Stroke

BASIC AND TRANSLATIONAL SCIENCES

Uric Acid Stroke Cerebroprotection Transcended Sex, Age, and Comorbidities in a Multicenter Preclinical Trial

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BACKGROUND: Past failures in translating stroke cerebroprotection provoked calls for a more rigorous methodological approach, leading to the stroke preclinical assessment network SPAN (Stroke Preclinical Assessment Network), where uric acid (UA) treatment exceeded a prespecified efficacy boundary for the primary functional outcome. Still, successful translation to humans requires confirmation of the effect of UA across key biological variables relevant to patients with stroke.

METHODS: We measured the effects of intravenous UA treatment (16 mg/kg) versus intravenous saline in groups of animals enrolled in the SPAN network with diverse comorbidities, sex, and age. The masked study drug or placebo was administered during reperfusion in rodents undergoing a transient middle cerebral artery filament occlusion. The primary outcome was the modified corner test index at day 30 poststroke, and numerous secondary outcomes were collected. A modified intention-to-treat population was used in the analysis. We tested for any interactions with sex, age, and comorbidities (obesity-induced hyperglycemia and hypertension).

RESULTS: In total, 710 animals were randomized to receive either intravenous UA or saline. After accounting for procedural dropouts and exclusions from treatment, a total of 687 animals were qualified and analyzed, including 458 assigned to UA and 229 to intravenous saline control. UA-treated animals exhibited a better primary functional outcome at day 30 (probability, 0.56 [95% CI, 0.52–0.60]; P=0.006). UA-treated animals also had a better corner test index at day 7 (probability, 0.55 [95% CI, 0.5–0.59]; P=0.035) and a higher survival rate at day 30 (hazard ratio, 1.41 [95% CI, 1.08–1.83]; P=0.011). Brain morphometry at day 2 and 30 was comparable between the treatment groups. The improved functional outcome and survival in UA-treated animals were preserved across different species, sexes, ages, and comorbidities.

CONCLUSIONS: UA provides ischemic stroke cerebroprotection across key relevant biological variables, making it a promising intervention to be further tested in human clinical trials.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: intention to treat analysis ■ ischemic stroke ■ middle cerebral artery ■ sex ■ uric acid

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Nonstandard Abbreviation and Acronym

AIS	acute ischemic stroke
CONSORT	Consolidated Standards of Reporting Trials
ITT	intention-to-treat
MRI	magnetic resonance imaging
MT	mechanical thrombectomy
NDS	neurological deficit score
SPAN	Stroke Preclinical Assessment Network
tMCAo	transient middle cerebral artery occlusion
UA	uric acid

Stroke is the second-leading cause of death and the third-leading cause of combined death and disability worldwide.[1] The currently approved therapies for acute ischemic stroke (AIS), intravenous thrombolysis, and mechanical thrombectomy (MT) focus exclusively on recanalizing the occluded artery. Although pursuing recanalization is critical, it is not sufficient because only less than half of the patients with AIS treated with MT achieve long-term functional independence.[2] This degree of functional dependence after MT creates a critical need to identify additional strategies to maximize the benefits of reperfusion. Previous cerebroprotection trial failures might be blamed on having been administered without concomitant reperfusion therapy[3] or have chosen therapies with unclear pathophysiological relevance,[4] because not all events of the ischemic cascade contribute equally to the fate of brain cells.[5] A substantial amount of early brain damage that occurs through the ischemic cascade is attributed to the generation of free radicals.[5] Current evidence suggests that peroxynitrite (ONOO−), a compound resulting from the simultaneous generation of nitric oxide and superoxide in anatomic proximity, is the most relevant free radical during AIS.[6,7] Furthermore, free radical production increases if there is reperfusion of the occluded vessel, contrary to what happens with other ischemic pathophysiological mechanisms such as glutamate excitotoxicity.[8] Importantly, unlike nitric oxide and superoxide, peroxynitrite does not have any known physiological functions that could be disrupted while being targeted.

Uric acid (UA), the end-product of the catabolism of purines, is a potent natural scavenger of oxidative species in humans, including peroxynitrite, hydroxyl radicals, and hydrogen peroxide. UA accounts for as much as two-thirds of the total antioxidant plasma capacity.[9] The endogenous UA provides some cerebroprotection against an AIS, but this natural antioxidant capacity is insufficient due to the rapid decrease of endogenous UA levels after a stroke.[10] This justifies the rationale for administering exogenous UA, to compensate for its consumption, as a cerebroprotectant strategy.[11] A meta-analysis of 14 preclinical studies showed that UA treatment significantly reduced infarct volume and neurofunctional deficit in animal models of ischemic stroke.[12] These promising preclinical results might still be seen with skepticism given the history of 114 translation failures from rodents to humans that were blamed on insufficient methodological rigor.[13] In fact, only 57%, 21%, and 50% of those studies of UA reported randomization, group allocation, or allocation concealment, respectively.[12] To maximize the chances of successful translation of UA treatment to patients, we sought additional supportive evidence for UA through our involvement with the SPAN (Stroke Preclinical Assessment Network).[14] In this platform, UA treatment exceeded the prespecified efficacy boundary to SPAN while tested in parallel with the other 5 interventions.[15] Here, we report the specific results of a randomized trial comparing UA versus placebo within the SPAN platform. To assess the reproducibility of these preclinical results to patients with stroke,[16] we specifically measured the effect of UA across relevant baseline characteristics such as sex, age, and traditional stroke comorbidities.

METHODS

The data will be available upon request from the corresponding authors.

Study Population

The SPAN 1.0 study included a total of 2615 animals and 6 interventions.[15] For this analysis, we selected a sample from the modified intention-to-treat population that included all animals who underwent the transient middle cerebral artery occlusion (tMCAo) procedure and were randomized to receive either intravenous (IV) UA treatment or intravenous saline control. Although SPAN combined intravenous and intraperitoneal controls, for this analysis, we used the intravenous saline controls as a comparison to avoid potential confounding related to the route of administration. This modified intention-to-treat population included male and female young healthy mice, aging mice, and obesity-induced hyperglycemic mice, as well as young healthy rats and spontaneously hypertensive rats. We used mice of the C57BL/6J strain, purchased from Jackson Laboratory. Young healthy mice were 10 to 12 weeks old and aging mice (donated by National Institute on Aging) were 15 to 17 months old on the day of tMCAo. To induce obesity, mice were fed a high-fat diet (60% high-fat, TD.06414; Teklad) starting at 4 to 5 weeks of age for 12 weeks before tMCAo. Young healthy rats and spontaneous hypertensive rats were purchased from Charles River and were 10 to 12 weeks on the day of tMCAo. All animal procedures were approved by the institutional animal care and use committee at each testing laboratory. All studies were performed according to the current ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiment; https://www.nc3rs.org.uk/arriveguidelines).

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Experimental Procedures

Filament Model of Cerebral Ischemia

Focal cerebral ischemia was induced by transiently occluding the right middle cerebral artery, as previously described.[14] Briefly, animals were anesthetized with isoflurane (4% induction, 1% to 2% maintenance in 70:30 N_2O: O_2). After a midline incision, the right common carotid artery was temporarily ligated, and a silicon monofilament (Doccol Corp, Sharon, MA) was inserted via the external carotid artery into the internal carotid artery up to the origin of the middle cerebral arter. The common carotid artery was opened during the ischemic period. Reperfusion was achieved by removing the filament after 1 hour (for all mice and spontaneous hypertensive rats) or 2 hours (for young healthy rats). These times were chosen a priori to get adequate infarct size in young healthy rats and reduce mortality in spontaneous hypertensive rats. Perisurgical pain management procedures have been previously described.[14] Bupivacaine and postoperative fluids were given subcutaneously and water-softened chow was offered to animals that could not properly maintain hydration or feed.

Study Intervention

All the interventions used for this study were previously lyophilized, bottled, labeled, and shipped in a blinded way from the SPAN Coordinating Center. The vials assigned to active treatment contained UA with the same formulation and excipients used by clinical trials. It consisted of 0.2 g of UA (No. 1056800250; Sigma), 0.1 g of lithium carbonate (No. 1056800250; Sigma), 5.0 g of mannitol (No. M8429-100G; Sigma), 0.01 g of disodium ethylenediaminetetraacetic acid (No. E1644-100G; Sigma), 100 mL of water, and CO_2: pH 7.0 to 7.5.[17] The vials assigned to the control group contained saline and had an identical appearance. After reconstitution in water, study material contained either 16 mg/kg of UA or saline, and a single intravenous treatment was initiated 5 minutes before middle cerebral artery reperfusion. The infusion was maintained for a total of 20 minutes.

Magnetic Resonance Imaging of the Brain

Magnetic resonance imaging (MRI) was performed at all sites following previously described standardized acquisition procedures[14,15] at days 2 and 30 poststroke under isoflurane anesthesia (3% induction, 1% to 2% maintenance in 70:30 N_2O:O_2). The T2 and apparent diffusion coefficient maps were obtained by a series of spin-echo and diffusion-weighted images (field of view 19.2 mm in-plane, 15 mm in-slice, 0.5 mm slice thickness). Images were uploaded to the Image and Data Archive (powered by LONI) for blinded and automated analysis. The day 2 MRI scans were used to assess infarct volume, brain volume, and ventricular (ie, cerebrospinal fluid) volume, whereas the day 30 MRI scans were used to assess brain volume and ventricular (ie, cerebrospinal fluid) volume. The midline shift was calculated to estimate swelling or atrophy.

Survival

Animals were observed for survival daily up to day 30 poststroke, which included day 30 MRI.

Functional Outcomes

All functional outcomes were measured by investigators blinded to the treatment assignment.[14,15] Before the behavior tests, animals were acclimated in the behavior testing room for at least 1 hour. Tests were conducted as early or late in the day as possible to minimize disrupting the sleep/wake cycle.

Corner Test

The sensorimotor outcomes were recorded and analyzed at baseline (1-week preceding tMCAo), day 7 (±1 day), and day 30 (±2 days) poststroke, as previously described.[14,15] Briefly, the test surfaces were thoroughly cleaned with 70% v/v ethanol between animals. Mice/rats were placed between the boards of the appropriately sized apparatus facing the corner top. Animals were carefully maneuvered to avoid overhandling or unilateral whisker contact with the boards, which could influence the turning preference. A turn was considered complete when the animal entered far enough into the corner, both vibrissae touched the boards, and then its head turned ≥90 degrees. The test ended when the animal successfully completed 10 turns. All corner tests were video recorded and uploaded to the Image and Data Archive for offline analyses by the network. The Coordinating Center anonymized and assigned each video to 3 certified raters from another site for blinded offline analysis. Raters recorded the number of right and left turns. Results were expressed as a corner test index (asymmetry index), calculated as the absolute value of [(left turns—right turns)/(left turns+right turns)]. This index represents both inversive and contraversive turning as abnormal.[15] The corner test recorded at day 30 was selected as the primary outcome measure, whereas the test recorded at day 7 was selected as the secondary outcome.

Neurological Deficit Score

SPAN used a modified neurological deficit score (NDS),[14] which included weight bearing and barrel rolling as abnormal ratings. The NDS was measured at days 1 and 2 poststroke.

Grid Walk Test

To assess the deficit of limb movements in poststroke animals, the grid walk test was performed at day 7 and day 30 poststroke, as previously described.[15] Briefly, the animal was placed in the middle of the grid with 1-inch (for mice) or 2-inch (for rats) square openings on sturdy supports. The animal was allowed to explore the surface freely for 5 minutes without any stimulation. The grid surface was thoroughly cleaned with 70% v/v ethanol between the animals. All grid walk tests were video recorded and uploaded to the Image and Data Archive for offline analyses by the network. The Coordinating Center anonymized and assigned each video to a certified rater from another site for blinded offline analysis. Raters recorded the number of steps and foot faults made by the animal. Results were expressed as a grid walk test index, calculated as the % of foot faults made by the left rear foot out of the total number of steps made by the left rear foot. The left foot was chosen as the affected (contralateral) side by right-sided tMCAo.

Statistical Analysis

The overall statistical plan and sample calculation of the trial were previously described.[14] Data for functional and imaging end points were displayed using violin plots combined with

box plots. Imputed missing data was not included in the plots. Kaplan-Meier curves were generated for the UA-treated and control groups. Treatment effects for functional and imaging end points were estimated using probabilistic index models,[18] which are a generalization of the Mann-Whitney *U* test that incorporates covariates. Based on the probabilistic index models, we estimated the probability that a random animal from the UA group has a better outcome than a random animal from the IV saline control group for both all animal subpopulations jointly and each subpopulation specifically. When the relative effect size is 0.5, there is no difference between study arms. The treatment effect for overall survival was modeled using Proportional Cox regression models to estimate hazard ratios with 95% CIs. Interaction and stratified analyses were performed to estimate the treatment effect within subgroups. In the interaction analysis, the differential treatment effect was assessed among mouse animal models and rat animal models when appropriate. *P* values for the interaction effect were obtained using a Wald test for (1) the models with interaction between sex and treatment, (2) the models with interaction between rat models and treatment, and a likelihood ratio test for the model with interaction between mouse models and treatment. Stratified analysis assessed treatment effect (1) for each animal model separately, (2) for each sex separately, and (3) for the combination of each animal model and sex separately. All models incorporated the following covariates as fixed effects when not used as a stratification factor in the analysis: site, sex, and animal model. For the corner test index, the models were also adjusted for the baseline corner test index. Treatment effects were conditional. In accordance with the best practices for multicenter blinded clinical trials, data analysis for behavioral outcomes where the death of the animal has an inseparable effect, we have implemented for the analysis of functional outcomes the worst-rank score imputation approach,[19] whereas in the case of NDS and brain imaging (MRI), missing data were not imputed. The worst-rank score imputation implies that we would apply the worst possible outcome for the animals that died during the study for any reason in both study groups. The worst-rank score was calculated within each study arm, animal model, and sex. For corner test and grid walk test indexes at day 30, outcomes at day 7 were used when animals had missing data at day 30 for any other reason that is not death. For all other cases, the animal was deleted from the analysis.

Calculations were performed using R, version 4.0 or higher. All hypotheses were tested at the 5% significance level. No multiplicity adjustment was performed.

RESULTS

Study Population

To address any concerns for attrition biases we reported this modified intention-to-treat analysis using the recommendations of the CONSORT guidelines (Consolidated Standards of Reporting Trials; Figure 1).[20] Out of the

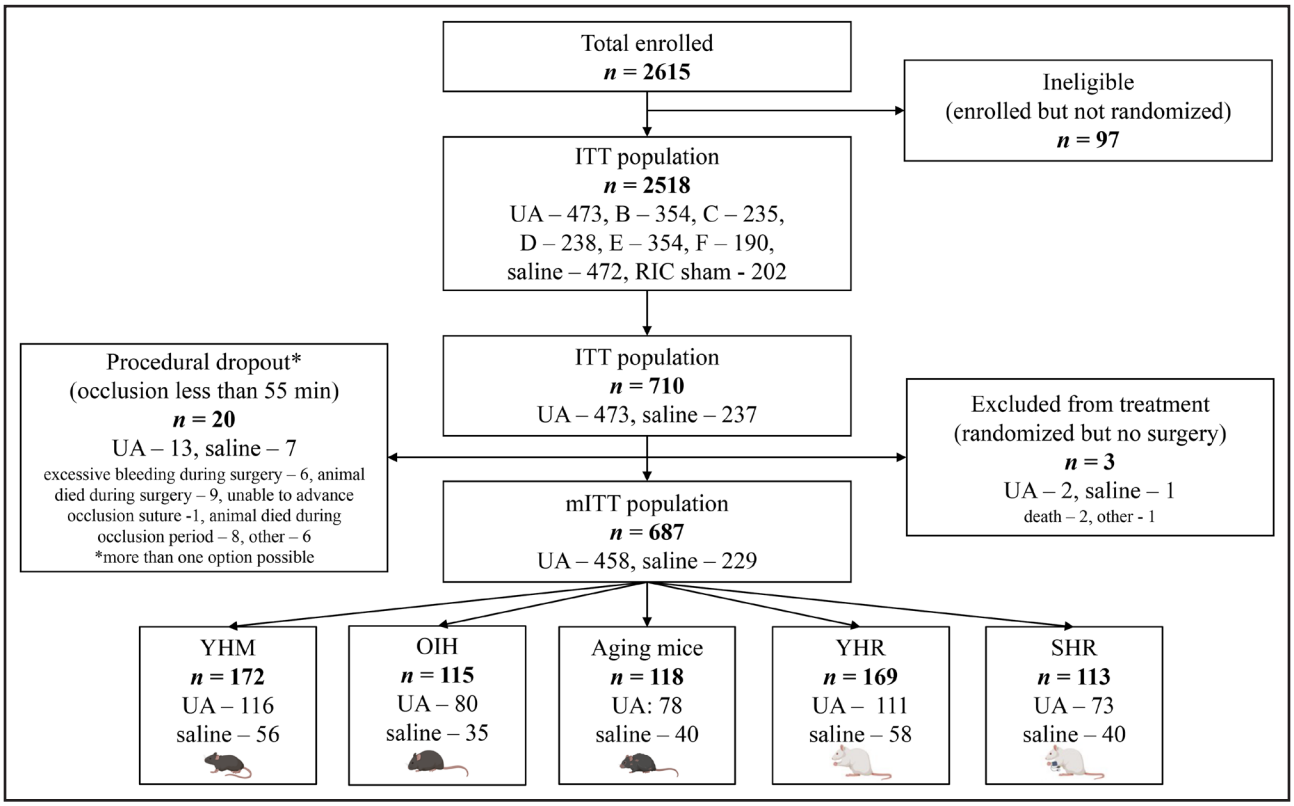

Figure 1. CONSORT diagram (Consolidated Standards of Reporting Trials) for study subpopulations.
B, C, D, E, and F denote other study interventions; RIC sham denotes control group for the RIC study group. ITT indicates intention-to-treat; mITT, modified intention-to-treat; n, number; OIH, obesity-induced hyperglycemia; RIC, remote ischemic conditioning; SHR, spontaneous hypertensive rats; UA, uric acid; YHM, young healthy mice; and YHR, young healthy rats.

2615 animals enrolled in the global SPAN 1.0 study,[15] we first identified all 710 animals randomized to receive either intravenous UA or vehicle (saline). After accounting for procedural dropouts and animals excluded from treatment due to death during surgery or occlusion, inability to advance filament, or no change in blood flow, a total of 687 (96.7%) animals were qualified and analyzed, including 458 assigned to UA and 229 to IV saline control. There were no baseline characteristic differences between the animals randomized to the UA-treated group and control (Tables S1 and S2). The smaller size of the IV control group compared with the UA intervention is explained by the SPAN trial design, which included a mixture of IV and intraperitoneal controls to adequately test different interventions across all study stages.

Effect of UA Treatment on the SPAN Primary Functional Outcome

By a priori consensus, SPAN decided to choose the modified corner test index at day 30 poststroke as the primary long-term outcome measure of this trial. The corner test measures sensorimotor asymmetry and can discriminate functional outcomes as evaluated in pilot studies (not shown). The UA-treated animals demonstrated a higher probability of a better (lower) corner test index compared with the saline at day 30 poststroke (probability, 0.56 [95% CI, 0.52–0.6]; *P*=0.006; Figure 2).

UA Treatment Improved Primary Outcome Across Different Species, Age, Sexes, and Comorbidities

We analyzed whether the beneficial effect of UA treatment on corner test outcomes was preserved among 5 distinct study subpopulations that differed in species, ages, and comorbidities. The analysis showed no difference in UA effect on the corner test index between mouse models (treatment-by-subpopulation interaction, *P*=0.253; Figure 3A) and rat model (treatment-by-subpopulation interaction, *P*=0.634; Figure 3B), suggesting that the cerebroprotective effect was preserved in young, aging, and obese mice, as well as young and hypertensive rats. Likewise, the evaluation of sex-specific treatment effects of UA on corner test outcomes revealed no difference between sexes (treatment-by-sex interaction, *P*=0.391; Figure 3C), indicating that UA was equally effective in improving the corner test index in both male and female animals.

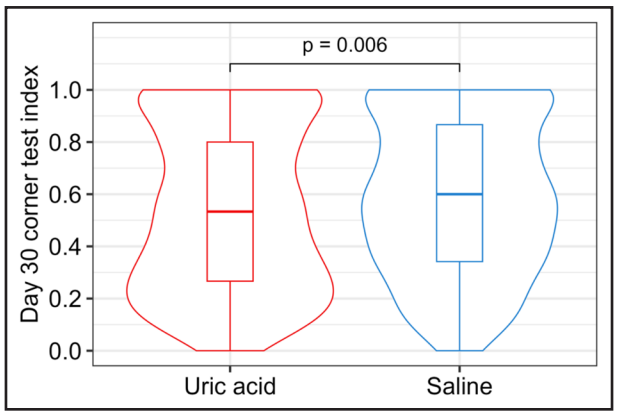

Figure 2. The effect of uric acid treatment on SPAN (Stroke Preclinical Assessment Network) primary outcome (day 30 corner test index) for the overall study population.
The data are presented as median and range without missing data and displayed as violin plots combined with box plots. Statistical analysis: probability index using a worst-rank score missing data imputation.

Effect of UA Treatment on Secondary Functional and Morphological Outcomes

We also evaluated the cerebroprotective effect of UA on secondary SPAN outcome measures. This included functional outcomes, like the modified corner test index at day 7 poststroke. We found a similar higher probability of a better (lower) corner test index compared with the saline (probability, 0.55 [95% CI, 0.5–0.59]; *P*=0.035; Figure 4A). The grid walk test showed similar scores in both study groups at day 7 and day 30 poststroke (Figure 4BC). UA treatment improved (lowered) the corner test index at day 7 poststroke to a similar extent in both mice (treatment-by-subpopulation interaction, *P*=0.283) and rats (treatment-by-subpopulation interaction, *P*=0.657). Likewise, the evaluation of sex-specific treatment effects of UA on corner test outcomes revealed no difference between sexes (treatment-by-sex interaction, *P*=0.654; Figure S1). MRI analysis of brain morphometry, including lesion volume, tissue volume, cerebrospinal fluid volume, and midline shift, at day 2 and day 30 poststroke did not reveal statistically significant differences between the UA and saline control groups (Figure 5; Figure S2). The NDS evaluated at day 1 and day 2 poststroke was also comparable between the groups (Figure S3).

UA Treatment Improves Long-Term Survival After Ischemic Stroke

UA infusion demonstrated a treatment effect on survival compared with saline control within day 30 poststroke. The overall 30-day hazard ratio was 1.41 ([95% CI, 1.08–1.83] *P*=0.011; Figure 6), indicating that the UA-treated animals had a significantly higher survival probability compared with the control. Further analysis showed no differences in survival across different animal subpopulations (treatment-by-subpopulation interaction, *P*=0.264 for mice and *P*=0.766 for rats) and sexes (treatment-by-sex interaction, *P*=0.481; Figures S4 through S6). Thus, the effect of UA treatment on poststroke survival was independent of species, age, comorbidities, and sex.

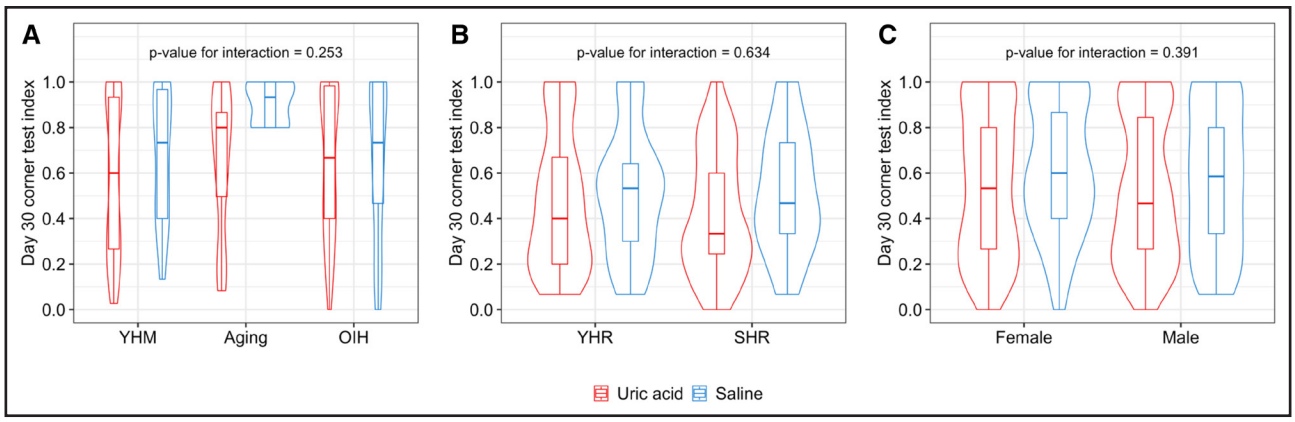

Figure 3. Effect of uric acid (UA) treatment on the day 30 corner test index according to different animal characteristics.
A, Effect of UA treatment in young healthy mice (YHM), aging mice, and obesity-induced hyperglycemic (OIH) mice. **B**, Effect of UA treatment in young healthy rats (YHR) and spontaneous hypertensive rats (SHR). **C**, Effect of UA treatment in female and male rodents (combined mice and rats). The data are presented as median and range without missing data and displayed as violin plots combined with box plots. Statistical analysis: probability index with worst-rank score missing data imputation.

DISCUSSION

The SPAN infrastructure is an enhanced paradigm for preclinical research, which revives interest in stroke cerebroprotection. Following a clinical trial approach, SPAN was led by a national Coordinating Center that prepared and masked the interventions and similar-looking intravenous placebos to prevent ascertainment bias. Adequate power was ensured using a prior sample size calculation. All animals were randomized to treatment or placebo to prevent selection biases. Attrition biases were prevented by using a modified intention-to-treat analysis with missing data imputation. The primary outcome was assessed by blinded investigators at independent centers to prevent detection biases. The purpose of SPAN was to select candidate cerebroprotectants that succeeded in such a rigorous testing platform and propose them as more likely to translate to humans undergoing ischemia-reperfusion.

In this new rigorous research context, UA treatment demonstrated stroke cerebroprotection, as shown by the results of the primary outcome. Importantly, the cerebroprotective effect of UA treatment was independent of sex, animal species, age, obesity and hyperglycemia, and hypertension. The significant effect of UA on the corner test, which assesses sensorimotor function and postural asymmetry, suggests that the cerebroprotective action of UA may impact brain regions crucial for these functions, such as the striatum and sensorimotor cortex.[21] Similar to a clinical trial, this outcome measure was chosen before initiating the trial by consensus among the SPAN investigators. This choice of a functional primary outcome was responsive to recommendations from the stroke community of giving priority in preclinical experiments to long-term functional outcomes to better replicate the patient experience.[22] The clear relevance of the long-term functional primary outcome, the magnitude of the effect of this intervention, and the consistency of

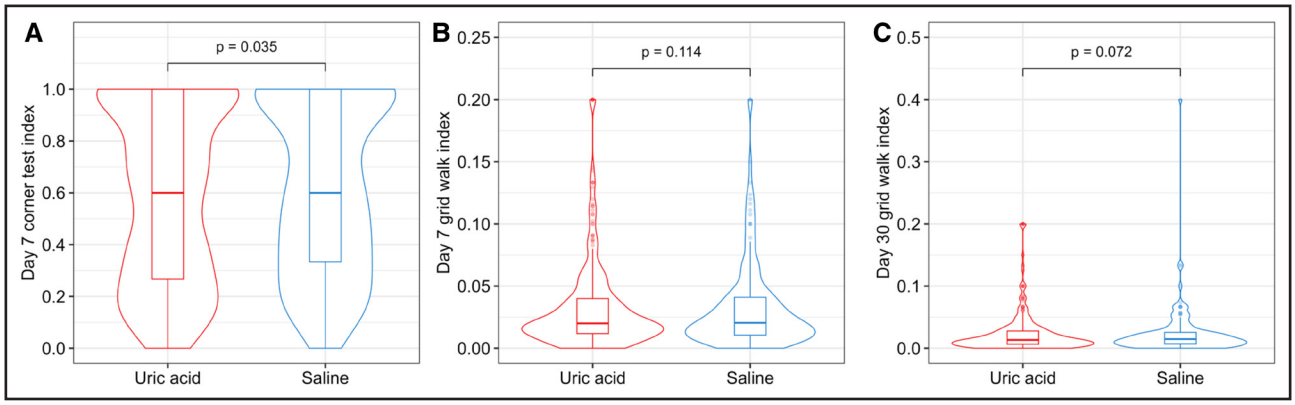

Figure 4. The effect of uric acid (UA) treatment in secondary functional outcomes.
A, Effect of UA treatment in corner test index at day 7 poststroke. **B** and **C**, Effect of UA treatment in grid walk index at days 7 and 30 poststroke. The data are presented as median and range without missing data and displayed as violin plots combined with box plots. Statistical analysis: Probability index with worst-rank score missing data imputation.

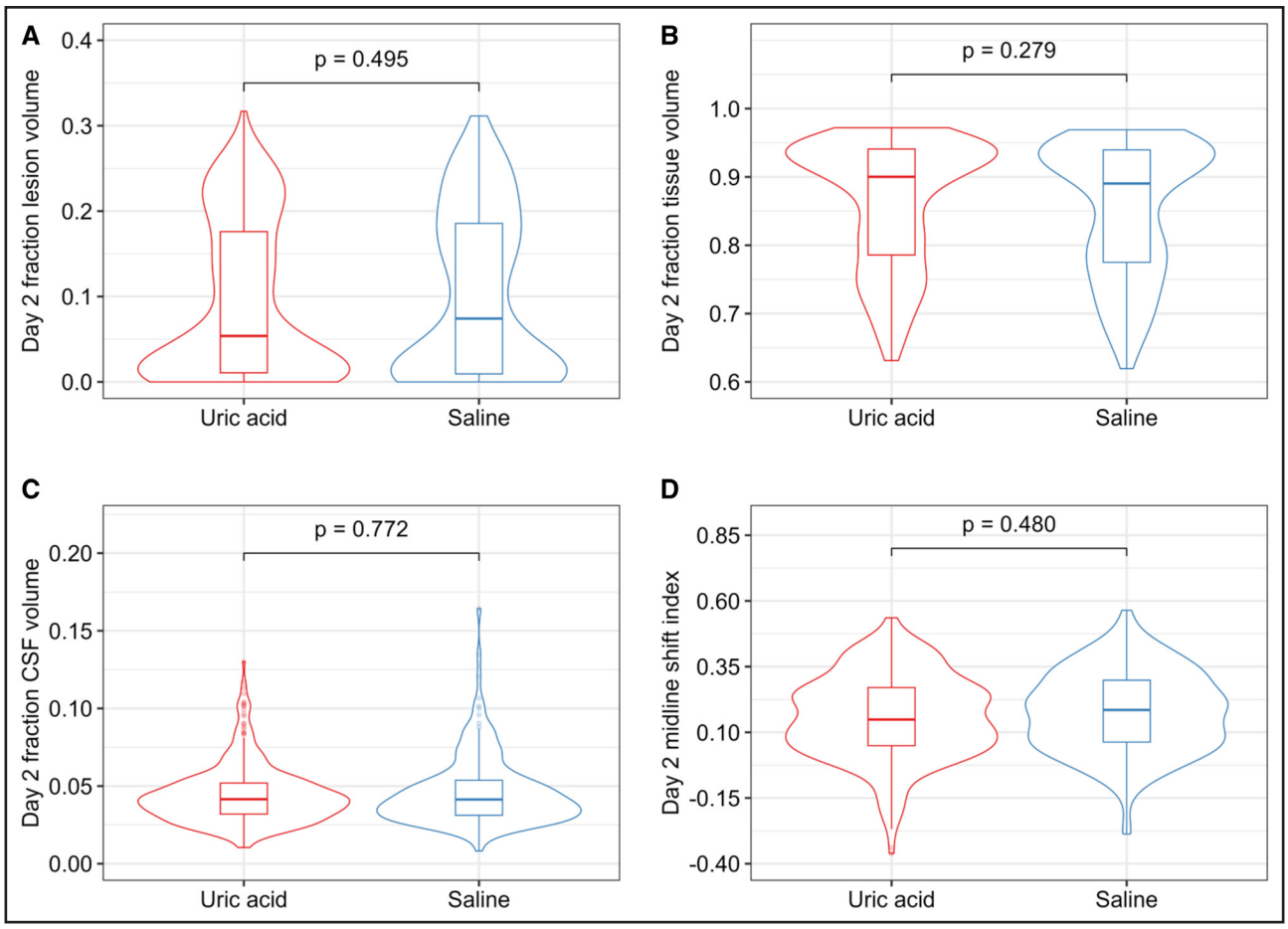

Figure 5. Effect of uric acid (UA) treatment on brain morphometry outcomes at day 2 poststroke.
Effect of UA treatment in (**A**) fraction lesion volume; (**B**) fraction tissue volume; (**C**) cerebrospinal fluid; and (**D**) the midline shift index. The data are presented as median and range without missing data and displayed as violin plots combined with box plots. Statistical analysis: Probability index without missing data imputation.

effect across different subgroups of subjects enhance the confidence in these positive results.[23]

Importantly for the translational potential, these results are not only consistent with the previous preclinical experiments[12] but also with the findings of human experiments. Previous epidemiological observational evidence indicates a clear beneficial association between higher endogenous levels of UA at the onset of stroke, with a significantly more favorable prognosis. A meta-analysis of 8131 patients with AIS confirmed that high serum UA levels at stroke onset were associated with significantly better outcomes.[24] A recent meta-analysis of 23 dose-response studies, including a total of 15 733 patients with AIS, revealed that a 50-µmol/L incremental increase in UA concentration was associated with a 7% lower risk of 90-day unfavorable outcome (modified Rankin Scale score of ≥2; odds ratio, 0.930 [95% CI, 0.875–0.990]; I^2=0%; n=3), after accounting for the relevant covariates.[25] These observations in patients with stroke led to intervention studies. URICO-ICTUS (Efficacy Study of Combined Treatment With Uric Acid and r-tPA in Acute Ischemic Stroke) was a phase II/III double-blind, randomized, placebo-controlled study that assessed the combination of intravenous 1000 mg of UA (equivalent to the 16 mg/kg used in rodents) in 411 patients with acute stroke also treated with intravenous thrombolytics. The intervention

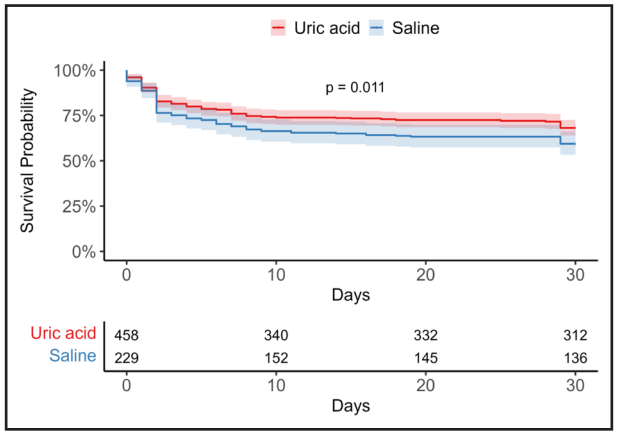

Figure 6. Effect of uric acid (UA) treatment on survival in the first 30 days after a stroke.
Kaplan-Meier curve for the UA treatment and control groups.

was well tolerated. The overall study results were neutral; 53% of patients in the UA group and 46% in the placebo group achieved good functional outcome (modified Rankin Scale score of 0–2 at 90 days).[17] However, UA therapy doubled the odds of attaining an excellent outcome in women (odds ratio, 2.088 [95% CI, 1.050–4.150]; *P*=0.036), but not in men (odds ratio, 0.999 [95% CI, 0.516–1.934]; *P*=0.997).[26] One of the reasons for better stroke outcomes in women could be due to lower uric levels at baseline compared with men. In contrast, this is not the case in rodents, which may partly explain the lack of an observed sex difference in SPAN.[27,28] Forty-five patients in the URICO-ICTUS trial also underwent MT, a de facto human model of ischemia-reperfusion.[29] In that context, an excellent functional outcome (modified Rankin Scale score of 0–2 at 90 days) was obtained in 67% of patients treated with UA and 48% treated with placebo (adjusted odds ratio, 6.12 [95% CI, 1.08–34]).[30] Altogether, these secondary analysis findings and our results highly support the cerebroprotective effect of UA in the specific clinical context of ischemia and reperfusion. These data may inform the design of future trials of UA by prioritizing patients treated with MT,[29] which is also aligned with the stroke community recommendations.[31]

The evaluation of secondary outcomes showed mixed results. While the corner test at day 7 and day 30 survival showed positive results, the grid test walk analysis, NDS, and MRI outcomes analysis demonstrated no difference. The positive corner test results at an earlier time point (day 7) enhance the validity of the primary outcome results using the same functional outcome measure. In addition, UA reduced stroke mortality at 30 days. Mortality is a highly relevant and objective outcome measure, particularly in a context driven by ischemic stroke and its complications. Conversely, we found no statistically significant difference between the groups in the grid walk test, which evaluates sensorimotor coordination of the performance of the 4 limbs. This test requires the integrity of more complex neural circuitry, including the cerebellum and corticospinal tracts.[32] It is also possible that the cerebroprotective effects of UA may be less pronounced in the subcortical brain structures that mostly contribute to the grid walking.[32] Another limitation of the grid walk data analysis is that, unlike the corner test, we lack baseline data to appropriately adjust the subsequent poststroke results. There could also be an issue with timing. Furthermore, there were also no differences in the NDS[33] in the first 2 days after stroke. Brain morphometry was also comparable between the UA and saline control groups. In addition, by only using IV controls, the most appropriate to evaluate an intravenous intervention such as UA, we might have lost some of the initial power that in SPAN was calculated based on a mixture of IV and intraperitoneal controls. As such, the early assessment of the neuroscore may not reflect the UA long-term therapeutic potential. We recognize that the absence of an effect on infarct volume is not consistent with the findings of prior studies.[12] The methodology to measure infarct volume in SPAN, however, was radically different.[14] Unlike the less detailed methods used by prior studies, SPAN used a complex infrastructure to automatically calculate those volumes.[14] In addition to that, the discrepancies between infarct volume and long-term clinical outcomes in stroke are well known,[34] and brain morphometric measures are not traditionally accepted as valid outcomes in stroke clinical trials. As such, the stroke field is prioritizing functional outcomes over morphometry. Based on the above, we suggest that the antioxidant effects of UA improved long-term functional outcomes in the absence of significant changes in gross brain morphometry.

CONCLUSIONS

The detailed analysis of the SPAN 1.0 multicenter preclinical trial demonstrates that the long-term cerebroprotective effect of UA on functional outcomes and survival is preserved across animals of different sexes, age groups, and comorbidities. These results validate the usefulness of including diverse animal subpopulations in translational stroke research and further support UA as a promising cerebroprotectant warranting evaluation in clinical trials of patients treated with MT that mimic the ischemia-reperfusion model.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S2
Figures S1–S6
The ARRIVE Checklist

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