Discovery of biomarkers to predict the clearance and remission of psoriasis in response to a course of Narrowband UVB phototherapy

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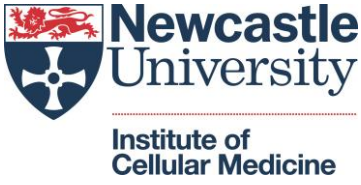
British Association of Dermatologists Intercalated Degree Grant

Master of Research: 2015-2016

Word count: 999



Background

Chronic plaque psoriasis, which represents 90% of psoriasis cases, is typified by recurring and remitting, elevated, erythematous, and scaly plaques and is seen in 1.3 – 2.2% of the United Kingdom population [1-5]. The pathophysiology of psoriasis involves keratinocyte hyperproliferation, loss of differentiation in the epidermis and T cell infiltration of the dermis.

The current guidelines from National Institute of Health and Care Excellence (NICE) for psoriasis treatment recommend using narrowband ultraviolet B (NbUVB) (311-312nm) phototherapy if topical treatments have failed [6].

A key mechanism of action of UVB involves induction of keratinocyte and T lymphocyte apoptosis from direct DNA photodamage [7-10]. Caspase 3/7 enzyme activation can be used to measure NbUVB induced apoptosis keratinocytes [9].

Concentrations of specific nutrients which have been shown to be potential biomarkers for psoriasis such as Selenium, Zinc, Magnesium and Calcium, have rarely been investigated to establish if *in vitro* supplementation of skin cells can alter NbUVB induced apoptosis [11-14]. Supplementation with these nutrients would be a cheap, convenient and safe method of augmenting psoriasis phototherapy treatment.

Using the above nutrients as biomarkers to predict clearance and remission of psoriasis following NbUVB phototherapy would allow for personalised treatment plans and save vital resources. It currently costs £1800 per NbUVB course for the National Health Service (NHS) and requires a large time commitment from the patient with irradiation occurring three-times weekly for 8-10 weeks. Inflammatory biomarkers as well as demographic variables have also rarely been assessed as potential predictors of clearance and remission length.

Aims

- Establish if selected nutrients have a detrimental/beneficial effect on NbUVB induced keratinocyte apoptosis
- Determine factors that predict clearance and remission duration

Methodology

Immortalised keratinocytes (HaCaTs) were irradiated with NbUVB using Standard Erythemal Doses as the unit of measurement. These HaCaTs were supplemented with derivatives of Selenium, Zinc, Magnesium and Calcium in cell viability and caspase (apoptosis) induction experiments. An MTS and Caspase-Glo 3/7 (luminescence based caspase 3/7 activation) assay were used respectively.

Data from the 'Predicting Remission of Psoriasis After Phototherapy' (PROPAP) pilot study was used for the second aim. One hundred chronic plaque psoriasis patients were recruited to the pilot study between April 2014 and April 2015 from the Royal Victoria Infirmary Dermatology Outpatients department, who had been prescribed routine NbUVB phototherapy. Nutritional/inflammatory biomarkers were measured before and after the NbUVB course. Demographic variables were measured prior to commencing phototherapy. Psoriasis Area and Severity Index (PASI) was used to measure the severity of psoriasis on a weekly basis during the course. Follow-up was monthly following completion of treatment until the patient relapsed.

Results and discussion

Preliminary studies were used to optimise the methodology of the lab based aspect of the project. 8 SEDs of NbUVB could induce caspase activation in a 24 hour period. Supplementation of HaCaTs with selected nutrient concentration ranges did not reduce cell viability. Selenium, Zinc and Magnesium could all induce caspase activation, see Figure 1.

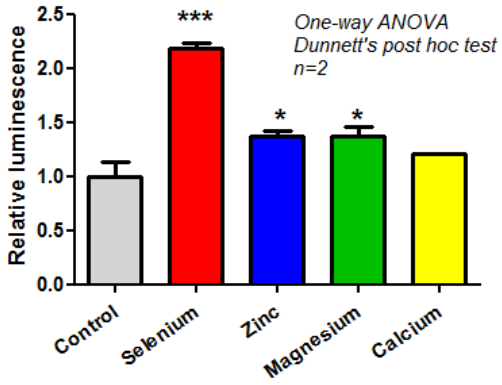


Figure 1. Induction of caspase activation in HaCaTs 48 hours after nutrient supplementation
The Caspase-Glo 3/7 assay measured the caspase enzyme activation 48 hours after treatment with Sodium Selenate, Zinc, Magnesium Sulphate and Calcium Chloride. The data shows the relative mean ±SEM from 3 repeats. One-way ANOVA with Dunnett's post-hoc test compared relative caspase 3/7 activation following treatment with the minerals to an untreated control. *($p<0.05$), *** ($p<0.001$).

In the final set of tests, four experiments were set up each lasting 48 hours. Each experiment exposed HaCaTs to a specific set of conditions as demonstrated in Table 1, with the aim of establishing whether pre or post supplementation of the HaCaTs with the specific nutrient concentration ranges established influenced the level of NbUVB induced apoptosis.

Table 4. Methodology investigating how pre and post treatment with the nutrients affects NbUVB induced caspase 3/7 activation
This table explains the structure of the experiments used to discover whether pre or post treatment with Sodium Selenate, Magnesium Sulphate, Zinc or Calcium Chloride effects NbUVB induced caspase 3/7 enzyme activity. There were three possible conditions that could be used for each experiment. In the first 24 hours, the HaCaTs could be pre treated (+) with the suitable ranges of concentrations of the compounds that did not reduce cell viability. At 24 hours, some cells were irradiated (+) with 8 SEDs of NbUVB to induce apoptosis. Following irradiation, specific experiments had their cells retreated (+) for another 24 hours. Experiments that did not receive a specific condition (-) for the final 24 hours of the experiment still had a caspase 3/7 activation measurement at 48 hours via a Caspase-Glo 3/7 assay.

Experiment	Pre treatment (0-24 hours)	NbUVB irradiation (at 24 hours)	Post treatment (24-48 hours)
1	+	-	+
2	+	+	+
3	-	+	+
4	+	+	-

Zinc and Calcium supplementation had no effect on NbUVB induced caspase activation. Figure 2 demonstrates that post supplementation of Selenium following NbUVB exposure significantly amplified caspase activation whilst pre treatment seemed protective. Pre Selenium treatment has consistently been found to be protective in the literature however post selenium supplementation has never been found to amplify NbUVB induced apoptosis [15-17].

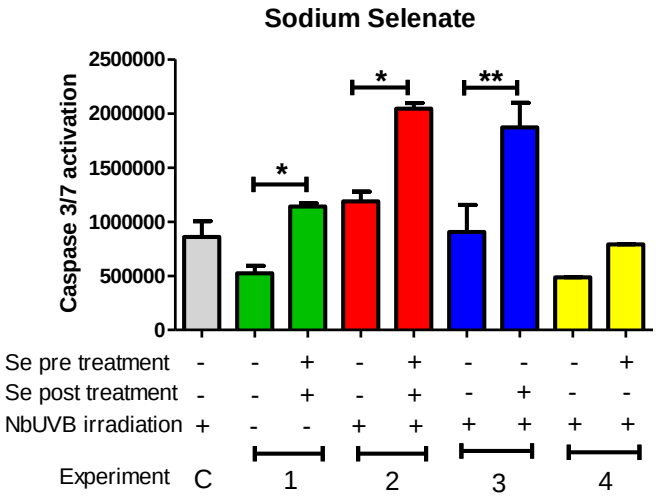


Figure 2. Pre and post Sodium Selenate treatment on NbUVB caspase 3/7 activation
HaCaTs were exposed to concentrations of Sodium Selenate and a specific set of conditions as set out in Table 1. The target was to investigate the effect of Selenium supplementation on NbUVB induction of caspase enzymes as measured by the Caspase-Glo 3/7 assay. The data shows the mean ±SEM from 2 repeats. One-way ANOVA with Bonferonni's post-hoc test compared caspase 3/7 activation following irradiation and treatment to an untreated control in each individual experiment. *($p<0.05$), **($p<0.01$).

Pre Magnesium supplementation of HaCaTs also seemed to amplify NbUVB induced caspase activation as seen in Figure 3 below. Again, amplification of NbUVB induced caspase activation has never been recorded before. Further study into the mechanism by which Magnesium effects NbUVB induced apoptosis is needed.

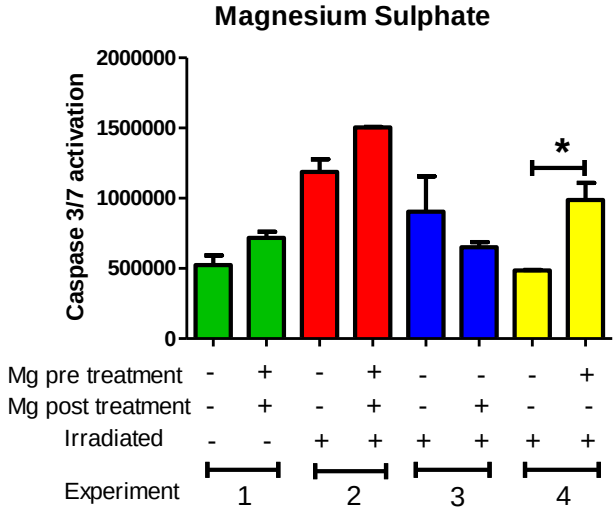


Figure 3. Pre and post Magnesium Sulphate treatment on NbUVB caspase 3/7 activation
HaCaTs were exposed to concentrations of Magnesium Sulphate and a specific set of conditions as set out in Table 1. The target was to investigate the effect of Magnesium supplementation on NbUVB induction of caspase enzymes as measured by the Caspase-Glo 3/7 assay. The data shows the mean ±SEM from 2 repeats. One-way ANOVA with Bonferonni's post-hoc test compared caspase 3/7 activation following irradiation and treatment to an untreated control in each individual experiment.*($p<0.05$).

Clinical results from the PROPAP study suggest that a low Selenium and Magnesium at baseline could mean a patient is less likely to achieve high levels of clearance, as shown in Figure 4, which are novel to the field. These results correspond well with the laboratory results adding to the argument that supplementation with Selenium and Magnesium during a course of NbUVB phototherapy may improve clearance rates.

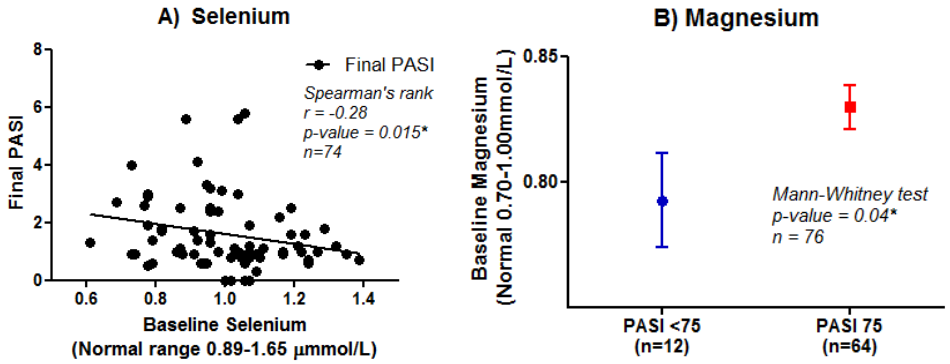


Figure 4. Low baseline Selenium (A) and Magnesium (B) reduce clearance rates
The Spearman's rank correlation analysis and the Mann-Whitney test investigate how baseline levels of Selenium and Magnesium influenced clearance rates following NbUVB phototherapy. To be included in these studies a patient had to have at least six weeks of NbUVB and a record of the baseline Magnesium or Selenium before NbUVB phototherapy. The data on the Magnesium graph represents the mean ±SEM. *(p<0.05).

The significant chi-squared test in Figure 5 establishes that only 23% (n=5) of patients who had a C-reactive protein (CRP) >5mg/L at baseline achieved PASI 90 compared to the 49% (n=34) who had a CRP of <5mg/L at baseline. CRP has been previously found to influence remission length before but never clearance [18, 19].

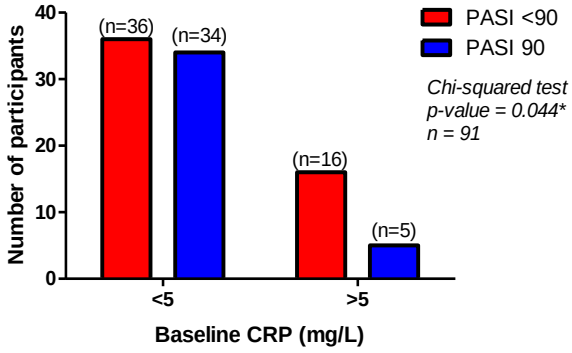


Figure 5. Baseline CRP and PASI 90
The chi-squared test in this figure analyses whether patients who had a high baseline CRP had a significantly different chance of achieving PASI 90. To be included in this study a patient had to have at least six weeks of NbUVB phototherapy and a baseline CRP had to be recorded. *(p<0.05).

Early Identification of poor responders to phototherapy would allow alternative treatments to be selected. Different time points were investigated throughout the PROPAP study to investigate whether a specific week during phototherapy predicts final PASI improvement. Figure 6A and 6B shows that only 46% (n=7) and 7% (n=1) of those who do not achieve PASI 50 after four weeks of phototherapy go on to achieve PASI 75 and PASI 90, respectively. This is an unprecedented result and has never been seen before.

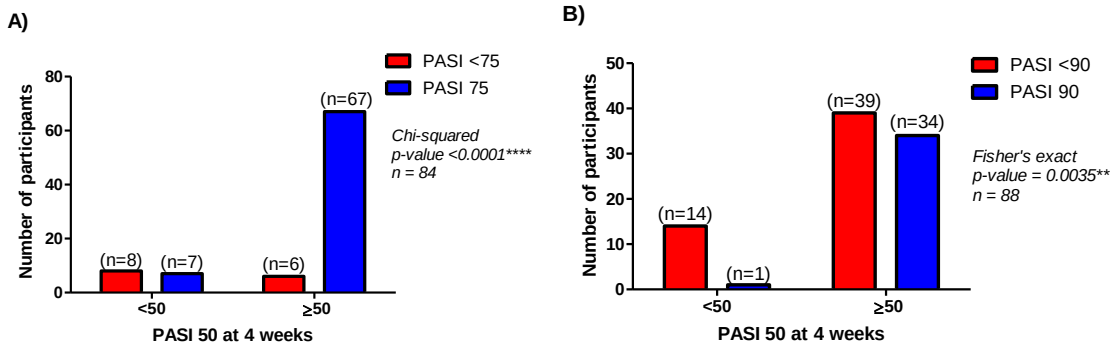


Figure 6. Four week PASI during a course of NbUVB phototherapy predicting PASI 75 (A) and PASI 90 (B) Chi-squared and Fisher's exact tests were used to research whether a patient's four week PASI improvement level could predict PASI 75 (A) and PASI 90 (B). To be included in this analysis a patient had to have a minimum of six weeks of NbUVB phototherapy. ** ($p<0.01$), **** ($p<0.0001$).

Figure 7 demonstrates the significant positive Spearman's rank correlation coefficient between age and remission following phototherapy. This novel result suggests that the older a patient begins NbUVB phototherapy, the longer their remission length. The effects of ageing on the immune system manifest on multiple levels including reduction of T lymphocyte proliferation which play a major role in cytokine production and plaque formation and may explain the result in Figure 8 [20, 21].

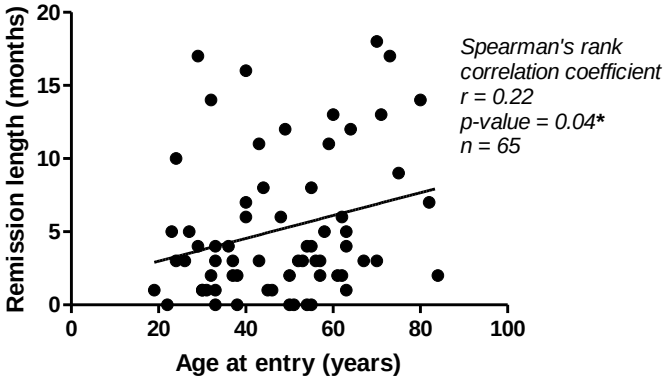


Figure 7. Age at entry and remission length
The Spearman's rank correlation test was used to correlate age against remission length. To be included in this analysis a patient had to have a minimum of six weeks of NbUVB phototherapy. Patients were included even if they had not yet relapsed following treatment but were excluded if they had been lost during the follow up process. *($p<0.05$).

Conclusion

The laboratory results of this project are novel, however, the use of HaCaTs hampers the significance of the results since psoriatic keratinocytes have been found to behave differently from HaCaTs. Further confirmation of the results is needed in follow-up studies with psoriatic keratinocytes. Many novel biomarkers for predicting clearance and remission length were identified in this research project however the PROPAP study was a pilot study that was never powered to predict individual biomarkers. Recruitment is needed to sufficiently power the PROPAP analysis.

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