Title:
Sunscreen and adhesive provide 24 hour durable photoprotection in human and mouse skin

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Abstract
The objective of this study was to determine whether sunscreen bonded to skin with an adhesive prevents the development of erythema in human skin and skin tumors in mice exposed to ultraviolet light, 24 hours after the application of sunscreen and adhesive. A second objective was to detect whether toxicity was observed in organs of mice, whose skin was treated with sunscreen and adhesive.

Bullfrog SPF 50 Water Armor Quik Dry Sunblock was applied to skin and covered with an adhesive and allowed to dry. 24 hours after application, the treated areas were exposed to UVB light. Human skin was observed 24 hours later for erythema. Mouse skin was harvested after 21 weeks of exposure 6 days per week to UVB light. Skin tumors were counted in control and sunscreen-adhesive (SS/Adh) groups.

The sunscreen and adhesive prevented erythema of human skin when exposed to UVB light 24 hours after sunscreen application. The SPF was at least 10, when measured 24 hours after sunscreen and adhesive application.

Mice developed more carcinomas in situ and total carcinomas than SS/Adh treated mice at the 95% confidence interval and P<0.05. No organ toxicity was observed in lungs, liver or kidneys of the SS/Adh treated mice.

The application of an adhesive to sunscreen on skin provides 24 hour photoprotection against skin erythema in human skin, and protection from carcinoma formation in mice without organ toxicity. Additional studies are needed to determine the exact SPF, 24 hours after application, in human subjects to determine the extent of durable photoprotection. Additional studies are needed to determine safety of adhesive application to human skin.
**Introduction**

The durability of sunscreen photoprotection after application is less than one day. Most sunscreens provide less than half their labeled SPF several hours after application in typical use. Most individuals apply too little sunscreen and do not reapply it. Reapplication of sunscreen every 2 hours is recommended. Sunscreens can be removed by rubbing; and water exposure enhances removal.

Photocarcinogenisis is a cumulative process related to total dose. If a more durable sunscreen could be applied prior to ultraviolet radiation (UVR) exposure, more of the total daily sun UVR dose could be reduced.

The purpose of the study was to determine whether sunscreen bonded to skin with an adhesive prevents the development of erythema in human testing and skin tumors in mice exposed to ultraviolet light 24 hours after the application of sunscreen and adhesive (SS/Adh). The second purpose of the study was to determine whether the adhesive sunscreen combination was toxic to mouse skin, lungs, liver or kidneys.

Skh:2 hairless mice exposed to UVB light develop skin tumors within 20 weeks. Sunscreens applied immediately before UVB exposure, block the development of skin tumors in this mouse model. Previous studies have only tested the sunscreen protective effect on mice exposed within 1 hour after application of the sunscreen. All existing sunscreens are only rated for several hours of sun protection and up to 80 min. of water resistance. The water resistance rating means that more than half of the sites tested have the same level of UVB protection as before water exposure. One study of sunscreen durability in humans showed that sunscreen maintained the same SPF on the skin for 8 hours if the site was protected from physical removal of the sunscreen and not exposed to water.

Loss of sunscreen protection with typical use, may occur because of removal by rubbing and washing of the skin. There is a need for a sunscreen that can last for 24 hours or longer on the skin with normal rubbing of the site and water exposure. Some occupations do not allow sufficient time for workers to reapply sunscreen every few hours as recommended. Many individuals chose not to reapply sunscreen. If a safe and effective SS/Adh can be developed, workers and individuals may choose to have such a protective coating applied by a sunscreen specialist at their place of work or recreation.

**Methods**

**Human tests**

The sunscreen used in this study was Bull Frog SPF 50 Water Armor Quik Dry Sunblock (SS) obtained from a local pharmacy. The adhesive, called Inland Marine Sealant, was obtained from Inland Marine USA.

All human tests were performed on the author, who has blue eyes, brown hair, and a Fitzpatrick skin phototype 2. Minimal erythematous dose (MED) on human skin was determined by exposing 1 cm² squares to UVB light. 24 hours after light exposure, the test sites were identified with a template and erythema was noted. MED was determined to be 1.5 min, which corresponds to 19 mJ/cm². The MED of skin 24 hours after application of sunscreen was also determined. The test sites were coated with the sunscreen until wet. The test sites were covered with clothing and normal activities were performed for 24 hours before testing.

For testing of the effect of adhesive over sunscreen, test sites were treated with either no sunscreen (-SS), sunscreen (SS), or sunscreen with adhesive (SS/Adh). The skin was marked with permanent marker to indicate the test sites and orientation of a template. After sunscreen application, the skin was
allowed to dry for 10 minutes. The adhesive was then applied over the sunscreen with a brush and
dried for 10 min. with a hair dryer. The tests sites were covered with regular clothing for 24 hours prior
to UVB exposure. Regular activities, including 15 min of aerobic activity, occurred. Prior to exposure,
the test sites were showered with water, and patted dry with a towel. A paper template with 1 cm$^2$
holes was taped to the skin over the test sites. The skin was then exposed to UVB light. Irradiation
was conducted with 4 UBL FSX72T12-UVB-HO bulbs. The output was measured with a radiometer,
which measures $\lambda = 290$ to 320 nm. The UVB exposure was 38 mJ/cm$^2$ (3min.) for sites with no
sunscreen and sunscreen without adhesive. Sites exposed to sunscreen and adhesive were exposed to
214 mJ/cm$^2$ (17 min.) Photography was performed 24 hours after UVB exposure.

Mouse tests
Ten female Skh:1 hairless mice 4 weeks of age; were purchased from Charles River Laboratories and
housed 5 mice per wire cage with rodent adsorbent bedding/litter in a temperature-controlled (24°C)
room. Identifying marks were punched on their ears. All mice were fed a nutritionally adequate diet of
mice and rat chow (Kaylee Forti-Diet Pro Health Mouse and Rat Food) and water from rodent
dispensing bottles. Diets were stored refrigerated, and food replenished every two days. Wood chews
and cardboard tubes were provided weekly for dental and psychological health.
The weights of the mice were measured. The mice were randomized to 2 separate treatment groups, 5
mice per group. One group, the control group, had no sunscreen applied. The other group had
sunscreen and adhesive applied.

Three days a week, Monday, Wednesday and Friday, before UV exposure, the dorsal surfaces of the
mice were painted with Bull Frog SPF 50 Water Armor Quik Dry Sunblock and a layer of the adhesive
from Inland Marine, and allowed to dry for 15 minutes. Both groups were exposed daily, six days per
week, Monday through Saturday, to UVB light, for 21 weeks. The sunscreen and adhesive treated
groups were therefore exposed 3 days a week, Tuesday, Thursday, and Saturday without additional
sunscreen application. Each group of mice was placed in a separate plastic tub and exposed to
ultraviolet light at the same time.

Ultraviolet irradiation
Irradiation was conducted with 4 UBL FSX72T12-UVB-HO bulbs. The output was measured with a
radiometer, which measures $\lambda = 290$ to 320 nm. The mice were irradiated with the lights suspended 40
cm above their backs in tubs. The bulbs provided a homogeneous field of irradiation to both tubs of
mice. The exposure time was 1 minute per session, 6 times a week. The dose of UVB light per
treatment was 12.6 mJ/cm$^2$. This irradiation was continued for 21 weeks. This dose of UV radiation
has been shown to induce skin cancer in this mouse model.

All mice were examined weekly to determine the degree of short-term sun damage, specifically
erythema.

Necropsy
At the end of the study, the mice were euthanized with isoflurane inhalation. After euthanasia, two skin
biopsies were performed on each tumor bearing area and one skin biopsy of clinically tumor free dorsal
back skin. Biopsies of the lungs, livers, and kidneys were also performed. Standard slides for
microscopy were stained with hemotoxylin and eosin. Histologic examination was performed by a
dermatopathologist, at Cole Diagnostics, who did not know the treatment group of each specimen.

Statistical analysis
The number of carcinomas per animal was compared between treatment groups by the two sided,
Results

Human tests on abdomen and buttock skin exposed with and without SS/Adh demonstrated photoprotection by the sunscreen and adhesive treatment (SS/Adh), 1 day after application.

Human tests on abdomen and buttock skin exposed with and without SS/Adh are shown in Figure 1 in the power point presentation.

The skin which had SS/Adh applied 24 hours before 17 min of UVB exposure had little erythema compared to the control sites of sunscreen without adhesive and no sunscreen for 3 min. of exposure. Since the MED was 1.5 min. (18mJ/cm^2), the SPF for the SS/Adh was at least 10. The exact SPF of the SS/Adh was not determined. The sunscreen alone, 24 hours after application, had an SPF of 2.

Figure 2 shows the appearance of MED test sites.

Mice tolerated treatments and gained weight equally in both groups. One mouse died in the control group from unknown causes at week four of the experiment. The mice attempted to groom off the sunscreen within 20 minutes of sunscreen application. All four of the mice in the control group (no sunscreen) developed at least one skin carcinoma. These were 4 squamous carcinomas and 3 carcinomas in situ in the control group. The photo of a typical mouse with tumors is shown in Figure 3. The histology of a typical carcinoma is shown in Figure 4.

Table 1 shows the number of squamous carcinomas, squamous carcinomas in situ and total carcinomas. There were significantly fewer carcinomas and carcinomas in situ in the SS/Adh group.

Table 1 Carcinomas in mice groups.

<table>
<thead>
<tr>
<th>Carcinomas</th>
<th>No sunscreen</th>
<th>Sunscreen-adhesive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinomas</td>
<td>4</td>
<td>0</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>3</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Total carcinomas</td>
<td>7</td>
<td>1</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

None of the mice in the SS/Adh developed invasive carcinomas, but did develop one carcinoma in situ. The SS/Adh group had significantly fewer skin tumors than the control group. The non-tumor bearing skin on both the control and SS/Adh mice clinically showed no erythema. The typical benign
appearance of the histology of skin from the SS/Adh mice is shown in Figure 5.

Other organs showed little histological difference. Two of the control mice had a lymphocytic perivascular infiltrate near the collecting tubules in the kidneys. One of the SS/Adh mice had a similar infiltrate. The kidneys otherwise appeared normal in both groups. The lungs and livers had normal histology in both groups.

**Conclusion**

Sunscreen with adhesive prevented the development of skin erythema in a human and skin tumors in mice from UVB light exposure 24 hours after sunscreen application. The sunscreen and adhesive prevented erythema of human skin when exposed to UVB light 24 hours after sunscreen application. The SPF was at least 10 after 24 hours, but SPF was not specifically determined. Human testing on the author show that this combination SS/Adh provides durable, water resistant protection from UVB light. The adhesive extended the SPF from 2 to a value greater than 10. No sunscreen formulation has demonstrated this duration of UVB protection.

The non-sunscreen treated control mice developed significantly more total carcinomas than the sunscreen and adhesive treated mice. These were squamous carcinomas as was found in other experiments.\(^6\)

The sunscreen treated mice received half of their ultraviolet light exposure 1 day after the application sunscreen. This suggests that the sunscreen may have had a protective effect that lasted for 24 hours. Another study, with more mice, and for longer duration, comparing the effect of UVB exposure only one day after application of the SS/Adh combination to a group with no sunscreen would help to determine whether the sunscreen stayed on mice for 24 hours. Another control group of mice treated with sunscreen, but no adhesive would also help to determine the benefit from the adhesive.

The duration of the current study was chosen based on a prior study which showed that mice exposed to UVB, 3 days per week, induced tumors in half the mice in 20 weeks.\(^7\) Since a study of UVB exposure only 24 hours after sunscreen application could only have UVB exposures 3 days a week, it would have to be a longer study lasting 30-40 weeks, in order to expect over half the control mice to develop carcinomas.

Substances have been included in sunscreen formulations to increase durability, the resistance to removal by wear; and substantivity, the ability to bind to the skin and resist removal by water, swimming, or sweating. These substances include film forming polymers, dimethicone crosspolymers or other copolymers. Oil and wax containing vehicles are very substantive to skin as most sunscreen active ingredients are oil soluble. The sunscreen used in this study was chosen partly because it has a low oil content and dries quickly. The adhesive may not have bonded as well to a sunscreen with a high oil content.

The application of adhesive over sunscreen in this study, increases durability and substantivity. The mechanism by which this adhesive holds the sunscreen active ingredients to the skin is unknown, but may involve covalent bonds to skin molecules. Further research could determine the chemical reactions which form the bonds and which formulations provide optimal UVR photoprotection. Further research could be done to measure UVA photoprotection by SS/Adh in an in vitro model.

None of the animals which had the sunscreen and adhesive had any histologic abnormality in their
lungs, livers or kidneys. Since the skin on the mice treated with sunscreen and adhesive never
developed erythema, there was no sunburn or irritation in the SS/Adh group. This suggests that the
adhesive is not irritating to mouse skin when applied daily. The MSDS sheet lists irritancy to skin and
eyes as a potential effect of the adhesive and directs users to avoid skin and eye contact. The adhesive
used in this study contains 5 ppm vinylidine chloride, a carcinogen when inhaled. The mice in this
study ingested large amounts of the adhesive, because they were observed grooming themselves shortly
after application of the adhesive. Vinylidine chloride is metabolized in the liver and excreted in the
kidneys and as carbon dioxide via the lungs. Histology of organs revealed no toxic effect from the
adhesive. No irritancy on human skin was noted. Vinylidine chloride has not been shown to be toxic
when ingested at the concentration in the adhesive. Perhaps this adhesive can be safely used in the
future. A larger long term study should be performed and with mice and other animal species to test for
safety and skin carcinogenicity before this adhesive could be recommended for general use.

Various adhesives were tried on skin prior to this study. Common household glues are not water
resistant or are too rigid. Some adhesives are too irritating to skin. Methyl- and octyl-methacrylate
adhesives form films which are too rigid on the skin. The adhesive selected for this study is intended to
repair holes in inflatable boats. It is flexible and has an acceptable texture on skin. The adhesive is not
apparent when dried on skin. Another sunscreen mineral makeup from L'Oreal was tested with
adhesive and found to be effective 24 hours after application at blocking UVB induced erythema, but
has an opaque appearance on the skin and can be rubbed off more easily than Bullfrog spray sunscreen.

Other sunscreen active ingredients may have even greater durability on the skin. SUNSPHERES
(Rohn and Haas, Philadelphia) are made from styrene/acrylate copolymers formed into hollow
spheres. These spheres cause light to scatter sideways through a thicker level of UVR filters in a
sunscreen formulation, rather than allowing penetration of UVR straight down to the skin surface.
Theoretically, these spheres could be bonded to skin with adhesive.

There may be instances where the risk and inconvenience of using this adhesive with sunscreen may
outweigh the risk of sunburn. Such instances might occur with the military, or with persons who must
work outdoors in the summer and are unable to reapply sunscreen. Men especially would benefit, as
they are less likely to apply sunscreen. In such instances, a trained person might apply the sunscreen
and adhesive with a brush and dry the product with a hair dryer.

In summary, this study suggests that the application of an adhesive over a spray sunscreen can
significantly increase the duration of sunscreen photoprotection on skin. Additional research on the
safety of the adhesive used in this study needs to be performed.

References

748-758
http://www.cancer.org/Cancer/CancerCauses/SunandUVExposure/SkinCancerPreventionandEarlyDete
3. Forbes PD, Davies RE, Sambuco CP, Urbach F Inhibition of Ultraviolet Radiation-Induced skin
tumors in Hairless Mice by Topical application of the sunscreen 2-ethyl hexyl-p methoxycinnamate, J