Uric acid therapy for vasculoprotection in acute ischemic stroke

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Abstract:

Uric acid (UA) is a product of the catabolism of purine nucleotides, the principal constituents of DNA, RNA, and cellular energy stores, such as adenosine triphosphate. The main properties of UA include scavenging of hydroxyl radicals, superoxide anion, hydrogen peroxide, and peroxynitrite that make this compound to be the most potent antioxidant in the human plasma. As the result of two silencing mutations in the gene of the hepatic enzyme uricase which degrades UA to allantoin, humans have higher levels of UA than most mammals. However, these levels rapidly decrease following an acute ischemic stroke (AIS), and this decrement has been associated to worse stroke outcomes. This review highlights the safety and potential clinical value of UA therapy in AIS, particularly in patients more exposed to redox-mediated mechanism following the onset of ischemia, such as women, hyperglycemic patients, or patients treated with mechanical thrombectomy. The clinical findings are supported by preclinical data gathered in different laboratories, and in assorted animal species which include male and female individuals or animals harboring comorbidities frequently encountered in patients with AIS, such as hyperglycemia or hypertension. A remarkable finding in these studies is that UA targets its main effects in the brain vasculature since available evidence suggests that does not seem to cross the blood–brain barrier. Altogether, the available data with UA therapy extend the importance of vasculoprotection for effective neuroprotection at the bedside and reinforce the role of endothelial cells after brain ischemia for an increased survival of the whole neurovascular unit.

Keywords:

Ischemic stroke, treatment, uric acid

Introduction

Despite the continuous progress made in the general care of patients with acute ischemic stroke (AIS) including the implementation of reperfusion therapies, up to one-third of stroke survivors remain with substantial disability. Therefore, improved approaches including effective neuroprotectants are imperative to diminish the burden of AIS worldwide. Neuroprotection in AIS refers to "any strategy, or combination of strategies, that antagonizes, interrupts, or slows the sequence of injurious biochemical and molecular events that, if left unchecked, would eventuate in irreversible ischemic injury."[1] Likewise, treatment approaches that primarily target the cerebral vasculature rather than the brain parenchyma are described under the term of vasculoprotection.[2]

Arguably, a better understanding of how drugs affect the different components of the neurovascular unit could assist in the design and implementation of a more successful clinical translation. However, a majority of the available studies that used neuroprotectant drugs did not specifically address neither the patency nor the structure of the brain microvasculature, which are essential components for effective brain reperfusion, and hence, successful parenchymal protection.[3] On the contrary, an improved knowledge of preclinical drugs effects before the conduct of clinical studies could allow the design

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of enriched randomized clinical trials (RCTs), where more homogeneous treatment populations could be evaluated, namely, the enriched studies could facilitate the prospective use of any patient characteristic to select the best responder individuals in RCT, in which detection of a drug effect (if one i, in fact, present) is more likely than it would be in an unselected population. In addition, enrichment strategies could also allow a treatment effect to be more readily discerned in smaller study populations, thus reducing the high costs of clinical trials. In this review, we argue that one appealing strategy would be the study of neuroprotectants in patients who reperfuse the occluded vessel after AIS either by the use of thrombolytic agents or mechanical devices, as brain reperfusion renders the ischemic brain vulnerable to mechanisms otherwise less germane in patients who harbor permanent vessel occlusions.[4] It also highlights the contribution of the vasculature for the normal function of the whole neurovascular unit in search of a more successful neuroprotection in AIS.

Search Strategy

Review of the literature was conducted through Internet search on public access website PubMed and Medline databases until 2018. Keywords utilized included uric acid (UA), neuroprotection, vasculoprotection, and stroke therapy. Titles that were felt to meet criteria were subjected to further review.

The Ischemic Cascade

Numerous investigations on the mechanisms of the brain ischemic cascade have singled out the role of excitotoxicity, inflammation, oxidative-nitrosative stress, apoptosis, and their complex bidirectional or multidirectional relationships.[5-9] Excitotoxicity, mitochondrial dysfunction, and reactive oxygen/nitrogen species (ROS/RNS) production are linked processes after brain ischemia, as failure in energy production from mitochondria after the ischemic insult, leads rapidly to ROS/RNS production, membrane depolarization, removal of the voltage-dependent Mg^{2+} block of the N-methyl-d-aspartate receptor, and its subsequent activation leading to increased intracellular Ca^{2+} levels. The latter contributes to proteinase activation and free radical generation during the early ischemic phase,[10] and a much larger rise of these toxic compounds during early reperfusion, both in neurons and endothelial cells.[11] In agreement with the available evidence, most therapeutic approaches developed in the laboratory and translated into RCT, focused on protecting the brain from these stressors, although hitherto with disappointing results.[12] A full analysis of the reasons for the failure of neuroprotection in AIS is beyond the scope of this

review,[13] but likely contributing factors would be that heterogeneous populations of patients were assessed and that few patients enrolled in the studies received concomitant reperfusion therapies.[14] Arguably, only a minority of patients reperfused after stroke in these studies, and in consequence, the neuroprotectants that were evaluated did not reach the ischemic brain or the vasculature or otherwise did it at inadequately low concentrations. Currently, these limitations could be overcome given the growing use of pharmacological and/or mechanical reperfusion therapies in patients with AIS, which may facilitate the design of RCT where all patients would require having an adequate brain reperfusion before testing the value of any putative new neuroprotectant drug.[15]

Endothelial Cells: A Cornerstone in the Neurovascular Unit

It is accepted that vasculogenic endothelial cells and nascent vessels in the developing heart, lung, pancreas, stomach, or gut, are critical for the earliest stages of organogenesis, before blood vessel function.[16] A similar relationship is likely to operate between the brain parenchyma and its circulatory system, and that interference of signals originated at the local vasculature may result in brain dysfunction. In the central nervous system, the vascular basement membrane separates the endothelial cells from neurons and glial cells and also contributes to vessel development and formation and maintenance of the blood–brain barrier (BBB).[17] The function of cerebral arteries is critical to maintain cerebrovascular resistance and minimize damage to ischemic brain regions after focal cerebral ischemia/reperfusion (I/R).[18] Endothelial cells are the site of the BBB and control the traffic of ions, molecules, and cells into and out of the brain.[19] Under physiological conditions, endothelial cells determine the thromboresistance property of vessels,[20] suppress pro-inflammatory gene expression, the recruitment of monocytes, and the development of atherosclerosis.[21] Endothelial cells also affect resting cerebral blood flow (CBF) and mediate vasodilator responses to shear stress, neurotransmitters, metabolic factors, and therapeutic agents, and contribute to the normal function of neural and glial cells.[22] Effects of endothelial cells on the underlying smooth muscle at the vessel wall are major regulators of vessel tone, either by the release of vasoactive molecules that diffuse into the smooth muscle or through endothelium-dependent hyperpolarization of vascular muscle.[23] Given this panoply of effects, endothelial dysfunction may represent a keystone event in the pathogenesis of neurovascular injury, and effective vasculoprotection might translate into clinically relevant therapeutic tactics.[24]

Endothelial Cells and Brain Ischemia

Brain I/R impairs both basal and receptor-mediated endothelium-dependent vasodilation of large arteries, and parenchymal arterioles alike, despite it results in an increase in both endothelial nitric oxide (NO) synthase expression and endothelium-derived hyperpolarization-type dilations.[25,26] I/R also result in loss of proportional and spatially controlled changes in CBF elicited by neural activity (neurovascular coupling), and a loss of myogenic tone and autoregulation, making the CBF to follow passively the changes in arterial pressure.[27] ROS and RNS as well as vasoactive factors such as NO, endothelin-1, vascular endothelial growth factor (VEGF), and angiopoietin I, play important roles in regulation of vascular tone and structure in the acute phase of the brain ischemia.[28] Restoring nutritive blood flow to the ischemic brain is essential for tissue survival, but reentry of oxygen and glucose into the ischemic region also represents a double sword that brings about an excess production of ROS and RNS, which may trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative/nitrosative injury, committing cells to necrosis or apoptosis.[10,29-32] Sources of high concentrations of ROS and RNS are the mitochondria,[33] the activity of cyclooxygenase enzymes,[34] nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expressed by vessel wall cells (e.g., endothelial cells),[35] brain pericytes,[36] and infiltrating neutrophils,[37] as well as the hypoxic-dependent conversion of xanthine dehydrogenase into xanthine oxidase (XOR).[38] Further, whole-tissue homogenates of the human brain have failed to exhibit significant XOR activity, immunohistochemical studies have revealed high levels of XOR antigen in brain vascular endothelium. Endothelial dysfunction is in part attributed to the effects of superoxide anion,[39] which is formed when oxygen acquires an additional electron and can react, among others, with arachidonic acid forming isoprostanes, or react with NO to produce peroxynitrite (ONOO⁻). The latter is particularly toxic as it crosses readily biological membranes and interacts with most critical biomolecules.[40,41] Under experimental conditions, reactive species are largely generated in the ischemic penumbra for about 6–12 h after stroke onset,[42] facilitating the demise of the penumbra by lipid peroxidation, mitochondrial damage, protein nitration and oxidation, depletion of antioxidant reserves, activation or inhibition of various signaling pathways, DNA damage, and BBB breakdown.[43] Recent evidence suggests that ONOO⁻ formed during I/R can disrupt endothelial filamentous-actin augmenting endothelium-derived hyperpolarization-type dilations of cerebral arteries, a mechanism whereby ONOO⁻ could contribute to promote postischemic brain injury.[26] Ischemia also induces sustained contraction of pericytes on microvessels despite successful recanalization in mice, and suppression of ONOO⁻ relieves pericyte contraction, reduces erythrocyte entrapment, restores microvascular patency, and improves the tissue survival.[44] Interestingly, by comparing the ROS-suppressing effect of N-tert-butyl-α-phenylnitrone (PBN) with its BBB impermeable analog 2-sulfophenyl-N-tert-butylnitrone (S-PBN) in mice, Taskiran-Sag *et al.* found that PBN and S-PBN completely suppressed the reperfusion-induced increase in ROS signal within vasculature;[45] PBN readily suppressed ROS produced in parenchyma and S-PBN suppressed the parenchymal ROS sometimes later. Yet, both compounds comparably reduced the size of the ischemic area and S-PBN restored the microvascular patency and perfusion after recanalization, suggesting that its delayed parenchymal antioxidant effect could be secondary to improved microcirculatory reperfusion.[45]

After ischemia, the BBB loses parts of its barrier properties, driving endothelial cell induction of selectins and integrins, and secretion of pro-inflammatory compounds such as tumor necrosis factor-α, interleukin-1 (IL-1) β, IL-6, monocyte chemoattractant protein-1, cytokine-induced neutrophil chemoattractant, and prostaglandins.[46,47] These events primarily affect postcapillary venules, where leukocytes adhere to swollen endothelium and infiltrate the brain parenchyma across the BBB.[48] Neutrophils have a remarkable destructive potential, either through the direct neurotoxic effects from the release of proteolytic enzymes[49,50] or through indirect effects that result from intravascular neutrophil accumulation, capillary blood flow obstruction, the no-reflow phenomenon,[51] or activation of the complement system.[52] Neutrophils can also extravasate from the leptomeningeal vessels and reach the brain in experimental animal models and human studies of permanent arterial occlusion.[53] Finally, a vicious cycle of inflammatory activation on both endothelial cells and leukocytes may contribute to increased vascular permeability and vasogenic edema.[54]

Uric Acid: A Potent Extracellular Antioxidant

UA is a product of the catabolism of purine nucleotides, the principal constituents of DNA, RNA, and cellular energy stores, such as adenosine triphosphate. In most mammals, UA is rapidly degraded by the hepatic enzyme uricase (urate oxidase) to allantoin,[55] but in humans, the uricase gene is nonfunctional. As a consequence, humans have higher UA levels than most mammals, in concentration almost tenfold higher than other antioxidants that contribute up to 60% of the total plasma antioxidant activity in healthy controls.[56] The main properties of UA include scavenging of hydroxyl

radicals, superoxide anion, hydrogen peroxide, and peroxynitrite; suppression of the Fenton reaction; chelation of transition metals; and prevention of lipid peroxidation.[57]

Uric Acid Therapy Following Brain Ischemia: Preclinical Data

Accumulating data attest the beneficial role of UA to minimize the negative consequences of the ischemic cascade. Yu *et al*. first described the dose-dependent effects of UA therapy to prevent cell death induced by exposure to glutamate and cyanide in cultured rat hippocampal neurons and showed that UA was neuroprotective administered following I/R in rats.[58] Romanos *et al.* identified the synergistic effects of UA and recombinant tissue plasminogen activator (rtPA) in a model of thromboembolic brain ischemia in rats.[59] In mice, Haberman *et al.* showed improved outcome, smaller infarcts, and reduced ROS production with UA therapy following I/R or permanent middle cerebral artery (MCA) occlusion,[60] and Ma *et al.* confirmed smaller infarcts, improved behavior, and reduced superoxide anion production and nitrotyrosination in brain, following I/R.[61] Preclinical studies have also demonstrated improved stroke outcomes following UA therapy in hyperglycemic mice,[62] female mice,[63] and in female rats.[64]

Uric Acid Mainly Targets the Vasculature

Onetti *et al*. showed in male rats that UA therapy attenuated the MCA wall thickening following I/R, and induced passive lumen expansion, reduced brain damage, and reduced nitrotyrosination in both the MCA and the brain tissue.[65] UA treatment prevented the increased wall and adventitial volume, and the augmented number of adventitial cells, but not smooth muscle cells. Remarkably, all these effects were more significant in animals with hyperemia after I/R.[66] A decrease in the myogenic response occurs during ischemia and is considered an important factor involved in the functional dysregulation of CBF after I/R.[66] However, UA treatment during reperfusion did not restore the myogenic response. Further, quantitative analysis of mRNA levels of NADPH oxidase (major source of vascular superoxide anion) subunits showed augmented mRNA expression in hyperemic animals, and this effect in conjunction with the enhanced oxidative stress was attenuated by UA.[65]

UA therapy after I/R in normotensive rats also exerted long-term brain protective effects which were associated with attenuation of the short-term rise in both circulating levels of IL-18 and cerebrovascular oxidative stress.[67] Strikingly, UA treatment attenuated both short- and long-term brain damage in hypertensive rats, an effect associated with abolishment of the acute oxidative stress response and prevention of stroke-induced long-lasting MCA remodeling. Further, in BBB permeability assays, the permeability of UA was poor, suggesting that it did not cross the BBB by passive transport. Indeed, UA plasma levels increased 10 min after intravenous (IV) UA administration, whereas UA levels in brain tissue were unaffected by UA infusion, suggesting that the main protective target of this compound was the brain vasculature.[67]

Beneficial actions of UA following I/R have been associated with the activation of the Nrf2 transcription factor and regulation of neurotrophic factor expression.[68] Our group further showed in spontaneously hypertensive rats that UA treatment induced Kruppel-like factor 2 (KLF2) expression, and lowered VEGF-A levels while reduced BBB leakage, and improved endothelial cell barrier integrity.[69] KLF2 is a transcription factor that modulates essential cellular functions, including regulation of endothelial cell growth, differentiation and activation,[70] and protects mice from thrombus formation by a decreased expression of endothelial thrombotic factors.[71] Human VEGF-A is a Janus-faced molecule, since together with its proangiogenic and neuroprotective effects, it disrupts the BBB integrity, leading to edema, hemorrhage, and brain damage.[72] Previously, it was shown that UA inhibits angiogenesis of cultured endothelial cells through KLF2-induced negative regulation of VEGF-A expression.[73] Altogether, these findings strongly support the concepts that UA is primarily a vasculoprotective compound and that this effect may be ultimately responsible for the overall neuroprotection and improved outcome shown in experimental studies.

Uric Acid Therapy Following Brain Ischemia: Clinical Data

The beneficial effects of UA found in preclinical models have also a promising translation into the clinic. In particular, Chamorro *et al.* first described in patients with AIS that higher endogenous levels of UA at clinical onset were associated with better stroke outcome.[74] Reassuringly, this finding was later confirmed in a meta-analysis of 8131 patients with AIS.[75] Waring *et al.* showed that IV administration of UA was safe in healthy volunteers, increased serum free-radical scavenging capacity at rest and during acute physical exercise, and abolished lipid peroxidation.[76] We conducted a pilot study that showed the safety of IV UA administration in 24 patients with AIS treated with rtPA treated within 3 h of clinical onset,[77] established in 44 h the half elimination life of the compound and found that UA treated patients had lower increments of activated

metalloproteinase-9 at follow-up.[78] Importantly, this pilot study also described a rapid consumption of the circulating levels of endogenous UA in untreated patients.[77]

The Phase 2 URICO-ICTUS trial confirmed the safety of a single 90-min infusion of 1 g UA administration in 421 patients with AIS treated with rtPA within 4.5 h of symptom onset.[79] The trial showed an overall nonsignificant 6% absolute increment in the rate of good outcome at follow-up but showed a highly significant reduction in the incidence of early stroke worsening stroke in treated patients.[80] Preplanned analyses in subgroups of patients more vulnerable to oxidative/nitrosative stress, demonstrated highly significant effects of UA therapy in the primary outcome of URICO-ICTUS (modified Rankin Scale score at day 90). These important subgroups included women,[81] hyperglycemic patients,[82] and patients treated with mechanical thrombectomy (MT).[83] A greater benefit of UA therapy in females could be attributed to lower endogenous levels of UA in this sex group and therefore greater exposure than males to unopposed toxic-free radicals.[81] The greater benefit of UA therapy observed in hyperglycemic patients accord with preclinical studies establishing that glucose exacerbates ischemic brain injury because it is the main electron donor for reperfusion-induced neuronal superoxide production in AIS.[84] Indeed, inactivation of neuronal superoxide production in mice counterbalances the deleterious effects of hyperglycemia after brain ischemia.[84] Finally, the greater benefit of UA therapy versus placebo in the subpopulation of patients treated with MT, which has the greatest brain reperfusion rate, is justified by the abundant experimental evidence indicating a much higher production of ROS and RNS, whenever brain reperfusion occurs. Confirmation of these provocative findings is planned in a pivotal RCT of patients treated with MT.

Conclusions

This review highlights the safety and potential clinical value of UA therapy in AIS, particularly in patients more exposed to deleterious redox-mediated mechanisms following ischemic brain damage, such as females, patients with moderate increase of glucose levels at stroke onset, or patients treated with MT. This assumption is supported by preclinical data gathered in different laboratories, assorted species, and male and female animals harboring comorbidities that frequently affects patients with AIS, such as hyperglycemia or hypertension. Altogether, the available data with UA therapy extend the importance of vasculoprotection for effective neuroprotection at the bedside and reinforce the role of endothelial cells after brain ischemia for an increased survival of the whole neurovascular unit.

Conflicts of interest
Dr. Chamorro owns stock in FreeOx Biootech SL.

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