Uric acid quenches singlet oxygen physically or reacts with it, but the oxidation product has not been previously characterized. The present study determined that the product is parabanic acid, which was confirmed by LC/TOFMS analysis. Parabanic acid was stable at acidic pH (<5.0), but hydrolyzed to oxaluric acid at neutral or alkaline pH. The total yields of parabanic acid and oxaluric acid based on consumed uric acid were ~100% in clean singlet oxygen production systems such as UVA irradiation of Rose Bengal and thermal decomposition of 3-(1,4-dihydro-1,4-epidioxy-4-methyl-1-naphthyl)propionic acid. However, the ratio of the amount of uric acid consumed to the total amount of singlet oxygen generated was less than 1/180, indicating that most of the singlet oxygen was consumed to the total amount of singlet oxygen generated. Parabanic acid was shown to be an oxidation product specific to ROS (Fig. 1): free radical-induced oxidation gives 6-aminouracil; nitric oxide (NO) gives 6-aminouracil; ONOO crossing of UA and PA was dissolved in 100 mM phosphate buffer (pH 7.4) and methanol, respectively, and stored at 4°C until use. The OUA was prepared by hydrolysis of PA upon addition of NaNO2 and PA was produced in high yield. However, the yield of PA was less than a few percent from peroxyl radical-, hypochlorite- or peroxynitrite-induced oxidation of uric acid. These results suggest that parabanic acid could be an in vivo probe of singlet oxygen formation because of the wide distribution of uric acid in human tissues and extracellular spaces. In fact, sunlight exposure significantly increased human skin levels of parabanic acid.

Key Words: singlet oxygen, uric acid, parabanic acid, oxaluric acid, sunlight exposure

Oxidative stress induces lipid peroxidation, DNA damage, and protein carbonylation, which can lead to diseases such as cancer, diabetes, Alzheimer’s disease, and ischemia reperfusion injury. Since initial oxidative stress is caused by various reactive oxygen species (ROS), the importance of identifying ROS in vivo is of interest in clinical investigations.

Identifying ROS in vivo can be done by monitoring an oxidation product as a marker. The oxidized substrate must show high reactivity toward different ROS and yield a specific oxidation product from an individual ROS. Uric acid (UA, Fig. 1) is an adequate substrate for this purpose. Uric acid is a terminal metabolite of purine in primates including humans. It is also a water-soluble antioxidant that can scavenge many types of ROS: free radicals, peroxynitrite (ONOO−), hypochlorous anion (ClO−), and singlet oxygen (1O2). Furthermore, its oxidation products are specific to the ROS (Fig. 1): free radical-induced oxidation gives allantoin (AL); ONOO−-induced oxidation yields triuret; and nitric oxide (NO) gives 6-aminouracil. However, the 1O2 induced-oxidation product has not been identified.

Singlet oxygen (1O2) is a prominent ROS that plays an important role in bactericidal action. Nakano et al. showed that 1O2 killed E. coli effectively, although it was not harmful against human umbilical vein endothelial cells. Because the respiratory chains of eukaryotic cells are enclosed in mitochondria, whereas those of prokaryotic cells are contained in the cell membrane, 1O2 penetrating from the cell surface turns into harmless triplet molecular oxygen (1O2) before it reaches the mitochondria. Therefore, 1O2 can be considered a relatively innocuous ROS against eukaryotic cells. However, an excess amount of 1O2 can damage organisms, and some reports indicate that it causes oxidative damage to lipids, proteins, and DNA and also induces apoptosis. Photosensitization is usually used to produce 1O2, but two-electron oxidation of H2O2 also can generate 1O2. The oxidation of H2O2 mimics myeloperoxidase (MPO), which produces ClO− from H2O and Cl2. The ClO− ion is a strong oxidant that can oxidize H2O2 to 1O2, which suggests that 1O2 production may occur in vivo without sunlight exposure.

The current study demonstrated that parabanic acid (PA, Fig. 1) was formed specifically by 1O2-induced UA oxidation. Production of 1O2 resulted from thermal decomposition of 3-(1,4-dihydro-1,4-epidioxy-4-methyl-1-naphthyl)propionic acid (NEPO), photodestruction of Rose Bengal, and H2O2 oxidation by ClO− or ONOO−, and PA was produced in high yield. However, the yield of PA was less than a few percent from peroxyl radical-, hypochlorite- or ONOO−-induced oxidation of UA. These results strongly suggest that PA is an oxidation product specific to 1O2 oxidation, and that PA and its hydrolysis product, oxaluric acid (OUA, Fig. 1), are suitable indicators of 1O2 production in vivo.

Materials and Methods

Chemicals. UA, PA, and other chemicals were purchased from Wako Pure Chemical Industries, Co., Ltd. (Osaka, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), or Waken B Tech Co., Ltd. (Kyoto, Japan), and used as received. An ONOO− generator, 3-(4-morpholinyl)sydnonimine hydrochloride (SIN-1), was purchased from Dojindo (Kumamoto, Japan). Authentic standard solutions of UA and PA were dissolved in 100 mM phosphate buffer (pH 7.4) and methanol, respectively, and stored at 4°C until use. The OUA was prepared by hydrolysis of PA upon addition of aqueous NH3 and then the solution was neutralized using 1 M HCl. The OUA formation was confirmed by LC/time-of-flight mass spectrometry (TOFMS) analysis using an ion corresponding to OUA (m/z = −131) and its fragment ion (m/z = −59).

ONOO− was synthesized using a modified procedure described by Kato et al. Briefly, an ice-cold 0.7 M H2O2 solution containing 0.6 M HCl (10 ml) was added to a well-stirred 0.6 M NaNO2 solution (10 ml) in an ice bath, immediately followed by addition of 1.5 M NaOH (20 ml). The excess H2O2 was removed by addition of MnO2. The solution was then frozen at −25°C. The ONOO− formed a yellow top layer due to frozen fractionation. This layer was collected and its concentration was determined as 330 mM by measuring its UV absorbance at 302 nm (ε = 1,670 M−1·cm−1).

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Oxidation of UA with \( ^1O_2 \) produced from the photo-irradiation of Rose Bengal. An aqueous mixture containing 50, 100, 150 or 200 µM UA and 10 µM Rose Bengal was irradiated by UVA light (1.12 mW/cm²) for ~3 h until all the UA was consumed. Next, the UVA light was turned off and the reaction solution was left at room temperature for ~9 h. Concentrations of UA and products were analyzed by LC/TOFMS and HPLC as described below.

Oxidation of UA with \( ^1O_2 \) produced from NEPO. Thermal decomposition of NEPO produces \( ^1O_2 \). The purity of the NEPO was determined as 78% by the comparison of the UV absorption at 288 nm before and after the thermal decomposition of a methanolic solution of NEPO. Most of the NEPO was decomposed within 3 h at 35°C. A mixture of 50 or 100 µM UA and 8.0 mM NEPO in 50% aqueous methanol was incubated at 35°C for 3 or 12 h. Concentrations of UA and products were analyzed by LC/MS/MS and HPLC as described below.

Oxidation of UA with \( ^1O_2 \) produced from \( H_2O_2 \) and \( NaClO \) or ONOO⁻. UA (130 µM) was dissolved with 2.5 mM \( H_2O_2 \) in 100 mM phosphate buffer (pH 7.4) containing 100 µM diethylenetriamine-\( N,N',N'',N''\)-pentaacetic acid (DTPA) for chelation of transition metal ions. Next, 33 µl of 182 mM aqueous NaClO solution was added into 20 ml of the well-stirred reaction mixture at a constant rate (2 µM NaClO/min) using a syringe pump (Harvard Apparatus, Holliston, MA) over 2.5 h.

A methanolic SIN-1 solution (100 mM) or an aqueous ONOO⁻ solution (330 mM) was added to 100 mM phosphate buffer (pH 7.4) containing 150 µM UA, 2.5 mM \( H_2O_2 \), and 100 µM DTPA and incubated at room temperature for 3 h.

In the absence of \( H_2O_2 \), the oxidation of UA by NaClO or ONOO⁻ was carried out at room temperature for 3 h. Concentrations of UA and products were determined by HPLC as described below.

UA oxidation by peroxyl radicals from 2',2-azobis(2-amidinopropane) dihydrochloride (AAPH). A mixture of 150 µM UA and 10 mM AAPH in phosphate buffer solution (pH 7.4) containing 100 µM DTPA was incubated at 37°C for 3 h. Concentrations of UA and products were determined by HPLC as described below.

Hydrolysis of PA to OUA. Phosphate buffers at pH 4.0 to 8.5 were prepared by adding 1 M NaOH or 1 M \( H_3PO_4 \) to the buffer solutions. Each 500 µM PA solution at various pHs was incubated under aerobic conditions at room temperature for 6 h. Concentrations of UA and OUA were determined by HPLC as described below.

PA detection on human skin surface. Five healthy volunteers participated in this study after giving informed consent. Skin surface UA and OUA were collected from their forearms before and after exposure to sunlight for 2 h. The collection procedure was as follows. Five glass tubes containing 1.0 ml of methanol were prepared. The open end of each tube (ø 20 mm) was pressed tightly against the skin at different locations on the forearm and then rotated carefully to allow the methanol to contact the skin for 1 min. The extracts were combined and the solvent removed using a nitrogen gas flow. The residue was re-dissolved into methanol and analyzed using LC/MS/MS.

HPLC analysis. The amounts of UA and its oxidative metabolites, PA, OUA, and AL, were determined by monitoring the absorption at 210 nm using a reversed-phase HPLC. The mobile phase was aqueous ammonium acetate (40 mM) and delivered at a rate of 1.0 ml/min. An ODS column (Tosoh, Tokyo, Japan; 5 µm, 4.6 mm × 250 mm) or a Develosil C30-UG (Nomura Chemical Co., Ltd., Tokyo, Japan; 5 µm, 250 mm × 4.6 mm) was used for separation. Retention times for UA, PA, OUA and AL were 7.8, 3.2, 11.0 and 2.5 min, respectively, using the ODS column, and 14.0, 7.0, 11.5 and 4.1 min, respectively, using the C-30 column.

Fig. 1. Reported oxidation products of UA induced by reactive oxygen species: AL is produced by free radical-induced oxidation; triuret by ONOO⁻; 6-aminouracil by *NO; and PA by \( ^1O_2 \) (this study).
LC/TOFMS analysis. To obtain accurate mass-to-charge ratios (m/z) of UA oxidative metabolites, HPLC combined with TOFMS (JMS-T100LC, JEOL Ltd., Tokyo, Japan) was used. Negative ionization was performed at an ionization potential of −2,000 V. The optimized applied voltages to the ring lens, outer orifice, inner orifice, and ion guide were −5 V, −10 V, −5 V and −500 V respectively. To obtain accurate m/z values, trifluoroacetic acid (TFA) was used as an internal standard for m/z calibration.

Fig. 2. MS spectra of 200 μM UA (A) before and (B) after 60-min photooxidation induced by UVA irradiation (1.12 mW/cm²) using 10 μM Rose Bengal as a sensitizer. The TOFMS analysis was conducted in negative ESI mode with an ionization potential of −2,000 V. The measured m/z values were corrected using TFA as an internal standard. The chemical formula of PA and its candidates of U1, U2 and U3 are shown in the insert table with measured and calculated m/z values.
LC/MS/MS analysis. An LC/MS/MS system (LCMS-8040, Shimadzu, Kyoto, Japan) was used to determine the amounts of PA and OUA at the picomole level. Aqueous formic acid (0.2 ml/min, pH 3.5) was used as the mobile phase with a Develosil C30-UG column (Nomura Chemical Co., Ltd., Tokyo, Japan; 5 μm, 250 mm × 2.0 mm). Negative ionization was performed at ~3.2 kV using an electrospray probe. For identification and quantification of each compound, multiple reaction monitoring (MRM) measurements were obtained. Optimized combinations of product and precursor ions for PA and OUA were determined as ~42/113 and ~59/131 respectively. Chromatographic retention times for PA and OUA were 6.0 and 10.5 min, respectively.

Results and Discussion

Identification of O\textsubscript{2} induced oxidation products of UA. O\textsubscript{2} was produced from UVA irradiated 10 μM Rose Bengal. Figure 2 shows the changes in the MS spectra in the presence of 200 μM UA before (Fig. 2A) and 60 min after irradiation (Fig. 2B), as determined by negative electrospray ionization (ESI) mode TOFMS. The UA concentration was reduced to 35% and three unidentified anions, U1, U2 and U3, appeared in the MS spectrum after 60 min. These products were not seen in the three unidentified anions, U1, U2 and U3, appeared in the MS spectra of U2 and authentic OUA were identical (data not shown), suggesting that U3 is OUA. U3 (C\textsubscript{4}H\textsubscript{2}N\textsubscript{2}O\textsubscript{4}) was shown to be an O\textsubscript{2} adduct of UA. The chemical formula of U2 was also determined to be C\textsubscript{6}H\textsubscript{5}N\textsubscript{2}O\textsubscript{4}, by its m/z value. This chemical formula is identical with that of PA and the m/z value of authentic PA is ~112,99868. Furthermore, the retention times of U1 and authentic PA were identical (data not shown). We therefore concluded that U1 is PA.

The chemical formula of U2 was also determined to be C\textsubscript{4}H\textsubscript{2}N\textsubscript{2}O\textsubscript{4}, by its m/z value. This chemical formula is identical with OUA, a hydrolysate of PA. The retention times and MS spectra of U2 and authentic OUA were identical (data not shown), indicating that U2 is OUA. U3 (C\textsubscript{4}H\textsubscript{2}N\textsubscript{2}O\textsubscript{4}) was shown to be an O\textsubscript{2} adduct of UA.

Hydrolysis of PA to OUA. The effect of pH on the stability of aqueous PA was examined. The rates of PA hydrolysis and OUA formation increased with increasing pH (Fig. 3). The formation of OUA was stoichiometric with the decomposition of PA. The OUA formed was stable in solution at all pHs (4–8.5) for at least 1 week (data not shown).

The above results indicate that the O\textsubscript{2} induced oxidation products of UA are PA and its hydrolysate OUA. We next examined whether this is true in other O\textsubscript{2} formation systems such as thermal decomposition of NEPO and H\textsubscript{2}O\textsubscript{2} plus ClO\textsuperscript{−} or ONOO\textsuperscript{−}.

Time course changes in PA and OUA levels during the oxidation of UA in various O\textsubscript{2} production systems. First, we employed NEPO which gives O\textsubscript{2} by its thermal decomposition. Figure 4A shows time course changes in UA, PA and OUA when 100 μM UA and 8 mM NEPO were incubated in methanol/water (50/50) at 35°C for 3 h. The major product was PA with a little OUA. The total yield of PA and OUA to consumed UA was 66.6%. When 1 mM NaN\textsubscript{3}, an O\textsuperscript{2} scavenger, was added to the reaction system, the rates of UA consumption and PA formation were reduced (Fig. 4B). The total yield of PA and OUA was also reduced to 13.2%, indicating that O\textsubscript{2} is a key oxidant of UA. All the NEPO was decomposed within 4 h at 35°C. However we incubated the reaction solution for another 9 h and found an increase of PA and OUA formation (Table 1), indicating that intermediates such as U3 slowly decomposed to PA. Therefore, the total yield of PA and OUA increased to 99.1%, indicating that PA and OUA are the exclusive products of O\textsubscript{2} induced oxidation of UA. This was also the case in the UVA-irradiated Rose Bengal system (Fig. 5A and Table 1).

Since all NEPO was converted to O\textsubscript{2}, we knew how much O\textsubscript{2} was produced. We could then calculate the ratio of the amount of UA consumed to the total amount of O\textsubscript{2} generated in the system. This ratio was 1/370 or 1/180, respectively, when 50 or 100 μM UA was oxidized (Table 1), indicating that O\textsubscript{2} was predominantly quenched physically by UA or solvents. In fact, the rate constant for the quenching of O\textsubscript{2} by UA was reported to be 3.6 × 10\textsuperscript{6} M\textsuperscript{−1} s\textsuperscript{−1} (23) while the rate constant for the reaction of O\textsubscript{2} with UA was determined to be 2.3 × 10\textsuperscript{7} M\textsuperscript{−1} s\textsuperscript{−1} (24).

The mechanism of PA formation remains unclear. The isolated U3 decomposed under neutral conditions to form PA (data not shown), suggesting that U3 is a direct precursor of PA formation. Identification of U3 is under investigation.

Hydrogen peroxide is known to be converted to O\textsubscript{2} by the reaction with ClO\textsuperscript{−} (20,21). To confirm this, we incubated aqueous UA in the presence of H\textsubscript{2}O\textsubscript{2} with ClO\textsuperscript{−}. Figure 5B shows the time course changes in UA, PA and OUA when 130 μM UA was incubated with 2.5 mM H\textsubscript{2}O\textsubscript{2} and NaClO. NaClO was added at a rate of 2 μM/min from 30 min. Thereafter, UA was decreased and PA was increased. The total yield of PA and OUA reached 56.1% for 2.5 h oxidation. In the absence of H\textsubscript{2}O\textsubscript{2}, the rate of UA consumption was slower and a little formation of PA and OUA (1.3% yield) was observed (Table 1 and Fig. 5E). These results suggest that ClO\textsuperscript{−} induced oxidation of UA only produced slight PA and OUA and ClO\textsuperscript{−} converted H\textsubscript{2}O\textsubscript{2} to O\textsubscript{2}.

Fig. 3. Hydrolysis of (A) PA to (B) OUA at room temperature at various pHs (4.0–8.5).
This may suggest that a small amount of yield of PA and OUA was only 1.9% but this was significant. UA resulted in UA decay and AL formation (Fig. 5D). The total tert-alkoxy radical occurred.\(^{(27-29)}\) However, this requires further investigation.

As shown before, the total yield of PA and OUA in CIO\(^-\) and ONOO\(^-\)-induced oxidation of UA was below 2%. Therefore, we concluded that PA is the \(^1\)O\(_2\) specific oxidation product of uric acid. We next tried to detect PA in biological samples.

**Detection of PA on human skin surface.** Human skin surface was selected as a candidate of PA detection since UA is present there and the level of squalene hydroperoxide (\(^2\)O\(_2\) oxidation product of squalene) increases after sunlight exposure.\(^{(30)}\) Methanol extracts of human skin were analyzed by LC/MS/MS. The analysis revealed the presence of UA and PA in skin lavage samples, but no OUA was detected. It is interesting that the PA and UA levels increased upon sunlight exposure (Table 2). The latter should be a protective response of human skin surface against photooxidation.

We are currently applying this method to human plasma samples. We believe our method is useful to determine the importance of \(^1\)O\(_2\) and its significance in many diseases under oxidative stress.

**Table 1.** Formation of PA and OUA and their yields during UA oxidation induced by different types of ROS [μM, mean ± SD (n = 3)]

| ROS | UA | T | Δ | PA | OUA | PA + OUA | Yield (%) |
|-----|----|---|---|----|-----|----------|-----------|
| \(^1\)O\(_2\) from 8.0 mM NEPO | 50 | 12 | 21.3 ± 1.3 | 20.4 ± 1.3 | 0.29 ± 0.07 | 20.7 ± 1.4 | 97.0 ± 2.0 |
| \(^1\)O\(_2\) from 8.0 mM NEPO | 100 | 3 | 40.0 ± 2.2 | 26.4 ± 0.9 | 0.18 ± 0.01 | 26.6 ± 0.9 | 66.6 ± 1.3 |
| \(^1\)O\(_2\) from 8.0 mM NEPO + 1.0 mM NaN\(_3\) | 100 | 3 | 26.4 ± 0.4 | 3.21 ± 0.04 | 0.25 ± 0.01 | 3.5 ± 0.04 | 13.2 ± 0.2 |
| \(^1\)O\(_2\) from 8.0 mM NEPO | 100 | 12 | 44.6 ± 1.1 | 42.4 ± 0.8 | 0.57 ± 0.13 | 44.2 ± 1.2 | 99.1 ± 0.3 |
| \(^1\)O\(_2\) from UVA-irradiated Rose Bengal | 50 | 12 | 50 | 7.2 ± 0.2 | 41.6 ± 0.4 | 48.8 ± 0.4 | 97.6 ± 0.8 |
| \(^1\)O\(_2\) from UVA-irradiated Rose Bengal | 100 | 12 | 100 | 18.3 ± 0.4 | 78.6 ± 0.5 | 96.9 ± 0.2 | 96.9 ± 0.2 |
| \(^1\)O\(_2\) from UVA-irradiated Rose Bengal | 150 | 12 | 150 | 33.1 ± 0.2 | 109.4 ± 0.1 | 142.5 ± 0.3 | 95.0 ± 0.2 |
| \(^1\)O\(_2\) from UVA-irradiated Rose Bengal | 200 | 12 | 200 | 55.7 ± 0.7 | 129.8 ± 0.1 | 185.4 ± 0.7 | 92.7 ± 0.4 |
| \(^1\)O\(_2\) from 2.5 mM H\(_2\)O\(_2\) + 300 μM CIO\(^-\) | 130 | 2.5 | 127 ± 0.5 | 70.9 ± 3.8 | 0.12 ± 0.003 | 71.0 ± 3.8 | 56.1 ± 3.3 |
| \(^1\)O\(_2\) from 2.5 mM H\(_2\)O\(_2\) + ONOO\(^-\) (1.0 mM SIN-1) | 150 | 3 | 150 | 0.04 ± 0.01 | 56.2 ± 0.3 | 56.3 ± 0.3 | 37.0 ± 0.2 |
| Peroxyl radicals from 10 mM AAPH | 150 | 3 | 85.9 ± 4.2 | 1.2 ± 0.1 | 0.5 ± 0.1 | 1.7 ± 0.1 | 1.9 ± 0.1 |
| CIO\(^-\) (360 μM) | 260 | 3 | 243 ± 9.9 | 1.6 ± 0.2 | 1.5 ± 0.2 | 3.1 ± 0.2 | 1.3 ± 0.1 |
| ONOO\(^-\) (1.0 mM SIN-1) | 200 | 3 | 132 ± 15 | ND | ND | ND | 0 |

ROS, reactive oxygen species; UA, uric acid; PA, parabanic acid; OUA, oxaluric acid; NEPO, 3-(4-morpholinyl)sydnonimine, hydrochloride; ND, not detected.

Similarly, ONOO\(^-\) converts H\(_2\)O\(_2\) to \(^1\)O\(_2\).\(^{(25,26)}\) Figure 5C shows the time course changes in UA, PA and OUA when 150 μM UA was incubated with 2.5 mM H\(_2\)O\(_2\) and 1 mM SIN-1, an ONOO\(^-\)generator. The major product was OUA rather than PA because the pH of the reaction solution was ~8, which accelerated the hydrolysis of PA. The total yield of PA and OUA reached 37.0% for 3 h oxidation. In the absence of H\(_2\)O\(_2\), the rate of UA consumption became slower and no formation of PA and OUA (0% yield) was observed (Table 1 and Fig. 5F). These results suggest that ONOO\(^-\)-induced oxidation of UA produced no PA and OUA and ONOO\(^-\) converted H\(_2\)O\(_2\) to \(^1\)O\(_2\). It is noteworthy that similar results were obtained when synthetic ONOO\(^-\) was used instead of SIN-1 (data not shown).

**Oxidation of UA induced by peroxyl radical, CIO\(^-\), or ONOO\(^-\).** Thermal decomposition of AAPH produces two tert-carbon-centered radicals which are immediately converted to two tert-peroxyl radicals. Peroxyl radical-induced oxidation of aqueous UA resulted in UA decay and AL formation (Fig. 5D). The total yield of PA and OUA was only 1.9% but this was significant. This may suggest that a small amount of \(^1\)O\(_2\) was formed by the termination of two tert-peroxyl radicals;\(^{(27)}\) and/or the Russell-reaction of two methylperoxyl radicals formed by β-scission of tert-alkoxy radical occurred.\(^{(27-29)}\) However, this requires further investigation.

As shown before, the total yield of PA and OUA in CIO\(^-\) and ONOO\(^-\)-induced oxidation of UA was below 2%. Therefore, we concluded that PA is the \(^1\)O\(_2\) specific oxidation product of uric acid. We next tried to detect PA in biological samples.
Conclusions

We identified PA as the $^{1}\text{O}_2$ specific oxidation product of UA. PA is slowly hydrolyzed to OUA under neutral conditions. Therefore, PA and OUA can serve as novel $^{1}\text{O}_2$ markers in vivo. We detected PA on human skin surface and its level increased upon sunlight exposure, indicating that sunlight exposure induced the formation of $^{1}\text{O}_2$ on human skin surface.
Abbreviations

AAPH 2,2-azobis(2-amidinopropane) dihydrochloride
AL allantoin
DTPA diethylenetriamine-pentaacetic acid
ESI electrospray ionization
NEPO 3-(1,4-dihydro-1,4-epidioxo-4-methyl-1-naphthyl)propionic acid
OUA oxaluric acid
PA parabanic acid
POS reactive oxygen species
SIN-1 3-(4-morpholinyl)sydnonimine, hydrochloride
TFA trifluoroacetic acid
TOFMS time-of-flight mass spectrometry
UA uric acid

Conflict of Interest

No potential conflicts of interest were disclosed.

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| PA (pmol/cm²) | Before exposure | After exposure | After/Before | Before exposure | After exposure | After/Before |
|--------------|----------------|---------------|--------------|----------------|---------------|--------------|
|              | 0.020 ± 0.010  | 0.065 ± 0.040*| 3.1 ± 1.2    | 13.9 ± 15.1    | 46.2 ± 30.8   | 4.8 ± 4.2    |

PA, parabanic acid; UA, uric acid; Each value represents mean ± SD (n = 5); *p<0.05.