

# Anti-inflammatory effects of sunscreens – wonder or science?

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## Introduction

The suppression of sun-induced erythema by addition of anti-inflammatory “excipients” has been a controversial issue with the attractiveness of this benefit claim countered by questions of ethics. Some formulators of sunscreens have intentionally incorporated ingredients with anti-inflammatory properties into products in the belief that this incorporation will enhance the Sun Protection Factor (SPF) and/or act to reduce sun induced inflammatory skin responses. Ingredients used with this objective include Ammonium glycyrrhizinate (1, 2), Chamomile extracts, aloe vera, and essential oils such as Tea tree oil. Coutreau et al (3) have identified the potential anti-inflammatory activity of  $\alpha$  bisabolol, allantoin and 18- $\beta$ -glycyrrhetic acid based emulsions.

Recently, Coutreau et al also reported (4) that anti-inflammatory activity, measured as mouse ear oedema, was apparent in 13 of 21 actives tested at up to their maximum (E.U.) permitted concentration, suggesting that this may impact on SPF, resulting in an artificially higher *In vivo* value. However, these findings are based on *In vitro* SPF testing and with the anti-inflammatory activity

measured using the mouse ear model, rather than erythema of human skin. In March 2013, Sayer (5) submitted a Citizens Petition to the FDA, providing evidence of such activity and proposing the removal of 5 sunscreen actives (from the FDA already depleted list of approved chemicals).

As none of these reports were based on experimental design equivalent to an internationally recognized SPF full panel test protocol (6), we set out an experimental schedule in order to investigate this potential, based on a combination of *in vivo* SPF and erythema regression methodology and utilizing a high SPF commercial formulation.

We firstly conducted an experiment, based on traditional *In vivo* SPF in order to measure the impact of a commercial SPF 100 formulation (7), which contained a high level of sunscreen actives (39%). We utilized the same SPF 100 product in our experimentation as was provided in the Sayer evidence to FDA. This methodology is similar to that reported by Kolbe et al (8). In order to further qualify validate the experimental design, a subsequent experiment was completed. For this, a therapeutic anti-inflammatory active was added as a positive control and compared with a reference sunscreen without this

addition.

## Background

Actives used in sunscreens include a number cinnamates, benzophenones and salicylates (9). Chemicals with similar structures to these chemical families of actives are recognized for their anaesthetic activities. One active, Trolamine salicylate, is also sold as an active in local anaesthetic creams (10). Salicylic acid is a non-steroidal anti-inflammatory (NSAID).

Salicylates such as methyl salicylate (Oil of Wintergreen) are traditionally used as a counter irritant for soothing inflamed skin. Anti-inflammatory activity of cinnamic acid esters has been reported (11).

## Experimental

In order the potential for the commercial formulation (7), to express greater than expected SPF activity, an *In vivo* experimental protocol was devised which incorporated all of the principles of the ISO SPF test (6) and, as well included more precise measurement of UV light induced erythema development. The objective of this study was to determine the efficacy of a test product in its erythema regression potential when topically applied product to the skin of human panelists following

stimulation of mild irritation by light.

Ten test subjects were enrolled for each of study. At recruitment, test subjects were assessed for qualification according to the ISO inclusion criteria for the study. Each completed and signed an informed consent. The study was conducted according to Declaration of Helsinki guidelines. Individual test subjects were aged between 18 and 65 and were of Fitzpatrick Skin Type I to III (ITA values > 20°). Race was Caucasian or Asian (first or second generation in Australia).

A Solar Simulator was used to induce a mild erythema (Minimal Erythema Dose “MED”) on designated areas of the back of test subjects. The experimental principles were consistent with those normally applied for the measurement of sun protection factor (SPF) and as described in ISO 24444. The response post treatment with the product was observed and compared with positive and negative controls as described below. Left to right randomization was applied.

## Determination of Anti-inflammatory effect of Commercial SPF 100 product

Site 1: Unprotected exposure – to serve as negative control.

Site 2: Unprotected Exposure followed by SPF 100 – to assess anti-inflammatory potential by sunscreen active.

Site 3: ISO P2 Reference Sunscreen then exposure as a control for Site 4.

Site 4: ISO P2 Reference Sunscreen then exposure, then remove P2 and apply SPF 100 imitated sunscreen use and erythema development just beyond protection level, followed by additional sunscreen imitating re- application.

The progression/ regression of erythema was evaluated at 1, 5 and 18 days, with the 4 hr post irradiation colour development being used as the baseline.

One and the same assessor was used for each individual instrumental measurement throughout the study in order to rule out variations occurring from different gradings by different assessors

The Minimum Erythema Dose (MED) necessary to induce a mild erythema response in each test participant was determined prior to commencement of the test. The target treatment area was identified for each test subject. The areas were mapped for future reference. On the day of the study, the product was applied to the skin of the target sites at a rate of 2mg/cm<sup>2</sup> according to the schedule set out in Section 2.

Test sites were delineated for each challenge patch series of UV light exposures. Reference SPF 15 sunscreen was applied to sites 3 and 4 at a rate of 2mg/cm<sup>2</sup>. A delay of 15 minutes was then completed. A Solar Simulator Model 16S was used to apply a series of irradiation doses over all selected sites.

Site 1: For the unprotected sites, this was a series between 0.5 and 2 MED's.

Site 2: Immediately after irradiation the clinician applied the SPF 100 test product to the previously exposed skin at a rate of 2mg/cm<sup>2</sup>. The product was left in place up to the 4 hr timepoint.

Site 3. The exposures were SPF 8 (i.e. 0.5 X SPF 16), SPF 16, SPF 19 and SPF 23. Following the irradiation, the SPF 15 product was removed by gentle wiping.

Site 4: For the SPF 15 protected sites ,the exposures were SPF 8, SPF 16, SPF 19 and SPF 23. Following the irradiation, the SPF 15 product was removed by gentle wiping and then the SPF 100 product applied. The SPF 100 product was left in place up to the 4 hr time point.

Panelists were required to remain in the laboratory area for a period of 4 hrs. At 4 hrs, visual, photographic and spectrophotometric measurements were made of all exposed spots for each test site.

Test subjects were instructed to return to the test lab at 1, 5 and 18 days for further measurement (t=2, 3 & 4). At all visits, test subjects were instructed to remain passively seated in a controlled environment with the test area of the back exposed until their skin was considered to have stabilized. Visual assessments were made and the erythema scored on by an expert, according to the Standard Scalar Ratings.

[0] = 0 = no erythema present

[--/+ ] = 1 = minimal faint (light pink), uniform or spotty erythema

[-/+ ] = 2 = mild erythema, pink uniform erythema covering most of contact site

[-/+ + ] = 3 = moderate erythema, pink/red erythema visibly uniform in entire contact area.

[+ ] = 4 = marked bright red erythema

[+ + ] = 5 = severe deep red erythema.

The visual assessment of the erythema intensity was scored for each set of exposures. The score for the SPF 100 treated spot was subtracted from the untreated in each case.

## Results

Results for visual assessments are shown in Fig. 1 and Fig. 2 below.

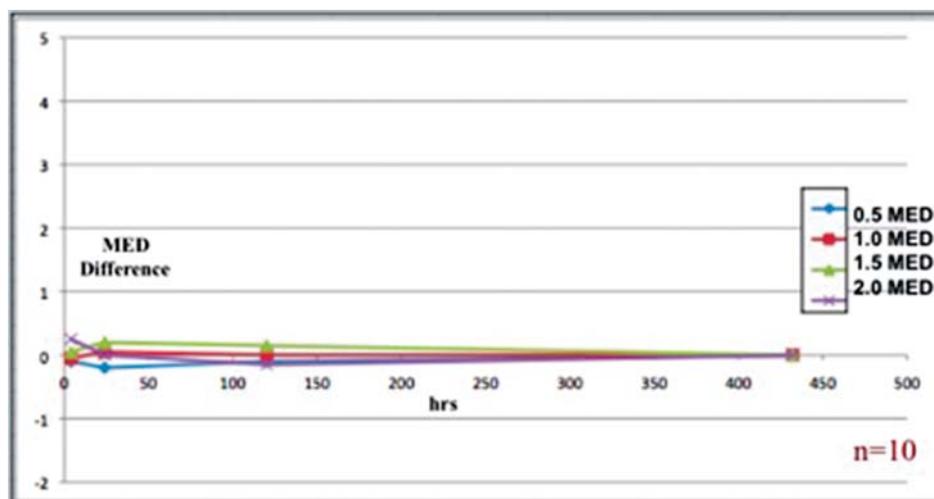


Fig 1. MED Difference SPF 100 Treated vis Untreated – Visually Assessed

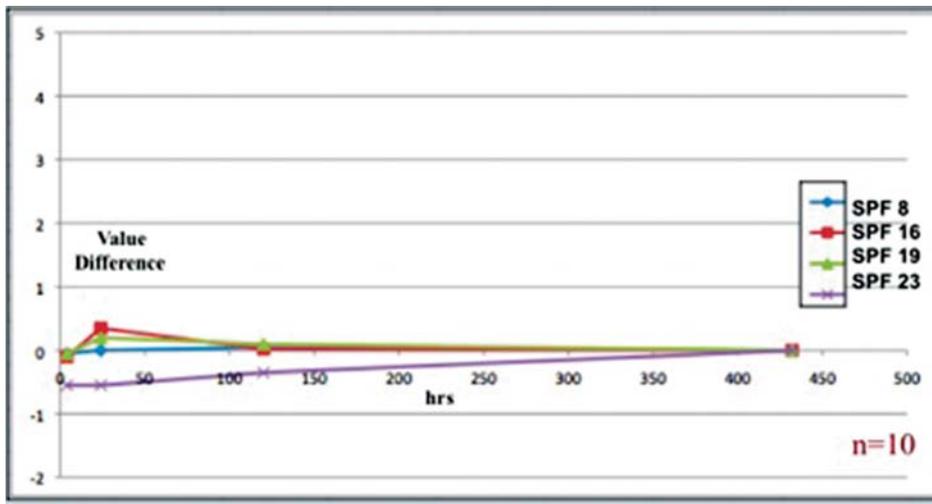


Fig 2. Value Difference SPF 100 Treated applied over P2 SPF 16 Sunscreen vis P2 untreated – Visual Assessment.

Colour measurements were taken. The  $L^*a^*b^*$  colour space values were recorded. A Minolta Chromometer hand held spectrophotometer was utilized to determine colour values and changes versus the corresponding negative control (untreated) area.

Changes in skin condition were reported with the objective of quantifiably measuring Skin Colour: Tristimulus light values – shift in each component of  $L^*a^*b^*$  values. – pigmentation colour reduction against the background indicates skin lightening. The L value gives the measurement of lightening of the spot, and the  $b^*$  value is the yellow –blue component. The most relevant value is the  $\Delta a^*$ , as this is the red (erythema) component. Specular Component Included (SCI) values are used for calculation of changes. Visually observations of the presence of any erythema or more severe response was made according to the Standard Scalar Ratings.

Similarly, the measured  $a^*$  value from the  $L^*a^*b^*$  instrumental readings were scored for each set of exposures. The score for the SPF 100 treated spot was subtracted from the untreated in both the MED and P2 exposure series. Results are set out in Fig 3 & 4.

## Results

With the exception of the highest exposure series over the P2 i.e. to SPF 23, the mean difference was not significant. In this final set, which was

overexposure over P2 sunscreen and then SPF 100 applied post radiation, the erythema was observed to be slightly less for the SPF 100 treated spots.

The Mean difference did not exceed an  $a^*$  value of 1 at any exposure time for either the MED or P2 exposure series.

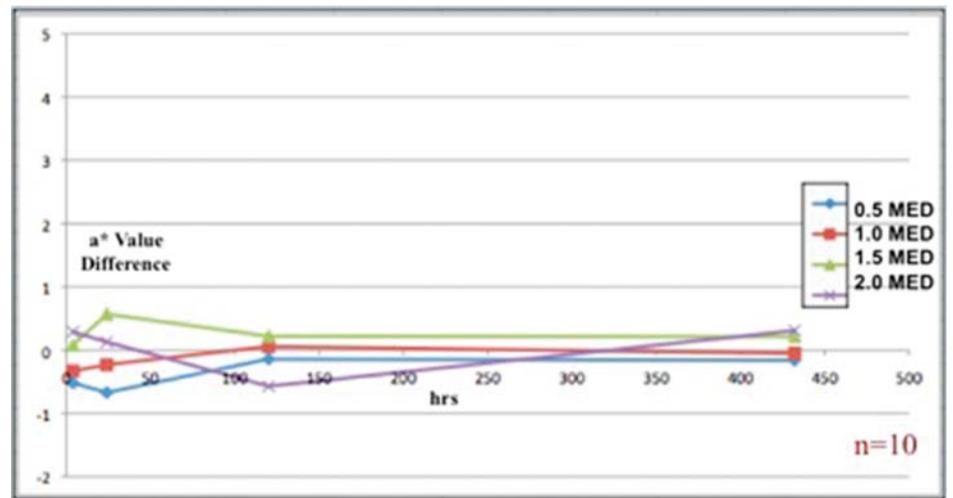


Fig 3.  $a^*$  Value Difference SPF 100 Treated vis Untreated – Instrumentally Measured

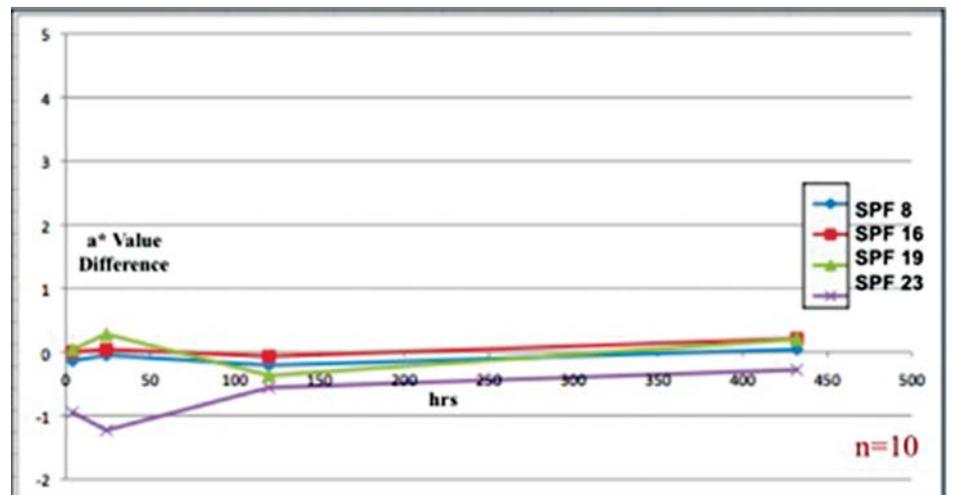


Fig 4. Value Difference SPF 100 Treated applied over P2 SPF 16 Sunscreen vis P2 untreated – Visual Assessment.

This represents not more than a minimal change in the reduction in erythema response.

## Confirmation of Potential Activity

Can we expect a mainstream therapeutic active to perform effectively if intentionally added to a sunscreen? In order to determine this, we conducted a further experiment in which the active hydrocortisone 17 butyrate was added to the same P2 sunscreen base as was used in the previous experiment. A further 10 subjects were enrolled and the protocol for applications was as follows.

Site 1: Unprotected exposure series from 0.5 to 2 MED's with Solar Simulator irradiation – to serve as base line control – exactly as per the ISO 24444 SPF Protocol.

Site 2: Unprotected Exposure as above, followed by application of P2

SPF 16 sunscreen to which 0.1% of hydrocortisone 17-butyrate was added. This post irradiation application was then left in place.

A comparative result is shown in Fig 5 at right.

Site 3: ISO P2 (SPF 16) Reference Sunscreen, then Solar simulator exposure in the range of 0.5, 1, 1.18 and 1.44 times expected SPF of 16. Sunscreen was then left in place.

Site 4: ISO P2 Reference Sunscreen including 0.1% Hydrocortisone 17 butyrate then exposure as above. Sunscreen was left in place.

The erythema for the MED series was also evaluated visually according to the ISO methodology. Values for each test subject, all unchanged, are shown in Fig 7.

As the SPF exposure series was not conducted on the same scale as the the MED series, due to risk of high overexposure, it was not relevant to assess this visually.

Finally, activity of both test lotions was confirmed by HPLC following the completion of the study.

## Discussion

An SPF 100 product, with 39% of actives, as tested in this experiment, is beyond the usual high end of the concentration of sunscreen actives typical for a primary sunscreen. For most markets, the SPF limit is half of this – SPF 50 or SPF 50 +, i.e around 60. The proportionality of actives of concern is thus likely to be around half of that used in this challenge. The SPF 15 product used in our experimentation has a long history of use in SPF test monographs as a reference control sunscreen.

It is possibly unrealistic to expect that sunscreen actives will express anti-inflammatory activity at a level above mainstream drugs, which have been more targeted for this therapeutic indication. Both of our experiments support the conclusion that such a secondary activity is at best limited.

The mechanism of erythema development is complex and visible skin response develops over an extended

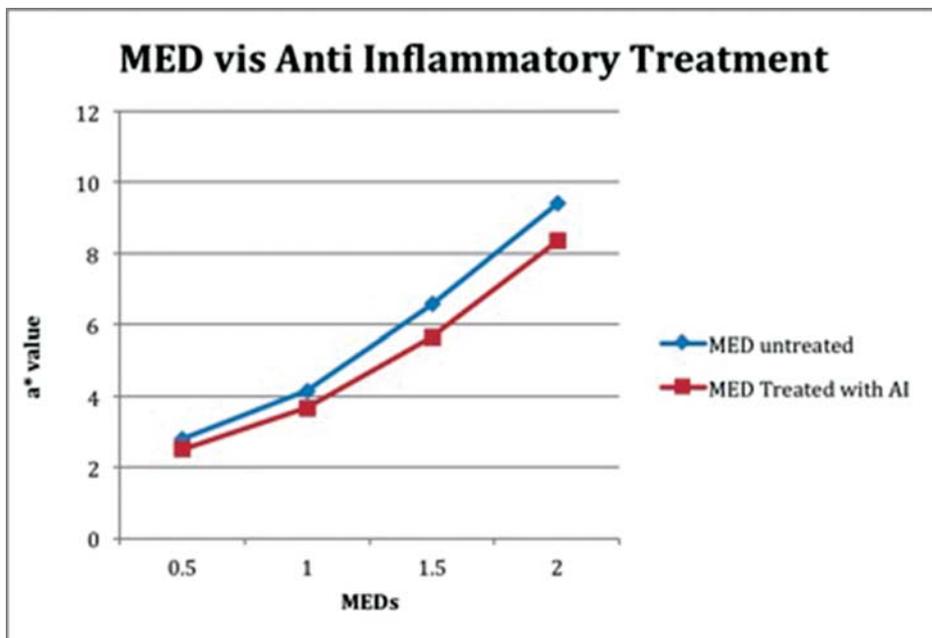


Fig 5. a\* value change in MED measured with and without post-treatment with Hydrocortisone anti-inflammatory (0.1%).

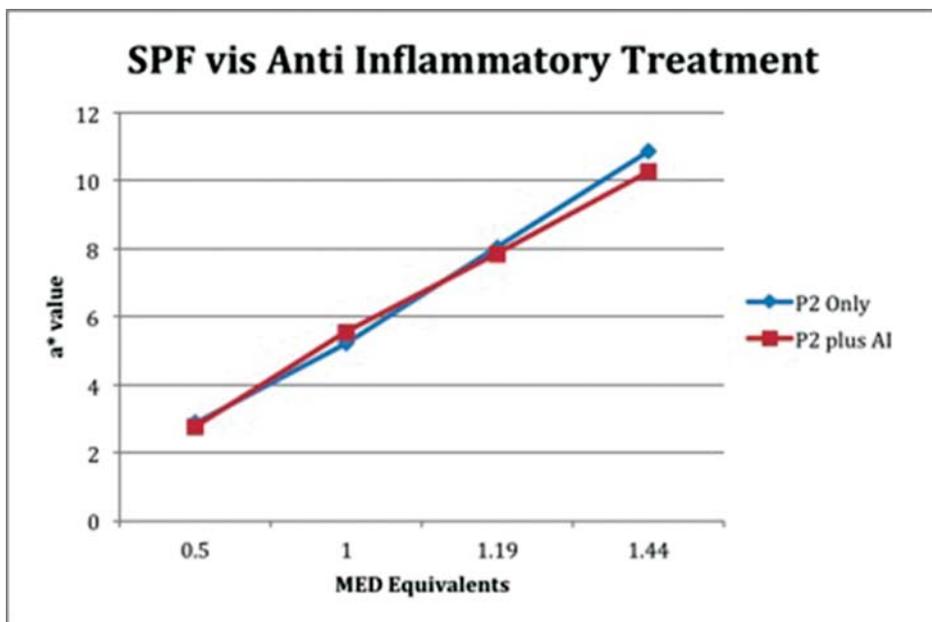


Fig 6. SPF 16 sunscreen comparative a\* value change vis added Hydrocortisone.

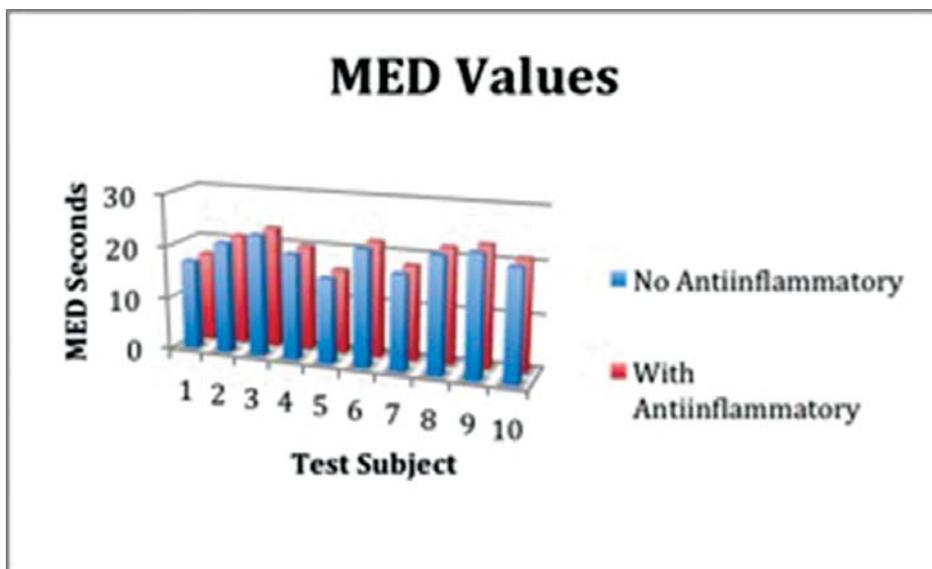


Fig 7 MED values for test subject – (visually assessed with and without cortico-steroid)



Fig 8. a\* value progression single value steps – 5 to 9

period, typically peaking at 24 hrs (12). Topical anti-inflammatory drugs have an activity, which is duration limited and reapplication is normal and indicated on commercial over the counter products such as Diclofenac. Typically, reapplication is required after 4 to 6 hrs. Additionally, a higher application rate is indicated – 4 mg/sq cm or more for efficacy. The measurement of *In vitro* SPF is not reliable indicator of *In vivo* performance (13). The experimental design described above was based on the ISO *In vivo* method now universal as a designator of in use SPF and reflects the start of the art for sunscreen product performance qualification. At this time, an equivalent *In vitro* SPF methodology does not exist.

Visual assessment of erythema, as used in the ISO SPF test, does not discriminate change in erythema as accurately as the instrumental spectrophotographic method. A change of one a\* unit is almost not perceivable. Fig 8 shows this change in an example series against a tanned background, in single a\* value steps.

## Conclusion

There was no significant difference between erythematous responses when measured according to ISO 24444 Procedure. Similarly, there was no significant difference between erythematous responses measured instrumentally.

Moderation of erythematous response by the SPF 100 sunscreen containing high levels (39%) of actives was not observed.

Evidence of anti-inflammatory activity of sunscreen actives was not apparent when a commercial very high SPF product was tested under standard Internationally accepted test conditions intended to reflect normal use.

When a mainstream therapeutic anti-inflammatory active, was incorporated into a standard SPF 15 sunscreen, there was an a\* value change of 0.48 at 1 x MED exposure, increasing to 1.1 at

2 x MEDs. The impact of this effect on SPF 16 protected skin was at best incremental, and extrapolation from the percentage decrease in erythema could only, at best be additive.

The changes could not be observed visually when assessed according to the ISO 24444 SPF protocol, but could only be shown with instrumental measurement with a colour computing spectrophotometer.

Significant difference could not be confirmed for any of the test series for treated vis untreated challenges.

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