

ORIGINAL ARTICLE

Oral gliadin-protected superoxide dismutase in addition to phototherapy for treating non-segmental vitiligo: A 24-week prospective randomized placebo-controlled study

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Abstract

Background Despite a solid rationale, the usefulness of antioxidants in treating vitiligo has not been clearly demonstrated. Combining superoxide dismutase (SOD) with a wheat gliadin biopolymer protects it during the passage through the gastrointestinal tract.

Objective To evaluate the efficacy of gliadin-protected SOD (GP-SOD), associated with narrowband ultraviolet B (NB-UVB), for treating vitiligo.

Methods We conducted a 24-week monocentric interventional prospective randomized placebo-controlled trial in the tertiary center for vitiligo care in the department of Dermatology of Nice University hospital, Nice, France. Subjects with non-segmental vitiligo affecting more than 5% of the total body surface were included. The subjects received gliadin-protected SOD (GP-SOD; 1 g/day for 12 weeks followed by 0.5 g/day for 12 weeks) or placebo in combination with twice-weekly sessions of NB-UVB. The primary endpoint was the total repigmentation rate at 24 weeks, compared with baseline, as assessed by investigator-assessed Vitiligo Extent Score (VES) on standardized pictures.

Results A total of 50 patients were included. After 24 weeks, a greater improvement in VES was observed in the GP-SOD group (19.85%; SE 4.63, $P < 0.0001$) compared with the placebo group (8.83%; SE 4.72, $P = 0.0676$). Tolerance was good in both groups. No related side-effect was reported.

Conclusions The use of GP-SOD appears to be a useful add-on to phototherapy in the treatment of vitiligo patients.

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Conflicts of interest

None of the authors received any financial compensation, and none report any conflict of interest.

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Introduction

Vitiligo is an acquired depigmentation of the skin and hair, affecting 1%–2% of the global population. A marked impact on quality of life of affected individuals has been demonstrated.^{1,2} The therapeutic demand is high but, despite significant advances in the understanding of the pathophysiology of the disease, the treatment of vitiligo remains unsatisfactory in most cases. Vitiligo lesions are due to an immune-mediated loss of melanocytes

in genetically predisposed patients.^{3,4} Although an oxidative imbalance in vitiligo skin has previously been reported, the link between the oxidative stress and the triggering of the immune response against melanocytes has only been elucidated recently.^{5–9} Interestingly, the oxidative stress has also been demonstrated to prevent repigmentation by downregulating the WNT pathway and subsequently by impairing the differentiation of melanocyte stem cells.¹⁰

Superoxide dismutase (SOD) is a key enzyme in the antioxidative machinery of the cell. As the first antioxidant mobilized by the cell, it catalyses the dismutation of the superoxide anion

The patients in this manuscript have given written informed consent to the publication of their case details.

Clinicaltrial.gov registration: NCT03941808

and decreases the oxidative stress and the downstream activation of inflammatory mediators.^{11–13} There are conflicting results in the literature concerning the levels of oxidants and antioxidants, including SOD levels, in vitiligo patients (some reporting an increase, others no difference and some a decrease).¹⁴ A recent study showed a significant decrease in SOD levels in the blood of vitiligo patients as compared to controls, with a greater decrease in generalized patients as compared to localized.¹⁵ Although interesting, all these results must be taken with great care as they might not reflect the redox level in the melanocytes and keratinocytes. Several studies have shown the protective effects of SOD in mice and humans against UV-induced DNA damage, neural degeneration and in decreasing the severity of inflammatory diseases.¹³ Importantly, oral administration of SOD and other antioxidant enzymes is ineffective under normal conditions since the enzyme is deactivated as it passes through the gastrointestinal tract. However, combining SOD with a wheat gliadin biopolymer protects it during the passage through the gastrointestinal tract.¹⁶ Studies, performed in animal models and in humans, have confirmed the superiority of using gliadin-protected SOD (GP-SOD), when compared to placebo or other antioxidants in neuroprotection, atherosclerosis or UV-induced erythema.^{17–20}

The objective of this study was to evaluate the efficacy of a combination treatment comprising narrowband ultraviolet B (NB-UVB) associated with GP-SOD for widespread non-segmental vitiligo.

Subjects and Methods

We conducted a prospective, randomized, double-blind, placebo-controlled study in France. The study was approved by the Ethics Committee (n° 2019-A00339-48) and registered at Clinicaltrial.gov (NCT03941808).

Patients aged ≥ 18 years old with vitiligo affecting at least 5% of the body surface area were included. Excluded were patients with segmental or mixed vitiligo, active infections, congenital or acquired immunosuppression, medical history of malignancy within the last 5 years, photodermatoses, coeliac disease or allergy to gluten, having received any topical therapy within 2 weeks prior to randomization or any phototherapy or systemic steroids or immunosuppressive drugs within 4 weeks prior to randomization, supplement / antioxidant intake and pregnant or breastfeeding women. All subjects provided written informed consent.

Subjects were randomized to receive 2 hard capsules of 250 mg of Glisodin® (Isocell laboratory, Paris, France) in the morning and 2 before lunch (30 min before meals) for the first 12 weeks and 2 hard capsules in the morning for the following 12 weeks (GP-SOD arm) or to receive the same oral regimen with capsules containing no SOD (Placebo arm). Both treatment arms received NB-UVB twice-weekly for 24 weeks according to current recommendations.²¹ Patients were advised to avoid sun

exposure during the entire study. Subjects in both arms were given the same number of capsules with identical labelling, and all capsules had the same taste, appearance and smell. Centralized block randomization was conducted by the Department of Clinical Research and Innovation (DRCI) at the Centre Hospitalier Universitaire (CHU) Nice. Randomization was balanced (1:1) and stratified by skin phototype group (fair skin types (Fitzpatrick I, II, III) and dark skin types (Fitzpatrick IV, V, VI)). Lists were created using Nquery® Advisor v 7.0. software.

The primary evaluation criterion was investigator-assessed Vitiligo Extent Score (VES).²² Although VASI and VES are two validated and useful scores, an international vitiligo expert meeting in 2019 validated the VES as the gold standard score for assessing the vitiligo extent.²³ Evaluations were performed by two physicians, blinded to the treatment received, on standardised pictures taken at baseline, week 12 (W12) and W24. If the two scores differed by more than 20%, a joint assessment was performed to reach an agreement. The quality of life was assessed using the Dermatology life Quality Index (DLQI).²⁴

The side effects were graded accordingly to the Common Terminology Criteria for Adverse Events. (CTCAE).

Sample size calculation

According to a previous study performed in our centre,²⁵ we conservatively estimated an improvement in mean VES score of $28\% \pm 25$ at W24 for the placebo arm receiving only NB-UVB. Anticipating a clinically relevant improvement of 50% (same standard deviation (SD)) in the GP-SOD arm, we determined that 22 subjects per arm were required to detect a difference between the groups (80% power and 5% type I error Nquery® Advisor v 7.0). Assuming 10% lost to follow-up, 50 subjects were required.

Statistical analysis

Analysis of the primary objective was performed on the modified intention-to-treat (ITTm) principle. All subjects who underwent randomization and took at least one dose of medication were included in the analysis. Missing values were imputed according to the last-observation-carried-forward (LOCF) procedure.

VES score change from baseline at W12 and W24 for the GP-SOD arm was compared with the placebo arm using an ANCOVA model adjusted for the baseline score and the phototype group. DLQI score improvements between the groups were studied in the same way.

All tests were two-sided and *P* values < 0.05 were considered statistically significant. We used SAS Enterprise Guide software version 7.1 (SAS institute, Inc, Cary, North Carolina, USA) for statistical analyses.

Results

A total of 50 patients were included in the study, and all were analysed; one was lost to follow-up and one discontinued due to

patient decision in the GP-SOD group, three patients discontinued due to patient decision in the placebo group (Fig. 1). The demographic and clinical characteristics of the patients are summarized in Table 1. The mean age was 48.4 years old in the GP-SOD group and 50.9 in the placebo group. The mean duration of vitiligo was 20.1 years in the GP-SOD group and 25.9 years in the placebo group. Prior to inclusion in the study, 23 patients in each group (92%) had previously been treated and failed to respond, including 12 and 17 patients, in the GP-SOD and placebo groups, respectively, who had failed to respond to at least two previous lines of treatment.

The adjusted mean percentage improvement in VES after 12 weeks of treatment compared with baseline was 9.74% (SE 2.36, $P = 0.0002$) in the GP-SOD group versus 4.81% (SE 2.40, $P = 0.0515$) in the placebo group. After 24 weeks of treatment, the adjusted mean improvement in VES was 19.85% (SE 4.63, $P < 0.0001$) in the GP-SOD group and 8.83% (SE 4.72, $P = 0.0676$) in the placebo group (Table 2 and Fig. 2). However, the between-group difference in the repigmentation rate was not statistically significant ($P = 0.089$). In the GP-SOD group, 6 (24%) and 4 (16%) out of the 25 patients reached 30% improvement in VES (VES 30) and VES 50, respectively. In the placebo group, 3/25 (12%) and 1/25 (4%) patients reached VES 30 and VES 50, respectively (Fig. 3). Clinical representative examples are provided in Figures S1 and S2.

The adjusted mean improvement in the DLQI score at week 12 compared with baseline was 1.51 (SE 0.71, $P = 0.0386$) in the GP-SOD group versus 0.84 (SE 0.79, $P = 0.2935$) in the placebo group. After 24 weeks, the mean improvement in the DLQI score was 2.47 (SE 0.56, $P < 0.0001$) in the GP-SOD arm versus 1.85 (SE 0.62, $P = 0.004$) in the placebo arm.

A total of 18 adverse events were reported in SOD group and 16 in the placebo group; all were mild (grade 1) and transient

and none of them were considered as related to the study treatment. Overall, tolerance was excellent in both groups.

Discussion

Despite a solid rationale, the usefulness of antioxidants in treating vitiligo has not been clearly demonstrated. A recent meta-analysis reported conflicting results, often supported by low-evidence studies.²⁶ The authors not only concluded that interesting data support the added value of oral antioxidants in vitiligo treatment, but also underlined that confirmatory studies were

Table 1 Demographic and clinical baseline characteristics

	NB-UVB + GP-SOD (n = 25)		NB-UVB placebo (n = 25)	
Age (years), Mean (±SD)	48.4	±11.6	50.9	±14.0
Sex (n (%))				
Male	6	24%	7	28%
Female	19	76%	18	72%
Phototype (n (%))				
I	0	0%	0	0%
II	5	20%	2	8%
III	13	52%	17	68%
IV	5	20%	5	20%
V	1	4%	1	4%
VI	1	4%	0	0%
Vitiligo duration (years), Mean (±SD)	20.1	±12.5	25.9	±14.7
Patients who failed to respond to at least one line of treatment (n (%))	23	92%	23	92%

GP-SOD, gliadin-protected super oxide dismutase; NB-UVB, narrowband ultraviolet B; SD, standard deviation.

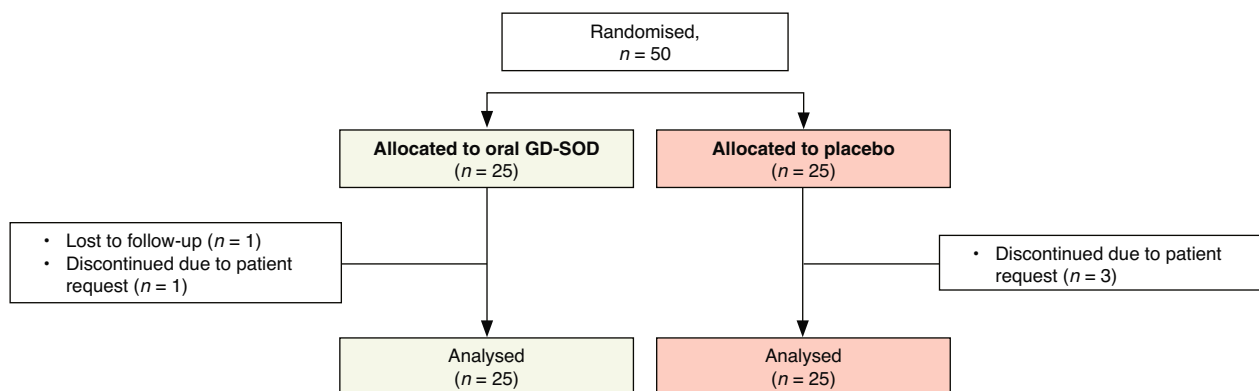


Figure 1 Study flowchart. GP-SOD, gliadin-protected super oxide dismutase.

Table 2 Evolution of vitiligo extent score

	Baseline (n = 25)	W12 (n = 25)	W24 (n = 25)	% change between baseline and W24 (n = 25)
VES	21.37 ±	19.96 ±	17.64 ±	-19.61 ±
NB-UVB + GP-SOD	16.24	16.21	15.38	24.26
Mean ± SD				
VES	18.75 ±	18.06 ±	17.29 ±	-8.92 ±
NB-UVB + placebo	16.38	15.51	15.00	18.07
Mean ± SD				

GP-SOD, gliadin-protected super oxide dismutase; SD, Standard deviation; VES, vitiligo extent score; W, week.

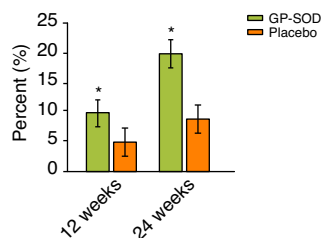


Figure 2 Percentage change in total repigmentation compared to baseline. GP-SOD, gliadin-protected super oxide dismutase. * $P < 0.05$.

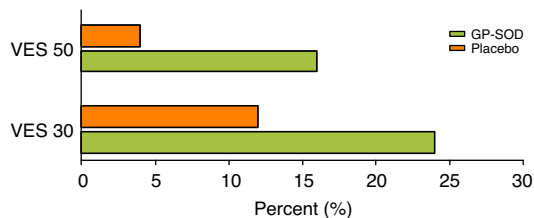


Figure 3 Proportion of patients achieving 30% and 50% improvement in vitiligo Extent Score. GP-SOD, gliadin-protected super oxide dismutase; VES, vitiligo extent score.

necessary. The discrepancy between variable clinical efficacy and solid fundamental evidence could be explained by the differences in effectiveness between all the antioxidants used and their digestive degradation. SOD is a key enzyme in the antioxidative process.^{11–13} The combination of SOD with a wheat gliadin biopolymer has been shown to protect it from degradation as it passes through the gastrointestinal tract. Furthermore, as the

activity of GP-SOD has been demonstrated in several animal models and clinical studies,^{16–20} it appears to be promising candidate for vitiligo treatment. Our results show almost 20% repigmentation after 24 weeks of treatment with GP-SOD combined with NB-UVB compared to 9% when NB-UVB was associated with placebo. The GP-SOD combination significantly improved repigmentation by week 24 compared with baseline ($P < 0.0001$), whereas the improvement observed for NB-UVB alone was not statistically significant ($P = 0.0676$). A trend was also observed when the repigmentation rate was compared between the two groups, but the 11-point difference in repigmentation rate (20% vs 9%, $P = 0.089$) was not statistically significant. This is probably due to the discrepancy with our initial hypothesis of a 28% and a 50% improvement after 24 weeks of treatment, respectively, in the group treated only with UVB and the group with UVB and GP-SOD. However, the higher repigmentation rate in patients treated by GP-SOD and NB-UVB was corroborated by a greater improvement in quality of life compared with those treated with only NB-UVB and placebo.

The main limitation of this study is the fact that it was conducted in a single specialized centre for vitiligo care so the vast majority of patients (92%) had already failed to respond to at least one previous line of treatment and the mean duration of the disease in the study population was over 20 years. Thus, most patients of this study had stable disease, and all of them had at least several years of duration of their disease. It would be interesting to see whether better results could be achieved in a population with a recent onset of vitiligo. Further studies are required to answer to this very important point. The relatively low response rate in the NB-UVB group compared with similar studies in the literature²⁷ presumably reflects the fact that the study population had somewhat more recalcitrant disease. Furthermore, the frequency of NB-UVB sessions in our study (twice-weekly), compared to 3 times weekly in most previous studies,²⁷ may also have contributed to the low response rate we observed. Finally, the relatively short period study has also probably more weight in our population with recalcitrant cases who tend to respond more slowly. Interestingly, in a recent phase 2 study evaluating ruxolitinib cream in non-segmental vitiligo, 12.1% of patients achieved a $\geq 50\%$ improvement from baseline in Vitiligo Area Scoring Index (VASI 50) after 24 weeks of treatment with the highest concentration of ruxolitinib.²⁸ Although we cannot directly compare the two trials, it is interesting to note that 16% of the patients treated with GP-SOD in combination with NB-UVB in the present study reached VES 50, which is comparable to VASI 50. Importantly, the topical ruxolitinib was used in monotherapy so we could reasonably expect that a combination of topical or systemic JAK inhibitors with phototherapy would result in a higher rate of repigmentation. One other limitation of this study is the lack of measuring SOD levels at baseline and at 24 weeks. Also, the doses used in our study were based upon our clinical experience. Thus, it might not

reflect the optimal dosage. Further studies comparing different dosing schedules might help to answer to this point.

Together these data highlight that vitiligo remains a difficult disorder to treat. It requires many months of treatment associating several approaches in order to obtain the best repigmentation rate. Therefore, the use of GP-SOD combined with NB-UVB could be a useful add-on in the treatment of vitiligo patients with excellent tolerability and safety.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1a. Representative clinical pictures. (a) Vitiligo on the face and arms at baseline (VES at baseline: 12.53).

Figure S1b. Representative clinical pictures. (b) After 24 weeks of narrowband UVB and Gliadin-protected Super Oxide Dismutase (VES at W24: 3.51).

Figure S2a. Representative clinical pictures. (a) Widespread vitiligo on the back (VES at baseline: 65.38).

Figure S2b. Representative clinical pictures. (b) After 24 weeks of narrowband UVB and Gliadin-protected Super Oxide Dismutase (VES at W24: 24.01).