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Effects of sulforaphane on cognitive function in patients with frontal brain damage: study protocol for a randomised controlled trial

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ABSTRACT

Introduction Many patients with frontal brain damage show serious cognitive function deficits, which hamper their quality of life and result in poor clinical outcomes. Preclinical research has shown that sulforaphane can significantly improve spatial localisation and working memory impairment after brain injury. The primary aim of this double-blind randomised controlled clinical trial is to assess the efficacy of sulforaphane for improving cognitive function in patients with frontal brain damage.

Methods and analysis Ninety eligible patients will be randomly allocated to an active treatment or a placebo group in a 2:1 ratio. Participants will undergo a series of cognitive and neuropsychiatric tests at baseline (week 0) and after 12 weeks to determine the effect of sulforaphane on cognition. Magnetic resonance spectrum of the brain will be studied using the 3T MRIs of the brain to detect brain metabolites markers, including N-acetyl aspartate, glutamate (Glu), glutathione (GSH) and γ-aminobutyric acid (GABA). Blood brain-derived neurotrophic factor, Glu, GSH and GABA levels and gut microbiota will also be assessed over this period. This study will also evaluate long-term outcomes of brain trauma, brain tumours and cerebrovascular disease via exploratory analyses. The primary outcome will be the difference in scores of a battery of cognitive tests after 12 weeks of sulforaphane treatment. The secondary outcomes will be changes in the Functional Activities Questionnaire (FAQ), the Patient Health Questionnaire (PHQ-9), the Self-Rating Anxiety Scale, the changes in T1-weighted MRI and resting-state functional MRI findings, and changes in brain and blood metabolic markers and gut microbiota at weeks 0 and 12. We expect that sulforaphane will yield favourable results in treating memory and learning deficits for patients with frontal brain damage. Cognitive functional treatment may also improve brain trauma, brain tumours and cerebrovascular outcomes.

Ethics and dissemination The study protocol has been approved by the Medical Ethics committee of the Xiangya Hospital of Central South University (No. 2017121019). The results will be disseminated in peer-reviewed journals and at international conferences.

Trial registration number This trial was registered on Clinicaltrials.gov on 31 January 2020 (NCT04252261). The protocol version is V.1.0 (20 December 2019).

Strengths and limitations of this study

► This trial will be the first randomised controlled trial to assess the effect of sulforaphane on the cognitive performance of patients with brain damage.
► The use of resting-state functional MRI and brain metabolite markers in conjunction with serum markers is unique and provides an innovative approach.
► The results of this study will help elucidate whether sulforaphane can be used in cognitive rehabilitation strategies in brain trauma, brain tumours and other types of brain damage.
► The various causes of frontal brain damage may affect the results of cognitive performance.
► This preliminary study will be limited to Chinese population, and studies on other population groups will subsequently be required.

BACKGROUND

The frontal brain, located in the front of the cerebral cortex, is involved in cognitive functions such as attention, working memory, language, spatial orientation and execution.[1–3] Frontal lobe lesions resulting from conditions, such as trauma, tumour, cerebrovascular disease or parasitic disease, can damage the cortical networks and lead to local neuronal necrosis, degeneration, ischaemia, and inflammation, and cognitive deficits.[1 4–6] Studies have shown that 42%–80% of the patients with frontal brain damage show serious cognitive function deficits, particularly in attention, learning, memory and executive function, and the damage can be sustained for more than 4–9 months.[2 7–10] In addition, the cognitive and functional impairment has been proved to be associated with poor clinical outcomes in patients with glioma and stroke.[11 12] Cognitive rehabilitation and treatment interventions can improve attention, executive function and decision-making abilities, but are poorly effective for abstract thinking, memory and visual structural

abilities.[9] [13] As such, it is of great clinical significance to develop and study treatments for cognitive impairment after frontal lobe damage.

Sulforaphane, an active extract of cruciferous vegetables such as broccoli and cabbage, upregulates genes that suppress oxidative stress, inflammation and DNA damage. Sulforaphane also enhances mitochondrial function, promotes glutathione (GSH) synthesis and crosses the blood–brain barrier to reduce nerve inflammation.[14] [15] Sulforaphane has shown significant antioxidant and cellular protective effects in animal models associated with oxidative stress, such as focal cerebral ischaemia, brain inflammation and intracranial haemorrhage,[16–19] and it could improve attention-focusing function in patients with schizophrenia.[20] In rat models, sulforaphane can significantly improve spatial localisation and working memory impairment after traumatic brain damage by inhibiting oxidative stress.[21] We, therefore, hypothesise that sulforaphane is useful as an early intervention to reduce neuroinflammation and improve cognitive function in patients with cognitive impairment after frontal lobe damage.

Previous studies have shown that neuron-specific metabolite abnormalities in N-acetyl aspartate (NAA), brain-derived neurotrophic factor (BDNF), GSH, glutamate (Glu) and γ-aminobutyric acid (GABA) levels could modulate and predict different aspects of cognitive performance.[22–28] Research has shown that oxidative stress can act on BDNF in the injured brain to affect synaptic plasticity and cognition;[29] therefore, the sulforaphane-mediated antioxidative effect might influence BDNF levels to improve cognitive function. Several studies also show that sulforaphane augments whole brain and peripheral GSH levels to attenuate oxidative stress, which also supports its potential application in cognitive improvement.[30] [31] Reportedly, gut microbiota is closely linked to memory, mood and cognitive function, through the interkingdom signalling system and the bidirectional communication of neuroendocrine and enteric nervous systems.[32–34] Members of genera such as *Escherichia*, *Lactobacillus* and *Trichuris* release neurotransmitters and neuropeptides (including GABA and BDNF) to affect brain function by modulating signalling within the enteric nervous system.[35] Probiotic administration has been shown to restore cognitive function and frontoparietal connectivity in various animal disease models, including hepatic encephalopathy, vascular dementia and cerebral ischaemia/reperfusion injury.[36] On the basis of these theories, we will also assess brain and blood BDNF, NAA, GSH, Glu and GABA levels and gut microbiota over the study period to reveal possible mechanisms by which sulforaphane alters cognition performance.

STUDY GOALS AND OBJECTIVES

The primary hypothesis is that sulforaphane treatment improves cognitive function, especially learning and memory in patients with frontal brain damage. We will explore associations between changes in cognitive function and changes in functional MRI (fMRI) and T1-weighted MRI as well as changes in the brain and blood BDNF, NAA, Glu, GSH and GABA levels. The associations between changes in cognitive function and gut microbiota over time will also be investigated. Finally, we will assess the effect of the improvement of in cognitive function on brain trauma, brain tumours and cerebrovascular outcomes in an exploratory analysis.

METHODS AND ANALYSIS
Patient and public involvement

Patients and public were not involved in the development of the research questions, study design or study conduct.

Study design

This is a prospective, single-centre, randomised controlled clinical trial that will be held at the Xiangya Hospital, Central South University, Changsha, China. Study recruitment will begin on 31 May 2020 and the estimated study completion date for the primary outcomes is 31 May 2022. We aim to assess 90 participants using a series of cognitive tests at baseline and 12 weeks later to determine the effect of sulforaphane (compared with placebo) on the cognition of patients with frontal brain damage. Baseline would be at least 2 days after surgery and less than 21 days after surgery. Participants will also be imaged using 3T MRI at these time points to obtain T1-weighted MRI, and resting-state fMRI (rs-fMRI), and to determine the MRI markers of brain metabolites, including NAA, Glu, GSH and GABA. Blood BDNF, Glu, GSH and GABA levels will also be assessed over this period. Gut microbiome and microbial DNA will be extracted from a gut sample according to the standard protocol.[37] After that, bacterial 16S rRNA gene sequencing and metagenomic technique will be applied to assess the changes in gut microflora over time. Figure 1 depicts the design of the study.

Subjects and recruitment

We will recruit 90 patients aged 18–65 years, and of both sexes, who have incurred cognitive deficits after frontal brain damage. All participants will be recruited from the Neurosurgery Department at the Xiangya Hospital between 10 July 2020 and 31 May 2022. Frontal brain injury will be verified using CT or MRI. Cognitive deficits will be diagnosed using the Chinese version of the Montreal Cognitive Assessment (MoCA-C), which is an eligibility test to assess multiple cognitive domains.[38] [39] Participants scoring <26 will be assessed as having cognitive deficits (<25 for patients who have received education for <12 years).[39] All eligible recruited patients will then be randomly allocated to either the sulforaphane group or the placebo group in a 2:1 ratio. During the trial period, standard clinical treatment and follow-up will be used to measure patient conditions and maintain compliance. All patients must be able to answer a series of cognitive tests, and complete measures including cognitive tests, the
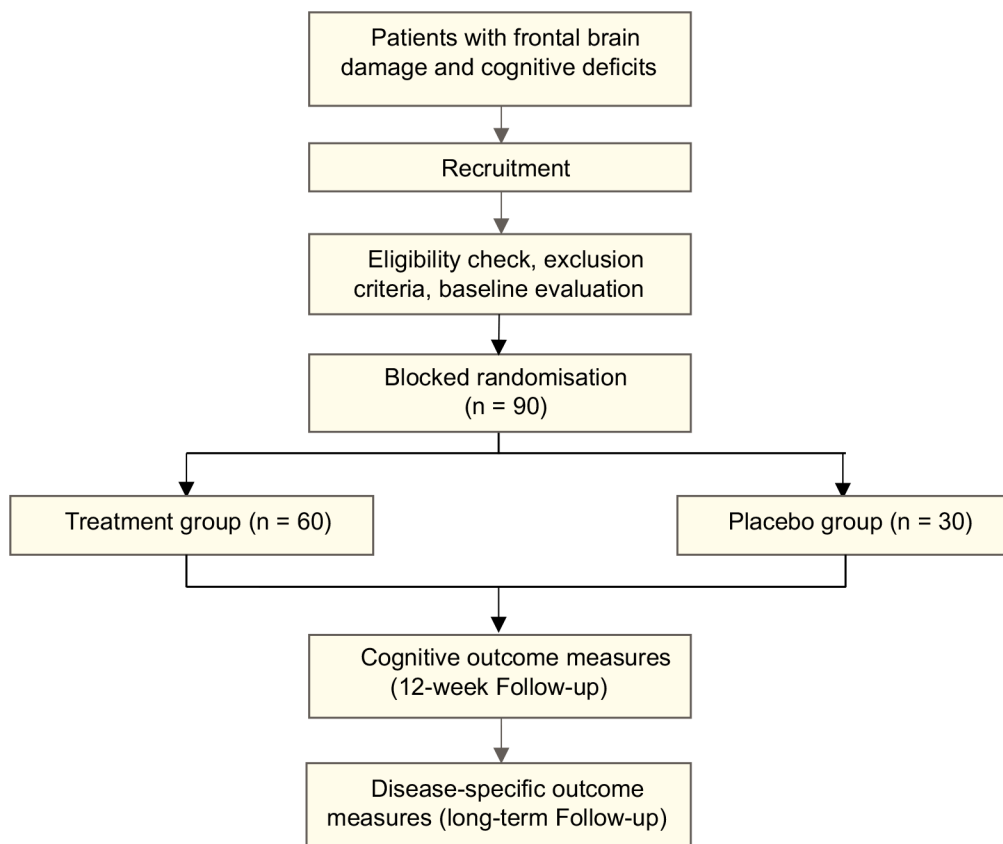
Figure 1 Study flow diagram: after recruitment, eligibility check and baseline evaluation, 90 participants will be randomised to sulforaphane group or placebo group in a 2:1 ratio. Baseline and 12-week follow-up evaluation include neurocognitive evaluation, T1-weighted MRI, resting-state functional MRI, magnetic resonance spectrum, Blood brain-derived neurotrophic factor, N-acetyl aspartate, glutathione, glutamate and γ-aminobutyric acid levels and gut microbiota. Long-term outcomes for brain trauma, brain tumours and cerebrovascular disease will also be assessed.

Functional Activities Questionnaire (FAQ), the Patient Health Questionnaire-9 (PHQ-9), the Self-Rating Anxiety Scale (SAS), MRI of the brain, blood metabolites and gut microbiota. After 12-week double-blind treatment phase, participants will be encouraged to follow-up until 24 weeks for further cognitive evaluation.

Exclusion criteria

1. History of cognitive impairment by clinical diagnosis, such as dementia and intellectual disability.
2. History of seizures owing to the potential of sulforaphane to increase seizure susceptibility.[40]
3. A current or prior significant DSM-5 (Diagnostic and Statistical Manual of Mental Disorder, Fifth Edition) diagnosis of psychiatric disease, chronic neurological disorders or active substance abuse.
4. Inability to cooperate while undergoing MoCA-C Test owing to disturbances in consciousness or mental disorder.
5. Pregnancy or lactation.
6. Life expectancy <3 months.
7. CO (carbon monoxide) poisoning, autoimmune encephalitis, intracranial infection or other types of diffuse encephalopathies.
8. Plan to receive radiotherapy during the trial period.

9. Laboratory examination showing liver and kidney insufficiency or other severe complications; presence of diseases which may interfere with the results of the evaluation.
10. Contraindications to MRI scanning, such as metallic implants or prosthetics, pacemakers, dependence on benzodiazepine medication, prohibitive claustrophobia or other medical conditions with potential safety risk.
11. Involvement in other trials 1 month prior to the start of the trial or during the trial period

Randomisation

Block randomisation will be conducted using the SAS (vesion is v9.4 software. Ninety patients will be randomised in a 2:1 ratio into a treatment group and a placebo group. Neuropsychologists, data analysts and patients will be blinded to the group assignment in this trial. Only two of the authors involved with the block randomisation will assign participants to interventions.

Study intervention

Sulforaphane or an identical placebo medication will be used as a treatment intervention. Patients will be given a specific number of sulforaphane tablets (Avmacol, 60

Table 1 Overview of cognitive tests, MRIs of the brain, magnetic resonance spectrum, blood metabolites and gut microbiota tested in the study

Cognitive tests	Cognitive domain(s)	Baseline	12 weeks
Hopkins Verbal Learning Test—Revised	Verbal learning and memory	✔	✔
Trail Making Test Parts A and B	Speed of processing and executive function	✔	✔
Digit Span Test	Concentration and working memory	✔	✔
The Chinese version of the Montreal Cognitive Assessment	Multiple cognitive domains	✔	✔
Neuropsychological Assessment Batteries	Reasoning and problem solving	✔	✔
Wisconsin Card Sorting Test	Reasoning and problem solving	✔	✔
Brief Visuospatial Memory Test—Revised	Visual learning	✔	✔
Animal Verbal Fluency Test	Verbal fluency	✔	✔
Examination	**Items**	**Baseline**	**12 weeks**
Neuropsychiatric Scale	Functional Activities Questionnaire, Patient Health Questionnaire-9, Self-Rating Anxiety Scale	✔	✔
MRI of the brain	T1-weighted MRIs of the brain (T1-weighted MRI), and resting state functional MRI	✔	✔
Magnetic resonance spectrum of the brain	N-acetyl aspartate, Glu, GSH, and GABA	✔	✔
Blood metabolites	brain-derived neurotrophic factor, Glu, GSH, and GABA	✔	✔
Gut microbiota		✔	✔

GABA, γ-aminobutyric acid; Glu, glutamate; GSH, glutathione.

tablets) once a day with dosage adjusted according to body weight, with patients weighting <45 kg will receive two tablets (1750 mg) per day, patients weighing 45–90 kg will receive three tablets (2550 mg) per day and patients weighing >90 kg will receive four tablets (3400 mg) per day. Study medication will be dispensed at baseline. For standard care, all patients will attend their clinical follow-up at the Xiangya Neurosurgery Outpatient Center independent of the study and group assignment and will be examined by other physicians not involved in this study.

Measures

Outcome measures for the study, including cognitive and neuropsychiatric tests, MRI of the brain and magnetic resonance spectrum (MRS) of the brain findings, blood metabolite levels and gut microbiota, are listed in table 1.

Cognitive testing

Cognitive function tests will be performed at baseline and again at 12 weeks after randomisation. These include the following:

1. Hopkins Verbal Learning Test—Revised to assess verbal learning and memory.[41]
2. Trail Making Test Parts A and B to assess the speed of processing and executive function.[42]
3. Digit Span Test to measure concentration and working memory.[43]
4. The MoCA-C to assess multiple cognitive domains.[38 39]

5. Neuropsychological Assessment Batteries to assess reasoning and problem solving.[44]
6. Wisconsin Card Sorting Test to detect frontal lobe dysfunction, and assess reasoning and problem solving.[45]
7. Brief Visuospatial Memory Test—Revised to measure visual learning.[46]
8. Animal Verbal Fluency Test to assess verbal fluency and frontal brain function.[47]

MRIs of the brain

All MRIs will be performed using a 3T Skyra scanner (Siemens, Erlangen, Germany). The MRI examination takes approximately 30 min to complete and comprises standardised sequences used for the analysis of brain morphometry, microstructure and function, including:

1. T1-weighted MRI of the brain, grey and white matter morphometry and anatomical reference.
2. rs-fMRI to investigate the functional connectivity between the prefrontal cortex (PFC) and other brain regions.
3. MRS with spectra recorded from the PFC;

Processing and interpretation of neuroimaging data will be conducted by an author who will be blinded to participant information and intervention. All scans will be reviewed and reported by a neuroradiologist.

Sample size

Sample size calculation will be carried out using the SAS software. Since there were no previous studies of

sulforaphane in patients with frontal brain damage, we estimated the sample size based on our unpublished study of sulforaphane treatment on cognitive impairment for other diseases (ClinicalTrials.gov: NCT02880462). 90 was calculated as the number necessary to detect an average difference of 18.5 in multiple cognitive domains between baseline and 3 months in the treatment group, and of 11.9 in the placebo groups at 80% power with a two-sided p value of 0.05. These calculations include a 20% inflation for dropout and loss of patients to follow-up. SD in total scores are estimated at 14 at 0 and 3 months. However, without knowledge of the within-person correlation between data points, it is impossible to predict the increase in power calculation.

Trial status

Trial status is currently in the preparation phase for recruitment as of 16 May 2020.

Safety considerations

There are negligible risks for patients treated with sulforaphane (Avmacol, 60 tablets), which meets the requirements of the US Food and Drug Administration. Serious adverse events will be recorded and properly managed, and then reported to the Ethics committee of the Xiangya Hospital.

Follow-up

Outcome measures including cognitive and neuropsychiatric tests, T1-weighted MRI, rs-fMRI, brain and blood metabolic markers and gut microbiota, will be tested after the 12-week double-blind treatment phase. This study will also evaluate long-term outcomes for brain trauma, brain tumours and cerebrovascular disease via exploratory analyses. All patients in the double-blind treatment phase will be encouraged to be re-evaluated at week 24. Study medicine will not be administered from week 12 to week 24. Further clinical treatment will be arranged by the study investigator and/or the subject's treating physician during this period. Participants who discontinue the intervention will be followed up for further statistical analysis according to the protocol because they have been randomly allocated.

Data management and statistical analysis

Every participant will receive a unique identification number for the duration of the study to maintain treatment anonymity. Neuropsychologists, data analysts and patients will be blinded to the treatment assignment in this trial. Only two of the authors involved with the block randomisation will assign participants to interventions. All cognitive, MRI, blood metabolite and sequencing data will be collected and stored by direct members of the research team before the end of the trial.

Statistical analysis will be performed from the obtained results by using SPSS V.24. The results of the continuous scale will initially be evaluated for normality and logarithmic conversions will be performed where appropriate. Results changes over time will be determined by mixed linear or non-linear longitudinal modelling, to determine whether each group behaves differently over time. Additional sensitivity analyses will be performed based on known predictors of the outcome (age, sex and other risk factors) and any baseline imbalances between groups. Subgroup analysis was conducted according to the causes of frontal brain injury. A two-sided p value of 0.05 or less will be considered to be statistically significant. The relationship between changes in brain and blood GSH, GABA, NAA and BDNF levels, gut microbiota, and cognitive function will also be determined using the mixed linear longitudinal model for cognitive function measurements over time.

Quality assurance

Test assessors for these protocols undergo standard training and assessment and are recertified annually. Annual monitoring will also be conducted by a senior neuropsychologist. This trial will adhere to Consolidated Standards of Reporting Trials guideline.

Outcomes and measurements
Primary outcome

The difference in scores of a battery of cognitive tests after sulforaphane treatment between week 12 and week 0.

Secondary outcomes

Besides evaluating the efficacy of sulforaphane on the cognitive performance of patients with frontal brain damage, we will analyse the changes in the FAQ, the PHQ-9, the SAS, the changes in T1-weighted MRI findings, rs-fMRI findings and changes in gut microbiota and metabolic markers (including BDNF, GSH, GABA, Glu and NAA) in the central and peripheral expressions at week 0 and week 12. We will determine whether any brain and blood metabolite changes are associated with changes in cognitive function and whether cognitive function is associated with altered brain structure and basic function.

Duration of the project

Patient recruitment was started on 31 May 2020 and will be completed by approximately 30 May 2022.

Ethics and dissemination

The study protocol has been approved by the Medical Ethics committee of the Xiangya Hospital of Central South University (No. 2017121019). All substantial amendments to the protocol should be reviewed by the medical ethics committee.

Written informed consent will be obtained from the patient's legal representative and/or the patients. Before inclusion, the investigator or an authorised member of the study will explain to the potential subjects and their legal representatives about the purpose of the study, the methods, strict data storage protocol, reasonably anticipated benefits and the potential hazards of the study. Subjects will be informed about the study's voluntary nature that they have the right to cease participation at any time. Choosing not to participate will not in any way

prejudice the care that the subject will receive for the treatment of his or her disease. The investigator will give sufficient time for the subject to read the consent form, ask questions and make decisions. Patients will only be included if their legal representatives provide written informed consent.

The results of this study will be disseminated via an international peer-reviewed publication. Further, it will be presented at national and international conferences as posters or oral presentations for communication and discussion.

DISCUSSION

This trial, to the best of our knowledge, is the first randomised controlled trial to assess the effect of sulforaphane on the cognitive performance of patients with brain damage. This is important as cognitive performance impairment leads to poor quality of life and has previously been shown to increase the risk of clinically significant outcomes and predict poor cancer-related outcomes. This study will also ascertain the clinical significance of sulforaphane by measuring the improvement in cognitive outcomes and by quantifying the relationship between cognitive outcomes and changes in brain fMRI and T1-weighted MRI, as well as with changes in brain and blood BDNF, NAA, Glu, GSH and GABA levels, and gut microbiota. In addition, we will assess the effect of cognitive function improvement in brain trauma, brain tumours and cerebrovascular outcomes in an exploratory analysis.

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