

# Salate derivatives found in sunscreens block experimental autoimmune encephalomyelitis in mice

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Contributed by Hector F. DeLuca, June 15, 2017 (sent for review March 22, 2017; reviewed by Margarita Contorna, Michael F. Holick, and Rajiv Kumar)

**UV light suppresses experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS, in mice and may be responsible for the decreased incidence of MS in equatorial regions. To test this concept further, we applied commercially available sunblock preparations to mice before exposing them to UV radiation. Surprisingly, some of the sunblock preparations blocked EAE without UV radiation. Furthermore, various sunblock preparations had variable ability to suppress EAE. By examining the components of the most effective agents, we identified homosalate and octisalate as the components responsible for suppressing EAE. Thus, salates may be useful in stopping the progression of MS, and may provide new insight into mechanisms of controlling autoimmune disease.**

multiple sclerosis | experimental autoimmune encephalomyelitis | sunscreen | homosalate | octisalate

**M**ultiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system, affecting 2.5 million people worldwide (1). In 1974, Agranoff and Goldberg observed that the incidence of MS is inversely related to sun exposure in both hemispheres (2). Because vitamin D is synthesized on UV irradiation of 7-dehydrocholesterol present in skin, Goldberg suggested that increased vitamin D production caused by sunlight exposure may be responsible for the reduction of MS incidence (3). More recent results have made this hypothesis unlikely, however, and instead narrow-band (NBUVB) radiation at 300–315 nm has been shown to suppress experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS characterized by demyelination of the central nervous system as well as infiltration of mononuclear cells (4). This NBUVB produces little vitamin D from 7-dehydrocholesterol because it does not increase blood levels of 25-hydroxycholecalciferol (5).

During the course of our studies, we used commercial sunblock preparations, presumably to prevent the suppression of EAE by NBUVB. Quite unexpectedly, some sunblock preparations themselves completely prevented EAE without UV radiation. We found that commercial sunblock preparations varied widely in their ability to suppress EAE. By examining the components of the preparations, we identified two components as responsible for the suppression of EAE: two esters of salicylic acid, homosalate and octisalate. We report these findings in this paper.

## Results

**Topical Application of Sunscreen (Coppertone Spray) Completely Suppressed EAE Development.** When mice were treated with NBUVB, the average disease severity was dramatically decreased similar to previous studies (Fig. 1*A*). Sunscreen application before NBUVB did not prevent the suppression by NBUVB (Fig. 1*A* and *B*). Surprisingly, topical administration of sunscreen itself completely blocked EAE (Fig. 1*B*).

**Commercial Sunscreens Differ Markedly in Their Ability to Suppress EAE.** Of the six brands of sunscreen tested on EAE (Table 1), four—Hawaiian Tropic, Coppertone Spray, Kiss My Face, and

Blue Lizard—produced significant suppression (Fig. 2*A*). The other two brands—Banana Boat and CoTZ Face—had no effect (Fig. 2*A*). To be sure that the effect of the sunscreen simply did not prevent a total block of all wavelengths, total darkness (–7 to 30 d) was tested and found to have no effect on EAE regardless of when initiated (Fig. 2*B*).

Interestingly, additional testing indicated the need for sunscreen application at the time of immunization (Fig. 3*A*). The suppression of disease by sunscreen was dose-dependent (Fig. 3*B*). Analysis of the numerous components of the sunscreen preparations tested revealed that the effectiveness was exactly correlated with the presence of the salate derivatives homosalate and octisalate. We immediately tested these compounds directly at concentrations found in the sunscreens.

**Homosalate and Octisalate Produced Significant EAE Suppression.** Doses of 30  $\mu$ L homosalate (1.5 g/kg) and 10  $\mu$ L octisalate (0.5 g/kg) were calculated to be the amount delivered by Coppertone Spray sunscreen. This quantity was applied to the mice. Sunscreen spray at 200  $\mu$ L [containing homosalate 15% (vol/vol) and octisalate 5% (vol/vol)] was applied to EAE mice as a positive control. Homosalate only and the combination of homosalate and octisalate dramatically suppressed EAE severity (Fig. 4*B* and Table 2). Octisalate at a dose of 0.5 g/kg produced moderate suppression that did not reach statistical significance (Fig. 4*B* and Table 2). The other two ingredients (avobenzone and oxybenzone) and the combination produced little to no EAE suppression (Fig. 4*A*).

**Homosalate and Octisalate Suppression was Dose-Dependent.** When tested at three different doses (0.5, 1.0, and 1.5 g/kg), both homosalate and octisalate exhibited dose-dependent suppression of EAE (Fig. 5*A*). When homosalate was applied less frequently each day, its effectiveness diminished (Fig. 5*B*).

## Discussion

The finding that sunblock creams themselves can prevent the development of EAE came as a surprise; we had expected to find

### Significance

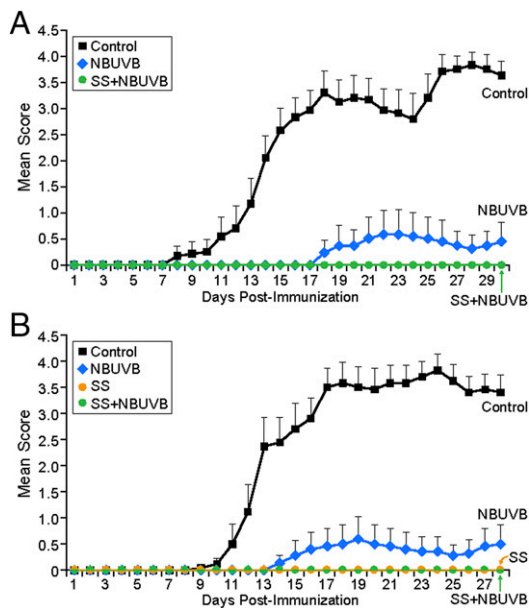
**Multiple sclerosis (MS) is an autoimmune disease that is difficult to manage and for which there is no cure. We have discovered that certain specific sunblock preparations can prevent the development of experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS. Salate esters in the sunblock preparations were found to be responsible for preventing EAE. Continued exploration of this finding may lead to new approaches to the management of symptoms of MS.**

Author contributions: Y.W., S.J.M., L.A.P., and H.F.D. performed research; and Y.W. and H.F.D. wrote the paper.

Reviewers: M.C., The Pennsylvania State University; M.F.H., Boston University; and R.K., Mayo Clinic.

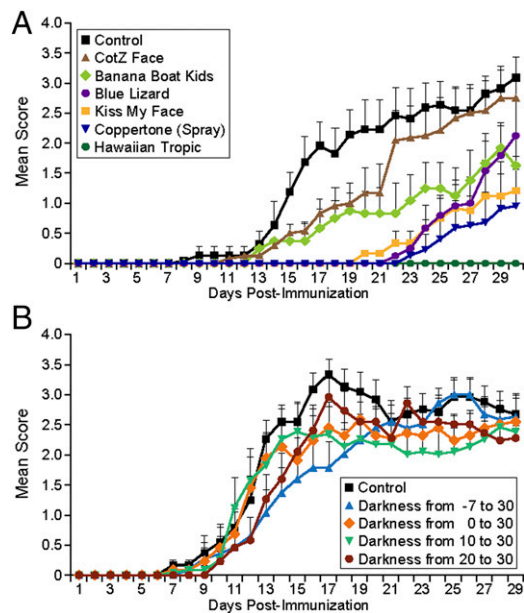
The authors declare no conflict of interest.

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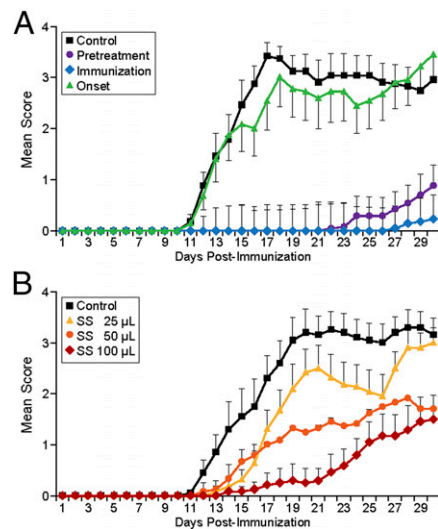


**Fig. 1.** Topical application of Coppertone Spray sunscreen (SS) completely blocks EAE. (A) Topical application of sunscreen before NBUVB. Mean score was recorded daily after induction of EAE. (B) Topical application of sunscreen with or without NBUVB completely blocked EAE development. Mean score was measured daily after immunization. Data are expressed as mean  $\pm$  SEM. All treatment groups in A and B were statistically significantly different from controls ( $n = 12$ ;  $P < 0.05$ ).

that the sunblock agents would prevent the suppression of EAE by UV light. Analysis revealed that not all sunblock preparations have this property. Furthermore, the complete absence of light

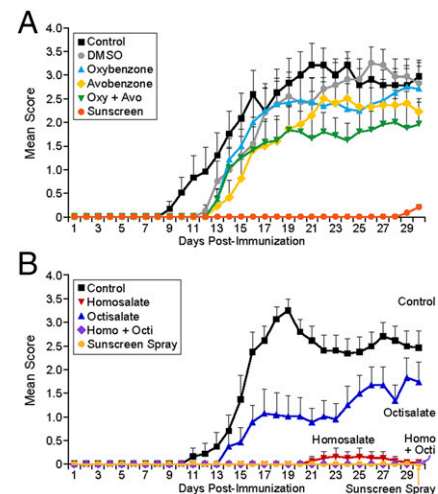


**Fig. 2.** Commercial sunscreen preparations differentially block EAE, whereas darkness itself does not. (A) Six different sunscreens were topically administered daily, and the mean score was recorded. (B) The effect of total darkness on EAE was determined. Treated mice were kept in total darkness for various periods, as indicated. Mean score was measured daily. Data are expressed as mean values  $\pm$  SEM. In A, all treatment groups except Banana Boat Kids and CotZ Face were statistically different from controls ( $n = 12$ ;  $P < 0.05$ ). In B, there were no statistically significant differences among the groups ( $n = 12$ ).



**Fig. 3.** Suppression of EAE by Coppertone Spray sunscreen (SS) is both time- and dose-dependent. (A) Disease scores in relation to time of administration of Coppertone Spray sunscreen. Mean score was determined daily. Mice were treated topically with 200  $\mu$ L/d for the entire experiment (day 30) with various starting treatment times. Pretreatment, treatment started at day -7 before immunization; immunization, treatment started at the time of immunization; onset, treatment initiated when the animal first exhibited a score  $\geq 1.0$ . (B) Dose-dependent suppression of EAE by Coppertone Spray sunscreen. Data are expressed as mean  $\pm$  SEM. Pretreatment and immunization treatments were significantly different from controls in A ( $n = 12$ ;  $P < 0.05$ ). (C) The two highest levels of SS (50 and 100  $\mu$ L) were significantly different from controls ( $n = 12$ ;  $P < 0.05$ ).

had no effect on EAE. Thus, we focused on the component(s) of the active sunblock preparations. The suppression of EAE by the active sunblock preparations was traced to two salate esters, homosalate and octisalate. When tested directly, both salates



**Fig. 4.** Two ingredients of effective sunscreen, homosalate and octisalate, significantly suppress EAE. Two other ingredients, avobenzone and oxybenzone, fail to suppress EAE. (A) Mice were treated with a solution of (12% avobenzone, 16% oxybenzone, or a combination in 25  $\mu$ L of DMSO) topically each day. Coppertone Spray (100  $\mu$ L) served as a positive control. Each mouse was scored daily. (B) Mice were treated topically with 30  $\mu$ L of homosalate (1.5 g/kg), 10  $\mu$ L of octisalate (0.5 g/kg), and a combination. Coppertone Spray (200  $\mu$ L) served as a positive control. Daily mean score was recorded. Data are expressed as mean  $\pm$  SEM. In A, all groups were significantly different from the sunscreen group ( $n = 12$ ;  $P < 0.05$ ). All treatment groups except octisalate alone were significantly different from the control groups ( $P < 0.05$ ).

**Table 1. Sunscreen brands tested**

Trade name	SPF	Active ingredients
Banana Boat Kids	50	Titanium dioxide 3.1%, zinc oxide 4.0%
Blue Lizard	30	Octinoxate 7.5%, octocrylen 2.0%, oxybenzone 3.0%, zinc oxide 6.0%
Coppertone Spray	50	Avobenzone 3.0%, homosalate 15.0%, octisalate 5.0%
CoTZ Face	40	Titanium dioxide 8.0%, zinc oxide 3.8%
Hawaiian Tropic	50	Avobenzone 2.7%, homosalate 8.0%, octisalate 4.5%, octocrylen 5.0%
Kiss My Face	50	Avobenzone 4.0%, homosalate 5.0%, octinoxate 7.5%, octisalate 5.0%, zinc oxide 1.7%

were equally active at ~1.5 g/kg in suppressing EAE. It is likely that these compounds are not acting by blocking or absorbing UV light. Simply keeping mice in the complete absence of light did not affect the development of EAE. Furthermore, some sunscreens that are effective as sunblockers do not suppress EAE. Only sunblocks that contain salicylate esters are effective, and the salates themselves clearly block EAE. The only adverse effect of the homosalate and octisalate is a temporary mild skin irritation.

Salicylates are well-known nonsteroidal anti-inflammatory drugs (NSAIDs) (6). However, a previous study revealed that acetylsalicylic acid had no effect on MS (7, 8). On the other hand, homosalate suppressed the ear edema response to dinitrobenzene (9). Because NSAIDs are well-known inhibitors of

**Table 2. Effects of sunscreen ingredients on EAE mice**

Treatment	Incidence	Day of onset	Mean severity	CDI
Control	100% (12/12)	16 ± 1	2.5 ± 0.8	42 ± 1
Homosalate	8% (1/12)*	22 ± 0*	1.3 ± 0.0*	1 ± 0*
Octisalate	75% (9/12)	18 ± 3*	2.0 ± 0.8*	20 ± 1*
Homosalate + octisalate	0% (0/12)*	0 ± 0*	0.0 ± 0.0*	0 ± 0*
Coppertone	0% (0/11)*	0 ± 0*	0.0 ± 0.0*	0 ± 0*

Data are expressed as mean ± SD. *n* = 11–12.

\**P* < 0.05 vs. control.

cyclooxygenase (COX) (10), and COX-2 has been observed in MS lesions (11), a possible mechanism of action of the salicylates is suppression of COX. COX inhibitors suppress EAE (12, 13); whether the salates suppress EAE by inhibiting COX is unknown. Regardless, our findings here present a clear opportunity to explore mechanisms and possible approaches to treating MS.

### Materials and Methods

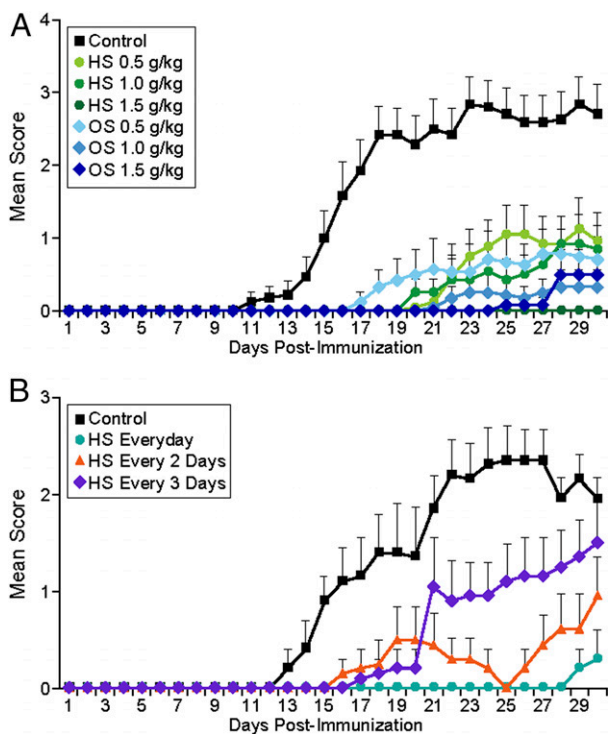
**Animal Husbandry.** Female C57BL/6 mice (age 8–10 wk), purchased from Jackson Laboratory, were fed a standard laboratory chow 5008 (Purina Mills). The mice were exposed to 12-h light-dark cycles. In one experiment, the animals were kept in darkness at all times. All procedures were approved by the Institutional Animal Care and Use Committee of the College of Agricultural and Life Sciences, University of Wisconsin–Madison.

**EAE Induction.** Mice were immunized with a MOG<sub>35–55</sub> kit (EK-2110; Hooke Laboratories). Each mouse was immunized with a 20-μL s.c. injection of MOG<sub>35–55</sub>/CFA emulsion and an i.p. injection of 200 ng of pertussis toxin (List Biological Laboratories) diluted in sterile PBS (14). A second booster pertussis toxin injection was given 48 h later. Each mouse was scored daily for clinical signs of EAE using the following scale: 0, no clinical disease; 1, loss of tail tone; 2, unsteady gait; 3, hind limb paralysis; 4, forelimb paralysis; 5, death (15).

**Treatment with Sunscreens and Active Ingredients.** The following sunscreen creams or spray were applied topically to the shaved back skin of the mice daily (Table 1): Banana Boat Kids (SPF 50; Energizer Personal Care); Blue Lizard Australian Sunscreen (SPF 30; Crown Laboratories); Coppertone Spray (SPF 50; MSD Consumer Care); CoTZ Face (SPF 40; CoTZ Skincare); Hawaiian Tropic (SPF 50; Energizer Personal Care); and Kiss My Face (SPF 50; Kiss My Face). Each sunscreen was applied daily to cover the shaved skin (100–200 μL). Avobenzone, oxybenzone, homosalate, and octisalate were purchased from Spectrum Chemical. Avobenzone and oxybenzone were dissolved in DMSO, whereas homosalate and octisalate were applied directly. The doses of avobenzone and oxybenzone administered topically to each mouse was adjusted to equal the concentration in the respective sunscreen product.

**NBUVB Treatment.** For NBUVB treatment, a set of four TL20W/01 UVB 311 narrow-band 2-ft bulbs wavelength centered at 311–313 nm were used daily at (10 KJ/m<sup>2</sup>) (14). The radiation output was measured using a UV radiometer equipped with a 302-nm sensor (UVP). A 16-chamber Plexiglass cage was used for daily UV radiation. Each chamber contained one mouse. The mice were rotated through the different chambers so that each mouse received equal light exposure. The mice were UV-irradiated beginning on the same day of immunization continuing through day 30 postimmunization.

**Statistical Analysis.** Data are expressed as mean, mean ± SD, or mean ± SEM. Onset was calculated by averaging the first day when the clinical score was ≥1.0 for 2 consecutive days. Mean severity was determined by averaging the clinical scores during the entire experiment. The cumulative disease index was calculated by summing the clinical scores for the group divided by the number of mice per group. Statistical analyses were performed using the two-tailed Fisher exact probability test for incidence, the Kruskal–Wallis nonparametric test with Dunn’s multiple comparison test for EAE severity (mean scores) and body weight. The unpaired Student’s *t* test was used for the other measurements. A *P* value < 0.05 was considered statistically significant.



**Fig. 5. Dose-dependent suppression of EAE by homosalate and octisalate.** (A) Mice were treated with various doses (10, 20, and 30 μL or 0.5, 1.0, 1.5 g/kg) of homosalate or octisalate topically. The mean score was recorded. B, Mice treated with homosalate (30 μL or 1.5 g/kg) topically at various times. Daily mean score was recorded. Data are expressed as mean value ± SEM. In A, all treatment groups were significantly different from control (*P* < 0.05). In B, the mean scores of homosalate every day and every 2 d were significantly lower than those of control (*P* < 0.05). HS, homosalate; OS, octisalate.

**ACKNOWLEDGMENTS.** We thank Wendy Hellwig for technical support and Debra Noltner for assistance with manuscript preparation. This

work was supported by funding from the Wisconsin Alumni Research Foundation.

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