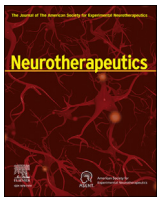
Neurotherapeutics 21 (2024) e00365

Contents lists available at ScienceDirect

Neurotherapeutics

journal homepage: www.sciencedirect.com/journal/neurotherapeutics



Original Article

Rapid Intravenous Glyceryl Trinitrate in Ischemic Damage (RIGID): A potential neuroprotection strategy for acute ischemic stroke (AIS) patients

Lipeng Cai [a], Yuchuan Ding [b,*], Gary Rajah [c], Yanna Tong [a], Honglian Duan [a], Zhenzhen Han [a], Jie Gao [a], Zhe Cheng [a], Ruiqiang Xin [d], Shangqian Jiang [e], Xiaokun Geng [a,b,e,*]

[a] Department of Neurology, Beijing Luhe Hospital, Capital Medical University, Beijing, China
[b] Department of Neurosurgery, Wayne State University School of Medicine, Detroit, MI, USA
[c] Department of Neurosurgery, Munson Medical Center, Traverse City, Mi, USA
[d] Department of Medical Imaging, Luhe Hospital, Capital Medical University, Beijing, China
[e] China-America Institute of Neuroscience, Beijing Luhe Hospital, Capital Medical University, Beijing, China

ARTICLE INFO

Keywords:
Neuroprotection
Nitric oxide
Intravenous thrombolysis
Randomized controlled trial

ABSTRACT

Despite advances in intravenous thrombolysis and endovascular thrombectomy, numerous acute ischemic stroke survivors continue to experience various disability levels. The nitric oxide (NO) donor, Glyceryl Trinitrate (GTN), has been identified as a potential neuroprotective agent against ischemic damage. We evaluated the safety and feasibility of intravenous GTN in AIS patients. Subsequently, we conducted a secondary analysis to assess for possible efficacy of GTN as a neuroprotectant. We conducted a prospective, double-blind, randomized controlled trial in the Stroke Intervention & Translational Center (SITC) in Beijing Luhe Hospital, Capital Medical University (ChiCTR2100046271). AIS patients within 24 h of stroke onset were evenly divided into GTN or control groups ($n = 20$ each). The GTN group received intravenous GTN (5 mg in 50 ml saline at a rate of 0.4 mg/h for 12.5 h/day over 2 days), while controls were administered an equivalent volume of 0.9% saline. Both groups followed standard Stroke Guidelines for treatment. Safety measures focused on SBP<110 mmHg and headache occurrence. Efficacy was assessed via the 90-day modified rankin score (mRS) and the national institutes of health stroke score (NIHSS). Of the 40 AIS patients, baseline characteristics such as age, gender, risk factors, and pre-mRS scores showed no significant difference between the groups. Safety measures of SBP<110 mmHg and headache occurrence were comparable. Overall, 90-day mRS (1 vs. 1) and NIHSS (1 vs. 1) did not significantly differ between groups. However, the GTN-treated group had a benefit in enhancing NIHSS recovery (△NIHSS 4.5 vs. 3, $p = 0.028$), indicating that GTN may augment recovery. Subgroup analyses revealed a benefit in the GTN group at the 90-day NIHSS score and △NIHSS follow up for non-thrombolysis patients (1 vs. 2, $p = 0.016$; 5 vs. 2, $p = 0.001$). Moreover, the GTN group may benefit mild stroke patients in NIHSS score at 90 day and △NIHSS observed at 90 days (1 vs. 1, $p = 0.025$; 3 vs. 2 $p = 0.002$). Overall, while preliminary data suggest GTN might aid recovery in NIHSS improvement, the evidence is tempered due to sample size limitations. The RIGID study confirms the safety and feasibility of intravenous GTN administration for AIS patients. Preliminary data also suggest that the GTN group may provide improvement in NIHSS recovery compared to the control group. Furthermore, a potential benefit for non-thrombolysis patients and those with mild stroke symptoms was identified, suggesting a possible potential role as a tailored intervention in specific AIS subgroups. Due to the limited sample size, further larger RCT will be necessary to replicate these results.
Trial Registration: www.chictr.org.cn, identifier: ChiCTR2100046271.

Introduction

Ischemic stroke remains a pivotal public health challenge due to its association with high mortality, morbidity, and the consequent socio-economic burden [1–5]. While reperfusion strategies, including mechanical thrombectomy and intravenous thrombolysis, has enhanced the therapeutic landscape for acute ischemic stroke (AIS), a significant portion of stroke patients continue to grapple with disabilities (46%) and

* Corresponding authors.
E-mail addresses: yding@med.wayne.edu (Y. Ding), xgeng@ccmu.edu.cn (X. Geng).

https://doi.org/10.1016/j.neurot.2024.e00365
Received 20 November 2023; Received in revised form 28 March 2024; Accepted 15 April 2024

mortalities (15.3%) post-treatment [6–9]. This underscores the extreme need for exploration of neuroprotection strategies that can be utilized to ameliorate functional deficits in AIS patients.

Nitric oxide plays a crucial role in hypoxic signaling, and its physiological and therapeutic levels have been linked to significant cytoprotective effects in ischemic-reperfusion injuries, particularly in the brain [10,11]. Glyceryl Trinitrate (GTN), an FDA-approved vasodilator and an NO donor, has showed potential benefits in this setting [12–14]. However, its application has faced challenges, particularly with the delivery method. Transdermal administration, despite proving effective for blood pressure modulation, has not yielded consistent results for functional improvement in AIS [15,16]. This inconsistency is due to various factors, such as dosage, time, and delivery method, which all play crucial roles in determining the neuroprotective effects of NO donors [17].

Considering the short half-life of GTN and the challenges associated with transdermal patches in achieving effective cerebrovascular concentrations, this study posits the potential benefits of intravenous administration of GTN [17]. Given that continuous 24-h GTN administration can induce tolerance, leading to sub-therapeutic levels, an intermittent therapeutic approach could potentially produce better outcomes [18–20]. Here we report a single-center, prospective and randomized controlled study on "Rapid Intravenous Glyceryl Trinitrate in Ischemic Damage (RIGID). This study primarily focuses on assessing the safety and feasibility of intravenous GTN in AIS patients, subsequently, a secondary analysis was conducted to evaluate its possible efficacy, in order to establish RIGID as a possible transformative neuroprotective strategy in stroke management.

Methods

Study design

The study protocol (previously published) and informed consent received approval from the Ethics Committee at Beijing Luhe Hospital. Between May 15, 2021, and June 30, 2022, patients were continuously admitted to the Stroke Intervention & Translational Center (SITC) in Beijing Luhe Hospital, Capital Medical University (ChiCTR2100046271). Over the course of the study, a total of 40 patients were randomized into either the GTN group or the control group, following a 1:1 allocation ratio. Safety endpoints, specifically SBP<110 mmHg and headache occurrences were carefully monitored. For evaluating efficacy, assessments were conducted using the 90-day modified rankin scale/score (mRS) and the National Institutes of Health Stroke Scale (NIHSS) score. Additionally, the rate of 90-day mRS scores between 0 and 2 and the recovery differential based on the baseline NIHSS score (△NIHSS) were measured.

Patient population: inclusion and exclusion criteria

We conducted a prospective, double-blind, randomized controlled trial at Beijing Luhe Stroke Center, focusing on stroke patients who are not suitable for EVT. The inclusion criteria are (1) ≥18 and ≤ 80 years old; (2) clinical diagnosis of AIS, (3) systolic blood pressure (SBP)≥120 mmHg; (4) NIHSS score ≥3 and ≤ 16; (5) patients with time from onset to treatment ≤24 h that did not receive endovascular treatment (EVT); (6) pre-stroke mRS ≤2; (7) informed consent provided by participant or legally authorized representative.

Exclusion criteria are as follows: (1) Patients who were suitable for EVT; (2) Severe anemia, HGB ≤60 g/L; (3) allergy to GTN; (4) glaucoma; (5) participant in another ongoing clinical trial; (5) life expectancy of fewer than 1 year due to comorbidities.

Randomization and blindness

During the recruitment period, participants were allocated 1:1 to two groups (n = 40) by computer-generated randomization procedures using opaque envelopes. During the study, an assistant who was not involved in the study prepared the envelopes. Patients were randomly assigned to either the intervention or the control group by treating physicians opening sealed opaque envelopes. Patients and assessors involved in the trial were blinded to treatment allocation to minimize selection bias. The outcomes were evaluated by two blinded observers. Lastly, blinded independent investigators collected and analyzed group outcomes and information as described previously [21,22].

Interventions

All participants, regardless of their group allocation, underwent standard care as per the established guidelines [23]. Within 24 h of displaying symptoms, those in the GTN group were given intravenous GTN, dosed at 5 mg in 50 ml saline, delivered at a rate of 0.4 mg/h for 12.5 h each day over a 2-day period. In contrast, the control group received an equivalent volume of 0.9% saline (refer to Fig. 1 for illustration). Throughout the treatment duration, physicians closely monitored and documented any adverse reactions resulting from the intravenous therapy.

Outcomes

Primary outcomes (safety assessment)

The chief safety measurement under evaluation was SBP<110 mmHg. This primary outcome, delineated as SBP<110 mmHg, was characterized by an average SBP reading of less than 110 mmHg within the initial 24 h following the commencement of GTN treatment. During the starting 2 h of GTN application, blood pressure was assessed at 15-min intervals, then at 30-min intervals from the 2nd to the 12th hour, and subsequently every 120 min from the 12th to the 48th hour. Post the 48-h mark, measurements were taken twice daily.

Blood pressure targets, as set out in the AIS guidelines, differ based on several parameters, encompassing whether a patient has been treated with alteplase, has undergone mechanical embolectomy, or has experienced a hemorrhage. The scientific community has not reached a unanimous agreement on the ideal blood pressure levels post-stroke. Prior research [24–27] has highlighted a U-shaped correlation between blood pressure levels and functional outcomes, identifying both exceedingly low and high blood pressure readings as predictors of adverse outcomes. Consequently, for the purpose of this study, we introduced a threshold of 110 mmHg for systolic blood pressure (SBP) to denote a low SBP.

Headache was our secondary safety outcome. According to GTN responder definitions, mild to moderate headaches (headache score 3–6 score) occur within 5–15 min with a short lasting duration (maximum of 30 min), and spontaneously recover within 1 h after administration of GTN without the need for any rescue medication [28]. Therefore, severe headaches (score 7–10) and analgesic use for GTN headaches were included as secondary safety outcomes. When patients experienced severe headaches, they were closely monitored for vital signs (blood pressure, heart rate, body temperature, breathing rate) and a CT scan was obtained to determine the cause of the headaches.

Secondary outcomes (efficacy assessment)

The main efficacy measurement was based on the 90-day modified Rankin Scale (mRS), with a score ranging from 0 to 2 indicative of functional independence. Other efficacy measurement included: the rate of mRS scores between 0 and 2 and NIHSS scores at the 90-day mark,and the recovery differential based on the NIHSS score (△NIHSS), as well as the incidence of death within this 90-day period. Assessment of mRS and NIHSS scores were conducted by personnel blinded to the study specifics. For accuracy in these evaluations, face-to-face consultations were employed within the study environment to determine both mRS and NIHSS scores.

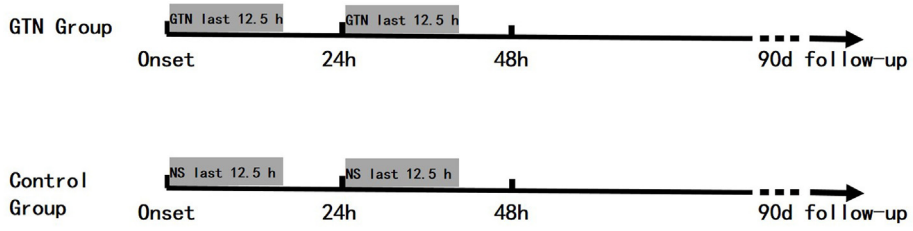


Fig. 1. Timelines for experimental procedure Abbreviations: NS, normal saline.

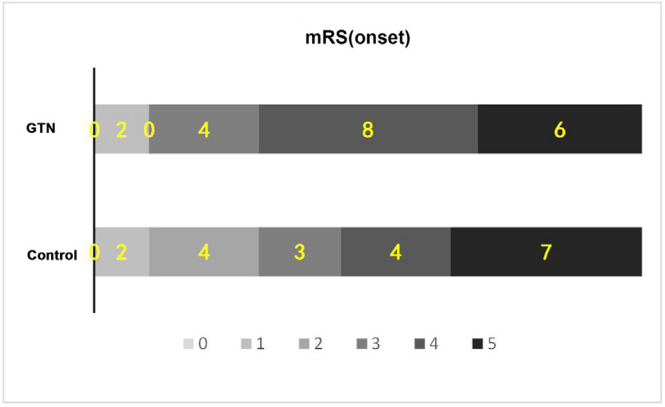


Fig. 2. mRS shift: the percentage of patients achieving each mRS score at onset.

Fig. 3. mRS shift: the percentage of patients achieving each mRS score at 90 day.

Estimation of sample size

This was a phase II study to determine safety and feasibility. Currently, there are no completed clinical studies on intravenous GTN in AIS patients, so there was no data available for reference. Nevertheless, Hertzog suggested that a pilot study with 10–20 patients in each group would be sufficient to assess feasibility [29]. According to Dobkin, 15 patients in each group were usually enough to decide whether a larger multicenter trial is needed [30]. The sample size for each group was determined by conducting a power analysis based on the results of prior research that compared to placebo groups, GTN had reduced SBP by 10 mmHg[12]. For the difference in blood pressure at 10 mmHg, standard deviation at 10 mmHg, in order to have alpha exceed 95%, and beta = 0.8, a sample size of 16 patients per group has been calculated. Due to treatment dropouts, crossovers, and losses to follow-up, we increased this sample size with 20% and recruited 40 patients. Results from this study can be used to determine whether intravenous GTN infusion is safe and feasible for patients with AIS. Based on the data, a sample size estimate and power calculation were conducted to plan a phase-III trial.

Statistical analyses

For categorical variables, the number and the proportions were presented and the groups were compared using chi-square tests or the continuity correction chi-square tests. For continuous variables, the mean and standard deviations or medians with IQRs are presented and the groups were compared using the Mann-Whitney *U* test if not normally distributed or the *t*-test if normally distributed. The significance level was set at $p < 0.05$. SPSS 22.0 software (Armonk, NY, IBM Inc.) was used for statistical analysis.

Results

From May 15, 2021 to June 30, 2022, 40 consecutive patients in our Stroke Center were randomly assigned (1:1) to the two groups. All patients finished the treatment and follow-up assessment. No patients were lost to follow up.

Baseline characteristics

Evaluating the GTN and control groups, there were no notable differences in terms of age, gender, and past medical histories, which included conditions, such as hypertension, diabetes, hyperlipidemia, prior stroke, coronary heart disease, atrial fibrillation, smoking habits and pre-mRS (Table 1). In our study, the median ages in the control and GTN groups were 66 and 63, respectively, yielding a p-value of 0.068. This marginal difference suggested minimal age-related impact on the analysis outcome from the study. Although the prior history of stroke was 35% in the control group and 10% in the GTN group, this difference did not reach statistical significance with a p-value of 0.058, suggesting that

Table 1
Demographic and clinical characteristics of all patients.

Characteristics	GTN group (n = 20)	Control group (n = 20)	*P* Value
Age, years (median [IQR])	63 (50.0–68.0)	66 (58.2–74.8)	0.068
Sex, male (%)	13 (65)	14 (70)	0.736
Previous history			
Hypertension n (%)	18 (90)	16 (80)	0.453
Diabetes n (%)	8 (40)	5 (25)	0.311
Hyperlipidemia n (%)	8 (40)	10 (50)	0.525
Coronary heart disease n (%)	0 (0)	8 (40)	NA
Atrial fibrillation	3 (15)	2 (10)	0.633
Stroke n (%)	2 (10)	7 (35)	0.058
Smoking n (%)	4 (20)	6 (30)	0.465
Pre-mRS score (median [IQR])	0 (0–0)	0 (0–0)	0.799
Clinical data			
NIHSS score (onset) (median [IQR])	6 (3.3–9.5)	4 (3–8.5)	0.049
rt-PA treatment n (%)	6 (30)	5 (25)	0.723
mRS score (onset) (median [IQR])	4 (3–5)	4 (3–5)	0.316
mRS 0–2 rate (onset) n (%)	2 (10)	6 (30)	0.114

Abbreviations: NIHSS, the National Institute of Health Scale Score; mRS, modified Rankin score; rt-PA, intravenous recombinant tissue plasminogen activator; IQR, Interquartile range.

history of stroke has no effect on the efficacy of GTN (Table 1). No significant differences were observed in pre-mRS score between the GTN and control groups (0 vs. 0, p = 0.799), suggesting that stroke history has no effect on the outcome of GTN and randomization was adequate (Table 1).

Safety

Neither the GTN group nor the control group exhibited occurrences of SBP<110 mmHg or headaches. The findings suggest that low-dose IV GTN is well-tolerated. No significant adverse reactions were reported (Table 2).

The administration of intravenous GTN resulted in a reduction of systolic blood pressure by an average of 10 mmHg and a decrease in diastolic blood pressure by 2 mmHg over a span of 24 h when compared to baseline readings. However, a slight increase of 3 mmHg was observed in both systolic and diastolic blood pressures between the 24-h and 48-h marks (Fig. 4). In contrast, the control group, when compared to their baseline readings, experienced a decline of 7 mmHg in systolic and 3 mmHg in diastolic blood pressures at the 24-h mark. This was followed by a marginal drop of 1 mmHg for both systolic and diastolic readings between 24 and 48 h (Fig. 5).

Efficacy

Upon examining the 90-day mRS and NIHSS outcomes, no significant differences were observed between the GTN and control groups, with both measures standing at 1 vs. 1. Importantly, an initial difference in stroke severity was noted between the groups as determined by the NIHSS score, with the GTN group at 6 and the control group at 4 (p = 0.049). Because of this barely significant level, we further analyzed the change in NIHSS scores (ΔNIHSS) to assess neurological function improvement post-treatment. Patients treated with GTN exhibited a more pronounced recovery as assessed by NIHSS (△NIHSS of 4.5 compared to 3 in the control group, p = 0.028), suggesting GTN's potential role in facilitating recovery over this duration (Table 2). The distribution of mRS scores at onset and 90 days (mRS shift data) indicated equal results across both groups (Figs. 2 and 3). In addition, the GTN group had apparent benefit at the 90-day NIHSS scores and △NIHSS for those who had not undergone thrombolysis (1 vs. 2, p = 0.016; 5 vs. 2, p = 0.001) (Table 3). Impressively, the GTN group with milder strokes, represented by NIHSS scores under 6, had significant improvements in NIHSS score and △NIHSS observed at 90 days (1 vs. 1, p = 0.025; 3 vs. 2 p = 0.002) (Table 4). In Patients with atherosclerosis, GTN also enhanced recovery on the NIHSS scale (△NIHSS of 6 versus 2.5 in the control, p = 0.005) (Table 5). Overall, while preliminary data suggest GTN might aid recovery in NIHSS improvement, the evidence is tempered due to the small sample size. Finally, when patients with stroke history and non-stroke history were evaluated after GTN adminstration, there was no significant difference between the two groups at the 90-day mRS score, NIHSS scores and △NIHSS. These results suggest that a history of previous stroke has no effect on the efficacy of GTN (Table 6).

Table 2
Comparison of safety and efficacy outcomes.

Endpoints	GTN group (n = 20)	Control group (n = 20)	*P* Value
Safty endpoints n (%)			
SBP<110 mmHg	0 (0)	0 (0)	NA
headache	0 (0)	0 (0)	NA
Efficacy endpoints n (%)			
mRS at 90d (median [IQR])	1 (1–2)	1 (1–2)	0.488
mRS 0–2 rate at 90d n (%)	19 (95)	17 (85)	0.292
90d NIHSS(median [IQR])	1 (1-1)	1 (1–2)	0.108
NIHSS recovered (△NIHSS) (median [IQR])	4.5 (3–10.5)	3 (2–6)	0.028

Abbreviations: SBP, systolic blood pressure.

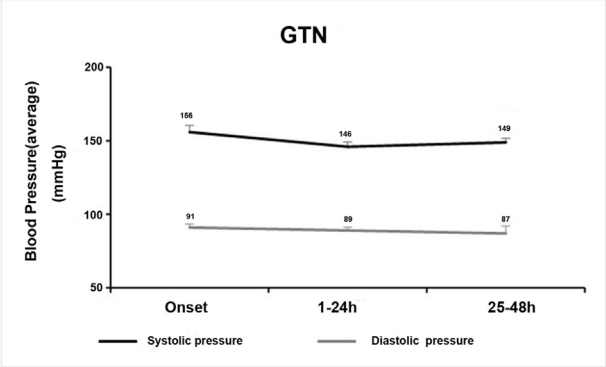


Fig. 4. Blood pressure changes in GTN group within 48 h of treatment.

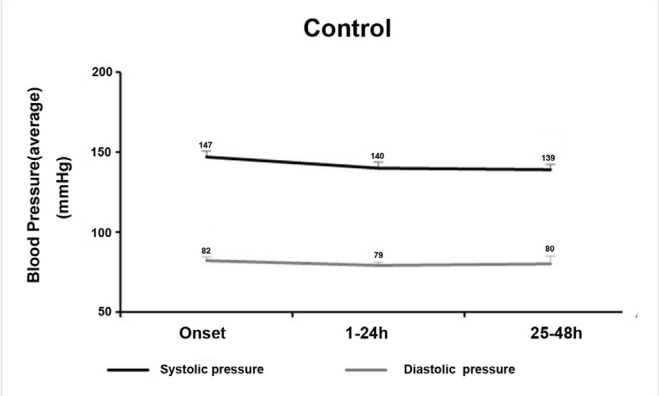


Fig. 5. Blood pressure changes in control group within 48 h of treatment.

Discussion

In summary, RIGID was designed to primarily identify the safety and feasibility of intravenous administration of GTN in AIS patients, a secondary analysis was conducted to evaluate its effectiveness. We found that GTN was well-tolerated and safe in the early stage of stroke without any significant adverse effects. However, the higher average NIHSS score in the GTN group suggests that GTN conferred benefits in patients with more severe stroke symptoms, indicating a potential therapeutic advantage that warrants further investigation. Notably, while overall 90-day mRS and NIHSS scores between the GTN and control groups did not significantly vary, the GTN-treated group had a benefit in enhancing superior NIHSS recovery. Additionally, the GTN group demonstrated a potential benefit for non-thrombolysis patients and those with mild stroke symptoms, suggesting a potential role as a tailored therapeutic in specific AIS subgroups.

In patients with AIS, the NIHSS is commonly used to measure stroke severity, which is a 15-item scale that quantifies neurological deficits in several domains. High NIHSS scores indicate a severe patient condition and a higher disability rate [31–33]. Results revealed that NIHSS scores of patients in the GTN group were significantly higher than those in the control group at the beginning of stroke and randomization. The GTN-treated group revealed a superior NIHSS recovery (△NIHSS = 90-day NIHSS-onset NIHSS), indicating that GTN may allow patients to recover faster through neuroprotective mechanisms. Although there was no significant difference in mRS between the GTN and control groups at 90 days, a higher percentage of patients had a favorable outcome in the GTN group compared to the control group (95% in GTN group vs. 85% in

Table 3
Efficacy outcomes in non-rt-PA treatment and rt-PA treatment patients.

	Non-rt-PA treatment			rt-PA treatment		
	GTN (n = 14)	Control (n = 15)	*P* Value	GTN (n = 6)	Control (n = 5)	*P* Value
NIHSS score (onset) (median [IQR])	5.5 (4.7–10.5)	4 (3–6)	0.010	7.5 (3–14)	9 (5–10)	1.000
NIHSS score (90d) (median [IQR])	1 (0.75–1)	2 (1–2)	0.016	1 (0.75–3)	1 (0–2)	0.546
△NIHSS (median [IQR])	5 (3.7–9.5)	2 (1–3)	0.001	3.5 (1.8–13)	6 (4.5–9.5)	0.537
mRS score (onset) (median [IQR])	1 (0.75–1)	3 (2–5)	0.056	4 (2–5)	5 (2.5–5)	0.686
mRS score (90 d) (median [IQR])	1 (1–2)	1 (1–2)	0.148	1.5 (0.75–2.25)	1 (0.5–1.5)	0.916

Table 4
Efficacy outcomes in NIHSS ≥ 6 and NIHSS<6 patients.

	NIHSS ≥ 6			NIHSS<6		
	GTN (n = 10)	Control (n = 8)	*P* Value	GTN (n = 10)	Control (n = 12)	*P* Value
NIHSS score (onset) (median [IQR])	12 (7.8–14)	9 (6.2–10.5)	0.080	4.5 (3.8–5)	3 (3–4)	0.027
NIHSS score (90d) (median [IQR])	1 (1–1.5)	1.5 (0.2–2.8)	0.602	1 (0–1)	1 (1–2)	0.025
△NIHSS (median [IQR])	10 (4.7–11.5)	7 (5.2–9.5)	0.360	3 (3–4)	2 (2–3)	0.002
mRS score (onset) (median [IQR])	5 (4–5)	5 (4.25–5)	5 (4–5)	3 (2.5–4)	2.5 (2–3.8)	0.414
mRS score (90 d) (median [IQR])	1 (1–2)	1.5 (1–2.8)	1 (1–2)	3 (1.8–3)	2 (1.3–3.0)	0.389

Table 5
Efficacy outcomes in large-artery atherosclerosis patients.

Large-artery atherosclerosis	GTN group (n = 15)	Control group (n = 16)	*P* Value
NIHSS score (onset) (median [IQR])	8 (5–12)	4 (3–6)	0.005
NIHSS score (90 d) (median [IQR])	1 (1–1)	1.5 (1–2)	0.113
△NIHSS (median [IQR])	6 (4–11)	2.5 (2–4.5)	0.005
mRS score (onset) (median [IQR])	4 (4–5)	3.5 (2–4.75)	0.110
mRS score (90 d) (median [IQR])	1 (1–2)	1 (1–2)	0.725

control). GTN administration demonstrated particular efficacy for those with mild stroke symptoms (NIHSS <6), suggesting its potential as a role as tailored intervention in specific AIS subgroups. There was no significant different between GTN and control groups for patient with NIHSS >6. This result was similar to the results reported in a previous small phase 2 ambulance-based trial (RIGHT) [16], in which GTN was associated with a tendency for a worse functional outcome in patients with severe stroke (NIHSS >12). The potential risks of GTN therapy associated with severe large territory stroke include nitro donors that have been experimentally and clinically been documented to elevate intracranial pressure, especially when this pressure is already high [34]. One anticipated mechanism behind the rise in intracranial pressure due to sodium nitroprusside is its ability to expand the intracranial blood volume by inducing cerebrovascular dilation. In smaller stroke territories and lower NIHSS strokes GTN therapy may allow for improved luxury perfusion via dilated collaterals. Furthermore, GTN has been shown to be particularly effective for patients who did not undergo thrombolysis, and to promote faster patient recovery. This observation suggests that GTN offers a promising adjunctive treatment strategy, potentially enhancing recovery outcomes for AIS patients who have received or not received intravenous thrombolysis.

NO, a multifaceted molecule in human, plays a multitude of physiological roles. These roles encompass acting as a vasodilator, serving as a neurotransmitter, modulating immune responses, and acting as an antagonist to both platelets and leucocytes [35]. Its versatility demonstrates the importance of NO in maintaining the physiological balance and overall health.

Ischemia/reperfusion injury is a complex phenomenon that can lead to organ damage and failure, primarily driven by inflammation that stems from intracellular injuries [36–39]. When tissues are deprived of oxygen, typically due to a blocked blood supply, it triggers a chain of events known as ischemic injury. The most immediate consequence of this deprivation is anoxic injury, characterized by diminished production of adenosine triphosphate (ATP), a molecule essential for energy transfer within cells. This depletion hinders the cell's ability to uphold homeostatic functions, thereby increasing membrane permeability and causing an influx of unwanted substances into the cytosol [40]. During reperfusion, the restoration of blood flow and oxygen supply paradoxically introduces another set of complications. The sudden surge in oxygen levels can lead to the production of free radicals, which are highly reactive molecules capable of damaging cells. To exacerbate the situation, the reperfusion phase is also marked by a decline in NO production. This decrease in NO, especially associated with endothelial nitric oxide synthase activity, is a contributing factor to the damage experienced in ischemia/reperfusion injury [41].

NO plays a pivotal role in ischemia reperfusion injury. The protective effects have been consistently highlighted in stroke. NO not only inhibits oxidative stress but also reduces leukocyte-endothelial adhesion, which can lead to inflammatory responses and damage [42]. Additionally, NO prevents programmed cell death or apoptosis. NO can diminish the size of infarctions and inflammation following an ischemic stroke, bolster cerebral blood flow and metabolism, and aid in neural function recovery [43].

From a clinical perspective, past observational studies have unveiled a correlation between ischemic strokes and NO levels [44]. Patients suffering from ischemic stroke generally exhibited considerably reduced

Table 6
Efficacy outcomes in Non-stroke history and stroke history patients.

	Non-stroke history			Stroke history		
	GTN (n = 18)	Control (n = 6)	*P* Value	GTN (n = 2)	Control (n = 14)	*P* Value
NIHSS score (onset) (median [IQR])	5.5 (4.8–12)	9 (5.5–11.3)	0.537	7 (4.24)	3.5 (3–5.3)	0.200
NIHSS score (90d) (median [IQR])	1 (1–1)	1 (0–2.3)	0.923	4.5 (6.36)	1.5 (1–2)	1.000
△NIHSS (median [IQR])	5 (3–11)	7 (5.3–10.3)	0.581	3.5 (0.71)	2 (1–3)	0.933
mRS score (onset) (median [IQR])	4 (3–5)	5 (3–5)	0.137	2.5 (2.12)	3 (2–4.3)	0.600
mRS score (90 d) (median [IQR])	1 (1–2)	1 (0.8–2.3)	0.923	2.5 (2.12)	2.5 (2.12)	0.600

Abbreviations: In the GTN treatment group, there were 2 cases with a history of stroke, presented as Mean ± SE.

plasma NO levels compared to healthy counterparts. Furthermore, an apparent relationship was established between decreased NO levels and the severity of strokes; those with low NO levels tended to have more severe strokes and consequently, poorer outcomes upon discharge [43]. This may represent autoregulation by the body to lower NO levels in the setting of profound ischemia to avoid or slow intracranial pressure problems from vasodilation.

Limitations

This study comes with certain limitations that need to be acknowledged. 1) Single-Center and Sample Size. The study was conducted in a single center with a relatively small sample size. This raises concerns regarding the generalizability of the findings to a wider population, spurious results and different clinical settings. 2) Population representativeness. The cohort in this study was not entirely indicative of the broader stroke patient demographic. Our focus was primarily on patients with mild to moderate AIS, as reflected in the NIHSS scores ranging between 3 and 16. 3) A bias related to unblinding should be noted. Although we implemented a double-blind approach, the observable changes in blood pressure might inadvertently hint at the treatment being administered. We have therefore ensured that only patients and outcome assessors were kept blinded to minimize this potential bias. Lastly despite randomization, GTN vs. control groups reached near significance in patient age and NIHSS.

Future Direction

The RIGID study provides valuable insights into the safety and efficacy of intravenous GTN for AIS patients. Low-dose intravenous GTN treatment after AIS has no significant impact on blood pressure, and no other adverse effects of GTN treatment have been identified. These results suggest that low-dose intravenous GTN is safe and well-tolerated in patients with AIS. Although the overall 90-day mRS and NIHSS scores did not exhibit significant differences between the GTN and control groups, there was a modest improvement in NIHSS recovery for those treated with GTN. This finding becomes even more pronounced when considering specific patient categories, in which GTN seemed particularly effective for non-thrombolysis patients and those showing mild stroke symptoms. These results hint at the potential of GTN as a targeted therapeutic strategy for specific AIS subpopulations. Due to the limited sample size, further larger RCT will be necessary to replicate these results.

Future research will need to elucidate the effects of GTN on moderate to severe stroke patients, while the current study was primarily focused on mild to moderate AIS patients. Multi-center randomized controlled trials encompassing a variety of clinical settings, demographics, and geographies will offer a full understanding of GTN's therapeutic potential and its generalizability. The optimal dosage, frequency, and duration of GTN administration remain to be determined. Further research into the molecular and cellular mechanisms by which GTN exerts its neuroprotective effects can further refine our understanding, and thus allowing us to modify the drug's application more effectively. Beyond the immediate post-stroke phase, understanding the long-term implications of GTN treatment on patients' neurological recovery, cognitive functions, and overall quality of life can provide a more comprehensive consideration.

Author Contributions

Lipeng Cai, Yanna Tong, Honglian Duan, Zhenzhen Han, Zhe Cheng and Ruiqiang Xin performed the study, analyzed data, and prepared the manuscript. Xiaokun Geng, Yuchuan Ding, Gary Rajah designed the study and revised the manuscript. Shangqian Jiang and Jie Gao evaluated the subjects. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Nature Science Foundation of China (No. 21707095, No. 82072549 and No. 81871838) and Science and Technology Plan of Beijing Tongzhou District (No. KJ2022CX020 and No. KJ2023CX030).

Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Beijing Luhe Hospital, Capital Medical University, Beijing, China. The participants will provide their written informed consent to participate in the study.

Declaration of competing interest

Research was conducted without any commercial or financial relationships that could be construed as conflicts of interest.

Acknowledgment

None.

References

[1] Wu S, Wu B, Liu M, Chen Z, Wang W, Anderson CS, et al. Stroke in China: advances and challenges in epidemiology, prevention, and management. Lancet Neurol 2019;18:394–405.
[2] Uzuner N, Uzuner GT. Risk factors for multiple recurrent ischemic strokes. Brain circulation 2023;9:21–4.
[3] Kurisu K, Sakurai J, Wada H, Takebayashi S, Kobayashi T, Takizawa K. Effects of clinical outcomes by modification of patient selection protocol based on premorbid independence for mechanical thrombectomy in older adult patients. Brain circulation 2022;8:24–30.
[4] Zhou J, Fangma Y, Chen Z, Zheng Y. Post-stroke neuropsychiatric complications: types, pathogenesis, and therapeutic intervention. Aging and disease 2023;14:2127–52.
[5] Eren F, Yilmaz SE. Neuroprotective approach in acute ischemic stroke: a systematic review of clinical and experimental studies. Brain circulation 2022;8:172–9.
[6] Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. Lancet 2014;384:1929–35.
[7] Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. Lancet 2016;387:1723–31.
[8] Yang N, Lee H, Wu C. Intravenous thrombolysis for acute ischemic stroke: from alteplase to tenecteplase. Brain circulation 2023;9:61–3.
[9] Deng G, Chu YH, Xiao J, Shang K, Zhou LQ, Qin C, et al. Risk factors, pathophysiologic mechanisms, and potential treatment strategies of futile recanalization after endovascular therapy in acute ischemic stroke. Aging and disease 2023;14:2096–112.
[10] Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov 2008;7:156–67.
[11] Isabella E, Indu S, Roselyn BR. The role of cortisol in the development of post-stroke dementia: a narrative review. Heart and Mind 2022;6:151–8.
[12] Bath PM, Pathansali R, Iddenden R, Bath FJ. The effect of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure and platelet function in acute stroke. Cerebrovasc Dis 2001;11:265–72.
[13] Willmot M, Ghadami A, Whysall B, Clarke W, Wardlaw J, Bath PM. Transdermal glyceryl trinitrate lowers blood pressure and maintains cerebral blood flow in recent stroke. Hypertension 2006;47:1209–15.
[14] Ankolekar S, Fuller M, Cross I, Renton C, Cox P, Sprigg N, et al. Feasibility of an ambulance-based stroke trial, and safety of glyceryl trinitrate in ultra-acute stroke: the rapid intervention with glyceryl trinitrate in hypertensive stroke trial (right, isrctn66434824). Stroke; a journal of cerebral circulation 2013;44:3120–8.
[15] Investigators ET. Efficacy of nitric oxide, with or without continuing antihypertensive treatment, for management of high blood pressure in acute stroke (enos): a partial-factorial randomised controlled trial. Lancet 2015;385:617–28.
[16] Prehospital transdermal glyceryl trinitrate in patients with ultra-acute presumed stroke (right-2): an ambulance-based, randomised, sham-controlled, blinded, phase 3 trial. Lancet 2019;393:1009–20.

[17] Maniskas ME, Roberts JM, Trueman R, Learoyd AE, Gorman A, Fraser JF, et al. Intra-arterial nitroglycerin as directed acute treatment in experimental ischemic stroke. J Neurointerventional Surg 2018;10:29–33.
[18] Bergbauer M, Weber K. Haemodynamic studies with a phasic release nitroglycerin patch system. Eur Heart J 1989;10(Suppl A):30–5.
[19] Reiniger G, Menke G, Boertz A, Kraus F, Rudolph W. Interval therapy in effective treatment of angina pectoris using nitroglycerin patch systems. A controlled study with determination of nitroglycerin plasma levels. Herz 1987;12:68–73.
[20] Reiniger G, Rudolph W. Discontinuous drug release as an alternative to interval therapy in the treatment of coronary heart disease with nitroglycerin patches. Herz 1987;12:348–53.
[21] Cai L, Rajah G, Duan H, Gao J, Cheng Z, Xin R, et al. Rapid intravenous glyceryl trinitrate in ischemic damage (rigid) after stroke: rationale, design and protocol for a prospective randomized controlled trial. Front Neurol 2021;12:693330.
[22] Cheng Z, Gao J, Ding Y, Pang Q, Rajah GB, Geng X. Arterial glyceryl trinitrate in acute ischemic stroke after thrombectomy for neuroprotection (again): a pilot randomized controlled trial. Neurotherapeutics 2023;20:1746–54.
[23] Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the american heart association/american stroke association. Stroke; a journal of cerebral circulation 2019;50:e344–418.
[24] Mulder M, Ergezen S, Lingsma HF, Berkhemer OA, Fransen PSS, Beumer D, et al. Baseline blood pressure effect on the benefit and safety of intra-arterial treatment in mr clean (multicenter randomized clinical trial of endovascular treatment of acute ischemic stroke in The Netherlands). Stroke; a journal of cerebral circulation 2017;48:1869–76.
[25] Verschoof MA, Groot AE, Vermeij JD, Westendorp WF, van den Berg SA, Nederkoorn PJ, et al. Association between low blood pressure and clinical outcomes in patients with acute ischemic stroke. Stroke; a journal of cerebral circulation 2020;51:338–41.
[26] Castillo J, Leira R, García MM, Serena J, Blanco M, Dávalos A. Blood pressure decrease during the acute phase of ischemic stroke is associated with brain injury and poor stroke outcome. Stroke; a journal of cerebral circulation 2004;35:520–6.
[27] Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PA. Blood pressure and clinical outcomes in the international stroke trial. Stroke; a journal of cerebral circulation 2002;33:1315–20.
[28] Gazerani P, Cairns BE, Yassin H, Yousefi JT, Sherzaman AR, Nedergaard BS, et al. Amplification of glyceryl trinitrate-induced headache features by noxious craniofacial stimuli in pain-free healthy humans. Pain Manag 2019;9:17–35.
[29] Hertzog MA. Considerations in determining sample size for pilot studies. Res Nurs Health 2008;31:180–91.
[30] Dobkin BH. Progressive staging of pilot studies to improve phase iii trials for motor interventions. Neurorehabilitation Neural Repair 2009;23:197–206.
[31] Kwah LK, Diong J. National institutes of health stroke scale (nihss). J Physiother 2014;60:61.
[32] Eaton RG, Duru O, Powers CJ. Direct transfer for thrombectomy in patients with large vessel occlusions on computed tomography angiography results in safe revascularization. Brain circulation 2023;9:25–9.
[33] Johnson JN, Srivatsan A, Chueh J, Arslanian R, Gounis MJ, Puri AS, et al. Impact of histological clot composition on preprocedure imaging and mechanical thrombectomy. Brain circulation 2022;8:87–93.
[34] Turner JM, Powell D, Gibson RM, McDowall DG. Intracranial pressure changes in neurosurgical patients during hypotension induced with sodium nitroprusside or trimetaphan. Br J Anaesth 1977;49:419–25.
[35] Snyder SH, Bredt DS. Biological roles of nitric oxide. Sci Am 1992;266:68–71. 74-67.
[36] Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. Nat Med 2011;17:1391–401.
[37] Kalirawna TR, Rohilla J, Bairwa SS, Gothwal SK, Tak P, Jain R. Increased concentration of serum gamma-glutamyl transferase in ischemic stroke patients. Brain circulation 2021;7:71–6.
[38] Li L, Han Z, Yang Z, Ma Q, Zhao H, Wang R, et al. Circulating inflammatory biomarkers level before thrombolysis for acute ischemic stroke predicts symptomatic intracerebral hemorrhage. Aging and disease 2023;14:9–13.
[39] Guo S, Wehbe A, Syed S, Wills M, Guan L, Lv S, et al. Cerebral glucose metabolism and potential effects on endoplasmic reticulum stress in stroke. Aging and disease 2023;14:450–67.
[40] Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/reperfusion. Compr Physiol 2016;7:113–70.
[41] Koken T, Inal M. The effect of nitric oxide on ischemia-reperfusion injury in rat liver. Clin Chim Acta 1999;288:55–62.
[42] Phillips L, Toledo AH, Lopez-Neblina F, Anaya-Prado R, Toledo-Pereyra LH. Nitric oxide mechanism of protection in ischemia and reperfusion injury. J Invest Surg 2009;22:46–55.
[43] Fox-Robichaud A, Payne D, Hasan SU, Ostrovsky L, Fairhead T, Reinhardt P, et al. Inhaled no as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. J Clin Invest 1998;101:2497–505.
[44] Rashid PA, Whitehurst A, Lawson N, Bath PM. Plasma nitric oxide (nitrate/nitrite) levels in acute stroke and their relationship with severity and outcome. J Stroke Cerebrovasc Dis : the official journal of National Stroke Association 2003;12:82–7.