Glibenclamide Is Comparable to Target Temperature Management in Improving Survival and Neurological Outcome After Asphyxial Cardiac Arrest in Rats

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Background—We previously have shown that glibenclamide (GBC), a sulfonylurea receptor 1–transient receptor potential M4 (SUR1-TRPM4) channel inhibitor, improves survival and neurological outcome after asphyxial cardiac arrest and cardiopulmonary resuscitation (ACA/CPR). Here, we further compare the efficacy of GBC with target temperature management (TTM) and determine whether the efficacy of GBC is affected by TTM.

Methods and Results—Male Sprague-Dawley rats (n=213) subjected to 10-minute ACA/CPR were randomized to 4 groups after return of spontaneous circulation (ROSC): normothermia control (NT); GBC; TTM; and TTM+GBC. Survival, neurodeficit scores, histological injury, as well as the expressions of SUR1 and TRPM4 were evaluated. The 7-day survival rate was 34.4% (11 of 32) in the NT group, 65% (13 of 20) in the GBC group, 50% (10 of 20) in the TTM group, and 70% (14 of 20) in the TTM+GBC group. Rats that received either GBC, TTM alone, or in combination showed less neurological deficit than NT control at 24, 48, and 72 hours and 7 days after ROSC. Moreover, TTM or GBC ameliorated neuronal degeneration and glial activation in the hippocampal CA1 region with similar efficacy, whereas the combination of them had a trend toward better effect. The subunits of SUR1-TRPM4 heterodimers were both strongly upregulated after ACA/CPR and expressed in multiple types of brain cells, but partly suppressed by TTM.

Conclusions—GBC is comparable to TTM in improving survival and neurological outcome after ACA/CPR. When GBC is given along with TTM, less histological injury tended to be achieved. (***J Am Heart Assoc***. 2016;5:e003465 doi: 10.1161/JAHA.116.003465)

Key Words: cardiopulmonary resuscitation • glibenclamide • heart arrest • sulfonylurea receptor 1-transient receptor potential M4 channel • target temperature management

Survivors from out-of-hospital cardiac arrest (OHCA) are still facing high risks of death and disability.[1] Target temperature management (TTM), also known as therapeutic hypothermia, is the only approach proven to improve outcome after ventricular fibrillation-induced OHCA.[2–4] Despite that, the efficacy of TTM on comatose adult patients whose initial rhythms are nonshockable requires further confirmation.[5,6] In addition, the adverse effects of hypothermia, the increased nursing labor, and costs for management of hypothermia are also issues of concern.[7,8] Overall, TTM is still underutilized.[9] Development of alternative or additive therapeutic approaches are required to favor post-CA (cardiac arrest) patients.

Glibenclamide (US adopted name, glyburide, glibenclamide [GBC]) is a US Food and Drug Administration (FDA)-approved sulfonylurea for treating type 2 diabetes mellitus for decades. In recent years, GBC has been proved to be neuroprotective in several disease models, including cerebral infraction, traumatic brain injury, subarachnoid hemorrhage, hemorrhagic encephalopathy of prematurity, spinal cord injury, hepatic encephalopathy, and multiple sclerosis.[10–16] Moreover, the use of sulfonylureas (to treat diabetes) around the time of acute ischemic stroke has been associated with less neurological deficit and lower risk of hemorrhagic conversion.[17,18] Two clinical trials (ClinicalTrials.gov identifier: NCT01454154 and NCT01794182) testing the efficacy of GBC on traumatic

Figure 1. Experimental procedure and flow diagram of the study. A, Experimental procedure and measurements during baseline, asphyxial cardiac arrest and cardiopulmonary resuscitation (ACA/CPR), and return of spontaneous circulation (ROSC). B, Flow diagram of the experiment. The whole study consisted of 3 parts. In part 2, rats were euthanized at 3, 6, 24, or 72 hours after ROSC. ECG indicates electrocardiogram; GBC, glibenclamide; MAP, mean arterial pressure; NT, normothermia; TTM, target temperature management.

brain injury as well as malignant edema and stroke are underway. Although the mechanisms are not fully uncovered, it is suggested that GBC blocks the activity of the transient receptor potential M4 (TRPM4) through interfering with the sulfonylurea receptor 1 (SUR1) subunit and thus ameliorates the resulting injuries to the neurovascular unit.[19]

In our previous study, post-CA treatment with GBC improved survival and neurological outcome in a rat model of 8-minute asphyxial cardiac arrest and cardiopulmonary resuscitation (ACA/CPR).[20] In this study, we further compared the efficacy of GBC with TTM in a more-severe model of

ACA/CPR. From a viewpoint of clinical translation, we also examined whether the efficacy of GBC was affected by TTM.

Methods

Animal Preparation

This study was approved by the Animal Care and Use Committee of the Nanfang Hospital, Southern Medical University (Guangzhou, China) and followed the National Guidelines for Animal Experimentation. Male Sprague-Dawley rats weighing between

300 and 350 g were obtained from the Experimental Animal Center of Southern Medical University and housed on a 12-hour light/dark cycle with free access to water and food.

Rat Model of Asphyxial Cardiac Arrest

A rat model of 10-minute ACA/CPR was established with modification (Figure 1A).[20] In brief, rats were anesthetized with isoflurane (4% for induction and 2% for maintenance; RWD, Shenzhen, China), orotracheally intubated, and connected to a mechanical ventilator (RWD). Intravascular catheters (PE50; Smiths Medical, Ashford, UK) were inserted into the right femoral artery and vein for dynamic artery blood pressure monitoring and drug administration. Electrocardiogram was recorded by subcutaneous needle electrodes. After 10-minute stabilization, rats were chemically paralyzed by IV vecuronium (2 mg/kg) and inspired gas was changed to room air. Then, the ventilator was disconnected for 10 minutes, leading to circulatory arrest within 4 minutes, which was confirmed by cessation of arterial pulse and reduction of mean arterial pressure (MAP) to below 20 mm Hg. At the end of 10-minute asphyxia, CPR was initiated by reconnecting the ventilator and thoracic compressions were performed by a custom-made mechanical chest compressor at a rate of 400 compressions per minute. To prolong the duration of CPR, epinephrine (0.01 mg/kg) was administered after 1 minute of thoracic compressions and repeated at 3-minute intervals (0.02 mg/kg), if necessary. Return of spontaneous circulation (ROSC) was defined as increase of MAP beyond 60 mm Hg for 10 minutes. Rats failed to ROSC within 5 minutes were excluded from the continuing experiments.

Core temperature was monitored by a rectal temperature probe (RC-4; Elitech, Xuzhou, China) and maintained with a temperature feedback system (RWD) as appropriate temperature management. Brain temperature was monitored in a subgroup of animals by placing a temperature probe (Physitemp Instruments, Inc., Clifton, NJ) in the right brain cortex (3 mm posterior and 3 mm lateral to the bregma and 4 mm below the skull surface). Blood glucose was detected intermittently using a glucometer (Optium, Abbott, CA). After spontaneous breath started, animals were weaned from the ventilator. After 1-hour observation, all catheters were removed and surgical wounds were sutured. At the end of each experimental period, rats were returned to their cages with easily accessible food and water and were observed in a room of constant temperature (22°C). Saline (20 mL/kg) was administered daily to rats until they were able to feed themselves.

Study Protocol

Fifteen minutes after ROSC, rats were block randomized to 4 treatment groups by using a random number table

(Figure 1A): normothermia and vehicle (NT); normothermia and GBC (GBC); TTM and vehicle (TTM); and TTM and GBC (TTM+GBC). Rats in the GBC groups were intraperitoneally administered GBC (Sigma-Aldrich, St. Louis, MO) with a loading dose of 10 µg/kg at randomization and 4 maintenance doses of 1.2 µg per 6 hours after ROSC, whereas rats in the vehicle groups received an equal volume of DMSO and saline.[20] In the TTM groups, rapid cooling was initiated after randomization. By using ice packs and an electric fan, a target temperature of 33°C was reached within 15 minutes and then maintained for 4 hours, followed by slow rewarming to 37°C at a rate of 0.5°C/h. Normothermic rats were constantly maintained at 37°C. Rats that underwent all procedures except ACA and CPR were used as sham control.

The present study includes 3 parts (Figure 1B). In part 1, 92 rats that successfully resuscitated were followed up for 7 days and survival, neurological outcome ,and histological injury were evaluated. In parts 2 and 3, expressions of SUR1 and TRPM4 at various time points and under different treatments were examined.

Survival Study and Neurological Function Evaluation

Survival was recorded daily. Neurological function was assessed by 2 investigators unaware of animal grouping at 24, 48, and 72 hours and 7 days after ROSC, using the neurodeficit score (NDS),[21] a previously validated scale consisted of 7 components: general behavioral deficit; brainstem function; motor assessment; sensory assessment; motor behavior; behavior; and seizures. A total NDS of 80 was considered normal, whereas 0 was brain death. To avoid the confounding brought by mortality, NDSs were analyzed in survived rats only.

Table 1. Baseline Characteristics Among the 4 Experimental Groups

Parameters	NT (n=32)	GBC (n=20)	TTM (n=20)	TTM+GBC (n=20)
Body weight, g	327±23	332±20	332±38	331±34
Time from asphyxia to CA, sec	212±26	218±24	225±21	222±18
Time required for ROSC, sec	194±65	173±72	179±65	167±73
Total dose of epinephrine, µg	7.3±3.2	6.3±3.3	6.6±3.4	5.3±3.2

CA indicates cardiac arrest; GBC, glibenclamide; NT, normothermia; ROSC, return of spontaneous circulation; TTM, target temperature management.

Table 2. Physiological Parameters and Laboratory Results of the 4 Experimental Groups at Baseline and During Post-CA Care After ROSC

Parameters	Time Points	NT (n=32)	GBC (n=20)	TTM (n=20)	TTM+GBC (n=20)
MAP, mm Hg	Baseline	108±9	112±8	108±9	104±7
	10 minutes	114±9	116±11	123±9	119±5
	30 minutes	87±7	86±8	87±5	88±9
	60 minutes	87±5	82±12	80±7	85±11
Heart rate, bpm	Baseline	405±9	399±11	398±9	402±15
	10 minutes	436±20	425±16	417±20	421±17
	30 minutes	386±14	385±13	392±9	378±14
	60 minutes	388±7	379±14	376±11	376±15
PH	Baseline	7.41±0.01	7.40±0.04	7.38±0.03	7.39±0.03
	10 minutes	7.16±0.03	7.15±0.03	7.14±0.05	7.14±0.06
	30 minutes	7.20±0.05	7.19±0.03	7.19±0.03	7.17±0.07
	60 minutes	7.25±0.05	7.23±0.02	7.23±0.06	7.22±0.05
PaO_2, mm Hg	Baseline	85±5	88±4	86±5	86±7
	10 minutes	105±7	109±6	112±7	111±6
	30 minutes	112±8	118±6	117±10	115±9
	60 minutes	99±4	96±7	99±7	100±8
$PaCO_2$, mm Hg	Baseline	40±4	40±4	42±4	41±2
	10 minutes	59±7	65±10	66±6	69±8
	30 minutes	56±5	55±5	54±5	57±7
	60 minutes	51±3	46±3	48±4	51±6
Lactate, mmol/L	Baseline	1.20±0.37	1.28±0.37	1.34±0.26	1.34±0.26
	10 minutes	5.52±1.78	4.44±1.25	4.82±1.53	4.62±1.14
	30 minutes	4.00±0.42	3.78±0.89	4.24±0.52	3.98±0.72
	60 minutes	3.08±0.80	3.22±0.61	3.26±0.50	2.64±0.33

All values were expressed as mean±SD. There were no significant differences for treatment by time among the four groups by mixed-effects model for repeated-measures analyses. bpm indicates beats per minute; CA, cardiac arrest; GBC, glibenclamide; MAP, mean arterial pressure; NT, normothermia; $PaCO_2$, arterial carbon dioxide pressure; PH, potential of hydrogen; ROSC, return of spontaneous circulation; TTM, target temperature management.

Histological Examination

After 7-day follow-up, rats were euthanized and coronal brain sections (6-μm thickness) located at 3.5 mm posterior to bregma were obtained (Leica CM1800; Leica Microsystems GmbH, Heidelberg, Germany). Immunohistochemistry was performed with antibodies against neuronal nuclei (NeuN; Abcam, Cambridge, UK) for detection of neuron, microtubule-associated protein 2 (MAP2; Sigma-Aldrich) for dendrite, glial fibrillary acidic protein (GFAP, Abcam) for astrocyte, and ionized calcium-binding adapter molecule-1 (Iba-1; Wako, Osaka, Japan) for microglia, using the protocol suggested by the manufacturer (ZSGB-BIO, Beijing, China). In each brain section, 2 slide fields were randomly examined.[22] The number of NeuN-positive cells and the relative intensity of MAP2, Iba-1, and GFAP staining was determined with ImageJ software (1.49v; National Institutes of Health [NIH], Bethesda, MD).

For immunofluorescence, brain sections were incubated with primary antibodies directed against SUR1 (Sigma-Aldrich), TRPM4 (Abcam), NeuN, von Willebrand factor (vWF; Abcam), GFAP, and Iba-1. Bound primary antibodies were detected with Alexa Fluor dye of donkey antimouse or antirabbit (Abcam) and observed with a confocal microscope (FluoView; Olympus, Tokyo, Japan). The total number of SUR1- and TRPM4-positive cells as well as the percentages of SUR1-staining in different types of cells were calculated by ImageJ (NIH).

Western Blotting

Western blot was routinely performed as previously reported.[20] Antibodies against SUR1, TRPM4, and β-actin (CWBIO, Beijing, China) were used. The densities of protein
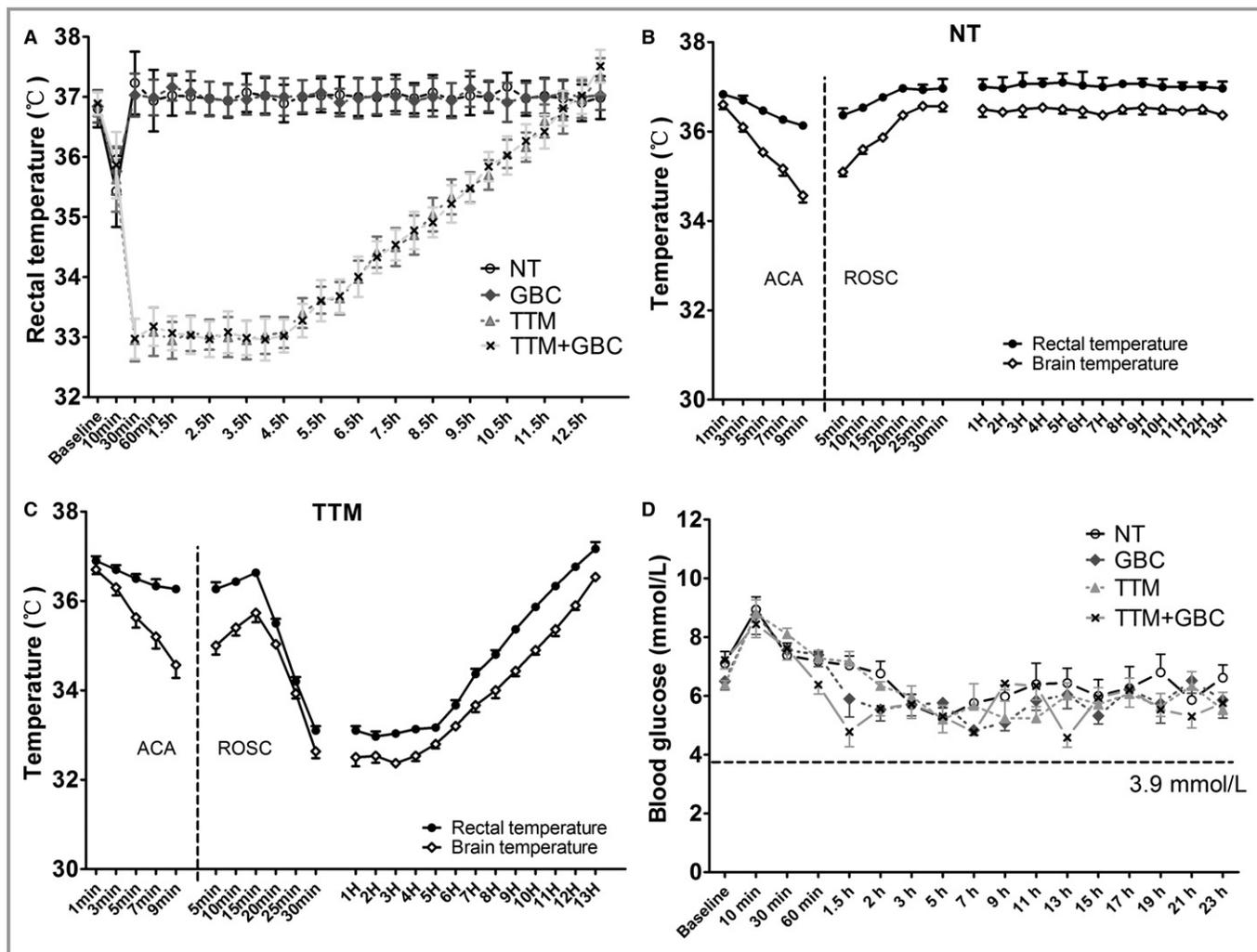
Figure 2. Core temperature and blood glucose monitoring at baseline and after ROSC. A, Rectal temperature monitoring of all rats in the experimental groups. B and C, Rectal temperature and brain temperature in the NT (n=3) and TTM (n=3) groups during ACA and after ROSC. D, Blood glucose monitoring at baseline and after ROSC. ACA indicates asphyxial cardiac arrest; GBC, glibenclamide; NT, normothermia; ROSC, return of spontaneous circulation; TTM, target temperature management.

blots were quantified by using ImageJ software (NIH) and normalized to level of β-actin.

Measurement of Gene Expression

mRNA levels of *Abcc8* (encoding SUR1), *Trpm4*, and *Gapdh* were routinely measured by quantitative real-time polymerase chain reaction.[20] Relative changes of mRNA expressions were normalized to levels of *Gapdh*.

Statistical Analysis

To calculate the sample size in survival analysis, a power analysis was performed based on our preliminary experiment. The survival rate of the NT group was estimated at 30% and the GBC group at 70%. Assuming an α error of 0.05 with a power of 0.85, a ratio of 1.6 for the NT-to-GBC group, we obtained a necessary sample size of 32 in the NT group and 20 in the GBC group to show a significant effect. The ratio of 1.6 for the NT-to-GBC group was determined based on estimated survival and to ensure sufficient brain samples for histological examination.

Data are presented as mean±SD. For multiple comparisons, one-way ANOVAs were used, followed by least-significant difference tests between multiple groups. Physiological variables were examined by repeated-measures analyses comprising treatment group, time, and treatment-by-time interaction. NDSs are presented as medians and 25th to 75th percentiles and were analyzed by Kruskal–Wallis with Mann–Whitney *U* analyses between multiple groups. Difference in survival rate was analyzed by Kaplan–Meier analysis with log-rank test. SPSS (version 20.0; IBM Corp, Armonk, NY)
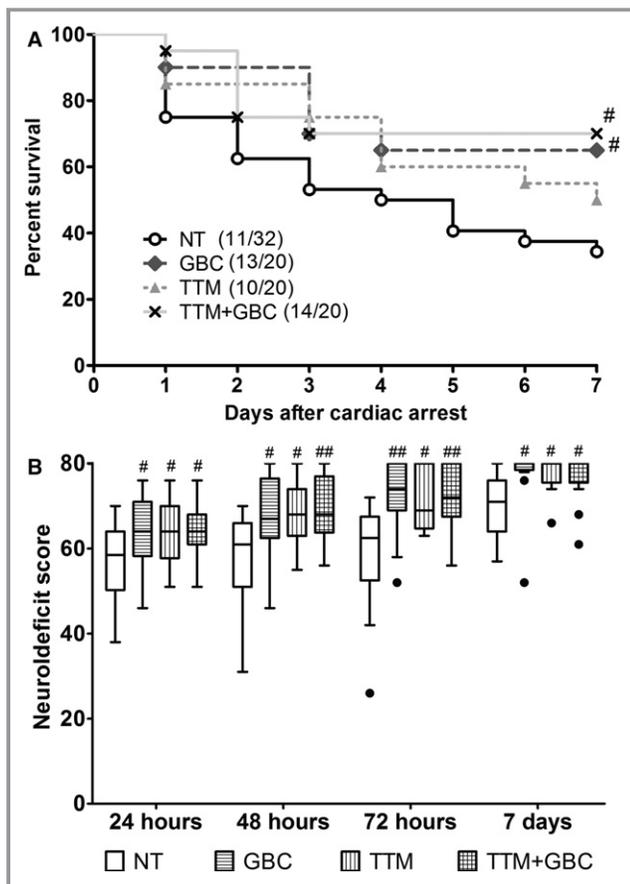
Figure 3. Effects of GBC and TTM on survival and neurological outcome after ACA/CPR. A, Kaplan–Meier analyses of cumulative survival during 7-day follow-up after ROSC. B, Neurodeficit scores (0=brain death; 80=normal) of survived rats at 24, 48, and 72 hours and at 7 days after ROSC. Filled circle indicates outlier. #P<0.05; ##P<0.01 versus NT group. ACA indicates asphyxial cardiac arrest; CPR, cardiopulmonary resuscitation; GBC, gliben-clamide; NT, normothermia; ROSC, return of spontaneous circulation; TTM, target temperature management.

and GraphPad Prism software (version 5.0; GraphPad Software Inc., La Jolla, CA) were used for statistical analyses. $P<0.05$ was considered statistically significant.

Results

GBC was Comparable to TTM in Improving Survival and Neurological Outcome After ACA/CPR

A total of 228 rats were prepared for the study (Figure 1B). Of 170 rats that successfully resuscitated, 15 died (3 in the 24 hours group, 2 in the 72 hours group, 3 in the GBC group, 5 in the TTM group, and 2 in the TTM+GBC group) before predefined time points were excluded. There were no significant differences in body weight, time from asphyxia to CA, time required for ROSC, and epinephrine usage among

the 4 experimental groups (Table 1). There were also no differences among the 4 experimental groups in MAP, heart rate, blood gases, or serum lactate at baseline or during post-CA care after ROSC (Table 2). Rectal temperature in hypothermic animals reduced rapidly to 33.0°C at 30 minutes after ROSC, whereas normothermic animals were maintained around 37.0°C during the whole period (Figure 2A). Brain temperature dropped faster than rectal temperature during asphyxia, but revealed linear trend with rectal temperature after ROSC (Figure 2B and 2C). Blood glucose levels in the GBC and TTM+GBC groups seemed lower than those in the NT and TTM groups, but no hypoglycemia (<3.9 mmol/L) was detected (Figure 2D).

The 7-day survival rate was 34.4% (11 of 32) in the NT group, 65% (13 of 20) in the GBC group, 50% (10 of 20) in the TTM group, and 70% (14 of 20) in the TTM+GBC group (Figure 3A). In addition, rats in the GBC, TTM, and TTM+GBC groups all showed higher NDSs than those in the NT group at 24, 48, and 72 hours and 7 days after ROSC (Figure 3B). These results suggest that GBC is comparable to, or even better than, TTM in improving survival and neurological outcome after 10-minute ACA/CPR.

GBC and TTM Ameliorated Histological Injury in the Hippocampal CA1 Region

Transient global cerebral ischemia could lead to delayed neuronal loss and activation of microglia and astrocytes in selectively vulnerable areas, such as the hippocampal CA1.[22] As indicated in the histological analysis of the hippocampal CA1 region in Figure 4, TTM or GBC alone significantly recovered numbers of neurons by NeuN staining compared to NT treatment, with their combination resulted in a further recovery (Figure 4A and 4E). In addition, dendritic injury revealing by MAP2 staining was partly prevented by GBC or TTM treatment (Figure 4B and 4F), with better preservation by TTM+GBC than GBC alone. Moreover, there were significantly more Iba-1-positive microglia and GFAP-positive astrocytes in the NT group than the other groups (Figure 4C, 4D, 4G and 4H). Together, these results imply that GBC is comparable to TTM in ameliorating histological injury after ACA/CPR, whereas their combination has a trend toward better effect.

TTM, but Not GBC, Inhibited the Upregulation of SUR1 and TRPM4 Induced by ACA/CPR

Given that the SUR1-TRPM4 channel is the putative therapeutic target of GBC,[10,23] we then examined expressions of this channel subunits before and after ACA/CPR. Results showed that both mRNA levels and protein levels of SUR1 and TRPM4 were significantly upregulated at 6 hours after ACA/CPR and reached a higher level at 24 hours (Figure 5A
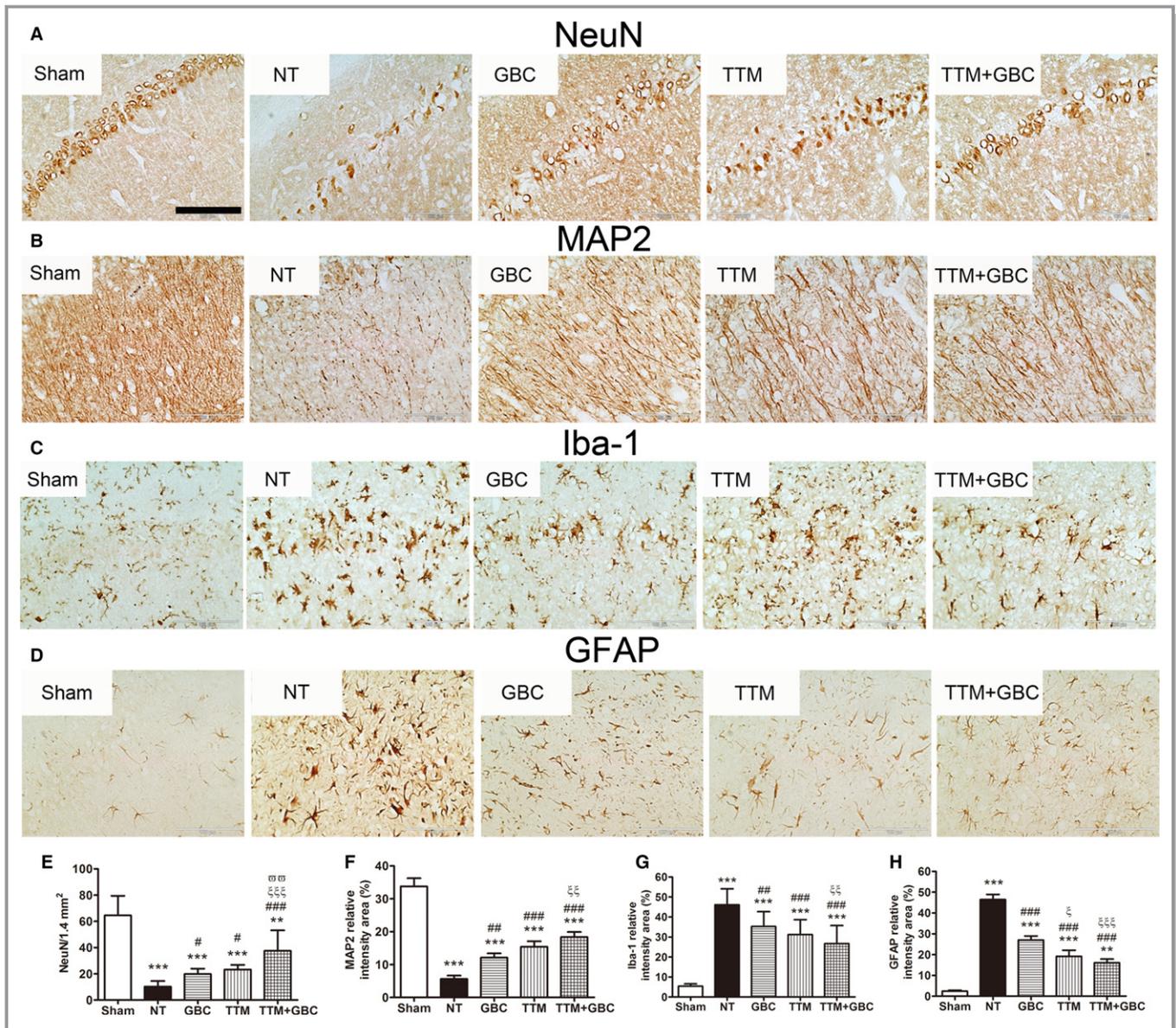
Figure 4. Effects of GBC and TTM on neuronal death and activation of microglia and astrocytes after ACA/CPR. A through D, Representative photomicrographs of immunohistochemistry for neuronal nuclei (NeuN), microtubule-associated protein 2 (MAP2), ionized calcium-binding adapter molecule 1 (Iba-1), and glial fibrillary acidic protein (GFAP) in the hippocampal CA1 region of the sham-operated and the experimental groups at 7 days after ROSC. Bar, 100 μm. E through H, Semiquantitative results of NeuN, MAP2, Iba-1, and GFAP, respectively. **$P<0.01$ and ***$P<0.001$ versus the sham-operated group; #$P<0.05$, ##$P<0.01$, and ###$P<0.001$ versus the NT group; ξ$P<0.05$, ξξ$P<0.01$, and ξξξ$P<0.001$ versus the GBC group; ϖϖ$P<0.01$ versus the TTM group. ACA indicates asphyxial cardiac arrest; CPR, cardiopulmonary resuscitation; GBC, glibenclamide; NT, normothermia; ROSC, return of spontaneous circulation; TTM, target temperature management.

through 5C). Immunofluorescent labeling also revealed more SUR1- and TRPM4-postive cells in the brain cortex and hippocampal CA1 region in post-CA rats (Figure 5D). Moreover, TTM, but not GBC, reduced the levels of SUR1 and TRPM4 (Figure 5E and 5F).

At 72 hours after ROSC, SUR1 was expressed in multiple types of brain cells, including neuron (NeuN-positive), endothelial cell (vWF-positive), microglia (Iba-1-positive), and astrocytes (GFAP-positive; Figure 6A). Besides, SUR1 colocalized closely

with TRPM4, in agreement with the fact that SUR1 and TRPM4 form a heterodimer.[24] TTM and TTM+GBC reduced the total number of SUR1- and TRPM4-positive cells (Figure 6B), without interfering the cellular distribution of the SUR1 (Figure 6C).

Discussion

GBC has been shown to improve survival and neurological outcome in a rat model of 8-minute ACA/CPR.[20] In this study,
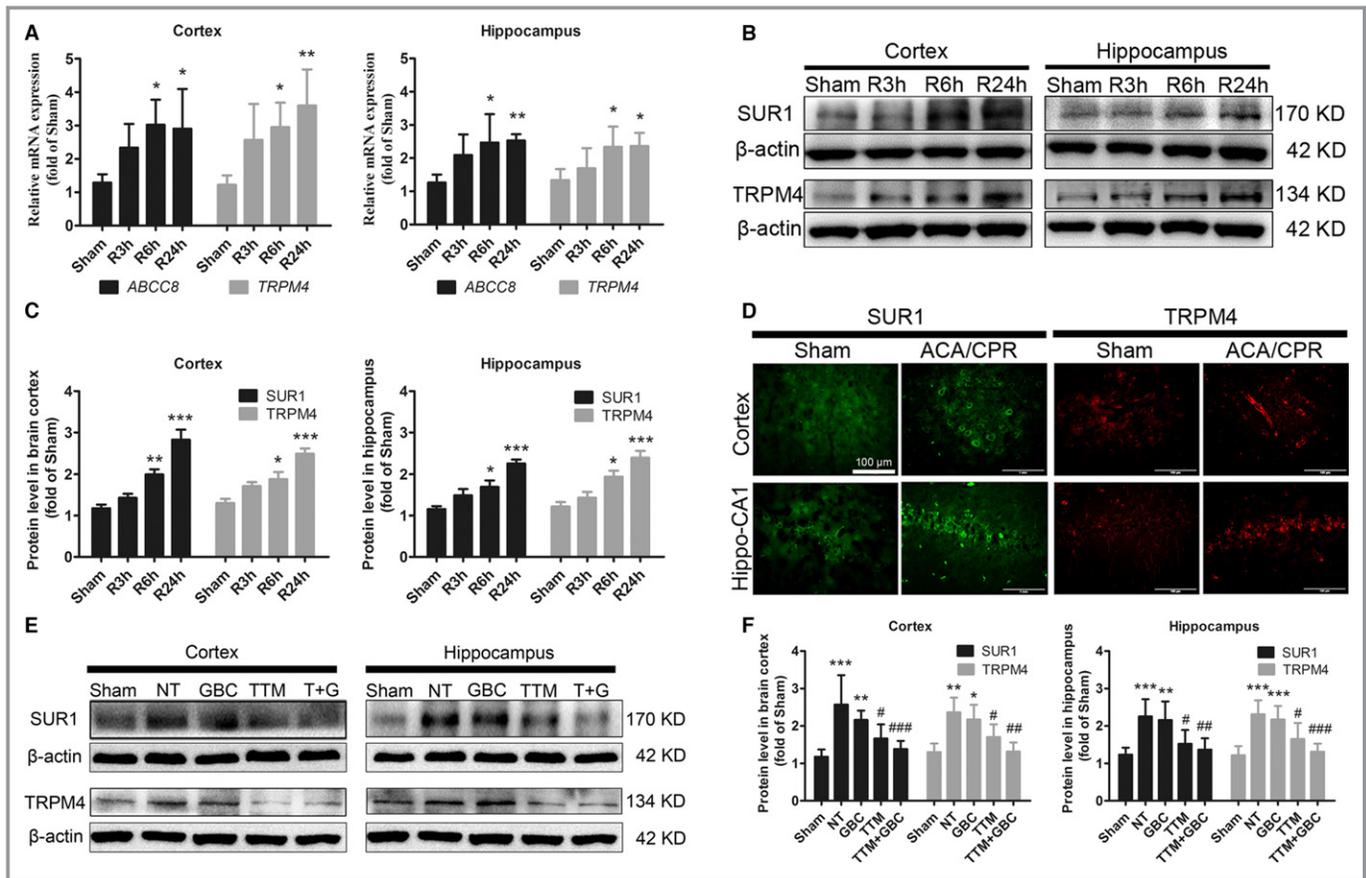
Figure 5. Effects of GBC and TTM on expressions of sulfonylurea receptor 1 (SUR1) and transient receptor potential M4 (TRPM4) after ACA/CPR. A, Quantitative real-time polymerase chain reaction results of message RNA expressions of *Abcc8* (encoding SUR1) and *Trpm4* in sham-operated rats and postcardiac arrest (post-CA) rats that euthanized at 3, 6, and 24 hours after ROSC. B and C, Western blot results of SUR1 and TRPM4 in sham-operated and post-CA rats that euthanized at different time points after ROSC. D, Representative photomicrographs of the immunofluorescence labeled with SUR1 (green) and TRPM4 (red) in brain cortex and hippocampal CA1 region from sham-operated and post-CA rats. Bar, 100 μm. E and F, Western blot findings of SUR1 and TRPM4 under different treatments detected at 24-hour after ROSC. T+GBC, TTM, and GBC. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ versus the sham-operated group; #$P<0.05$, ##$P<0.01$, and ###$P<0.001$ versus the NT group. ACA indicates asphyxial cardiac arrest; CPR, cardiopulmonary resuscitation; DAPI, 4′,6-diamidino-2-phenylindole; GBC, glibenclamide; NT, normothermia; ROSC, return of spontaneous circulation; TTM, target temperature management.

we demonstrated that GBC was comparable to TTM in improving survival and neurological outcome, attenuating neuronal injury and glial activation in a rat model of 10-minute ACA/CPR. When GBC was combined with TTM, there was a trend toward less histological injury than either of them alone. We also found paralleled upregulation of SUR1 and TRPM4 after ACA/CPR, which were inhibited by TTM.

TTM is the cornerstone of post-CA care.[2] However, there is a dispute on the target temperature, especially after the findings presented by Nielsen et al.,[25] that hypothermia at a temperature of 33°C in unconscious survivors of OHCA did not confer a benefit as compared to 36°C. Although the lack of benefit at the lower temperature might be ascribed to the improvement in critical care or diversity of illness severity, the concept of TTM, aiming to prevent hyperthermia, seems to have become an established paradigm of postresuscitative

care.[26,27] The current American Heart Association guideline recommends comatose adult patients with ROSC after CA to have TTM, by selecting and maintaining a constant temperature between 32°C and 36°C.[2] In this study, 33°C was selected and maintained for all TTM.

Besides the depth of hypothermia, the initiation, duration, and rate of rewarming are all crucial factors to optimize TTM.[28] In humans, TTM is suggested to be maintained for at least 24 hours after achieving target temperature.[2] However, shorter duration of TTM was commonly utilized in animal studies, because of the high metabolic rate of rodents and shorter time in reaching target temperature.[22,29,30] Ye et al.[31] reported that 2-hour duration of mild hypothermia induced rapidly after ROSC improved survival, myocardial and cerebral functions as well as prolonged duration of hypothermia. As for rewarming, it is recommended to be conducted at a rate of 0.25
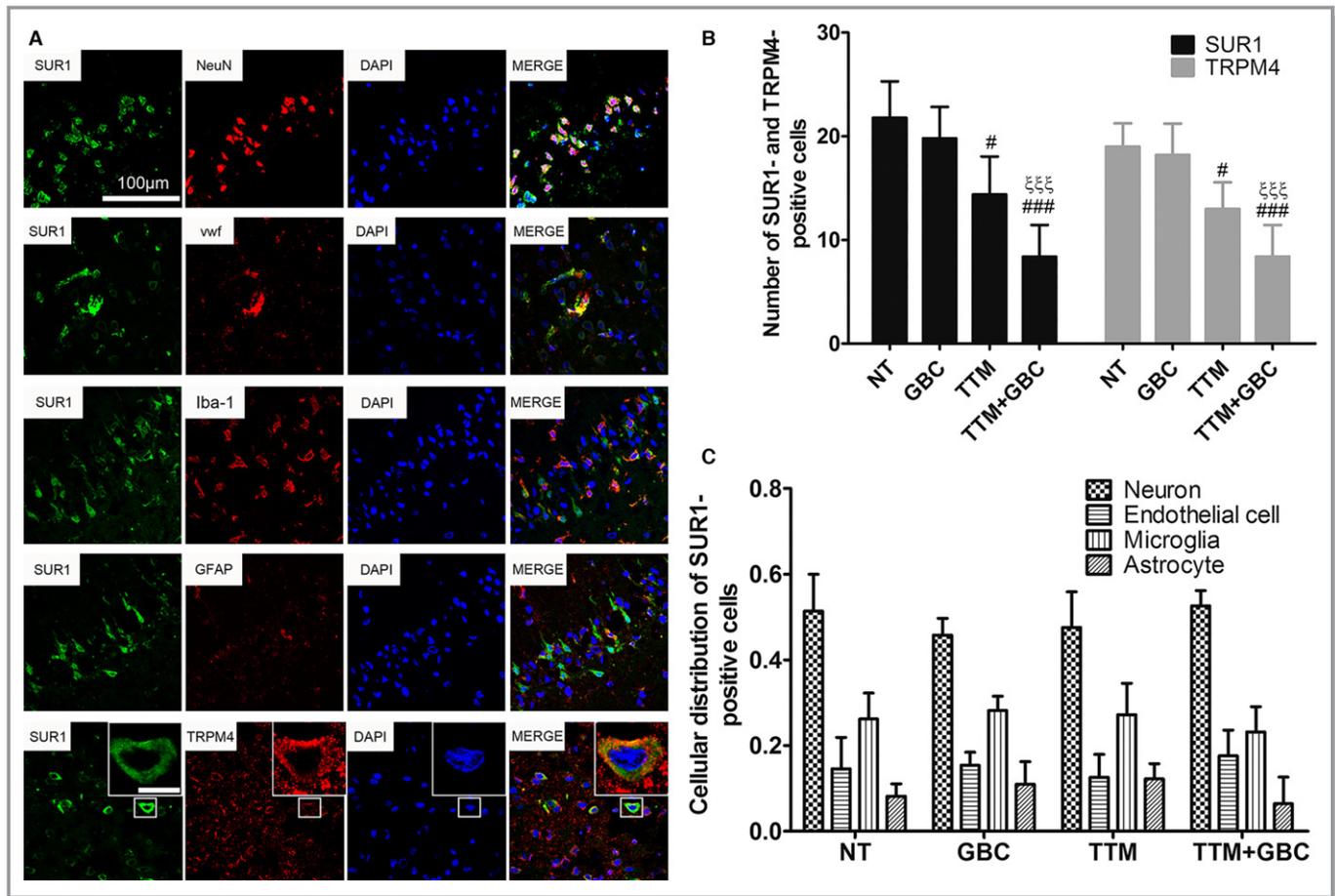
Figure 6. Cellular distribution of SUR1 in the brain at 72-hour after ROSC. A, Representative photomicrographs observed under confocal microscope are shown. SUR1 (green) colocalizes with multiple cellular markers (red), including neuronal nuclei (NeuN), von Willebrand factor (vWF), ionized calcium–binding adapter molecule 1 (Iba-1), and glial fibrillary acidic protein (GFAP). SUR1 also colocalizes with TRPM4 (red). Bar, 100 μm. Inserted bar, 5 μm. B, Number of SUR1- and TRPM4-positive cells in each group. C, Cellular distribution of SUR1in different groups. All n=5. [#]*P*<0.05 and [###]*P*<0.001 versus the NT group; [ξξξ]*P*<0.001 versus the GBC group. GBC, glibenclamide; NT, normothermia; ROSC, return of spontaneous circulation; TTM, target temperature management.

to 0.5°C/h.[1] A rate of rewarming at 2°C/h may abolish the beneficial effects of hypothermia and, at 4°C/h, can adversely affect the brain.[29,32] Taking these factors into account, we initiated TTM at 15 minutes after ROSC, maintained it for 4 hours after reaching 33°C, and followed by a rewarming rate at 0.5°C/h. As expected, TTM alleviated neurological deficit and histological injury after 10-minute ACA/CPR.

From the viewpoint of clinical translation, it is also important to note that the combined therapy of TTM and GBC had a trend of better effect than TTM alone in improving survival and alleviating histological injury. TTM moderates a variety of destructive processes after CA/CPR, but the exact mechanism is still unknown.[33] Given that the subunits of the SUR1-TRPM4 channel were upregulated after ACA/CPR and targeting this channel by GBC or gene manipulation have been shown to provide benefit in many neuropathological circumstances,[10,23] we checked here whether TTM would regulate this channel. As

indicated in Figure 5E and 5F, TTM inhibited the upregulations of SUR1 and TRPM4 induced by ACA/CPR. Although the causal relationship between the SUR1-TRPM4 channel and post-CA brain injury requires to be further determined, targeting the SUR1-TRPM4 channel might be one of the mechanism that leads to the neuroprotection of TTM.

The SUR1-TRPM4 channel is a heterodimer that exhibits pharmacological properties of SUR1 and biophysical properties of TRPM4.[24] Once it has been overactivated upon ischemia, hypoxia, trauma, or inflammation, the SUR1-TRPM4 channel could cause unchecked influx of extracellular sodium and water, leading to consequential cell swelling, cell death, release of inflammatory cytokines, and breakdown of the blood–brain barrier.[10–13] GBC is well documented to bind to the SUR1 subunit and increase the probability of long closed states of the TRPM4 channel,[16,19,34] without altering their structural expressions.[20] Consistent with our previous data,[20]

we showed paralleled upregulations of SUR1 and TRPM4 after ACA/CPR. However, because of technological limitation, we have no direct evidence for the blocking of this channel by GBC and thus are not able to rule out other possible mechanisms that might contribute to the salutary effect of GBC. Therefore, our findings suggest a possible role of the SUR1-TRPM4 channel in post-CA brain injury, but further studies are required to prove their causal relationship.

In histological examination, we demonstrated that not only neurons, but also dendrites were salvaged by GBC treatment. GBC also suppressed the activation of microglia and astrocytes, which could either be a reaction to tissue damage or a key contributor to the pathophysiology of brain ischemic injuries, such as delayed neuronal loss.[22,35] Similar to that found in ischemic stroke,[10] the upregulated SUR1 was evident in neurons, astrocytes, and endothelial cells after ACA/CPR, with paralleled upregulation of TRPM4. Moreover, the delayed upregulation of SUR1 and TRPM4 from 6 to 24 hours or even 72 hours post-ROSC might suggest a broader window for GBC therapy in ACA/CPR and better effects brought by longer repetitive application of GBC.[34,36]

It is worthy of note that no hypoglycemia was detected after GBC treatment, either alone or in combination with TTM. In 2 previous studies, GBC in triple dosage (33 µg/kg) of that used here did not induce a sharp reduction of serum glucose either, even when applied under hypothermia.[37,38] Therefore, the findings from our present and previous studies may have demonstrated the effectiveness and safety of GBC in different degrees of ACA/CPR. Although the SUR1-TRPM4 channel was supposed to be the therapeutic target of GBC, as stated above, our experiments did not prove a causal relationship here, which is the key limitation of this study.

Conclusion

GBC is comparable to TTM in improving survival and neurological outcome in a rat model of 10-minute ACA/CPR. When GBC is applied along with TTM, a trend toward better effects in ameliorating neuronal injury and activation of microglia and astrocytes is observed. As an FDA-approved medication, GBC merits further exploration in ACA for clinical translation.

Sources of Funding

Disclosures

None.

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