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Precision Effects of Glibenclamide on MRI Endophenotypes in Clinically Relevant Murine Traumatic Brain Injury

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OBJECTIVES: Addressing traumatic brain injury (TBI) heterogeneity is increasingly recognized as essential for therapy translation given the long history of failed clinical trials. We evaluated differential effects of a promising treatment (glibenclamide) based on dose, TBI type (patient selection), and imaging endophenotype (outcome selection). Our goal to inform TBI precision medicine is contextually timely given ongoing phase 2/planned phase 3 trials of glibenclamide in brain contusion.

DESIGN: Blinded randomized controlled preclinical trial of glibenclamide on MRI endophenotypes in two established severe TBI models: controlled cortical impact (CCI, isolated brain contusion) and CCI+hemorrhagic shock (HS, clinically common second insult).

SETTING: Preclinical laboratory.

SUBJECTS: Adult male C57BL/6J mice ($n = 54$).

INTERVENTIONS: Mice were randomized to naïve, CCI±HS with vehicle/low-dose (20 μg/kg)/high-dose glibenclamide (10 μg/mouse). Seven-day subcutaneous infusions (0.4 μg/hr) were continued.

MEASUREMENTS AND MAIN RESULTS: Serial MRI (3 hr, 6 hr, 24 hr, and 7 d) measured hematoma and edema volumes, T2 relaxation (vasogenic edema), apparent diffusion coefficient (ADC, cellular/cytotoxic edema), and 7-day T1-post gadolinium values (blood-brain-barrier [BBB] integrity). Linear mixed models assessed temporal changes. Marked heterogeneity was observed between CCI versus CCI+HS in terms of different MRI edema endophenotypes generated (all $p < 0.05$). Glibenclamide had variable impact. High-dose glibenclamide reduced hematoma volume ~60% after CCI ($p = 0.0001$) and ~48% after CCI+HS ($p = 4.1 \times 10^{-6}$) versus vehicle. Antiedema benefits were primarily in CCI: high-dose glibenclamide normalized several MRI endophenotypes in ipsilateral cortex (all $p < 0.05$, hematoma volume, T2, ADC, and T1-post contrast). Acute effects (3 hr) were specific to hematoma ($p = 0.001$) and cytotoxic edema reduction ($p = 0.0045$). High-dose glibenclamide reduced hematoma volume after TBI with concomitant HS, but antiedema effects were not robust. Low-dose glibenclamide was not beneficial.

CONCLUSIONS: High-dose glibenclamide benefitted hematoma volume, vasogenic edema, cytotoxic edema, and BBB integrity after isolated brain contusion. Hematoma and cytotoxic edema effects were acute; longer treatment windows may be possible for vasogenic edema. Our findings provide new insights to inform interpretation of ongoing trials as well as precision design (dose, sample size estimation, patient selection, outcome selection, and Bayesian analysis) of future TBI trials of glibenclamide.

KEY WORDS: cerebral edema; cytotoxic edema; glibenclamide/glyburide; hemorrhage progression; magnetic resonance imaging edema endophenotypes; traumatic brain injury; vasogenic edema

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KEY POINTS

Question: Is there a role for precision medicine in glibenclamide treatment after TBI?

Findings: In a randomized blinded preclinical murine trial, glibenclamide benefits varied with dose, TBI model, and MRI hematoma/endophenotype. Marked edema endophenotype heterogeneity was noted between two TBI models. High-dose glibenclamide was universally beneficial to isolated contusion. Addition of a clinically common second insult (hypotension) limited glibenclamide's benefit to hematoma volume, with a trend toward improving vasogenic edema, but no impact on cytotoxic edema. Low-dose glibenclamide was not beneficial to a potential signal toward harm.

Meaning: There is an important role for subject- and outcome-based endophenotyping in TBI (and potentially other acute brain injury) for trial design/analysis and precision medicine.

Sulfonylurea receptor 1 (SUR1) transient receptor potential melastatin-4 (TRPM4) is a nonspecific monovalent cation channel that was discovered ~2 decades ago (1). It is upregulated de novo after several types of central nervous system injury including stroke and traumatic brain injury (TBI) (1). Channel opening results in depolarization, sodium influx, cerebral edema, and oncotic cell death (1). Increased channel expression occurs in multiple cell types at different time points post-TBI including endothelial/microvascular cells, astrocytes, neurons, and microglia (1). Molecular expression patterns and timings in TBI are distinct from stroke and contribute not only to cellular swelling/cerebral edema but also to the reported (less familiar) impact on blood-brain-barrier (BBB) breakdown and hematoma progression (1–12). SUR1-TRPM4-associated BBB breakdown and hematoma progression have been related to channel expression and oncotic cell death in specific cell types like microvascular cells and astrocytes involved in BBB maintenance (1, 13). Both these molecularly linked secondary injury processes of cerebral edema and hematoma progression are major contributors to unfavorable outcome and critical care mortality in TBI (14–17). No targeted or preventive treatments are available for either; management remains reactive, nonspecific, and with known systemic side effects (18, 19).

Early preclinical and clinical work in TBI suggests that SUR1-TRPM4 inhibition, via glibenclamide (glyburide), may reduce both edema and hematoma progression and improve outcome; however, effects have varied (1, 11, 12, 20–26). An intravenous formulation of glibenclamide (BIIB093) is being evaluated in a phase 2 trial of contusional TBI (Antagonizing SUR1-TRPM4 To Reduce the progression of intracerebral hematoma And edema surrounding Lesions [ASTRAL], NCT03954041). Unlike the phase 3 trial in stroke (Cirara in large Hemispheric infarction Analyzing modified Rankin and Mortality [CHARM], NCT02864953), the primary end point for ASTRAL is contusion expansion based on the known pathobiology of this channel in TBI (12). However, several promising therapies in TBI have reached this stage only to fall short of anticipated benefits; thus, addressing heterogeneity in both injury (mechanism, intracranial compartment, and comorbid insults) and treatment (dose and timing) is key to successful translation.

The incidence and type of cerebral edema and hematoma progression post TBI are variable, as is SUR1-TRPM4 contribution depending on sex (5) and genetics (14, 27–29). Here, we hypothesize that edema endophenotype and response to targeted treatment (SUR1-TRPM4 inhibition) will vary based on type of injury. Understanding nuanced effects of SUR1-TRPM4 inhibition on clinically measurable edema endophenotypes and hematoma progression in clinically relevant TBI models, thus, has the potential to guide phase 3 trial design and precision medicine beyond the ongoing trials.

Cerebral edema endophenotypes are classically dichotomized into cytotoxic/cellular versus vasogenic. Although somewhat artificial (given overlapping molecular mechanisms [19]), this classification remains clinically informative to phenomenologically identify energy failure and cell swelling versus a leaky BBB to guide management and prognosis (19). In TBI, these endophenotypes have a variable spatiotemporal evolution with distinct prognostic implications. Cellular/cytotoxic edema develops acutely and is considered highly deleterious to outcome, whereas vasogenic edema is often more space-occupying/compressive with extracellular fluid/plasma protein extravasation (30–33). The two subtypes may require different

treatment targets and/or warrant adjustments in dose or timing of the same treatment. The extent (and potentially endophenotype) of cerebral edema post-TBI is markedly greater in the presence of hypotension, which is common in TBI, often from polytrauma and hemorrhagic shock (HS) (21, 34–38). Hypotension profoundly increases TBI morbidity and mortality (39–45). One episode of systolic blood pressure less than 90 mm Hg can double mortality; this threshold may underestimate clinically relevant hypotension (43–45). Additionally, the large volumes of resuscitation fluid (in both preclinical and clinical settings) may further impact the magnitude, type, and location of edema (21). In this randomized blinded preclinical trial, we, therefore, evaluate longitudinal effects of high- and low-dose glibenclamide after contusional TBI in two clinically relevant models of contusion (controlled cortical impact [CCI], with and without hypotension from HS). Our outcomes focus on clinically relevant imaging endophenotypes of these secondary injuries.

MATERIALS AND METHODS

Injury Models

The two injury models were CCI and CCI+HS (**Supplemental Methods**, http://links.lww.com/CCM/H260) (21). Experiments were approved by the Institutional Animal Care and Use Committee (20087786, August 27, 2020, Supplemental Methods, http://links.lww.com/CCM/H260) and compliant with Animal Research: Reporting of In Vivo Experiments guidelines. Adult C57/BL6 male mice were used (aged 12–15 wk, weighing 25–30 grams; Jackson Laboratories, Bar Harbor, ME)—given our prior work with sex differences (5), a separate powered study is warranted in females. Our established injury protocols were followed for CCI and CCI+HS (21). Fifty-four mice were randomized into seven groups (n = 5–9 per group, average, 8/group): naïve, CCI+vehicle, CCI+low-dose glibenclamide, CCI+high-dose glibenclamide, CCI+HS +vehicle, CCI+HS+low-dose glibenclamide, and CCI+HS+high-dose glibenclamide.

Glibenclamide Dosing

Intravenous glibenclamide was loaded 10 minutes after CCI or within 5 minutes of HS completion. High dose =10 μg/mouse and low dose = 20 μg/kg (1). Vehicle solutions contained everything except drug (Fixnal, DMSO, normal saline) (21). Subcutaneous infusions were continued for 7 d (0.4 μg/hr, Alzet mini-pump; Durect Corporation, Cupertino, CA) for both groups. Glibenclamide was the only treatment used in this study; no other treatments like hyperosmolar therapy were used.

Outcome Measures

The primary outcome was a priori identified as hematoma expansion (a key endophenotype target in ASTRAL [12]), with critical secondary outcomes being the two edema endophenotypes: cellular/cytotoxic edema, and vasogenic edema. These were quantified by MRI (below).

MRI Sequence Acquisition, Image Processing, and Quantification

Research personnel were blinded to injury model and treatment. Anesthetized mice (isoflurane, 36.8°C ± 0.2°C) underwent in vivo multiparametric MRI after injury at 3 hours, 6 hours, 24 hours, and 7 days using a 7-Tesla Bruker-BioSpec 70/30 USR spectrometer (Bruker, Billerica, MA). The same mouse was imaged at each time point. T1-post gadolinium images were obtained at 7 days. Sequences included multiplanar T_1- and T_2-weighted anatomical imaging for volumetric analyses (hematoma and edema); multiecho quantitative T_2 relaxometry (quantitative vasogenic edema), multiplanar diffusion/quantitative apparent diffusion coefficient (ADC, quantitative cytotoxic edema), and postgadolinium T_1-weighted images (BBB integrity) (30–32, 46–48) (Supplemental Methods, http://links.lww.com/CCM/H260). Aside from the contusion, regions of interest (ROIs) included ipsilateral and contralateral cortex, hippocampus, corpus callosum, and thalamus. These were manually segmented, quantified, and analyzed (Bruker ParaVision 5.1Xtip software [Burker]; Supplemental Methods, http://links.lww.com/CCM/H260).

Statistical Analyses

Based on preliminary data, a sample size of seven mice/group was estimated for $\alpha = 0.05$, $\beta = 0.80$ (Supplemental Methods, http://links.lww.com/CCM/H260). The study code was broken for analysis after acquisition,

processing, quantification, and quality control of MRI sequences. Hematoma volume, edema volume, quantified T2, and ADC intensities are presented as mean ± SD and 95% CIs. Comparisons were performed to evaluate imaging endophenotype differences between injury models (CCI vs CCI+HS) and to identify variable treatment responses between models (to low- and high-dose glibenclamide). Longitudinal differences between TBI models and/or treatment groups were assessed by linear mixed models (including generalized estimating equation [GEE] and generalized linear mixed-effects models [GLMM] with Bonferroni corrections used for post hoc comparisons; Supplemental Methods, http://links.lww.com/CCM/H260). Statistical significance was based on $\alpha = 0.05$. Analyses were performed using Stata 15.1 (StataCorp, College Station, TX) and R (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

All mice were adult C57/BL6 males 12–15 weeks old; there was no difference between groups in terms of weights, sodium levels, or osmolarity (**Supplemental Table 1**, http://links.lww.com/CCM/H260). No hypoglycemia less than 100 mg/dL was observed with glibenclamide. Mortality was higher after CCI+HS versus CCI (6 vs 2 mice) and occurred within 24 hours but did not differ with treatment.

Imaging Endophenotypes Varied Between the Two TBI Models CCI Versus CCI+HS

Hematoma volumes, cerebral edema volumes, ipsilateral, and contralateral T2 and ADC values serially quantified over 7 days after CCI and CCI+HS revealed endophenotype profiles that were unique to the two different models (**Supplemental Fig. 1, Supplemental Table 2**, and **Supplemental Results**, http://links.lww.com/CCM/H260). Acute hematoma volumes (≤24 hr) were double in CCI versus CCI+HS, reaching significance by 6 hours (16.3 ± 8.56 vs 8.78 ± 3.45 μL; $p = 0.04$; **Supplemental Fig. 1*Ai***, http://links.lww.com/CCM/H260). By 7 days, hematoma volumes were similar in both models. Conversely, cerebral edema volumes in CCI+HS were consistently/markedly higher versus isolated CCI, persisting through 7 days (**Supplemental Fig. 1*Aii***, http://links.lww.com/CCM/H260; $p_{GLMM} = 0.026$, $p_{GEE} = 0.01$). T2-hyperintensity and ADC diffusion restriction patterns also varied with model and region (Supplemental Results, http://links.lww.com/CCM/H260).

High-Dose Glibenclamide Decreased Hematoma Volume in Both TBI Models

Hematoma and cerebral edema volume trajectories after both glibenclamide doses versus vehicle were serially quantified at 3 hours, 6 hours, 24 hours, and 7 days after CCI±HS (**Fig. 1, *A*** and ***B;*** and **Table 1**).

High-dose glibenclamide reduced longitudinal hematoma volumes by ~60% in CCI ($\beta_{GEE} = -9.6$, $p_{GEE} = 0.0001$, $\beta_{GLMM} = -8.15$, $p_{GLMM} = 0.012$) and ~48% in CCI+HS ($\beta_{GEE} = -5.21$, $p_{GEE} = 4.1 \times 10^{-6}$, $\beta_{GLMM} = -5.01$, $p_{GLMM} = 0.009$) versus vehicle (Table 1 and Fig. 1*A*). In both models (CCI±HS), the reduction was noted within 3 hours of high-dose glibenclamide: hematoma volumes with high-dose glibenclamide were 60% lower after CCI and 70% lower after CCI+HS versus vehicle (**Table 2**). This acute substantial reduction was sustained over time. In CCI, the benefit remained at ~60% reduction at 7 days, whereas in CCI+HS, this decreased from ~70% (3 hr) to ~48% (7 d) hematoma volume reduction versus vehicle. Low dose had no impact on hematoma volume in either model. Post hoc analyses suggested a transient unfavorable trend toward increased hematoma volumes after CCI with low-dose glibenclamide versus vehicle at 3 hours ($p_{Bonferroni} = 0.089$). At 7 days, edema volume in CCI+HS was reduced to a similar extent by both glibenclamide doses versus vehicle (32.1 ± 5.25 μL and 34.1 ± 5.25 μL, respectively, versus 51.5 ± 5.25 μL; $p = 0.04$; Table 1 and **Figs. 1*Bii*** and **2**). Longitudinal models missed significance even between high-dose glibenclamide versus vehicle in both models ($p_{CCI(GEE)} = 0.06$ and $p_{CCI+HS(GEE)} = 0.07$; Table 1 and Fig. 1*B*).

High-Dose Glibenclamide Normalizes Vasogenic T2-Hyperintensity Signal Towards Naïve Levels in CCI but Not CCI+HS

Glibenclamide had a dose- and time-dependent reduction of T2-hyperintensity after CCI (Table 2 and **Fig. 3*Ai*** and ***Bi***). In CCI, T2-hyperintensity was markedly reduced over time in the ipsilateral cortex with high-dose glibenclamide versus vehicle ($\beta_{GEE} = -13.8$ ms, $p_{GEE} = 4.8 \times 10^{-5}$, $\beta_{GLMM} = -13$ ms, and $p_{GLMM} = 0.011$). A reduction was also found in the ipsilateral hippocampus ($\beta_{GEE} = -3.9$ ms, $p_{GEE} = 0.00078$, $\beta_{GLMM} = -3.85$ ms, and $p_{GLMM} = 0.005$). Both ipsilateral cortex and hippocampal T2-hyperintensity values with high-dose glibenclamide after CCI approached naïves (Fig. 3*Ai* and *Bi*). Low-dose had no effect. A similar trajectory was seen after CCI+HS (**Fig. 3*Aii*** and ***Bii***). However, here, effects of glibenclamide

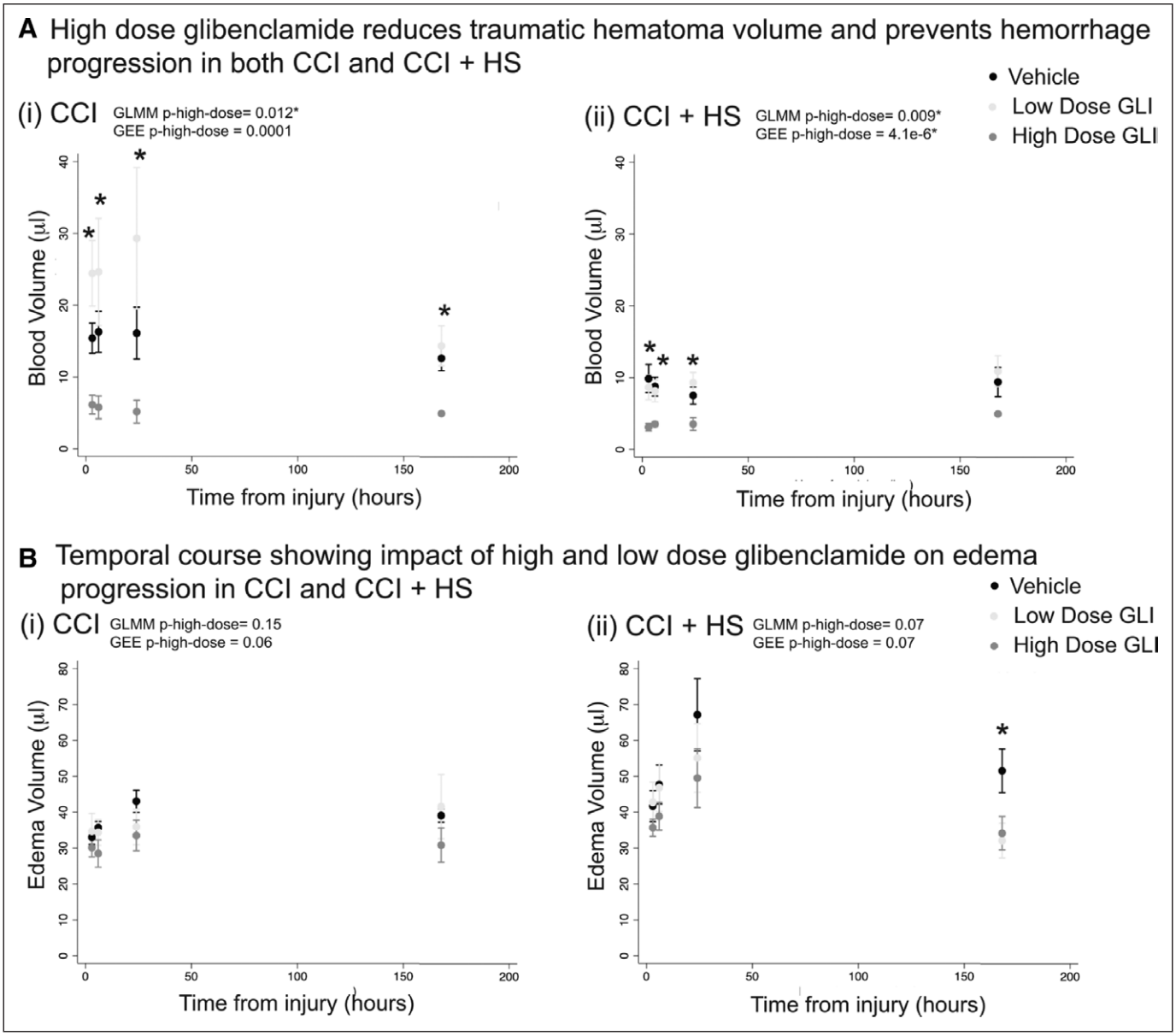

Figure 1. Longitudinal impact of low-dose glibenclamide and high-dose glibenclamide vs vehicle on hematoma and total edema volumes over 7 d after isolated contusional-traumatic brain injury (TBI) (controlled cortical impact [CCI]) vs contusional-TBI with concomitant hemorrhagic shock (CCI+hemorrhagic shock [HS]). *Dot-and whisker plots* over four time points (3 hr, 6 hr, 24 hr, and 7 d) demonstrating that high-dose glibenclamide but not low-dose glibenclamide reduces hematoma-volume after CCI (**Ai**) and CCI+HS vs vehicle (**Aii**). Despite a protective trend in CCI+HS, neither dose significantly decreases edema volume longitudinally in CCI (**Bi**) or CCI+HS (**Bii**). However, at 7 d, both high-dose glibenclamide and low-dose glibenclamide reduce edema volume vs vehicle in CCI+HS. Linear mixed model results including generalized linear mixed models (GLMM)and generalized estimating equation (GEE) are noted in the *top-left corner* of each subplot with the associated *p* values. *Statistically significant analysis of variance results at individual time points comparing high-dose vs low-dose vs vehicle ($p < 0.05$). *Dark gray* indicates high-dose glibenclamide, *light gray* indicates low-dose glibenclamide, and *black* indicates vehicle. GLI = glibenclamide.

on T2-hyperintensity were inconsistent. Although there was indication of potential T2-hyperintensity reduction, this was inconsistent (Table 2). Further nuances were noted regarding differences in glibenclamide response based on TBI model (Supplemental Results, http://links.lww.com/CCM/H260).

High-Dose Glibenclamide Normalizes Cytotoxic Diffusion Restriction (ADC) Signal Intensity Toward Naïve Levels in CCI but Not CCI+HS

A dose- and time-dependent reduction of diffusion restriction in ipsilateral cortex and hippocampus was observed with glibenclamide after CCI but not

TABLE 1.

Effect of Low- and High-Dose Glyburide on Hematoma and Edema Volumes Over Time in Two Models of Traumatic Brain Injury

Hematoma volume (μL) Longitudinal analyses	CCI		CCI+Hemorrhagic Shock	
	β (SE)	*p*	β (SE)	*p*
Generalized estimating equations				
LD glyburide vs vehicle (veh)	8.1 (5.7)	0.15	0.22 (1.7)	0.90
HD glyburide vs vehicle	−9.6 (2.5)	***0.0001***[a]	−5.21 (1.13)	***4.1 × 10^{-6}***[a]
Generalized linear mixed-effects model				
LD glyburide vs vehicle	2.11 (3.13)	0.50	0.38 (1.6)	0.82
HD glyburide vs vehicle	−8.15 (3.01)	***0.012***[a]	−5.01 (1.7)	***0.009***[a]

Individual time points	Group means (μL) (95% CI)	ANOVA *p*	Group means (μL) (95% CI)	ANOVA or KW *p*
3 hr	Veh: 15.4 (10.6–20.2) LD: 24.4 (18–30.9) HD: 6.1 (0.27–12)	***0.0014***[a]	Veh: 9.9 (6.5–13.2) LD: 8.7 (5.3–12.0) HD: 3.11 (−0.5 to 6.7)	***0.024***[a]
6 hr	Veh: 16.3 (8.9–23.7) LD: 24.6 (14.7–34.5) HD: 5.8 (−4.1 to 15.7)	***0.037***[a]	Veh: 8.8 (6.2–11.4) LD: 8.2 (5.6–10.8) HD: 3.5 (0.45–6.6)	***0.02***[a] ***(KW)***
24 hr	Veh: 16.1 (6.0–26.3) LD: 29.3 (16.5–42.1) HD: 5.2 (−7.7 to 18.0)	***0.04***[a]	Veh: 7.5 (5.0–10.1) LD: 9.3 (6.8–11.9) HD: 3.5 (0.75–6.3)	***0.017***[a]
7 d	Veh: 12.6 (9.1–16.1) LD: 14.3 (9.9–18.8) HD: 4.9 (0.45–9.4)	***0.012***[a]	Veh: 9.4 (5.6–13.2) LD: 10.9 (7.1–14.6) HD: 4.9 (1.2–8.7)	*0.076*

Edema volume (μL) Longitudinal analyses	β (SE)	*p*	β (SE)	*p*
Generalized estimating equations				
LD glyburide vs vehicle	−1.02 (4.8)	0.83	−6.7 (8.1)	0.41
HD glyburide vs vehicle	−6.8 (3.7)	0.06	−11.9 (6.7)	0.07
Generalized linear mixed-effects model				
LD glyburide vs vehicle	−3.4 (3.7)	0.37	−5.8 (7.8)	0.47
HD glyburide vs vehicle	−5.3 (3.5)	0.15	−11.8 (8.2)	0.07

Individual time points	Group means(μL) (95% CI)	ANOVA *p*	Group means(μL) (95% CI)	ANOVA or KW *p*
3 hr	Veh: 33.0 (27.7–38.3) LD: 34.6 (27.5–41.8) HD: 30.1 (23.6–36.6)	0.61	Veh: 41.7 (32.4–51.0) LD: 42.7 (33.4–52.0) HD: 35.7 (25.7–45.7)	0.52
6 hr	Veh: 35.7 (30.8–40.6) LD: 34.4 (27.8–41.0) HD: 28.5 (21.9–35.1)	0.20	Veh: 47.7 (36.0–59.4) LD: 46.9 (35.2–58.5) HD: 38.9 (25.1–52.7)	0.60 (KW)

(Continued)

TABLE 1. (*Continued*).

Effect of Low- and High-Dose Glyburide on Hematoma and Edema Volumes Over Time in Two Models of Traumatic Brain Injury

Individual time points	Group means(μL) (95% CI)	ANOVA *p*	Group means(μL) (95% CI)	ANOVA or KW *p*
24 hr	Veh: 43.0 (35.7–50.3)	0.21	Veh: 67.2 (47.4–86.9)	0.43
	LD: 35.9 (26.7–45.2)		LD: 55.1 (35.4–74.9)	
	HD: 33.5 (24.3–42.8)		HD: 49.5 (27.9–71.1)	
7 d	Veh: 39. 1 (29.9–48.3)	0.36	Veh: 51.5 (40.1–63)	***0.04***[a]
	LD: 41.6 (29.9–53.2)		LD: 32.1 (20.7–43.5)	
	HD: 30.8 (19.2–42.5)		HD: 34.1 (22.7–45.6)	

ANOVA = analysis of variance, CCI = controlled cortical impact, HD = high dose, KW = Kruskal-Wallis, LD = low dose.
[a]Italicized boldface values are statistically significant.

CCI+HS (Table 2, **Fig. 4**; and **Supplemental Fig. 2**, http://links.lww.com/CCM/H260). Reduced cellular swelling/diffusion restriction was limited to high-dose glibenclamide. ADC trajectories of ipsilateral cortex were higher (i.e., reduced diffusion restriction/cytotoxic edema) with high-dose glibenclamide versus vehicle after CCI ($\beta_{GEE} = 1.0 \times 10^{-1}$ 10^{-3} mm^2/s, $p_{GEE} = 0.0014$, $\beta_{GLMM} = 10 \times 10^{-2}$ 10^{-3}mm^2/s, and $p_{GLMM} = 0.03$). These approached naïve values at all acute time points (≤24 hr). This impact of high-dose glibenclamide on reducing cytotoxic edema after CCI was significant by 3 hours (vehicle = 0.64 ± 0.03 mm^2 10^{-3}/s, low dose = 0.55 ± 0.04 mm^2 10^{-3}/s, and high dose = 0.77 ± 0.04 mm^2 10^{-3}/s; $p = 0.0045$). Histograms quantified individual ADC pixel values in ROIs over six sequential images containing the contusion (**Supplemental Fig. 3**, http://links.lww.com/CCM/H260). These demonstrated overlapping ADC values in naïve and high-dose glibenclamide treated mice that were clearly distinct from vehicle and low-dose glibenclamide distributions at all time points less than 24 hours in several brain regions.

High-Dose Glibenclamide Normalizes T1-Post Contrast Signal Intensity Toward Naïve Levels in CCI but Not CCI+HS

Only high-dose glibenclamide reduced 7-day ipsilateral cortex T1-post gadolinium hyperintensity versus vehicle after CCI ($p_{anova} = 0.013$ [**Supplemental Fig. 4*A***, Supplemental Results, http://links.lww.com/CCM/H260]). In this model/treatment combination, ipsilateral cortex T1-post gadolinium values were not different from naïve ($p = 0.76$). Low-dose glibenclamide did not alter T1-post gadolinium values versus vehicle indicating no radiographic improvement with treatment ($p_{Bonferroni} = 0.22$) in CCI. Glibenclamide did not reduce T1-post gadolinium hyperintensity after CCI+HS at any dose/region (**Supplemental Fig. 4*B***, http://links.lww.com/CCM/H260).

DISCUSSION

In this randomized blinded preclinical trial of glibenclamide after contusional TBI, impact of glibenclamide varied depending on dose, radiographic/outcome endophenotype, timing, and injury model (presence/absence of a hypotensive insult). High-dose glibenclamide universally improved imaging endophenotypes in isolated contusion (CCI): it decreased hematoma volume, reduced ipsilateral T2-hyperintensity (vasogenic edema), ADC diffusion restriction (cytotoxic edema), and T1-post contrast sequences (BBB integrity) with values no different from naïve. Effects on hematoma and diffusion restriction were acute (within 3 hr); however, T2-improvement emerged by 24 hours and was most prominent by 7 days. Low-dose glibenclamide in CCI had no positive impact on these MRI parameters. Conversely, in CCI+HS, both glibenclamide doses reduced 7-day edema volume; however, only high dose reduced hematoma volume. Neither dose significantly affected T2-hyperintensity, ADC diffusion restriction, or T1-post contrast scans after CCI+HS. Although substantially greater edema volumes were generated in CCI+HS versus CCI, hematoma volumes were lower by ~50% after the second insult of HS. Our results, thus, suggest an important role for patient- and outcome-based endophenotypings in TBI (and potentially other

TABLE 2.

Regional Effects of Low- and High-Dose Glyburide on T2- and Apparent Diffusion Coefficient–Based Secondary Injury Endophenotypes in Two Models of Traumatic Brain Injury

MRI Sequence and Region	Longitudinal Mixed Models for Repeated Measures Over Time (Ref = Vehicle)							
	CCI				CCI+Hemorrhagic Shock			
	Generalized Estimating Equations		Generalized Linear Mixed-Effects Models		Generalized Estimating Equations		Generalized Linear Mixed-Effects Models	
	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
T2								
Ipsilateral cortex								
LD glyburide	−0.82 (5.3)	0.88	−0.83 (4.8)	0.87	−6.7 (5.3)	0.26	−5.4 (4.8)	0.26
HD glyburide	−13.8 (3.4)	***4.8 × 10−5***	−13.0 (4.6)	***0.011***	−11.1 (5.9)	*0.08*	−11.9 (5.2)	***0.02***
Ipsilateral hippocampus								
LD glyburide	0.91 (1.1)	0.40	0.43 (1.23)	0.74	−3.7 (1.8)	***0.05***	−3.7 (2.3)	0.12
HD glyburide	−3.9 (1.2)	***0.00078***	−3.85 (1.2)	***0.005***	−4.1 (2.5)	*0.10*	−3.8 (2.4)	0.14
Apparent diffusion coefficient								
Ipsilateral cortex								
LD glyburide	-7.9×10^{-2} (4.7×10^{-2})	*0.09*	-8.5×10^{-2} (4.4×10^{-2})	*0.07*	1.9×10^{-3} (3.2×10^{-2})	0.95	-1.4×10^{-3} (3.2×10^{-2})	0.97
HD glyburide	1.1×10^{-1} (3.5×10^{-2})	***0.0014***	10.0×10^{-2} (4.3×10^{-2})	***0.03***	-2.2×10^{-2} (2.3×10^{-2})	0.34	-2.7×10^{-2} (3.5×10^{-2})	0.56
Ipsilateral hippocampus								
LD glyburide	-3.8×10^{-2} (4.2×10^{-2})	0.37	-2.4×10^{-2} (3.8×10^{-2})	0.54	1.5×10^{-2} (3.2×10^{-2})	0.65	1.5×10^{-2} (3.4×10^{-2})	0.67
HD glyburide	1.0×10^{-1} (3.4×10^{-2})	***0.003***	9.7×10^{-2} (3.7×10^{-2})	***0.016***	2.5×10^{-3} (2.5×10^{-2})	0.92	-3.3×10^{-3} (3.6×10^{-2})	0.95
Contralateral cortex								
LD glyburide	-5.1×10^{-2} (3.7×10^{-2})	0.17	-5.1×10^{-2} (3.6×10^{-2})	0.18	4.2×10^{-2} (3.0×10^{-2})	0.16	4.09×10^{-2} (2.8×10^{-2})	0.17
HD glyburide	1.03×10^{-1} (2.8×10^{-2})	***0.00019***	1.1×10^{-1} (3.4×10^{-2})	***0.007***	2.5×10^{-4} (1.7×10^{-2})	0.99	1.3×10^{-3} (3.1×10^{-2})	0.97
Contralateral hippocampus								
LD glyburide	-4.7×10^{-3} (5.8×10^{-4})	0.94	-1.9×10^{-3} (4.8×10^{-2})	0.97	3.7×10^{-2} (3.0×10^{-2})	0.21	3.8×10^{-2} (3×10^{-2})	0.21
HD glyburide	9.4×10^{-2} (2.8×10^{-2})	***0.0009***	9.88×10^{-2} (4.68×10^{-2})	***0.049***	2.08×10^{-2} (1.98×10^{-2})	0.91	2.0×10^{-3} (3.2×10^{-2})	0.95

CCI = controlled cortical impact, HD = high dose, LD = low dose.
Italicized boldface values are statistically significant.

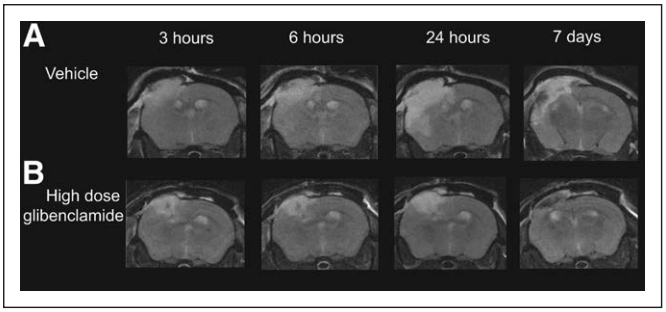

Figure 2. Serial representative MRI-T2-RARE images from median mice demonstrate evolution of T2 edema with vehicle (**A**) vs high-dose glibenclamide (**B**) at 3 hr, 6 hr, 24 hr, and 7 d, where high-dose glibenclamide treatment appeared to minimize edema progression. RARE = Rapid Imaging with Refocused Echoes.

acute brain injury) trials testing therapies targeting cerebral edema or hemorrhage progression.

SUR1-TRPM4, Edema Endophenotype, and Hemorrhage Progression

SUR1-TRPM4 facilitates cerebral edema by conducting sodium intracellularly, resulting in water influx (1, 49). This produces cellular/cytotoxic edema and oncotic cell death. In microvascular cells (endothelial and astrocyte podocytes) responsible for BBB integrity, cell swelling and oncotic cell death disrupt/degrade tight junctions, contributing to vasogenic edema; complete disruption facilitates hemorrhage progression—although the impact of SUR1-TRPM4 on hematoma/TBI lesion volume is less familiar than the well-known effects on cerebral edema, it has been reported by multiple independent groups (11, 13, 19, 20, 22, 33).

SUR1-TRPM4 is not constitutively expressed in normal brain; upregulation after contusional TBI is seen at various time points in neurons, astrocytes, microvessels, and endothelium (4, 6, 11, 50). Neuronal and microvascular upregulation are acute (6–24 hr)—consistent with the early hemorrhage progression and cytotoxic edema noted on ADC sequences in our study after CCI±HS. Glibenclamide may block hematoma expansion via SUR1-TRPM4 inhibition in these cell types; however, it may also act on alternate pathways of hemorrhage progression involving macrophage endocytosis/induction of a phagocytic microglial phenotype (51). SUR1-TRPM4 expression in astrocytes, microglia, and endothelium (>72 hr) may correlate with ongoing vasogenic edema, detectable on MRI even 7-day postinjury. This study, therefore, supports a measurable association between the known underlying molecular pathophysiology (i.e., SUR1-TRPM4 expression patterns) and clinically detectable imaging endophenotypes.

Translating time courses across species to identify a "therapeutic window" is challenging (52). Protein turnover in rodents is ~10× faster versus humans (52). Acute glibenclamide administration after contusional TBI is likely needed for maximal benefit on hematoma and cytotoxic edema—in mice, both processes approached their "worst" values within 3 hours after CCI. Although both cytotoxic and vasogenic edema are clinically seen in TBI (30, 32), recent work confirmed that cytotoxic edema was acute (24 hr) and associated with unfavorable outcome (31).

Conversely, glibenclamide's protective effect on vasogenic edema/BBB dysfunction emerged by 24 hours and persisted until 7 days, suggesting a longer timeframe to intervene. Vasogenic edema contributes to mass effect and herniation. In ASTRAL, BIIB093 infusion is initiated within 6.5 hours with primary endpoints including 96 hours hematoma and perihematomal edema expansion.

Glibenclamide and TBI Models

High-dose glibenclamide reduced hematoma volume by 3 hours in CCI and CCI+HS, without further expansion demonstrating a hyperacute and persistent benefit of glibenclamide. Effects were more pronounced in CCI versus CCI+HS, though absolute hematoma volumes were lower after HS mean arterial pressure reduction from HS may reduce cerebral perfusion, limiting acute hemorrhage progression (53). The degree of MRI-quantified hematoma reduction after CCI with glibenclamide mimics extravasated blood noted spectrophotometrically over 24 hours (11). Beyond acute benefit, glibenclamide was the only drug (of 12) in a multicenter rat study to reduce 21-day contusion volume after CCI (20).

Minimal glibenclamide impact on several edema metrics (T2-hyperintensity/vasogenic edema, ADC diffusion restriction/cytotoxic edema, or T1-post contrast sequences) regardless of dose in CCI+HS was unexpected. Diffusion restriction in CCI+HS mimicked CCI, although total edema volume and T2-hyperintensity were both markedly higher in CCI+HS. Glibenclamide did not reduce cytotoxic edema after CCI+HS. The trend toward reduced T2-hyperintensity by 7 days with both doses after CCI+HS possibly evaded significance due to overwhelming vasogenic edema. High-dose glibenclamide reduced longitudinal ipsilateral cortex T2-hyperintensity, but this was not robust to

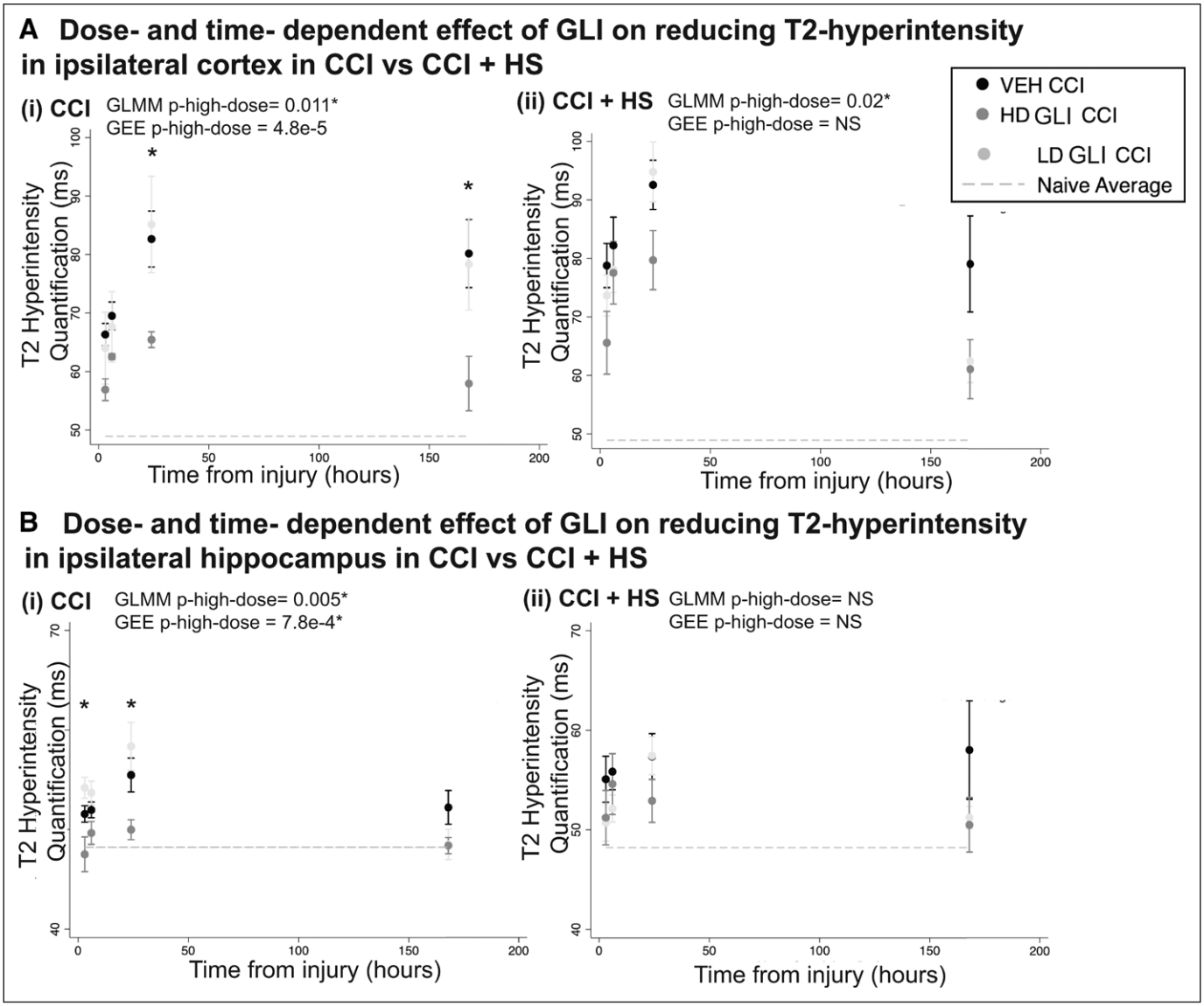

Figure 3. Variable spatiotemporal effects of high- and low-dose glibenclamide vs vehicle (VEH) on T2-hyperintensity after isolated contusional-traumatic brain injury (TBI) (controlled cortical impact [CCI]) vs contusional-TBI with concomitant hemorrhagic shock (CCI+hemorrhagic shock [HS]). **A** and **B**, *Dot and whisker plots* (mean ± 95% CIs) demonstrating consistently lower T2-hyperintensity with high-dose glibenclamide treatment after CCI in the ipsilateral-cortex (**Ai**) and ipsilateral-hippocampus (**Bi**). T2-hyperintensity in both these regions after high-dose glibenclamide approaches naïve levels. Differences emerge within 24 hr and in the ipsilateral-cortex are most prominent at 7 d. Conversely, no impact of either high- or low-dose glibenclamide is reliably noted after CCI+HS in either the ipsilateralcortex (**Aii**) or hippocampus (**Bii**). Linear mixed model results including generalized linear mixed models (GLMM) and generalized estimating equations (GEE) are noted in the *top-left corner* of each subplot with the associated *p* values. *Statistically significant analysis of variance results at individual time-points comparing high-dose, low-dose glibenclamide, and vehicle ($p < 0.05$). Naïve values are shown by the *dashed gray line*. *Dark gray* indicates high-dose glibenclamide, *light gray* indicates low-dose glibenclamide, and *black* indicates vehicle. GLI = glibenclamide, HD = high dose, LD = low dose, NS = nonsignificant.

sensitivity analyses. Mechanisms underlying edema generation after TBI with HS might also be glibenclamide-resistant. Importantly, in CCI+HS, aggressive fluid resuscitation is required to restore and maintain blood pressure (21, 54). This produces a much greater degree of edema versus CCI alone, which may overwhelm selective SUR1-TRPM4 blockade. Fluid resuscitation of HS after TBI requires larger than anticipated volumes—likely related to a disrupted sympathetic nervous system (55). ASTRAL excludes patients with hemodynamic instability requiring vasopressors or greater than 6-L resuscitation but lacks a specific cutoff for hypotension. Post hoc analyses of this subgroup could be informative.

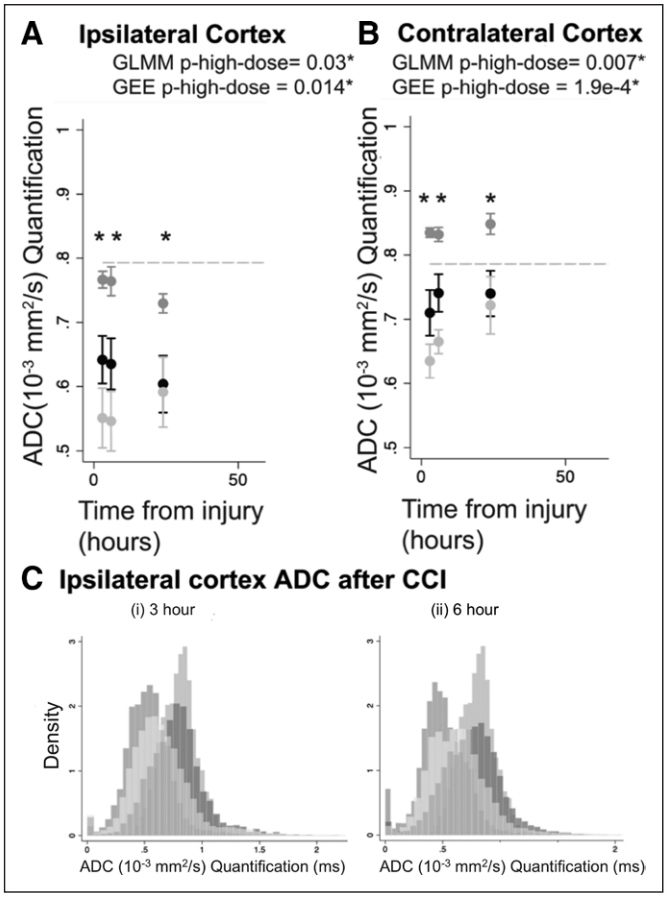

Figure 4. Variable spatiotemporal effects of high and low-dose glibenclamide vs vehicle apparent diffusion coefficient in the cortex after isolated contusional-traumatic brain injury (controlled cortical impact [CCI]). High-dose glibenclamide reduces diffusion-restriction vs vehicle at acute time points after CCI. *Longitudinal dot and whisker plots* (mean ± 95% CIs) of apparent diffusion coefficient (ADC) values in high-dose glibenclamide, low-dose glibenclamide, and vehicle groups in ipsilateral cortex (**A**) and contralateral cortex (**B**). High-dose glibenclamide reduced diffusion-restriction at all acute time points ≤24 hr in the ipsilateral and contralateral-cortex with normalization towards naïve levels. *Dark gray* indicates high-dose glibenclamide, *light gray* indicates low-dose glibenclamide, and *black* indicates vehicle. *Dashed gray line* indicates average naïve levels. *Statistically significant analysis of variance results at individual time points comparing high-dose glibenclamide and low-dose glibenclamide and vehicle ($p < 0.05$). **C**, Granular histograms demonstrating high-dose glibenclamide reduces diffusion-restriction vs vehicle in the ipsilateral cortex at acute time points up to 24 hr after CCI – two time points are shown here at (**i**) 3 hr and (**ii**) 6 hr. GEE = generalized estimating equations, GLI = glibenclamide, GLMM = generalized linear mixed models.

Glibenclamide Dosing

Reports of preclinical glibenclamide dosing in TBI have varied by species: rat loading dose 10 μg/kg versus mouse 20 μg/kg to 10 μg/mouse (~20× higher) (1). Even at the lower dose in mice in our study, plasma levels greater than 10,000 pg/mL were obtained 1 hour post load and persisted at steady state (21). Although the dosing was based on previously reported research with pharmacokinetic data (1, 21), it is critical to emphasize that it remains unknown how our dose range scales to those in humans for oral glibenclamide or the proprietary intravenous formulation (BIIB093) being tested in ASTRAL. Given the increased sensitivity of MRI to edema endophentoypes and the signal toward a reduction in diffuse/contralateral edema previously reported at low dose (21), we anticipated a dose response. This was not observed. This is relevant to ASTRAL, which randomizes patients to low dose (3 mg/d) versus high-dose (5 mg/d) BIIB093. In our study, only high-dose glibenclamide reduced hematoma volume across models. In CCI, effects on all MRI outcomes were binary: high-dose glibenclamide benefited hematoma volume and all edema endophenotypes, versus low-dose glibenclamide with no benefit across metrics. This contrasts CCI+HS, where effects of low- and high-dose glibenclamide on edema volume and T2-hyperintensity had similar directionality with trends toward benefit, but neither dose impacted cytotoxic/cellular edema. Mechanistic underpinnings merit further study.

The transient trend toward increased hematoma volume (by ~60%) at 3 hours with low-dose glibenclamide, if validated, could be clinically detrimental. However, it should be emphasized that this was not a statistically significant difference and has been noted solely given the potential clinical implications. The reasons for this remain unknown, and speculative mechanisms include effects of glibenclamide on other SUR1-regulated channels, or off target effects. Although mechanistically unclear, it warrants future exploration including into related transcriptome and protein networks affected by glibenclamide beyond SUR1-TRPM4 particularly given off-label drug use. Also of note, this finding is accompanied by a trend of worsened diffusion restriction ($p = 0.09$). To our knowledge, despite several studies of glibenclamide in TBI, both preclinical and clinical, contusion expansion has not been reported although it has also not been evaluated this acutely, using this modality, and at this dose/level of injury.

Strengths and Limitations

Our study has several strengths: it is a rigorous preclinical evaluation mimicking a blinded randomized controlled clinical trial. Clinically relevant injury models and MRI outcomes were selected to maximize translatability. We describe the impact of glibenclamide on "clinical" imaging outcomes that distinguish edema endophenotypes and enable spatial/temporal correlations of cytotoxic and vasogenic edema with established "molecular" patterns in distinct cell types, thereby connecting clinical measures with preclinical/molecular research.

There are also limitations. Although rigorous blinding with extensive quality control on imaging quantification prior to analysis makes artifactual findings unlikely, given the small animal model, it would be valuable to validate/replicate these findings in an independent laboratory. Our findings corroborate the histopathological findings of glibenclamide on reducing lesion volume in other independent studies of male rodent CCI (20, 22). Nonetheless, females may respond differently to SUR1-TRPM4 inhibition and merit dedicated study. We reported diminished benefit of genetic *Abcc8* (SUR1) knockout on 21-day lesion volume in female versus male mice after CCI (5). Here, we focused on males as a foundational reference for future work evaluating that sex-based differences, since dose, timing, and off-target effects may differ in genetic knockout versus pharmacological inhibition. Although we evaluated glibenclamide over 7 days, the therapeutic window was not defined. Despite the standard and clinically relevant/translatable MRI endophenotypes used in this study, additional imaging approaches would be valuable to evaluate including free-water assessment, arterial spin labeling, and blood oxygen level–dependent sequences to evaluate perfusion. That said, another limitation is that in severe TBI, acute MRIs are not always clinically feasible due to intracranial monitoring and/or physiologic instability. Importantly, hematoma expansion can be assessed using computed tomography–based similar quantification methods. Parallel with ASTRAL, we focused on imaging end points/endophenotypes reflecting underlying pathophysiology rather than behavioral outcomes, which have been previously reported (20, 50). It is important for future studies to evaluate the impact of different doses on a battery of behavioral outcomes that test different functions and to explore how these effects vary with TBI model. Both the CCI and CCI+HS models as used here generate robust behavioral targets. We did not evaluate glibenclamide across injury severity/other TBI models: most preclinical studies report benefit after CCI, but results in other models are mixed (1). Nonetheless, small human studies of glibenclamide suggest benefit across TBI endophenotypes (24–26).

CONCLUSIONS

In this murine study, glibenclamide was most beneficial to a higher dose and in isolated contusional TBI. Positive effects spanned MRI endophenotypes including hematoma and edema volume, T2-hyperintensity (vasogenic edema), diffusion restriction (ADC and cytotoxic edema), and T1-gadolinium enhancement (BBB integrity). High-dose benefit on hematoma volume and cytotoxic edema after contusional TBI was acute, with a longer treatment window for vasogenic edema. Hematoma reducing effects were retained in TBI plus HS (a common and devastating second insult), but antiedema effects were less robust. This suggests that relevant outcome measures for future clinical trials should be informed by key patient/injury characteristics. Low-dose glibenclamide was largely ineffective across insults. Our findings may inform trial design pertaining to dose, timing, prognosis, and high-specificity patient and outcome selection. This is relevant to analysis of ongoing trials and design of planned clinical trials of glibenclamide in TBI and other acute brain injuries.

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