Efficacy of broccoli and glucoraphanin in COVID-19: From hypothesis to proof-of-concept with three experimental clinical cases

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Short title: Fast-acting broccoli capsules in COVID-19

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Abstract

One clinical case describes COVID-19 in a patient who has proposed the hypothesis that Nrf2-interacting nutrients may help to prevent severe COVID-19 symptoms. Capsules of broccoli seeds containing glucoraphanin were being taken before the onset of SARS-CoV-2 infection and were continued daily for over a month after the first COVID-19 symptoms. They were found to reduce many COVID-19 symptoms rapidly and for a duration of 6-12 hours by repeated dosing. When the patient was stable but still suffering from cough and nasal obstruction when not taking broccoli, a double-blind induced cough challenge confirmed the speed of onset of the broccoli capsules (less than 10 minutes). A second clinical case with lower broccoli doses carried out during the cytokine storm confirmed the clinical benefits already observed. A third clinical case at the onset of COVID-19 symptoms showed similar effects. In the first clinical trial, we used a dose of under 600 micromoles per day of glucoraphanin but such a high dose may induce some pharmacologic effects that must be studied carefully before any study is carried out. It is likely that the fast onset of action is mediated through TRPA1 channel. These experimental clinical cases represent a proof-of-concept to confirm the hypothesis that Nrf2-interacting nutrients are effective in COVID-19 but cannot be used in practice before more safety data are available. They should be confirmed by proper trials on efficacy and safety.

Key words: COVID-19, Nrf2, broccoli, cough challenge

Introduction

The present report consists of the self-description of a COVID-19 case where the patient – who is also the author - describes his clinical course as well as the anecdotal evidence of symptom improvement after treatment with broccoli capsules. Since the patient himself developed the concept that Nrf2-interacting nutrients may help to prevent severe COVID-19 symptoms or, less likely, to prevent SARS-CoV-2 infection [1-3], he took low-dose broccoli capsules with glucoraphanin and myrosinase to prevent the infection, and higher doses when COVID-19 was clinically evident. Broccoli was chosen as it contains glucoraphanin. The most potent Nrf2 natural activator is sulforaphane [4-10], but it is difficult to deliver in an enriched and stable form for purposes of direct human consumption [11]. Thus, glucoraphanin, the precursor of sulforaphane, is administered orally with myrosinase, the enzyme that transforms glucoraphanin into sulforaphane.

After the cytokine storm, the patient continued to suffer from cough and nasal obstruction when he was not taking the broccoli capsules. A double-blind induced cough challenge was carried out to assess the speed of onset of the broccoli capsules.

1- Hypothesis: The heterogeneity of COVID-19 death rates between countries could be partially due to the consumption of Nrf2-interacting nutrients

There are large between- and within-country variations in COVID-19 death rates. Some very low death rate settings such as Eastern Asia, Central Europe, the Balkans and Africa have a common feature of eating large quantities of fermented foods and some specific vegetables such as cabbage. Although biases exist when examining ecological studies, fermented vegetables or cabbage were associated with lower death rates in European countries. Many foods have antioxidant properties and many mechanisms may be involved. However, the activation of the Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) anti-oxidant transcription factor may be of primary importance. Nrf2 is the main regulators of the antioxidant response in humans, modulating the expression of hundreds of genes, including not only the antioxidant enzymes (i.e. glutathione related), but large numbers of genes that control seemingly disparate physiopathological processes [12,13]. Nrf2 activity can block the AT_1R axis as well as endoplasmic reticulum stress.

Cabbage contains precursors of sulforaphane, the most active natural activator of Nrf2. Fermented vegetables contain many lactobacilli, which are also potent Nrf2 activators. Three examples where the association between COVID-19 mortality could have been influenced by the presence or lack of fermented vegetable consumption were proposed: Kimchi in Korea, westernized foods and the slum paradox. It was proposed that fermented cabbage is a proof-of-concept of dietary manipulations that may enhance Nrf2-associated antioxidant effects helpful in mitigating COVID-19 severity [3].

There are many Nrf2-interacting nutrients (berberine, curcumin, epigallocatechin gallate, genistein, quercetin, resveratrol, sulforaphane and many others) that all act similarly to reduce insulin resistance, endothelial damage, lung injury and cytokine storm (Bousquet et al., submitted). They also act on the same mechanisms (mTOR: Mammalian target of rapamycin, PPARγ: Peroxisome proliferator-activated receptor, NFκB: Nuclear factor kappa B, ERK: Extracellular signal-regulated kinases and eIF2α: Elongation initiation factor 2α). They may as a result be important in mitigating the severity of COVID-19, acting through the endoplasmic reticulum stress or ACE-Angiotensin-II-AT_1R axis (AT_1R) pathway. Interestingly, geographical areas with very low COVID-19 mortality are those with the lowest prevalence of obesity (Sub-Saharan Africa and Asia).

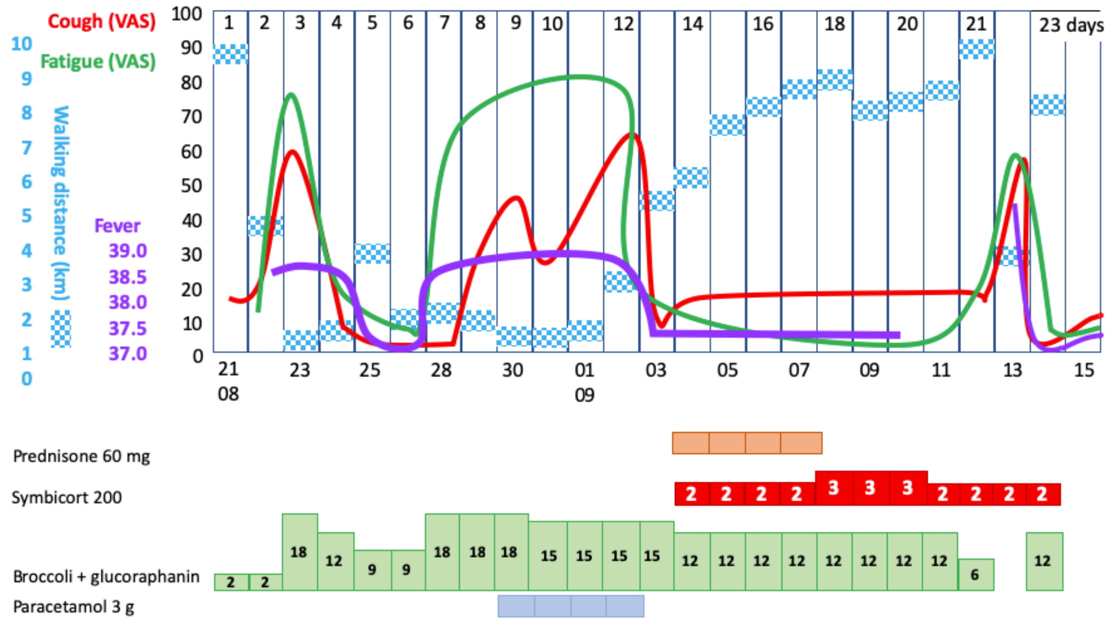
We proposed that Nrf2-interacting foods and nutrients can re-balance insulin resistance and have a significant effect on COVID-19 severity. It is therefore possible that the intake of these foods may restore an optimal natural balance for the Nrf2 pathway and may be of interest in the mitigation of COVID-19 severity. Understanding the balance between Nrf2-interacting foods and nutrients would help to: (i) better understand the mechanisms of the oxidative stress in insulin resistance associated diseases, (ii) develop optimal Nrf2-interacting nutrients and diets to reduce the prevalence and severity of IR diseases, (iii) optimize Nrf2 drug development and (iv) develop these strategies to mitigate COVID-19 severity.

2- Fast onset of broccoli capsules with glucoraphanin on COVID-19 symptoms

2-1- Clinical case 1

The hypothesis that oral administration of broccoli seeds and glucoraphanin capsules (from now on broccoli capsules) could beneficially ameliorate the COVID-19 course was tested on a 73-year old man, a former professor of respiratory medicine at the Montpellier University of France, (BMI 23, allergic rhinitis, intermittent untreated asthma and well-controlled type-2 diabetes under metformin, HbA1C: 6.2%). Before developing COVID-19, the patient self-prescribed broccoli capsules OD in the morning (Aerobiane, Pileje, France: broccoli seeds 300 mg + glucoraphanin 30 mg, around 70 µmol and mirosinase) in the hope to prevent the onset of COVID-19. But he did contract COVID-19 (Figure 1).

Figure 1: Clinical case



Only The highest daily score of symptoms for the day are shown

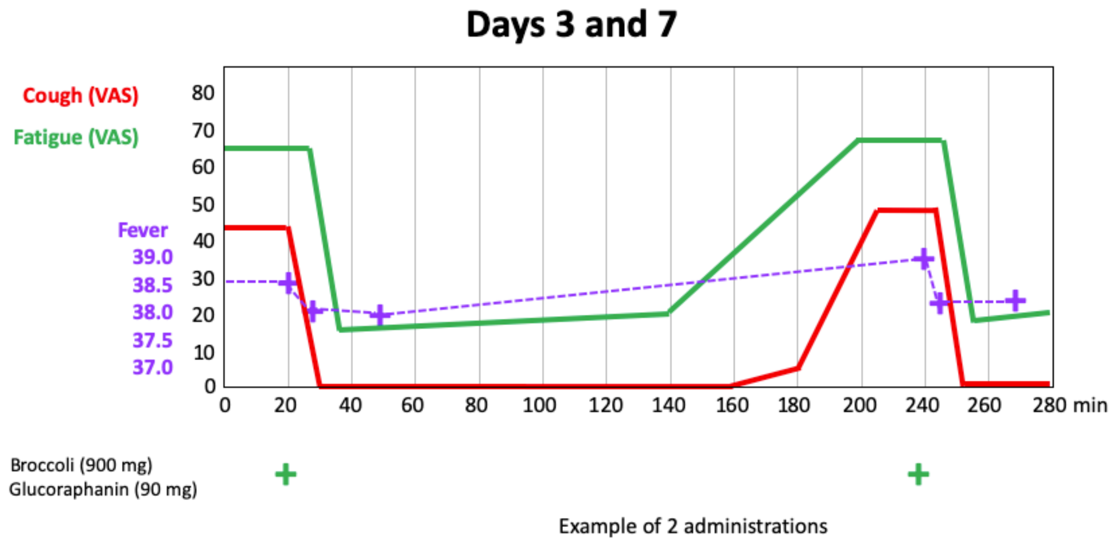
Day 1: He started to experience COVID-19 symptoms on the 22nd of August 2020 (Figure 1). A few months ago, the patient developed a COVID-19 app (MASK-COVID) - available on Android - using the estimation of symptom severity by visual analogue scale (VAS) based on his previous experience of developing an allergy app. [14] He used VAS to score his daily symptoms on paper, as the app is not yet available on iOS and the patient did not have access to Android. For each day, the maximal VAS was to be reported. For **days 1 to 3**, VAS was estimated retrospectively.

Days 2-4: The symptoms began with mild cough (VAS: 2/10), followed 12 hours later by severe fatigue (VAS: 6/10) and then, at 24 hours, fever (38.9°). At 36 hours, he also experienced loss of appetite (VAS: 7/10), nausea (VAS: 3/10), diarrhea (VAS: 2/10), hyposmia (VAS: 3/10), dysgeusia (VAS: 3/10) and nasal obstruction (VAS: 4/10) (Figure 1).

The patient increased the dosage of the broccoli capsules on day 3. The first dose of 600 mg of broccoli (4 capsules) (7:00) improved the symptoms incompletely and he therefore took a higher dose 5 hours later (900 mg). Interestingly, the ingestion of the capsules always induced the same effect with rapid disappearance of cough, nasal obstruction, nausea and diarrhea, improvement in fatigue and some reduction in fever (Figure 2). He repeated this dose when nasal obstruction reappeared and cough increased in severity (VAS>5/10), needing capsules every 6 to 8 hours (12:00, 18:00, 23:00, 7:00, 13:00) to achieve symptom control (Figure 2). At 14:30 on day 4, the patient felt well, had no fever and was asymptomatic.

Since the patient found the symptoms bothersome on day 3, he contacted Prof. H. Blain (HB: geriatrician, Montpellier) - with whom he is working on COVID-19 in home care services [15] - and discussed the Nrf2 hypothesis [2,3]. HB considered that the initial symptoms were severe and contacted (day 4) Prof. V. Le Moing (VLM: Director of the COVID-19 clinic of the Montpellier hospital). Then, for the next three weeks, the patient had daily contact with VLM by phone or SMS to adjust the treatment. Hospitalization would have been proposed at the very first sign of severity of the cytokine storm (e.g. respiratory rate >24/min). To optimize shared decision making, the patient scored all of the symptoms on a VAS scale (0-10) and reported them at least once daily to VLM. He also had regular contact with Dr. W Czarlewski with whom he developed the Nrf2 hypothesis [2,3]. Dr. Czarlewski is knowledgeable on natural medicine and was able to discuss the dosage of the broccoli capsules.

Figure 2: Evolution of symptoms after ingestion of broccoli capsules



Days 5-6: The patient took 3 broccoli capsules (450mg) 3 times per day and felt well.

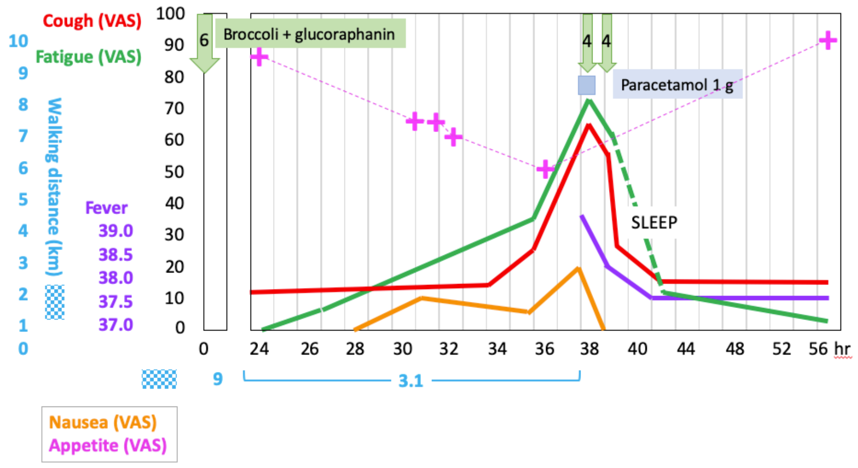
Days 7-12 (Figure 1)**:** The symptoms of the cytokine storm began on day 7 and the patient increased the dose of broccoli (900 mg). He noted the same rapid effect for the following 2 days (days 7 and 8) (Figure 2) and took a dose once every 6 hours when cough reappeared and increased in severity (VAS>5). The total daily dose of broccoli seeds was 2.7 g and of glucoraphanin 270 mg (around 600 micromoles).

On **day 8**, fever did not decrease and so he added paracetamol (3 g a day for 4 days). Fever reduced to 36.9° (morning) and 37.3° (evening) and remained the same up to day 13. Interestingly, the intervals between the broccoli doses (900 mg) could then be increased to 10-12 hours. The patient's respiratory rate was never >20/min. Since the symptoms were controlled (including fatigue VAS: 2-4/10) and the respiratory rate was not increased, the patient stayed at home, under the twice-daily supervision of VLM.

Days 13-20: On day 13, the patient felt well and had no fever. A dose of 900 mg of broccoli BID was ingested. However, he developed a productive cough (yellow eosinophil-like sputum). Further to shared-decision making with VLM, the patient took Prednisone (60 mg/day) from days 14 to 17 which resulted in translucent sputum. After day 22, the sputum production ceased.

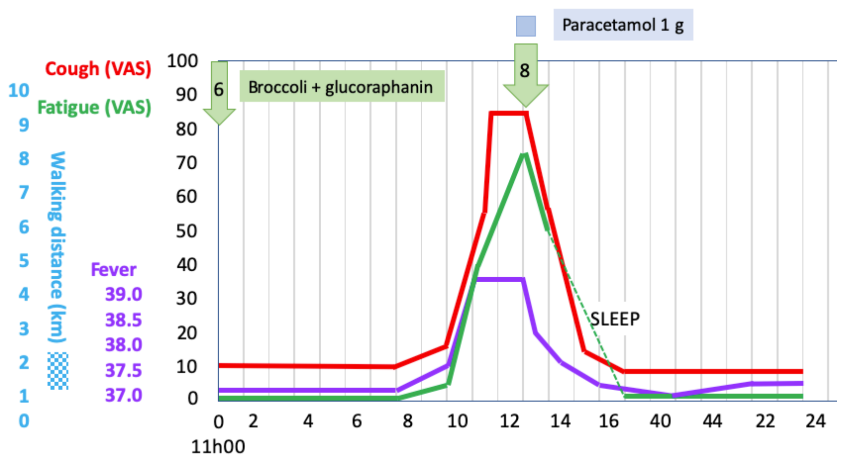
Days 21-24: On day 21, the patient stopped the broccoli capsules because he was travelling and had forgotten them (Figure 3). He experienced a recurrence of symptoms with increasing fatigue, loss of appetite, nausea and, later, within 24 hours, cough and fever. He was only able to ingest the capsules 3 hours after the onset of symptoms and had never had such a severe cough since the beginning of the symptoms. The patient took 600 mg of broccoli capsules and, within 5 minutes, the cough had reduced and the nausea had disappeared. Given that his fever was still at 38.9°, he took 1 g of paracetamol. The fever decreased to 38.1° within minutes. Since cough was not reduced, he took another 600 mg of broccoli capsules and it improved. After two hours of perspiring, the patient's temperature had fallen to 36.9°. He then felt perfectly well and experienced no further fatigue or cough. The next morning, the patient felt well with a return of appetite and no fatigue. He then took 300 mg of broccoli morning and evening (days 23-24).

Figure 3: Evolution of symptoms at days 21-22



Day 25: The patient purposely delayed the broccoli treatment to find out if it was needed, and the same symptoms returned (fatigue, cough, nausea, fever) (Figure 4). He then decided to wait for 2 hours before taking the broccoli capsules. The cough became very severe (VAS 8/10) and his temperature increased to 38.9°. He took the broccoli capsules (1200 mg) and paracetamol, and the same sequence of events was observed.

Figure 4: Evolution of symptoms at day 25



Days 26-38: The patient took 2 capsules (300mg) in the morning and 2 during the day every day. After the ingestion of the broccoli, he was rapidly almost symptom free (VAS 1-2 for nasal symptoms and spontaneous cough). After 10-12 hours, the cough and nasal obstruction returned. When cough VAS was over 5, he took the broccoli capsules. On days 30 and 31, the patient did not take the capsules and did a double-blind, placebo-controlled challenge.

Over the course of the disease, the patient attempted to improve cough using formoterol-budesonide low dose but there was no effect. The patient also attempted to control nasal obstruction using azelastine-fluticasone propionate without any effect.

Biological tests and CT-scan

The patient had a first positive PCR test to SARS-CoV-2 on August 24 (day 3). The test was repeated a second time on September 15 and was still positive. The patient had IgG antibodies to SARS-CoV-2. He had a moderate inflammation (September 15): CRP 30 mg/l, D-dimers (945 ng/ml), no lymphopenia, and normal hepatic and kidney biology.

The lung CT-scan carried out on September 18 showed that less than 25% of the lung was impacted with patchy images.

2-2- Clinical case 2

A 61-year old woman in perfect health and receiving no treatment, developed mild COVID-19 symptoms September 28 (nasal obstruction, cough, fatigue and headache) that disappeared on day 2 (Table 1).

Table 1: Clinical case 2

Day		Cough	Fever	Fatigue	appetite	smell loss	taste loss	nez bouché	BROCCOLI	Paracetamol
4		8	38,6	6	0	10	10	0		
5		8	38,5	10	5	10	10	5		
6	14h30	7	38,3	5	8	10	10	0	2	
	14h40	0	38	3	8	5	7	0		
	15h00	0	37,9	0	8	5	5	0		
	16h00	0	37,6	0	8	5	5	0		
	18h00	0	37,2	0	8	5	5	0		
	20h30	5	38,2	5	8	5	5	5	2	1000
	20h45	0	37,9	5	8	5	5	5		
7	08h00	0	37,2	8	5	5	5	5		
	10h20	7	37,8	6	8	5	5	5	2	
	10h40	0	37,7	3	8	3	3	0		
	18h45	7	37,8	5	8	5	5	5	2	500
	19h00	0	37,8	0	8	5	5	0		
8	10h15	7	37,2	0	5	5	5	5	2	
	10h30	0	37,2	0	5	3	5	3		
	14h00	2	37,2	0	4	4	4	0		
	16h30	3	37,3	0	3	3	3	0		
	19h15	7	37,2	0	3	3	3	0	2	500
	19h30	0	37,2	0	3	3	3	0		
	21h00	0	37,2	0	3	3	3	0		
9	9h00	0	37,8	5	4	8	8	8	2	500
	10h30	0	7,6	3	4	3	3	3		
	12h00	0	37,7	0	2	2	0	0		
	20h30	7	38	3	2	2	0	0	2	500
	21h00	0	37,9	3	2	2	0	0		
	22h00	0	37,6	3	2	2	0	0		

October 1 (day 4), she had most of the COVID-19 symptoms including loss of taste, smell and appetite. On day 6, she took 300 mg of broccoli capsules at 14h30. In 10 minutes, cough and nausea disappeared, and smell and taste improved. Fever decreased over 5 hours from 38.3° to 37.2°. After 6 hours, at 20h30, fever (38.2°), nasal obstruction and cough (VAS 5) reappeared. She took the same dose of broccoli with paracetamol (1000 mg) and the same improvements were noticed for a longer period of time (13 hours). The third episode occurred the next morning. She then took broccoli capsules (300 mg) and the same sequence of improvement was observed for 8 hours. The fourth episode occurred at 18h45 and she took broccoli (300 mg) and paracetamol (500 mg) and the duration of the effect was around 15 hours. The fifth episode occurred at 10h15. She took broccoli (300 mg) and symptoms re-occurred at

19h15. She then took broccoli (300 mg) and paracetamol 500 mg and the same improvement was found for 14 hours. Other episodes were less clear as she was improving. Interestingly, loss of smell and taste were improved by the broccoli but the extent of this improvement may have required specific testing for an accurate assessment although VAS is accurate.

The patient was tested positive for SARS-CoV-2 on day 8.

2-3- Clinical case 3

A 63-year-old man with controlled hypertension and receiving losartan had the first COVID-19 symptoms October 3. He had rhinorrhea, dry cough, incomplete loss of smell and taste and fatigue day 2. Day 3 at 11h00, he had severe cough and fever and took 300 mg broccoli. Cough and nasal obstruction disappeared very rapidly but fever was slower (Table 2). He took the same treatment with 500 mg paracetamol (after the third dose) five times and cough (VAS 5-8) always disappeared within 10 minutes. Nasal obstruction showed a similar trend in 3 case (in two there were no nasal symptom). Loss of smell and taste (VAS 6) the first two days were improved after the first broccoli capsule (VAS 5) and were overall largely improved (VAS 0 at the last evaluation day 5). Fatigue and fever appeared to be improved by broccoli but the results are less consistent. Paracetamol was given 5 times and increased the duration of the benefit.

The patient was tested positive for SARS-CoV-2 day 3.

Table 2: Clinical case 3

Day		Cough	Fever	Fatigue	appetite	smell loss	taste loss	nasal obst	BROCOLI	Paracetamol
1		0	0	8	6	5	5	5		
2	18h00	5	37,2	0	0	6	6	0		
3	09h30	8	38	5	0	5	5	0		
	11h00	8	37,8	6	0	5	5	0	2	
	11h15	0	37,7	6	0	5	5	0		
	12h30	0	37,6	3	0	5	5	0		
	15h15	8	37	2	0	5	5	0		
	16h30	8	37,8	5	0	5	5	5		
	19h00	8	38,6	5	0	5	5	4	2	1000
	19h15	0	39,2	7	0	5	5	0		
	21h00	0	38,8	9	5	5	5	0		
4	9h00	5	37,9	5	0	4	4	6	2	500
	10h30	0	37,5	5	0	0	0	0		
	12h00	0	37	5	0	0	0	0		
	21h00	0	37,9	6	5	4	4	0	2	1000
	21h15	0	37,7	0	5	4	4	0		
	22h00	0	37,4	0	5	4	4	0		
5	9h15	5	37,7	5	8	4	4	6	2	1000
	9h30	5	38,1	5	5	4	4	0		
	12h00	3	37,6	3	0	3	3	0		
	12h30	0	37,2	0	0	0	0	0		
	15h30	5	37,8	0	0	0	0	3		
	17h00	0	38,6	7	0	0	0	3		
	21h00	4	37,9	3	0	0	0	3	2	1000
	21h15	2	37,7	3	0	0	0	3		

3- Cough provocation test in clinical case 1

The patient is allergic to grass pollen and cat, and has moderate to severe allergic rhinitis which is perfectly controlled by PRN medications. Further to the onset of COVID-19, he experienced mild to moderate nasal obstruction (VAS up to 6/10) and occasional episodes of mild rhinorrhea (up to 3/10).

The rhinorrhea did not have the features of allergic rhinitis. The patient attempted to reduce nasal symptoms with azelastine-fluticasone propionate without any effect. The patient did not experience any sneezing, nasal pruritus or eye symptoms. He proposed a score for his symptoms according to the VAS for allergic rhinitis (from 0 to 10) [16-18]. He noticed that nasal symptoms and cough always improved within minutes of ingesting the broccoli capsules and then reappeared with cough when the treatment became ineffective.

3-1- Design of the test

The patient also suffers from mild intermittent asthma, especially when exposed to high levels of air pollution. He uses formoterol-budesonide low dose when needed and his maximal annual consumption is around 40 doses. He has a severe bronchial hyperreactivity. During the COVID-19, he had symptoms - that were not well defined - in the form of an intermittent pruritus of the tracheobronchial tree followed, within minutes, by dry cough. He had never experienced such symptoms. He used formoterol-budesonide low dose to reduce cough, without any effect. These symptoms were always followed by a more severe cough and the patient ingested the broccoli capsules when cough was over 4 or 5/10 (VAS). There are many scores for cough [19,20] and he used a VAS score for "cough". When he had pruritus, a deep inspiration followed by a rapid expiration led to immediate mild "wheezing" (not similar to asthma) and to cough that could be scored. He also used a score for induced cough during challenge, with some criteria. For spontaneous cough during challenge, he decided to count the number of coughs per episode (Table 3).

Table 3: Cough symptom score

Spontaneous cough	VAS	Induced cough challenge	score
≥5 coughs for one episode	3	persisting severe cough	9-10
2-4 coughs for one episode	2	>3 cough, intolerable	8
1 cough for one episode	1	>3 cough, severe	7
		>3 cough, mild	6
		2-3 cough severe	5
		2-3 cough mild	4
		1 cough	3
		Loud "wheezing"	2
		Some "wheezing"	1

3-2- Open label induced cough challenge with broccoli (days 26-28 and 31-38)

In order to test the induced cough challenge and the dose that could be effective, the patient performed three induced cough challenges with a dose of 300 mg broccoli (daily dose recommended). Similar to the FEV_1, he took the deepest breath he could, and then exhaled as fast and as hard as possible and counted the number of coughs according to Table 2. He found that induced cough was improved before 10 minutes after broccoli ingestion, and nasal obstruction even more rapidly. He then selected this dose for the DB, PC challenge.

After the DB, PC challenge, when the patient needed the broccoli capsules (VAS spontaneous cough >5/10) he performed, at least once a day, an open challenge. A total of seven challenges were carried out (Table 3).

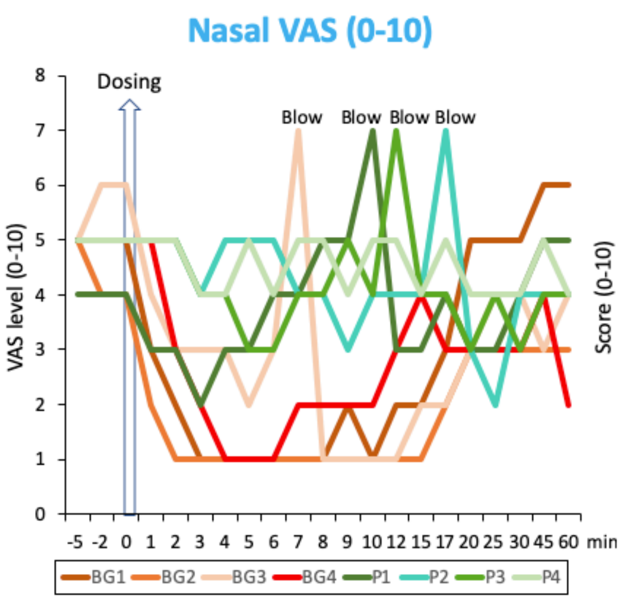
Table 4: Open label induced cough challenge with broccoli (N=10)

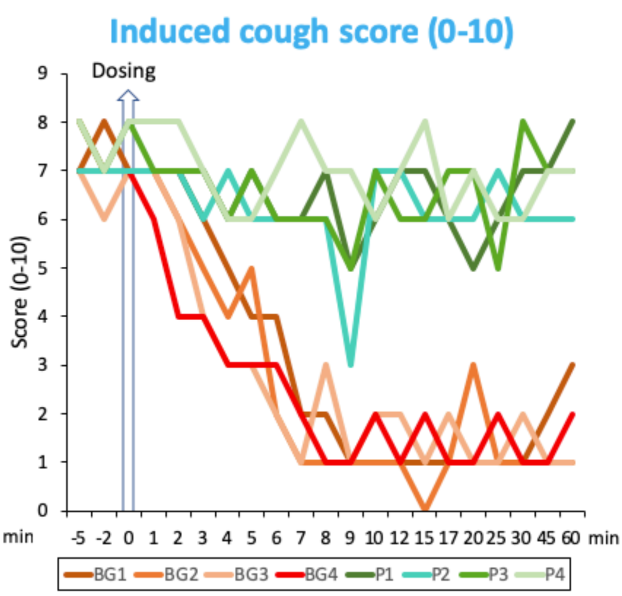
Time (min)	Mean score	SD	Minimum score	Maximum score
-5	7.5	0.53	7	8
Dosing	6.9	0.74	6	8
1	6.9	0.67	6	8
2	6.9	0.75	6	8
5	4.3	1.26	2	6
8	2.1	0.57	1	3
10	2.0	0.82	1	3
15	2.0	0.63	1	3
20	1.8	0.67	1	3
25	1.8	0.79	1	3
30	1.7	0.63	1	3
45	1.8	1.03	1	3
60	1.6	1.00	1	3

3-3- Double-blind induced cough challenge with broccoli (days 29-30)

A double-blind placebo-controlled trial was carried out on induced cough using 4 placebo capsules or 4 broccoli (300 mg) capsules. The placebo and broccoli capsules were prepared by the Pharmacie des Quatre Seigneurs (Montpellier). The investigator was not aware of the blinding or the code that was broken after the test. Similar to the open challenge, he took the deepest breath he could, and then exhaled as fast and as hard as possible. He started the test when spontaneous cough VAS was over 5/10. He first measured spontaneous and induced cough scores as well as nasal VAS, 5 minutes before ingesting the capsule. He ingested the capsule and then measured the 3 parameters every minute for 10 minutes, at 12, 15, 17, 20, 25, 30, 45 and 60 minutes (Figure 5).

Figure 5: Double-blind placebo-controlled challenge (300 mg broccoli (BG) or placebo (P)) on spontaneous nasal and induced bronchial symptoms at days 29-30





In the broccoli group, induced cough score was around 7-8 before the dose and below 3 after 6-9 minutes (Figure 5). Score levels remained at between 0 and 3 for the 60-minute duration of the test. In the placebo group, there was merely no effect. The results for both the active and placebo groups were very consistent. The results for the active group were consistent with open cough challenges (Table 3).

In the broccoli group, 6, 6, 10 and 10 episodes of spontaneous cough were recorded for over 60 minutes, compared to 21, 25, 26 and 28 in the placebo group.

In the broccoli group, nasal symptoms were reduced very rapidly (2-3 minutes), except for BG3. Nasal obstruction was the only symptom recorded during the baseline of BG1, BG2 and BG4, whereas both nasal obstruction and rhinorrhea (VAS: 3) were recorded during BG3 baseline. The patient needed to blow his nose at 7 minutes (VAS 7). After 12-15 minutes, symptoms increased. In the placebo group, symptoms were variable but were never below 2, and all three tests required nose blowing (indicated as VAS 7).

VAS spontaneous cough ≥5 was noticed at 4h30, 5h00, 6h00 and 7h45 after the ingestion of the broccoli capsules, whereas it was present at 60 minutes (two cases), 1h15 and 1h30 after the ingestion of the placebo capsules.

4- Discussion

These three experimental clinical cases should be considered carefully, like any clinical case, in terms of efficacy and with caution in terms of safety. Moreover, in the first clinical case, the patient and the investigator who proposed the treatment were the same person. On the other hand, the case has been well described and the study analyzed - as if it were to be published – right from the onset of symptoms and the fast-acting broccoli capsules of the first clinical case (day 3). Safety should be considered carefully as high doses are needed.

Several foods act on Nrf2 [4,21-27]. It has been proposed but not confirmed that Nrf2-interacting nutrients may be effective in COVID 19 [28-32] and the cases presented herein are a proof-of-concept for the clinical effects of Nrf2-interacting nutrients in patients with COVID-19 using broccoli and glucoraphanin. We have proposed that differences in death rates between and within countries are associated with Nrf2-inteacting nutrients. [1-3] In the present study, there are six levels of evidence suggesting that broccoli components are effective in COVID-19: (i) During COVID-19, with initial symptoms (days 3 and 4) and the cytokine storm (days 7-12), a high dose of broccoli was always effective within minutes; (ii) When the broccoli was stopped, the same COVID-19 symptoms reappeared and were controlled with the same speed using the same treatment; (iii) Broccoli appeared to be effective within minutes on most COVID-19 symptoms except fever. However, for clinical case 2, smell and taste were reduced but need specific tests to be fully appraised [33] ; (iv) The effect of broccoli was also found in clinical case 1 during the recovery period after the cytokine storm; (v) A double-blind, placebo-controlled induced cough challenge was performed at days 28 and 29 and the results were perfectly in line with 17 open induced cough challenges and with the clinical benefits observed on days 3, 4, 7, 8 (broccoli capsules only) and on days 9, 10, 11, 12 (broccoli capsules and paracetamol);

In the broccoli capsule, it is labelled that there is 10% of glucoraphanin. However, the exact dosage cannot be found, neither on the label nor on the website of the manufacturer. The capsule therefore needs to be analyzed. Glucoraphanin is an important component of broccoli because sulforaphane [1-isothiocyanato-4-(methylsulfinyl) butane], found in a stored form such as glucoraphanin in cruciferous vegetables, is the most potent natural activator of Nrf2. [34] Present in the plant as its precursor, glucoraphanin, sulforaphane is formed through the action of myrosinase, a β-thioglucosidase present in either the plant tissue or the mammalian microbiome [4,5]. Sulforaphane is a clinically relevant nutraceutical compound used for the prevention and treatment of chronic diseases and may be involved in ageing.[35] However, the very early effects of broccoli cannot be attributed to sulforaphane.

The mechanisms of action of broccoli are unclear. The immediate effect on cough in COVID-19 was noted at 5-10 minutes after ingestion in both clinical cases. It is interesting to note that most COVID-19 symptoms are rapidly improved, except cough which is either less well controlled or at a different kinetic (starts early and takes 2-3 hours to be improved but not fully controlled). The speed of onset of broccoli is surprising but highly consistent (the 4 DB-PC induced cough challenges and 10 open label challenges) and it is possible that changes in oxidative stress can be very rapid. Nasal effects are even faster. Broccoli has an inconstant effect on asthma [36] whereas sulforaphane from broccoli has a bronchoprotective response through Nrf2 [37] and reduces nasal response to diesel exhaust particulates [22]. Oxidant activation of the airway neurons induces respiratory depression, nasal obstruction, sneezing and cough [38,39].

However, other mechanisms may be proposed. The cough reflex is regulated by vagal, sensory afferent nerves which innervate the airway. The transient receptor potential (TRP) family of ion channels is expressed on sensory nerve terminals, and, when activated, can evoke cough [40]. Diesel exhaust can act on airway sensory neurons [41]. TRPA1 (Transient Receptor Potential Ankyrin type 1), a member of the TRP gene family, is a major oxidant sensor in airway sensory neurons [42,43]. It is possible that the early effects on the nose may be mediated through these mechanisms through a TRPA1 reflex. Another mechanism may be proposed. TRAP1 channel may be activated and desensitized at high doses within seconds by electrophilic pungent compounds [44]. Broccoli contains flavonoids [45,46] including quercetin [45], naringenin [47] and kaempferol [48] that are interacting with TRPA1. We suggest that electrophilic ligands activate and desensitize TRAP1 as well as they activate Nrf2 that will block the activation of TRPA1 by reactive oxygen species (ROS) produced by COVID-19.

Medications in capsules can be active after 1-2 minutes and pass into the blood stream within 2 minutes. The effects on cough are probably more complex. The early effects of the ingestion of the broccoli capsules can only be ascribed to broccoli, since sulforaphane cannot be produced so fast. The rapid but steady decrease in cough symptoms may be associated with the same mechanisms, but probably include other mechanisms. It is unlikely that cough in COVID-19 is associated mainly to eosinophilic inflammation, since inhaled corticosteroids appear to be ineffective in the first clinical case.

One question that cannot be answered is whether broccoli reduced the severity of the cytokine storm. The patient of the first clinical case was at high risk of developing a severe cytokine storm due to the number of symptoms he experienced during the first phase of COVID-19, his age and sex. Moreover, he developed an early cytokine storm on day 6 that was long lasting. However, symptoms were controlled by the treatment and he never suffered from dyspnea or from a respiratory rate of above 24. He had a mild inflammatory response and the CT-scan showed few COVID-19 typical lesions. It may be suggested that broccoli prevented a severe COVID-19 illness. The second clinical case was carried out during the cytokine storm and symptoms were improved by a lower dose of broccoli.

Using the dose of broccoli recommended by the manufacturer, there was no preventive effect on SARS-CoV-2. However, it is likely that the dose was insufficient for any effect. The doses required for effective action during COVID-19 symptoms of case 1 are quite high and should raise safety concerns. We used a daily dose of under 600 µmol of glucoraphanin, and trials with doses of up to 800 µmol daily have been reported without safety concerns [9,21,49]. However, such a high dose may have some pharmacologic effects such as the antagonism of aryl hydrocarbon receptors modulating the CYP1 (Cytochrome P450, family 1, subfamily A, polypeptide 1) family of cytochromes P450 [50] or a direct effect of CYP1 [51]. These effects must be studied carefully before any trial is carried out. Moreover, in broccoli seeds, there are many other compounds that may have pharmacologic properties when ingested at high doses [21]. Thus, this experimental clinical case is a proof-of-concept to confirm the hypothesis but cannot be used in practice before more safety data are available.

The patient of the first clinical case is allergic to grass pollen and cat and also suffers from mild intermittent asthma. It might be possible that some of the effects of Nrf2-interacting nutrients are related to bronchial hyperreactivity, but inhalation of formoterol-budesonide low dose did not reduce cough. However, it cannot be ruled out that the results observed on cough may be partly associated with asthma or allergy. The patient also attempted to reduce nasal symptoms with azelastine-fluticasone propionate but there was no effect. Moreover, nasal symptoms are mainly characterized by nasal obstruction alone that is not associated with allergic triggers.

These three clinical cases should be considered with care and confirmed by proper trials on efficacy and safety. However, it fits with the hypothesis that cabbage may reduce COVID-19 [1-3] and with many papers highlighting the role of nutrition in COVID-19. What is novel is the demonstration in three clinical cases that the hypothesis is supported and the speed of action of the nutrients. Research is needed to (i) confirm the case, (ii) investigate in which patients the treatment is safe and effective, (iii) differentiate the effects on Nrf2 and TRPA1, (iv) assess the different broccoli compounds that are active early and late, (v) assess the dose required for effective action, (vi) confirm the speed of onset of action and (vii) optimize the nutrients to be administered as nutrients or active molecules.

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Ethics approval and consent to participate: Not applicable since this was a food supplement used a regular doses by the patients of cases 2 and 3 and at higher doses (case 1) by the author of the study

Consent for publication: All authors gave their informed consent

Availability of data and materials: Not applicable

Competing interests: None of the authors declared any competing interest

Funding: None

Authors' contributions: JB proposed the treatment and was the patient. He discussed it with JMA WC and TZ. JB wrote the paper. VLM, HB and JR were the physicians of JB. AB, JPC, AAC, AF, TH, GI, LK, PK, EM, JM, BS and AV were the ARIA reviewers. RdlT and NPL discussed the broccoli content. All authors were requested to comment on the concept and to review the paper.

Acknowledgements : Prof Bernard Bataille (Pharmacy school of Montpellier) who gave advice for the absorption of drugs in capsules, the Pharmacie du Plan des Quatre Seigneurs (Montpellier) who prepared the placebo capsules for the cough challenge and Ms Anna Bedbrook who reviewed the English.