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Protocol

A randomized, double-blind, placebo-controlled, multicenter trial-DL-3-n-butylphthalide therapy for cerebral hypoperfusion from unilateral internal carotid system stenosis study: study protocol

Dawei Chen[1], Yanwei Yin[1], Jin Shi[1]*, Xiaoxuan Ma[2], Huiping Shi[2], Feng Huang[3], Feng Qiu[4], Yonghong Tang[5]

[1]Department of Neurology, Air Force Medical Center, Chinese PLA, Beijing, China
[2]Department of Radiology, Air Force Medical Center, Chinese PLA, Beijing, China
[3]Department of Medical Statistics, Air Force Medical Center, Chinese PLA, Beijing, China
[4]Department of Neurology, The Eighth Medical Center, Chinese PLA General Hospital, Beijing, China
[5]Department of Neurology, The Affiliated Nanhua Hospital, Hengyang Medical School, University of South, Hunan, China

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***Correspondence:**
Dr. Jin Shi,
E-mail: shijin_dr9@126.com

ABSTRACT

Background: Cerebral hypoperfusion caused by large-vessel stenosis is an important risk factor for ischemic stroke and vascular cognitive impairment. *In vitro*, animal and clinic studies demonstrated that DL-3-n-butylphthalide (NBP) can improve the collateral circulation and the cerebral perfusion. In this trial, the benefit of NBP to ameliorate the chronic cerebral hypoperfusion resulting from unilateral internal carotid system stenosis will be explored.
Methods: This trial is a randomized, double-blind, placebo-controlled, multicenter clinical study. A total 480 subjects with ≥70% stenosis or occlusion in unilateral internal carotid artery system, cerebral hypoperfusion in the ipsilateral middle cerebral artery (MCA) territory, and no transient ischemic attacks (TIA) or ischemic strokes within 2 weeks will be enrolled in China. Patients will be assigned in a 1:1 ratio to NBP and placebo groups. Patients in NBP or placebo group received 200 mg or 20 mg of NBP capsules three times daily for 4 weeks respectively. The cerebral perfusion will be assessed again after 12 weeks. The primary efficacy outcome is the proportion of patients with cerebral blood flow (CBF) amelioration, stabilization and deterioration after treatment.
Conclusions: This trial will provide a high-level of evidence for NBP to treat the cerebral hypoperfusion, and a novel strategy to improve the cerebral hemodynamic impairment due to large intracranial and extracranial atherosclerotic stenosis in the surgical high-risk patients, especially in the aged and Asians.
Trial registration: This trial is registered as ChiCTR2100053112 on November 12th, 2021.

Keywords: DL-3-n-butylphthalide, Cerebral large-vessel stenosis, Cerebral hypoperfusion, Computed tomography perfusion, Randomized double-blind controlled trial

INTRODUCTION

Cerebral hemodynamic impairment caused by large intracranial and extracranial atherosclerotic stenosis is an important risk factor for ischemic stroke and vascular cognitive impairment. Improving cerebral hypoperfusion may prevent ischemic stroke and cognitive impairment.[1] Although the surgical revascularization is the most effective method to increase perfusion in cerebral large artery diseases, it is not suitable to all the patients. First, for the extracranial stenosis, carotid endarterectomy (CEA) and carotid artery stenting (CAS) can normalize

cerebral hemodynamics by increasing the lumen diameter, but they are considered high risk to some patients.[2-3] Cheng et al found that about 17% symptomatic carotid stenosis patients were unfit for these surgeries.[4] Second, for the intracranial stenosis, stenting versus aggressive medical management therapy for intracranial arterial stenosis (SAMMPRIS) and Vitesse intracranial stent study for ischemic stroke therapy (VISSIT) trials did not provide evidence of benefit for percutaneous intracranial stenting over the intensive medical therapy (IMT) alone.[5-6] In addition, the indication and efficacy of extracranial-intracranial (EC-IC) bypass is still controversial for the intracranial stenosis.[7] Therefore, it is very necessary to explore the drug therapy to improve the cerebral hypoperfusion resulting from cervicocephalic large-vessel stenosis for the patients who are unfit to the surgical revascularization.

The cerebral perfusion distal to stenosis is not only determined by the blood flow of stenotic vessels, but also by the compensation of collateral circulation. Augmentation of cerebral collateral circulation is an alternative method to improve the cerebral hypoperfusion.[8] Some studies demonstrated a favorable role of better collateral circulation in the patients with acute ischemic stroke (AIS) receiving intravenous thrombolytic therapy and endovascular treatment.[9] In the patients with symptomatic intracranial artery stenosis (ICAS), the warfarin-aspirin symptomatic intracranial disease (WASID) and SAMMPRIS trials showed that the collateral status significantly altered the risk of recurrent stroke.[5,10] Recently, Wabnitz et al also showed that patients with borderzone infarcts and impaired collateral flow had the highest risk of recurrent stroke.[11]

DL-3-n-butylphthalide (NBP) is a synthetic chiral compound based on l-3-butylphthalide, which is originally isolated from seeds of Apium graveolens.[12] Many *in vitro* and animal studies had provided the evidences that NBP could improve the collateral growth and the cerebral microcirculation by arteriogenesis and angiogenesis to restore local CBF in AIS and chronic cerebral hypoperfusion (CCH).[13-17] A clinic study showed that NBP increased much more cerebral primary and secondary collaterals than the control in AIS patients.[18] Moreover, a randomized controlled trial including 170 patients reported that NBP plus IMT could enhance neurological recovery in AIS patients as compared with standard medical treatment alone, which was correlated with a significantly higher level of circulating endothelial progenitor cells that may promote angiogenesis and neovascularization in those treated with NBP.[19] Our previous randomized controlled trial also showed that on the basis of IMT，NBP could improve the CCH in the patients with severe carotid stenosis.[20] However, it only provided a low level of evidence for NBP to treat the CCH in cerebral large-vessel stenosis for some limitations, such as single-center, small sample size and high dropout rate.

In this randomized, double-blind, placebo-controlled, multicenter trial, we aim to further compare the efficacy of NBP versus placebo to ameliorate the CCH in the ipsilateral MCA territory due to unilateral severe internal carotid system stenosis. We hypothesize that on the basis of IMT, NBP group will have a significantly higher proportion of patients with cerebral perfusion improvement than placebo group. Therefore, this study will provide much more high-quality evidence for NBP to improve CCH resulting from large-vessel stenosis.

METHODS

Design

The DL-3-n-BUtylphthalide Therapy for Cerebral Hypoperfusion from unilateral internal carotid stenosis (BUTCH) trial is a randomized, double-blind, placebo-controlled, multicenter clinical study. The trial was designed by the principal investigators and discussed by a steering committee consisting of experts in cerebrovascular diseases, neuroradiologist and statistician. The BUTCH study protocol has been approved by the ethics committee of Air Force Medical Center, Chinese PLA. This trial was registered as No. ChiCTR2100053112 on November 12th, 2021, in the Chinese clinical trial registry (http://www.chictr.org.cn/index.aspx). The study flowchart was shown in Figure 1.

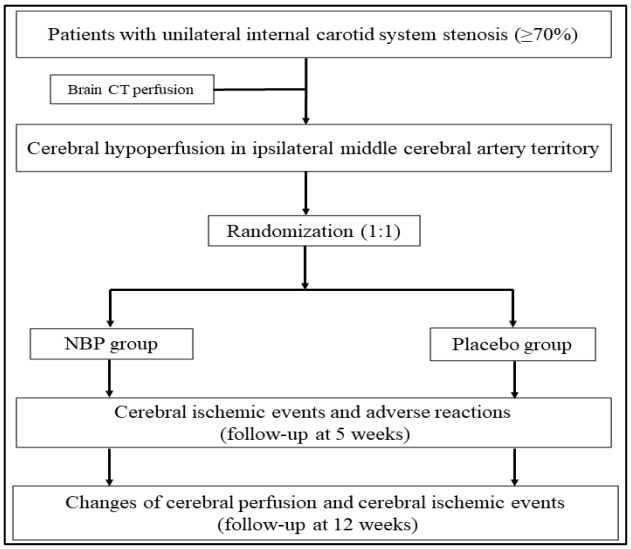

Figure 1: Study flowchart, NBP, DL-3-n-butylphthalide.

Patients

In order to be eligible to participate in this study, a subject must meet all of the following inclusion criteria: (1) 35～85 years of age; (2) ≥70% stenosis or occlusion in unilateral internal carotid artery (ICA) or horizontal segment (M1) of MCA; (3) cerebral hypoperfusion in the ipsilateral MCA territory; (4) no TIA or ischemic strokes

within 2 weeks; (5) written informed consent from patient or surrogate, if unable to provide consent. Exclusion criteria are: (1)≥50% stenosis or occlusion in contralateral ICA or M1 of MCA; (2)≥50% stenosis or occlusion in bilateral carotid arteries or innominate artery; (3) preferring to carry out CAS, CEA or other revascularization surgeries; (4)≥10 mm infarction in both MCA territories on CT or MRI; (5) other cerebral diseases (such as infection, degeneration, demyelination, tumor and trauma) which influence cerebral perfusion; (6) receiving NBP or vasodilatation therapy within 30 days; (7) pregnant or lactating women; (8) severe cardiac, pulmonary, alimentary and neoplastic diseases, and life expectancy ≤6 months; (9) serum creatinine≥140 umol/L or transaminase≥3 times the upper limit of normal value; (10) cerebral stenosis caused by any disease other than atherosclerosis, such as arterial dissection, vasculitis and moyamoya disease; (11) allergy to NBP or Apium graveolens; (12) participating in other clinical studies within 3 months; (13) other situations that are not suitable for inclusion.

Assessment of cerebral artery stenosis and perfusion

The cerebral artery stenosis will be assessed by magnetic resonance angiography (MRA), computed tomography angiography (CTA), or digital subtraction angiography (DSA). The extracranial artery stenotic degree is calculated according to North American symptomatic carotid endarterectomy trial collaborators (NASCET) and the ICAS degree is calculated according to WASID. The stenotic degree is divided into severe (70%-89%), high-grade (90%-99%) and occlusion.

The cerebral perfusion will be assessed by computed tomography perfusion (CTP).[21] The ipsilateral and mirror MCA territories to ICA or MCA stenosis are selected as region of interests (ROIs) to measure cerebral perfusion. The methods of drawing ROIs are not required identical among all the clinical centers. However, in every clinical center, the ROIs drawing must be carried out in a same equipment, using a same software, and by a same imaging technician for the two times CTP in all the patients during the trial.

The cerebral perfusion parameters include CBF, cerebral blood volume (CBV), mean transit time (MTT) and time to peak (TTP). CBF represents the volume of blood perfusing the given volume of brain per unit time (mL/100 g/min); CBV represents the total volume of blood in a given volume of brain tissue (mL/100g); MTT represents the average transit time for the blood to pass through the given volume of the brain (s); TTP represents the time between baseline and peak of enhancement (s). The ratios of above parameters obtained from the ROI in the ipsilateral MCA territory to those from the ROI in the mirror MCA territory are calculated for the cerebral perfusion normalization. These ratios are called as relative CBF (rCBF), relative CBV (rCBV), relative MTT (rMTT) and relative TTP (rTTP) respectively and will be used for data analysis. The rCBF is expressed by $CBF_{stenosis}/CBF_{mirror}$, rCBV is done by $CBV_{stenosis}/CBV_{mirror}$, rMTT is done by $MTT_{mirror}/MTT_{stenosis}$, and rTTP is done by $TTP_{mirror}/TTP_{stenosis}$. The cerebral hypoperfusion was defined as rCBF≤90%.

Randomization and treatment

Patients will be randomly assigned in a 1:1 ratio to NBP and placebo groups. An independent statistician from Chinese Beijing medical university creates the randomization list by the compute. Every kit of drug was labeled with sequential numbers corresponding to the randomization list. The successive patients will be randomly enrolled into any participating clinic center by competitive principle and distributed with the kits from lowest number to highest number by the drug administrator. All kits of drugs have identical appearance and similar smell. The patients, investigators and drug administrator will be blinded to the drug allocation.

The patients in NBP group will be given oral 200 mg NBP each time and three times daily. The patients in placebo group will be given oral 20 mg NBP (ineffective dose) each time and three times daily. The course of treatment is 4 weeks in both groups. In addition, all the patients are required to receive standard IMT to prevent ischemic stroke, including antiplatelet aggregation, lipid-lowering, antihypertension and hypoglycemia during the trial, according to 2021 AHA/ASA Guideline for the Prevention of Stroke.[22] However, any drug of volume expansion, vasodilatation, and induced hypertension will be prohibited to take during this trial.

Follow-up and withdrawal

There are two times follow-up during this trial. At the 5th week, the medication compliance and drug adverse reactions will be assessed. After 12 weeks, the cerebral perfusion will be assessed by CTP again. At same time, it will be investigated whether the risk factors related to ischemic stroke, such as smoking, body mass index, hypertension, diabetes mellitus, hyperlipidemia, have been controlled to the ideal level. In addition, the cerebral ischemic events in the ipsilateral MCA territory and in any territory will be recorded in first and second follow-ups.

Patients can leave the trial at any time for any reason. Every attempt will be made to complete follow-up in these patients. If the patients have some situations that influence the cerebral perfusion assessment, they will be withdrawn from the trial. If the patients appear serious adverse event (SAE), they must be asked to stop the trial.

Primary outcome measure

The primary efficacy outcome is the proportion of patients with rCBF change in the ipsilateral MCA

territory to ICA or MCA stenosis after treatment. The rCBF change is classified according to the difference of rCBF between pre-treatment and post-treatment. The rCBF amelioration was defined as $rCBF_{after}$ / $rCBF_{before}$-1≥10%, and the rCBF deterioration was defined as $rCBF_{after}$ / $rCBF_{before}$ −1≤-10%, and the rCBF stabilization was defined as -10%＜$rCBF_{after}$ / $rCBF_{before}$ −1＜10%.[23]

Secondary outcome measures

The secondary outcomes include the proportions of patients with rCBV, rMTT and rTTP changes in the ipsilateral MCA territory to ICA or MCA stenosis after intervention. Similar to rCBF, the rCBV, rMTT and rTTP changes are also classified into amelioration, stabilization and deterioration according to the difference between pre-treatment and post-treatment. In addition, the absolute difference values of rCBF, rCBV, rMTT and rTTP between pre-treatment and post-treatment for each patient are also recorded. The secondary outcomes also include the rates of cerebral ischemic events in the ipsilateral MCA territory and in any territory.

Safety measures

Adverse events are defined as any unexpected incidents disadvantageous to subjects during the study, whether or not they are related to the experimental treatment. A SAE is defined as any untoward occurrence or effect that causes death, is life-threatening, requires prolonged hospitalization or results in persistent significant disability. All adverse events will be reported spontaneously by the patients or observed by the investigators at the follow-up. The white blood cell count, platelet count, hemoglobin, and renal and hepatic functions will also be investigated at the follow-up. Incidence and severity of adverse events will be used for safety evaluations.

Data monitoring and management

Data monitoring and management will be performed in accordance with the international conference on harmonisation-good clinical practice guidelines (ICH-GCP). All adverse events will be reported to the data and safety monitoring board (DSMB), and SAEs will be reported to the ethics committee. DSMB composed of experienced neurologists, pharmacologists, neuroradiologists that do not participate in the trial. DSMB will monitor outcomes of the trial and be responsible to recommend whether to stop/continue the trial. Personal information will be protected and will only be available for the coordinating investigators. Every patient will be assigned a study number to maintain anonymity. All neuroimaging data will be either uploaded through website or collected by hard disk and stored in the Trial Imaging Center. Two independent neuroradiologists will monitor all the imaging data collected from each center to confirm the imaging inclusion and exclusion criteria from baseline CT/MRI, CTA/MRA/DSA and CTP, and to ensure the follow-up CTP valid. It will be decided by the third neuroradiologist for disagreements. After all data are entered into a central digitalized database using Epidata software by the investigators, the database will be checked and locked. Then the first unblinding will be done, and the statistician will conduct statistical analysis according to the plan formulated in advance. The second unblinding will be done once the statistical analysis is completed. This is an investigator-initiated study, no other parties and companies will influence the analysis or publication concerning study data.

Clinical centers

Thirty-three clinical centers across China will participate in this trial (Supplementary file 1). The clinical co-ordinating center and the trial imaging center are located at the Air Force Medical Center, Chinese PLA (People liberation army).

Sample size and statistical analysis

The sample size of this study is based on the primary outcome, the proportion of rCBF change after treatment. Our previous studies showed that there was about 15% higher percentage of CBF amelioration and stabilization for the patients with carotid stenosis and cerebral hypoperfusion after 12 weeks in NBP group than in placebo group.[20] Type 1 and type 2 error probabilities are set at 0.05 (two-sided) and 0.20, respectively. All patients are assigned in a 1:1 ratio. Based on the above assumptions, the sample size is about 400 with 200 patients in each arm using a two-sided z-test by the software of SPSS 16.0 (IBM, Amon, New York, USA). Given an expected dropout of 20%, the total number of patients to be randomized is increased to 480 (240 per group).

The primary and secondary outcome measures will be analyzed in the data from the intention-to-treat population and the per-protocol population. In this study, the intention-to-treat population will include all the patients who will complete the second CTP examination. The per-protocol population will consist of the patients with no protocol violations among the intention-to-treat population.

The proportions of CTP parameter changes (amelioration, stabilization and deterioration) after treatment will be compared using Chi-square test or Fisher's exact test between NBP group and placebo group. The absolute difference values of CTP parameters between pre-treatment and post-treatment will be compared using Student's t test or Mann-Whitney U test between two groups according to the normality test. In addition, the rates of cerebral ischemic events in the ipsilateral MCA territory and in any territory, will also be examined between two groups by Chi-square test or Fisher's exact test. Furthermore, multivariable regression model will be

used to adjust for possible imbalance of main prognostic variables between the NBP and placebo groups, such as demographics, cardiovascular risk factors, stenotic characteristics, IMT, modified cardiovascular health status. The safety outcome, such as incidence and severity of adverse events, will be compared between two groups using Chi-square test or Fisher's exact test. The statistical significance will be defined as $p \leq 0.05$. All the data will be analyzed by the software of SPSS 16.0 (IBM, Amon, New York, USA).

DISCUSSION

This trial is designed to compare the efficacy and safety of NBP with placebo, on basis of IMT, for cerebral hypoperfusion in the patients with unilateral internal carotid system stenosis. This trial may provide an evidence-based clinical strategy to prevent ischemic stroke and vascular cognitive impairment due to cerebral large-vessel stenosis by the amelioration of cerebral hypoperfusion, especially for the patients who have the high-risk features or are unfit for the surgical revascularization treatment.

Some facets in this trial design warrant discussion. First, compared with SPECT used in the previous single-center trial, CTP is the more commonly performed perfusion imaging technique for the evaluation of cerebral hemodynamics, given that CT is widespread availability in China, not influenced by tracer half-life, ease of patient monitoring, and provides more parameters for perfusion evaluation.[20,21] Therefore, CTP imaging technique will be selected to warrant this trial to be carried out in the different clinic centers.

Second, when a cerebral vessel is occluded, the MTT will increase. According to the central volume principle: CBF=CBV/MTT, if the collateralization is appropriate, there will be compensatory increase in CBV to maintain a normal CBF. However, when collateral blood flow is not sufficient to increase CBV and compensate for the prolonged MTT caused by the arterial occlusion or stenosis, CBF will decrease. Therefore, CBF can indirectly provide cerebral collateral information, although it does not directly assess the size and number of the collaterals.[24] The cerebral vascular reserve (CVR) is a more important cerebral hemodynamic parameter to predict future ischemic stroke in patients with carotid artery stenosis or occlusion, but it needs two times CTP, and acetazolamide injection or CO_2 inhalation which are uncomfortable to some subjects. In addition, CVR usually reflects the ability of cerebral vasodilation, but not collateral status.[2,25] In view of the above, this trial will select CBF as the primary efficacy outcome to verify the hypothesis that NBP can significantly ameliorate cerebral hypoperfusion by increasing the cerebral collaterals.

Third, the patients eligible to this trial have a unilateral severe internal carotid system stenosis, and the contralateral arteries are normal or have slight lesions that do not influence cerebral perfusion. In normal subjects, there is no significant difference between perfusion in the left and right sides of brain, and the variability in left- and right-sided cerebral perfusion is not over 10%.[26] Therefore, we defined the difference between ipsilateral perfusion and contralateral perfusion ≥10% as the cerebral hemodynamic impairment from the stenosis or occlusion. This definition will eliminate the bias from the difference of cerebral perfusion between the individuals or environment influence.

Fourth, one study showed that intra-observer and inter-observer variability were smaller for relative CTP values compared with absolute CTP values for CBF (10% versus 18%, p=0.000, and 13% versus 18%, p=0.000).[27] This suggested that the relative perfusion values (ratios of stenotic hemisphere to contralateral hemisphere) were more stable than absolute values. At same time, the MCA territory showed the least measurement variability among the 4 territories of anterior cerebral artery (ACA), MCA, posterior cerebral artery (PCA) and basal ganglia. Another study also demonstrated that the relative CTP values were more sensitive than absolute CTP values to detect the changes in cerebral perfusion after revascularization in patients with unilateral symptomatic carotid artery stenosis.[28] In addition, compared with absolute CTP values, the relative CTP values can also decrease the bias from CTP techniques between the different centers. Therefore, we will select the relative CTP values to follow up and detect the changes of cerebral perfusion between pre-treatment and post-treatment.

Last but not least, since NBP smells plant odor, the placebo medicine will be designed as a low and ineffective dose rather than a blank one, in order to make sure of an effective double-blind in this trial.

The CEA and CAS have their limitations to treat extra-canial artery stenosis. The high-risk features for CEA include the presence of severe cardiac disease, severe pulmonary disease, CEA restenosis, neck radiation/surgery, stenotic location too high/too low, contralateral occlusion. The high-risk features for CAS are heavy calcification, type III arch, carotid tortuosity, and thrombus. Although the age is not high risk per se to CEA and CAS, these high-risk conditions usually exist in the patients who are over 80 years old.[3] Unfortunately, it had been showed that the carotid stenosis increased consistently with age, and the prevalence of >50% carotid stenoses among ≥80 years of patients was 7.5% and 5.0% for males and females respectively.[2,29,30] In addition, ICAS is more prevalent in the aged and in Asians.[31-32] The current studies and guidelines do not support the percutaneous intracranial stenting and EC-IC employed as first-line treatment in patients with ICAS.[7,33] Therefore, it is reasonable to use the drug strategy to improve the cerebral hypoperfusion in the patients who are unfit for the surgical revascularization. This study

expands the upper limit of eligible age to 85 years old and does not exclude the above high-risk conditions for the surgical revascularization as possible as we can. In fact, this study does not enroll the subjects of more than 85 years old and severe systemic diseases, mainly considering the toxicity of the iodine contrast agent from CTP examination but not NBP to these subjects. Since the previous studies have showed that NBP was generally well tolerated and had little adverse events, such as gastrointestinal reactions and slight elevation of aminotransferase, NBP should be also safety for the very old and severe systemic diseases.[34-36] Therefore, a positive result in our trial will suggest that NBP can provide a novel strategy to improve the cerebral hemodynamic impairment due to large intracranial and extracranial atherosclerotic stenosis in the surgical high-risk patients, especially in the aged and Asians.

CONCLUSION

Our trial will provide a high-level of evidence for NBP to treat the CCH, which may consequently advance the guideline for the prevention of ischemic stroke and cognitive impairment in patients with cerebral large-vessel stenosis.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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