EXPERT REVIEWS

Uric acid administration in patients with acute stroke: a novel approach to neuroprotection

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Uric acid (UA) is the end product of purine catabolism in humans and is a powerful antioxidant whose generation is increased under ischemic conditions. However, both clinical and experimental studies reveal a gradual exhaustion of the antioxidant capacity after transient cerebral ischemia, and the magnitude of this consumption seems to be correlated with the extent of brain tissue injury, growth of the infarction, severity of neurological impairment in the acute phase, and long-term functional outcome. Growing evidence supports the neuroprotective effect of UA administration after brain ischemia. In experimental conditions, the administration of UA is neuroprotective both in mechanical models of brain ischemia (transient or permanent intraluminal occlusion of the middle cerebral artery) and in thromboembolic models of autologous clot injection. The administration of UA is feasible and safe in healthy volunteers. In acute stroke patients treated with recombinant tissue plasminogen activator (rt-PA), co-administration of UA has proven to reduce lipid peroxidation and to prevent the fall in UA blood levels that occur very early after stroke onset. Currently, a multicentric Phase III clinical trial is testing whether the administration of UA increases the clinical benefits of rt-PA, which represents the only approved therapy in patients with acute ischemic stroke. This review summarizes the available information justifying such a novel therapeutic approach in this devastating clinical condition.

KEYWORDS: cerebrovascular disorder • clinical trials • neuroprotection • oxidative stress • stroke

Uric acid

Uric acid (UA) is a product of the catabolism of purine nucleotides, the principal constituents of DNA, RNA and cellular energy stores, such as ATP. In most mammals, UA is degraded by hepatic enzyme uricase (urate oxidase) to allantoin. In humans, the uricase gene is nonfunctional, owing to two parallel but distinct mutations occurring approximately 20 to 5 million years ago and resulting in higher serum UA levels [1]. Consequently, serum UA concentrations in humans are much higher than in most animals and at physiologic pH values serum monoanionic UA concentrations are close to maximum solubility [2–4]. It has been argued that the mutation of uricase in humans represents an evolutionary advantage over other primates owing to the antioxidant properties of UA that favor the capacity to maintain blood pressure under the low sodium dietary conditions of early hominoids [5], lengthening lifespan and decreasing age-specific cancer rates [6]. It has also been conjectured that the increased levels of UA may be an evolutionary antioxidant substitute for the loss of ability to synthesize ascorbate in higher primates [6].

Uric acid homeostasis

UA is ubiquitously distributed in the organism, being present intracellularly and in all body fluids, although at lower levels than in plasma. UA is not an inert molecule and it has been shown to have several biological actions that could either be beneficial or detrimental to humans, depending not only on its concentration but also on the surrounding environment, including ambiental and genetic factors [7]. The levels of UA in the blood are physiologically maintained by the

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balance between generation and excretion. Hyperuricemia, usually defined as greater than 6.5 or 7.0 mg/dl in men, and greater than 6.0 mg/dl in women, is more frequent in men, postmenopausal women, kidney disease patients, metabolic syndrome sufferers, alcohol drinkers, patients treated with thiazides or in subjects that follow a high purine diet. Other factors that can increase the generation of UA include conditions with high cell turnover, or enzymatic defects in purine metabolism. Markedly elevated serum levels of UA combined with specific environmental (especially dietary) and genetic factors may give rise to pathological conditions, especially related to precipitation in the form of urate crystals, such as kidney stones and gout.

The biosynthesis of urate is catalyzed by the enzyme xanthine oxidase (XO) and its isoform, xanthine dehydrogenase (XDH) [3,8]. Owing to its high serum levels in humans UA can also be oxidized by nonenzymatic metabolism to allantoin and other final products such as parabanate and alloxan [9,10]. The excretion of UA is realized by specialized transporters located in renal proximal tubule cells, intestinal epithelial cells and vascular smooth muscle cells. After filtration, UA undergoes both reabsorption and secretion in the proximal tubule by means of a urate/anion exchanger and a voltage-sensitive urate channel [11,12]. This process can be modified by organic anions such as lactate, which decrease urate secretion by competing for urate through the organic anion transporter, or substances such as probenacid and benziodarone that increase urate secretion [13]. The combined effects of efficient mechanisms for urate reabsorption together with the loss of uricase during hominoid evolution are responsible for the higher levels of urate in humans [14].

Oxidative stress & cerebral ischemia

Oxidative stress is defined as the alteration of the physiological balance between oxidation and antioxidation in favor of the former, which frequently results from an increased production of free radicals and other reactive chemical species with potential to damage the organism. Free radicals, such as anion superoxide ($O^{\bullet}_2$), hydrogen peroxide (H_2O_2), hydroxil radical ($OH^{\bullet}$), and the anion peroxinitrite ($ONOO^-$) are unstable and highly reactive molecules owing to the presence of at least one unpaired electron on their surface. Free radicals may produce injury through lipid peroxidation, reaction with proteins, nucleotide basis and DNA-repair enzymes, and reduction of nitric oxide ($NO^{\bullet}$) levels. The steady levels of free radicals are determined by the rate of its production and their clearance by endogenous scavenging antioxidant mechanisms, which include enzymes such as superoxide dismutase (SOD), glutathione peroxidase or catalase, and molecules such as UA, vitamins C and E, and glutathione [15].

The brain is particularly susceptible to oxidative damage owing to its relatively high oxygen consumption, high content in iron and lipids, and low content of protective antioxidant systems. The role of reactive oxygen species (ROS) in the pathogenesis of brain ischemia and reperfusion injury is well known [16]. The increased production of reactive oxygen free radicals, particularly superoxide anion, occurs rapidly after experimental ischemia and reperfusion [17], and is accompanied by a drop in the concentrations of tissue antioxidant levels [18]. In addition to direct toxic effects, the enhanced release of free radicals resulting from ischemia and reperfusion can modify the expression of several genes upregulating the redox-sensitive transcription factor nuclear factor-κB [19]. This mechanism may result in an increased expression of proinflammatory cytokines and endothelial adhesion molecules, such as selectins and intercellular adhesion molecule (ICAM)-1, which play a relevant role in the fate of brain tissue under ischemic conditions [20]. Moreover, inflammatory infiltrating cells may elaborate more ROS that increase oxidative stress and which are able to facilitate further damage. Several observational studies have described decreased antioxidant capacity in patients with acute stroke in parallel with reduced levels of vitamin C, vitamin E and SOD [21,22]. Urate concentrations may also decrease significantly over time in stroke patients, and the plasma antioxidant capacity measured as the total peroxyl radical trapping potential of plasma has been inversely correlated in these patients with the volume of cerebral infarction and the severity of neurological impairment [23].

The evidence in support of the deleterious role of oxidative stress in acute brain ischemia has led to the design of a number of therapeutic strategies aimed at increasing the antioxidant capacity of patients with acute ischemic or hemorrhagic stroke. Four antioxidant agents with free radical scavenging or trapping activity (tirilazad, ebselen, edaravone and NXY-059) have progressed into clinical trials to evaluate their neuroprotective action. Edaravone showed signs of clinical efficacy in a single placebo-controlled trial in a relatively small number of patients, which lead to its approval in Japan as a neuroprotectant for the treatment of acute stroke [24]. The free radical trapping agent NXY-059 arrived to the clinical scenario with promising preclinical data, but the clinical efficacy observed in the first Phase III clinical trial (SAINT-I) [25] was not confirmed in a second trial required by the US FDA (SAINT-II) [26]. Likewise, tirilazad showed negative results in patients with acute stroke [27], and definitive data regarding efficacy of ebselen is lacking. Likely reasons for the failure of these therapeutic strategies, excepting edaravone, could be found in the excessively long treatment window allowed in these trials (at least 6 h), and the lack of concomitant use of reperfusion therapies in most of the patients investigated [28]. Overall, none of the antioxidant strategies have entirely passed the rigorous test of randomized controlled clinical trials. Nonetheless, we will defend in this review the rationale and supporting data for a novel antioxidant approach in which patients with acute ischemic stroke will receive UA in combination with recombinant tissue plasminogen activator (rt-PA).

Uric acid in physiological & ischemic conditions

The enzyme xanthine oxidoreductase (XOR) catalizes the oxidation of the purine bases hypoxanthine and xanthine, resulting in the production of high amounts of UA. XOR exists in

two forms, XDH, which is the predominant form in physiological conditions, and XO. Under ischemic conditions, ATP is degraded to adenine and xanthine, and there is an increased conversion of XDH to XO. As a product of the catabolism of hypoxhantine and xhantine, XO produces high amounts of UA and also considerable amounts of superoxide free radical [29]. XO is induced and increases its activity during ischemia and reperfusion, as it has been demonstrated in coronary endothelial cells [30] and in animal models of acute brain injury where local UA concentrations increased significantly [31]. Clinically, UA increases during interruption of limb arterial flow [32], after coronary angioplasty [33], during coronary artery bypass surgery [34] and in other hypoxic states such as chronic heart failure [35]. Likewise, the pharmacological inhibition of XO has shown beneficial effects in animal models of cerebral ischemia [36] and also in models of myocardial ischemia, where allopurinol limited infarction size [37] and enhanced recovery of stunned myocardium [38], perhaps by limiting the generation of toxic free radicals.

Uric acid is an antioxidant molecule

UA is one of the most important antioxidants in plasma and its concentration is almost tenfold higher than other antioxidants. It contributes as much as two-thirds of all free radical scavenging capacity in plasma [6,39]. As a putative protective mechanism, local UA concentrations increase in the brain tissue during acute oxidative stress and ischemia, trying to counteract the excess of free radicals. Amongst its multiple direct antioxidant actions, urate (the soluble form of UA in blood) is able to scavenge hydroxyl radicals and hydrogen peroxide, suppress the Fenton reaction, chelate transition metals and prevent lipid peroxidation [39]. UA also scavenges the radicals generated by the decomposition of peroxynitrite, a particularly toxic product that results from the reaction of superoxide anion with nitric oxide and that can injure cells by nitrosylating the tyrosine residues of proteins [40]. The formation of nitrotyrosine has been reported in a model of transient middle cerebral artery (MCA) occlusion/reperfusion, where it is believed to contribute to brain injury [41], mediating a variety of pathological changes including tyrosine nitration, lipid peroxidation and DNA strand breakage which may lead to dysfunction and cell death. Additionally, site-specific nitration of protein tyrosine residues may result in alteration of normal function and promotion of disease. Some examples are the loss of activity linked to nitration of Mn-SOD [42] or PGI2 synthase [43], or the facilitation of a prothrombotic state induced by nitration of fibrinogen [44]. In humans, an association between protein 3-nitrotyrosine levels in plasma and risk of symptomatic coronary artery disease has also been found [45].

UA also prevents the degradation of extracellular SOD3, an enzyme critical in maintaining normal endothelial and vascular function [46]. SOD3 is an extracellular enzyme that catalyzes the reaction of $O_2^{-\bullet}$ to H_2O_2, that helps to maintain NO levels and consequently endothelial function by removing $O_2^{-\bullet}$. In ischemic transgenic mice, the lack of SOD is detrimental [47], whereas overexpression of the enzyme is neuroprotective [48]. Consequently, an increased tissue biodisponibility of SOD may exert additional benefits to the direct effects of UA.

There are some *in vitro* data in favor of urate radical acting on a pro-oxidative molecule, especially when ascorbic acid is depleted in the experiments [49]. However, the expected consequences of this effect are modest because urate radical is significantly less reactive than other classic oxidants and it can be rapidly scavenged by ascorbate [50]. Furthermore, UA stabilizes ascorbate in serum, largely owing to its iron chelation properties [39,51]. More importantly, *in vivo* data obtained in animal experiments and in human trials point towards the preponderance of antioxidant actions of UA, even during highly pro-oxidant conditions such as brain ischemia [52–54].

UA could also have other properties in addition to its antioxidant effects. Indeed, a single dose of UA given to mice subjected to acute renal ischemia afforded significant renoprotection and caused a robust mobilization of endothelial progenitor cells (EPCs) [55]. These findings led the investigators to suggest that a transient surge in UA could serve as a harbinger to the ischemic tissue that would accelerate the recruitment of EPCs. In agreement with these findings, a recent study demonstrated an increase of circulating EPCs in patients with acute stroke that had better functional recovery and lesser infarct growth, suggesting that EPCs participated in neuroreparative processes [56].

Preliminary studies also suggest that soluble UA could have anti-inflammatory effects *in vivo* by down-regulation of neutrophil influx to sites of local inflammation [57]. The role of the immunomodulatory actions of systemic UA administration in acute ischemia deserves further investigation.

Detrimental effects of uric acid

UA may also have detrimental effects. In experimental and *in vitro* conditions it has been shown that UA can act as a proinflammatory molecule. UA increases platelet adhesiveness [58], stimulates the synthesis of monocyte chemoattractant protein-1 in rat vascular smooth muscle [59], and stimulates mice mononuclear cells to produce IL-lβ, IL-6 and TNF-α [60]. In the rat, UA stimulates vascular smooth muscle cell proliferation, and it activates specific protein kinases and transcription factors that can lead to the synthesis of thromboxane and PDGF [61]. Likewise, hyperuricemia may also be involved in the pathogenesis of hypertension and renal failure [62] by means of several mechanisms such as endothelial dysfunction, activation of the renin–angiotensin system and the development of microvascular disease [63,64]. A summary of the beneficial and detrimental effects of UA are shown in Table 1.

Uric acid & cardiovascular risk

Several studies have provided conflicting results about the clinical significance of elevated UA in patients with cardiovascular or cerebrovascular disease. Consequently, the current view of the role of UA as an independent marker of cardiovascular risk still remains controversial. Thus, while some studies show an independent association between elevated UA serum levels and increased cardiovascular disease or mortality, others suggest that the association is confounded by the effect of vascular risk factors.

Serum UA was found to be an independent predictor of coronary heart disease or excess mortality in several epidemiological studies in unselected populations [65–68] in studies of women [67,69,70], alcohol abstainers [71] and in high-risk groups, such as those with isolated systolic hypertension [72], essential hypertension [73,74] noninsulin-dependent diabetes mellitus [75], preexisting cerebrovascular disease [76] or heart failure [77]. Conversely, in other epidemiological studies [78–80] the association between UA and coronary heart disease did not remain statistically significant after adjustment for the effect of potential confounders. Indeed, hyperuricemia has been shown to be associated with well-established cardiovascular risk factors, such as those related with the metabolic syndrome (insulin resistance, obesity, hypertension and dyslipidemia) [81]. Some studies have assessed specifically the association between UA and stroke risk. An association between elevated UA and fatal or nonfatal strokes in unselected subjects [82,83], elderly patients [68] and diabetics [75] has been reported.

The disparate results of epidemiological studies can be attributed to substantial differences in study design, such as the use of sex-specific analyses, adjustment for confounding factors, definition of outcome events, or a variable inclusion of patients with cardiovascular disease at baseline, which may hamper a clear distinction between the progression of pre-existent disease or the effects of increased levels of UA [78].

The mechanisms by which hyperuricemia is associated with atherosclerotic vascular disease remain to be clarified. Three different explanations have been put forward to interpret this association. First, hyperuricemia represents a risk factor on its own, but as previously discussed, epidemiological studies are controversial and there is no definitive proof that treatment of hyperuricemia reduces cardiovascular events; second, hyperuricemia is an innocent bystander in individuals with other well-established cardiovascular risk factors; and third, hyperuricemia is a compensatory endogenous antioxidant mechanism attempting to counteract increased oxidative stress. The latter interpretation is sustained by recent observations of higher serum aqueous fraction antioxidant-capacity activity mostly owing to the effect of UA in individuals with progression of carotid atherosclerosis [84], and by the proposed link between atherosclerosis and increased oxidative stress [85].

Uric acid in experimental brain ischemia & other diseases of the CNS

Free radical production supervenes early after the onset of cerebral ischemia and is amplified following reperfusion [17]. Oxidative stress can be counterbalanced by a variety of antioxidant systems, but if these systems are depleted oxidative damage may ensue. An increase of lipid peroxidation products [86,87] and a decrease in tissue antioxidant levels such as glutathione, cysteine and ascorbic acid in the brain have been reported as fingerprints of oxidative stress in experimental ischemia [18]. Also, in the rat brain, there is a marked increase in UA after transient focal ischemia [18]. All these findings have led to the design of several neuroprotectant therapies aimed to heighten the antioxidant capacity in the setting of acute stroke. Disappointingly, a long list of neuroprotectants, including some antioxidants [88], has failed to show benefits in the clinic despite encouraging findings previously observed in the laboratory [89].

Table 1. Beneficial and detrimental effects of uric acid.

Uric acid beneficial effects	Ref.
Scavenging of hydroxyl radicals and hydrogen peroxide	[39]
Supression of Fenton reaction	[39]
Quelation of transition metals	[39]
Stabilization of serum ascorbate	[51]
Blockage of the formation of peroxynitrite and nitrotyrosine	[40]
Prevention of the degradation of SOD3	[46]
Uric acid detrimental effects	
Increase of platelet adhesiveness	[58]
Stimulation of the synthesis of MCP-1 in rat smooth muscle cells	[59]
Stimulation of mice mononuclear cells to produce IL-1ß, IL-6 and TNF-α	[60]
Stimulation of vascular smooth muscle cell proliferation and synthesis of tromboxane and PDGF	[61]

A novel antioxidant approach that deserves further testing is the exogenous administration of UA because in several experimental models of cerebral ischemia it has revealed neuroprotective effects. The addition of physiological concentrations of UA in *in vitro* models of ischemic neuronal injury has shown to protect hippocampal neurons against cell death induced by glutamate and metabolic injury [52]. UA treatment suppressed oxyradical accumulation, stabilized calcium homeostasis and preserved mitochondrial functions. Moreover, administration of UA to adult rats, either 24 h prior to MCA occlusion (62.5 mg UA/kg, intraperitoneally) or 1 h following reperfusion (16 mg UA/kg, intravenously) resulted in a highly significant reduction in ischemic damage to the cerebral cortex and striatum and led to improved behavioral outcome at 24 h in treated animals. Confocal laser scanning microscopy also showed that UA was able to suppress the accumulation of ROS and lipid peroxidation after cerebral ischemia, or exposure to glutamate [52]. In a thromboembolic model of stroke in the rat, a model closer to the situation at the bedside, UA was able to exert additive neuroprotection to the benefits of rt-PA. Thus, either UA or rt-PA given in isolation resulted in smaller infarctions and better neurological behavior from the onset of treatment in relation to controls. Yet, dual treatment with rt-PA and UA further increased these clinical and pathological benefits, suggesting that cytoprotection after early reperfusion explained the main findings of the study. UA also reduced neutrophil infiltration in the ischemic brain and protein tyrosine nitration into the infarcted tissue, indicating that this treatment attenuated the acute inflammatory response induced by ischemia [53]. Taken togheter, these findings suggest that an early elevation of the levels of UA during or shortly after the onset of acute ischemic stroke could confer significant protection against neurological deficit, especially if this treatment is associated with the administration of rt-PA [90]. A schematic diagram showing the proposed neuroprotective mechanism of action of UA within the ischemic neuronal injury pathway and reperfusion-mediated damage modulation is shown in FIGURE 1.

Other experimental models of CNS disorders give further ground to the potential therapeutic effects of UA. Thus, in experimental allergic encephalomyelitis in mice, an animal model of multiple sclerosis, the exogenous administration of UA is beneficial owing to the inhibition of peroxynitrite-induced tissue damage, blood–CNS barrier permeability changes and inflammatory cell invasion into CNS [91]. UA also protects against secondary damage after spinal cord injury by reducing tissue damage, nitrotyrosine formation, lipid peroxidation, activation of poly(ADP-ribose) polymerase and neutrophil invasion [92].

Uric acid & acute ischemic stroke

Observational studies have shown that the antioxidant activity of patients with acute stroke decreases in parallel with the levels of vitamin C, vitamin E and SOD [21,22]. Urate concentrations also decrease significantly over time in these patients, and their plasma antioxidant capacity (measured as the total peroxyl radical trapping potential of plasma) is inversely correlated with the volume of cerebral infarction and the severity of neurological impairment [23]. However, the clinical significance of serum UA levels in the setting of acute stroke is controversial. Several studies have found serum UA levels to be an independent predictor of poor outcome in stroke patients. In two different studies including unselected cohorts of stroke patients, UA levels were associated with subsequent cardiac death independently of diuretic treatment [93] and other cardiovascular risk factors [93,94]. In a larger prospective study, serum levels of urate measured within the first 24 h after stroke onset in 2498 patients were found to be directly and independently associated with poor outcome [76]. In this study, poor outcome was defined at 90 days in patients who after the index stroke were dead or alive in care, instead of alive at home. However, the study did not report which neurological changes occurred during the acute phase of stroke, or whether late-onset complications influenced final outcome. Moreover, this measurement of functional outcome is subject to unmeasured biases, including economical factors, social status and medical comorbidity, as recently discussed [95]. The relationship between increased serum urate concentration and poor outcome was also found in a cohort study of Type 2 diabetes patients with acute stroke [96]. Contrarily, a prospective study of 881 consecutive patients with acute ischemic stroke identified a 12% increase in the odds of good clinical outcome for each mg/dl increase of serum UA after adjustment for potential confounders [97]. Patients had repeated brain CT scan or MRI studies to delineate the course of the infarction, and an inverse correlation between the levels of UA and the growth of the infarction at follow-up was found [97].

Uric acid administration in healthy volunteers

The feasibility and impact on antioxidant function of the systemic administration of 1000 mg of UA or vitamin C was compared in a randomized, placebo-controlled, double-blind study in healthy volunteers [98]. In this study, a significant increase in serum free-radical scavenging capacity from baseline was observed during UA and vitamin C infusion, but the effect of the former was substantially greater. Serum UA concentration exhibited a two-component decay, with a mean elimination half-life of 10.8 h and no adverse effects of the study treatment were observed. In a subsequent randomized placebo-controlled double-blind crossover study, the effect of UA on excercise-induced oxidative stress was evaluated in healthy adults. Participants received UA (500 mg) dissolved in 250 ml 0.1% lithium carbonate/4% dextrose vehicle, or vehicle alone. In controls, a single bout of high-intensity exercise caused a significant increase in circulating 8-isoprostaglandin F 2α concentrations, a marker of oxidative stress that was abolished in UA-treated subjects. Similarly, in the previous study, no adverse reactions were encountered in UA treated subjects [99]. In another study,

UA was administered locally or systemically to healthy adult men and women to investigate whether UA impaired endothelial function [100]. No acute effects on hemodynamic variables, basal forearm blood flow, or nitric-oxide dependent endothelial function were observed in the study. Conversely, the intravenous administration of 1000 mg of UA to subjects exposed to chronic oxidative stress, such as regular smokers and Type 1 diabetes, has demonstrated an amelioration of endothelial dysfunction in the forearm vascular bed [101]. In summary, patients with a growing number of observations attest the feasibility, safety and antioxidant capacity of UA administration, as shown in Table 2.

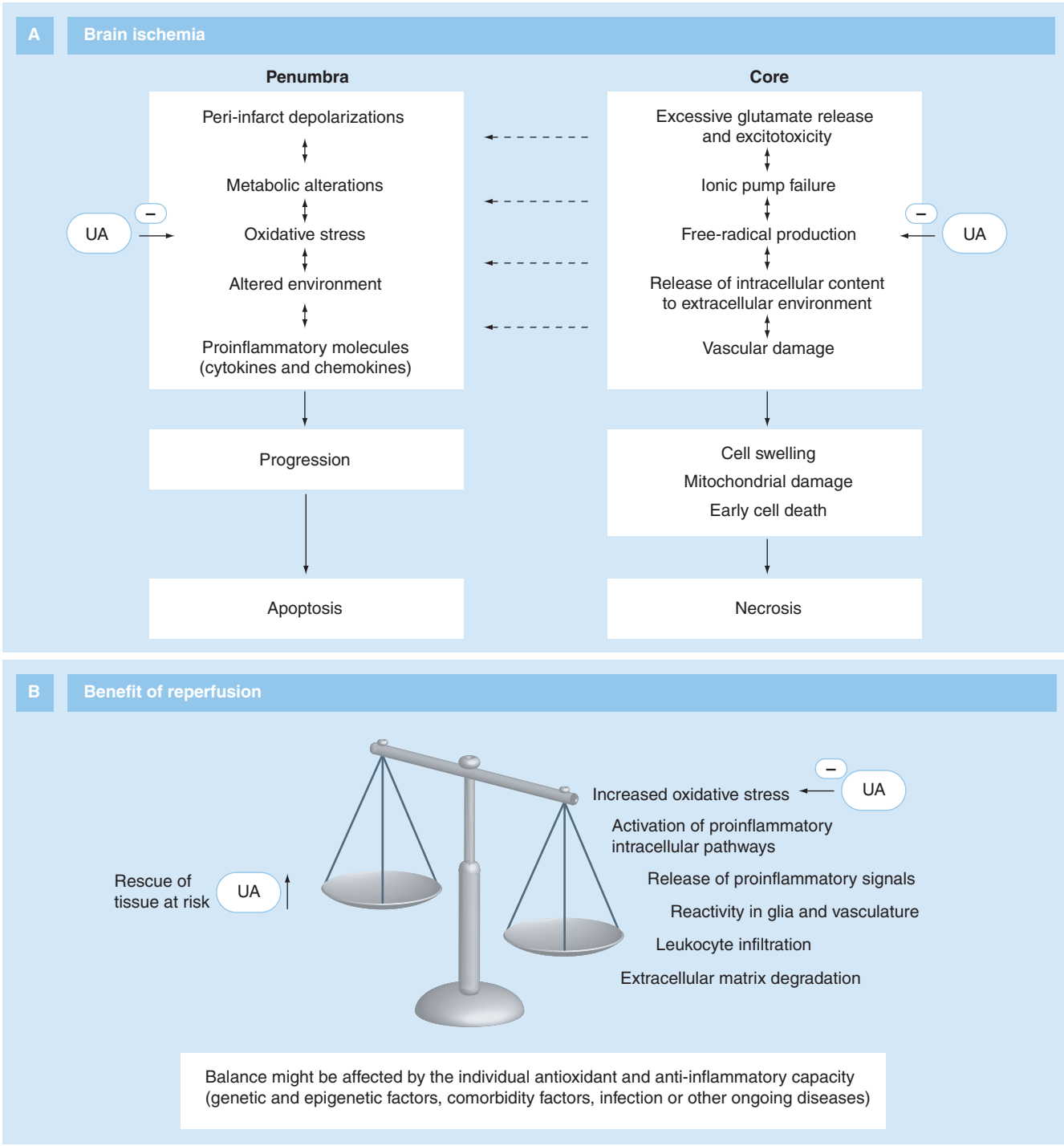

Figure 1. (A) Potential sites of action of UA within the ischemic cascade. **(B)** Oxidative stress and reperfusion-mediated damage. UA: Uric acid.

Table 2. Summary of the effects of exogenous administration of uric acid in neurological disease (*in vivo* animal and human studies) and in healthy volunteers.

Animal studies	Ref.
Neuroprotection in permanent and transient cerebral ischemia in rats	[52]
Neuroprotection in a thromboembolic model of brain ischemia in rats	[53]
Beneficial effects in experimental allergic encephalomyelitis in mice	[91]
Neuroprotection after spinal cord injury in mice	[92]
Healthy volunteers studies	
Increase of serum free-radical scavenging capacity	[98]
Reduction of circulating 8-isoprostaglandin F 2 concentrations after high-intensity anaerobic exercise	[99]
Improvement of endothelial dysfunction in diabetics and regular smokers	[101]
Stroke patients	
Clinical safety and reduction of lipid peroxidation in patients receiving rtPA	[54]

Uric acid administration in patients with acute ischemia

Recently, we have reported for the first time a Phase II, vehicle-controlled clinical trial of dual treatment with rt-PA and UA within the first 3 h of ischemic stroke onset [54]. Patients were randomized to an intravenous solution of 500 mg (n = 8) or 1000 mg of UA (n = 8), or vehicle. Vehicle consisted of a 500 mL solution of 5% mannitol and 0.1% lithium carbonate to assure the iso-osmolarity and solubility of the preparation. One patient with 11.0 mg/dl of UA on admission and peak levels of 16.1 mg/dl after the infusion of 1 g of UA experienced mild acute arthritis within 24 h that rapidly resolved with anti-inflammatory drugs. Otherwise, there were no serious adverse events related to the administration of UA. None of the patients of the study experienced signs or symptoms of acute renal toxicity and lithium concentration measured at the end of the infusion was far below toxic levels. UA showed a maximal decrease 6–7 h after the onset of stroke in patients allocated vehicle as shown in Figure 2. Conversely, patients treated with 500 mg of UA did not show this decrement of UA levels, and those treated with 1 g showed an increment of UA that remained above baseline levels for approximately 24 h (elimination half-life of 44 h). At day 5, patients allocated the high dose of UA showed a decrement of malondialdehyde levels, a marker of lipid peroxidation, whereas patients allocated the low dose or vehicle showed an increment (–32.5, 23.4 and 27.5%, respectively; $p < 0.003$). The study was not powered to evaluate whether dual therapy with UA and rt-PA improved the clinical outcome at follow- up. Yet, the early consumption of UA observed in untreated patients, the effect on lipid peroxidation and the lack of serious adverse effects support additional clinical assessment of UA administration in acute stroke. Indeed, an ongoing Phase III, multicentric, confirmatory trial is currently testing the clinical value of UA in acute stroke.

Expert commentary

The main targets of pharmacological therapy in acute ischemic stroke are early reopening of occluded vessels and salvage of the ischemic penumbra. Currently, rt-PA is the only approved therapy for selected patients with acute ischemic stroke, but the fear of bleeding complications and the short therapeutic window of rt-PA administration (3 h) means only a few eligible patients benefit from this therapy. Therefore, additional strategies in combination with the administration of reperfusion drugs are required [28]. Oxidative damage has been related to reperfusion injury in preclinical studies, but the role of dual therapy with antioxidant agents and thrombolytic drugs has provided conflicting results in clinical trials [25,26]. This dual approach is particularly attractive because free radicals are released early after the onset of brain ischemia and may contribute to reperfusion injury when the occluded vessel is reopened [102].

Growing evidence supports the neuroprotective effect of UA administration after brain ischemia. Both clinical and experimental studies reveal a gradual exhaustion of the antioxidant capacity after transient cerebral ischemia, and the magnitude of this consumption seems to be correlated with the extent of brain tissue injury, growth of the infarction, severity of neurological impairment in the acute phase and long-term functional outcome. In experimental ischemia in rats, the administration of UA is neuroprotective both in mechanical models of brain ischemia (transient or permanent intraluminal occlusion of the MCA) and in thromboembolic models of autologous clot injection. The administration of UA is feasible and safe in healthy volunteers and in acute stroke patients receiving rt-PA. Unlike in rats, the enzyme uricase that converts UA to oxidized end products is inactive in humans, hampering the extrapolation of the results obtained in rat models to humans. Nonetheless, a very early fall in UA blood levels during the first hours after stroke onset has been identified, which is probably related to a

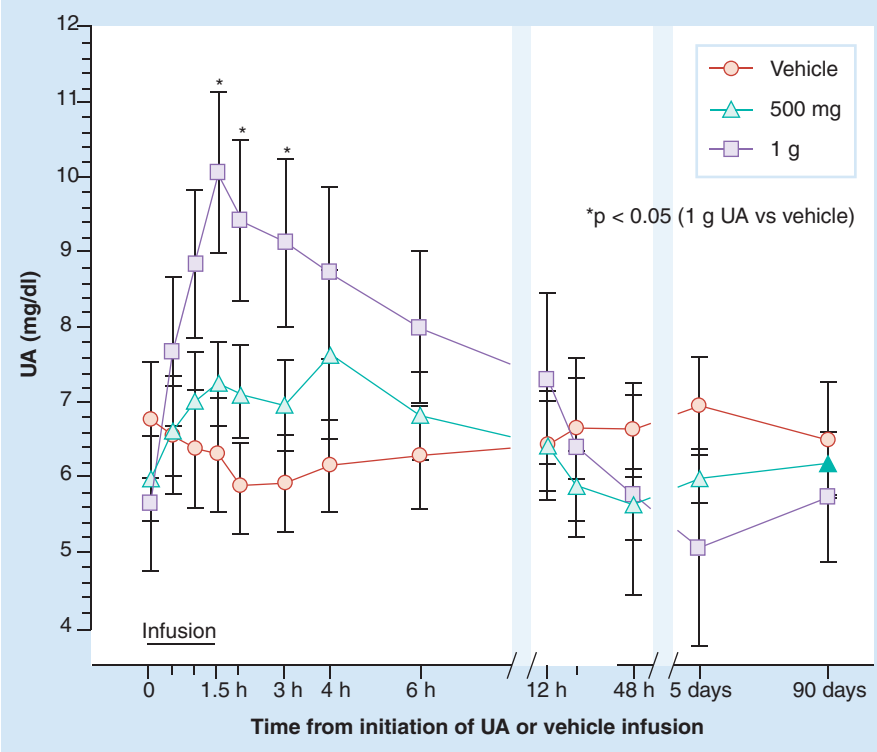

Figure 2. Serum levels of UA during the study course in patients allocated to receive only vehicle, or 500 mg or 1 g of UA. Time 0 indicates the end of recombinant tissue plasminogen activator infusion. Reproduced with permission from [54].
UA: Uric acid.

nonenzymatic oxidative consumption or to an increased uptake from plasma to the ischemic tissue. We argue that UA may function as a physiologic buffer trying to counteract the excess of free radicals produced during the ischemia and reperfusion, and that the exogenous supplementation of UA avoids this early decay without apparent safety concerns. Indeed, none of the 16 stroke patients [54], and none of the 70 healthy volunteers [98–101] who received exogenous UA (doses ranging from 500 to 1000 mg) in the different published clinical trials suffered acute renal failure or any other serious adverse event. As shown in Figure 2, this safety profile could be explained by the very transient nature (a few hours) of the increment of UA over its saturation threshold (~7.0 mg/dl at 37°C). Altogether, it is reasonable and biologically plausible to test the real value of this therapeutic approach in an adequately powered clinical study. The indeterminate relationship between chronic elevation of UA and long-term cardiovascular and cerebrovascular risk should be separated from the potential beneficial effects of a short-lived administration of UA aimed to prevent the early fall of this antioxidant during the acute setting of brain ischemia. Indeed, both effects are not mutually exclusive. Unquestionably, the low cost of UA is an additional bonus for the design of a larger trial because even a mild clinical benefit would be cost–effective.

Five-year view

A Phase III clinical trial testing if combined treatment with rt-PA and UA is better than rt-PA alone in acute ischemic stroke is ongoing and hopefully the results will be available within the next 3 years. The study has been designed to include 400 patients with acute ischemic stroke that will receive rt-PA within the first 3 h of clinical onset. At the end of the 1-hour rt-PA infusion patients will be randomized to receive 90 min intravenous infusion of vehicle or UA. The primary outcome measure will be the rate of good functional outcome at 3 months, defined as a modified Rankin Scale Score (mRS) of 0 or 1, or 2 in patients with a prior mRS of 2. Other testable hypotheses will be whether the administration of UA expands the time window of benefit of rt-PA without increasing the risk

Key issues

- Uric acid (UA) is the end product of purine catabolism in humans and is a powerful antioxidant whose production is increased under ischemic conditions.
- Oxidative damage has been related to reperfusion injury in preclinical studies, but dual therapy with antioxidant agents and thrombolytic drugs has provided conflicting results in clinical trials.
- Chronic hyperuricemia is associated with cardiovascular disease, but the role of UA as an independent marker of cardiovascular risk still remains controversial.
- In experimental conditions of brain ischemia the administration of UA is neuroprotective, both in mechanical and in thromboembolic models. In the latter model, UA exerts additive neuroprotection to the benefits of recombinant tissue plasminogen activator (rt-PA).
- In healthy volunteers, UA administration is safe, increases the antioxidant capacity of serum and decreases oxidative stress.
- In ischemic stroke patients treated with rt-PA, the administration of UA is safe, reduces lipid peroxidation and prevents a very early fall in blood levels of UA that occur after clinical onset.
- An ongoing Phase III, multicentric, confirmatory trial is currently testing the clinical value of UA in patients with acute stroke.

of hemorrhage. The administration of UA could be factored in trials investigating the benefit of rt-PA given within 6 h from the onset of symptoms. Another clinical scenario in which the value of exogenous UA could be tested in a randomized controlled trial is during cardiopulmonary bypass operations, as patients suffering this procedure may sustain neurocognitive changes mediated by oxidative stress in as many as 30–80% of the interventions.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Papers of special note have been highlighted as:

- • of interest
- •• of considerable interest

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