Treatment with Uric Acid Reduces Infarct and Improves Neurologic Function in Female Mice After Transient Cerebral Ischemia

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Background: Exogenous administration of uric acid, a naturally occurring antioxidant that scavenges reactive oxygen species in vasculature, has shown protective efficacy in both rodent models of stroke and human stroke patients in Spain as an adjuvant treatment to mechanical thrombectomy. Before clinical trials can be initiated in the United States, however, confirmation of efficacy in alternative preclinical models is required in accordance with stroke therapy academic industry roundtable-RIGOR criteria. To date, preclinical efficacy has only been established in the acute setting in male rodents. *Methods:* To address this need, we subjected 7- to 9-week old ovariectomized female mice to filament-induced right middle cerebral artery ischemia and reperfusion, an established preclinical model of mechanical thrombectomy. Fidelity of the procedure was monitored by laser Doppler flowmetry. A separate lab randomly assigned animals to vehicle versus uric acid infusion, which was initiated immediately after 45 minutes of reperfusion. Poststroke analysis of infarction size and neurologic function were conducted by investigators blind to treatment group, with a 7-day primary endpoint and a 3-day intermediary analysis at 1and. *Results:* Infarct size and neurologic function at 7 days poststroke were significantly improved in uric acid-treated animals, relative to vehicle. *Conclusion:* Efficacy of uric acid in preclinical models of stroke is now expanded to include female mice analyzed at a later time point than has been investigated previously. These results support stroke therapy academic industry

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roundtable-RIGOR driven determination of the suitability of acute administration of uric acid as an adjuvant to mechanical thrombectomy in clinical trials for patients with stroke. **Key Words:** Stroke—animal model—uric acid—neuroprotection. Published by Elsevier Inc. on behalf of National Stroke Association.

Introduction

Uric acid (UA), which is most well-known as a renally-excreted oxidation end-product of purine metabolism, also functions as an endogenously-generated antioxidant during hypoxia that scavenges peroxynitrite derivatives and hydroxyl radicals in blood vessels.[1] During severe ischemia, however, endogenous UA reserves are rapidly depleted, leading to failure of this natural defense system.[2] A novel strategy proposed for treating patients after stroke thus involves augmenting serum UA levels through exogenous administration. This is supported by epidemiologic data, including a meta-analysis of 8131 patients with ischemic stroke in which patients showing high baseline levels of serum UA at the time of stroke onset were noted to have better clinical outcome.[3] In addition, a phase 2b/3 intervention trial conducted in Spanish centers with 421 acute ischemic recombinant tissue plasminogen activator-treated patients with stroke who were randomized to acute intravenous supplementation of either 1 g of UA or placebo showed that UA was well-tolerated and conferred favorable outcome in 39% of patients, compared with 33% of patients treated with placebo (adjusted relative risk 1.23 (.96-1.56)).[4] Infusion of UA has also been shown to improve outcomes in a prespecified population of patients treated with both recombinant tissue plasminogen activator and mechanical thrombectomy (MT), with 67% of UA-assigned patients achieving a favorable outcome compared with 48% of placebo-assigned patients (odds ratio 6.12; 1.08-34.6).[1] These results are consistent with a role for UA in mitigating reperfusion-induced oxidative stress, and call for additional confirmatory trials in patients revascularized with MT.[4,5] In accordance with established STAIR-RIGOR criteria for National Institutes of Health-supported translational research in the United States,[6] a promising intervention like UA needs evaluation in multiple preclinical models that incorporate numerous variables, including longer periods of observation and both sexes.[6] In the case of UA, however, preclinical studies before this work evaluated only short-term efficacy in male animals.[7-9] Thus, we have now addressed this critical need by evaluating the neuroprotective efficacy of exogenously supplied UA in female mice with 1 week outcome measures.

Methods

Animals

Ovariectomized female C57BL/6J mice (7-9 weeks old; ~ 20-23 g) from Charles River, Wilmington, MA were housed in standard conditions with controlled temperature and humidity, and ad libitum food and water. University of Iowa Animal Care and Use Committee approved all procedures. Procedures were performed according to Animal Research: Reporting of *In Vivo* Experimental Guidelines (https://www.nc3rs.org.uk/arrive-guidelines).

Cerebral Ischemia and Reperfusion

Focal cerebral ischemia was induced by right middle cerebral artery occlusion for 45 minutes.[10] Mice were anesthetized with 1%-1.5% isoflurane mixed with medical air. After midline incision, the right common carotid artery (CA) was temporarily clamped and a 6.0 siliconized filament (Doccol Corporation, Sharon, MA) was inserted in the external CA and advanced to the internal CA up to the middle CA origin. Reperfusion was achieved by filament removal after 45 minutes, opening the common CA. Throughout surgery, body temperature was maintained at 37°C ± 1.0 using a heating pad. Buprenorphine (.1 mg/kg, subcutaneous) was administered every 6-12 hours for 48 hours postsurgery. Laser Doppler flowmetry (Perimed, Stockholm, Sweden) measured degree of ischemia and reperfusion. Inclusion criteria included (1) greater than 70% regional cerebral blood flow reduction during ischemia, (2) return of regional cerebral blood flow to 80%-120% of baseline after filament removal, and (3) animal survival at least 7 days after stroke.

Uric Acid (UA) Administration

Mice were randomized to 20-minute jugular vein infusion of UA (16 mg/kg body weight based on previous reports,[8,11] Sigma U0881, prepared daily) or vehicle (Locke's buffer) starting 45-minute postreperfusion. The investigator conducting the surgery was blinded to treatment group.

Magnetic Resonance Imaging (MRI) and Infarct Volume Quantification

MRI 1, 3, and 7 days after stroke was employed to monitor infarct evolution, with primary analysis at day 7. Animals were sedated with isoflurane (2.5% induction, 1.2% maintenance) and placed in 7.0 Tesla MRI (Agilent Technologies Inc., Santa Clara, CA) bore with 2-channel receive-only surface coil. Following scout scans to orient imaging planes, high-resolution images were acquired with 9-minute T2-weighted 2D fast spin–echo pulse sequence oriented coronally. Imaging parameters included TR/TE = 6380 ms/83 ms, echo train length of 12