in vivo, then the observation that the nitrated uric acid product releases NO in a continuous fashion suggests that the product could play a significant role in the pathophysiology of acute and chronic inflammation. In addition, the nitrated product may prove valuable to the rational design of a new class of mechanism-based, anti-inflammatory drugs that mimic the properties of endogenous uric acid. It is possible that a mimic of the nitrated uric acid product could be utilized to alleviate myocardial ischemic syndromes following ischemic/fibrinolytic, cardioprotective ischemic arrest, coronary artery thrombosis after thrombolysis, and restenosis after transluminal coronary angioplasty (63, 64). The uric acid nitrosation product may also exhibit anti-inflammatory properties by inhibiting platelet aggregation/adherence (67) as well as by attenuating leukocyte adhesion to the endothelial cell surface (68). The combined vasodilator and anti-inflammatory properties of such a NO donor may make it ideally suited for minimizing tissue damage associated with organ transplantation, ischemic bowel disease, and multiple organ dysfunction associated with systemic shock states such as burns, hypovolemia, and trauma.

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