Inhibition of free radical-induced DNA damage by uric acid

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Single-strand DNA breaks were produced in isolated rat liver nuclei incubated with 3 separate oxygen free radical generating systems: xanthine oxidase-acetaldehyde plus Fe(II); hematin-R(H)OOH; Fe(II)-H_2O_2. Uric acid inhibited the induction of damage in the first two systems only. At concentrations below those found in human plasma, it was particularly effective against strand breaks produced by hematin-cumene hydroperoxide. These results offer additional evidence that uric acid may function as a cellular protective agent against superoxide and hydroperoxyl free radical-induced cytotoxicity toxicity.

Urate *Free radical* *Inhibition* *DNA damage* *Liver* *Nucleus*

1. INTRODUCTION

The evolution of aerobic organisms has been accompanied by the development of numerous protective mechanisms against oxygen toxicity. These defense mechanisms function to reduce the deleterious effects of free radicals which may arise from the univalent reduction of oxygen during the course of several important metabolic sequences [1]. Oxygen-containing free radicals have been implicated as causative agents in aging, mutation, cancer, and heart disease [2].

The harmful effects of oxygen free radicals on membrane lipids can be diminished through the scavenging effects of α-tocopherol and β-carotene. Similarly, superoxide dismutase, glutathione and selenium-dependent glutathione peroxidase protect against free radicals generated in the aqueous environment of the cell. In addition, non-primates can utilize the antioxidant properties of ascorbic acid as a cellular protective mechanism.

As noted in [3], the loss of the ability to synthesize ascorbic acid in primates has been coincident with the loss of the enzyme uricase. This prompted the suggestion that uric acid may replace ascorbate as a defense mechanism against oxygen toxicity. The ability of urate to inhibit lipid peroxidation and the lysis of erythrocytes [4] is consistent with this proposal. More recently, authors in [5] provided direct evidence that urate provides a general defense mechanism against singlet oxygen and organic hydroperoxides. This report demonstrates that uric acid is also capable of inhibiting some forms of oxygen free radical-induced DNA damage.

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2. MATERIALS AND METHODS

2.1. *Reagents*

Xanthine oxidase (spec. act. 1.3 units/mg), superoxide dismutase (spec. act. 2800 units/mg) and hematin were obtained from Sigma. DNA-grade hydroxyapatite HTP was obtained from Bio-Rad. All other chemicals were of the highest purity available.

2.2. *Preparation of nuclei and incubation conditions*

Hepatic nuclei from male Sprague-Dawley rats were purified through 2.1 M sucrose/0.1 mM EDTA/0.1 mM EGTA in buffer (60 mM KCl/15 mM NaCl/0.15 mM spermine/0.5 mM spermidine/15 mM Tris–HCl, pH 7.6) as in [6].

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Reaction mixtures (1 ml) containing the indicated concentrations of test compounds and 2–4 × 10^7 nuclei were prepared in this buffer and incubated at 37°C for 30 min.

2.3. *Quantitation of DNA damage*

Following incubation, the nuclei were lysed by the rapid addition of 1 ml of 0.1 N NaOH. The DNA was allowed to unwind under subdued lighting conditions for 20 min at room temperature. HCl (0.1 N, 1 ml) was then added and the mixture was vortex mixed to achieve rapid neutralization. Single- and double-stranded DNA were separated by batch hydroxyapatite chromatography and quantitated spectrofluorometrically exactly as in [7]. Results are reported as changes in the percent double-stranded DNA since this has been shown to correlate directly with the extent of radiation and chemically induced DNA damage [8].

Table 1

Effect of uric acid on superoxide-mediated DNA damage

Additions	% double-stranded DNA
None	82.6
Xanthine oxidase (10 μg)	80.2
Acetaldehyde (2 mM)	77.4
Xanthine oxidase	
+ acetaldehyde	51.8
+ uric acid (0.5 mM)	68.6
+ SOD (10 μg)	70.2

Nuclei (2–4 × 10^7/ml) were incubated with the components shown at 37°C for 30 min. In all cases, 5 μM $FeCl_3$ and 50 μM ATP were present in the reaction mixture. DNA damage was quantitated as described in section 2

3. RESULTS

3.1. *Superoxide-mediated DNA damage*

Metabolism of acetaldehyde by xanthine oxidase leads to the production of the superoxide radical anion [10], a species which has been found to induce DNA damage [11]. The data in table 1 demonstrate that while neither component alone is effective, the action of xanthine oxidase on acetaldehyde, in the presence of $FeCl_2$-ATP, leads to demonstrable DNA damage. The role of superoxide radical in this process is inferred from the significant protection observed by inclusion of superoxide dismutase (SOD) in the reaction mixture. Table 1 also shows that a similar inhibition of superoxide-mediated DNA damage was produced by uric acid. The concentration used (0.5 mM) approximates that of human plasma [12].

3.2. *Hydroxyl radical-mediated DNA damage*

The author in [13,14] has recently demonstrated that Fe(II)–nucleotide complexes interact with H_2O_2 in a Fenton-type reaction to produce hydroxyl radicals. Accordingly, it was of interest to ascertain the effect of uric acid on the induction of DNA damage under these conditions. Table 2 il-

Table 2

Effect of uric acid on hydroxyl radical-mediated DNA damage

Additions	% double-stranded DNA
None	83.5
H_2O_2 (1 mM)	79.8
$FeCl_2$ (25 μM) + ATP (250 μM)	74.8
$FeCl_2$ (50 μM) + ATP (500 μM)	53.5
$FeCl_2$ (25 μM) + ATP (250 μM) + H_2O_2 (1 mM)	65.0
+ uric acid (0.5 mM)	67.9
$FeCl_2$ (50 μM) + ATP (500 μM) + H_2O_2 (1 mM)	34.5
+ uric acid (0.5 mM)	37.0

Nuclei (2–5 × 10^7/ml) were incubated with the components shown at 37°C for 30 min. DNA damage was quantitated as described in section 2

lustrates a finding noted by others [15,16], i.e., Fe(II) salts alone are capable of inducing DNA damage. However, the data also demonstrate the synergistic effect produced by the addition of H_2O_2 to the reaction mixture. More importantly, uric acid was found to be completely devoid of protective activity in this system.

3.3. *Peroxyl radical-mediated DNA damage*

ESR studies [17,18] indicate that peroxyl free radicals are produced upon the interaction of trivalent iron porphyrin-containing compounds and peroxides. The production of DNA damage under these conditions is clearly indicated by the results in table 3. As shown in the table, the interaction between hematin and cumene hydroperoxide resulted in marked DNA damage. Under these conditions uric acid produced 80% inhibition of the damage. A similar protective effect against damage induced by hematin-H_2O_2 was noted, although the total extent DNA degradation was far less with H_2O_2.

The concentration-dependent protective effect of uric acid against hematin-cumene hydroperoxide-induced DNA damage is shown in fig.1. The potency of uric acid in this system is apparent since a concentration as low as 10 μM resulted in 30% inhibition of damage. Near maximal inhibition was achieved at approx. 100 μM.

Table 3

Effect of uric acid on peroxyl free radical-induced DNA damage

Additions	% double-stranded DNA
None	82.4
Hematin (20 μM)	80.5
Cumene·OOH (0.1 mM)	81.6
H_2O_2 (1 mM)	79.4
Hematin + cumene·OOH	33.6
+ uric acid (0.5 mM)	72.4
Hematin + H_2O_2	70.1
+ uric acid (0.5 mM)	77.3

Nuclei (2–4 × 10^7/ml) were incubated with the components shown at 37°C for 30 min. DNA damage was quantitated as described in section 2

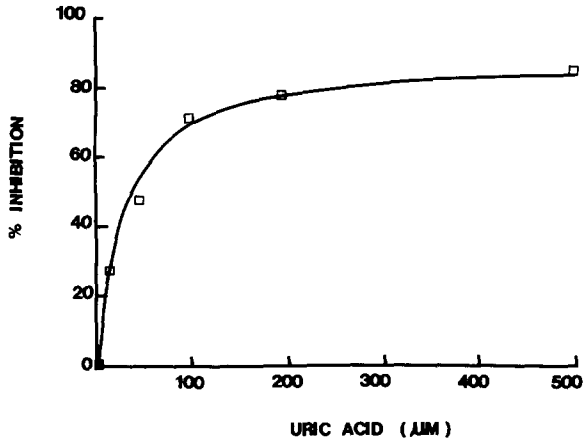

Fig.1. Inhibition by uric acid of DNA damage induced by hematin–cumene hydroperoxide. Reaction mixtures contained 2–4 × 10^7 nuclei, 20 μM hematin, 100 μM cumene hydroperoxide and the concentration of uric acid shown in a total volume of 1 ml buffer (pH 7.6). The reaction was initiated by the addition of the peroxide and incubation at 37°C for 30 min.

4. DISCUSSION

Authors in [5] recently proposed a cellular protective role for uric acid against oxygen toxicity in humans. Their hypothesis is based on the demonstrated reactivity of urate with potentially damaging species such as singlet oxygen [5,19], peroxyl free radicals [20], and possibly hydroxyl radicals [4]. The coincident loss of uricase and ascorbate biosynthetic activity in primates is consistent with the notion that urate may replace ascorbate as a primary antioxidant in humans. The high plasma urate:ascorbate ratio in humans [4] and the existence of mechanisms to transport urate into cells [21] are supportive of this concept.

This study demonstrates an inhibitory effect of uric acid on free radical-induced DNA damage. In particular, urate was effective against damage produced in association with superoxide generation by the xanthine oxidase-acetaldehyde system. Since $FeCl_2$-ATP was also present in the incubation mixture, it could be surmised that the ultimate damaging species is the hydroxyl radical produced by an iron-catalyzed Haber-Weiss reaction [22]. However, as shown in table 2, urate failed to exert a protective effect against damage resulting from the direct interaction between Fe(II) and H_2O_2.

Consequently, the protective effect of urate in the xanthine oxidase-acetaldehyde system may involve an interaction with singlet oxygen as proposed in [5].

The most pronounced inhibitory effect of uric acid occurred in the hematin–peroxide systems (table 3). This finding was not entirely surprising, since authors in [20] have reported that urate is rapidly oxidized in the presence of hematin and H_2O_2. Their results as well as those of authors in [17] and [18] indicate that hydroperoxyl radicals are the primary toxic species in this system. The high reactivity of urate with those radicals is also suggested by the very low concentrations (10–100 μM) required for inhibition of DNA damage (fig.1). The effective concentrations of urate are lower than those found in the plasma and erythrocytes [12]. Accordingly, these preliminary results suggest that further investigation into the role of uric acid as an intracellular cellular protective agent is warranted.

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