Abstract

Background Low catalase levels and cellular vacuolation in the epidermis of patients with vitiligo support major oxidative stress in this compartment. There is now in vivo evidence for increased epidermal hydrogen peroxide (H$_2$O$_2$) accumulation in this patient group by utilizing noninvasive Fourier Transform Raman spectroscopy (FT Raman). Epidermal H$_2$O$_2$ can be removed with a topical application of narrow band UVB activated pseudocatalase cream (PC-KUS). (Mn/EDTA-bicarbonate complex, patent No. EPO 584711 A), yielding initiation of repigmentation. Dead Sea climatotherapy is another successful treatment modality for vitiligo, but the mode of action has escaped definition so far.

Methods Epidermal hydrogen peroxide (H$_2$O$_2$) was assessed in vivo before and after 21 days treatment at the Dead Sea using noninvasive Fourier-Transform Raman spectroscopy. The effectiveness of repigmentation was followed in 59 patients with vitiligo by comparing Dead Sea climatotherapy alone with the combination of Dead Sea climatotherapy/pseudocatalase cream (PC-KUS) as well as Dead Sea climatotherapy/placebo cream. Clinical repigmentation was documented by standardized black/white photography using non-UV coated bulbs as flashlight and by color photography.

Results This study on 59 patients who had vitiligo for an average time of 17 years (range 3–53 years) confirmed in vivo H$_2$O$_2$ accumulation in mM concentrations in the epidermis of untreated patients. Furthermore, we demonstrated a pseudocatalase activity after 15 min of Dead Sea bathing, but the decrease of epidermal H$_2$O$_2$ levels was significantly less compared to narrowband UVB activated pseudocatalase cream (PC-KUS). Initiation of repigmentation was already observed between day 10 and day 16 after a combination of Dead Sea climatotherapy/pseudocatalase cream compared to conventional pseudocatalase monotherapy (8–14 weeks) and Dead Sea climatotherapy alone (5–6 weeks).

Conclusion The results of this study show a significantly faster initiation of repigmentation in vitiligo after a combination of short-term climatotherapy (21 days) at the Dead Sea in combination with a pseudocatalase cream (PC-KUS) compared to either conventional climatotherapy at the Dead Sea alone or with placebo cream in combination with climatotherapy. This combined therapy is significantly faster in repigmentation than narrowband UVB activated pseudocatalase cream (PC-KUS) treatment alone.

The results of this study support the necessity of epidermal H$_2$O$_2$ removal as well as the influence of solar UV-light in the successful treatment of vitiligo.
epidermal H$_2$O$_2$, together with low catalase levels have been identified in vivo and in vitro using Fourier Transform Raman spectroscopy (FT Raman). It has been shown that the missing catalase activities can be substituted with a narrow-band activated pseudocatalase cream (PC-KUS) which is based on a bis manganese EDTA bicarbonate complex (Patent No. EPO 58417 A). This complex successfully removes the high H$_2$O$_2$ levels from these patients leading to initiation of repigmentation and to recovery from vacuolation in the epidermal cells. The time course for repigmentation can vary considerably in the patients, but repigmentation can be achieved in long-term vitiligo even after 47 years (Fig. 1).

Since the combination of solar radiation and sea water bathing at the Dead Sea is a successful treatment modality in various skin diseases, such as psoriasis and atopic eczema, as well as vitiligo, we wanted to investigate whether we could enhance the effect of the conventional pseudocatalase cream (PC-KUS) monotherapy in combination with the Balneoclimatotherapy at the Dead Sea.

Until now it is generally believed that the high salt concentration (346 g/L) contributes to the efficacy of the therapy by several mechanisms. A release of pro-inflammatory and chemotactic mediators has been proposed. Only recently the effect of the high magnesium chloride (MgCl$_2$) concentration of this water has been further elucidated by Schempps et al., who were able to show that MgCl$_2$ dramatically influences the antigen presenting capacity of Langerhans cells. Furthermore, it is generally believed that the unique solar UV spectrum in the Dead Sea basin contributes to the sea water bathing. In this context an increased photosensitivity after the salt bathing has been implicated. Solar radiation at the Dead Sea is filtered by an additional 400 m below sea level atmosphere and it has been shown that the shortest part of UVB does not arrive at the Dead Sea. These conditions...
could foster activation of the inactive manganese-EDTA bicarbonate complex of the conventional pseudocatalase cream (PC-KUS) therapy.\textsuperscript{13,14}

\textbf{Patients and methods}

\textbf{Patient selection and study design}

The clinical observation was based on a randomised three-arm study, in order to compare the effect of climatotherapy alone (group 1/control arm) vs. placebo cream in combination with climatotherapy (group 2) and pseudocatalase cream (PC-KUS) in combination with climatotherapy (group 3) at the Dead Sea basin over 21 days.

The patients were eligible for the study when they had vitiligo according to a full body clinical examination in association with Wood’s light.

Fifty-nine English and German patients with vitiligo (14 men and 45 women) with a mean age of 38.6 years (range 18–62 years) from the Institute for Pigmentary Disorders in Greifswald, Germany were included in the study. The majority of the group (86\%) had the most common clinical subtype vulgaris and 14\% presented with acrofacial vitiligo. Only patients with photo skin type III (Fitzpatrick classification) were included in order to exclude any possible effect of other skin types.\textsuperscript{24}

The duration of the disease process varied from 3 to 53 years with a mean age of 16.7 years. Family history was positive in 28/59 patients (47.5\%). The patients were otherwise healthy. Written consent was obtained from the local ethics committee from each patient prior to this clinical study.

The study took place at the DMZ-Mor Clinic in Ein Bokek, Israel during the months of June and July.

\textbf{FT-Raman protocol to follow epidermal H$_2$O$_2$ removal in vivo in the skin}

All patients ($n = 59$) were tested in vivo before treatment and at the end of the 21-day stay at the Dead Sea basin for epidermal H$_2$O$_2$ concentrations using in vivo FT-Raman spectroscopy. The results were compared to 15 healthy photo skin-type matched controls.\textsuperscript{12} FT-Raman spectra were obtained with a Bruker RFS 100/S spectrometer equipped with a liquid nitrogen cooled germanium detector. Sample excitation was accomplished using a Nd$^{3+}$/YAG laser operating at 1064 nm with a laser power of 400 mW. Each spectrum was accumulated over 5 min with 300 scans and a resolution of 4 cm$^{-1}$. Total H$_2$O$_2$ was visualized as a well defined peak at 875 cm$^{-1}$ based on the oxygen-oxygen (O-O) stretch vibration.\textsuperscript{12}

The study design was approved by the local Ethics Committees.

\textbf{Atomic Absorption Spectroscopy}

In order to elucidate the content of transition metals in the Dead Sea water we utilized Atomic Absorption Spectroscopy. Total transition metal analyzes were carried out using a Perkin-Elmer model 1100 Atomic Absorption spectrometer. Samples of Dead Sea water were diluted 1 : 20 with de-ionized distilled water and made up to 1 L.

\textbf{Treatment protocol}

All patients had to bathe for 15 min in the Dead Sea twice daily. The bath was followed by a shower to wash off the salt. Group 1 ($n = 10$) then underwent directly total body sun exposure, whereas group 2 ($n = 10$) and group 3 ($n = 39$) applied their creams to the entire body surface prior to sun exposure.

The exposure times were slowly increased to a maximum of 1 h. The treatment took place in the morning between 7.30 am and 10.30 am and in the afternoon between 2.30 pm and 5.30 pm. The patients had to record the exposure times.

\textbf{Clinical assessment by standardized photography}

For a valid clinical assessment of the repigmentation of the face and hands we utilized black and white photographs using a fixed frame assembly holding the camera (Pentax MZ-M with a 90 mm lens and an Ilford B82 glass filter) and a Kodak T-MAX 400 film. The flashlight with non-UV coated bulbs was also fixed (Bowens Esprit 500). This technique assures a good quality comparison of subsequent photographs and allows, even in very fair skin, the objective evaluation of affected areas. In addition, we used whole body color photographs with a standard camera and flashlight (Minolta) to follow disease stability during this treatment period.

Patients were photographed before treatment and at the end of the therapy at the Dead Sea after 21 days.

\textbf{Assessment of efficacy}

The efficacy of each treatment modality was assessed after 21 days based on the special photographic documentation of the face and hands. The grading was based on the repigmentation of affected areas by comparison of the photos before and after 21 days.

\textbf{Statistical analysis}

For statistical analysis we used the Wilcoxon signed rank test for paired samples. Values $P < 0.05$ were significant.

\textbf{Results}

\textbf{The Dead Sea water has pseudocatalase activity}

The Dead Sea water analysis showed the presence of transition metals such as manganese (7440 p.p.m.), copper (581 p.p.m.), and iron (1398 p.p.m.), as well as high concentrations of sodium bicarbonate and calcium chloride. These results were obtained from analysis of the Dead Sea water using Atomic Absorption Spectroscopy. Since these transition metals can form the chemical base for pseudocatalases, we expected that the Dead Sea water bathing would act as a “pseudocatalase”.\textsuperscript{13–14} In order to test this assumption, we
followed \textit{in vivo} the reduction of $\text{H}_2\text{O}_2$ levels in 16 of 59 patients using FT Raman spectroscopy. The results showed a significant decrease of epidermal $\text{H}_2\text{O}_2$ removal in vitiligo using FT-Raman spectroscopy. Measurements were taken before and after the bath, showing a significant reduction of $\text{H}_2\text{O}_2$ ($p = 0.001$).

The outcome showed that patients treated with placebo cream/Dead Sea climatotherapy ($n = 39$) had significantly less follicular repigmentation in the face compared to the patient group treated with pseudocatalase cream (PC-KUS) in combination with climatotherapy (group 3).

One example of extensive follicular repigmentation in the face is shown in Fig. 4.

There was no significant difference between Dead Sea climatotherapy (group 1) and placebo in combination with climatotherapy (group 2).

**Discussion**

The positive healing effect of climatotherapy at the Dead Sea basin has been well established in the treatment of psoriasis and atopic eczema. The treatment protocol for these diseases usually lasts for 28–42 consecutive days and the response rates are described as high as 80%. Studies on UV-intensities at the Dead Sea area showed that 9.4% of UVB
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and 3.5% of UVA are filtered out yielding a unique environment in this region.29 The safety aspects of the solar phototherapy have been investigated and discussed extensively.30 It has been suggested that the UVB range at the Dead Sea could be a natural narrow-band therapy.29 In addition, the high mineral content of the Dead Sea has been considered a major factor in this successful treatment modality. The study presented here identified the Dead Sea water as a “pseudocatalase”. We were able to follow in vivo the reduction of epidermal H$_2$O$_2$ directly in the skin of patients with vitiligo. This reducing effect was much weaker than a narrow-band UVB activated pseudocatalase cream (PC-KUS) in combination with Dead Sea water bathing. However, whether this H$_2$O$_2$ reduction was indeed beyond the mM range cannot yet be concluded due to the limitation in sensitivity of in vivo FT Raman spectroscopy.12

A significantly faster initiation of repigmentation compared to conventional pseudocatalase cream (PC-KUS) monotherapy or climatotherapy alone was observed using the combined protocol Dead Sea/pseudocatalase cream (PC-KUS)/solar phototherapy twice daily in the early morning and late afternoon.12–14 It should be noted that the treatment protocol in the study presented here used much less sun exposure than the conventional treatment recommendation at the Dead Sea for vitiligo.29 (2 h compared to 7–8 h per day). In conclusion, from the results of this placebo controlled study, it appears that the combination of climatotherapy and conventional pseudocatalase cream (PC-KUS) mimates a faster repigmentation in all patients studied. A comparison of our own results using narrow-band UVB activated pseudocatalase cream (PC-KUS) and the combination therapy revealed that the combination therapy is much more effective in the onset of repigmentation.12–14 This fast repigmentation continues for at least up to 3 months (unpublished results).

This study did not investigate the contribution of the unique solar spectrum to the initiation of repigmentation in vitiligo. Since all patients (groups 1, 2, and 3) underwent their treatment at the same time of the year and during the same sun hours of the day, we believe that the data of this clinical observation are valid and support once more the importance of epidermal H$_2$O$_2$ removal and also underline the significant contribution of UV-light to initiating a successful repigmentation in this disease.12–14

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References
