

# Antioxidants and narrow band-UVB in the treatment of vitiligo: a double-blind placebo controlled trial

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## Summary

**Background.** Vitiligo is an acquired depigmenting disease with uncertain aetiopathogenesis, possibly associated with oxidative stress. Narrowband ultraviolet B phototherapy (NB-UVB) is the most widely used and effective treatment.

**Aim.** To evaluate the clinical effectiveness of NB-UVB and the repairing of oxidative stress-induced damage, using oral supplementation with an antioxidant pool (AP).

**Methods.** Patients ( $n = 35$ ) with nonsegmental vitiligo were enrolled in a randomized, double-blind, placebo-controlled multicentre trial. The treatment group received, for 2 months before and for 6 months during the NB-UVB treatment, a balanced AP containing  $\alpha$ -lipoic acid, vitamins C and E, and polyunsaturated fatty acids. The area and number of lesions, as well as some parameters of the oxidation–reduction (redox) status of the peripheral blood mononuclear cells (PBMCs) were estimated at the beginning, after 2 months, and at the end of the trial.

**Results.** In total, 28 patients completed the study. After 2 months of AP supplementation, the catalase activity and the production of reactive oxygen species (ROS) were 121% and 57% of the basal values ( $P < 0.05$  and  $P < 0.02$  vs. placebo, respectively). The AP increased the therapeutic success of NB-UVB, with 47% of the patients obtaining  $> 75\%$  repigmentation vs. 18% in the placebo group ( $P < 0.05$ ). An increase in catalase activity to 114% ( $P < 0.05$  vs. placebo) and decrease in ROS level of up to 60% ( $P < 0.02$  vs. placebo) of the basal value was observed in PBMCs. Finally, the AP intake maintained the membrane lipid ratio (saturated : unsaturated fatty acids 1.8 : 3.1;  $P < 0.05$ ), counteracting phototherapy-induced saturation.

**Conclusions.** Oral supplementation with AP containing  $\alpha$ -lipoic acid before and during NB-UVB significantly improves the clinical effectiveness of NB-UVB, reducing vitiligo-associated oxidative stress.

## Introduction

Vitiligo is an acquired pigmentary disorder, caused by several overlapping pathogenetic mechanisms leading

to loss of functional melanocytes. Immune-mediated and toxin-associated damage to melanocytes is the most probable pathological mechanism. The toxic damage can be due to dysregulation of some metabolic pathways (epidermal and systemic metabolism of biopterins and catechols and of the intracellular antioxidant system) because of genetic polymorphisms. The clinical findings are amelanotic areas that vary in size during the course of the disease, and are often symmetrically distributed, involving periorificial and frictional areas, sometimes with white hairs.

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Because of the incomplete understanding of the aetiopathogenesis of vitiligo, the current treatment remains unsatisfactory.

Phototherapy [ultraviolet (UV)B or psoralen plus UVA] is widely used. Open-label trials indicate that NB-UVB ( $312 \pm 2$  nm) is superior to other light treatment options (50–70% effectiveness after 6–12 months).<sup>1–3</sup> The aim is to stimulate growth and migration of the remaining perilesional and follicular melanocytes. UVB promotes the release of keratinocytic growth factors and induces melanocytic expression of focal adhesion kinase, involved in adhesion and migration processes.<sup>4</sup> In order to reduce exposure to and side-effects of UV exposure, and promote survival and migration of melanocytes through several simultaneous mechanisms, combined approaches have been proposed in association with different compounds.<sup>5–7</sup>

Oxidative stress, characterized by an increase in free-radical production exceeding the intracellular antioxidant defence, has been proposed as a possible pathogenetic mechanism, and administration of various antioxidants, alone or in combination with phototherapy, has been used, although definitive clinical evidence of its efficacy is lacking so far.<sup>8–13</sup> We previously demonstrated that (i) in active vitiligo patients, even peripheral blood mononuclear cells (PBMCs) show an imbalance in oxidative–reduction (redox) status, suggesting that vitiligo can be considered a systemic disease with circumscribed (mainly cutaneous) manifestations and (ii) the redox status in PBMCs mirrors that in epidermis.<sup>14–16</sup>  $\alpha$ -lipoic acid is a lipophilic and hydrophilic compound acting as a fatty-acid peroxy and hydroxyl radical scavenger, lipoxygenase inhibitor and glutathione synthesis promoter, and is involved in recycling vitamins C and E. Vitamin C is a hydrophilic antioxidant, while vitamin E is a free-radical scavenger that inhibits lipid peroxidation and helps maintain membrane integrity.

In this paper, we report the results of a pilot double-blind, placebo-controlled, multicentre study conducted to assess whether an antioxidant mixture containing  $\alpha$ -lipoic acid, vitamin E and vitamin C is able to improve the clinical effectiveness of NB-UVB phototherapy and to reduce the alteration in redox status caused by the treatment.

## Methods

The study comprised patients with vitiligo referred to the three dermatological institutes participating in the trial (Table 1). The medical ethics committee of the San Gallicano Dermatological institute approved the study

**Table 1** Enrolled patients: clinical features and summary of the clinical results after the overall protocol.

	AP	Placebo
Patients randomly assigned	21	14
Patients receiving intended treatment	21	14
Patients completing study	17	11
Patients analysed	17	11
Phenotypes		
I	0	0
II	2	2
III	16	7
IV	3	4
V	0	1
VI	0	0
Duration of the disease, years (range)	2–10	1–8
Extent of lesions (% of body surface)	15–42	15–38
Autoimmune diseases	3	4
Active : stable phase of the disease	14 : 7	9 : 5
Repigmentation		
Grade 2	4/17 (23.5%)	2/11 (18%)
Grade 3	8/17 (47%)	2/11 (18%)
No. of UVB treatments	18	23
UVB dosage (J/cm <sup>2</sup> )	19.4	25.7

Inclusion criteria were: age > 18 years, duration of the disease > 1 year, and lesions involving > 15% of the body surface. Exclusion criteria were: treatment with UVB or PUVA during the past 12 months; use of antioxidants, topical corticosteroids, vitamin D derivatives, or systemic immunomodulators during the past 6 months; and occurrence of renal insufficiency or pregnancy.

protocol, and all subjects provided written informed consent.

The trial was a prospective, randomized, double-blind, placebo-controlled multicentre study of NB-UVB in combination with an antioxidant pool (AP) vs. NB-UVB alone for treatment of generalized vitiligo in adults, carried out between January 2003 and April 2004. Patients were allocated using a randomized-numbers table in a one-to-one manner. The study included three follow-up visits: screening visit before therapy (T0), after 2 months of AP/placebo intake before beginning NB-UVB (T2), and after 6 months of NB-UVB +/- AP (T6).

## Treatment

AP is a commercially available drug (Lipoacid Combi; General Topics, Salò, Italy) containing  $\alpha$ -lipoic acid (50 mg), vitamin C (50 mg), vitamin E (20 mg), polyunsaturated fatty acids (12%), and cysteine monohydrate (50 mg). The patients took two tablets per day for 8 weeks before starting phototherapy. The taking of tablets continued for 6 months during the UVB treatment. Patients were treated twice weekly with

NB-UVB (Waldmann UV-8001 booth, equipped with 48 TL01/100 W Philips fluorescent tubes; Herbert Waldmann GmbH & Co, Villingen-Schwenningen, Germany) starting with 70% of the minimal erythema dose (MED) on unaffected skin, with subsequent increments of 30% (treatments 1–4), 20% (treatments 4–8), and 10% MED (treatment 8 onwards). The dose was held constant when minimal asymptomatic erythema occurred on the lesions. If symptomatic erythema developed, treatment was withheld and resumed at the last dose less 20%. Irradiance was measured with a portable UV meter (Herbert Waldmann GmbH & Co).

### Visual repigmentation grading

Two independent observers, blinded to the treatment, evaluated the size and the number of lesions, which were also photographed. Repigmentation was recorded as 'absent' (grade 0), 'moderate' (< 50%, grade 1), 'good' (between 50 and 75%, grade 2) and 'excellent' (> 75%, grade 3) for each lesion.

### Biochemical parameters measurement

PBMCs were isolated using a Ficoll-Hypaque gradient and stored, along with plasma, at  $-80^{\circ}\text{C}$ . Vitamin E was isolated from the plasma using hexane/ethanol extraction, with  $\gamma/\delta$ -tocopherol as internal standards. The organic phase containing the tocopherols was analysed by gas-chromatography mass-spectrometry (GC-MS) on an Ultra 2 column [ $30\text{ m} \times 0.2\text{ mm} \times 0.25\text{ mm}$ ; Hewlett-Packard (now Agilent Technologies) South Queensferry, West Lothian, UK], using the selected-ion monitoring technique.

Productive of reactive oxygen species (ROS) by PBMCs was assessed using a hydrogen-peroxide kit (AmplexRed; Molecular Probes, New Brunswick, NJ, USA), following the manufacturers' instructions. The catalase activity of PBMCs was assayed spectrophotometrically by measuring the rate of disappearance of hydrogen peroxide.<sup>17</sup> The analysis was always performed on frozen samples, thus preventing any possible difference due to sample manipulation.

Lipids were extracted from the cellular pellet using chloroform/methanol extraction in the presence of butylated hydroxytoluene as antioxidant. Phospholipid fatty acids were *trans*-methylated and analysed using GC-MS on a free fatty-acid phase (FFA-P) capillary column ( $60\text{ m} \times 0.32\text{ mm} \times 0.25\text{ mm}$ ; Hewlett-Packard). The results are reported as a percentage of the total fatty acids.<sup>18</sup>

### Statistical analysis

Data were reported as means  $\pm$  SD. Statistical analysis was performed using Student's *t*-test, with  $P < 0.05$  considered statistically significant.

## Results

### Clinical data

In total, 35 patients (21 women, 14 men; mean age 39.9 years, range 24–61) with generalized vitiligo were enrolled. Of these, 28 patients completed the 6 months of therapy with full compliance and were included in the statistical analysis. The remaining 20% of the patients dropped out for reasons unconnected with the therapy (fever, physical trauma, job changes).

At T2, the AP group exhibited stabilization of the disease and partial repigmentation of some lesions, whereas patients receiving placebo showed only stabilization of the disease.

At T6, 47% ( $P < 0.05$  vs. placebo group) and 23.5% of the patients in the AP group developed grade 3 and grade 1–2 repigmentation, respectively. In the placebo group, 18% achieved repigmentation of grades 2 and 3. Of the 10 patients with grade 3 repigmentation (8 in the AP group and 2 in the placebo group), 8 had stable vitiligo and 2 had active disease. The average number of treatments required to achieve 50% repigmentation was 18 in the AP group and 23 in the placebo group (Table 1). We did not observe any significant correlation between effectiveness of the therapy and skin phototype.

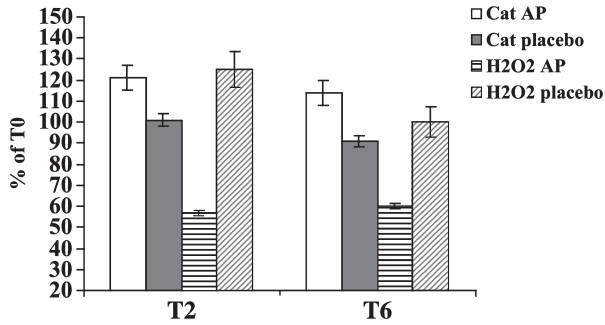
The best response was noted on the face, neck, proximal limbs excluding the elbow/knee regions, and trunk. Most of the lesions over the hands and feet showed mild to no response. No new lesions appeared in any patient during the course of the trial.

Adverse effects to phototherapy were minimal and none of the patients required suspension or discontinuation of the therapy.

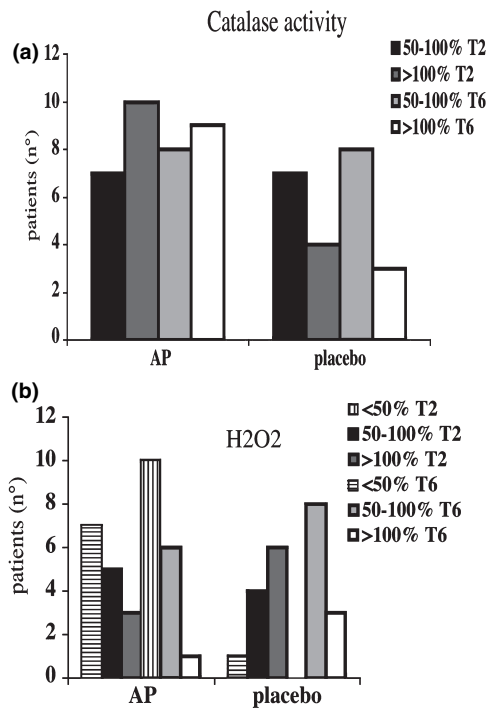
### Biochemical data

At T0 the catalase activity was  $147 \pm 36$  U/mg protein and  $149 \pm 34$  U/mg protein and the intracellular ROS amount corresponds to  $0.63 \pm 0.4$   $\mu\text{M}$  and  $0.42 \pm 0.2$   $\mu\text{M}$  in AP and the placebo group, respectively.

At T2 the catalase activity was 121% ( $168 \pm 73$  U/mg protein;  $P < 0.05$ ) and 101% ( $143 \pm 73$  U/mg protein) of the basal value in AP and placebo groups, respectively (Fig. 1 and 2A; Table 2).



**Figure 1** Increased catalase (Cat) activity and reduced intracellular H<sub>2</sub>O<sub>2</sub> production in PBMC of AP-treated compared with placebo-assigned patients with vitiligo. The values are reported as a percentage of the basal level at T0.



**Figure 2** Distribution of patients on the basis of the modification (< 50%, 50–100%, or > 100%) of the catalase activity and of the intracellular H<sub>2</sub>O<sub>2</sub> compared with the basal value (T0). Values correspond to the number of the patients in whom catalase activity and intracellular H<sub>2</sub>O<sub>2</sub> was < 50%, 50–100% or > 100% of the basal value (T0), respectively.

At T6 the catalase activity in the AP group was higher ( $164 \pm 43$  U/mg protein, 114% of the basal value) than that in placebo group ( $139 \pm 31$  U/mg protein,  $P < 0.05$ , 91% of the basal value) (Fig. 1).

Independent of clinical effectiveness, intracellular ROS production decreased in the treated group (57% and 60% of the starting value at T2 and T6, respectively;

$P < 0.02$ ), whereas it did not change in the placebo group (Figs 1 and 2B).

Finally, the phototherapy induces a progressive saturation of the membrane (saturated/unsaturated fatty acid ratio was 2.2, 2.3, 3.1, at T0, T2 and T6, respectively) but the oral administration of AP counteracts this process maintaining the membrane unsaturated (2.6, 2.1, and 1.8 at T0, T2 and T6, respectively;  $P < 0.05$  at T6) (Fig. 3).

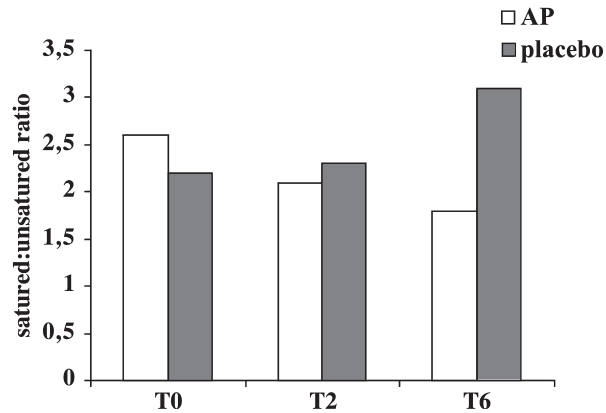
## Discussion

NB-UVB phototherapy is currently considered an effective treatment for generalized vitiligo, with a success rate of 50–70% depending on patient age and disease severity.<sup>1</sup> Various therapies have been proposed to increase the degree of repigmentation and reduce the length of treatment, including vitamin D analogues, tacrolimus, and antioxidants.<sup>5,6,9</sup> This controlled trial demonstrates that oral administration of an AP increases the clinical effectiveness of subsequent NB-UVB phototherapy and is associated with the restoration of the correct intracellular redox status. In accordance with our previous data,<sup>14</sup> we found lower catalase activity and higher intracellular ROS production in PBMCs from vitiligo patients compared with those from normal subjects. Intake of AP for 2 months restored the balance in intracellular redox status, allowing better response to the subsequent phototherapy. Our results show that the combined treatment significantly increased the degree of repigmentation compared with phototherapy alone (Fig. 4), and that the repigmentation occurred earlier and with lower cumulative UVB dosage. A better clinical result was obtained in subjects with stable disease, probably because their cellular redox status is only slightly compromised<sup>14</sup> and thus can be restored more easily. The clinical results were supported by biochemical parameters showing at T6 an increase in catalase activity (114% of basal activity), decrease in intracellular ROS production (60% of the basal value), and reduced membrane peroxidation (saturated/unsaturated fatty-acid ratio of 1.8) in the AP group. In the placebo group, a reduction in the antioxidant enzymatic activity at T6 was observed (91% of basal), probably due to phototherapy-associated oxidative stress, amplifying the difference from the treated group. Thus, the enzymatic activity measured at T6 in the AP group can be considered the result of the protective effect of the AP. Finally, in the AP group the number of patients with increased catalase activity and reduced H<sub>2</sub>O<sub>2</sub> was higher at both T2 and T6 (Fig. 2).

**Table 2** Biochemical results.

	AP			Placebo		
	T0	T2	T6	T0	T2	T6
Catalase (U/mg protein)	147 ± 36	168 ± 73	164 ± 43*	149 ± 34	143 ± 73	139 ± 31
Vitamin E (ng/mL)	10 ± 1.4	11 ± 3	9 ± 1.9	8 ± 2.3	7.7 ± 3.3	5.8 ± 3.2
PUFA (%)	22 ± 6	25 ± 4	28 ± 6	24 ± 6	24 ± 7	20 ± 8
H <sub>2</sub> O <sub>2</sub> (μmol/l)	0.63 ± 0.4	0.36 ± 0.2†	0.38 ± 0.2†	0.4 ± 0.2	0.5 ± 0.4	0.4 ± 0.3

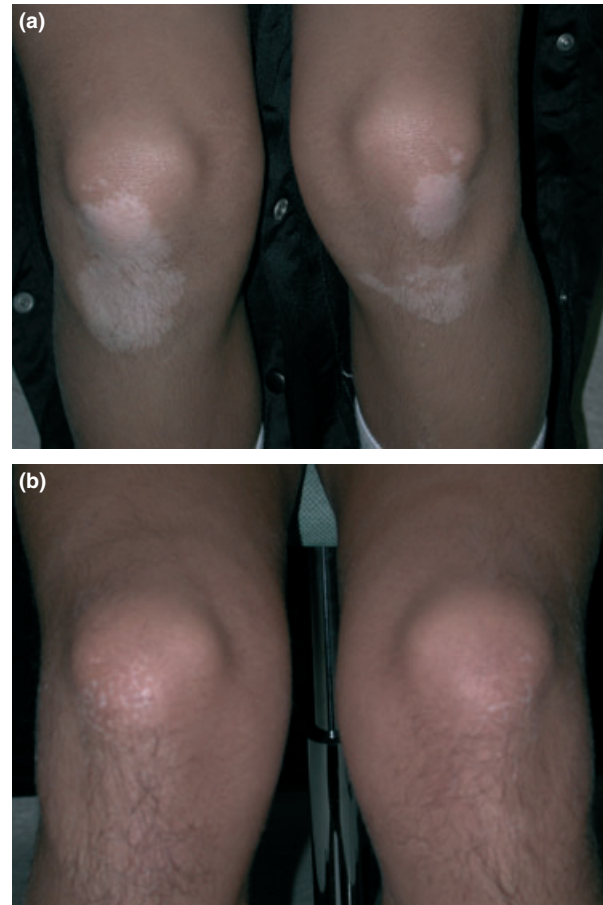
The values are reported as mean ± standard deviation. \**P* < 0.05 vs. placebo; †*P* < 0.02 vs. T0. PUFA, polyunsaturated fatty acids.



**Figure 3** Increased saturated : unsaturated fatty-acid ratio in PBMCs of patients assigned to the placebo group and exposed to NB-UVB can be reversed by the intake of AP.

Previous clinical trials and animal studies underline an *in vivo* antioxidant activity for most AP components. Supplementation with  $\alpha$ -lipoic acid results in restoration of the correct cellular redox status in diabetes, chemotherapy-induced oxidative stress, ageing and neurological diseases.<sup>19–21</sup> Vitamin E has been reported to reverse phototherapy-induced lipoperoxidation. Vitamin C, acting as a reducing agent, prevents damage by free oxygen radicals and slows the oxidative process. The different antioxidants act synergistically because of reciprocal recycling activity and a multistep process.<sup>9,22–25</sup> The present study is the first double-blind, placebo-controlled trial demonstrating that long-term administration of an AP is effective in improving the rate of repigmentation by NB-UVB phototherapy in patients with vitiligo. We suggest that the components of the AP can counteract the oxidative stress occurring in patients with vitiligo, and interfere with the possible pathogenetic mechanisms of the disease.

Thus, supplementation with antioxidants may represent a valuable adjunctive therapy, preventing the progression of the disease as well as increasing the effectiveness of NB-UVB. NB-UVB and antioxidants,



**Figure 4** AP-treated patient (a) before and (b) after overall treatment. A significant reduction in the size and number of the lesions was observed (score 3).

mainly vitamin E and  $\alpha$ -lipoic acid, work through different mechanisms: (i) regulating immunosuppression and stimulating melanocyte replication and proliferation and (ii) regulating the antioxidant balance, which is critical in vitiligo. This could mean that these two modalities are supplementary and synergistic. Further evaluations will be performed to establish whether the

adjuvant treatment with antioxidants can reduce the rate of relapse in vitiligo.

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