Uric acid inhibits L-DOPA-CU(II) mediated DNA cleavage

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Abstract

It has been proposed that considerable DNA damage may be caused by endogenous metabolites produced during the body's normal metabolic processes. We have previously shown that L-DOPA, in the presence of Cu(II) leads to oxidative DNA breakage in vitro. Uric acid is considered to be a naturally occurring antioxidant and is present in plasma at a relatively high concentration. In this paper we report that uric acid inhibits L-DOPA-Cu(II) mediated DNA cleavage at concentrations similar to or lower than those found in plasma. Xanthine, which is the structural analogue of uric acid is a more potent inhibitor of the reaction. Uric acid was also shown to directly quench the generation of hydroxyl radicals by L-DOPA-Cu(II). The results have been discussed in relation to the putative protective role of uric acid against endogenous DNA damage by oxygen radicals. © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved

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It has been proposed that considerable DNA damage may be caused by endogenous metabolites produced during the body's normal metabolic processes. For example, it has been shown that malondialdehyde which is the ubiquitous product of lipid peroxidation and iocosanoid metabolism reacts with cellular DNA to form a propanodeoxyguanosine adduct [2,4]. Evidence indicates that the adduct exists at significant levels in the hepatic DNA of rats and humans and is an efficient premutagenic lesion in E. coli [2,5]. Dopamine, formed by the decarboxylation of L-DOPA [11], condenses with acetaldehyde, a product of ethanol metabolism, to generate L-Methyl-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (Salsolinol) [7]. Salsolinol is considered to be involved in the etiology of Parkinson's and Huntington's diseases and has been detected in the cerebrospinal fluid of Parkinsonian patients [13]. Thus, dopamine can be considered a precursor of an endogenous neurotoxin. We have shown that L-DOPA causes DNA breakage in vitro in the presence of transition metals such as Cu(II) and that the reaction is catalysed by the formation of reactive oxygen species [10]. Copper has been shown to be a normal component of chromatin and is available for inter-nucleosomal DNA fragmentation in isolated nuclei [3]. Halliwell et al. [20] have proposed that copper ions released in the presence of L-DOPA and its metabolites may be an important mechanism of neurotoxicity in several neurological disorders [20]. A number of molecules present in human extracellular fluids are considered to have antioxidant function [9]. These include ascorbic acid and uric acid. Uric acid is present in human plasma at a relatively high concentration (up to 0.6 mM) and is capable of scavenging hydroxyl radical, lipid hydroperoxides, singlet oxygen and oxo-heme oxidants [1]. In this paper, we report that uric acid inhibits L-DOPA-Cu(II) mediated DNA cleavage and quenches the hydroxyl radical production by the same system at concentrations similar or lower to those found in plasma.

Calf thymus DNA (sodium salt; average MW: 1 x 10^6) and S1 nuclease were from Sigma (St. Louis, MO). L-DOPA was obtained from Fluka (Switzerland). Supercoiled plasmid pBR322 DNA was prepared according to standard methods [12]. All other chemicals were of analytical grade.

Formation of single strand breaks by L-DOPA-Cu(II) and their inhibition by uric acid was assayed by the extent of generation of S1 nuclease sensitive sites in Calf thymus DNA.
DNA [16]. Induction of DNA strand breaks and the inhibition of the reaction by uric acid and xanthine was further assessed by the decrease in superhelicity and linearization of supercoiled plasmid pBR322 DNA [10]. Reaction mixtures (30 μl) contained 10 mM Tris-HCl (pH 7.5), 0.50 μg plasmid DNA and varying concentrations of uric acid. Incubation at room temperature was for 20 min. To compare the effects of uric acid and other known hydroxyl radical scavengers on the generation of hydroxyl radicals by the L-DOPA-Cu(II) system, the method of Quinlan and Gutteridge [15] was followed. The reaction mixtures (0.5 ml) contained 2 mM deoxyribose, 50 μM L-DOPA and indicated concentrations of uric acid, mannitol and thiourea.

We have previously shown that L-DOPA in the presence of Cu(II) generates S$_1$ nuclease sensitive sites in calf thymus DNA [10]. The reaction records the proportion of DNA converted to acid soluble nucleotides by the nuclease. Fig. 1 gives the kinetics of such an experiment in the absence and presence of 300 μM uric acid. At the end of a 4 h incubation period, a decrease of about 30% in the amount of acid soluble material produced is observed. The percent inhibition caused by uric acid after 4 h of incubation was found to be significant ($P < 0.001$).

The inhibitory effect of uric acid was also examined on the cleavage of supercoiled plasmid DNA as the relaxation of such a molecule is a sensitive test for just one nick per molecule. Fig. 2a shows the effect of three different concentrations of uric acid on the conversion of supercoiled pBR322 DNA to open circular and linear forms. It is seen that the formation of linears is inhibited by 200 μM uric acid and completely inhibited at a 400 μM concentration (lane 4). As xanthine is the structural analogue and the metabolic precursor of uric acid, it was also of interest to observe its effect on single strand breakage in the plasmid by the L-DOPA-Cu(II) system. The results indicate that xanthine is a better inhibitor of the reaction as the full conversion of molecules to the relaxed form is prevented at even 100 μM concentration and the formation of linears is not seen at all.

![Fig. 1. Kinetics of degradation of calf thymus DNA by L-DOPA-Cu(II) in the presence (●) and absence (▲) of uric acid (300 μM). The concentrations of L-DOPA and Cu(II) were 100 μM each.](image1)

![Fig. 2. Effects of various antioxidants on cleavage of supercoiled plasmid DNA by L-DOPA and Cu(II) (50 μM of each). (a) Effect of different concentrations of uric acid and xanthine. Lanes: 1, DNA alone; 2, L-DOPA + Cu(II); 3–5, 100, 200 and 400 μM uric acid, respectively; 6–8, 100, 200 and 400 μM xanthine, respectively. (b) Effect of uric acid, xanthine, mannitol and thiourea. Lanes: 1, DNA alone; 2, L-DOPA + Cu(II); lanes 3–6, uric acid, xanthine, mannitol and thiourea, respectively (200 μM each).](image2)

The oxidative DNA breakage by L-DOPA-Cu(II) system was earlier shown to involve the hydroxyl radical as the proximal DNA cleavage agent [10]. We have, therefore, compared the inhibitory effect of uric acid and xanthine with two known scavengers of the hydroxyl radical, namely mannitol and thiourea. Results given in Fig. 2b confirm that xanthine exhibits a greater inhibitory effect than uric acid (lanes 3 and 4). However, both mannitol and thiourea (lanes 5 and 6) do not show any inhibition of the cleavage at the concentrations (200 μM) used for uric acid and xanthine. It may be noted that the concentrations of mannitol and thiourea required for observing in vitro protection against DNA cleavage by L-DOPA-Cu(II) is 50 mM [10]. This explains the effect of these two scavengers in the above experiment.

We directly tested the effect of uric acid on the generation of hydroxyl radicals by L-DOPA-Cu(II). The assay used involves the reaction of hydroxyl radicals with deoxyribose leading to a free radical intermediate that decomposes to form an aldehyde which in turn gives an adduct with TBA. The effect of two concentrations of uric acid on the kinetics of hydroxyl radical generation was studied and a
dose-response relationship was observed as the inhibitory effect of 50 μM uric acid was greater than 25 μM uric acid (results not shown). The quenching effect of uric acid was also compared with thiourea and mannitol. In the presence of uric acid, the rate of formation of hydroxyl radicals was reduced to about 50% at the end of a three hour incubation period. At the same concentration (25 μM), mannitol did not show any effect whereas thiourea was inhibitory to the extent of about 20% (Fig. 3). The inhibitory effects of mannitol, thiourea and uric acid at 180 min incubation were significant with \( P < 0.001 \), \( P < 0.001 \) and \( P < 0.001 \), respectively. Further, the inhibition by uric acid with respect to mannitol and thiourea was also found to be significant (\( P < 0.001 \)).

The normal physiological concentration of uric acid in plasma is 3–9 mg/dl or 0.2–0.6 mM [14]. Under certain diseased conditions such as hyperuricemia, gout and arthritis, the uric acid concentration is increased two to four times than normal. In the present studies, the inhibitory concentrations of uric acid for L-DOPA-Cu(II) mediated DNA cleavage is 0.2–0.4 mM. The concentration required for direct scavenging of L-DOPA-Cu(II) generated hydroxyl radicals is even lower (25–50 μM). Thus, these studies indicate that at the physiological concentrations found in blood, uric acid is capable of exerting a scavenging effect on L-DOPA-Cu(II) generated DNA damaging oxygen radicals. Further, the studies support the putative antioxidant role of uric acid in higher primates.

Having stated the above, it is to be recognised that it is not established whether uric acid is present in the cell nucleus. Although it is hydrophilic in nature, it is conceivable that as a complex with copper, uric acid is capable of traversing the cell or nuclear membrane. Uric acid binds metal ions such as copper and iron and therefore mobilizes free or loosely bound copper and iron [6]. In addition to chromatin, normal serum contains up to 8 μM loosely bound copper [3,17]. Loosely bound copper is defined by Gutteridge as that copper which is available for binding to the chelating agent 1, 10-phenanthroline [8]. It is possible that such loosely bound copper is also available for binding to uric acid. Several of the known antioxidants such as flavonoids in plants and ascorbate in animals are known to generate reactive oxygen species in the presence of transition metal ions and lead to DNA cleavage [18]. Similarly, we have shown that uric acid in the presence of Cu(II) is capable of causing strand scission in DNA and that this reaction is associated with the generation of hydroxyl radicals [19]. However, the concentration of uric acid required for any significant DNA cleavage is 1 mM. Irrespective of the physiological significance of our results it is suggested that whereas potential endogenous DNA damaging reactions may occur, the animal system also provides for preventive mechanisms against such damage.

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