Research Report

A pilot study of a broccoli-seed tea by Parkinson's Disease patients

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Running title: Pilot study of broccoli tea for Parkinson's

Abstract

Over the last two decades, research has demonstrated how the transcription factor Nrf2 can be activated to attenuate oxidative stress and inflammation in neurons, but this hypothesis has not been formally tested on Parkinson's disease patients. Here we report on a series of eight independent *(n-of-1*) experiments carried out at their own initiative by people with Parkinson's disease using a tea prepared from broccoli seeds following a method that optimises the production of the potent Nrf2 activator, sulforaphane. The results show a marked reduction in the mean scores for non-motor symptoms compared to motor symptoms over the six-week duration of the experiment. These results suggest that quite different processes are involved in the development of these two symptoms groups and that it may be possible to slow or interrupt the process driving non-motor symptoms. The results are provisionally interpreted in terms of a cascade of events in the pathogenesis of Parkinson's disease. In this model, age-related chronic oxidative stress triggers mitochondrial dysfunction in dopaminergic neurons and is the cause of non-motor symptoms. Upregulating Nrf2 can potentially reverse this step. The second step of the cascade is the loss of the neurons as a consequence of energy shortages due to the mitochondrial dysfunction. This step is believed to be the cause of motor symptoms and is most likely irreversible.

Keywords: Nrf2, oxidative stress, mitochondrial dysfunction, motor symptoms, non-motor symptoms, isothiocyanate, sulforaphane, clinical, glucoraphanin.

The scientific context

Neurodegenerative diseases are commonly associated with oxidative stress and mitochondrial dysfunction. Indeed, the breakdown of cellular redox homeostasis is thought to play a central role in the decline and eventual loss of dopaminergic neurons of the *substantia nigra* of Parkinson's disease patients. The transcription factor Nrf2 (nuclear factor Erythroid 2 p45-related factor 2), is the master regulator of redox homeostasis and can effectively counter oxidative stress damage to mitochondria by activating the antioxidant response elements (ARE) of genes encoding detoxification and antioxidant enzymes, and promoting their transcription.[1–4] In 2010, pre-clinical research on an experimental animal model of Parkinsonism established that sulforaphane upregulated the transcription factor Nrf2, activated the so-called phase 2 complex of cytoprotective and antioxidant genes in the brain and protected nigral dopaminergic neurons from attack by the neurotoxin MPTP,[5,6] In spite of this much-cited discovery which outlined a strategy to intervene on causes of PD,[7,8] this theory has never been subjected to its most critical examination; *applying it to people with Parkinson's Disease.*

The natural isothiocyanate sulforaphane has shown great promise as a potent activator of Nrf2 and can easily be made from broccoli sprouts or seeds (*B. oleracea,* var. *italica*).[9] On the basis of earlier work and preliminary results, the author, a retired research scientist diagnosed with Parkinson's disease in 2018, developed a method to produce a broccoli-seed tea rich in sulforaphane.[9–12]

The objective of the preparation method was to optimise the yield of the hydrolysis of glucoraphanin to sulforaphane prior to ingestion. The former is very stable whereas the latter is reactive and unstable, thus the goal was to produce sulforaphane just prior to ingestion. Glucoraphanin is hydrolysed by the enzyme myrosinase, both of which are present in the seeds but only enter into reaction when the seeds are crushed in water. During hydrolysis the reaction can take several paths of which only one delivers a high yield of sulforaphane. The yield is dependent on the hydrolysis temperature, the degree of dilution and the presence or not of an "epithiospecifier" protein (ESP), which, when active, orients the final step of the hydrolysis reaction to produce an inactive by-product. To ensure inactivation of ESP, the protocol included a step to heat-treat dry broccoli seeds at 60°C followed by adding a few % of untreated white mustard seeds (*Sinapis alba*) as a replacement source of highly potent myrosinase. (Of note, mustard seeds are not a significant source of ESP.) The seeds were then ground to a course powder in a domestic coffee grinder. This powder is quite stable if kept dry.

Although natural sulforaphane sources have been used in clinical trials for conditions other than Parkinson's disease and is generally considered to be safe and beneficial to health, in most cases it has been tested in the form of its untransformed precursor molecule glucoraphanin, rather than sulforaphane itself.[12] The untransformed glucoraphanin delivery route is biologically less direct, less rapid and has a lower bioavailability than the direct sulforaphane delivery method described herein. [9]

Experiment design

Preliminary results on the use of sulforaphane obtained by the author over the previous 12 months [13] spurred interest from other people with Parkinson's disease, a number of whom expressed their wish to do their own self-experimentation. Although all the information required to prepare sulforaphane from broccoli seeds is freely available in the publications cited,[9,11,12] in order to avoid accidents if unwitting Parkinsonians were to indulge in excessive consumption of the brew, details of the preparation method were not made public. Instead, discussions began with a small group of well-informed Parkinsonians on how to exploit this situation safely based on the scientific literature available and known risks.

This evolved over several weeks to become "The broccoli-seed tea experiment". Together, participants of this group were invited to contribute to establishing a protocol for preparing and using the tea which included clear warnings and recommendations to seek advice from a medical practitioner before going further. It became a collaborative study of which the initial aim was very modest: To identify any symptoms that showed a response to the broccoli tea. Identifying one or more symptoms that might be susceptible to improvement over a short time was considered to be a more realistic and more useful goal for the group than trying to demonstrate an average or quantitative change over many symptoms.

The protocol

The protocol was primarily concerned with safety, seed sourcing and the preparation of the tea. Seed choice is very important since the seeds of each variety or cultivar of broccoli contain a different quantity of glucoraphanin, [14] and environmental conditions (year, climate, soil, etc.) also affect its concentration.[15] The following points were discussed at length by potential participants before the final version was adopted:

- ensuring that participants understood the risks involved and sought appropriate medical advice,
- the dosing plan, not exceeding the lowest dose that produces any effect, positive or negative,
- ensuring rapid feedback to all participants in case of adverse effects.
- standardising the seed procurement and an easy to follow preparation method,
- ensuring that participants understood the importance of each step in the preparation method,
- establishing the scale for reporting symptom changes,

Identity code: Fdd/mm/yy			Starting date: 10/25/20				
Symptom	**Baseline**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
Daily product quantity (g)	0	0,5	0,5	0,5	0,5	0,6	0,6
Number of doses per week	7	7	7	7	7	7	7
Daytime urinary urgency	1	1	1	1	1	1	1
Nocturnal urinary frequency	1	1	1	1	1	1	1
Constipation	1	1	1	1	0	0	0
Mood changes	1	1	0	0	0	0	0
Brain fog	1	1	0	0	0	0	0
Lack of motivation	1	0	0	0	0	0	0
General fatigue	1	0	1	0	0	0	0
Sleep quality	1	1	1	1	1	0	0
Dreams, Rem sleep	0	0	0	0	0	0	0
Memory	1	1	1	1	1	0	0
Sense of smell	1	1	1	1	1	1	1
Total non-motor	**10**	**8**	**7**	**6**	**5**	**3**	**3**
Dyskinesia,	0	0	0	0	0	0	0
Dystonia	0	0	0	0	0	0	0
Slowness	1	1	1	1	1	1	1
Rigidity	1	1	1	1	1	1	1
Hand pain	1	1	1	1	1	1	1
Leg pain	0	0	0	0	0	0	0
Balance	1	1	1	1	1	1	1
Speech difficulty	0	0	0	0	0	0	0
Soft voice	0	0	0	0	0	0	0
Eye fatigue	0	0	0	0	0	0	0
Swallowing	0	0	0	0	0	0	0
Coordination	1	1	1	1	1	1	1
Typing ability	1	1	1	1	1	1	1
Walking	0	0	0	0	0	0	0
Tremor	1	1	1	1	1	1	1
Total motor	**7**	**7**	**7**	**7**	**7**	**7**	**7**
Total symptom score	**17**	**15**	**14**	**13**	**12**	**10**	**10**
Overall evaluation							
Notes	a		b				
Events		1		2			

Fig. 1: *Example of the sorted data sheet for a participant after exclusion of the low incidence symptom records.*

Participants were requested to keep all other medications and supplements unchanged and to record their assessment of 30 common symptoms of Parkinson's disease prior to consuming the tea and each week thereafter for a period of six weeks. A 4-point scale of symptom severity was adopted with the following scores: *0: insignificant or absent, 1: moderate but manageable, 2: severe or difficult to manage, 3: major handicap or disabling*. This even-numbered Likert-type [16,17] rating scale which had no comfortable default rating, was chosen to constrain participants to make unambiguous assessments. Furthermore Chang [18] contends that a 4-point scale gives a more reliable outcome with less variance than a 6-point scale when assessing empirical observations of this kind.

With hindsight, this scale was not well adapted to the actual symptom scores of those participants who managed to complete the experiment. Parkinson's disease patients often suffer from high levels of "fatigue" and "lack of motivation" which means that they are unable to commit to a long program [19–21]. Unfortunately this was also the case here. Participants with more severe symptoms dropped out quite early in the program. As a result, those who did complete the program were patients whose disease was relatively well controlled and who rated most of their symptoms with a score of 0 or 1, more rarely, 2. Any reported changes in these symptom scores were therefore mostly of a binary nature.

The recommended starting dose was set at 0.5g of ground seed powder used in the daily dose of tea. This was deliberately set well below the dose range expected to produce any adverse effects based on the author's experience. If no adverse effects were noted and no improvement in symptoms was observed, participants were free to modify the quantity of seed powder by 0.1g or 0.2g each week. In the event that a symptom improvement was observed, the protocol recommendation was that the dose to be maintained at that level. In case of adverse effects (the most common being minor digestive problems and nausea and in some cases an increase in Parkinson's Disease symptoms), the recommendation was to interrupt the tea for a few days and subsequently restart at a lower dose once the symptoms had declined.

Participants

There were no selection criteria other than having been diagnosed with Parkinson's disease, wishing to participate and declaring no other major health conditions. Those who chose to participate were requested to note their weekly assessment of a pre-defined list of symptoms according to a simple scale and to share this record after consuming a daily dose of the tea for 6 weeks.

Participants were entirely free to make their own decisions about proceeding further and conducting their own (*n-of-1*) self-monitored experiment. Once they had completed the 6-week program, participants who declared having adhered closely to the protocol, then shared their data and observations. This article reports on the observations of eight "Patient-Researchers" who successfully completed this experiment.

Data analysis

The eight symptom data sheets were coded for anonymity before being analysed jointly by the author and by a microbiologist who assumed no responsibility for this experiment but whose expertise complemented that of the author. The primary objective of this experiment; to identify any early response symptoms was achieved and surprisingly, exceeded. Indeed, the data shows that a group of symptoms commonly called non-motor symptoms were strongly impacted by the tea, even at the lowest doses, whereas motor symptoms were largely unchanged.

To investigate this further, the data were sorted into two groups, representing 15 motor symptoms and 11 non-motor symptoms. The remaining four symptoms in the original list had very low or zero incidence for these participants and so were excluded from further tabulation. Figure 1 shows an example of a completed data sheet after sorting into two symptom groups and exclusion of the low incidence symptoms.

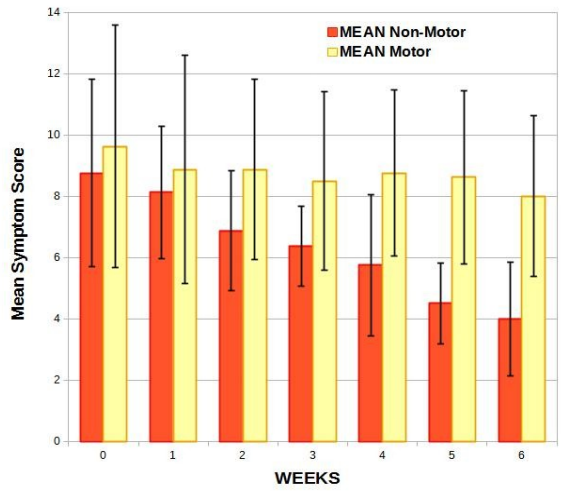

Fig. 2. *Evolution of mean symptom scores of non-motor and motor symptoms for all 8 participants. Error bars are ±1 standard deviation.*

Symptom differentiation

Each validated patient data sheet comprised 26 symptom assessments monitored over seven steps starting before ingesting the tea and for six weeks thereafter. For each period the symptom data was grouped into motor and non-motor symptoms. The sum of each symptom-group score was calculated for each patient each week. Data from the eight individual reports were then used to calculate the mean values for all eight participants. The results are shown graphically in Figure 2.

Patient differentiation

Further examination of the data revealed that five patients showed strong and rapid attenuation of their total non-motor symptoms, whereas those of the three remaining patients were practically unchanged. A closer examination showed that all the non-responders had recorded very much lower scores for non-motor symptoms at baseline, equal to about half of those for the five responders. Their potential for improvement of their non-motor scores was therefore extremely low.

Individual symptom responses

The symptoms most strongly attenuated over the course of the experiment were: a) fatigue and b) lack of motivation. Every participant reported an improvement in fatigue and the overall score for this symptom was reduced by 90% after six weeks. These are symptoms known to be related to compromised energy production by mitochondria. Urinary urgency and frequency, symptoms which have a strong impact on quality of life also responded well. Loss of sense of smell, a non-motor symptom which occurs in the very early stages of Parkinson's Disease, failed to respond.

Discussion

Notwithstanding the subjective nature of the symptom reporting, and the small number of participants, this experiment has shown that some Parkinson's Disease patients can experience strong and sustained attenuation of non-motor symptoms when consuming broccoli tea regularly.

Although these results demonstrate an association between the consumption of the broccoli seed tea and the symptom relief observed, this does not establish a relation of cause and effect. We can however consider the consistency of these results in the light of various hypotheses.

Possible placebo effect

Previous work has established that Parkinson's disease patients can produce a strong placebo response when taking part in placebo-controlled drug trials. This patient-executed experiment did not have the resources to incorporate a placebo-controlled arm. Research into the origin of the placebo effect in PD indicates that a positive response may be due to the expectation of reward (benefit from the drug) which increases the production of endogenous dopamine in the striatum.[22,23] This has been demonstrated by PET scans using ^{11}C-labelled Raclopride.[24] The increased dopamine production was associated with improved motor function and decreased motor symptoms. Other trials to generate a placebo effect have produced similar results.[25,26] Although the placebo effect cannot be ruled out when considering the results of this broccoli-seed tea experiment, they are not consistent with previous observations of the placebo effect.

Hypothesis: Oxidative-stress triggers mitochondrial dysfunction which in turn causes energy shortages in dopaminergic neurons and their eventual loss

The impact of oxidative stress on the integrity of mitochondria in neurons is emerging as a major component that can explain, and is associated with the early phase of Parkinson's Disease. Oxidative stress, mitochondrial dysfunction and energy deficiency are implicated in many neurological and neurodegenerative diseases, despite not being classified as mitochondrial diseases.[27–31] Oxidative stress associated with mitochondrial dysfunction has been demonstrated by post-mortem examination of brains and peripheral tissues of patients with PD.[32] Furthermore, the role of oxidative stress as a major contributing factor for mitochondrial dysfunction and compromised bioenergetic performance is now well established.[33,34]

The brain accounts for a large amount of the energy expenditure in humans. Comprising only 2% of body weight, the brain consumes 20% of the oxygen and 25% of available glucose. Energy is generated via oxidative phosphorylation which produces adenosine triphosphate (ATP) from nutrients and oxygen and involves the multistage electron transport chain across the inner mitochondrial membrane. Oxidative phosphorylation also generates reactive oxygen species (ROS) such as superoxide and hydrogen peroxide which, if not immediately neutralised, can cause damage to the mitochondria in which they are produced. This makes neurons in the brain particularly susceptible to both oxidative stress and energy deficits.

Chronic oxidative stress develops when ROS and reactive nitrogen species (RNS) production exceeds the capacity of the cell's antioxidant defence system to rapidly neutralise them. Under normal conditions, Nrf2, the master regulator of redox homeostasis, constantly monitors ROS and activates the glutathione and thioredoxin systems to protect mitochondria from ROS damage. In dopaminergic neurons of PD brains, there is ample evidence that this protection is defective. Oxidative stress also activates transcription factors that generate pro-inflammatory cytokines and the proliferation of lymphocytes and other immune cells which in turn leads to the production of more ROS. This interconnection between oxidative stress and chronic inflammation creates a self-sustaining and self-amplifying condition.

There is a wealth of evidence that oxidative stress is a common underlying condition driving cellular dysfunction in genetic and idiopathic Parkinson's Disease.[35] This is evidenced by increased levels of oxidised lipids, proteins and DNA, and decreased levels of reduced GSH in the *substantia nigra pars compacta* (SNpc) of PD patients.[36] Impairment of Complex 1 (NADH ubiquinone oxidoreductase) of the electron transport chain is a common feature of mitochondria isolated from PD patients.[37] Impairment of Complex 1 also leads to increased ROS production and further amplifies mitochondrial dysfunction. Magnetic Resonance Spectrometry *in vivo* has revealed that mitochondrial dysfunction is not restricted to the *substantia nigra,* but can occur in almost all regions of the brains of Parkinson's disease patients.[38,39]

The link between Parkinsonism and mitochondria was identified in the early 1980s. The neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) which causes a Parkinsonian syndrome, inhibits complex I of the electron transport chain. Complex I is reported to be reduced by about 30% in the SN and frontal cortex of Parkinson's disease patients at autopsy.[40] Mitochondrial dysfunction is well documented as contributing to the pathogenesis of Parkinson's disease.[38,39,41–43] Mitochondria in dopaminergic distal neuron axons are more susceptible to complex 1 impairment than their counterparts in the neuron body.[44]

The ability of sulforaphane to upregulate Nrf2 and activate the antioxidant response (ARE) defence processes is well established. Activation of the Nrf2/ARE pathway to neutralise oxidative stress is the most likely interpretation of the impact of sulforaphane on mitochondrial dysfunction. However, Nrf2 is also able improve mitochondrial performance in other ways such as by increasing the mitochondrial membrane potential and the availability of substrates for respiration and ATP production. [45–47] In fact, alterations in mitochondrial function have recently been documented in a neurodevelopmental condition following administration of sulforaphane. [48,49]

Persistent fatigue, a sensation of global exhaustion unrelated to physical effort, is one of the earliest and most common symptoms reported by Parkinson's disease patients, often occurring well before diagnosis and remaining throughout its progression. Fatigue has a major impact on quality of life of PD patients, but remains one of the least documented and least researched symptoms of PD.[19,20,50] Fatigue is also a hallmark symptom of mitochondrial disease.[51] Markers of elevated oxidative stress and mitochondrial dysfunction correlate with disease severity of patients diagnosed with Chronic Fatigue Syndrome.[49]

In the broccoli-seed tea experiment, the presence of fatigue at baseline was reported by all eight participants. During the experiment the score for fatigue was progressively attenuated in all eight participants over the six-week period. The impact was similar for lack of motivation (apathy), a behavioural symptom strongly influenced by available energy. Although many non-motor symptoms were attenuated by this experiment, the attenuation of fatigue may be the most important and significant observation.

Given the wealth of experimental data relating oxidative stress to mitochondrial dysfunction and compromised energy production in DA neurons,[42,52] the omnipresence of fatigue reported by Parkinson's disease patients is unsurprising. The remarkable attenuation of fatigue by the participants in the broccoli seed tea experiment is consistent with the hypothesis that sulforaphane, by upregulating the Nrf2/ARE pathway and attenuating oxidative stress may be able to reduce mitochondrial dysfunction and restore normal energy production in some types of neurons. The dynamics of non-motor symptom relief also appears to be consistent with the dynamics of mitochondrial regeneration, a process that takes days or weeks.[53,54] More research is needed to confirm this hypothesis.

Implications and perspectives

The differentiation between motor and non-motor symptoms and especially between fatigue and motor symptoms, strongly indicates that the conditions responsible for these two symptom groups are generated by different processes and could therefore benefit from different therapeutic approaches.

If the hypothesis that chronic oxidative-stress triggers mitochondrial dysfunction which in turn causes the loss of dopaminergic neurons can be confirmed, then a number of questions regarding the current understanding and treatment of Parkinson's disease need to be raised.

The current therapy of dopamine replacement is almost universally directed at attenuating motor symptoms due to the loss of dopaminergic neurons. If the cause of this loss can be attributed to mitochondrial dysfunction then it could open a window to explore disease modifying therapies targeting non-motor symptoms. Non-motor symptoms create a heavy burden carried by both caregivers and Parkinson's Disease patients. A recent cross-sectional analysis of the impact of Parkinson's Disease non-motor symptoms revealed that quality of life was most severely impacted by three domains; sleep/fatigue, mood/apathy and urinary dysfunction.[21] Despite this observation, there is currently no approved therapy to specifically target non-motor symptoms of the disease.

The theory that up to 70% of dopamine-producing neurons are dead at the time of diagnosis needs to be revisited. The possibility that a proportion of them may simply be in a diminished condition for lack of available mitochondrial energy cannot be dismissed. It is of course possible that the recovery from fatigue noted here, could also be due to improved energy production in cells other than those involved in dopamine production. Both of these possibilities require further investigation.

The results of this experiment indicate that many non-motor symptoms of Parkinson's Disease may be attenuated by upregulating the transcription factor Nrf2. However, until we understand the mechanism for this observation in more detail, we cannot know whether this corresponds to slowing down, interrupting or reversal of a process involved in this disease. Nevertheless, if at this stage, it can be proven that these two quite different mechanisms are involved in the pathogenesis of Parkinson's Disease, this information alone should help define the fields where new research for disease-modifying therapies could be focused.

Limitations of this study and recommended future improvements

This study had many limitations which the investigator and the participants readily acknowledge. It was designed as a patient-led, self-funded experiment with a very modest objective: To identify any symptoms which might respond to the consumption of broccoli seed tea. It was purely investigative in nature and did not have any quantitative objectives relative to symptom changes. Fortunately, the initial symptom-tracking plan provided enough data to undertake a more detailed analysis once the results were obtained. Armed with this knowledge, any future study should be designed to integrate better calibration and documentation of the preparations and more ambitious objectives that include the measurement of crucial biomarkers to shine light on mechanisms. Several design improvements should be considered:

- Any future study should aim to use a single broccoli seed cultivar from a single large seed-lot (harvest) with a high glucoraphanin content that has been reliably measured. This pilot investigation used 2 easily-available broccoli seed cultivars: Waltham 29 and Calabrese Don Vito. The glucoraphanin content of Waltham 29 has been reported to be in the region of 33µmol/g, [55] whereas no quantitative data was found for that of Calabrese Don Vito. Unfortunately, there is abundant evidence that year-to-year and location-to-location glucoraphanin content of the same cultivar can vary substantially. [14,15] Several broccoli cultivars have been developed with much higher levels of glucoraphanin in the range 100µmol/g to 120µmol/g. These include the open pollinated cultivars "Hopkins" and "Hi-Tech"[56] and the F1 hybrid "Premium Crop".[55] The use of high-glucoraphanin seeds would enable the sulforaphane content of the tea to be increased whilst simultaneously reducing the ingestion of other seed components such as erucic acid. F1 hybrids are not normally sold for seed consumption as they would be extraordinarily expensive for such a purpose. Furthermore some F1 hybrids may be incapable of producing second-generation seed either due to natural self-incompatibility or through artificially engineered Cytoplasmic Male Sterility (CMS), a condition which results in sterile pollen.[57] The author is actively seeking to procure access to large reliable batch of high glucoraphanin broccoli seed to support future investigations.

- Chemical analysis of the glucosinolate content of source seeds and the yield of sulforaphane delivered by the hydrolysis reaction is critical. The challenges of using broccoli seeds or sprouts as a source of sulforaphane in clinical trials has recently been discussed by Fahey & Kensler.[58]
- This pilot investigation did not have a placebo arm. As a collaborative project run by patients themselves, a blinded placebo-controlled study would not be feasible. However, an alternative, although less-satisfactory approach could be to run treatment and control phases alternatively as in a recent trial using broccoli sprouts by M T López-Chillón et al.[59]
- A major non-motor symptom of advanced Parkinson's disease is apathy.[21] Indeed, patients who suffer from lack of motivation are very likely to drop out of trials which last several weeks. This was the case for this pilot study. It is therefore important to establish appropriate selection rules to exclude patients who suffer from severe apathy. Such selection rules will very likely exclude patients with other severe symptoms. The symptom grading system should therefore be reviewed to take this into account.
- It should be recognised that collaborative studies of this kind lie outside of the institutionalised framework of the Helsinki Declaration and patient groups have neither the financial capacity nor the influence to convene an ethics committee before the study starts. Any group of patients intending to carry out grouped self experimentation studies should however make every effort to respect the principles that apply to other studies. This means that all prospective participants should be fully aware of the risks of self experimentation and declare their informed consent for all stages of the study. This has been discussed at some length by ethicists and clinical trials experts [60–62] and though not the gold standard in clinical interventions, it could be an acceptable alternative in this case.

How did this self-experimentation study come about?

This research began after the author was diagnosed with Parkinson's Disease and tried to learn how best to manage his own condition. As a new patient I was informed that the causes of PD were unknown, at the point of diagnosis the average patient had lost about 70% of dopamine-producing neurons and there was currently no way to slow down the progress of neurodegeneration. It was a distressing announcement.

I therefore turned to look for opinions from the research community. As a retired physicist, I am familiar with research methodology and scientific method, though I needed self-training in specific areas of biology and biological research. I found considerable consensus among researchers that oxidative stress, inflammation, mitochondrial dysfunction and axon damage to DA neurons were involved in the pathogenesis of Parkinson's Disease, but no clear consensus concerning how these processes were linked. The contrast between what I had been told and what I discovered for myself was instructive.

A small community of PD patients take a keen interest in their condition and share information on the current state of scientific research via specialized forums. In my early months of PD, I learnt a lot from this community and decided to follow their example. These patient-researchers regularly carry out (*n-of-1*) self-experiments, trying various combinations of vitamins, plant extracts or food supplements and share their observations. The first (*n-of-1*) study that I carried out using broccoli-seed tea was typical of these self-experiments, the only difference being that product used was a home-made preparation rather than an off-the-shelf supplement. As soon as the results of this first experiment were shared[13], other informed PD patients shared a range of complementary articles, videos and personal opinions. Many requests were received asking me to publish details of the preparation method and the dose used, but this raised fears of possible abuse, disinformation and accidents.

The chosen solution was to enable a limited number of Parkinson's disease patients to work together and carry out their own individual self-experimentation studies using more or less the same method so that the results could be compared. The idea was to try to learn more as a group rather than as individuals. The

experimental protocol was inspired by the author, discussed at length with prospective participants and modified several times before being adopted. All prospective participants were given access to the same information and asked to check with their doctor before going further. The protocol was designed to help participants obtain the appropriate supplies and equipment, use the same method of preparation and dose, and to report back in a standardised and anonymous way. It was a collaborative effort; all email addresses were shared for transparency. Once the protocol was finalised, the author had no control of how or when the experiments were actually carried out. The program was run on trust.

Consent by participants was intrinsic to the protocol. No materials, supplies or equipment of any kind were supplied to any participants by the investigator. Each member was entirely responsible for purchasing his/her own supplies and equipment and for carrying out the operations as and when they pleased. The participants were also extremely well informed. All the information about the potential benefit/risks of preparing and drinking this tea had been shared and discussed. The individuals in this group were fully committed to the experiment. The potential benefit/risk associated with participating in the experiment was considered by these participants to be higher than that of not doing so.

The question of self-experimentation, regulation and ethics has recently been reviewed by Hanley, Bains and Church.[60] They conclude that self experimentation by scientists is widespread and carries little risk when carefully designed by scientists. It has been shown to be valuable for scientific discovery and attempts should not be made to regulate it. Interestingly, they point out that grouped self-experimental studies are collaborative in nature rather than managed top-down by the investigator who has limited control once the idea is launched. This was the case here. What started out as a personal initiative was transformed into a team effort. This group of well-informed patients clearly understood that working together and sharing data might provide better information about their disease than doing self-experimentation alone. Even three patients who saw no improvement in their symptoms completed the 6-week program and submitted their data. Alone, they would have stopped long before the end.

Collaborative self-experimentation studies of this kind which produce a consistent result are extremely rare. They can only occur when enough potential participants, already belonging to an established group with a common objective, become strongly motivated by a desire to take collective action. The idea crystallises when the potential benefit/risk is judged to be favourable, when they realise that no-one else is going to do it for them and where the practical obstacles are relatively easy to overcome. Internet and social media have enabled such groups to develop, to share individual experiences and to build collective knowledge and trust. These conditions are therefore likely to be fulfilled by other groups in the future.

Despite the limitations of this pilot study, it has provided the very first indications of the efficacy of sulforaphane in attenuating the symptoms of Parkinson's Disease, an area of research that was abandoned more than ten years ago by the institutions and the pharmaceutical industry, but has now been revived through the actions of a small group of Parkinson's Disease patients. We hope that this pilot study will stimulate further, more rigorous studies leading to a proof of concept and so improve our understanding, not only of Parkinson's Disease, but of related neurological diseases.

Abbreviations

ARE	Antioxidant Response Element
ATP	Adenosine triphosphate
CNS	Central Nervous System
ESP	Epithiospecifier protein
Nrf2	Nuclear factor erythroid 2-related factor 2
PD	Parkinson's disease
OS	Oxidative stress
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SN	S*ubstantia Nigra*

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Original data

The de-identified original data is available from the author or can be deposited with the publisher.

Conflict of interest

The author declares being diagnosed with Parkinson's disease in 2018.

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