CLINICAL STUDY PROTOCOL

Title: Preventive Effects of Ginseng against Atherosclerosis and SUbsequent ischemic Stroke: A randomized controlled trial

(PEGASUS)

Study Type: Single-center, Randomized, Double-blind, Placebo-controlled trial; Investigator-initiated trial

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1. Ethical considerations

This trial will be conducted in compliance with Good Clinical Practice (GCP) and Declaration of Helsinki. This trial will start after the written approval of the institutional review board (IRB) of our institution is obtained.

2. Introduction or background information

Atherosclerotic stenosis in a cerebral artery progressing more than 70% caused cerebral ischemia, leading to developing collateral circulation. Ischemic symptoms of a patient are varying by the development of collateral circulation, which is caused by not only anatomical differences but also angiogenesis factors. Recent study reported that Korean red ginseng (KRG) had a good effect on cardiovascular system by affecting nitric oxide (NO). Endothelial nitric oxide synthase (eNOS) was studied a lot because that NO produced by eNOS was associated with anti-inflammation and anti-atherosclerosis. Moreover, KRG was reported that it had a vasodilation effect, a blood pressure stabilizing effect and an anti-platelet effect such as reducing platelet aggregation. Several effects mentioned above suggests that KRG had the preventive effect in cardiovascular system and ischemic cerebral stroke.

Therefore, we hypothesize that ginseng has prevention effects against atherosclerosis and subsequent ischemic stroke. The preventive effects of ginseng on cerebral ischemic stroke will be evaluated in the group of the patients with advanced atherosclerosis such as severe stenosis or occlusion in the intracranial arteries. In addition, the changes of collateral perfusion state on an ischemic territory caused by atherosclerosis will be evaluated using

quantitative magnetic resonance angiography as a surrogate marker.

3. Literature review

A. Some reports showed that ginseng had a good effect on cardiovascular system through affecting NO.
B. It was reported that NO produced by endothelial nitric oxide synthase (eNOS) was related to anti-inflammation and anti-atherosclerosis.
C. Ginseng had effects of reducing platelet aggregation, lowering mean systolic pressure, and vasodilatation.

4. Study objectives and purpose

The aim of this trial is to study prevention effects of ginseng against atherosclerosis and subsequent cerebral ischemic stroke in high-risk patients with severe steno-occlusive lesions in internal carotid artery, middle cerebral artery, and posterior circulation.

5. Expected study duration

From the date of institutional review board (IRB) approval to July 31, 2018.

6. Study subjects

A. Selection criteria

1) Inclusion criteria

i. Aged between 20 and 80.

ii. Severe stenosis or occlusion at an internal carotid artery, middle cerebral artery and vertebrobasilar system in cerebral angiography.

iii. Any stroke risk factors such as hypertension, diabetes, hyperlipidemia, smoking and alcohol drinking.

iv. No adverse effect history of ginseng administration.

2) Exclusion criteria

i. Genetic cerebrovascular diseases.

ii. Adverse reaction to contrast medium.

iii. Pregnant women.

iv. History of previous intra-arterial thrombolysis, cerebral angioplasty and/or stenting, carotid endarterectomy, and bypass surgery.

v. History of cardioembolic stroke or possibility of cardioembolic stroke.

vi. Possibility of cancer stroke.

vii. Renal failure (GFR< 30 ml/min) or liver disease.

B. Risk and benefit evaluation

1) Potential risk of this study: Although additional risks are considered as minimal, potential risks participating in this trial are showed as follows: any adverse effects related to ginseng; pain, bruise or hematoma at puncture site, dizziness, syncope, or infection related to cerebral catheter angiography.

2) Potential benefit of this study: Although there would be no potential benefit for

participants, their participations in this trial would help medical advancements.

3) Conclusion of risk and benefit analysis: It is considered that a potential benefit would be bigger than a potential risk because of less adverse effect of ginseng on subjects, and an important suggestion to future treatment.

C. Eligible patients

Any inpatients or outpatients who are referred to the department of radiology for transfemoral cerebral angiography will become eligible patients. Among them fulfilled selection criteria, investigators will obtain written informed consent individually.

7. Study design

A. Type of a trial design

A single-center, double-blind, parallel-group, placebo-controlled randomized trial.

B. Outcomes

1) Primary outcome:

i. Presence of ischemic stroke or TIA (Transient Ischemic Attacks) assessed by symptom history during one-year follow-up and measured by number of patients in each group.

ii. Presence of other cerebro-cardiovascular morbidity or mortality assessed by aggravation of patient status (mRS, modified Rankin Scale) at out-patient clinic during one-year follow-up and measured by the number of patients.

2) Secondary outcome:

i. Changes in volumetric blood flow (ml/Sec) in quantitative MRA measured

by percentage change from the baseline study.

ii. Changes in velocity (cm/Sec) in quantitative MRA measured by percentage change from the baseline study.

iii. New FLAIR MR lesions assessed on one-year follow-up MRI and measured by the number of patients.

C. Sample size calculation

Assuming the proportion of 0.1 in the ginseng group and the proportion of 0.4 in placebo group in the analyses of primary and secondary outcomes, calculated sample size would be 29 patients each group for determining difference between two groups in the level of alpha of 0.05, power of 0.8 and 1:1 sampling ratio (equality test).

D. Randomization and follow-up of outcomes

There are two study groups as follows: a ginseng group as an intervention group, a promising placebo group as a control group. The participants will be randomly allocated to either the ginseng group or the placebo group by 1:1 ratio according to random permuted block with a block size of 20. Both groups will be followed up at 1, 3, 6 and 12 months after randomization. At each follow-up, history of the primary outcome, any adverse effects, any co-administration drugs, and study drug compliance will be evaluated. The secondary outcomes will be evaluated by comparison of the results of quantitative MR angiography studies performed at baseline and 12 months after randomization.

E. Study drugs and administration

Two study drugs are identical in appearance. In the intervention group, 2-gram ginseng per day (0.5 grams/capsule, 2 capsules twice a day) will be administrated for

12 months. In the control group, 2-gram placebo per day (0.5 grams/capsule, 2 capsules twice a day) will be administrated for 12 months.

F. Safety evaluation and reporting

If any adverse effects are occurred, the participants will report the problem to an investigator. The investigator will appoint outpatient clinic. When the subject visits the outpatient clinic, the investigator will evaluate allergic symptoms such as redness, swelling, respiratory difficulty and grade the symptom severity. The investigator will gather other subject symptoms as patient says.

G. Primary outcome evaluation

The presence of any cerebral ischemic symptoms is the primary outcome. The history of cerebral ischemic symptoms includes transient ischemic attack (TIA) and cerebral ischemic infarction. If the related history is reported, a diffusion-weight MRI will be performed. It will be evaluated whether new ischemic lesion is related to the steno-occlusive lesion confirmed by cerebral angiography at baseline.

H. Secondary outcome evaluation

A quantitative MR angiography (QMRA) with noninvasive optimal vessel analysis (NOVA) will be performed for evaluating secondary outcomes at baseline and 12 months after randomization. QMRA with NOVA will measure blood flow at the predetermined sites. The results would become an indirect measurement of collateral flow state of a steno-occlusive vascular lesion.

8. Data collection forms and items

Subject No.	Sex	Age	Results of cerebral catheter angiography			The presence of acute ischemic lesions
			The presence of a steno-occlusive lesion at internal carotid artery, middle cerebral artery, and vertebrobasilar system	Stenosis severity (Severe/moderate/mild)	Complete total occlusion	

Medication Information				
Duration (mo)	Dose	Date Start	Date End	Compliance

※ Compliance

Drug name	Expected administration days	Actual administration days	Compliance (%)
Ginseng			
Placebo			

Non-invasive Optimal Vessel Analysis (NOVA) (at baseline)	Non-invasive Optimal Vessel Analysis (NOVA) (at 12 months after randomization)

Brain-MRI (New lesions on FLAIR) (at baseline)	Brain-MRI (New lesions on FLAIR) (at 12 months after randomization)

The presence of any ischemic stroke history at 3 months follow-up	The presence of any ischemic stroke history at 6 months follow-up	The presence of any ischemic stroke history at 9 months follow-up	The presence of any ischemic stroke history at 12 months follow-up

The follow-ups can be conducted in outpatient clinics or by telephone interview performed by experienced nurses since the presence of ischemic stroke or TIA history is asked, and mRS is evaluated in the interview. The investigators can do neurologic evaluations if needed.

9. Blood laboratory test and imaging test

A. Blood laboratory test

none

B. Imaging test

1) A quantitative MR angiography (QMRA) with noninvasive optimal vessel analysis (NOVA) for the changes of cerebral blood flow.

2) A brain MRI including FLAIR for the presence of new cerebral ischemic lesions.

10. Subject discontinuation (Drop-out)

1) Difficulty in administration of study drugs.

2) Impossible to follow up.

11. Patient data safety

Any identifiable human material and data will be excluded in the research. And a subject will be given a subject number. Collected data will be managed with a password by only the principle investigator and investigators. The data will be discarded after 3-year mandatory storage period.

12. Audit

The principle investigator will carry out inspections at finishing data collection and at

finishing data analysis.

13. Statistical analysis

The results will be analyzed in intention-to-treat method. For analysis of primary outcome, Fisher's exact test will be used for a dichotomized variable.

14. Detailed study schedule

Visits	Screening baseline	1st F/U (Telephone/ OPD visit)	2nd F/U (Telephone/ OPD visit)	3rd F/U (Telephone/ OPD visit)	4th F/U (Telephone/ OPD visit)
Date	0 day	1 month (± 2 weeks)	3 months (± 2 weeks)	6 months (± 2 weeks)	12 months (± 2 weeks)
Obtaining consent	●				
MR	●				●
Drug compliance		●	●	●	●

15. References

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