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Glibenclamide in Cerebral Ischemia and Stroke

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Abstract

The sulfonylurea receptor 1 (Sur1)–transient receptor potential 4 (Trpm4) channel is an important molecular element in focal cerebral ischemia. The channel is upregulated in all cells of the neurovascular unit following ischemia, and is linked to microvascular dysfunction that manifests as edema formation and secondary hemorrhage, which cause brain swelling. Activation of the channel is a major molecular mechanism of cytotoxic edema and “accidental necrotic cell death.” Blockade of Sur1 using glibenclamide has been studied in different types of rat models of stroke: (i) in conventional non-lethal models (thromboembolic, 1–2 h temporary, or permanent middle cerebral artery occlusion), glibenclamide reduces brain swelling and infarct volume and improves neurological function; (ii) in lethal models of malignant cerebral edema, glibenclamide reduces edema, brain swelling, and mortality; (iii) in models with rtPA, glibenclamide reduces swelling, hemorrhagic transformation, and death. Retrospective studies of diabetic patients who present with stroke have shown that those whose diabetes is managed with a sulfonylurea drug and who are maintained on the sulfonylurea drug during hospitalization for stroke have better outcomes at

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discharge and are less likely to suffer hemorrhagic transformation. Here, we provide a comprehensive review of the basic science, preclinical experiments, and retrospective clinical studies on glibenclamide in focal cerebral ischemia and stroke. We also compare the preclinical work in stroke models to the updated recommendations of the Stroke Therapy Academic Industry Roundtable (STAIR). The findings reviewed here provide a strong foundation for a translational research program to study glibenclamide in patients with ischemic stroke.

Keywords

Cerebral ischemia; Stroke; Sur1; Sur1–Trpm4 channel; Glibenclamide; RP-1127; Stroke Therapy Academic Industry Roundtable (STAIR)

Introduction

Stroke accounts for 5.5 million deaths each year [1, 2]. “Malignant infarction” occurs in 10–12 % of stroke victims, 70,000 patients per year in the USA alone [3], and is characterized by the formation of rapidly accumulating cerebral edema. It is the syndrome of a large stroke causing progressive edema and tissue swelling that compromises arterial inflow to surrounding tissue, culminating in further ischemic damage, enlargement of the infarct, brain herniation, and death. The prognosis for these patients is poor, with case fatality rates as high as 60–80 % [3, 4].

The multidecade-long effort to identify pharmacotherapeutic targets to reduce death and disability after ischemic stroke has yet to succeed. Recombinant tissue plasminogen activator (rtPA) currently is the only medication approved by national regulatory agencies for use in acute ischemic stroke, but for many reasons it is used in <20 % of stroke victims in developed countries [5–8].

Despite the great morbidity and mortality that are a direct consequence of brain swelling following stroke, novel approaches for the prevention of cerebral edema are lacking. Current practice for treating brain swelling is based on unproven, largely reactive drug interventions such as mannitol and hypertonic saline, and mechanical interventions such as decompressive craniectomy. Treatments to prevent swelling are lacking even though, from a physiological and clinical perspective, preventing swelling is preferable to decompressing the already swollen brain.

Sulfonylurea receptor 1 (Sur1), which is encoded by *Abcc8*, serves as the regulatory subunit for two distinct ion channels. Sur1 co-associates with the ATP-sensitive K⁺ channel, Kir6.2, with which it forms K_{ATP} channels in pancreatic β cells and neurons [9–11]. Sur1 also co-associates with the ATP- and calcium-sensitive non-selective cation channel, transient receptor potential melastatin 4 (Trpm4), with which it forms Sur1–Trpm4 channels [12] (previously named Sur1-regulated NC_{Ca}-ATP channels). Both K_{ATP} (Sur1–Kir6.2) and Sur1–Trpm4 channels are regulated by Sur1, but the two have opposite functional effects—opening of K_{ATP} channels hyperpolarizes the cell [13], whereas opening of Sur1–Trpm4 channels depolarizes the cell (Fig. 1) which, if unchecked, results in cytotoxic edema and necrotic cell death [14, 15].

Sur1 is transcriptionally upregulated in neurons, astrocytes, oligodendrocytes, and microvascular endothelial cells after focal ischemia in animal models [16, 17] and humans [18]. Upregulation of Sur1 is paralleled by upregulation of Trpm4 [19], and is associated with expression of Sur1–Trpm4 channels [16].

Sur1 contains binding sites for therapeutic sulfonylurea drugs and related compounds. Drugs such as glibenclamide (US adopted name, glyburide) and repaglinide bind with nanomolar or subnanomolar affinity, and are potent inhibitors of Sur1-regulated channel activity. Sur1 is the target of sulfonylurea drugs used to treat diabetes mellitus type 2 (DM-2), neonatal diabetes, and some forms of congenital hyperinsulinism [11]. Growing evidence from animal models and retrospective human studies shows that inhibition of Sur1 with glibenclamide may have important therapeutic effects in focal cerebral ischemia.

Sur1 and “Accidental Necrotic Cell Death”

Activation of the Sur1–Trpm4 channel is a major molecular mechanism of “accidental necrotic cell death” in the CNS [15, 16, 20]. The other principal mechanism, NMDA-receptor-mediated excitotoxicity, is specific to neurons, whereas the mechanism mediated by the Sur1–Trpm4 channel involves all members of the neurovascular unit, including neurons, astrocytes, oligodendrocytes, and endothelial cells. “Accidental” cell death is substantially different from “programmed” cell death (apoptosis, autophagy, and necroptosis) [15]. The designation programmed implies that a specific molecular sequence is initiated by an identified stimulus, with the explicit purpose of causing cell death. By contrast, accidental implies that molecular machinery that normally serves a role unrelated to death is inadvertently transformed into a cell executioner.

In stroke, the overwhelming form of cell death is not programmed, but is accidental necrosis, due to factors external to the cell or tissue. Transcriptional upregulation of the Sur1–Trpm4 channel in CNS injury is not directed to inducing cell death. As reviewed elsewhere [12, 14], the normal function of the channel is to protect against a pathological rise in intra-cellular calcium, one of the hallmarks of CNS injury [21, 22]. However, because Sur1–Trpm4 channels also are sensitive to the intracellular concentration of ATP, extreme depletion of ATP, as occurs in stroke, can result in persistent activation of the channel. Unchecked channel opening, in turn, leads to persistent sodium influx, resulting in oncotic cell swelling (cytotoxic edema) and accidental necrotic cell death [15].

Sur1 in CNS Ischemia

Sur1 is constitutively expressed in some but not all neurons of the CNS, where it forms K_{ATP} channels exclusively [23, 24]. Sur1 normally is absent in oligodendrocytes, astrocytes, endothelium, and many neurons [25]. The Sur1–Trpm4 channel is not expressed in uninjured tissues [12].

Animal Models of Stroke

Sur1 is upregulated progressively during several hours after the onset of ischemia. Following permanent mechanical middle cerebral artery occlusion (MCAo) in the rat, 3 h are required before *Abcc8* mRNA is increased 2.5-fold, and 8 h are required before Sur1 protein is increased 2.5-fold [16]. In a model with 105 min of mechanical MCAo followed by 1 h of reperfusion, upregulation of Sur1 is observed only in microvascular endothelial cells [26]. After 3 h of reperfusion, upregulation becomes evident in neurons [26]. By 24 h, Sur1 is upregulated in all members of the neurovascular unit in the penumbra, including neurons, astrocytes, oligodendrocytes and endothelium, whereas Sur1 expression is undetectable in the necrotic core [16, 17]. In a model with 4.5 h ischemia followed by a 30-min infusion of rtPA, Sur1 upregulation after infusion is apparent, but it increases 7- to 11-fold over the subsequent 19 h, especially in watershed regions [27]. Expression of Sur1 protein in neurons and endothelial cells after ischemia has been corroborated by in situ hybridization for *Abcc8* mRNA [16,17]. In cerebral ischemia, transcriptional upregulation of Sur1 takes place without upregulation of the K_{ATP} channel protein Kir6.2 or its

corresponding mRNA [16]. However, cerebral ischemia is associated with upregulation of *Trpm4* and its mRNA [19].

Cell Culture

Sur1 also is upregulated in cultured cells under conditions that simulate injury. Exposing cultures of brain microvascular endothelial cells, both primary cultures and bEnd.3 cells, to hypoxia or TNF α results in upregulation of Sur1 protein and *Abcc8* mRNA [26, 28, 29].

Human Stroke

A comprehensive analysis of human brain autopsy specimens showed that Sur1 is transcriptionally upregulated in neurons, astrocytes, and vascular endothelial cells in patients with ischemic stroke [18], corroborating previous case reports [30]. Postmortem specimens obtained from 15 patients within the first 31 days after focal infarcts were examined. Significantly elevated levels of Sur1 were detected in all cases, with three distinct temporal patterns of expression: (i) neurons and endothelium showed the greatest elevation during the first week, after which levels declined; (ii) astrocytes and microglia/macrophages showed progressive increases during the first 31 days; (iii) neutrophils near the infarct showed persistently elevated degrees of immunoreactivity that did not change with time. Upregulation of Sur1 was corroborated using *in situ* hybridization for *Abcc8* mRNA.

Targeting Sur1 in CNS Ischemia

The pharmacological properties of Sur1 in Sur1–Trpm4 channels are indistinguishable from those of Sur1 in K_{ATP} (Sur1–Kir6.2) channels [31]. The Sur1–Trpm4 channel is blocked by first and second generation sulfonylureas, tolbutamide (EC₅₀, 16.1 μ M at pH 7.4), and glibenclamide (EC₅₀, 48 nM at pH 7.4) [31]. Blockade by sulfonylurea drugs is due to a prolongation of, and an increase in the probability of long closed states of the channel, with no effect on open channel dwell times or channel conductance. The inhibitory effect of glibenclamide on channel opening is prevented by an antibody directed against one of the cytoplasmic loops of Sur1 [31]. The potency of blockade by glibenclamide is increased 8-fold at pH 6.8 (EC₅₀, 6 nM), compared to pH 7.4, consistent with a weak acid needing to enter the lipid phase of the membrane to cause blockade [16, 32], paralleling observations with K_{ATP} channels [33].

Normally, glibenclamide and other sulfonylureas do not accumulate in the brain in physiologically relevant concentrations [34], accounting for the observation that, despite decades of clinical use in diabetic patients, there are no reports of side effects involving the CNS that are attributable directly to sulfonylureas (as distinct from CNS effects of hypoglycemia), even though the brain contains neurons that express K_{ATP} channels [14, 23]. However, penetration of glibenclamide into the brain is enhanced after an ischemic insult. With ischemia, lactic acidosis of the tissues creates a relatively low pH environment [35]. Glibenclamide is a weak acid and, as such, its lipid solubility, and therefore its ability to penetrate the blood–brain barrier (BBB), is enhanced at low pH. This factor, combined with dysfunction of the BBB that also occurs with an ischemic insult, results in glibenclamide being “selectively” taken up by ischemic tissues while being excluded from normal CNS tissues. Studies using the fluorescent BODIPY-glibenclamide (constant infusion, 75 ng/h) [16], as well as [³H]glibenclamide (single injection, 100 μ Ci IP; specific activity not specified) [36], have shown that glibenclamide is undetectable in normal brain but accumulates preferentially in the ischemic brain. As a result, relatively low doses of drug can be used to obtain a favorable therapeutic effect, while minimally affecting insulin secretion in the pancreas [14].

Blockade of Sur1 in Rat Models of Stroke

Focal ischemic injury involving the brain is associated with progressive microvascular dysfunction [37, 38], which is characterized initially by the formation of ionic edema and subsequently by the formation of vasogenic edema. Edema fluid that accumulates in the parenchyma when the tight junctions of the BBB are intact is largely devoid of protein but is rich in ions, and hence is called ionic edema. When the tight junctions of the BBB become disrupted, the edema fluid that accumulates is essentially a plasma filtrate, and is called vasogenic edema. Microvascular dysfunction may evolve into the catastrophic structural failure of capillaries. The structural failure of microvessels can lead to isolated microscopic (petechial) hemorrhages, or in the worst cases, to the formation of a parenchymal hematoma. Ionic and vasogenic edema, as well as hemorrhage, add mass to the brain that causes tissue swelling. Swollen tissues can compromise adjacent tissues and worsen the original insult. If severe, as in malignant cerebral edema following a large stroke, death of the organism may ensue.

The molecular events responsible for these various forms of microvascular dysfunction are complex, but they include the upregulation of Sur1 and subsequent Sur1-mediated microvascular dysfunction. Several preclinical studies utilizing a variety of different models of stroke originating from several independent laboratories have demonstrated that Sur1 inhibition by glibenclamide reduces adverse secondary manifestations and increases favorable outcomes in rat models of focal cerebral ischemia.

Conventional Non-lethal Rat Models of Stroke

The effects of glibenclamide have been studied in three conventional non-lethal rat models of ischemic stroke (Table 1): thromboembolic [16, 39], temporary MCAo (tMCAo) [36, 39, 40], and permanent MCAo (pMCAo) [39, 41] models.

In a thromboembolic model [16], glibenclamide (no loading dose; 75 ng/h) was associated with cortical sparing that was attributed to improved leptomeningeal collateral blood flow due to reduced mass effect from edema (Fig.2a–c). In the same model [39], glibenclamide (33 –g/kg loading dose plus 75 ng/h constant infusion) administered 6 h after the onset of ischemia was associated with 50 % reduction in lesion volume.

In a tMCAo (60 min) model [36], glibenclamide (0.2 µg/rat IV at 7, 13, and 25 h after onset of ischemia) was associated with beneficial effects on neuroscores, on necrotic volume and on neuronal preservation at 3 days, including reduced subcortical (ventral pallidum) necrosis, reduced neuronal loss, and reduced pathological calcium deposition. In another tMCAo (105 min) model [39], glibenclamide (10 µg/kg loading dose plus 200 ng/h constant infusion) administered at 5.75 h after the onset of ischemia was associated with 41 % reduction in lesion volumes. In this study, smaller lesions were associated with less hemispheric swelling.

In a pMCAo model [41], glibenclamide (10 µg/kg loading dose plus 200 ng/h constant infusion) administered shortly after the onset of ischemia, was associated with a 26 % reduction in infarct volume, 50 % reduction in hemispheric swelling, better neurological severity scores and improved grip strength. In the same model [39], glibenclamide (33 µg/kg loading dose plus 200 ng/h constant infusion) administered 4 h after the onset of ischemia was associated with a 51 % reduction in lesion volume.

In another study with tMCAo (60 min), Ortega et al. [40] analyzed long-term outcomes with glibenclamide (0.2 µg/rat IV; ~0.6 µg/kg) administered at 7, 13, and 25 h after onset of ischemia. They observed neuronal preservation, as in their first paper [36], and they reported

significant improvements in sensorimotor and cognitive functions up to 1 month. In addition, glibenclamide was accompanied by an increase in BrdU and NeuN labeling in and around the infarct, consistent with an enhancement in neurogenesis in the cortex. Also, glibenclamide enhanced angiogenesis in the cortex and hippocampus, which is a well characterized hallmark of recovery following ischemic stroke.

Lethal Rat Models of Stroke

The effect of glibenclamide also has been examined in rat models of malignant infarction/malignant cerebral edema associated with high mortality rates (Table 2). In a model that utilized particle embolization in male and female rats [16], malignant edema was manifested as an increase in brain tissue water from a normal value of 78–83 % at 8 h, and was associated with 65 % mortality at 7 days (Fig. 2d). Administration of glibenclamide (no loading dose; 75 ng/h) beginning shortly after the onset of ischemia decreased tissue water to 80 % and reduced mortality at 7 days to 24 %. Another model utilized 6 h tMCAo [17] and was associated with thrombosis of MCA branches that required up to 2–3 h to recanalize. Malignant edema was manifested as a 21 % increase in hemispheric volume and was associated with 67 % mortality at 24 h. Administration of glibenclamide (10 µg/kg loading dose plus 200 ng/h) at 6 h, at the time of occluder withdrawal, decreased hemispheric swelling to 8 % and reduced mortality to 5 %. Using this model, the effect of glibenclamide was compared to the effect of a large decompressive craniectomy. Both treatments administered at 6 h were equally effective in essentially eliminating mortality. However, neurological function during the subsequent 2 weeks showed that glibenclamide was superior to decompressive craniectomy. The superior effect of glibenclamide on neurological function was associated with better tissue (white matter) preservation of the corpus callosum and of the watershed cortex of the anterior and middle cerebral arteries, consistent with the aphorism that preventing swelling is preferable to decompressing the already swollen brain.

An important, albeit poorly studied outcome in animal models of stroke is the general health of the organism. tMCAo in rats is associated with severe weight loss of unknown etiology that is not explained by dehydration, stress, or altered hormonal secretions [42–44]. In the aforementioned study [17], weight gain quickly resumed its normal trajectory and endured for the 2 weeks of observation in the rats treated with glibenclamide, but not in those treated with decompressive craniectomy. Several reasons potentially could account for this, including better sparing of cortico-striatal and hypothalamic tissues in the rats treated with glibenclamide.

Rat Models of Stroke with rtPA

The effect of glibenclamide was examined in a rat model of malignant cerebral edema involving 4.5 h MCAo plus administration of rtPA (0.9 mg/kg IV, as in humans) at the time of occluder withdrawal [27]. Malignant edema was manifested as 8 and 15 % increases in hemispheric volume measured at 10 and 24 h, respectively, and was associated with 53 % mortality at 48 h. Administration of glibenclamide (10 µg/kg loading dose plus 200 ng/h) at 4.5 h, at the time of recanalization and administration of rtPA, decreased hemispheric swelling to 4 and 8 % at 10 and 24 h, respectively, reduced mortality to 17 %, and yielded significant improvements in neurological function. Administration of glibenclamide at 10 h after the onset of ischemia (5.5 h after recanalization plus administration of rtPA), decreased hemispheric swelling to 8 % at 24 h, reduced 48 h mortality to 12 %, and yielded significant improvements in neurological function. In these experiments, lesion volumes were not changed by glibenclamide, consistent with 4.5 h MCAo producing a maximum ischemic insult. However, examining the relationship between infarct volume and neurological function was revealing. In the controls, a steep, highly significant negative correlation was

found. By contrast, with glibenclamide, the association was much less steep and was not statistically significant, indicating that glibenclamide essentially dissociated functional outcome from infarct volume, due to the significant reductions in edema and swelling.

The effect of glibenclamide was studied in Wistar and SHR models of malignant cerebral edema involving 6 h MCAo plus administration at the time of occluder withdrawal of a dose of rtPA (10 mg/kg IV) that is expected to be thrombolytic in rats [45]. Malignant edema was manifested as a 26 % increase in hemispheric volume and a high incidence of hemorrhagic transformation at 24 h. Administration of glibenclamide (10 µg/kg loading dose plus 200 ng/h) at 6 h, at the time of recanalization and administration of rtPA, decreased hemispheric swelling to 12 % and significantly reduced scores of hemorrhagic transformation. Mortality and functional outcomes were equally poor in rats without or with treatment with rtPA, with neurological scores of 7-8 in both groups (8 signifies death, 7 signifies coma). Glibenclamide significantly reduced mortality and improved neurological scores, both without or with treatment with rtPA, with scores of 3-4. The best scores were observed in rats treated with a thrombolytic dose of rtPA plus glibenclamide. The beneficial effects of glibenclamide endured for the 2 weeks of observation.

K_{ATP} Channels and Neuronal Protection

In acute ischemic injury to the CNS, the principal target of glibenclamide is the Sur1–Trpm4 channel. However, some CNS neurons and microglia constitutively express K_{ATP} (Sur1–Kir6.2) channels [23, 24], which also could be blocked by glibenclamide. Protective effects of glibenclamide in the context of cerebral ischemia have been reported in two studies in which the effect was unlikely to be due to Sur1–Trpm4 channels, since there was no time for transcriptional up-regulation of the channel.

The first study involved a model of global ischemia/reperfusion (15 min/60 min) in Wistar rats [46]. Ischemia/reperfusion increased myeloperoxidase activity (a marker of neutrophil activity), lipid peroxides, TNFα and PGE2, while it decreased glutathione, total antioxidant capacity, nitric oxide and IL-10 levels in the hippocampus. Glibenclamide (1 mg/kg) pre-treatment prevented all of these alterations, with neutrophil recruitment reduced by 66 %. Notably, glibenclamide previously was reported to ameliorate ischemia/reperfusion injury due to neutrophil recruitment in other organs [47–50].

The second study involved an in vitro model of cerebral ischemia [hippocampal brain slices subjected to 14 min oxygen/glucose deprivation (OGD)] [51]. This study found that glibenclamide (10 –M in the bath) superfused for 30 min before and during the “hypoxic” period, prevented the usually irreversible loss of field potentials produced by 14 min OGD. By comparison, no protection was observed when the ischemic insult was induced in slices treated with the mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate, consistent with glibenclamide acting on plasmalemmal K_{ATP}, not mito-K_{ATP}. In this study, glibenclamide also significantly reduced cell loss of CA1 hippocampal pyramidal neurons.

Conventional thinking suggests that *opening* K_{ATP} channels should be protective, because the associated cell hyperpolarization would reduce influx of calcium via voltage-dependent calcium channels [13]. However, this hypothesis discounts the fact that voltage-dependent calcium channels undergo voltage-dependent inactivation, which diminishes the potential harm from prolonged cell depolarization. In addition, as Nistico et al. [51] point out, opening of K_{ATP} channels might be harmful, if it results in an excess efflux of potassium that would then have to be compensated by ATP-consuming sodium–potassium pumps (Na⁺/K⁺-ATPase). Also, hyperpolarization increases the electrochemical driving force for calcium, increasing its influx via non-voltage-dependent pathways. Thus, in the setting of ischemia, neuronal *depolarization* may be more protective than hyperpolarization [52].

K_{ATP} Channels and Microglia

In the two studies by Ortega and colleagues [36, 40] cited above, as well as in a recent review [53], these authors proposed that glibenclamide exerts its protective effect in cerebral ischemia due to modulation of microglial K_{ATP} channels. In culture, they found that BV2 cells (a transformed microglial cell line) exposed to the proinflammatory stimulus of lipopolysaccharide (LPS) plus interferon (IFN) γ showed upregulation of the K_{ATP} channel subunits, Sur1, Kir6.1 and Kir6.2, both protein and mRNA. Adding glibenclamide to the medium augmented the reactive morphology, the phagocytic capacity and the release of TNF α [36]. Also, in resting microglia, binding of BODIPY-glibenclamide was confined to the perinuclear space (endoplasmic reticulum), whereas after activation with LPS+IFN γ , BODIPY-glibenclamide labeling was present in the plasmalemmal membrane [40], suggesting functional channel localization. Based on these findings, the authors postulated that protection observed with glibenclamide in their MCAo model (see above) was due, in part, to increased microglial phagocytic capacity after microglial K_{ATP} channel inhibition.

Glibenclamide and Ischemic Preconditioning

Ischemic preconditioning is a biological response with an important adaptive advantage [54]. Preconditioning, i.e., the induction of tolerance, is obtained when a subcritical episode of ischemia activates endogenous protective mechanisms and thereby renders the brain resistant to a subsequent, more severe ischemic event [55–57]. Preconditioning is produced not only by subcritical ischemia but also by several non-ischemic stimuli, including cortical spreading depression, hypoxia, hyperthermia, and a number of chemical substances such as isoflurane, diazoxide, adenosine, and others. A considerable body of literature has addressed this important biological response (reviewed in [54]). Here, we consider only ischemia-induced preconditioning, due to its relevance to transient ischemic attacks (TIAs) in humans.

In a rat model, preconditioning induced by 3 min global ischemia 3 days before 6 min global ischemia was largely negated by administering glibenclamide (1 μ mol ~500 μ g) intracerebroventricularly 20 min prior to the first ischemia [58] (This dose is many times greater than what is administered subcutaneously post-insult to reduce the effects of focal ischemia/reperfusion). That experiment recently was revisited using a gerbil model in which the duration of ischemic depolarization was used to define the severity of the insults [59]. When equivalent insult severity was maintained during priming insults, the depolarization thresholds for ischemic neuronal injury were identical in vehicle- and glibenclamide-treated animals, and glibenclamide did not affect preconditioning. Doubt about the potential involvement of Sur1 in ischemic preconditioning also has arisen from experiments with knock-out mice. *Abcc8*^{-/-} mice exhibit normal ischemic preconditioning, but yet are susceptible to the same augmentation of neurodegeneration by 5-hydroxydecanoate, the selective blocker of mitoK_{ATP} channels, as are wild-type mice [60].

The importance of ischemic preconditioning in human stroke remains to be clarified [61]. Concern regarding this phenomenon applies primarily to diabetic patients taking a sulfonylurea agent who have a TIA, i.e., an ischemic preconditioning event, a few days before their stroke. In the retrospective study by Kunte et al. [62], patients were on sulfonylureas before and after stroke, and if they had a preconditioning event (TIA) before their stroke, preconditioning should have been blunted if sulfonylurea agents affect this response. However, analysis directed to this question revealed no interaction between prior TIA and the beneficial effect of glibenclamide in non-lacunar stroke (additional details below).

Stroke Therapy Academic Industry Roundtable

The Stroke Therapy Academic Industry Roundtable (STAIR) was originally undertaken to help address the translation of animal studies to human clinical trials and to facilitate successful translation. STAIR provided recommendations in 1999, updated in 2009, for the preclinical development of acute ischemic stroke treatments [63, 64]. The most recent STAIR recommendations for translational experiments in stroke are listed below (in *italics*), along with comments on how the available preclinical studies on glibenclamide address these recommendations.

Dose Response

Minimum effective and maximum tolerated dose; a target concentration; a tissue level of effect identified from animal histology and behavioral studies; the drug in these ranges should access the target organ—Effects on infarct

volume, edema, swelling, and neurological function were assessed in non-lethal models of stroke using loading doses of 0, 3.3, 10, or 33 $\mu\text{g/kg}$ plus a constant infusion dose of 75 or 200 ng/h [16, 39, 41]. In the thromboembolic model, where loading doses of 3.3 and 33 $\mu\text{g/kg}$ administered at 6 h were compared directly, the higher loading dose was more efficacious. Effects on neurological function and on mortality were studied in lethal models of stroke using loading doses of 0 or 10 $\mu\text{g/kg}$ and constant infusion doses of 75 or 200 ng/h [16, 17]. A clear benefit on mortality was observed with the higher dose.

Constant infusion doses of 200 and 400 ng/h subcutaneously in rats are devoid of apparent toxicity and yield serum glucose levels that are within the normal clinical range [16, 28, 29]. The higher infusion dose (400 ng/h) is efficacious in a model of global cerebral ischemia [65], but has not been evaluated systematically in focal cerebral ischemia.

Steady-state serum levels of glibenclamide were measured using liquid chromatography and mass spectroscopy by a commercial firm (Celerion, Lincoln, NE). Drug levels were measured 24 h after the start of infusion in rats administered 400 ng/h subcutaneously, a dose twice that used in published studies on focal ischemia (to improve the fidelity of detecting low serum concentrations). This dosing regimen yielded steady-state serum levels of glibenclamide of $16 \pm 3 \text{ ng/mL}$ ($n = 3$) (Simard and colleagues, unpublished). These serum levels are below those observed in humans administered 3 mg/day IV infusion of injectable glibenclamide (RP-1127; Remedy Pharmaceuticals, Inc.), which is associated with minimal or no hypoglycemia (additional details below).

Studies using fluorescent BODIPY-glibenclamide (75 ng/h subcutaneously) [16] as well as [^3H]glibenclamide (100 μCi IP; specific activity not specified) [36] in animals with cerebral infarctions have shown that glibenclamide reaches and accumulates in its intended target. The doses of drug found to accumulate in the target were protective, but these doses were below those found in other studies to have more robust protective effects (see above).

Therapeutic Window

The [time] window for neuroprotection—Beneficial effects on infarct volume have been observed in non-lethal models of stroke with treatment delivered up to 4–6 h after the onset of ischemia [39]. Beneficial effects on neurological function, swelling and on mortality have been observed in lethal models of stroke with treatment delivered at 6 and 10 h after the onset of ischemia [17, 27].

Outcome Measures

Multiple endpoints; histological and behavioral outcomes; behavioral studies at least 2–3 weeks after stroke—The multiple endpoints evaluated include edema formation, brain swelling, infarction volume, neuronal preservation, white matter preservation, neurological scores, Garcia scores, grip strength, weight gain and mortality, as well as evidence of neurogenesis and angiogenesis. Studies have examined endpoints out to 2 weeks [17, 45] and 4 weeks [40].

Physiological Monitoring

Blood pressure, temperature, blood gases, and blood glucose; cerebral blood flow using Doppler flow or perfusion imaging—Temperature, blood gases, and blood glucose have been monitored and reported [17, 27, 39, 41]. Laser Doppler flowmetry has been monitored and reported [17, 27, 39, 41].

Multiple Species

Rodents or rabbits are acceptable for initial testing and gyrencephalic primates or cats are desirable as a second species, but the cost, availability, and ethical acceptability may be problematic—To date, all studies have been carried out in rats [Wistar, Sprague–Dawley, spontaneously hyper-tensive rat (SHR)]. There has been no study of glibenclamide in gyrencephalic non-human primates (NHP) or cats. There are two reasons for this. First, in some respects, glibenclamide is akin to decompressive craniectomy—it is being studied largely for its promise in reducing swelling and death, two endpoints that were not considered by STAIR [63, 64]. Second, there is no accepted model of the malignant MCA syndrome in NHP or cat. A non-survival model of MCA ischemia/reperfusion (3/6 h) has been described in the cat [66, 67], and several models of stroke have been described in NHP [68–73], including two with potentially high mortality in baboons [68, 74], depending on timing of reperfusion. Meaningful implementation of this recommendation in the spirit of STAIR would require developing an ethically acceptable, high mortality model of the malignant MCA syndrome in cat or NHP. However, this may be unachievable. It was recognized by the STAIR committee that “cost, availability and ethical acceptability may be problematic” [64]. It is difficult to gain IACUC (Institutional Animal Care and Use Committee) approval for high mortality studies with any species, but especially with sentient species, due to perceived suffering. Given these considerations, current thinking is that the study of a gyrencephalic model would not be warranted in a setting such as this.

Reproducibility

There are five items in this category:

Fundamentals of good scientific inquiry—These recommendations have been met in part or in full, as documented in several studies, with the most comprehensive description given in Simard et al. [27].

Studies in aged animals and animals with comorbidities—One study reported the effect of glibenclamide on hemorrhagic transformation in SHR [45].

Studies in male and female animals—One study reported the effect of glibenclamide on mortality in males and females [16].

Interaction studies with medications used in stroke patients—Quantitative analysis of clot lysis induced by rtPA showed that glibenclamide has no effect on rtPA activity [45].

In four retrospective studies of diabetic patients with stroke [30, 62, 75, 76], many of the patients would be expected to have been taking other commonly prescribed medications (some are listed in the original papers), suggesting no adverse interactions in stroke between sulfonylurea drugs and other commonly prescribed medications.

Relevant biomarker endpoints—No preclinical study has reported the effect of glibenclamide on serum biomarkers, although one study reported reduced MMP-9 activity in brain tissues [45].

Additional Comments in the STAIR Document

Apart from the above-mentioned formal recommendations, the updated STAIR document [64] makes a number of other observations relevant to preclinical studies, including:

No model precisely mimics clinical stroke in that truly permanent occlusion rarely occurs in humans because of spontaneous recanalization, and the transient models are restricted to relatively short time windows, otherwise risking fatal edema and hemorrhage—STAIR addresses non-lethal models of stroke exclusively. Several studies on glibenclamide have gone beyond the traditional assessment of infarct size and neurological function in non-lethal models to look at fatal outcomes from swelling [16, 17, 27] and from hemorrhagic transformation [45] (Table 2). Models with occlusion times up to 6 h have been studied, with drug administered at the time of recanalization [17].

Lack of direct linkage between the model and the clinical situation—One study was specifically designed to mimic the clinical situation by studying 4.5 h MCAo with rtPA (0.9 mg/kg) administered at the time of filament withdrawal, and glibenclamide given 5.5 h later, 10 h after the onset of ischemia. These conditions were designed to reflect salient features of anticipated clinical trials.

Treatment that targets only neurons and that does not also salvage white matter tracts—The study of Simard et al. [17] documented that, in a model of malignant cerebral edema, white matter preservation was better with glibenclamide than with decompressive craniectomy.

Neurovascular unit—Several studies have shown that all members of the neurovascular unit upregulate Sur1 de novo, including neurons, astrocytes, oligodendrocytes and endothelium [16, 17, 39], and that Sur1 also is expressed by microglia [40].

Many of the neuroprotective targets may have a negative effect on the recovery process—The study by Ortega et al. [40] showed that glibenclamide is associated with improved neurogenesis and angiogenesis at 1 month.

Summary of the Preclinical Material

The preclinical studies on glibenclamide have fulfilled both the spirit of STAIR as well as almost all of its specific recommendations, in some cases, numerous times. However, STAIR addresses non-lethal models of stroke exclusively. The preclinical studies of glibenclamide have gone beyond the vision of STAIR to examine lethal models of stroke intended to mimic malignant cerebral infarction in humans.

Retrospective Studies in Humans with Stroke

There is a natural experiment that can be exploited to examine the potential benefit of Sur1 inhibition in human stroke—compare the outcomes of patients with DM-2 presenting with stroke whose diabetes is managed with a second generation sulfonylurea drug versus those whose diabetes is managed without a sulfonylurea drug (controls). In recent years, two types of such studies have been reported. The first type examined outcomes in patients who were on a sulfonylurea drug at the time of stroke, but who did not necessarily continue on a sulfonylurea after presenting with stroke. The second type of study examined patients who were on a sulfonylurea drug at the time of stroke and who were maintained on the sulfonylurea after hospitalization for stroke. The first type of study can ascertain whether the *presence of drug at the onset of ischemia* has an effect on outcome. The second type of study can ascertain whether the presence of drug during the days following the ischemic insult has an effect on outcome. The first type of study addresses an important safety issue, i.e., should patients with DM-2 at risk for stroke be treated with a sulfonylurea drug. The second type of study addresses a very different issue, one of efficacy, i.e., might patients with an acute ischemic stroke benefit from being on a sulfonylurea drug.

The first retrospective study of diabetic patients on second generation sulfonylurea drugs was conducted by Weih et al. [75]. That study was of the first type, i.e., a safety study. It did not take into account whether patients were maintained on a sulfonylurea drug after stroke. They studied 146 patients with diabetes and hemispheric stroke, 60 of whom were using a sulfonylurea drug at the time of stroke onset, and 86 of whom were not (diabetes managed with diet alone, insulin, or other oral agent such as acarbose or metformin). The study found that the use sulfonylurea drugs in diabetic patients presenting with stroke is not associated with increased stroke severity, mortality, or a worse in-hospital outcome. Notably, there was a nonsignificant trend toward neurological improvement in the sulfonylurea group, and more patients without sulfonylurea drugs had unimproved neurological deficits in the first 3 days.

A similar safety study was reported by Favilla et al. [76] based on an analysis of the Virtual International Stroke Trials Archive (VISTA), a compilation of unrelated data sets that has important limitations for analysis. They analyzed outcomes in 1,050 diabetic patients, 298 with sulfonylurea use before stroke onset. Similar to Weih et al. [75], they concluded that sulfonylurea use before stroke onset did not affect stroke severity or long-term functional outcome compared with other treatments for DM-2. However, in a subset of 28 patients in whom sulfonylureas were continued following stroke, trends favored sulfonylureas.

Because the Sur1–Trpm4 channel is transcriptionally upregulated beginning several hours after the onset of ischemia [26], and is expressed in neurons and endothelium for 1 week or more after stroke [18], only patients maintained on a sulfonylurea drug after stroke would be expected to benefit. The importance of continued use of a sulfonylurea drug after stroke is underscored by the findings of Kunte et al. [62]. They began their analysis with precisely the same cohort of patients previously reported by Weih et al. [75] (see above). They excluded patients hospitalized later than 24 h after the onset of acute ischemic stroke, and after making other balancing exclusions, they ended up with a cohort comprised of 33 patients who were taking a second generation sulfonylurea (glibenclamide, glimepiride, or glibornuride) at admission through discharge (treatment group), and 28 patients who were not on a sulfonylurea drug (control group). The primary outcome was a decrease in National Institutes of Health Stroke Scale (NIHSS) of 4 points or more from admission to discharge or a discharge NIHSS score = 0, either of which is considered a “major neurological improvement.” The secondary outcome was a discharge modified Rankin Scale (mRS) score of 2 or less, which signifies functional independence. No significant differences, other than stroke subtype, were observed in baseline variables between control and treatment groups.

The primary outcome was reached by 36 % of patients in the treatment group and 7 % in the control group [odds ratio (OR) = 7.4; 95 % confidence interval (CI) 1.5-37; $P = 0.007$]. The secondary outcome was reached by 82 versus 57 % (OR = 3.4; CI 1.1-11; $P = 0.035$). Subgroup analysis showed that the benefit of drug was observed only in patients with non-lacunar strokes, with 42 % of patients on sulfonylureas who harbored a non-lacunar stroke achieving the primary outcome, versus 0 % of controls. Outcomes were independent of sex, previous TIA (see section above on ischemic preconditioning), and blood glucose levels. Thus, comparing the reports of Weih et al. [75] and of Kunte et al. [62], the same population of patients analyzed to reflect continued use of sulfonylurea drug after stroke yields a very different conclusion compared to an analysis that omits this critical factor.

A second retrospective study was carried out by Kunte et al. [30] involving a different cohort of patients. A review of medical records was performed on diabetic patients admitted to Charité Hospital (Berlin) with an acute ischemic stroke from January 2005 to December 2006. After exclusions, the cohort consisted of 177 control patients not on a sulfonylurea and 43 patients in the treatment group, who were treated with a sulfonylurea (glibenclamide, glimepiride, or gliquidone) at admission through discharge. In this study, the primary outcome measure was evidence of symptomatic hemorrhagic transformation prior to discharge or within 21 days of the ischemic stroke, and the secondary outcome measures were any hemorrhagic transformation (symptomatic or asymptomatic), death in hospital, mRS score of 2 or less, and a decrease in NIHSS score of 4 points or more from admission to discharge, or a discharge NIHSS score = 0.

Patients treated with a sulfonylurea drug were significantly more likely to survive hospitalization [no patient in the sulfonylurea group died, compared to 18 (10 %) in the control group ($P = 0.027$)] and significantly less likely to experience hemorrhagic transformation [20 control patients (11 %) experienced symptomatic hemorrhagic transformation, whereas no patient in the sulfonylurea group experienced symptomatic hemorrhagic transformation ($P = 0.016$)]. In agreement with their prior observations [62], they also found that patients treated with a sulfonylurea were significantly more likely to have better neurological outcomes. Even after matching for imbalances in baseline variables and excluding outliers, the influence of sulfonylurea treatment remained significant.

Another retrospective study of diabetic patients from the Registry of the Canadian Stroke Network was reported at the International Stroke Conference in 2009 [77] (Dr. Frank Silver, University of Toronto, personal communication). Of 15,814 patients screened, 2,448 with diabetes were admitted to hospital within 24 h of symptom onset. For patients who had been on and continued on sulfonylurea drugs, compared to controls not on sulfonylureas, the likelihood of in-hospital mortality was less (OR = 0.51; CI 0.37–0.71; $P < 0.05$), and the likelihood of neurological worsening was less (OR = 0.70; CI 0.55–0.89; $P < 0.05$). A subgroup of patients was analyzed that were treated with IV rtPA. For patients who had been on and continued on sulfonylurea drugs, compared to controls not on sulfonylureas, the likelihood of in-hospital mortality was less (OR = 0.30; CI 0.12–0.73; $P < 0.05$), and the likelihood of neurological worsening was less (OR = 0.52; CI 0.28–0.98; $P < 0.05$).

Overall, the available data on diabetic patients presenting with acute ischemic stroke suggest that, if a patient is on a sulfonylurea drug, this drug should be continued, not stopped, unless a contraindication is present.

Injectable Glibenclamide (RP-1127)

Glibenclamide has been used safely for three decades as an enteral (*per os*) formulation for the treatment of DM-2. However, oral administration of glibenclamide for other indications

in non-diabetic patients is not appropriate. In all patients, diabetic or not, episodic oral administration of drug leads to high peak serum levels that reach a maximum 1–2 h after ingestion, followed by low serum levels (troughs) at times between peaks. Peak serum levels of drug trigger insulin release that is considerably more robust in non-diabetic patients with a normally functioning pancreas compared to diabetic patients. As a result, non-diabetics taking oral glibenclamide are at risk for hypoglycemia, which can become symptomatic. Also, the systemic absorption of drug taken orally depends on stomach pH. Stomach pH varies widely, especially in gravely ill patients, leading to unpredictable serum levels. Thus, oral administration of glibenclamide in acutely ill stroke patients can be problematic, and is not recommended.

The undesirable pharmacokinetic features and the undesirable side-effect of hypoglycemia associated with orally administered glibenclamide in non-diabetic patients can be avoided by parenteral administration. Intravenous administration can be used to rapidly reach and maintain steady serum concentrations of drug that, for reasons discussed above, are non-hypoglycemogenic in non-diabetic patients but may be therapeutic for cerebral ischemia. In acute cerebral ischemia, two goals are paramount: (i) rapidly achieving the desired serum concentration—“time is brain;” (ii) maintaining constant receptor occupancy to optimize channel inhibition. The importance of these precepts is underscored by the preclinical data. The most robust protection observed in preclinical studies was obtained using a constant subcutaneous infusion of glibenclamide [17, 27, 39, 45]. (Bioavailability with subcutaneous infusion closely approximates that with IV infusion [78]). By contrast, less robust protection was reported with episodic drug delivery, either IV [36] or IP [79].

Given the forgoing, an injectable formulation of glibenclamide, RP-1127, was developed by Remedy Pharmaceuticals Inc. (New York, NY) that can be administered IV to non-diabetic patients with acute ischemic stroke (see Conflict of interest statement). RP-1127 has been studied in a Phase I clinical trial assessing safety in normal volunteers.

Phase I Clinical Trial of RP-1127 in Normal Volunteers

Healthy volunteers were enrolled in a Phase I study of RP-1127 ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01132703) identifier: NCT01132703). The primary objective was to evaluate the safety and tolerability of RP-1127 administered as a bolus dose followed by a 72 h continuous infusion (Table 3). The secondary objectives were to assess the pharmacokinetics of RP-1127 and blood glucose and serum insulin pharmacodynamic responses to RP-1127.

Study subjects were housed in an in-patient facility to allow for continuous safety monitoring. Blood glucose was measured throughout the study to obtain pharmacodynamic information and for safety reasons. Safety labs, vital signs, and 12-lead electrocardiograms (ECG) were collected at baseline and at various times after initiation of study drug. Patients were monitored for adverse events (AEs) including IV irritation.

There were no serious AEs during the study. No ECG abnormality, including no evidence of QT interval prolongation, was observed. No clinically relevant IV irritation was noted. The incidence of blood glucose levels above 80 mg/dL was more frequent in the placebo group than in the 3 mg/day group, while there was no difference in the incidence of blood glucose levels below 70 mg/dL, implying a reduction in blood glucose at the dose of 3 mg/day without hypoglycemia (defined as <70 mg/dL) [80–82].

Two subjects (in the 6.00 and 10.00 mg/day treatment groups) were discontinued from the study due to persistent hypoglycemia. The subject in the 6.00 mg/day treatment group that was discontinued also experienced transient increases in ALT levels (reported as a severe AE) and increases in AST levels (reported as a moderate AE) beginning 3 days after study

drug discontinuation. This subject was asymptomatic at all times. After peaking on day 6, her liver enzyme levels decreased and were no longer clinically significantly elevated by day 15, and by day 22 both were normal. (Liver function abnormalities, including elevations in liver enzymes, have been reported in patients taking oral glyburide, as described in package inserts for approved oral glyburide drug products.) There were no other clinically significant drug-related AEs.

Steady-state plasma levels for the dose of 3.0 mg/day were 27.3 ± 8.4 ng/mL (mean $\pm$ SD). Plasma glibenclamide concentrations >25 ng/mL resulted in decreased blood glucose levels relative to baseline.

Based on the findings in the Phase I study, the dose of 3 mg/day IV of RP-1127 may be safe and least likely to lead to refractory hypoglycemia, yet appears to yield serum levels of drug that exceed those observed to be therapeutic in the animal models of stroke (see above).

Conclusion

Recent advances in molecular neurobiology have broadened our understanding of the role of the Sur1-Trpm4 channel in acute focal cerebral ischemia, not only in oncotic cell swelling and necrotic (oncotic) cell death, but also in microvascular dysfunction marked by edema formation and delayed secondary hemorrhage. As reviewed here, a wealth of evidence implicates this channel in cerebral ischemia, and indicates that channel inhibition with glibenclamide results in robust protection in animal models of stroke. Numerous putative neuroprotectants previously were shown to be beneficial in typical non-lethal animal models [83], but to our knowledge no agent previously studied exhibits the life-saving effect observed with glibenclamide in lethal models of malignant cerebral edema. The relevance of the Sur1-Trpm4 channel to human disease is underscored by recent findings that the channel is upregulated in humans with stroke, and by retrospective studies showing beneficial effects of channel inhibition in diabetics with stroke. As stated in an editorial in *Stroke* by Professor Arboix [84], “a prospective randomized trial of sulfonyleureas is justified and [is] urgently needed.”

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Fig. 1. Schematic diagrams of the K_{ATP} (Sur1–Kir6.2) and the Sur1–Trpm4 channels. The heterooctameric structure comprised of 4 Sur1 subunits and 4 Kir6.2 subunits depicted for K_{ATP} is widely accepted. The structure depicted for the Sur1–Trpm4 channel is hypothesized by analogy. Also shown are the principal physiological actions of the two channels when they are activated by ATP depletion: (i) outward flux of K^+ via the K^+ -selective pore-forming subunit, Kir6.2, resulting in hyperpolarization with the K_{ATP} channel; (ii) inward flux of Na^+ via Trpm4, resulting in depolarization with the Sur1–Trpm4 channel. Adapted from Simard et al. [14]

Fig. 2.

Glibenclamide reduces infarct volume and mortality in non-lethal and lethal MCA models of stroke. **a, b** Images of TTC-stained coronal sections 2 days after thromboembolic MCAo in a rat treated with saline (**a**) and another treated with glibenclamide (**b**), showing cortical sparing with glibenclamide. **c** Infarct volumes after thromboembolic MCAo were assessed at 2 or 7 days in three groups, consisting of 9, 9, and 7 male rats, respectively, treated with either saline (SAL; 2 days) or glibenclamide (GLIB; 2 and 7 days); ** $P < 0.01$. **d** In a malignant cerebral edema model, mortality was assessed during 7 days in two treatment groups, each consisting of 19 female and 10 male rats, treated with either saline (*open symbols*) or glibenclamide (*filled symbols*). Mortality at 7 days was significantly different ($P < 0.002$); subgroup analyses for sex showed similar results. Adapted from Simard et al. [16]

Table 1

Effects of glibenclamide in conventional non-lethal models of stroke

Model (Tx delay)	<i>N</i> ^a	Effects observed (all effects listed were statistically significant: 0.001 <i>P</i> < 0.05)
Thrombo-embolic ^b (none)	7—9/9	Reduced infarct volume by 53–57 % at 48 h and 7 days, respectively Improved laser Doppler flowmetry signals by 51 % at 48 h
Thrombo-embolic ^c (4 and 6 h)	9—5/5	Reduced infarct volume by ~50 % at 48 h
60 min MCAO ^d (7 h)	15—15/15/15	Reduced subcortical (ventral pallidum) necrosis by 55 % at 72 h Reduced neuronal loss by 30–40 % at 72 h Reduced pathological calcium deposition by ~50 % at 72 h
60 min MCAO ^e (7 h)	12—12	Neuronal preservation in striatum and cortex Improved sensorimotor and cognitive function Neurogenesis and angiogenesis
105 min MCAO ^c (1¾ and 5¾ h)	11—8/7	Reduced infarct volume by 41 % at 48 h
Permanent MCAO ^f (none)	9—8	Reduced infarct volume by 26 % at 48 h Reduced hemispheric swelling by >50 % at 48 h Improved neuroscores from 6.5 to 4.0, and grip strength from 41 to 61
Permanent MCAO ^c (4 h)	12—14	Reduced infarct volume by 51 % at 48 h

MCE malignant cerebral edema, *rtPA* recombinant tissue plasminogen activator^a*N*, number of rats per group (numbers on the left, vehicle control; numbers on the right, glibenclamide; multiple series separated by “/”)^bSimard et al. [16]^cSimard et al. [39]^dOrtega et al. [36]^eOrtega et al. [40]^fWali et al. [41]

Table 2

Effects of glibenclamide in models of malignant cerebral edema

Model (Tx delay)	N ^a	Effects observed (all effects listed were statistically significant: 0.001 P < 0.05)
MCE ^b (none)	29—29	Reduced mortality from 65 to 24 % at 7 days Reduced excess brain water by 42 % at 8 h
MCE ^f (6 h)	21/10—22/10	Reduced mortality from 67 to 5 % at 24 h Reduced hemispheric swelling from 21 to 8 % at 24 h Better preservation of watershed cortex and white matter, better neuroscores, Garcia scores, and the trajectory of weight gain compared to decompressive craniectomy during 2 weeks post-insult
MCE+rtPA ^d (4.5 and 10 h)	51—41/25	Reduced mortality from 53 to 17 or 12 % at 48 h (vehicle vs. drug @4.5 or 10 h) Reduced swelling from 14.7 to 8.1 or 8.8 % at 24 h (same groups) Improved Garcia scores from 3.8 to 7.6 or 8.4 at 48 h (same groups)
MCE+rtPA ^e (6 h)	7/9/22—8/13/20	Reduced hemispheric swelling from 26 to 12 % at 24 h Reduced scores for hemorrhagic transformation from 2.4 to 0.6 at 24 h Improved neuroscores from 7 to 3 at 2 weeks

MCE malignant cerebral edema, rtPA recombinant tissue plasminogen activator, Tx treatment

^aN, number of rats per group (numbers on the left, vehicle control; numbers on the right, glibenclamide; multiple series separated by “/”)^bSimard et al. [16]^cSimard et al. [17]^dSimard et al. [27]^eSimard et al [45]

Table 3

Number of patients in RP-1127 Phase I dosing groups

Dose (mg/day)	RP-1127	Placebo
0.4	8	2
3.0	16	4
6.0	1	1
10.0	1	1