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Short communication

Experimental meningitis in the rat: protection by uric acid at human physiological blood concentrations

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Abstract

The natural peroxynitrite scavenger uric acid was previously shown to be protective in a rat model of pneumococcal meningitis; however, rats have much lower blood uric acid levels than humans. Therefore, we evaluated its therapeutic effect at human physiological blood concentrations. Intraperitoneal pretreatment with uric acid increased its blood concentrations from 44.9 ± 10.0 μM in untreated rats to 169.8 ± 122.6 μM and reduced the cerebrospinal fluid (CSF) pleocytosis from 12 767 ± 2520 to 8376 ± 2450 cells/μl ($P < 0.05$) and the intracranial pressure from 11.6 ± 3.0 to 4.3 ± 1.2 mm Hg ($P < 0.05$). Coadministration of oxonic acid, an inhibitor of urate oxidase, increased the blood uric acid levels to 355.0 ± 79.6 μM and further reduced the CSF pleocytosis (4190 ± 1749 cells/μl, $P < 0.05$) and the intracranial pressure (1.4 ± 2.4 mm Hg). Uric acid + oxonic acid also had a beneficial effect when adminstered 2 or 4 h after the induction of meningitis. We demonstrate a dose-dependent anti-inflammatory effect of uric acid at blood levels in the human physiological range.

Keywords: Meningitis; Oxidative stress; Peroxynitrite; Uric acid

1. Introduction

The unfavourable outcome of acute bacterial meningitis is often due to cerebral complications comprising cerebrovascular insults, brain edema, hydrocephalus, and increased intracranial pressure (Pfister et al., 1993). In an adult rat model of pneumococcal meningitis, we recently demonstrated that pretreatment with the peroxynitrite scavenger uric acid attenuated meningeal inflammation, blood–brain barrier disruption, and intracranial hypertension (Kastenbauer et al., 1999). However, blood uric acid levels were not determined in that study, and rats, like most non-primates, have much lower blood levels of uric acid than humans (rats: approximately 20–100 μM, humans: 100–400 μM) due to its rapid degradation by the hepatic enzyme urate oxidase (Becker, 1993). Therefore, in the present investigation, we used a rat model of pneumococcal meningitis that allowed exact control of blood uric acid levels and studied whether uric acid further attenuates pathophysiological alterations during bacterial meningitis when its blood concentrations are increased to levels such as those found in humans.

2. Materials and methods

2.1. Rat model of pneumococcal meningitis

We used a well-characterized rat model of pneumococcal meningitis, which was previously described in detail (Lorenzl et al., 1996). Adult male Wistar rats (270–350 g) were anesthetized with 100 mg/kg intraperitoneal (i.p.) thiopental (Trapanal, Byk Gulden, Germany), tracheotomized, and artificially ventilated with a small animal ventilator (Model Ap-10, Effenberger, Pfaffing, Germany). A catheter was inserted into the left femoral artery for continuous monitoring of mean arterial blood pressure, for blood gas analyses, repeated blood sampling (usually 200 μl), and for Evans Blue administration to evaluate blood–brain barrier permeability (Uyama et al., 1988). To apply uric acid and oxonic acid (an inhibitor of urate oxidase), a catheter was inserted into the peritoneal cavity for i.p. injection. A burr hole was made in the occipital bone and a catheter was introduced into the cisterna magna for continuous intracranial pressure monitoring, injection of pneumococci (pneumococcal challenge, p.c.), and collection of

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CSF samples (usually 100–200 μl). Both the mean arterial blood pressure and the intracranial pressure were measured through fluid-filled plastic tubes connecting the respective catheter to a Gabarith DT-XX transducer (Ohmeda, Erlangen, Germany); the pressure was displayed using an analogous pressure monitor (Siemens, Munich, Germany). The CSF leukocytes were counted in the Neubauer chamber. The increase of intracranial pressure was calculated as the difference of the intracranial pressure 1 and 6 h after intracisternal injection of pneumococci (intracisternal injection caused a transient increase of intracranial pressure which normalized within 1 h). Meningitis was induced by intracisternal (i.c.) injection of 100 μl phosphate-buffered saline (PBS) containing 10^9 colony-forming units (cfu)/ml of heat-killed (60 °C for 4 h) unencapsulated pneumococci. Control rats were injected intracisternally with 100 μl sterile PBS. CSF protein was quantified according to Bradford (Nanoquant, Roth, Karlsruhe, Germany). At the end of the experiment, the rats were killed by an overdose of thiopental.

The following experimental groups were studied: controls (i.c. PBS), meningitis (i.c. pneumococci), meningitis + uric acid pre (i.c. pneumococci, 300 mg/kg i.p.uric acid 1 h before and 2 h after p.c.), meningitis + uric acid + oxonic acid pre (i.c. pneumococci, 300 mg/kg i.p. uric acid before and 2 h after p.c. plus 250 mg/kg i.p. oxonic acid 1 h before p.c. and 120 mg/kg every hour thereafter), meningitis + uric acid + oxonic acid post 2 h (i.c. pneumococci, 300 mg/kg i.p. uric acid plus 250 mg/kg i.p. oxonic acid 2 h after p.c. and 120 mg/kg oxonic acid every hour thereafter) and meningitis + uric acid + oxonic acid post 4 h (i.c. pneumococci, 300 mg/kg i.p. uric acid plus 250 mg/kg i.p. oxonic acid 4 h after p.c. and 120 mg/kg oxonic acid every hour thereafter).

2.2. Determination of blood–brain barrier permeability

One hour before the end of the experiment, 1 ml 1% Evans Blue was injected intraarterially. Evans Blue concentration in the CSF was determined by measuring the absorbance at 650 nm spectrophotometrically, using serial dilutions of Evans Blue in PBS as a standard.

2.3. Measurement of uric acid in CSF and blood samples

CSF and blood were deproteinated with 3 vol. 0.5 M $HClO_4$, centrifuged at 14 000 rpm for 10 min, and the supernatants were collected. Uric acid levels were determined by high pressure liquid chromatography (HPLC) using a slightly modified, previously published protocol (Becker, 1993). In brief, 10 μl of supernatant were applied to a 5-μm C-18 nucleosil 4 × 250-mm column (Macherey-Nagel, Dueren, Germany). $HClO_4$/methanol/water at a ratio of 90:6:4 (vol.) served as eluent. At a flow rate of 1 ml/min, the retention time was 4.7 min. Uric acid was detected by its ultraviolet absorbance at 280 nm.

2.4. Statistical analysis

Statistical analysis was performed by nonparametric procedures including the Kruskal–Wallis ranking test as well as the Mann–Whitney *U*-test (combined with α-adjustment for multiple comparisons) to detect differences between experimental groups. Experimental groups were compared as follows: (a) controls (PBS) were compared with meningitis (untreated); (b) meningitis (untreated) and the pretreatment groups (meningitis + uric acid pre, meningitis + uric acid + oxonic acid pre) were compared with each other; (c) meningitis (untreated) was compared with each of the post-treatment groups (meningitis + uric acid + oxonic acid post 2 h and meningitis + uric acid + oxonic acid post 4 h).

2.5. Reagents

Unless otherwise stated, all reagents and chemicals were obtained from Sigma (Deisenhofen, Germany).

3. Results

Intracisternal injection of heat-killed pneumococci caused a significant increase of CSF uric acid concentra-

Table 1
Blood and CSF uric acid concentrations (μM)

Group (*n*)	Plasma uric acid at 2 h	Plasma uric acid at 4 h	Plasma uric acid at 6 h	CSF uric acid at 6 h
Controls (3)	37.7 ± 8.0	28.7 ± 5.3	41.1 ± 28.6	9.0 ± 2.5
Meningitis (7)	31.1 ± 8.6	31.2 ± 10.3	44.9 ± 10.0	30.7 ± 6.3[a]
Meningitis + uric acid pre (5)	115.9 ± 47.4[b]	161.9 ± 29.6[b]	169.8 ± 122.6[b]	71.4 ± 25.8[b]
Meningitis + uric acid + oxonic acid pre (6)	260.8 ± 57.2[b,c]	326.6 ± 201.6[b,c]	355.0 ± 79.6[b]	585.4 ± 187.7[b,c]
Meningitis + uric acid + oxonic acid post 2 h (4)	45.3 ± 20.4	274.9 ± 126.8[b]	369.0 ± 119.1[b,c]	432.8 ± 95.3[b,c]
Meningitis + uric acid + oxonic acid post 4 h (4)	28.7 ± 3.9	64.6 ± 41.1	370.1 ± 83.0[b,c]	674.7 ± 216.7[b,c]

Data are mean values ± S.D.
[a] $P < 0.05$ compared with controls.
[b] $P < 0.05$ compared with untreated meningitis.
[c] $P < 0.05$ compared with uric acid-pretreated animals.

Table 2
CSF leukocyte counts, increase of intracranial pressure, parameters of blood–CSF barrier disruption, and mean arterial blood pressure 6 h after pneumococcal challenge

Group (n)	CSF leukocyte count (cells/μl)	Increase of intracranial pressure (mm Hg)	CSF Evans Blue (μg/ml)	CSF protein (g/l)	Mean arterial blood pressure (mm Hg)
Controls (3)	23 ± 13	0.2 ± 0.7	0.52 ± 0.81	0.45 ± 0.21	113 ± 10
Meningitis (7)	12 767 ± 2520[a]	11.6 ± 3.0[a]	28.8 ± 9.0[a]	2.87 ± 0.58[a]	109 ± 26
Meningitis + uric acid pre (5)	8376 ± 2450[b]	4.3 ± 1.2[b]	17.1 ± 8.3	2.31 ± 0.48	113 ± 9
Meningitis + uric acid + oxonic acid pre (6)	4190 ± 1749[b,c]	1.4 ± 2.4[b]	18.6 ± 3.9[b]	1.38 ± 0.85[b]	97 ± 22
Meningitis + uric acid + oxonic acid post 2 h (4)	3848 ± 4208[b]	0.4 ± 4.2[b]	11.8 ± 9.1[b]	1.96 ± 0.53[b]	91 ± 9
Meningitis + uric acid + oxonic acid post 4 h (4)	6375 ± 1969[b]	6.0 ± 0.9[b]	12.3 ± 3.5[b]	2.22 ± 1.12	112 ± 28

Data are mean values ± S.D. Detection limit for Evans Blue: 0.5 μg/ml.
[a] $P < 0.05$ compared with controls.
[b] $P < 0.05$ compared with untreated meningitis.
[c] $P < 0.05$ compared with uric acid-pretreated animals.

tions (Table 1). Furthermore, induction of meningitis caused an increase of CSF leukocyte counts, intracranial pressure, and parameters of blood–brain barrier disruption (CSF Evans Blue and protein concentration, Table 2).

Pretreatment of rats with intraperitoneal injections of uric acid significantly increased the plasma and further increased CSF uric acid concentrations (Table 1). Moreover, treatment with uric acid reduced the CSF leukocyte counts and the intracranial pressure compared with untreated animals with meningitis (Table 2).

Pretreatment with uric acid combined with oxonic acid (an inhibitor of urate oxidase) significantly further increased plasma and CSF uric acid concentrations (Table 1). In this experimental group, the CSF leukocyte count was even further reduced compared with animals treated with uric acid alone (Table 2). Furthermore, combined uric acid and oxonic acid significantly reduced the intracranial pressure and parameters of blood–brain barrier disruption (Table 2).

Post-treatment with uric acid and oxonic acid initiated 2 or 4 h after intracisternal injection of pneumococci caused an increase of plasma uric acid concentrations after the respective times of initiation of therapy (Table 1). Post-treatment significantly reduced CSF leukocyte counts, intracranial pressure, and CSF Evans Blue concentration at 6 h compared with untreated meningitis (Table 2).

4. Discussion

Over the last decade, there has been a substantial body of work implying that reactive oxygen species, such as superoxide anion, hydrogen peroxide, or hydroxyl radicals, and reactive nitrogen species, such as peroxynitrite, play a central role in the development of intracranial complications and brain damage in bacterial meningitis (reviewed in Koedel and Pfister, 1999). The strong oxidant peroxynitrite ($ONOO^-$) forms at sites where superoxide anion (O_2^-) and NO are simultaneously produced (Beckman and Koppenol, 1996). Peroxynitrite can be cytotoxic in bacterial meningitis by a number of mechanisms, including tyrosine nitration, lipid peroxidation, release of interleukin-8, and activation of matrix metalloproteinases (Koedel and Pfister, 1999; Filep et al., 1998; Maeda et al., 1998). Peroxynitrite was shown to be efficiently detoxified by uric acid (Hooper et al., 1998), which comprises 30–65% of the total peroxyl radical-scavenging ability of human plasma (Wayner et al., 1987).

Rats, like most non-primates, have low blood levels of uric acid due to its rapid metabolism by the hepatic enzyme urate oxidase to allantoin, which does not have antioxidant properties (Whiteman and Halliwell, 1996). The low plasma concentrations of uric acid in uninfected and infected rats are in the range reported for rats (20–100 μM) (Nomikos et al., 1994). In control animals, CSF urate was much lower than in plasma, confirming that the blood–brain barrier is normally relatively impermeable to uric acid (Hooper et al., 2000). Induction of meningitis caused an increase of CSF uric acid, probably due to an increase in purine metabolism or leakage through the disrupted blood–brain barrier. Repeated i.p. injections of 300 mg/kg uric acid caused a significant additional increase of its CSF concentration, suggesting that exogenously administered uric acid penetrates the already compromised blood–central nervous system barrier during meningitis. In all animals treated with uric acid plus oxonic acid, the concentration of uric acid in CSF exceeded that in plasma, signifying an intracranial net production of uric acid. The only reported functions of oxonic acid are the competitive inhibition of the hepatic peroxisomal enzyme urate oxidase and the blockade of an electrogenic uric acid transporter/channel that has some homology with urate oxidase (Leal et al., 1999). The mRNA of this uric acid transporter/channel has been detected in the

rat brain (Leal et al., 1997). Therefore, it is conceivable that oxonic acid contributes to the increase of CSF uric acid by inhibiting uric acid transport from the central nervous system. In view of these findings, we attribute the greater beneficial effects of the combination therapy (uric acid + oxonic acid) to the increase of plasma and/or CSF concentrations of uric acid.

In conclusion, our findings indicate that uric acid can attenuate meningitis-associated intracranial complications at blood levels in the human physiological range, suggesting that uric acid may also be an efficient endogenous modulator of central nervous system inflammation and complications during human bacterial meningitis.

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