Promotion of wound healing by photodynamic therapy. A phase II, randomised, placebo-controlled trial in chronic leg and diabetic foot ulcers.

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1 ABSTRACT

Background: Photodynamic therapy (PDT) uses light activation of drugs to obtain a therapeutic effect. It has mainly been applied in oncology and ophthalmology and is now being developed for treatment of infection. There is laboratory evidence that PDT may promote wound healing by reduction in bacterial load and also by stimulation of growth factors.
Purpose: To determine if PDT in bacterially colonised chronic leg (CL) ulcers and chronic diabetic foot (CDF) ulcers can reduce bacterial load and lead to accelerated wound healing.
Methods: 16 patients with CL ulcers and 16 patients with CDF ulcers (each 8 active treatment/8 placebo) were recruited into a blinded, randomised, placebo-controlled, single treatment, Phase IIa trial. All patients had ulcer duration > 3 months, bacterially colonised with $>10^4$ cfu/ml. After sampling for determination of pre-treatment bacterial load (swabbing), 3,7-Bis(di-n-butylamino) phenothiazin-5-ium bromide in Unguentun M or placebo was applied topically to wounds for 15 minutes, followed by 50 J/cm^2 of broad band red light (approximately 15 minutes) and the wound again sampled for microbiology.
Results: Treatment was well tolerated with no reports of pain or other safety issues. Compared with placebo, patients on active treatment showed a statistically significant reduction in bacterial load after treatment (p=0.017). After 3 months 50% (4 of 8) actively treated CL ulcer patients showed complete healing, compared to 12.5% (1 of 8) placebo patients. 3-month healing could not be assessed in the CDF patients because of the number of withdrawals.
Conclusions: This first controlled study of PDT in chronic wounds demonstrated significant reduction in bacterial load and a strong trend towards wound healing. Trials with larger patient numbers are required to confirm this acceleration of wound healing and are ongoing.

2 INTRODUCTION

Antimicrobial photodynamic therapy: With increasing occurrence of antibiotic resistant microorganisms, there is a clinical need for novel antimicrobial treatments. One potential such treatment is antimicrobial photodynamic therapy (PDT), which uses a combination of a photosensitiser and visible light. The photosensitiser can be applied locally, for example to a wound, and subsequently activated by visible light to generate singlet oxygen (a reactive oxygen species, but not a free radical), that damages and kills microorganisms. The multi-targeted cell kill mechanism reduces the chances for the development of microbial resistance.
The photosensitiser PPA904: PPA904 (3,7-bis(di-n-butylamino) phenothiazin-5-ium bromide) is a novel photosensitiser that is under clinical development for topical PDT (Figure 1). In combination with visible light, it is highly effective *in vitro* against a broad spectrum of microorganisms including Gram positive and Gram negative bacteria, resistant bacteria such as MRSA and yeast (Figure 2).
Early clinical studies of PDT and chronic ulcers: Healing of wounds may be impeded by a high bacterial load. As well as its antimicrobial effects, PDT has also been shown to accelerate wound healing by stimulation of growth factors. There is therefore a dual rationale for treatment of wounds by PDT. The first clinical study using PDT in chronic ulcers was performed in 10 subjects with either a chronic leg ulcer or a diabetic foot ulcer given a single topical treatment with 100µM or 500µM PPA904 in Unguentum M : water (1:2) and 50J/cm^2 visible light. This demonstrated a statistically significant reduction in bacterial load immediately post treatment compared to pre treatment, with some evidence of wound improvement.
Aim: The aim of the current study was to perform a randomised, placebo controlled, proof of principle trial in subjects with either a chronic leg ulcer or a diabetic foot ulcer to demonstrate that photodynamic therapy treatment with PPA904 and visible light can reduce the bacterial burden of chronic ulcers and to examine the effect of treatment on wound healing.

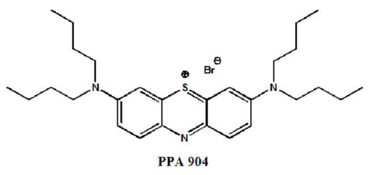

Figure 1: Structure of PPA904

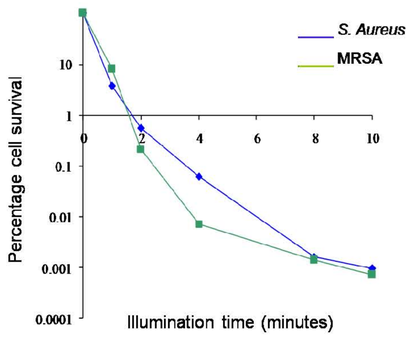

Figure 2: Effect of PPA904 on *S.Aureus*. 3.5x10^8 CFU/mL bacteria were treated with 10 µM PPA904 and illuminated with 665nm laser illumination at 5.3 mW/cm^2

3 PATIENTS AND STUDY DESIGN

Recruitment and randomisation: Thirty two subjects were recruited: 16 with chronic leg ulcers and 16 with diabetic foot ulcers. All subjects had ulcers of at least 3 months duration and with a bacterial load of $>10^4$ CFU/cm^2. After screening, 8 subjects with each ulcer type were randomised to receive PPA904 and light and 8 of each type to receive placebo and light. Subjects on antibiotics were excluded. The study was blinded for subjects and all staff performing post dose procedures.
Treatment: PPA904 cream (500µM PPA904 in Unguentum M : water 1:2) or placebo cream (Unguentum M:water, 1:2) was applied topically to the ulcer surface and surrounding ulcer margin (5mm) giving a cream depth of 1mm. The ulcer was occluded under film, shielded from light, for 15 minutes, excess cream was then removed and the ulcer illuminated at a light dose of 50J/cm^2. After treatment chronic leg ulcers were dressed with standard absorbent dressings and multilayer bandaging using a similar compression to that routinely used in their normal clinical care. Diabetic foot ulcers were dressed with standard dressings.
Endpoints: The primary endpoint of the study was bacterial load of the ulcer immediately post treatment compared to immediately pre treatment and the secondary endpoint was reduction in ulcer area at 3 months.
Safety : Safety assessments were carried out at screening and at 1 week and 1 month after treatment, including vital signs, 12-lead ECG and clinical laboratory evaluations.
Microbiology: The whole area of the wound, or the area within a 4cm by 4cm template for large ulcers, was swabbed using a moistened sterile swab and the bacterial load of the ulcer was subsequently calculated in CFU/cm^2 using ulcer area data.
Ulcer topography: Ulcers were photographed and the area was measured by tracing the ulcer using a plastic film grid and measuring the area of the grid using a Visitrak™ system.

4 RESULTS

No treatment-associated safety issues were identified in the study. Patients experienced no discomfort or pain.

Table 1 shows the demographics of patients in the study in terms of ulcer duration, ulcer size and pre-treatment bacterial load. For the leg ulcers, 12 were venous, 3 were mixed and there was only one arterial ulcer.

		Chronic Leg Ulcers		Diabetic Foot Ulcers	
		Placebo	PPA904	Placebo	PPA904
Number of subjects		8	8	8	8
Ulcer duration (months)	Median	9	10	9	10.5
Ulcer area (cm^2)	Median	14.8	4.1	0.5	0.8
Bacterial load (screening) (CFU/cm^2)	Median	$1.34x10^6$	$3.14x10^5$	$8.35x10^6$	$7.50x10^7$

Table 1: Patient demographics

Table 2 shows the results of treatment on bacterial load for all patients and for the leg ulcer patients and the diabetic foot ulcer patients separately. The data clearly show that there was a significant reduction in bacterial load following treatment with PPA904, whether the data were analysed for all patients, for leg ulcer patients or for diabetic foot ulcer patients, but no significant reduction in placebo patients. When the data were analysed for all patients in terms of reduction in PPA 904 treated patients versus placebo directly, the reduction was again highly significant (p = 0.017). Although reduction in bacterial load persisted after 1 week when data for all patients were analysed, this was not statistically significant.

Patient group	Treatment	Median reduction in bacterial counts (log_{10}cfu/cm^2)*	P value
All patients	PPA904	1.01	<0.001
	Placebo	0.29	0.277
Leg ulcer patients	PPA904	1.09	0.016
	Placebo	0.42	0.313
Diabetic foot ulcer patients	PPA904	1.04	0.016
	Placebo	0.22	0.813

* Pre-PDT – post-PDT

Table 2: Statistical analysis of the effect of PPA904 compared to placebo on bacterial counts in all subjects and in leg ulcer and diabetic foot ulcer patients separately.

Of the 8 leg ulcer patients on active treatment, 4 healed completely compared with only 1/8 patients in the placebo group. Figure 3 shows the change in wound area with time for the treatment and placebo groups for the leg ulcer patients. It is clear that, underlying the increased complete healing, there appears to be a steeper reduction in the treated patients than in the placebo patients, although the numbers are too small to allow statistical analysis. Because of the numbers of withdrawals in the diabetic foot ulcer patients due to the need for antibiotics within the 3 month follow up, it was not possible to evaluate wound area reduction in this group. Figure 4 shows the healing obtained after 2 months in one patient with ulcer duration of 14 months.

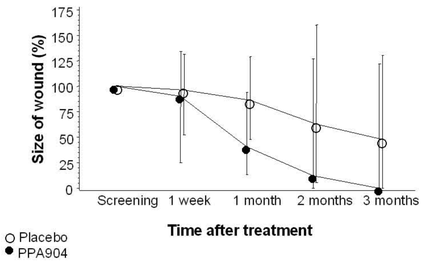

Figure 3: Median % size of wound in leg ulcer subjects (with minimum and maximum values), relative to screening data

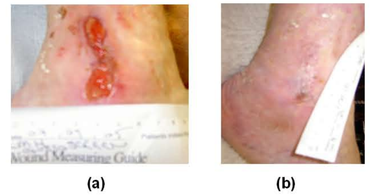

Figure 4: The chronic leg ulcer (a) had existed without healing for 18 months and was treated with PDT using non-coherent light. Complete healing occurred (b) two months after PDT treatment.

Three patients in the study had available wound area data for the period immediately before treatment. Figure 5 shows the change in wound area for these three patients (two on active drug, one on placebo). This clearly shows the change in healing gradient following treatment with PPA904 compared to no change with placebo.

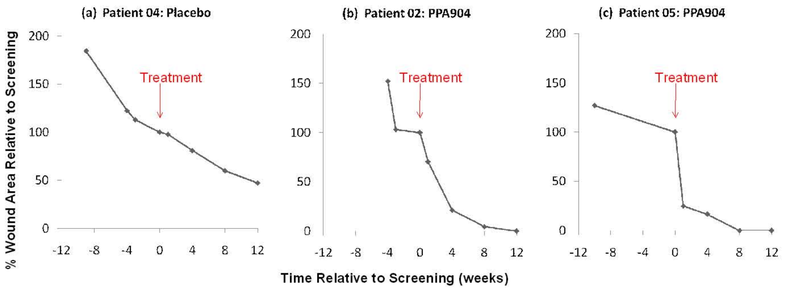

Figure 5: Change in wound area after PDT treatment with placebo (a) or PPA905 (b&c).

5 DISCUSSION

This is the first placebo-controlled study to be carried out using PDT to reduce bacterial load in chronic ulcers. Bearing in mind that the data refer to only a single treatment, it is clear that PPA904 PDT is effective in statistically reducing bacterial load in both chronic leg ulcers and chronic diabetic foot ulcers.

Whilst patient numbers did not permit demonstration of significance for reduction of wound area, there was a strong trend towards accelerated healing in the PPA904-treated patients. Particularly striking was the change in the slope of area reduction shown in Figures 3 and 4 in treated patients compared with the placebo patients. If this effect is confirmed, it is likely to be due to a combination of bacterial load reduction and positive promotion of wound healing by PDT.

This work reports data from only a single treatment. A Phase IIb multi-treatment, multi-centre, trial in patients with venous leg ulcers is planned to commence in August 2008..

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