Safety and efficacy of glibenclamide combined with rtPA in acute cerebral ischemia with occlusion/stenosis of anterior circulation (SE-GRACE): a randomized, double-blind, placebo-controlled trial

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eClinicalMedicine 2023;65: 102305
Published Online xxx
https://doi.org/10.1016/j.eclinm.2023.102305

Summary

Background Glibenclamide alleviates brain edema and improves neurological outcomes in experimental models of stroke. We aimed to assess whether glibenclamide improves functional outcomes in patients with acute ischemic stroke treated with recombinant tissue plasminogen activator (rtPA).

Methods In this randomized, double-blind, placebo-controlled trial, patients with acute ischemic stroke were recruited to eight academic hospitals in China. Patients were eligible if they were aged 18–74 years, presented with a symptomatic anterior circulation occlusion with a deficit on the NIHSS of 4–25, and had been treated with rtPA within 4.5 h of symptom onset. We used web-based randomization (1:1) to allocate eligible participants to the glibenclamide or placebo group, stratified according to endovascular treatment and baseline stroke severity. Glibenclamide or placebo was taken orally or via tube feeding at a loading dose of 1.25 mg within 10 h after symptom onset, followed by 0.625 mg every 8 h for 5 days. The primary outcome was the proportion of patients with good outcomes (modified Rankin Scale of 0–2) at 90 days, assessed in all randomly assigned patients who had been correctly diagnosed and had begun study medication. The study is registered with ClinicalTrials.gov, NCT03284463, and is closed to new participants.

Findings Between January 1, 2018, and May 28, 2022, 305 patients were randomly assigned, of whom 272 (142 received glibenclamide and 130 received placebo) were included in the primary efficacy analysis. 103 (73%) patients in the glibenclamide group and 94 (72%) in the placebo group had a good outcome (adjusted risk difference 0.002, 95% CI −0.098 to 0.103; p = 0.96). 12 (8%) patients allocated to glibenclamide and seven (5%) patients allocated to placebo died from any cause at 90 days (p = 0.35). The number and type of adverse events were similar between the two groups. There were no drug-related adverse events and no drug-related deaths.

Interpretation The addition of glibenclamide to thrombolytic therapy did not increase the proportion of patients who achieved good outcomes after stroke compared with placebo, but it did not lead to any safety concerns.

Funding Southern Medical University and Nanfang Hospital.

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Keywords: Acute ischemic stroke; Recombinant tissue plasminogen activator; Glibenclamide; Brain edema; Neuroinflammation

Research in context

Evidence before this study

Whether glibenclamide improves functional outcomes in patients with acute ischemic stroke remains controversial. We searched PubMed for randomized controlled trials published in English between January 1, 2010, and July 1, 2023, using the search terms "stroke" AND either "glyburide" OR "glibenclamide" OR "sulfonylurea". The GAMES-RP Trial is the only trial found to evaluate the benefit of glibenclamide for patients with acute ischemic stroke and at risk of malignant brain edema. However, this trial used an intravenous form of glibenclamide and included a large proportion of patients who had not received reperfusion therapy such as alteplase thrombolysis or endovascular therapy.

Added value of this study

To the best of our knowledge, the SE-GRACE trial is the first randomized, double-blind, a placebo-controlled trial designed to evaluate the safety and efficacy of oral glibenclamide for patients with acute anterior circulation ischemic stroke receiving intravenous thrombolysis with rtPA. Treatment was well tolerated, hypoglycemia was uncommon. Although the percentage of people who had a modified Rankin Scale score of 0–2 at 90 days was not significantly different in the glibenclamide and placebo groups, the glibenclamide group had lower levels of MMP-9 among those who had their serum tested.

Implications of all the available evidence

Our findings do not support routine use of oral glibenclamide in patients with acute anterior circulation ischemic stroke, at least not in a non-selective population.

Introduction

Intravenous thrombolysis using recombinant tissue plasminogen activator (rtPA) increases the likelihood of early reperfusion for patients with acute ischemic stroke.[1] However, due to the restricted time window, the relatively low rate of complete early recanalization in proximal occlusion, and the noteworthy incidence of hemorrhagic transformation, the number of patients benefiting from rtPA thrombolytic therapy is limited.[1] Recent advance in endovascular therapy has greatly increased the likelihood of early recanalization and thus improved functional outcomes, but about 56% of patients are still unable to live independently after endovascular treatment.[2] Secondary brain injury, including brain edema and neuroinflammation, may be the main cause of poor prognosis after recanalization therapy.[3,4] Therefore, adjunct treatment targeting brain edema and neuroinflammation may help improve the overall efficacy of rtPA thrombolysis and endovascular treatment.

Glibenclamide (US-adopted name, glyburide) is a long-acting sulfonylurea that has been used safely for decades to treat type 2 diabetes. Over the past 20 years, glibenclamide has been repeatedly shown to prevent brain edema and neuroinflammation and improve neurological outcomes in different animal models of stroke, presumedly by blocking a *de novo* synthesis ion channel called sulfonylurea receptor 1-transient receptor potential M4 (SUR1-TRPM4).[5–7] In a clinically relevant rodent stroke model treated by rtPA, glibenclamide was also effective and its therapeutic window was 10 h after ischemia.[8] A well-designed trial in patients with large anterior circulation infarction has presented a protective role of intravenous glibenclamide in alleviating brain edema and improving functional outcomes in patients aged ≤70 years.[9,10] For oral glibenclamide, two retrospective studies show that type 2 diabetics who take sulfonylureas such as glibenclamide before a stroke and during hospitalization tend to have better functional outcomes and fewer hemorrhagic conversions.[11,12] Our exploratory study showed that oral glibenclamide was well tolerated in patients with acute ischemic stroke.[13] These findings lend support to the scrutiny of the coadministration of glibenclamide and rtPA thrombolytic therapy in patients with acute ischemic stroke.

The SE-GRACE trial was set up to evaluate whether oral glibenclamide would safely improve functional outcomes at 90 days in patients receiving rtPA for acute ischemic stroke of anterior circulation.

Methods

Study design and participants

SE-GRACE is an investigator-initiated, randomized, double-blind, placebo-controlled, 1:1 parallel-group trial conducted at 8 academic hospitals in China (eFig. 1). The study protocol of SE-GRACE has been previously reported (Appendix).[14] This trial has been approved by the Medical Ethics Committee of Nanfang Hospital and all participating institutions have obtained Local Ethics

Committee approval. Written informed consent was obtained from all participants or their legally authorized representatives at enrolment. Due to the impact of the COVID-19 pandemic, the study's enrollment was postponed from September 2020 to May 2022.

Patients were eligible if they were aged 18–74 years, diagnosis of acute ischemic stroke for which they had received intravenous rtPA within the first 4.5 h of symptom onset, presented with a symptomatic anterior circulation occlusion with a deficit on the National Institute of Health Stroke Scale (NIHSS) of 4–25. At enrollment, the diagnosis of acute ischemic stroke of anterior circulation was based on clinical manifestations and cranial CT, and if indicated, brain MRI. Therefore, some patients with posterior circulation infarction were mistakenly included before an MRI examination or angiography was done. Once these subjects were confirmed as having an acute ischemic stroke of posterior circulation after being included, they stopped using the study drugs immediately but continued to receive other standard treatments and follow-ups. These participants were excluded from the modified intention-to-treat (mITT) population when performing statistical analysis. The key exclusion criteria included a previous ischemic stroke with significant disability exist (modified Rankin Scale [mRS] >1), blood glucose <3.0 mmol/L at enrollment, or a known history of severe heart disease. The full exclusion criteria are listed in the eTable 1. Site investigators were responsible for enrolling participants, obtaining informed consent and managing randomization.

Randomization and masking

Eligible participants were allocated in a 1:1 ratio to receive glibenclamide or placebo using a web-based randomization process. The randomization was stratified by center and the Pocock and Simon's minimization method was implemented to balance two important prognostic factors, endovascular treatment and baseline stroke severity (NIHSS ≥14 *vs* <14). Glibenclamide and placebo were prepared as tablets and packaged in plastic medical bottles. These bottles and tablets were visually identical, except for a unique bottle number, so that all trial personnel, patients, and outcome assessors were fully masked to treatment allocation.

Procedures

All eligible patients received full-dose intravenous alteplase (Actilyse;® 0.9 mg/kg; maximum dose, 90 mg; 10% administered as 1-min bolus, remaining infused over 1 h; Boehringer Ingelheim Co) before randomization.[15] Endovascular therapy was allowed for the study and interpretation of treatment guidelines was at the discretion of the treating team.[16] After randomization, the study drug was administered as soon as possible, with the target time of administration being less than 10 h from the onset of stroke.[9,14] The study drug (glibenclamide/placebo, 2.5 mg/tablet) was divided evenly with a pill splitter and given orally by trained hospital nurses. For the patients who were unable to ingest orally due to dysphagia or altered consciousness, the study drug was administered through a nasogastric tube. To rapidly achieve steady-state concentration, a loading dose of 1.25 mg was given, then 0.625 mg was administered every 8 h for 5 consecutive days. All participants received their study drugs at a scheduled time and took the medicine under the supervision of trained nurses. All participants were hospitalized for at least 5 days unless the patient strongly requested to be discharged or died. Patients who were required to be discharged within 5 days were no longer treated with the study drug but were included in the mITT population. The rationale of the dosing regimen is detailedly addressed in the eMethods of the Appendix.

All patients had standard assessments of demographic characteristics, medical history, laboratory values, and NIHSS scores at enrollment, and NIHSS scores were repeated at 24 h, 72 h, and 7 d. For participants who were discharged within 7 days, the examinations were performed on the day of discharge instead. Cranial CT examination was performed before treatment and repeated at 24 h, 72–96 h after treatment, and as clinically indicated. Two independent neuroradiologists masked to the clinical data reviewed the CT images, calculated the midline shift, and scored the hemorrhagic transformation.[17] The serum concentrations of matrix metalloproteinase-9 (MMP-9) were measured by ELISA with a commercially available kit (Human MMP-9 Quantikine ELISA, R&D Systems, Minneapolis, MN, USA).

Considering that glibenclamide may cause hypoglycemia, blood glucose levels were checked every 2 h during the first 24 h, followed by every 4 h in the absence of any decline below 3.9 mmol/L during the period of study drug administration. In the case of hypoglycemia, the blood glucose level was corrected by administration of glucose or glucagon, as appropriate. For safety reasons, the study drug was suspended when two consecutive events of hypoglycemia (<3.9 mmol/L) or one incidence of serious hypoglycemia (<2.2 mmol/L) occurred. Blood glucose and corrective glucose administration were recorded.

Participants were followed up 30 ± 7 days, 90 ± 14 days, 180 ± 30 days and 360 ± 30 days after the onset of stroke, by trained neurologists, to collect the scores of mRS, Barthel Index (BI), and Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE).[18] To ensure reliable scoring on mRS, a standardized questionnaire was used to assist the evaluation in person or through telephone review.[19] Details can be found in the study protocol (Appendix).[14]

Outcomes

The primary outcome was the proportion of good outcomes defined as an mRS score of 0–2 at 90 days.

Secondary outcomes included the ratio of NIHSS decreased ≥4 at 7 days, the ratio of parenchymal hemorrhagic transformation in cranial CT within 96 h, the ratio of midline shift ≥6 mm in cranial CT within 96 h, the mRS shift at 90 days, the ratio of BI of 60–100 points at 90 days, the proportion of IQCODE of ≤3.40 at 6 months and 1 year after the stroke onset, and the serum concentration of MMP-9 before treatment, 24, 48, and 72 h after treatment. Parenchymal hemorrhagic transformation (PH) included parenchymal hematoma type 1 and 2 of the Heidelberg classification.[17] Midline shift was measured in the axial section (craniocaudal direction, level of the Foramen of Monro; anteroposterior direction, level of maximum midline shift) and was defined as deviation from midline structures (eg, the septum pellucidum).[20]

Exploratory subgroup analyses were provided for the primary outcome measure, by sex (male *vs* female), age (≤60 years *vs* 61–74 years), endovascular treatment (yes *vs* no), NIHSS (4–13 *vs* 14–25), time window in rtPA thrombolysis (<3.5 h *vs* 3.5–4.5 h), and study drug treatment time (<6 h *vs* 6–10 h).

Safety outcomes included all-cause mortality at 90 days, the ratio of neurological deterioration (NIHSS increased ≥4) within 24 h, the incidence of hypoglycemia (random blood glucose <3.9 mmol/L), the incidence of cardiac events in cardiac examination (ECG, echocardiography), and the incidence of pulmonary infection. Cardiac events were defined as new-onset arrhythmias (atrial fibrillation, ventricular flutter, and ventricular fibrillation) and ischemic changes (including STEMI, NSTEMI, unstable angina) after enrollment. All of the above events, together with brain herniation, were considered adverse events, while all-cause death and brain herniation were defined as serious adverse events.

Statistical analysis

Our prespecified statistical analysis plan, power estimations, and futility interim analyses have been published previously.[14] The sample size calculation was based on previous findings, assuming an effect size of 17 percentage points (58% responders with glibenclamide vs 41% with placebo).[12] Using nQuery + nTerim 4.0 (Statistical Solutions Ltd, Farmer's Cross, Cork, Ireland) with a 2-sided χ^2 test, a two-sided significance level of 0.05, a power of 80%, the O'Brien-Fleming alpha spending function (2 equally spaced looks with nominal alpha at interim as 0.003 and at the final look as 0.049) and a dropout rate of 10%, a total sample size of 306 was calculated (153 in each group).

The primary and secondary outcomes were analyzed based on the mITT population which excluded those patients who did not receive treatment or control and had no efficacy outcome data, and those subjects who were diagnosed with posterior circulation occlusion alone after being included. Patients with posterior circulation occlusion alone were excluded to maintain the homogeneity of the study population since the severity of disease and cerebral edema in these patients is quite different from that of patients with anterior circulation infarction. Per-protocol patients who finished the treatment protocol with good compliance were analyzed as a sensitivity analysis. Safety analyses were done in patients receiving allocated treatment. Since we have no missing data for the primary outcome in our mITT population, we didn't perform the last observation carried forward method and the multiple imputation method that planned to deal with the missing data of the primary outcome. A predefined interim analysis was conducted at September 21, 2020 and the Independent Data Safety Monitoring Board decided to proceed with the trial after reviewing the interim results (eResults; eTables 2–5; eFig. 2).

The primary outcome was compared between 2 groups using χ^2 tests, and the confidence interval (CI) of the risk difference was calculated using Newcombe's method.[21] Common risk differences stratified by endovascular treatment and baseline NIHSS (≥14 *vs* <14) were reported. The Mantel-Haenszel estimate, confidence limits, and test for the common risk differences by using Mantel-Haenszel stratum weights and the Sato variance estimator were used.

For baseline variables, comparisons between normally distributed continuous variables, expressed as mean ± SD, were performed using 2-sample t-tests; non-normally distributed continuous variables, presented as a median and interquartile range, were analyzed using Wilcoxon rank sum tests. Fisher's exact tests were used for categorical data expressed as percentages. For secondary outcomes, the binary outcomes were analyzed by using the same methods for the primary outcome, while the mRS shift was analyzed using an ordinal logistic regression model and a common odds ratio with 95% CI adjusted for endovascular treatment and baseline NIHSS was derived after validation of the proportional odds assumption. The stratified Wilcoxon score test with the Hodges-Lehmann estimation of location shift was also performed as a sensitivity analysis in case the proportional odds assumption was not met. MMP-9 data was analyzed by using a linear mixed effects model with data assessed at 24–72 h as a response value, treatment group, time (24 h interval), and baseline data as fixed effects without any interaction item while treating the subject as a random effect. The coefficient for the treatment represented the average difference in MMP-9 between groups adjusted for baseline data. The interaction effect of the treatment group and time were also evaluated by including their interaction item in the model. The average differences of all visit times were used to show the average effect, and the profile plots were used to show the difference at each visit. For safety analysis, all adverse events and serious adverse events, including deaths, were summarized and the frequencies from the two groups were reported. Fisher's exact tests

were used to compare the incidence of adverse events between the two groups.

All analyses were 2-tailed and a significance level of 0.05 was used for all outcomes. All data analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). The SE-GRACE study is registered at the clinicaltrials.gov (identifier NCT03284462).

Role of the funding source

The funders had no influence on the study design, the collection, management, analysis, and interpretation of data. The principal investigator (SP) and primary statistician (PC) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During January 1, 2018, and May 28, 2022, 305 patients were randomly assigned. Due to an error in the network response of the randomization system, a participant was assigned two random numbers and used the latter one. Of the 305 participants enrolled, 153 were allocated to the glibenclamide group and 152 to the placebo group. Seven participants (five in the placebo group and two in the glibenclamide group) withdrew their informed consent after randomization, and five participants (four in the placebo group and one in the glibenclamide group) missed their first medication within 10 h of the onset due to surgical procedures and changes in clinical condition after randomization. None of the above 12 participants ended up receiving the study drug and they were excluded from the mITT sample. Eight patients in the glibenclamide group and 13 in the placebo group were confirmed as posterior circulation occlusion alone, as determined by brain MRI, and were also excluded from the mITT sample. A total of 272 participants remained in the mITT analysis (Fig. 1). Demographics and baseline characteristics were similar in the two treatment groups (Table 1). In the mITT study population, the median age was 62 (IQR 54–69) years, the median NIHSS was 8 (IQR 5–12), and 47 (17%) participants received endovascular treatment. The median time from symptom onset to receiving rtPA treatment and study drug did not differ between the glibenclamide and placebo groups.

No patients were lost to follow-up at the primary endpoint. At 90 days, 103 (73%) of the 142 patients in the glibenclamide group had a good outcome, compared with 94 (72%) of the 130 patients in the placebo group (risk difference 0.002, 95% CI –0.103 to 0.108; adjusted risk difference 0.002, 95% CI –0.098 to 0.103; p = 0.96; Table 2). In the analysis of the mRS shift, the proportional odds assumption for the ordinal logistic regression model was not met (p < 0.001). Therefore, stratified Wilcoxon scores (p = 0.094) and Hodges-Lehmann estimation of location shift (0.00, 95% CI 0.00–1.00) were used for the mRS shift analysis, showing no significant difference between the glibenclamide and placebo groups (Fig. 2A; eResults). For participants whose serum MMP-9 concentration was measured, the glibenclamide group had lower mean MMP-9 concentrations at 24–72 h compared with the placebo group (106 ng/mL *vs* 126 ng/mL; mean difference –20.16, 95% CI –33.28 to –7.05; Fig. 2B; Table 2). However, there were no statistically significant differences between the glibenclamide and placebo groups in the other prespecified and exploratory secondary clinical endpoints (Table 2; eTable 6). All participants in the mITT set completed the treatment protocol, so the number of per-protocol patients and their outcomes were consistent with that of the mITT.

In the exploratory analysis of subgroups, including age, sex, endovascular treatment, baseline NIHSS, the time window in rtPA thrombolysis, and study drug treatment time, we did not note any statistically significant differences in the proportion of good outcomes between the glibenclamide and placebo groups (eFig. 3; eTable 7).

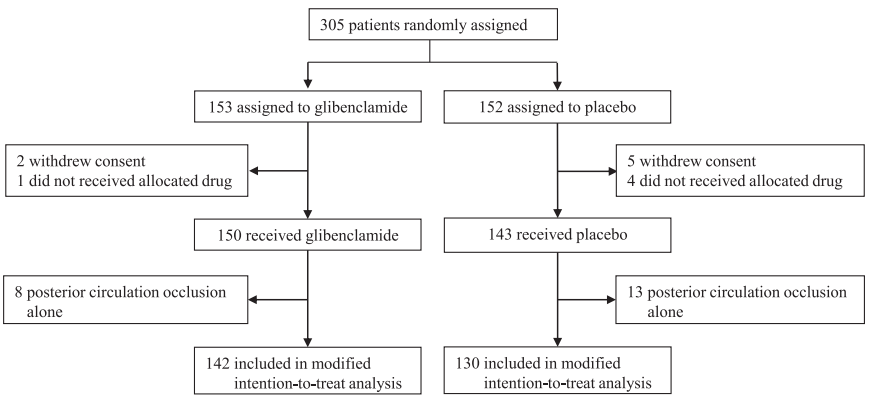

Fig. 1: Trial profile.

	Glibenclamide (n = 142)	Placebo (n = 130)
Demographics		
Age (years)	61 (11)	61 (12)
Female	39 (27%)	39 (30%)
Medical history		
Hypertension	85 (60%)	66 (51%)
Type 2 diabetes	20 (14%)	19 (15%)
Ischemic stroke or transient ischemic attack	19 (13%)	22 (17%)
Atrial fibrillation	13 (9%)	11 (8%)
Stroke characteristics		
Cause of stroke		
Large artery atherosclerosis	68 (48%)	66 (51%)
Cardio-aortic embolism	29 (20%)	23 (18%)
Small artery	37 (26%)	35 (27%)
Other	3 (2%)	2 (2%)
Unknown	5 (4%)	4 (3%)
Baseline NIHSS	9 (5–12)	8 (5–11)
NIHSS ≥14	25 (18%)	20 (15%)
Baseline blood glucose (mmol/L)	6.4 (5.7–7.7)	6.5 (5.5–7.9)
Treatment		
Endovascular treatment	26 (18%)	21 (16%)
Time intervals		
Symptom onset to intravenous rtPA (h)	2.8 (2.1–3.7)	2.6 (1.8–3.5)
Symptom onset to baseline CT (h)	2.4 (1.5–3.3)	2.2 (1.4–3.2)
Symptom onset to endovascular treatment (h)	4.0 (3.1–6.2)	4.8 (3.1–6.2)
Symptom onset to study drug treatment (h)	6.2 (4.5–8.9)	6.2 (4.7–8.4)

Data are mean (SD), n (%), and median (IQR). NIHSS, National Institutes of Health Stroke Scale Score; rtPA, recombinant tissue plasminogen activator.

Table 1: **Demographics and baseline characteristics.**

As shown in Table 3 and eTable 8, after enrollment, 12 (8%) patients in the glibenclamide group died within 90 days, compared with seven (5%) in the placebo group. Of them, three (two in the glibenclamide group and one in the placebo group) were considered cardiac death. There was no statistically significant difference in the ratio of neurological deterioration (NIHSS increased ≥4) within 24 h between the glibenclamide and placebo groups (12 [8%] *vs* 9 [6%]). Ten (7%) episodes of hypoglycemia (glucose level <3.9 mmol/L) occurred in the glibenclamide group, compared with 16 (11%) in the placebo group. Besides, the incidence of cardiac events, pulmonary infection, any AE, and any SAE were similar between the glibenclamide and placebo groups.

Discussion

In the SE-GRACE trial, glibenclamide did not improve functional outcomes at 90 days among individuals presented with symptomatic anterior circulation occlusion/stenosis and treated with intravenous rtPA. This finding was consistent across all prespecified subgroups. However, the results indicated the safety of oral glibenclamide for patients with acute ischemic stroke.

Two retrospective studies found an association between sulfonylureas and better functional outcomes or fewer hemorrhage transformation.[11,12] However, these retrospective studies used other types of sulfonylureas besides glibenclamide, such as glimepiride and glibornuride, with different doses and courses of treatment, making these results difficult to extrapolate. Therefore, we conducted the SE-GRACE trial, which, to our knowledge, is the first randomized controlled trial specifically designed to evaluate the safety and efficacy of oral glibenclamide for patients with acute anterior circulation ischemic stroke receiving intravenous thrombolysis with rtPA. The results of SE-GRACE are in line with a retrospective study that sulfonylurea pretreatment and in-hospital use do not impact acute ischemic stroke outcomes following intravenous thrombolysis.[22] Another retrospective study using data from a large prospective cohort of patients with non-reperfusion ischemic stroke combined with diabetes mellitus also suggests that sulfonylurea use before stroke has a neutral effect on 90-day functional outcomes.[23]

The findings of SE-GRACE also partly agree with a previous randomized controlled trial of individuals

	Glibenclamide (n = 142)	Placebo (n = 130)	p value	Effect size[c] (95% CI)
Primary outcome[a]				
mRS 0–2 at 90 days	103 (73%)	94 (72%)	0.96	RD 0.002 (−0.098 to 0.103)
Secondary outcomes				
NIHSS decreased ≥4 at 7 days compared with baseline	69 (51%)	64 (52%)	0.88	RD −0.010 (−0.131 to 0.112)
Parenchymal hemorrhagic transformation in cranial CT within 96 h	17 (12%)	13 (10%)	0.47	RD 0.026 (−0.042 to 0.094)
Midline shift ≥6 mm in cranial CT within 96 h	8 (6%)	4 (3%)	0.21	RD 0.030 (−0.015 to 0.075)
Barthel Index of 60–100 at 90 days	117 (86%)	106 (84%)	0.68	RD 0.018 (−0.066 to 0.101)
IQCODE of ≤3.40 at 6 months	107 (79%)	90 (73%)	0.26	RD 0.059 (−0.042 to 0.159)
IQCODE of ≤3.40 at 1 year	104 (77%)	88 (73%)	0.41	RD 0.043 (−0.060 to 0.146)
Mean MMP-9 (ng/mL) during 24–72 h[b]	105.9 (96.6–115.3)	126.1 (116.9–135.3)	0.003	MD −20.16 (−33.28 to −7.05)

Data are n (%) except MMP-9. mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale Score; IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly; MMP-9, matrix metalloproteinase-9; RD, risk difference. MD, mean difference. [a]No missing data. [b]MMP-9 data collected at 24 h, 48 h, and 72 h was averaged for each patient in the mITT and are presented as mean (95% CI). [c]Data were adjusted for endovascular treatment and baseline NIHSS (≥14 or <14).

Table 2: **Efficacy outcome measures (mITT).**

treated with intravenous glibenclamide (GAMES-RP trial), with similarly neutral results of functional outcomes. The GAMES-RP trial, which enrolled patients with large hemispheric infarction for less than 10 h and baseline diffusion-weighted MRI image lesion volume of 82–300 cm^3 on MRI, found a non-significant reduction in mortality and reduced brain swelling, but no significant difference in functional outcome.[9] Nevertheless, it should be noted that our findings cannot be directly compared with those of the GAMES-RP trial because of substantial differences in the dosage of glibenclamide, route of administration (oral *vs* intravenous), and severity of the study population.

Several explanations for the findings showing no efficacy of oral glibenclamide might be discussed. First, the patients included in this study had relatively mild disease severity, the median baseline NIHSS score was only 8 (5–12), and the proportion of small-vessel

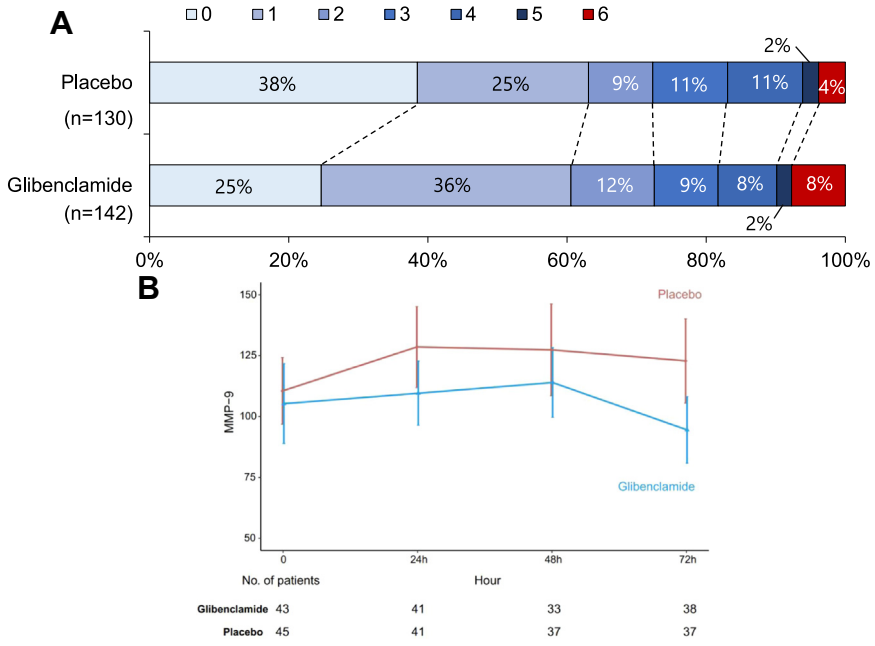

Fig. 2: **Secondary clinical outcomes in the glibenclamide and placebo groups**. (A) Distribution of modified Rankin Scale scores in the mITT population at 90 days (Table 2). (B) Mean total plasma matrix metalloproteinase-9 (MMP-9) over time for the mITT sample. The error bars are 95% confidence intervals at baseline and 24 h, 48 h, and 72 h from start of study drug. The number of patients measured MMP-9 at each timepoint is listed.

	Glibenclamide (n = 150)	Placebo (n = 143)	p value[c]
All-cause death at 90 days	12 (8%)	7 (5%)	0.35
The ratio of neurological deterioration (NIHSS increased ≥4) within 24 h	12 (8%)	9 (6%)	0.65
The incidence of hypoglycemia (random blood glucose <3.9 mmol/L)	10 (7%)	16 (11%)	0.22
Cardiac death	2 (1%)	1 (1%)	1.00
Cardiac events[a]	18 (12%)	15 (11%)	0.72
The incidence of pulmonary infection[b]	28 (19%)	29 (20%)	0.77
Any AE	51 (37%)	46 (32%)	0.80
Any SAE	13 (9%)	8 (6%)	0.37

Data are n (%). NIHSS, National Institutes of Health Stroke Scale Score; AE, adverse event; SAE, severe adverse event. [a]Cardiac events include new-onset arrhythmias and ischemic changes on EEG after enrollment. [b]New-onset pulmonary infection within one week after enrollment. [c]Fisher's exact test.

Table 3: **Safety outcomes.**

occlusion was large, leading to a low probability of severe cerebral edema in the study population, while one of the main mechanisms by which glibenclamide takes effect is to prevent cerebral edema. In addition, the inclusion of too many patients with mild stroke may make the clinical benefit of glibenclamide difficult to detect.[24] As implied from the secondary analysis of the GAMES-RP trial, glibenclamide may be more effective in patients with malignant edema.[25] In fact, our data showed that glibenclamide significantly reduced the levels of MMP-9, an indicator of blood-brain barrier destruction and brain edema, suggesting that glibenclamide has the potential in alleviating brain edema.[9] However, MMP-9 was only tested in about 30% of the participants (non-random) who were willing to take blood, so the results should be interpreted with caution.

Second, we used a relatively lower dose regimen of glibenclamide compared with that of the GAMES-RP trial and a recent trial of oral glibenclamide therapy for intracerebral hemorrhage (GATE-ICH, 1.25 mg tid).[9,26] The dose regimen of SE-GRACE has been found to be well tolerated in patients with acute ischemic stroke in our preliminary study, without an increased risk of hypoglycemia and early neurological deterioration.[13] In addition, our unpublished data has shown that this dose regimen was able to result in average steady-state plasma glibenclamide levels of 19 ng/mL, which was not inferior to the concentrations reached by the effective therapeutic dose in rats (16 ng/mL).[27] Unlike diabetics, non-diabetics have a higher risk of hypoglycemia even with lower doses of glibenclamide than diabetes treatment,[9,26] which may impair or even counteract the neuroprotective effects of glibenclamide.[28] SE-GRACE did not find an increased risk of hypoglycemia in the glibenclamide group, suggesting that this dosing regimen may be safer. Nevertheless, the neutral results of this trial suggest that further studies might consider a higher dose of glibenclamide to produce a stronger effect. It should be noted that, given the potential hypoglycemic risk of glibenclamide, we strictly required all subjects to initiate enteral nutrition support while receiving the study drugs, so the low incidence of hypoglycemia in this study may also be related to this factor. Since the peak of cerebral edema usually occurs 2–3 days after the onset of the disease, it should be reasonable to choose 5 days of administration in this study.

Third, although we aimed to evaluate glibenclamide in patients undergoing reperfusion therapy by intravenous rtPA and endovascular therapy, the proportion of endovascular therapy was low and there was a lack of adequate assessment of the rate of vascular recanalization after thrombolytic therapy, which is a key factor in determining whether the drug has an adequate cytoprotective effect.[28] Recanalization therapy may conflict with the evaluation of neuroprotective agents. Successful recanalization reduces injury cascades such as brain edema and inflammation, making the effects of neuroprotective agents such as glibenclamide hard to detect, while the failure of recanalization prevents glibenclamide from fully reaching the target to take action. Designing a study that considers both recanalization and the severity of brain damage immediately after recanalization may be beneficial to adequately evaluate the neuroprotective effects of the study drug, but this would be challenging.

Our trial has several strengths. This is a randomized, double-blind, placebo-controlled study, and the primary endpoint (mRS) was obtained by rigorously trained staff using a standardized questionnaire. No patients were lost to follow-up at the primary endpoint. Besides, our study endpoints included clinical, neuroimaging, and biomarkers, and covered both the acute and chronic phases. Finally, this trial was conducted in patients who had received reperfusion therapy so that glibenclamide had a higher chance to reach the area of ischemic injury.[24]

Our study has limitations. First, the site investigators preferred to include patients with mild to moderate neurological impairment, resulting in a low proportion of patients with NIHSS scores ≥14 and a high proportion of good outcomes, which may

underestimate the efficacy of glibenclamide. Second, we used pre-thrombolytic NIHSS rather than post-thrombolytic NIHSS as a screening criterion, so the influence of thrombolytic efficacy cannot be ruled out, which may be an important confounding factor. Third, although this study allowed subjects to receive endovascular therapy when they were eligible, the proportion of these subjects was so small that it is impossible to know whether glibenclamide is effective in patients who are also receiving adequate recanalization. Fourth, our definition of mITT and the per-protocol estimates may bring bias.

In summary, oral glibenclamide did not increase the absolute rate of a favorable outcome at 90 days in patients with acute ischemic stroke and treated with rtPA, although it was safe and led to reduced concentrations of MMP-9, a biomarker of brain edema. These findings do not support the routine use of oral glibenclamide in patients with acute anterior circulation ischemic stroke, at least not in a non-selective population. Further studies aiming to evaluate the cytoprotection of glibenclamide might consider higher dose regimens and advanced brain imaging to select patients who are most likely to benefit from cytoprotective treatment.

Contributors

SP is the principal investigator of the SE-GRACE trial. SP, KH, YW, and ZJ conceived the study. SP, KH, YW, ZJ, JH, and PC designed the trial protocol and supervised the trial conduct. KH, XLZ, YZ, GY, and SZ collected the data and wrote the original draft with no external writing assistance. SP, YM, ZJ, and JH critically reviewed the manuscript and decided to submit the manuscript. ZY, WH, GW, ZL, SW, XL, YH, JSZ, XZ, HL, SY, YG, MZ, WC, WQ, NL, QC, and YC were investigators who contributed to the enrolment of patients, data collection, and integrity of the data at each site. All authors had access to all the data in the study. ZJ and YW reviewed the CT images, calculated the midline shift, and scored the hemorrhagic transformation. JH verified the data. CD performed statistical analysis and interpretation of the data. All authors have approved the final version of the manuscript to be published.

Data sharing statement

The authors are committed to responsible data sharing regarding this clinical trial, including access to anonymized, individual-level, and trial-level data (analysis datasets). The clinical trial data, study protocol, and statistical analysis plan can be requested from the corresponding author, by any qualified researchers who engage in rigorous independent scientific research. All data will be available from the time of publication, with no end date.

Declaration of interests

The authors declare no conflict of interest.

Acknowledgements

This trial was supported by the Initiative Project of Clinical Research of Southern Medical University (LC2016ZD001) and the Clinical Research Program of Nanfang Hospital, Southern Medical University (2018CR025).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102305.

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