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Benefits and safety of transdermal glyceryl trinitrate in acute stroke: a systematic review and meta-analysis of randomised trials (Protocol)

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ABSTRACT:

Introduction: High blood pressure (BP) is common in acute stroke and has adverse outcomes. Transdermal glyceryl trinitrate (GTN) has beneficial properties and can be easily administered to control BP. There is an urgent need for an updated meta-analysis as the previous review in 2016 has important limitations. We report the protocol for a systematic review and meta-analysis on the safety and benefits of transdermal GTN in acute stroke.

Methods and Analysis: We will search Medline, Pubmed, Embase, CINAHL and Cochrane Library from inception until June 2020 for randomised trials that report the efficacy and safety of transdermal GTN vs placebo/control therapy among adult patients with acute stroke. Primary outcomes include in-hospital mortality, BP lowering and late functional status. Secondary outcomes include early, late, resource utilisation and surrogate outcomes. Safety outcomes include reported adverse events. Reviewers will first screen titles and abstracts, and then full texts, to identify eligible studies. Independently and in duplicate, they will extract data, assess risk of bias (RoB) using a modified Cochrane RoB tool and quality of evidence using GRADE. Disagreement will be resolved by discussion and consultation with an external reviewer if necessary. Using a random-effects model, we will report effect sizes using relative risks and 95% confidence intervals. We will perform pre-defined subgroup analyses: intracerebral haemorrhage vs ischaemic stroke; minor (NIHSS ≤ five) vs major (NIHSS > five) ischaemic stroke; ischaemic stroke with vs without thrombolysis; pre-hospital vs non pre-hospital settings; time from stroke to randomisation ≤ six vs > six hours and high vs low overall RoB studies. We will also perform trial sequential analysis for the primary outcomes of in-hospital mortality and late functional status.

Ethics and Dissemination: Ethics board approval is unnecessary. PROSPERO registration has been obtained (CRD42020173093). The results will be disseminated through publication in a peer-reviewed journal.

ARTICLE SUMMARY

Strengths and Limitations of Study

- This is the first update since the previous meta-analysis in 2016 on the use of transdermal GTN in acute stroke which included several small trials (n≤ 90). This meta-analysis will include the large (n> 1000), multi-centre RIGHT-2 trial published in 2019.
- This study will examine an important gap identified by the previous meta-analysis in 2016; i.e benefits of transdermal GTN vs placebo/control therapy in ultra-acute stroke (presentation ≤ six hours) in a pre-planned subgroup analysis with limited sample size (n= 312). The data from the RIGHT-2 trial includes > three times the total sample size of acute stroke patients with presentation < four hours used in the 2016 review.
- Other strengths of this protocol include a comprehensive search strategy of published and unpublished literature, an extensive predefined subgroup analysis plan and inclusion of GRADE methodology to assess certainty of evidence.
- This study will be the first to use trial sequential analysis on the important primary outcomes of in-hospital mortality and late functional status associated with transdermal GTN use in acute stroke.
- Limitations to this protocol include the anticipated high clinical heterogeneity given the haemorrhagic and ischaemic subtypes of acute stroke, variation in timing of

randomization from onset of stroke to transdermal GTN or placebo/control therapy and reporting of outcome measures across trials.

INTRODUCTION

High blood pressure (BP) is present in greater than 70% of patients with acute ischaemic stroke.[1] It is associated with poor outcomes including acute stroke recurrence, death within a few weeks or combined death and dependency after several months.[1-4] High BP is similarly common in acute intracerebral haemorrhage (ICH)[5] and may be associated with haematoma expansion and increased mortality.[6-8]

It is recommended to lower BP in ICH[9-10] although controversy exists regarding optimal BP target in patients with ICH and there is no current literature on the role of pre-hospital BP reduction. The management of high BP in acute ischaemic stroke and the decision to treat or not to treat has been a constant debate since 1985. Current available guidelines recommend withholding antihypertensive therapy in the early post-stroke period unless there is markedly elevated BP (>220/120mmHg) or with BP >185/110mmHg for patients eligible for thrombolysis or BP >180/105mmHg during the 24-hour period following reperfusion.[11-13]

Nitric oxide (NO) donors are candidate agents to lower BP in acute stroke because NO is a cerebral and systemic vasodilator, modulates vascular and neuronal function, is neuroprotective, and inhibits apoptosis.[14] In addition, vascular NO concentrations are low in acute stroke which are associated with increased severity of stroke, mortality and institutionalization.[15] These observations support that NO supplementation might be beneficial.

Glyceryl trinitrate (GTN) is an example of a NO donor. Transdermal GTN administration offers a constant release of the drug across the skin into the systemic circulation for 24 hours which achieves sustained steady-state plasma concentrations.[16] Transdermal GTN offers a formulation which is easily administered in many clinical settings [pre-hospital, Emergency Department (ED) and inpatient] managing acute stroke which may help to minimise fluctuations in drug concentrations and hence BP.

The only meta-analysis published in 2016; using data from five completed transdermal GTN trials (n= 4197); reported no improvement in outcomes across a range of domains (death, disability, cognition, mood, and quality of life with transdermal GTN vs placebo or control therapy.[17] However, in a pre-specified subgroup analysis of patients with time from stroke to randomisation ≤ six vs > six hours (n= 312), this meta-analysis reported a favourable functional outcome as measured by modified Rankin scale (mRS) at 90 days with transdermal GTN. There were important limitations in this meta-analysis. Four out of five selected trials had small sample sizes (n≤ 90). The remaining multi-centre ENOS trial[18] recruited 4011 patients and it dominated the pooled analysis (95.6% of all patients and 86.9% of those randomised within six hours of onset). In addition, all the included trials were conducted by a single research group and it is important that other research groups study the role of transdermal GTN in acute stroke. Finally, a relatively small number of patients (n= 312) were treated within six hours of stroke onset and these patients came from just two of the five trials.

The recently published multi-centre RIGHT-2 trial randomised 1149 participants with acute stroke within four hours of onset to receive transdermal GTN vs sham therapy.[19] The data from this study more than triples that used to examine the role of transdermal GTN in ultra-early stroke (onset ≤ six hours). There is an urgent need to update the evidence behind the

efficacy and safety of transdermal GTN in acute stroke especially among those patients with ultra-early (≤ six hours) presentation.

The aim of this systematic review and meta-analysis is to examine; using recent data; whether transdermal GTN improves important patient centred outcomes and is safe among patients with acute stroke in the pre-hospital and in-hospital settings compared to placebo or control therapy by reviewing randomised controlled trials (RCTs).

METHODS

Study Registration

This systematic review and meta-analysis protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO). The reference number is CRD42020173093. We will adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement for reporting systematic review and meta-analysis.[20]

Eligibility Criteria

We will include randomised trials investigating the efficacy and safety of transdermal GTN vs placebo or control therapy among adult patients presenting with acute stroke.

Patients aged ≥ 16 years presenting with either acute ischaemic or haemorrhagic stroke in the pre-hospital, ED and inpatient clinical settings will be considered. Acute stroke patients are defined as those with presentation within five days of onset of symptoms. We select five days since onset of symptoms as the inclusion cut-off criterion for this review because there can be significant delay in presentation after an acute stroke; especially for less severe ischaemic strokes.[21] Patients with ischaemic stroke are included regardless of whether they

receive thrombolysis. The comparator arms will include transdermal GTN patch, sham patch and control with existing standard therapy.

Primary outcomes are important patient centred outcomes including in-hospital mortality, lowering of BP measurements and late functional status.

Secondary outcomes are classified as early, late, resource utilisation and surrogate outcomes. Early secondary outcomes include development of ICH, recurrent stroke and change in calculated National Institutes of Health Stroke Scale (NIHSS). Late secondary outcomes include reported changes in activities of daily living, cognition, quality of life and mood. Resource utilisation secondary outcomes include length of hospital stay and discharge destination. Surrogate secondary outcomes include changes in cerebral haemodynamics and laboratory parameters like platelet aggregation.

Safety outcomes include any adverse events reported by the authors.

Search Strategy

We will search Medline, Pubmed, Embase, CINAHL and Cochrane Library from inception until June 2020 without language restrictions. We will review reference lists for eligible new trials and also search ClinicalTrials.gov for ongoing or unpublished trials and for additional data from published trials. The search strategy will include the following keywords: stroke, ischaemic stroke, haemorrhagic stroke, intracerebral haemorrhage, transdermal glyceryl trinitrate, transdermal nitroglycerin, glyceryl trinitrate patch, nitroglycerin patch, trinitroglycerin, pre-hospital, mortality, blood pressure, functional outcome, humans and randomised clinical trials.

Study Selection

Reviewers (LBL, CWL, LWF and NWM) will independently and in duplicate screen the titles and abstracts of all identified studies to generate a list of eligible trials from which full texts will be obtained. Subsequently, the same reviewers will independently assess eligibility of these full texts of published trials to decide on the final included studies. Discrepancies between reviewers will be resolved through discussion and consensus or, if needed, by adjudication from an external reviewer and/or contact with authors of the original trials for clarification.

Data Extraction

Two pairs of reviewers (LBL and CWL; LWF and NWM) will extract data from included studies both independently and in duplicate. Data extracted will include the following: general study information [authors, publication year and study location(s)]; study population details [clinical setting- pre-hospital vs ED vs inpatient, sample size, types of strokes- ischaemic vs haemorrhagic; subgroup of ischaemic strokes with thrombolysis]; details on the comparator arms [different doses and duration of GTN patch; sham patch and control] as well as the primary, secondary and safety outcomes as listed above.

In randomized trials that included more than one arm of GTN dosing and duration, we will extract data from the arm closest to a single dose regimen that is comparable to other primary studies to be used for analysis.

Discrepancies in data extraction will be resolved through discussion and consensus or, if needed, via an external reviewer and/or contact with authors of the original trials for clarification.

Risk OF Bias Assessment

We will assess the risk of bias (RoB) for each outcome of the individual studies using a modified Cochrane RoB instrument.[22] The instrument assesses biases in the following five

domains: selection bias (random sequence generation and allocation concealment); performance bias (blinding of participants and researchers); detection bias (blinding of outcome assessment); attrition bias (incomplete outcome data) and reporting bias (selective reporting). Within each domain, we will classify the RoB as high, unclear or low. Reviewers will also judge to determine whether any particular domain is impossible to achieve in any of the primary studies (like blinding in trials comparing GTN patch vs existing standard therapy) and likely or unlikely to affect the reported effect size of the outcome.

Primary studies will be classified as having an overall high RoB when they have been rated at least one domain as having high risk after exclusion of certain domain that is judged to be logistically impossible to achieve for that particular trial and unlikely to affect reported effect size of outcome.

Quality of Evidence

We will also assess the quality of evidence for each outcome using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach that classifies evidence as high, moderate, low or very low quality based on considerations of RoB, consistency, directness, precision and publication bias.[23]

Assessment of the individual and overall RoB categories as well as the quality of evidence will be performed independently by the two pairs of reviewers (LBL and CWL; LWF and NWM) with any discrepancies resolved by discussion and consensus or if necessary, via consultation with an external reviewer.

Data Analysis

All analyses will be performed using RevMan 5.3 (Cochrane Collaboration, Oxford) software. We will use DerSimonian and Laird random-effects model a priori to conduct the data analysis and meta-analysis. We chose the random-effects model as it produces more

conservative confidence intervals and it considers both within- and between-study variability.[20]

For continuous outcomes, we will calculate the mean difference and its corresponding 95% confidence intervals (CIs) whenever possible. For dichotomous outcomes, we will calculate the relative risk (RR) and its corresponding 95% CIs. We will generate forest plots to demonstrate the individual and pooled effect sizes for the outcome of interest if there are at least two studies. We will assess for heterogeneity between studies by first visual inspection of the forest plots and then using the I^2 statistic. I^2 measures the percentage of the total variation in estimated effects of the outcome across studies that is due to heterogeneity rather than to chance.[24] A I^2 value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity.

Regardless of the observed statistical heterogeneity (I^2 values), we plan to conduct the following a priori subgroup analyses for each outcome when each subgroup is represented by at least two studies. These subgroup analyses will be: ICH vs ischaemic stroke; minor (NIHSS ≤ five) vs major (NIHSS > five) ischaemic stroke; ischaemic stroke with vs without thrombolysis; pre-hospital vs non pre-hospital (ED and inpatient) settings; time from stroke to randomization ≤ six vs > six hours and high vs low overall RoB studies.

Meta-analyses may result in Type I errors due to an increased risk of random error when sparse data are collected and repeated significance testing when a cumulative meta-analysis is updated with new trials.[25-26] We will perform trial sequential analysis (TSA) using a random-effects model for the primary outcomes of in-hospital mortality and late functional status. In the TSA, we will use a statistical significance level of 5%, a power of 80% and an estimated effect size difference between transdermal GTN vs placebo or control therapy as reported by the included trials. TSA generates the required information size calculated as

diversity-adjusted information size (DIS)[27] suggested by the estimated effect size difference; thereby providing important information on how many more patients need to be included in further trials. TSA also creates adjusted thresholds for statistical significance (trial sequential monitoring boundaries) with addition of each new trial.[25-26] The cumulative Z curve which includes the selected trials; if it crosses the trial sequential monitoring boundary, will signify that a sufficient level of evidence has been reached and no further trials are needed.[25-26.]If the Z curve fails to cross the trial sequential monitoring boundary, the required information size is not reached and there is insufficient evidence to reach a conclusion.

TSA will be performed using Trial Sequential Analysis v.0.9.5.10 beta (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark, www. ctu.dk/tsa).

PATIENT AND PUBLIC INVOLVEMENT

We have not and will not involve new patients or the public in this protocol.

DISCUSSION

Acute ischaemic and haemorrhagic strokes are frequently managed in various clinical settings; from pre-hospital, ED to inpatient. High BP is common in both types of strokes and is associated with short-term poor outcomes (acute stroke recurrence, death within a few weeks[1-4] and haematoma expansion[6-8]) and adverse effects in the longer-term (delayed death and dependency after several months[1-4]). BP control is an essential part of the management of acute ischaemic and haemorrhagic strokes.

NO donors are candidate agents to lower BP in acute stroke because of its various beneficial properties ranging from vasodilatation to neuroprotection and inhibition of apoptosis.[14] GTN is an example of a NO donor. Transdermal GTN offers an easily

administered formulation which is valuable especially in the pre-hospital and ED settings to provide constant drug release.

There was only a meta-analysis published in 2016[17] that investigated the effects of transdermal GTN in acute stroke which reported no overall benefits. However, it reported a favourable functional outcome (improvement in mRS at 90 days) with transdermal GTN vs placebo or control therapy in a pre-specified subgroup of patients with ultra-acute stroke (time from stroke to randomization ≤ six hours). The meta-analysis had important limitations. Apart from the ENOS trial[18], the remaining four included trials had small sample sizes (n≤ 90) and all these trials were conducted by a single research group. In addition, that subgroup analysis involving ultra-acute stroke patients also suffered from a small sample size (n= 312).

With the inclusion of the recently published multi-centre RIGHT-2 trial which recruited 1149 patients with acute stroke within four hours of onset[19], our systematic review and meta-analysis will significantly increase the sample size available for pooling of studies; especially so when it will more than triple that used to examine the role of transdermal GTN in ultra-acute stroke (onset ≤ six hours). Our planned subgroup analysis of patients with ultra-acute stroke will address a significant gap in the literature that arose from the previous meta-analysis in 2016.

In addition, our TSA for the important primary outcomes of in-hospital mortality and late functional status will reduce Type I error. Our TSA will determine whether the DIS and trial sequential monitoring boundaries for these outcomes have indeed been reached in our meta-analysis; signifying that a sufficient level of evidence has been attained to reach a conclusion.

Other strengths of our protocol include a comprehensive search strategy of published and unpublished literature, extensive subgroup analyses involving clinically important patient subgroups and using GRADE methodology to assess certainty of evidence.

Limitations to our protocol include the anticipated high clinical heterogeneity given the haemorrhagic and ischaemic subtypes of acute stroke as well as variation in timing of randomization from stroke onset to transdermal GTN or placebo/control therapy and reporting of outcome measures across trials even within a subtype of acute stroke. We will address clinical heterogeneity by evaluating for statistical heterogeneity, explore pre-defined clinically important subgroup analyses and to account for inconsistencies in our GRADE evaluation. In order to address for differences in reporting of outcome measures across included trials, we will include a spectrum of primary and secondary outcomes. We will assess reporting of these outcomes independently and in duplicate and if there are discrepancies, we will resolve through discussion, consensus, potentially involving an external reviewer and contact the primary authors for clarification.

In conclusion, this protocol describes the details and methodology of a planned systematic review and meta-analysis addressing the safety and benefits of transdermal GTN in acute stroke. The results of this meta-analysis are expected to fill the gap in the literature on the subgroup of patients with ultra-acute stroke (onset ≤ 6 hours), inform daily practice, clinical practice guidelines and guide areas of investigation for future RCTs.

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COMPETING INTERESTS

There are no completing interests to disclose.

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CONTRIBUTIONS

LBL, CWL and LWF conceived the study. LBL, CWL and LWF also wrote the study protocol to be registered with PROSPERO.

All authors contributed to protocol development. LBL, CWL and LWF drafted the study protocol and this manuscript. All authors contributed to refinement of the study protocol and manuscript as well as approved the final manuscript.

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Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1,2,6
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	2,3,5

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	6
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	14
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	NA
Support			
Sources	#5a	Indicate sources of financial or other support for the review	14
Sponsor	#5b	Provide name for the review funder and / or sponsor	14
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	14
Introduction			

Rationale	#6	Describe the rationale for the review in the context of what is already known	4,5,6
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6,7
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
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	7
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	7,8
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8

Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
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	8,9
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	9,10,11
Data synthesis	#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 (such as I2, Kendall's τ)	9,10,11
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10

Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	NA
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	9

BMJ Open

Benefits and safety of transdermal glyceryl trinitrate in acute stroke: a systematic review and meta-analysis of randomised trials (Protocol)

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Benefits and safety of transdermal glyceryl trinitrate in acute stroke: a systematic review and meta-analysis of randomised trials (Protocol)

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ABSTRACT:

Introduction: High blood pressure (BP) in acute stroke has adverse outcomes. Transdermal glyceryl trinitrate (GTN) has beneficial properties in controlling BP. The 2016 meta-analysis and 2017 Cochrane review showed transdermal GTN was beneficial in a small patient subgroup with stroke onset ≤ six hours. Larger studies focusing on this patient subgroup have since been conducted. We report the protocol for an updated systematic review and meta-analysis on the safety and benefits of transdermal GTN in acute stroke.

Methods and Analysis: We will search Medline, Pubmed, Embase, CINAHL and Cochrane Library from inception until June 2020 for randomised trials that report the efficacy and safety of transdermal GTN vs placebo/control therapy among adult patients with acute stroke. Primary outcomes include in-hospital mortality, BP lowering and late functional status. Secondary outcomes include early, late, resource utilisation and surrogate outcomes. Safety outcomes include reported adverse events. Reviewers will first screen titles and abstracts, and then full texts, to identify eligible studies. Independently and in duplicate, they will extract data, assess risk of bias (RoB) using a modified Cochrane RoB tool and quality of evidence using GRADE. Disagreement will be resolved by discussion and consultation with an external reviewer if necessary. Using a random-effects model, we will report effect sizes using relative risks and 95% confidence intervals. We will perform pre-defined subgroup analyses: intracerebral haemorrhage vs ischaemic stroke; minor (NIHSS ≤ five) vs major (NIHSS > five) ischaemic stroke; ischaemic stroke with vs without thrombolysis; pre-hospital vs non pre-hospital settings; time from stroke to randomisation ≤ six vs > six hours and high vs low overall RoB studies. We will also perform trial sequential analysis for the primary outcomes.

Ethics and Dissemination: Ethics board approval is unnecessary. PROSPERO registration has been obtained (CRD42020173093). The results will be disseminated through publication in a peer-reviewed journal.

ARTICLE SUMMARY

Strengths and Limitations of Study

- This is an updated meta-analysis which includes more recent larger trials.
- This study will examine an important gap on the benefits of transdermal GTN in ultra-acute stroke (≤ six hours) identified by previous reviews.
- Other strengths include a comprehensive search strategy, an extensive predefined subgroup analysis plan and inclusion of GRADE methodology to assess certainty of evidence.
- This study will be the first to use trial sequential analysis on important primary outcomes.
- Limitations include high clinical heterogeneity given the different subtypes of acute stroke, variation in timing of randomization from onset of stroke to transdermal GTN or placebo/control therapy and reporting of outcome measures across trials.

INTRODUCTION

High blood pressure (BP) is present in greater than 70% of patients with acute ischaemic stroke.[1] It is associated with poor outcomes including acute stroke recurrence, death within a few weeks or combined death and dependency after several months.[1-4] High BP is similarly common in acute intracerebral haemorrhage (ICH)[5] and may be associated with haematoma expansion and increased mortality.[6-8]

It is recommended to lower BP in ICH[9-10] although controversy exists regarding optimal BP target in patients with ICH and there is no current literature on the role of pre-hospital BP reduction. The management of high BP in acute ischaemic stroke and the decision to treat or not to treat has been a constant debate since 1985. Current available guidelines recommend withholding antihypertensive therapy in the early post-stroke period unless there is markedly elevated BP (>220/120mmHg) or with BP >185/110mmHg for patients eligible for thrombolysis or BP >180/105mmHg during the 24-hour period following reperfusion.[11-13]

Nitric oxide (NO) donors are candidate agents to lower BP in acute stroke because NO is a cerebral and systemic vasodilator, modulates vascular and neuronal function, is neuroprotective, and inhibits apoptosis.[14] In addition, vascular NO concentrations are low in acute stroke which are associated with increased severity of stroke, mortality and institutionalization.[15] These observations support that NO supplementation might be beneficial.

Glyceryl trinitrate (GTN) is an example of a NO donor. Transdermal GTN administration offers a constant release of the drug across the skin into the systemic circulation for 24 hours which achieves sustained steady-state plasma concentrations.[16] Transdermal GTN offers a formulation which is easily administered in many clinical settings [pre-hospital, Emergency

Department (ED) and inpatient] managing acute stroke which may help to minimise fluctuations in drug concentrations and hence BP.

The latest meta-analysis published in 2016[17] and Cochrane review in 2017[18], using data from five completed transdermal GTN trials (n= 4197), reported no improvement in outcomes across a range of domains (death, disability, cognition, mood, and quality of life with transdermal GTN vs placebo or control therapy. However, in a pre-specified subgroup analysis of patients with time from stroke to randomisation ≤ six vs > six hours (n= 312), these two reviews reported a favourable functional outcome as measured by modified Rankin Scale (mRS) at 90 days with transdermal GTN. There were important limitations in these reviews. Four out of five selected trials had small sample sizes (n≤ 90). The remaining multi-centre ENOS trial[19] recruited 4011 patients and it dominated the pooled analysis (95.6% of all patients and 86.9% of those randomised within six hours of onset). In addition, all the included trials were conducted by a single research group and it is important that other research groups study the role of transdermal GTN in acute stroke. Finally, a relatively small number of patients (n= 312) were treated within six hours of stroke onset and these patients came from just two of the five trials.

The recently published multi-centre RIGHT-2 trial randomised 1149 participants with acute stroke within four hours of onset to receive transdermal GTN vs sham therapy.[20] The data from this study more than triples that used to examine the role of transdermal GTN in ultra-early stroke (onset ≤ six hours). There is an urgent need to update the evidence behind the efficacy and safety of transdermal GTN in acute stroke especially among those patients with ultra-early (≤ six hours) presentation.

The aim of this systematic review and meta-analysis is to examine, using recent data, whether transdermal GTN improves important patient centred outcomes and is safe among

patients with acute stroke in the pre-hospital and in-hospital settings compared to placebo or control therapy by reviewing randomised controlled trials (RCTs).

METHODS

Study Registration

This systematic review and meta-analysis protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO). The reference number is CRD42020173093. We will adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement for reporting systematic review and meta-analysis.[21]

Eligibility Criteria

We will include randomised trials investigating the efficacy and safety of transdermal GTN vs placebo or control therapy among adult patients presenting with acute stroke.

Patients aged ≥ 16 years presenting with either acute ischaemic or haemorrhagic stroke in the pre-hospital, ED and inpatient clinical settings will be considered. Acute stroke patients are defined as those with presentation within five days of onset of symptoms. We select five days since onset of symptoms as the inclusion cut-off criterion for this review because there can be significant delay in presentation after an acute stroke, especially for less severe ischaemic strokes.[22] Patients with ischaemic stroke are included regardless of whether they receive thrombolysis. The comparator arms will include transdermal GTN patch, sham patch and control with existing standard therapy.

Primary outcomes are important patient centred outcomes including in-hospital mortality, lowering of BP measurements and late functional status. BP parameters will include systolic BP, diastolic BP and mean arterial pressure measured at intervals stated by the authors.

Late functional status will involve assessment using the mRS within three months of stroke onset or later (as reported by the authors); the preferred outcome measurement for acute stroke trials.[23] The hierarchical mRS scores range from 0 to 6, with a score of 0 indicating no symptoms, 1 indicating some symptoms but no significant disability, 2–5 indicating increasing levels of disability and dependency, and 6 indicating death.[23]

Secondary outcomes are classified as early, late, resource utilisation and surrogate outcomes. Early secondary outcomes include development of ICH, recurrent stroke and change in calculated National Institutes of Health Stroke Scale (NIHSS). Late secondary outcomes include reported changes in activities of daily living, cognition, quality of life and mood. Resource utilisation secondary outcomes include length of hospital stay and discharge destination. Surrogate secondary outcomes include changes in cerebral haemodynamics and laboratory parameters like platelet aggregation.

Safety outcomes include any adverse events reported by the authors.

Search Strategy

We will search Medline, Pubmed, Embase, CINAHL and Cochrane Library from inception until June 2020 without language restrictions. We will review reference lists for eligible new trials and also search ClinicalTrials.gov for ongoing or unpublished trials and for additional data from published trials. The search strategy will include the following keywords: stroke, ischaemic stroke, haemorrhagic stroke, intracerebral haemorrhage, transdermal glyceryl trinitrate, transdermal nitroglycerin, glyceryl trinitrate patch, nitroglycerin patch, trinitroglycerin, pre-hospital, mortality, blood pressure, functional outcome, humans and randomised clinical trials. Medical Subject Heading (MeSH) terms will include acute stroke, brain infarction, brain haemorrhage, prehospital emergency care, nitroglycerin, nitric oxide

donors, blood pressure, haemodynamics and cerebral haemodynamics. A proposed search strategy on Medline using the Pubmed interface is attached as Appendix 1.

Study Selection

Reviewers (LBL, CWL, LWF and NWM) will independently and in duplicate screen the titles and abstracts of all identified studies to generate a list of eligible trials from which full texts will be obtained. Subsequently, the same reviewers will independently assess eligibility of these full texts of published trials to decide on the final included studies. Discrepancies between reviewers will be resolved through discussion and consensus or, if needed, by adjudication from an external reviewer and/or contact with authors of the original trials for clarification.

Data Extraction

Two pairs of reviewers (LBL and CWL; LWF and NWM) will extract data from included studies both independently and in duplicate. Data will be extracted using a pre-designed data extraction form adapted from the Cochrane Collaboration.[24] The data collection form is attached as Appendix 2. Data extracted will include the following: general study information [authors, publication year and study location(s)]; study population details [clinical setting- pre-hospital vs ED vs inpatient, sample size, types of strokes- ischaemic vs haemorrhagic; subgroup of ischaemic strokes with thrombolysis]; details on the comparator arms [different doses and duration of GTN patch; sham patch and control] as well as the primary, secondary and safety outcomes as listed above.

In randomized trials that included more than one arm of GTN dosing and duration, we will extract data from the arm closest to a single dose regimen that is comparable to other primary studies to be used for analysis.

Discrepancies in data extraction will be resolved through discussion and consensus or, if needed, via an external reviewer and/or contact with authors of the original trials for clarification.

Risk OF Bias Assessment

We will assess the risk of bias (RoB) for each outcome of the individual studies using a modified Cochrane RoB instrument.[25] The instrument assesses biases in the following five domains: selection bias (random sequence generation and allocation concealment); performance bias (blinding of participants and researchers); detection bias (blinding of outcome assessment); attrition bias (incomplete outcome data) and reporting bias (selective reporting). Within each domain, we will classify the RoB as high, unclear or low. Reviewers will also judge to determine whether any particular domain is impossible to achieve in any of the primary studies (like blinding in trials comparing GTN patch vs existing standard therapy) and likely or unlikely to affect the reported effect size of the outcome.

Primary studies will be classified as having an overall high RoB when they have been rated at least one domain as having high risk after exclusion of certain domain that is judged to be logistically impossible to achieve for that particular trial and unlikely to affect reported effect size of outcome. The overall RoB for each individual trial will be considered low if RoB is judged to be low in all domains and unclear if RoB is judged to be unclear in any of the domains.

Quality of Evidence

We will also assess the quality of evidence for each outcome using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach that classifies evidence as high, moderate, low or very low quality based on considerations of RoB, consistency, directness, precision and publication bias.[26] We attach a summary of findings

table (Appendix 3) which is adapted using the GRADEpro software to demonstrate how we will present our GRADE assessment for the main outcomes.

Assessment of the individual and overall RoB categories as well as the quality of evidence will be performed independently by the two pairs of reviewers (LBL and CWL; LWF and NWM) with any discrepancies resolved by discussion and consensus or if necessary, via consultation with an external reviewer.

Data Analysis

All analyses will be performed using RevMan 5.3 (Cochrane Collaboration, Oxford) software. We will use DerSimonian and Laird random-effects model a priori to conduct the data analysis and meta-analysis. We chose the random-effects model as it produces more conservative confidence intervals and it considers both within- and between-study variability.[21]

For continuous outcomes, we will calculate the mean difference and its corresponding 95% confidence intervals (CIs) whenever possible. For dichotomous outcomes, we will calculate the relative risk (RR) and its corresponding 95% CIs. We will generate forest plots to demonstrate the individual and pooled effect sizes for the outcome of interest if there are at least two studies. We will assess for heterogeneity between studies by first visual inspection of the forest plots and then using the I^2 statistic. I^2 measures the percentage of the total variation in estimated effects of the outcome across studies that is due to heterogeneity rather than to chance.[27] A I^2 value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity.

Regardless of the observed statistical heterogeneity (I^2 values), we plan to conduct the following a priori subgroup analyses for each outcome when each subgroup is represented by at least two studies. These subgroup analyses will be: ICH vs ischaemic stroke; minor

(NIHSS ≤ five) vs major (NIHSS > five) ischaemic stroke; ischaemic stroke with vs without thrombolysis; pre-hospital vs non pre-hospital (ED and inpatient) settings; time from stroke to randomization ≤ six vs > six hours; time from stroke to randomization ≤ two vs > two hours and high vs low overall RoB studies.

Missing data in the primary studies will be addressed in several ways. We will evaluate for rates of missing data in these primary studies, reasons for missing data and to contact primary authors for clarification if necessary. We will determine whether authors of these primary studies attempted to address the impact of missing data by using intention-to-treat analysis and performing sensitivity analyses through methods like imputation, best- and worst-case scenario analyses to investigate how their reported effect size estimates had changed. We will then make judgement independently, through consensus and/or consultation with an external reviewer whether the reported effect size estimates (including any sensitivity analyses) by the primary authors will likely or unlikely be affected by their missing data. We will perform separate sensitivity analyses of our pooled results by including and excluding those studies that are judged likely to be affected by missing data to investigate how the pooled effect size estimates will be affected. Finally, we will also assess the risk of missing data (attrition bias) of the primary studies through our RoB and GRADE assessment.

Meta-analyses may result in Type I errors due to an increased risk of random error when sparse data are collected and repeated significance testing when a cumulative meta-analysis is updated with new trials.[28-29] We will perform trial sequential analysis (TSA) using a random-effects model for the primary outcomes (in-hospital mortality, BP lowering and late functional status). In the TSA, we will use a statistical significance level of 5%, a power of 80% and an estimated effect size difference (or mean difference for continuous outcomes) between transdermal GTN vs placebo or control therapy as reported by the included trials.

TSA generates the required information size calculated as diversity-adjusted information size (DIS)[30] suggested by the estimated effect size difference; thereby providing important information on how many more patients need to be included in further trials. TSA also creates adjusted thresholds for statistical significance (trial sequential monitoring boundaries) with addition of each new trial.[28-29] The cumulative Z curve which includes the selected trials; if it crosses the trial sequential monitoring boundary, will signify that a sufficient level of evidence has been reached and no further trials are needed.[28-29] If the Z curve fails to cross the trial sequential monitoring boundary, the required information size is not reached and there is insufficient evidence to reach a conclusion.

TSA will be performed using Trial Sequential Analysis v.0.9.5.10 beta (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark, www. ctu.dk/tsa).

PATIENT AND PUBLIC INVOLVEMENT

We have not and will not involve new patients or the public in this protocol.

DISCUSSION

Acute ischaemic and haemorrhagic strokes are frequently managed in various clinical settings; from pre-hospital, ED to inpatient. High BP is common in both types of strokes and is associated with short-term poor outcomes (acute stroke recurrence, death within a few weeks[1-4] and haematoma expansion[6-8]) and adverse effects in the longer-term (delayed death and dependency after several months[1-4]). BP control is an essential part of the management of acute ischaemic and haemorrhagic strokes.

NO donors are candidate agents to lower BP in acute stroke because of its various beneficial properties ranging from vasodilatation to neuroprotection and inhibition of apoptosis.[14] GTN is an example of a NO donor. Transdermal GTN offers an easily

administered formulation which is valuable especially in the pre-hospital and ED settings to provide constant drug release.

There was a meta-analysis in 2016[17] and Cochrane review in 2017[18] that investigated the effects of transdermal GTN in acute stroke which reported no overall benefits. However, they reported a favourable functional outcome (improvement in mRS at 90 days) with transdermal GTN vs placebo or control therapy in a pre-specified subgroup of patients with ultra-acute stroke (time from stroke to randomization ≤ six hours). The meta-analysis and Cochrane review had important limitations. Apart from the ENOS trial[19], the remaining four included trials had small sample sizes (n≤ 90) and all these trials were conducted by a single research group. In addition, that subgroup analysis involving ultra-acute stroke patients also suffered from a small sample size (n= 312).

With the inclusion of the recently published multi-centre RIGHT-2 trial which recruited 1149 patients with acute stroke within four hours of onset[20], our systematic review and meta-analysis will significantly increase the sample size available for pooling of studies; especially so when it will more than triple that used to examine the role of transdermal GTN in ultra-acute stroke (onset ≤ six hours). Our planned subgroup analysis of patients with ultra-acute stroke will address a significant gap in the literature that arose from these previous reviews.

In addition, our TSA for the important primary outcomes will reduce Type I error. Our TSA will determine whether the DIS and trial sequential monitoring boundaries for these outcomes have indeed been reached in our meta-analysis; signifying that a sufficient level of evidence has been attained to reach a conclusion.

Other strengths of our protocol include a comprehensive search strategy of published and unpublished literature, extensive subgroup analyses involving clinically important patient subgroups and using GRADE methodology to assess certainty of evidence.

Limitations to our protocol include the anticipated high clinical heterogeneity given the haemorrhagic and ischaemic subtypes of acute stroke as well as variation in timing of randomization from stroke onset to transdermal GTN or placebo/control therapy and reporting of outcome measures across trials even within a subtype of acute stroke. We will address clinical heterogeneity by evaluating for statistical heterogeneity, explore pre-defined clinically important subgroup analyses and to account for inconsistencies in our GRADE evaluation. In order to address for differences in reporting of outcome measures across included trials, we will include a spectrum of primary and secondary outcomes. We will assess reporting of these outcomes independently and in duplicate and if there are discrepancies, we will resolve through discussion, consensus, potentially involving an external reviewer and contacting the primary authors for clarification.

In conclusion, this protocol describes the details and methodology of a planned systematic review and meta-analysis addressing the safety and benefits of transdermal GTN in acute stroke. The results of this meta-analysis are expected to fill the gap in the literature on the subgroup of patients with ultra-acute stroke (onset ≤ 6 hours), inform daily practice, clinical practice guidelines and guide areas of investigation for future RCTs.

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COMPETING INTERESTS

There are no completing interests to disclose.

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CONTRIBUTIONS

LBL, CWL and LWF conceived the study. LBL, CWL and LWF also wrote the study protocol to be registered with PROSPERO.

All authors (LBL, CWL, LWF, NWM and TWL) contributed to protocol development. LBL, CWL and LWF drafted the study protocol and this manuscript. All authors (LBL, CWL, LWF, NWM and TWL) contributed to refinement of the study protocol and manuscript as well as approved the final manuscript.

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Appendix 1. Medline Search Strategy Through Pubmed Interface

Filters:

Time period: 1st Jan 1966 to 30th June 2020

Article type: Clinical Study, Clinical Trial Phases 1 to IV, Controlled Clinical Trial, Pragmatic Clinical Trial

Species: Humans

Language restrictions: No

Age limits: 13 to 80+ years

#	Searches
1.	Acute stroke/
2.	Ischaemic stroke (tw)
3.	Haemorrhagic stroke (tw)
4.	Intracerebral haemorrhage (tw)
5.	Brain infarction/
6.	Brain haemorrhage/
7.	Prehospital emergency care/
8.	1-7 or
9.	Transdermal glyceryl trinitrate (tw)
10.	Nitroglycerin/
11.	Trinitroglycerin (tw)
12.	GTN (tw)
13.	Nitric oxide donor*/
14.	9 -13 or
15.	Mortality (tw)
16.	Death (tw)
17.	Blood pressure/
18.	Functional outcome* (tw)
19.	Haemodynamic*/
20.	Cerebral haemodynamic*/
21.	15-20 or
22.	8 and 14 and 21

Appendix 2. Data Collection Form (Adapted from Cochrane Collaboration)

General Information

Date form completed	
Name of reviewer extracting data	
Contact details of reviewer extracting data	
Title of publication	
Publication ID (first author and year of publication)	
Country in which study was conducted	
Study funding source	
Possible conflicts of interests for study authors	

Primary Study Details

1. Methods

Study Characteristics	Review Inclusion Criteria
Design (Type of randomized trial)	Blinded vs non-blinded Cross-over present
Method(s) of recruitment of participants	
Unit of allocation (individual vs cluster/group)	
Clinical setting	Pre-hospital vs Emergency Department vs Hospital
Types of intervention	Different dosing regimens of transdermal GTN

Types of comparator	Standard therapy vs placebo
Types of outcome measures	Primary: Secondary: Safety:

2. Study Population and Setting

Study population description	Stroke subtypes: haemorrhagic vs ischaemic Stroke onset to randomization Other stroke subgroups (like IV thrombolytics, etc)
Inclusion criteria	
Exclusion criteria	
Start date	
End date	
Duration of participation (recruitment to last follow-up)	

3. Participants (in intervention vs control/placebo groups)

Total number of individuals randomized	Intervention group: Control/ placebo group:
Total number of clusters randomized (if applicable)	Intervention group: Control/ placebo group:
Number of withdrawals/exclusions	Intervention group: Control/ placebo group:
Number of cross-overs	Intervention group:

	Control/ placebo group:
Baseline imbalances	
Other treatments (apart from intervention vs control/placebo)	Intervention group: Control/ placebo group:
Subgroups measured	
Subgroups reported	

4. Outcomes (create a separate section for each outcome)

Outcome name	
Outcome type (Primary vs secondary vs safety)	
Time points when outcome was measured (from start or at end of intervention or control/placebo)	
Time points reported	
Outcome definition	
Method(s) of outcome assessment (using any tool/scale, etc)	
Is the outcome assessment tool validated?	
Persons measuring and /or reporting outcome	
Imputation of missing data	
Analysis via intention-to-treat or per-protocol or both	

5. Results (create a separate section for each outcome)

Outcome	

Dichotomous or continuous	
Subgroup	
Time point	
Results (may have more than two arms)	Intervention group: Control/placebo group
Number of missing participants	Intervention group: Control/placebo group:
Number of cross-over	Intervention group: Control/placebo group:
Statistical methods used and appropriateness of these methods	

Risk of Bias Assessment (create a separate section for each outcome)

Domain	Risk of bias (High/Low/Unclear)	Support for Judgement
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)		
Other bias		

Appendix 3. Summary of findings table

Certainty assessment							No of patients		Effect		Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Transdermal GTN	Control/placebo therapy	Relative (95% CIs)	Absolute (95% CIs)		
In-hospital mortality (follow up: range 1 day to 3 months) [Primary Outcome]												
Mean arterial pressure (follow up: range 1 day to 10 days) [Primary Outcome]												
Modified Rankin Scale (follow up: range 3 months to 12 months) [Primary Outcome]												
Development of intracerebral haemorrhage (follow up: range 1 day to 10 days) [Secondary Outcome]												
Deterioration of NIHSS scores by at least 4 points during hospitalization (follow up: range 1 day to 10 days) [Secondary Outcome]												
Length of hospital stay (follow up: range 1 day to 3 months) [Secondary Outcome]												
Number of hypotensive episodes requiring intervention* (follow up: range 1 day to 10 days) [Safety Outcome}												

GTN: Glyceryl trinitrate; CIs: Confidence intervals; NIHSS: National Institutes of Health Stroke Scale
*Interventions include discontinuing transdermal GTN, administration of intravenous fluids and/or inotropic drugs

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1,2,6
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	2,3,5

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	6
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	14
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	NA
Support			
Sources	#5a	Indicate sources of financial or other support for the review	14
Sponsor	#5b	Provide name for the review funder and / or sponsor	14
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	14
Introduction			

Rationale	#6	Describe the rationale for the review in the context of what is already known	4,5,6
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6,7
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
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	7
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	7,8
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8

Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
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	8,9
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	9,10,11
Data synthesis	#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 (such as I2, Kendall's τ)	9,10,11
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10

Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	NA
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	9

BMJ Open

Benefits and safety of transdermal glyceryl trinitrate in acute stroke: a systematic review and meta-analysis of randomised trials (Protocol)

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Benefits and safety of transdermal glyceryl trinitrate in acute stroke: a systematic review and meta-analysis of randomised trials (Protocol)

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ABSTRACT:

Introduction: High blood pressure (BP) in acute stroke has adverse outcomes. Transdermal glyceryl trinitrate (GTN) has beneficial properties in controlling BP. The 2016 meta-analysis and 2017 Cochrane review showed transdermal GTN was beneficial in a small patient subgroup with stroke onset ≤ six hours. Larger studies focusing on this patient subgroup have since been conducted. We report the protocol for an updated systematic review and meta-analysis on the safety and benefits of transdermal GTN in acute stroke.

Methods and Analysis: We will search Medline, Pubmed, Embase, CINAHL and Cochrane Library from inception until June 2020 for randomised trials that report the efficacy and safety of transdermal GTN vs placebo/control therapy among adult patients with acute stroke. Primary outcomes include in-hospital mortality, BP lowering and late functional status. Secondary outcomes include early, late, resource utilisation and surrogate outcomes. Safety outcomes include reported adverse events. Reviewers will first screen titles and abstracts, and then full texts, to identify eligible studies. Independently and in duplicate, they will extract data, assess risk of bias (RoB) using a modified Cochrane RoB tool and quality of evidence using GRADE. Disagreement will be resolved by discussion and consultation with an external reviewer if necessary. Using a random-effects model, we will report effect sizes using relative risks and 95% confidence intervals. We will perform pre-defined subgroup analyses: intracerebral haemorrhage vs ischaemic stroke; minor (NIHSS ≤ five) vs major (NIHSS > five) ischaemic stroke; ischaemic stroke with vs without thrombolysis; pre-hospital vs non pre-hospital settings; time from stroke to randomisation ≤ six vs > six hours and high vs low overall RoB studies. We will also perform trial sequential analysis for the primary outcomes.

Ethics and Dissemination: Ethics board approval is unnecessary. PROSPERO registration has been obtained (CRD42020173093). The results will be disseminated through publication in a peer-reviewed journal.

ARTICLE SUMMARY

Strengths and Limitations of Study

- This is an updated meta-analysis which includes more recent larger trials.
- This study will examine an important gap on the benefits of transdermal GTN in ultra-acute stroke (≤ six hours) identified by previous reviews.
- Other strengths include a comprehensive search strategy, an extensive predefined subgroup analysis plan and inclusion of GRADE methodology to assess certainty of evidence.
- This study will be the first to use trial sequential analysis on important primary outcomes.
- Limitations include high clinical heterogeneity given the different subtypes of acute stroke, variation in timing of randomization from onset of stroke to transdermal GTN or placebo/control therapy and reporting of outcome measures across trials.

INTRODUCTION

High blood pressure (BP) is present in greater than 70% of patients with acute ischaemic stroke.[1] It is associated with poor outcomes including acute stroke recurrence, death within a few weeks or combined death and dependency after several months.[1-4] High BP is similarly common in acute intracerebral haemorrhage (ICH)[5] and may be associated with haematoma expansion and increased mortality.[6-8]

It is recommended to lower BP in ICH[9-10] although controversy exists regarding optimal BP target in patients with ICH and there is no current literature on the role of pre-hospital BP reduction. The management of high BP in acute ischaemic stroke and the decision to treat or not to treat has been a constant debate since 1985. Current available guidelines recommend withholding antihypertensive therapy in the early post-stroke period unless there is markedly elevated BP (>220/120mmHg) or with BP >185/110mmHg for patients eligible for thrombolysis or BP >180/105mmHg during the 24-hour period following reperfusion.[11-13]

Nitric oxide (NO) donors are candidate agents to lower BP in acute stroke because NO is a cerebral and systemic vasodilator, modulates vascular and neuronal function, is neuroprotective, and inhibits apoptosis.[14] In addition, vascular NO concentrations are low in acute stroke which are associated with increased severity of stroke, mortality and institutionalization.[15] These observations support that NO supplementation might be beneficial.

Glyceryl trinitrate (GTN) is an example of a NO donor. Transdermal GTN administration offers a constant release of the drug across the skin into the systemic circulation for 24 hours which achieves sustained steady-state plasma concentrations.[16] Transdermal GTN offers a formulation which is easily administered in many clinical settings [pre-hospital, Emergency

Department (ED) and inpatient] managing acute stroke which may help to minimise fluctuations in drug concentrations and hence BP.

The latest meta-analysis published in 2016[17] and Cochrane review in 2017[18], using data from five completed transdermal GTN trials (n= 4197), reported no improvement in outcomes across a range of domains (death, disability, cognition, mood, and quality of life with transdermal GTN vs placebo or control therapy. However, in a pre-specified subgroup analysis of patients with time from stroke to randomisation ≤ six vs > six hours (n= 312), these two reviews reported a favourable functional outcome as measured by modified Rankin Scale (mRS) at 90 days with transdermal GTN. There were important limitations in these reviews. Four out of five selected trials had small sample sizes (n≤ 90). The remaining multi-centre ENOS trial[19] recruited 4011 patients and it dominated the pooled analysis (95.6% of all patients and 86.9% of those randomised within six hours of onset). In addition, all the included trials were conducted by a single research group and it is important that other research groups study the role of transdermal GTN in acute stroke. Finally, a relatively small number of patients (n= 312) were treated within six hours of stroke onset and these patients came from just two of the five trials.

The recently published multi-centre RIGHT-2 trial randomised 1149 participants with acute stroke within four hours of onset to receive transdermal GTN vs sham therapy.[20] The data from this study more than triples that used to examine the role of transdermal GTN in ultra-early stroke (onset ≤ six hours). There is an urgent need to update the evidence behind the efficacy and safety of transdermal GTN in acute stroke especially among those patients with ultra-early (≤ six hours) presentation.

The aim of this systematic review and meta-analysis is to examine, using recent data, whether transdermal GTN improves important patient centred outcomes and is safe among

patients with acute stroke in the pre-hospital and in-hospital settings compared to placebo or control therapy by reviewing randomised controlled trials (RCTs).

METHODS

Study Registration

This systematic review and meta-analysis protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO). The reference number is CRD42020173093. We will adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement for reporting systematic review and meta-analysis.[21]

Eligibility Criteria

We will include randomised trials investigating the efficacy and safety of transdermal GTN vs placebo or control therapy among adult patients presenting with acute stroke.

Patients aged ≥ 16 years presenting with either acute ischaemic or haemorrhagic stroke in the pre-hospital, ED and inpatient clinical settings will be considered. Acute stroke patients are defined as those with presentation within five days of onset of symptoms. We select five days since onset of symptoms as the inclusion cut-off criterion for this review because there can be significant delay in presentation after an acute stroke, especially for less severe ischaemic strokes.[22] Patients with ischaemic stroke are included regardless of whether they receive thrombolysis. The comparator arms will include transdermal GTN patch, sham patch and control with existing standard therapy.

Primary outcomes are important patient centred outcomes including in-hospital mortality, lowering of BP measurements and late functional status. BP parameters will include systolic BP, diastolic BP and mean arterial pressure measured at intervals stated by the authors.

Late functional status will involve assessment using the mRS within three months of stroke onset or later (as reported by the authors); the preferred outcome measurement for acute stroke trials.[23] The hierarchical mRS scores range from 0 to 6, with a score of 0 indicating no symptoms, 1 indicating some symptoms but no significant disability, 2–5 indicating increasing levels of disability and dependency, and 6 indicating death.[23]

Secondary outcomes are classified as early, late, resource utilisation and surrogate outcomes. Early secondary outcomes include development of ICH, recurrent stroke and change in calculated National Institutes of Health Stroke Scale (NIHSS). Late secondary outcomes include reported changes in activities of daily living, cognition, quality of life and mood. Resource utilisation secondary outcomes include length of hospital stay and discharge destination. Surrogate secondary outcomes include changes in cerebral haemodynamics and laboratory parameters like platelet aggregation.

Safety outcomes include any adverse events reported by the authors.

Search Strategy

We will search Medline, Pubmed, Embase, CINAHL and Cochrane Library from inception until June 2020 without language restrictions. We will review reference lists for eligible new trials and also search ClinicalTrials.gov for ongoing or unpublished trials and for additional data from published trials. The search strategy will include the following keywords: stroke, ischaemic stroke, haemorrhagic stroke, intracerebral haemorrhage, transdermal glyceryl trinitrate, transdermal nitroglycerin, glyceryl trinitrate patch, nitroglycerin patch, trinitroglycerin, pre-hospital, mortality, blood pressure, functional outcome, humans and randomised clinical trials. Medical Subject Heading (MeSH) terms will include acute stroke, brain infarction, brain haemorrhage, prehospital emergency care, nitroglycerin, nitric oxide

donors, blood pressure, haemodynamics and cerebral haemodynamics. A proposed search strategy on Medline using the Pubmed interface is attached as Appendix 1.

Study Selection

Reviewers (LBL, CWL, LWF and NWM) will independently and in duplicate screen the titles and abstracts of all identified studies to generate a list of eligible trials from which full texts will be obtained. Subsequently, the same reviewers will independently assess eligibility of these full texts of published trials to decide on the final included studies. Discrepancies between reviewers will be resolved through discussion and consensus or, if needed, by adjudication from an external reviewer and/or contact with authors of the original trials for clarification.

Data Extraction

Two pairs of reviewers (LBL and CWL; LWF and NWM) will extract data from included studies both independently and in duplicate. Data will be extracted using a pre-designed data extraction form adapted from the Cochrane Collaboration.[24] The data collection form is attached as Appendix 2. Data extracted will include the following: general study information [authors, publication year and study location(s)]; study population details [clinical setting- pre-hospital vs ED vs inpatient, sample size, types of strokes- ischaemic vs haemorrhagic; subgroup of ischaemic strokes with thrombolysis]; details on the comparator arms [different doses and duration of GTN patch; sham patch and control] as well as the primary, secondary and safety outcomes as listed above.

In randomized trials that included more than one arm of GTN dosing and duration, we will extract data from the arm closest to a single dose regimen that is comparable to other primary studies to be used for analysis.

Discrepancies in data extraction will be resolved through discussion and consensus or, if needed, via an external reviewer and/or contact with authors of the original trials for clarification.

Risk OF Bias Assessment

We will assess the risk of bias (RoB) for each outcome of the individual studies using a modified Cochrane RoB instrument.[25] The instrument assesses biases in the following five domains: selection bias (random sequence generation and allocation concealment); performance bias (blinding of participants and researchers); detection bias (blinding of outcome assessment); attrition bias (incomplete outcome data) and reporting bias (selective reporting). Within each domain, we will classify the RoB as high, unclear or low. Reviewers will also judge to determine whether any particular domain is impossible to achieve in any of the primary studies (like blinding in trials comparing GTN patch vs existing standard therapy) and likely or unlikely to affect the reported effect size of the outcome.

Primary studies will be classified as having an overall high RoB when they have been rated at least one domain as having high risk after exclusion of certain domain that is judged to be logistically impossible to achieve for that particular trial and unlikely to affect reported effect size of outcome. The overall RoB for each individual trial will be considered low if RoB is judged to be low in all domains and unclear if RoB is judged to be unclear in any of the domains.

Quality of Evidence

We will also assess the quality of evidence for each outcome using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach that classifies evidence as high, moderate, low or very low quality based on considerations of RoB, consistency, directness, precision and publication bias.[26] We attach a summary of findings

table (Appendix 3) which is adapted using the GRADEpro software to demonstrate how we will present our GRADE assessment for the main outcomes.

Assessment of the individual and overall RoB categories as well as the quality of evidence will be performed independently by the two pairs of reviewers (LBL and CWL; LWF and NWM) with any discrepancies resolved by discussion and consensus or if necessary, via consultation with an external reviewer.

Data Analysis

All analyses will be performed using RevMan 5.3 (Cochrane Collaboration, Oxford) software. We will use DerSimonian and Laird random-effects model a priori to conduct the data analysis and meta-analysis. We chose the random-effects model as it produces more conservative confidence intervals and it considers both within- and between-study variability.[21]

For continuous outcomes, we will calculate the mean difference and its corresponding 95% confidence intervals (CIs) whenever possible. For dichotomous outcomes, we will calculate the relative risk (RR) and its corresponding 95% CIs. We will generate forest plots to demonstrate the individual and pooled effect sizes for the outcome of interest if there are at least two studies. We will assess for heterogeneity between studies by first visual inspection of the forest plots and then using the I^2 statistic. I^2 measures the percentage of the total variation in estimated effects of the outcome across studies that is due to heterogeneity rather than to chance.[27] A I^2 value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity.

Regardless of the observed statistical heterogeneity (I^2 values), we plan to conduct the following a priori subgroup analyses for each outcome when each subgroup is represented by at least two studies. These subgroup analyses will be: ICH vs ischaemic stroke; minor

(NIHSS ≤ five) vs major (NIHSS > five) ischaemic stroke; ischaemic stroke with vs without thrombolysis; pre-hospital vs non pre-hospital (ED and inpatient) settings; time from stroke to randomization ≤ six vs > six hours; time from stroke to randomization ≤ two vs > two hours and high vs low overall RoB studies.

Missing data in the primary studies will be addressed in several ways. We will evaluate for rates of missing data in these primary studies, reasons for missing data and to contact primary authors for clarification if necessary. We will determine whether authors of these primary studies attempted to address the impact of missing data by using intention-to-treat analysis and performing sensitivity analyses through methods like imputation, best- and worst-case scenario analyses to investigate how their reported effect size estimates had changed. We will then make judgement independently, through consensus and/or consultation with an external reviewer whether the reported effect size estimates (including any sensitivity analyses) by the primary authors will likely or unlikely be affected by their missing data. We will perform separate sensitivity analyses of our pooled results by including and excluding those studies that are judged likely to be affected by missing data to investigate how the pooled effect size estimates will be affected. Finally, we will also assess the risk of missing data (attrition bias) of the primary studies through our RoB and GRADE assessment.

Meta-analyses may result in Type I errors due to an increased risk of random error when sparse data are collected and repeated significance testing when a cumulative meta-analysis is updated with new trials.[28-29] We will perform trial sequential analysis (TSA) using a random-effects model for the primary outcomes (in-hospital mortality, BP lowering and late functional status). In the TSA, we will use a statistical significance level of 5%, a power of 80% and an estimated effect size difference (or mean difference for continuous outcomes) between transdermal GTN vs placebo or control therapy as reported by the included trials.

TSA generates the required information size calculated as diversity-adjusted information size (DIS)[30] suggested by the estimated effect size difference; thereby providing important information on how many more patients need to be included in further trials. TSA also creates adjusted thresholds for statistical significance (trial sequential monitoring boundaries) with addition of each new trial.[28-29] The cumulative Z curve which includes the selected trials; if it crosses the trial sequential monitoring boundary, will signify that a sufficient level of evidence has been reached and no further trials are needed.[28-29] If the Z curve fails to cross the trial sequential monitoring boundary, the required information size is not reached and there is insufficient evidence to reach a conclusion.

TSA will be performed using Trial Sequential Analysis v.0.9.5.10 beta (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark, www. ctu.dk/tsa).

PATIENT AND PUBLIC INVOLVEMENT

We have not and will not involve new patients or the public in this protocol.

DISCUSSION

Acute ischaemic and haemorrhagic strokes are frequently managed in various clinical settings; from pre-hospital, ED to inpatient. High BP is common in both types of strokes and is associated with short-term poor outcomes (acute stroke recurrence, death within a few weeks[1-4] and haematoma expansion[6-8]) and adverse effects in the longer-term (delayed death and dependency after several months[1-4]). BP control is an essential part of the management of acute ischaemic and haemorrhagic strokes.

NO donors are candidate agents to lower BP in acute stroke because of its various beneficial properties ranging from vasodilatation to neuroprotection and inhibition of apoptosis.[14] GTN is an example of a NO donor. Transdermal GTN offers an easily

administered formulation which is valuable especially in the pre-hospital and ED settings to provide constant drug release.

There was a meta-analysis in 2016[17] and Cochrane review in 2017[18] that investigated the effects of transdermal GTN in acute stroke which reported no overall benefits. However, they reported a favourable functional outcome (improvement in mRS at 90 days) with transdermal GTN vs placebo or control therapy in a pre-specified subgroup of patients with ultra-acute stroke (time from stroke to randomization ≤ six hours). The meta-analysis and Cochrane review had important limitations. Apart from the ENOS trial[19], the remaining four included trials had small sample sizes (n≤ 90) and all these trials were conducted by a single research group. In addition, that subgroup analysis involving ultra-acute stroke patients also suffered from a small sample size (n= 312).

With the inclusion of the recently published multi-centre RIGHT-2 trial which recruited 1149 patients with acute stroke within four hours of onset[20], our systematic review and meta-analysis will significantly increase the sample size available for pooling of studies; especially so when it will more than triple that used to examine the role of transdermal GTN in ultra-acute stroke (onset ≤ six hours). Our planned subgroup analysis of patients with ultra-acute stroke will address a significant gap in the literature that arose from these previous reviews.

In addition, our TSA for the important primary outcomes will reduce Type I error. Our TSA will determine whether the DIS and trial sequential monitoring boundaries for these outcomes have indeed been reached in our meta-analysis; signifying that a sufficient level of evidence has been attained to reach a conclusion.

Other strengths of our protocol include a comprehensive search strategy of published and unpublished literature, extensive subgroup analyses involving clinically important patient subgroups and using GRADE methodology to assess certainty of evidence.

Limitations to our protocol include the anticipated high clinical heterogeneity given the haemorrhagic and ischaemic subtypes of acute stroke as well as variation in timing of randomization from stroke onset to transdermal GTN or placebo/control therapy and reporting of outcome measures across trials even within a subtype of acute stroke. We will address clinical heterogeneity by evaluating for statistical heterogeneity, explore pre-defined clinically important subgroup analyses and to account for inconsistencies in our GRADE evaluation. In order to address for differences in reporting of outcome measures across included trials, we will include a spectrum of primary and secondary outcomes. We will assess reporting of these outcomes independently and in duplicate and if there are discrepancies, we will resolve through discussion, consensus, potentially involving an external reviewer and contacting the primary authors for clarification.

In conclusion, this protocol describes the details and methodology of a planned systematic review and meta-analysis addressing the safety and benefits of transdermal GTN in acute stroke. The results of this meta-analysis are expected to fill the gap in the literature on the subgroup of patients with ultra-acute stroke (onset ≤ 6 hours), inform daily practice, clinical practice guidelines and guide areas of investigation for future RCTs.

ETHICS AND DISSEMINATION

Ethics board approval is unnecessary. PROSPERO registration has been obtained (CRD42020173093). The results will be disseminated through publication in a peer-reviewed journal.

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We have no acknowledgments to make.

COMPETING INTERESTS

There are no completing interests to disclose.

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CONTRIBUTIONS

LBL, CWL and LWF conceived the study. LBL, CWL and LWF also wrote the study protocol to be registered with PROSPERO.

All authors (LBL, CWL, LWF, NWM and TWL) contributed to protocol development. LBL, CWL and LWF drafted the study protocol and this manuscript. All authors (LBL, CWL, LWF, NWM and TWL) contributed to refinement of the study protocol and manuscript as well as approved the final manuscript.

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Appendix 1. Medline Search Strategy Through Pubmed Interface

Filters:

Time period: 1st Jan 1966 to 30th June 2020

Article type: Clinical Study, Clinical Trial Phases 1 to IV, Controlled Clinical Trial, Pragmatic Clinical Trial

Species: Humans

Language restrictions: No

Age limits: 13 to 80+ years

#	Searches
1.	Acute stroke/
2.	Ischaemic stroke (tw)
3.	Haemorrhagic stroke (tw)
4.	Intracerebral haemorrhage (tw)
5.	Brain infarction/
6.	Brain haemorrhage/
7.	Prehospital emergency care/
8.	1-7 or
9.	Transdermal glyceryl trinitrate (tw)
10.	Nitroglycerin/
11.	Trinitroglycerin (tw)
12.	GTN (tw)
13.	Nitric oxide donor*/
14.	9 -13 or
15.	Mortality (tw)
16.	Death (tw)
17.	Blood pressure/
18.	Functional outcome* (tw)
19.	Haemodynamic*/
20.	Cerebral haemodynamic*/
21.	15-20 or
22.	8 and 14 and 21

Appendix 2. Data Collection Form (Adapted from Cochrane Collaboration)

General Information

Date form completed	
Name of reviewer extracting data	
Contact details of reviewer extracting data	
Title of publication	
Publication ID (first author and year of publication)	
Country in which study was conducted	
Study funding source	
Possible conflicts of interests for study authors	

Primary Study Details

1. Methods

Study Characteristics	Review Inclusion Criteria
Design (Type of randomized trial)	Blinded vs non-blinded Cross-over present
Method(s) of recruitment of participants	
Unit of allocation (individual vs cluster/group)	
Clinical setting	Pre-hospital vs Emergency Department vs Hospital
Types of intervention	Different dosing regimens of transdermal GTN

Types of comparator	Standard therapy vs placebo
Types of outcome measures	Primary: Secondary: Safety:

2. Study Population and Setting

Study population description	Stroke subtypes: haemorrhagic vs ischaemic Stroke onset to randomization Other stroke subgroups (like IV thrombolytics, etc)
Inclusion criteria	
Exclusion criteria	
Start date	
End date	
Duration of participation (recruitment to last follow-up)	

3. Participants (in intervention vs control/placebo groups)

Total number of individuals randomized	Intervention group: Control/ placebo group:
Total number of clusters randomized (if applicable)	Intervention group: Control/ placebo group:
Number of withdrawals/exclusions	Intervention group: Control/ placebo group:
Number of cross-overs	Intervention group:

	Control/ placebo group:
Baseline imbalances	
Other treatments (apart from intervention vs control/placebo)	Intervention group: Control/ placebo group:
Subgroups measured	
Subgroups reported	

4. Outcomes (create a separate section for each outcome)

Outcome name	
Outcome type (Primary vs secondary vs safety)	
Time points when outcome was measured (from start or at end of intervention or control/placebo)	
Time points reported	
Outcome definition	
Method(s) of outcome assessment (using any tool/scale, etc)	
Is the outcome assessment tool validated?	
Persons measuring and /or reporting outcome	
Imputation of missing data	
Analysis via intention-to-treat or per-protocol or both	

5. Results (create a separate section for each outcome)

Outcome	

Dichotomous or continuous	
Subgroup	
Time point	
Results (may have more than two arms)	Intervention group: Control/placebo group
Number of missing participants	Intervention group: Control/placebo group:
Number of cross-over	Intervention group: Control/placebo group:
Statistical methods used and appropriateness of these methods	

Risk of Bias Assessment (create a separate section for each outcome)

Domain	Risk of bias (High/Low/Unclear)	Support for Judgement
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)		
Other bias		

Appendix 3. Summary of findings table

Certainty assessment							No of patients		Effect		Certainty	Importance
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Transdermal GTN	Control/placebo therapy	Relative (95% CIs)	Absolute (95% CIs)		
In-hospital mortality (follow up: range 1 day to 3 months) [Primary Outcome]												
Mean arterial pressure (follow up: range 1 day to 10 days) [Primary Outcome]												
Modified Rankin Scale (follow up: range 3 months to 12 months) [Primary Outcome]												
Development of intracerebral haemorrhage (follow up: range 1 day to 10 days) [Secondary Outcome]												
Deterioration of NIHSS scores by at least 4 points during hospitalization (follow up: range 1 day to 10 days) [Secondary Outcome]												
Length of hospital stay (follow up: range 1 day to 3 months) [Secondary Outcome]												
Number of hypotensive episodes requiring intervention* (follow up: range 1 day to 10 days) [Safety Outcome}												

GTN: Glyceryl trinitrate; CIs: Confidence intervals; NIHSS: National Institutes of Health Stroke Scale
*Interventions include discontinuing transdermal GTN, administration of intravenous fluids and/or inotropic drugs

Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1,2,6
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	2,3,5

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	6
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	14
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	NA
Support			
Sources	#5a	Indicate sources of financial or other support for the review	14
Sponsor	#5b	Provide name for the review funder and / or sponsor	14
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	14
Introduction			

Rationale	#6	Describe the rationale for the review in the context of what is already known	4,5,6
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
Methods			
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	6,7
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
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	7
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	7,8
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8

Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
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	8,9
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	9,10,11
Data synthesis	#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 (such as I2, Kendall's τ)	9,10,11
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10

Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	NA
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	9