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Effectiveness and safety of Glibenclamide for Stroke: Protocol for a systematic review and meta-analysis

Lihong Wen[1], Bin Huang[1], Rong Tu[2],Kunzhen Wan[3], Hong Zhang[4],Xiaoyun Zhang[5]

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ABSTRACT

Introduction: Despite the continuous improvement in modern medical treatment, stroke is still a leading cause of death and disability worldwide. How to effectively improve the survival rate and reduce disability in patients with stroke has become the focus of many investigations. Recent findings concerning the benefits of glibenclamide as a neuroprotective drug have initiated a new area for prospective studies on the effects of sulfonylureas. Given the high mortality and disability associated with stroke, it is essential to weigh the benefits of neuroprotective drugs against their safety. Therefore, the objective of the current study is to conduct a systematic review using meta-analysis to assess the benefits and safety of glibenclamide as a neuroprotective drug.

Methods and analysis: This study will analyze randomized clinical trials (RCTs) and observational studies published up to December 31, 2020, and include direct or indirect evidence. Studies will be retrieved by searching PubMed, EMBASE, Web of Science, the Cochrane Library, as well as CNKI and WanFang Databases. The outcomes of this study will be mortality, scores from the modified Rankin scale (mRS), and the occurrence of hypoglycemic events. The risk of bias will be assessed using the Cochrane Risk of Bias assessment instrument for RCTs. A random-effect/fixed-effect model will be used to summarize the estimates of the mean difference (MD)/risk ratio (RR) using a 95% confidence interval (CI).

Dissemination: The results of this study will be submitted to a peer-reviewed journal for publication.

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PROSPERO registration number CRD42020144674.

Strengths and limitations of this study

► This is the first systematic review and meta-analysis to analyze the benefits and safety of glibenclamide in stroke patients based on data from both randomized clinical trials (RCTs) and observational studies.

►As is common with most meta-analyses, significant and unexplained heterogeneity may exist.

► The risk for ecological fallacy exists in this study as for any aggregate data meta-analysis.

INTRODUCTION

Rationale

Stroke is a common disease with high mortality and morbidity worldwide, and often leaves survivors with severe neurological impairments and long term disability[1-2]. According to data from The Third Cause of Death Survey in China, stroke is the leading cause of mortality and disability in adults in China. The stroke burden in China currently exhibits an explosive growth trend, which is characterized by rapid increases in stroke in low-income and younger individuals[3]. At present, the average incidence for the first stroke for residents in China aged 40 to 74 years has increased by an annual rate of 8.3%. The prevalence of stroke in adults in China aged 40 years or older has increased from 1.89% in 2012 to 2.19% in 2016, and it is estimated that 1.96 million people die due to the consequences of stroke every year[4]. Better prevention and treatment of stroke still face enormous challenges, and the medical system needs further improvement and optimization. Therapies targeting the underlying pathophysiology of central nervous system (CNS) ischemia and hemorrhage are conspicuously lacking. Several neuroprotective agents have been studied, but their clinical efficacy has been unsatisfactory.

Recent findings concerning the benefits of glibenclamide as a neuroprotective drug have initiated a number of new prospective studies[5]. Glibenclamide is a member of the sulfonylurea class of drugs and has been used in the clinic as an oral hypoglycemic agent[6].

It exerts its pleiotropic protective effects on acute central nervous system (CNS) injury by inhibiting the recently characterized Sur1-Trpm4 channel (formerly, the Sur1-regulated nonselective cation (NCCa-ATP) channel). Glibenclamide improves functional neurological outcomes in stroke models by protecting the

microvascular endothelium, reducing edema formation and secondary hemorrhage, inhibiting neuronal cell death, maintaining the integrity of the blood-brain-barrier (BBB), and promoting neurogenesis by blocking the Sur1-Trpm4 channel[7-8]. Thus, glibenclamide has received renewed attention as a CNS treatment for ischemic hemorrhagic stroke and subarachnoid hemorrhage[9-13]. When studied in models of ischemic stroke, glibenclamide significantly reduced the mortality rate to 5%, whereas the vehicle-treated group exhibited 67% mortality at 24 h. Compared to a decompressive craniectomy (DC) group, glibenclamide significantly improved neurological function[14-16].

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Objective

The primary objective of this study is to conduct a systematic review and meta-analysis of randomized clinical trials and observational studies to assess the safety and efficacy of glibenclamide as a component of the medical treatment for patients with stroke and develop supporting evidence for effective clinical strategies.

METHODS

Study registration

This protocol is being conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[20] or meta-analyses of healthcare interventions. The protocol report for this study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols[21]. This protocol has been registered in the PROSPERO network (registration number: CRD42020144674).

Eligibility criteria

Studies that meet the following criteria will be included in the analysis: (1) Randomized trials (RCTs) and observational studies; (2) patients diagnosed with stroke using computed tomography (CT) and magnetic resonance imaging (MRI), including ischemic, hemorrhagic stroke, and subarachnoid hemorrhage; (3) an intervention group will be treated with intravenous or oral glibenclamide; (4) a comparison control group will be included in the randomized trials, which will consist of a placebo, blank, or others; (5) data concerning mortality and other major adverse events will be included; (6) studies to be included will be published up to December 31, 2020.

Direct comparisons will be made from the identified studies comparing a placebo or other control compounds. Both RCTs and observational studies will be included because it has been suggested recently that RCTs and observational studies should not be analyzed in isolation[22].

Information sources

The following databases will be searched from their inception forward for potentially eligible studies without language restrictions and published up to December 31, 2020: (1) PubMed; (2) Scopus; (3) Web of Science; (4) Cochrane Central Register of Controlled Clinical Trials; (5) CNKI and WanFang Databases.

Search strategy

Search strategies will be adapted from previous research[23-24] and will be developed using text words and medical subject headings. Electronic databases will be searched for studies on the effects and safety of glibenclamide in adults with stroke. The first author will conduct all database searches without language restrictions. The search strategy for all other databases will be adapted based on the requirements of each database.

Study records

Study selection

All studies will be extracted from electronic databases using the search strategy described above and imported into EndNote X7 software (Thomson Reuters, Canada). Duplicate studies will be removed. Two authors will select studies independent of each other. Complete articles will be retrieved for all titles and abstracts that appear to meet the inclusion criteria or where any uncertainty exists. The two reviewers will list all the studies to be included and document the primary reasons for excluding studies that do not conform to the inclusion criteria. Disagreements between the two authors will be resolved by discussing with the third author, and if necessary, consulting with the fourth author. The overall agreement rate prior to correcting for discrepancies will be calculated using Cohen's kappa (κ) statistics. A flow diagram will be constructed that depicts the search process. An online supplementary file containing a reference list of all excluded studies, including the reason(s) for exclusion, will be included in the study. We will show the details of the selection process in the PRISMA flow chart. The proposed structure for the flow diagram is shown in Figure 1[25].

Data acquisition

Before initiating data acquisition, a codebook will be developed in Microsoft Excel 2013. Two independent researchers will extract data. The following data will be extracted from each eligible study using a standardized data collection form: (1) Study characteristics, including publication year, author, country of the study, type of study (RCT, cohort, case-control, and others), sample size, follow-up duration, and others; (2) participant characteristics, including age, sex, stroke type (ischemic, hemorrhagic stroke, or subarachnoid hemorrhage), and baseline condition of participants (e.g., disease severity and others); (3) intervention characteristics, including name of the drug(s), dose, route of administration, and others; (4) control characteristics, including the type of drug(s) used, dose, route of administration, and others; and (5) outcome data for mortality, modified Rankin scale (mRS), and occurrence of hypoglycemic events. The first two authors will acquire the data from the selected studies, independent of each other, using the codebook in Microsoft Excel. After data acquisition, both authors will review the codebooks and resolve discrepancies by consensus. If a consensus cannot be reached, the third author will provide a recommendation. A complete list of covariates that we will include is shown in Table 1.

Outcomes

The outcomes will include mortality, modified Rankin scale (mRS), and the occurrence of hypoglycemic events.

Assessment of the quality of the evidence

According to the Grading of Recommendations Assessment Development and Evaluation (GRADE), the quality of included studies will be assessed using the online guideline development tool http://gdt.guideline-development.org/), and divided into four levels of quality: high, moderate, low, and very low[26].

Risk of bias assessment in individual studies

Risk of bias for RCTs will be assessed using the Cochrane Risk of Bias instrument[27], which contains seven specific domains, random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other biases. This instrument assesses the methodological quality of the RCTs concerning low risk, high risk, or unclear risk of bias[28]. If any domain is scored high/low risk of bias, the study will be considered high/low risk of bias. No study will be excluded based on the results of the risk of bias assessment[29]. The first two authors will conduct all risk of bias assessments independent of each other. Then the two authors will review the results for the risk of bias assessment and resolve any discrepancies by consensus. If a consensus cannot be reached, the third author will be consulted.

The Nine-item Newcastle–Ottawa Quality scale is widely used to assess the quality of non-randomized trials using risk evaluation for the adequacy of selection, comparability, and outcomes assessment[30]. The high-quality studies will be defined as studies with a score that is greater than or equal to six.

Data synthesis and analysis

All analyses will be conducted using the natural log of OR and transformed back to ORs for presentation. If an OR is not reported, it will be calculated from the data reported in the study. If data are not available to calculate OR, it will be requested from the study authors. Secondary outcomes will be calculated using the same procedure used for our primary outcome.

The measures of the effects of interest will be the risk ratio (RR)/mean difference (MD) with 95% confidence intervals (CI). A subgroup analysis will be performed that is stratified by patients based on the location of the hemorrhage. We will use the Cochrane Chi-Square test (Q test) and the I^2 test to evaluate the level of heterogeneity across studies[31-32]. When significant heterogeneity ($P<0.05$ or $I^2>50\%$) is detected, we will pool the data using a random-effect model. Otherwise, a fixed-effect model will be used. We will explore the source of heterogeneity using sensitivity analysis. Funnel plots will be visually inspected to identify any potential publication bias. All statistical analyses will be performed using Stata software (version 14.0; Stata Corp., College Station, TX, USA).

If there is considerable heterogeneity, and the data are sufficient, subgroup analyses will be conducted to identify potential causes for the heterogeneity. Subgroup analyses will be performed based on the type of stroke, including ischemic stroke, hemorrhagic stroke, or subarachnoid hemorrhage.

Sensitivity analysis

We will conduct sensitivity analyses of the primary results to explore the robustness of the review conclusions if feasible, after considering the impact of methodological quality, missing data, and sample size. According to the Cochrane Handbook, when a sufficient number of original studies are included (generally more than ten trials), publication bias analysis will be performed using a funnel plot. A symmetrical funnel plot indicates low publication bias. If the funnel plot is asymmetric, that will be an indication of high risk for publication bias.

Software used for data analysis

All data will be analyzed using Stata/IC for Mac V.14.0.

REGISTRATION

In accordance with the Primary Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P), our systematic review with network meta-analysis was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on April 28, 2020 (registration number: CRD42020144674).

Contributors

WLH and HB drafted the manuscript. TR and WKZ contributed to the development of the data sources to search for relevant literature, including the search strategy, selection criteria, data extraction criteria, and risk of bias assessment strategy. WLH provided statistical expertise, and ZXY, BH, and ZH provided content expertise on glibenclamide and stroke. All four authors read, provided feedback, and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for data publication

All participants consented to data publication. The privacy for all individuals has been preserved.

Ethics issues

This meta-analysis is a secondary research project that is based on previously published data. Therefore, ethical approval or informed consent was not required for this meta-analysis.

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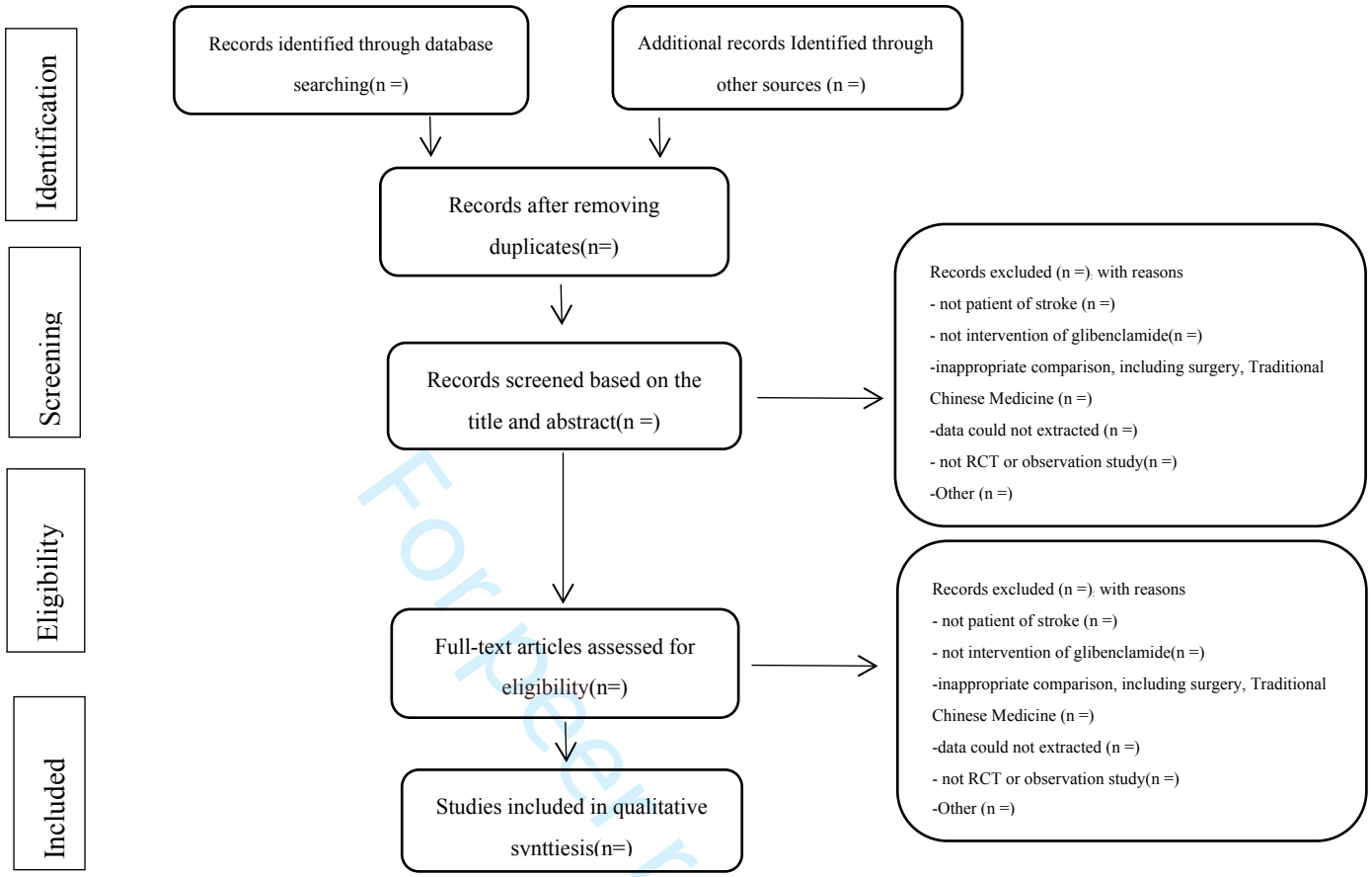
Table1 Covariates that will be included in the study

Studies characteristics	publication year, author, country of the study, type of study (RCT, cohort, case–control, etc), sample size, follow-up duration,etc).
Participant characteristics	Age, sex, stroke type(ischemic ,hemorrhagic stroke or subarachnoid hemorrhage ） , baseline condition of participants (eg, disease severity), etc)
intervention characteristics	name of the drug,dose, route of administration, etc);
control characteristics	type of the drug,dose, route of administration, etc);
Outcome	mortality,modified Rankin scale (mRS) and occurrence of hypoglycemia events events.

Figure1 Flow diagram of study selection process.



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microvascular endothelium, reducing edema formation and secondary hemorrhage, inhibiting neuronal cell death, maintaining the integrity of the blood-brain-barrier (BBB), and promoting neurogenesis by blocking the Sur1-Trpm4 channel[7-8]. Thus, glibenclamide has received renewed attention as a CNS treatment for ischemic hemorrhagic stroke and subarachnoid hemorrhage[9-13]. When studied in models of ischemic stroke, glibenclamide significantly reduced the mortality rate to 5%, whereas the vehicle-treated group exhibited 67% mortality at 24 h. Compared to a decompressive craniectomy (DC) group, glibenclamide significantly improved neurological function[14-16].

Glibenclamide scavenges free radicals, reduces activated-caspase-3 expression, increases the Bcl-2/Bax ratio, and inhibits apoptosis by blocking NC channels to improve functional neurological outcomes in a rat model of intracranial hemorrhage(ICH)[17-18].Also,glibenclamide can significantly reduce BBB permeability, markers of cell injury or cell death, protect the normal junctional localization of ZO-1, as well as reduce inflammation and markers of inflammation, vasogenic edema, and caspase-3 activation to improve functional neurological outcomes after subarachnoid hemorrhage[19].

Several retrospective studies suggest that taking a sulfonylurea drug and continuing it following an ischemic CNS insult significantly improves outcomes, including survival, greater functional independence, lower NIH stroke scale scores, and less hemorrhagic transformation[20-21]. Administration of a sulfonylurea drug also improved long-term cognitive function in clinically relevant models of subarachnoid hemorrhage[22]. A prospective study suggested that intravenous glibenclamide (GBC) reduced water accumulation and mass effects after large hemispheric infarctions[23]. Another prospective study suggested that oral GBC is safe in treating acute hemispheric infarctions and potentially could prevent brain edema and subsequent severe disability and death. Two studies [24-25]have suggested that GBC reduced edema, protected blood-brain barrier (BBB) integrity, and improved long-term neurological deficits. Another study reported that GBC reduced oxidative stress, inhibited apoptosis, and improved neurological deficits[26]. However, a recent study tested a widely-used GBC dose shown to be effective in other studies (10 μg/kg loading dose followed by 200 ng/hr for up to seven days), and the result suggested that recovery from neurological impairments was not improved by GBC and also did not improve ICH outcomes[27]. Ghasami et al. compared the use of GBC and insulin when given to diabetic hemorrhagic stroke patients and reported that GBC had no benefit compared to the insulin group[28]. However, we note that this was a small, non-randomized, non-placebo-controlled trial. Thus, further clinical work in hemorrhage is needed.

Objective

The primary objective of this study is to conduct a systematic review and meta-analysis of randomized clinical trials and observational studies to assess the safety and efficacy of glibenclamide as a component of the medical treatment for patients with stroke and develop supporting evidence for effective clinical strategies.

METHODS

Study registration

This protocol is being conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[29] or meta-analyses of healthcare interventions. The protocol report for this study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols(PRISMA-P) [30]. The PRISMA-P checklist is presented in online supplemental appendix 1. This protocol has been registered in the PROSPERO network (registration number: CRD42020144674).

Patient and Public Involvement statement

No patient involved.

Eligibility criteria

Studies that meet the following criteria will be included in the analysis: (1) Randomized trials (RCTs) and observational studies; (2) patients diagnosed with stroke using computed tomography (CT) and magnetic resonance imaging (MRI), including ischemic, hemorrhagic stroke, and subarachnoid hemorrhage; (3) an intervention group will be treated with intravenous or oral glibenclamide; (4) a comparison control group will be included in the randomized trials, which will consist of a placebo, blank, or others; (5) data concerning mortality and other major adverse events will be included; (6) studies to be included will be published up to December 31, 2020.

Direct comparisons will be made from the identified studies that include a placebo or other control compounds. RCTs and observational studies will be screened for eligibility. Whether both types of studies should be included to conduct the meta-analysis is dependent on the circumstances because it has been suggested recently that RCTs and observational studies should not be analyzed in isolation[31]. Therefore, the inclusion of selected RCTs and observational studies in the meta-analysis is dependent on the quality assessment.

Information sources

The following databases will be searched from their inception forward for potentially eligible studies without language restrictions and published up to December 31, 2020: (1) PubMed; (2) Scopus; (3) Web of Science; (4) Cochrane Central Register of Controlled Clinical Trials; (5) CNKI and WanFang Databases. We also will manually search references from relevant randomized controlled trials identified through systematic reviews, meta-analyses, and studies included in this review.

Search strategy

A systematic search of six public domain databases above mentioned will be performed. The first author will conduct all database searches without language restrictions. The search strategies will be adapted from previous research and developed using text words and medical subject headings. We will use exploded Medical Subject Headings and appropriate corresponding keywords related to the population, combined with exposure and outcomes such as :'Stroke' OR 'intracerebral hemorrhage' OR 'ischemic Stroke ' OR ' cerebral infarction' OR 'Hemorrhagic stroke' OR 'subarachnoid hemorrhage' AND 'Glibenclamide' AND 'prognostic' OR 'modified Rankin Scale'.A sample search strategy for PubMed is shown in online supplemental appendix 2.

Study records

Study selection

All studies will be extracted from electronic databases using the search strategy described above and imported into EndNote X7 software (Thomson Reuters, Canada). Duplicate studies will be removed. Two authors will select studies independent of each other. Complete articles will be retrieved for all titles and abstracts that appear to meet the inclusion criteria or where any uncertainty exists. The two reviewers (WLH and HB)will list all the studies to be included and document the primary reasons for excluding studies that do not conform to the inclusion criteria. Disagreements between the two authors will be resolved by discussing with the third author(TR), and if necessary, consulting with the fourth author(WKZ). The overall agreement rate prior to correcting for discrepancies will be calculated using Cohen's kappa (κ) statistics. A flow diagram will be constructed that depicts the search process. An online supplementary file containing a reference list of all excluded studies, including the reason(s) for exclusion, will be included in the study. We will show the details of the selection process in the PRISMA flow chart. The proposed structure for the flow diagram is shown in Figure 1[32].

Data acquisition

Before initiating data acquisition, a codebook will be developed in Microsoft Excel 2013. Two independent researchers will extract data. The following data will be extracted from each eligible study using a standardized data collection form: (1) Study characteristics, including publication year, author, country of the study, type of study (RCT, cohort, case-control, and others), sample size, follow-up duration, and others; (2) participant characteristics, including age, sex, stroke type (ischemic, hemorrhagic stroke, or subarachnoid hemorrhage), and baseline condition of participants (e.g.,disease severity:NIHSS will be used to gauge disease severity); (3) intervention characteristics, including name of the drug(s), dose, route of administration, and others; (4) control characteristics, including the type of drug(s) used, dose, route of administration, and others; and (5) outcome data for mortality, modified Rankin scale (mRS), and occurrence of hypoglycemic events. The first two authors will acquire the data from the selected studies, independent of each other, using the codebook in Microsoft Excel. After data acquisition, both authors will review the codebooks and resolve discrepancies by consensus. If a consensus cannot be reached, the third author will provide a recommendation. A complete list of covariates that we will include is shown in Table 1.

Outcomes

The outcomes will include mortality, modified Rankin scale (mRS), and the occurrence of hypoglycemic events.

Assessment of the quality of the evidence

According to the Grading of Recommendations Assessment Development and Evaluation (GRADE), the quality of included studies will be assessed using the online guideline development tool http://gdt.guideline-development.org/), and divided into four levels of quality: high, moderate, low, and very low[33].

Risk of bias assessment in individual studies

Risk of bias for RCTs will be assessed using the Cochrane Risk of Bias instrument[34], which contains seven specific domains, random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other biases. This instrument assesses the methodological quality of the RCTs concerning low risk, high risk, or unclear risk of bias[35]. If any domain is scored high/low risk of bias, the study will be considered high/low risk of bias. No study will be excluded based on the results of the risk of bias assessment[36]. The first two authors will conduct all risk of bias assessments independent of each other. Then the two authors will review the results

for the risk of bias assessment and resolve any discrepancies by consensus. If a consensus cannot be reached, the third author will be consulted.

The Nine-item Newcastle–Ottawa Quality scale is widely used to assess the quality of non-randomized trials using risk evaluation for the adequacy of selection, comparability, and outcomes assessment[37]. The high-quality studies will be defined as studies with a score that is greater than or equal to six.

Data synthesis and analysis

Data synthesis

Review Manager V.5.3 (Cochrane Collaboration) and Stata V.16.0 software will be used to conduct this meta-analysis. The mean difference or standardised mean difference with 95% CIs is used to calculate continuous variables.

Assessment of heterogeneity

Statistical heterogeneity among included studies will be assessed using the $\chi 2$ test and I^2 test. Initially, we will use a fixed-effect model for data analysis. If $I^2 > 0.5$ or $p < 0.1$, this will indicate the presence of significant heterogeneity among the studies, and a random-effect model will be used without examining the probable cause for the high heterogeneity.

Subgroup analysis

If there is considerable heterogeneity, and the data are sufficient, subgroup analyses will be conducted to identify potential causes for the heterogeneity. Subgroup analyses will be performed based on the type of stroke, including ischemic stroke, hemorrhagic stroke, or subarachnoid hemorrhage.

Assessment of publication bias

Publication bias will be examined according to the funnel plot method. Also, Egger's test and Begg's test will be conducted to assess the publication bias quantitatively using Stata V. 16.0 software.

Sensitivity analysis

We will conduct sensitivity analyses of the primary results to explore the robustness of the review conclusions, if feasible, after considering the impact of methodological quality, missing data, and sample size. According to the Cochrane Handbook, when a sufficient number of original studies are included (generally more than ten trials), publication bias analysis will be performed using a funnel plot. A

symmetrical funnel plot indicates low publication bias. If the funnel plot is asymmetric, that will indicate a high risk for publication bias.

Software used for data analysis

All data will be analyzed using Stata/IC for Mac V.16.0.

REGISTRATION

In accordance with the Primary Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P), our systematic review with network meta-analysis was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on April 28, 2020 (registration number: CRD42020144674).

Contributors

WLH and HB drafted the manuscript. TR and WKZ contributed to the development of the data sources to search for relevant literature, including the search strategy, selection criteria, data extraction criteria, and risk of bias assessment strategy. WLH provided statistical expertise, and ZXY, BH, and ZH provided content expertise on glibenclamide and stroke. All four authors read, provided feedback, and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for data publication

All participants consented to data publication. The privacy for all individuals has been preserved.

Ethics issues

This meta-analysis is a secondary research project that is based on previously published data. Therefore, ethical approval or informed consent was not required for this meta-analysis.

figure 1 Flow diagram of study selection process.

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Studies characteristics	publication year, author,country of the study, type of study (RCT, cohort, case–control, etc), sample size, follow-up duration,etc).
Participant characteristics	age, sex, stroke type(ischemic ,hemorrhagic stroke or subarachnoid hemorrhage),baseline condition of participants(eg,underlying disease, medication history)
intervention characteristics	name of the drug,dose, route of administration(oral or intravenous);
control characteristics	type of the drug,dose, route of administration);
Outcome	mortality,modified Rankin scale(mRS) and occurrence of hypoglycemia events.

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Table1 Covariates that will be included in the study

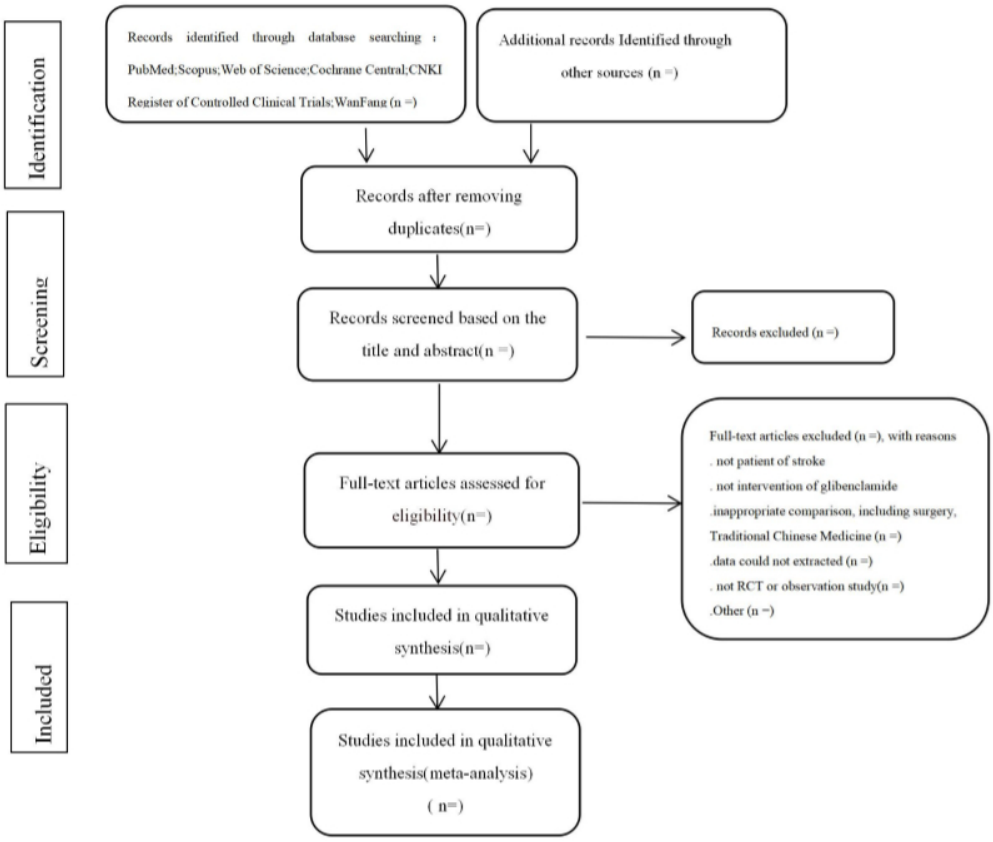

figure 1 Flow diagram of study selection process.

PRISMA-P Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☐	☐	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☐	
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☐	☐	1
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☐	☐	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☐	☐	7
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☐	
Support					
Sources	5a	Indicate sources of financial or other support for the review	☐	☐	7
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☐	7
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☐	
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☐	☐	2,3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☐	☐	3

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☐	☐	4
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☐	☐	4
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☐	☐	4
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☐	☐	5
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☐	☐	5
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☐	☐	5
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☐	☐	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☐	☐	5
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☐	☐	6
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	☐	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☐	6
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	☐	6
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☐	☐	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	☐	6

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	☐	5

Appendix 1.A sample search strategy for PubMed

Stroke	1.“Cerebrovascular Disorders”[Mesh] OR stroke*[tiab] OR poststroke*[tiab] OR cerebral vascular OR accident*[tiab] OR cva*[tiab] OR brain injur*[ti] OR apoplex*[tiab] 2.“Brain”[Mesh] OR brain*[tiab] OR cerebr*[tiab] OR cerebell*[tiab] OR intracran*[tiab] OR intracerebral*[tiab] OR vertebrobasilar*[tiab] 3.“Blood vessels”[Mesh] OR blood vessel*[tiab] OR vascular*[tiab] OR “arteries”[Mesh] OR arter*[tiab] 4.“Intracranial Aneurysm”[Mesh] OR “Intracranial Hemorrhages”[Mesh] OR “Intracranial OR Hemorrhage, Hypertensive”[Mesh] OR “Hematoma, Subdural, Intracranial”[Mesh] OR “Subarachnoid Hemorrhage”[Mesh] OR “SAH”[tiab] 5.“Hemorrhage”[Mesh] OR hemorrhag*[tiab] OR haemorrhag*[tiab] OR “hematoma” [Mesh] OR hematoma*[tiab] OR haematoma*[tiab] OR bleed*[tiab] 6.“Intracranial Embolism and Thrombosis”[Mesh] OR “Intracranial Embolism”[Mesh] OR “Vasospasm, OR Intracranial”[Mesh] OR “Ischemic Attack, Transient”[Mesh] OR “TIA”[tiab] OR “Brain Ischemia”[Mesh] 7.“Ischemia”[Mesh] OR ischemi*[tiab] OR ischaemi*[tiab] OR thrombo*[tiab] OR “embolism”[Mesh] OR emboli*[tiab] OR oclus*[tiab] 8.(#2 AND #7) OR #6 9.(((#2 OR subarachnoid*[tiab]) AND #5) OR #4) 10.#1 OR #8 OR #9
Glybenclamide	11.“diabetic medications” [Mesh] OR “Sulfonylurea” [Mesh] OR “Glybenclamide” [Mesh] OR “Glyburide” [Mesh] OR “Glibornuride"
Type of studies	12.Randomised controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab] 13.Animals [mh] NOT humans [mh] 14.#12 and #13
	15.#10 and #11 and #14

BMJ Open

Effectiveness and safety of Glibenclamide for Stroke: Protocol for a systematic review and meta-analysis

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Neurology
Keywords:	Stroke < NEUROLOGY, Neurological injury < NEUROLOGY, Neurotology < OTOLARYNGOLOGY

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Effectiveness and safety of Glibenclamide for Stroke: Protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction: Despite the continuous improvement in modern medical treatment, stroke is still a leading cause of death and disability worldwide. How to effectively improve the survival rate and reduce disability in patients with stroke has become the focus of many investigations. Recent findings concerning the benefits of glibenclamide as a neuroprotective drug have initiated a new area for prospective studies on the effects of sulfonylureas. Given the high mortality and disability associated with stroke, it is essential to weigh the benefits of neuroprotective drugs against their safety. Therefore, the objective of the current study is to conduct a systematic review using meta-analysis to assess the benefits and safety of glibenclamide as a neuroprotective drug.

Methods and analysis: This study will analyze randomized clinical trials (RCTs) and observational studies published up to December 31, 2020, and include direct or indirect evidence. Studies will be retrieved by searching PubMed, EMBASE, Web of Science, the Cochrane Library, as well as CNKI and WanFang Databases. The outcomes of this study will be mortality, scores from the modified Rankin scale (mRS), and the occurrence of hypoglycemic events. The risk of bias will be assessed using the Cochrane Risk of Bias assessment instrument for RCTs.A random-effect/fixed-effect model will be used to summarize the estimates of the mean difference (MD)/risk ratio (RR) using a 95% confidence interval (CI).

Dissemination: The results of this study will be submitted to a peer-reviewed journal for publication.

Ethics issues: This meta-analysis is a secondary research project, which is based on previously published data. Therefore, ethical approval and informed consent were not required for this meta-analysis.

Keywords:Glibenclamide;stroke;meta-analysis, protocol

PROSPERO registration number CRD42020144674.

Strengths and limitations of this study

► This is the first systematic review and meta-analysis to analyze the benefits and safety of glibenclamide in stroke patients based on data from both randomized clinical trials (RCTs) and observational studies.

►As is common with most meta-analyses, significant and unexplained heterogeneity may exist.

► The risk for ecological fallacy exists in this study as for any aggregate data meta-analysis.

INTRODUCTION

Rationale

Stroke is a common disease with high mortality and morbidity worldwide, and often leaves survivors with severe neurological impairments and long term disability[1-2]. According to data from The Third Cause of Death Survey in China, stroke is the leading cause of mortality and disability in adults in China. The stroke burden in China currently exhibits an explosive growth trend, which is characterized by rapid increases in stroke in low-income and younger individuals[3]. At present, the average incidence for the first stroke for residents in China aged 40 to 74 years has increased by an annual rate of 8.3%. The prevalence of stroke in adults in China aged 40 years or older has increased from 1.89% in 2012 to 2.19% in 2016, and it is estimated that 1.96 million people die due to the consequences of stroke every year[4]. Better prevention and treatment of stroke still face enormous challenges, and the medical system needs further improvement and optimization. Therapies targeting the underlying pathophysiology of central nervous system (CNS) ischemia and hemorrhage are conspicuously lacking. Several neuroprotective agents have been studied, but their clinical efficacy has been unsatisfactory.

Recent findings concerning the benefits of glibenclamide as a neuroprotective drug have initiated a number of new prospective studies[5]. Glibenclamide is a member of the sulfonylurea class of drugs and has been used in the clinic as an oral hypoglycemic agent[6].

It exerts its pleiotropic protective effects on acute central nervous system (CNS) injury by inhibiting the recently characterized Sur1-Trpm4 channel (formerly, the Sur1-regulated nonselective cation (NCCa-ATP) channel). Glibenclamide improves functional neurological outcomes in stroke models by protecting the microvascular endothelium, reducing edema formation and secondary hemorrhage, inhibiting neuronal cell death, maintaining the integrity of the blood-brain-barrier (BBB), and promoting neurogenesis by blocking the Sur1-Trpm4 channel[7-8]. Thus, glibenclamide has received renewed attention as a CNS treatment for ischemic hemorrhagic stroke and subarachnoid hemorrhage[9-13]. When studied in models of ischemic stroke, glibenclamide significantly reduced the mortality rate to 5%, whereas the vehicle-treated group exhibited 67% mortality at 24 h. Compared to a decompressive craniectomy (DC) group, glibenclamide significantly improved neurological function[14-16].

Glibenclamide scavenges free radicals, reduces activated-caspase-3 expression, increases the Bcl-2/Bax ratio, and inhibits apoptosis by blocking NC channels to improve functional neurological outcomes in a rat model of intracranial hemorrhage(ICH)[17-18].Also,glibenclamide can significantly reduce BBB permeability, markers of cell injury or cell death, protect the normal junctional localization of ZO-1, as well as reduce inflammation and markers of inflammation, vasogenic edema, and caspase-3 activation to improve functional neurological outcomes after subarachnoid hemorrhage[19].

Several retrospective studies suggest that taking a sulfonylurea drug and continuing it following an ischemic CNS insult significantly improves outcomes, including survival, greater functional independence, lower NIH stroke scale scores, and less hemorrhagic transformation[20-21]. Administration of a sulfonylurea drug also improved long-term cognitive function in clinically relevant models of subarachnoid hemorrhage[22]. A prospective study suggested that intravenous glibenclamide (GBC) reduced water accumulation and mass effects after large hemispheric infarctions[23]. Another prospective study suggested that oral GBC is safe in treating acute hemispheric infarctions and potentially could prevent brain edema and subsequent severe disability and death. Two studies [24-25]have suggested that GBC reduced edema, protected blood-brain barrier (BBB) integrity, and improved long-term neurological deficits. Another study reported that GBC reduced oxidative stress, inhibited apoptosis, and improved neurological deficits[26]. However, a recent study tested a widely-used GBC dose shown to be effective in other studies (10 μg/kg loading dose followed by 200 ng/hr for up to seven days), and the result suggested that recovery from neurological impairments was not improved by GBC and also did not improve ICH outcomes[27]. Ghasami et al. compared the use of GBC and insulin when given to diabetic hemorrhagic stroke patients and reported that GBC had no benefit compared to the insulin group[28]. However, we note that this was a small, non-randomized, non-placebo-controlled trial. Thus, further clinical work in hemorrhage is needed.

Objective

The primary objective of this study is to conduct a systematic review and meta-analysis of randomized clinical trials and observational studies to assess the safety and efficacy of glibenclamide as a component of the medical treatment for patients with stroke and develop supporting evidence for effective clinical strategies.

METHODS

Study registration

This protocol is being conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[29] or meta-analyses of healthcare interventions. The protocol report for this study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols(PRISMA-P) [30]. The PRISMA-P checklist is presented in online supplemental appendix 1. This protocol has been registered in the PROSPERO network (registration number: CRD42020144674).

Patient and Public Involvement statement

No patient involved.

Eligibility criteria

Studies that meet the following criteria will be included in the analysis: (1) Randomized trials (RCTs) and observational studies; (2) patients diagnosed with stroke using computed tomography (CT) and magnetic resonance imaging (MRI), including ischemic, hemorrhagic stroke, and subarachnoid hemorrhage; (3) an intervention group will be treated with intravenous or oral glibenclamide; (4) a comparison control group will be included in the randomized trials, which will consist of a placebo, blank, or others; (5) data concerning mortality and other major adverse events will be included; (6) studies to be included will be published up to December 31, 2020.

Direct comparisons will be made from the identified studies that include a placebo or other control compounds. RCTs and observational studies will be screened for eligibility. Whether both types of studies should be included to conduct the meta-analysis is dependent on the circumstances because it has been suggested recently that RCTs and observational studies should not be analyzed in isolation[31]. Therefore, the

inclusion of selected RCTs and observational studies in the meta-analysis is dependent on the quality assessment.

Information sources

The following databases will be searched from their inception forward for potentially eligible studies without language restrictions and published up to December 31, 2020: (1) PubMed; (2) Scopus; (3) Web of Science; (4) Cochrane Central Register of Controlled Clinical Trials; (5) CNKI and WanFang Databases. We also will manually search references from relevant randomized controlled trials identified through systematic reviews, meta-analyses, and studies included in this review.

Search strategy

A systematic search of six public domain databases above mentioned will be performed. The first author will conduct all database searches without language restrictions. The search strategies will be adapted from previous research and developed using text words and medical subject headings. We will use exploded Medical Subject Headings and appropriate corresponding keywords related to the population, combined with exposure and outcomes such as :'Stroke' OR 'intracerebral hemorrhage' OR 'ischemic Stroke ' OR ' cerebral infarction' OR 'Hemorrhagic stroke' OR 'subarachnoid hemorrhage' AND 'Glibenclamide' AND 'prognostic' OR 'modified Rankin Scale'.A sample search strategy for PubMed is shown in online supplemental appendix 2.

Study records

Study selection

All studies will be extracted from electronic databases using the search strategy described above and imported into EndNote X7 software (Thomson Reuters, Canada). Duplicate studies will be removed. Two authors will select studies independent of each other. Complete articles will be retrieved for all titles and abstracts that appear to meet the inclusion criteria or where any uncertainty exists. The two reviewers (WLH and HB)will list all the studies to be included and document the primary reasons for excluding studies that do not conform to the inclusion criteria. Disagreements between the two authors will be resolved by discussing with the third author(TR), and if necessary, consulting with the fourth author(WKZ). The overall agreement rate prior to correcting for discrepancies will be calculated using Cohen's kappa (κ) statistics. A flow diagram will be constructed that depicts the search process. An online supplementary file containing a reference list of all excluded studies, including the reason(s) for exclusion, will be included in the study. We

will show the details of the selection process in the PRISMA flow chart. The proposed structure for the flow diagram is shown in Figure 1[32].

Data acquisition

Before initiating data acquisition, a codebook will be developed in Microsoft Excel 2013. Two independent researchers will extract data. The following data will be extracted from each eligible study using a standardized data collection form: (1) Study characteristics, including publication year, author, country of the study, type of study (RCT, cohort, case-control, and others), sample size, follow-up duration, and others; (2) participant characteristics, including age, sex, stroke type (ischemic, hemorrhagic stroke, or subarachnoid hemorrhage), and baseline condition of participants (e.g.,disease severity:NIHSS will be used to gauge disease severity); (3) intervention characteristics, including name of the drug(s), dose, route of administration, and others; (4) control characteristics, including the type of drug(s) used, dose, route of administration, and others; and (5) outcome data for mortality, modified Rankin scale (mRS), and occurrence of hypoglycemic events. The first two authors will acquire the data from the selected studies, independent of each other, using the codebook in Microsoft Excel. After data acquisition, both authors will review the codebooks and resolve discrepancies by consensus. If a consensus cannot be reached, the third author will provide a recommendation. A complete list of covariates that we will include is shown in Table 1.

Outcomes

The outcomes will include mortality, modified Rankin scale (mRS), and the occurrence of hypoglycemic events.

Assessment of the quality of the evidence

According to the Grading of Recommendations Assessment Development and Evaluation (GRADE), the quality of included studies will be assessed using the online guideline development tool http://gdt.guideline-development.org/), and divided into four levels of quality: high, moderate, low, and very low[33].

Risk of bias assessment in individual studies

Risk of bias for RCTs will be assessed using the Cochrane Risk of Bias instrument[34], which contains seven specific domains, random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other biases. This

instrument assesses the methodological quality of the RCTs concerning low risk, high risk, or unclear risk of bias[35]. If any domain is scored high/low risk of bias, the study will be considered high/low risk of bias. No study will be excluded based on the results of the risk of bias assessment[36]. The first two authors will conduct all risk of bias assessments independent of each other. Then the two authors will review the results for the risk of bias assessment and resolve any discrepancies by consensus. If a consensus cannot be reached, the third author will be consulted.

The Nine-item Newcastle–Ottawa Quality scale is widely used to assess the quality of non-randomized trials using risk evaluation for the adequacy of selection, comparability, and outcomes assessment[37]. The high-quality studies will be defined as studies with a score that is greater than or equal to six.

Data synthesis and analysis

Data synthesis

Review Manager V.5.3 (Cochrane Collaboration) and Stata V.16.0 software will be used to conduct this meta-analysis. The mean difference or standardised mean difference with 95% CIs is used to calculate continuous variables.

Assessment of heterogeneity

Statistical heterogeneity among included studies will be assessed using the $\chi 2$ test and I^2 test. Initially, we will use a fixed-effect model for data analysis. If $I^2 > 0.5$ or $p < 0.1$, this will indicate the presence of significant heterogeneity among the studies, and a random-effect model will be used without examining the probable cause for the high heterogeneity.

Subgroup analysis

If there is considerable heterogeneity, and the data are sufficient, subgroup analyses will be conducted to identify potential causes for the heterogeneity. Subgroup analyses will be performed based on the type of stroke(ischemic stroke, hemorrhagic stroke, or subarachnoid hemorrhage),and time to treatment.

Assessment of publication bias

Publication bias will be examined according to the funnel plot method. Also, Egger's test and Begg's test will be conducted to assess the publication bias quantitatively using Stata V. 16.0 software.

Sensitivity analysis

We will conduct sensitivity analyses of the primary results to explore the robustness of the review conclusions, if feasible, after considering the impact of methodological quality, missing data, and sample size. According to the Cochrane Handbook, when a sufficient number of original studies are included (generally more than ten trials), publication bias analysis will be performed using a funnel plot. A symmetrical funnel plot indicates low publication bias. If the funnel plot is asymmetric, that will indicate a high risk for publication bias.

Software used for data analysis

All data will be analyzed using Stata/IC for Mac V.16.0.

REGISTRATION

In accordance with the Primary Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P), our systematic review with network meta-analysis was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on April 28, 2020 (registration number: CRD42020144674).

Contributors

WLH and HB drafted the manuscript. TR and WKZ contributed to the development of the data sources to search for relevant literature, including the search strategy, selection criteria, data extraction criteria, and risk of bias assessment strategy. WLH provided statistical expertise, and ZXY, BH, and ZH provided content expertise on glibenclamide and stroke. All four authors read, provided feedback, and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for data publication

All participants consented to data publication. The privacy for all individuals has been preserved.

Ethics issues

This meta-analysis is a secondary research project that is based on previously published data. Therefore, ethical approval or informed consent was not required for this meta-analysis.

figure 1 Flow diagram of study selection process.

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Studies characteristics	publication year, author,country of the study, type of study (RCT, cohort, case–control, etc), sample size, follow-up duration,etc).
Participant characteristics	age, sex, stroke type(ischemic ,hemorrhagic stroke or subarachnoid hemorrhage),baseline condition of participants(eg,underlying disease, medication history)
intervention characteristics	name of the drug,dose, route of administration(oral or intravenous);
control characteristics	type of the drug,dose, route of administration);
Outcome	mortality,modified Rankin scale(mRS) and occurrence of hypoglycemia events.

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Table1 Covariates that will be included in the study

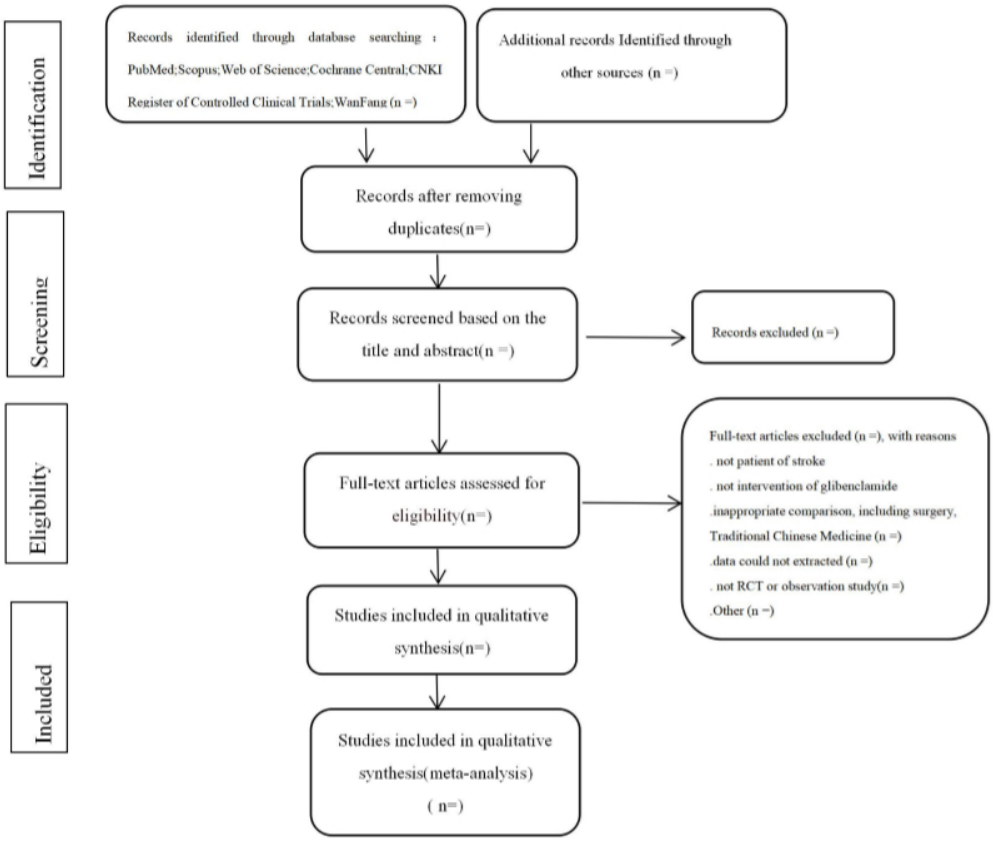

figure 1 Flow diagram of study selection process.

Appendix 1.A sample search strategy for PubMed

Stroke	1.“Cerebrovascular Disorders”[Mesh] OR stroke*[tiab] OR poststroke*[tiab] OR cerebral vascular OR accident*[tiab] OR cva*[tiab] OR brain injur*[ti] OR apoplex*[tiab] 2.“Brain”[Mesh] OR brain*[tiab] OR cerebr*[tiab] OR cerebell*[tiab] OR intracran*[tiab] OR intracerebral*[tiab] OR vertebrobasilar*[tiab] 3.“Blood vessels”[Mesh] OR blood vessel*[tiab] OR vascular*[tiab] OR “arteries”[Mesh] OR arter*[tiab] 4.“Intracranial Aneurysm”[Mesh] OR “Intracranial Hemorrhages”[Mesh] OR “Intracranial OR Hemorrhage, Hypertensive”[Mesh] OR “Hematoma, Subdural, Intracranial”[Mesh] OR “Subarachnoid Hemorrhage”[Mesh] OR “SAH”[tiab] 5.“Hemorrhage”[Mesh] OR hemorrhag*[tiab] OR haemorrhag*[tiab] OR “hematoma” [Mesh] OR hematoma*[tiab] OR haematoma*[tiab] OR bleed*[tiab] 6.“Intracranial Embolism and Thrombosis”[Mesh] OR “Intracranial Embolism”[Mesh] OR “Vasospasm, OR Intracranial”[Mesh] OR “Ischemic Attack, Transient”[Mesh] OR “TIA”[tiab] OR “Brain Ischemia”[Mesh] 7.“Ischemia”[Mesh] OR ischemi*[tiab] OR ischaemi*[tiab] OR thrombo*[tiab] OR “embolism”[Mesh] OR emboli*[tiab] OR oclus*[tiab] 8.(#2 AND #7) OR #6 9.(((#2 OR subarachnoid*[tiab]) AND #5) OR #4) 10.#1 OR #8 OR #9
Glybenclamide	“diabetic medications” [Mesh] OR “Sulfonylurea” [Mesh] OR “Glybenclamide” [Mesh] OR “Glyburide” [Mesh] OR “Glibornuride"OR “Glibenclamide” [Mesh] OR “HB-419”[Mesh] OR “Maninil” [Mesh] OR “Adiab”[Mesh]
Type of studies	12.Randomised controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab] 13.Animals [mh] NOT humans [mh] 14.#12 and #13
	15.#10 and #11 and #14

PRISMA-P Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☐	☐	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☐	
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☐	☐	1
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☐	☐	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☐	☐	7
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☐	☐	
Support					
Sources	5a	Indicate sources of financial or other support for the review	☐	☐	7
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☐	7
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☐	
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☐	☐	2,3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☐	☐	3

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☐	☐	4
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☐	☐	4
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☐	☐	4
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☐	☐	5
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☐	☐	5
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☐	☐	5
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☐	☐	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☐	☐	5
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☐	☐	6
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	☐	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☐	6
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	☐	6
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☐	☐	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	☐	6

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	☐	5