Sunscreens Suppress Cutaneous Vitamin D₃ Synthesis*

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ABSTRACT. Sunscreens block the cutaneous absorption of UV-B radiation and prevent sunburning, premature aging, and cancer of the skin. Inasmuch as UV-B radiation is also responsible for the photosynthesis of vitamin D₃, we investigated the effect of sunscreens on the cutaneous formation of vitamin D₃ in vivo and in vitro. Eight normal subjects, four of whom had been protected with the sunscreen para-aminobenzoic acid (sun protection factor 8), were exposed to one minimal erythema dose of UV radiation. The mean serum vitamin D₃ concentration increased from 1.5 ± 1.0 (±SEM) to 25.6 ± 6.7 ng/mL in unprotected subjects, whereas it was 5.6 ± 3.0 and 4.4 ± 2.4 ng/mL at these times in the subjects who were protected with para-aminobenzoic acid. Para-aminobenzoic acid also prevented the photosomeration of 7-dehydrocholesterol to previtamin D₃ in human skin slices in vitro. These results indicate that the sunscreen interfered with the cutaneous production of vitamin D₃. (J Clin Endocrinol Metab 64: 1165, 1987)

A variety of biological effects occur in human skin after exposure to sunlight. Of particular concern are the photochemical reactions triggered by the high energy wavelengths 290-320 nm (UV-B). Acute exposure (minutes to hours) to solar UV irradiation (UVR) causes erythema, whereas chronic exposure (years to decades) produces cancer and premature aging of the skin (1, 2). These adverse effects of sunlight can be prevented by the routine topical use of sunscreens, such as para-aminobenzoic acid (PABA), esters of para-aminobenzoic acid, cinnamates, and salicylates (3). Topical sunscreens absorb UV-B radiation, thereby interfering with the penetration of solar radiation to the skin.

Exposure to solar UVR also promotes vitamin D₃ synthesis in the skin (4). After solar irradiation, the UV-B photons absorbed by the epidermis cause the sterol 7-dehydrocholesterol to be photolysed to previtamin D₃ (4-6). This thermally labile photoproduct spontaneously isomerizes over several days to vitamin D₃ (4), which enters the circulation. Vitamin D-binding protein may help translocate vitamin D₃ into the circulation (4). Once in the circulation, vitamin D₃ is metabolized sequentially in the liver and kidney to its biologically active form, 1,25-dihydroxyvitamin D₃ (5).

Because sunscreens act by absorbing high energy solar UVR, we investigated the effect of topical application of PABA on the photosynthesis of previtamin D₃ in human skin in vitro. We also determined the effect of a sunscreening agent on the circulating vitamin D concentrations in normal subjects exposed to whole body UV radiation.

Materials and Methods

In vitro studies

A piece of normal human skin that had been surgically removed during a reduction mammoplasty from a woman was obtained from the Department of Pathology and divided into nine pieces (each 6.2 cm²). Triplicate samples of skin received an application of either 0.1 mL ethanol or ethanol with 5% (wt/vol) PABA. These six skin specimens were then exposed to 1 MED (~30 mjoules/cm²) of simulated solar UVR, as previously described (6). The three remaining pieces of skin served as controls. All skin samples were then separated into dermis and epidermis (6). The basal layer of the epidermis was further isolated, using techniques previously described in detail (4). The lipid fraction of the basal layer was extracted in 8% ethyl acetate in n-hexane, and the 7-dehydrocholesterol and previtamin D₃ were separated and quantitated by high-performance liquid chromatography (HPLC) (4-6).

In vivo studies

Effect of a single application of PABA on serum vitamin D concentrations. The study population was comprised of eight normal subjects (six women and two men), all Caucasian, ranging in age from 21-45 yr. The study was performed during a
period of 21 days in early spring (April through May). The participants were asked to avoid direct exposure to sunlight during the experiment. All had normal hepatic and renal function, and none was taking vitamin D, anticonvulsant drugs, or steroids. Each gave written informed consent, and the study was approved by the Springfield Committee for Research on Human Subjects.

UVR was delivered in a walk-in irradiation chamber (National Biological Corp., Cleveland, OH) equipped with eight vertically arranged fluorescent sunlamps (Westinghouse model FS72T12, Bloomfield, NJ). The total spectral distribution ranged from 260–360 nm, with a peak emission at 313 nm; the energy delivered by the wavelengths in the band 260–330 nm was 0.8 mwatts, determined at a distance 30 cm from the source.

The minimal erythema dose (MED) for each subject was determined 1 week before the study. Five test areas of 1 cm² each on the skin of the lower aspect of the back were left unprotected and were successively exposed to UVR for variable periods. The lowest dose was one third of the estimated MED, with subsequent stepwise increments of 40% for the other squares. Twenty-four hours later, the exposed sites were examined, and the lowest dose of UVR that caused a faint pink coloration with four distinct borders was recorded as the MED for that individual.

Each subject was then randomly assigned to one of the two study groups. Four subjects received whole body exposure to 1 MED UVR. The other four subjects received a total body application of approximately 10 mL 5% PABA in 55% ethanol with water and emollients (PreSun, Westwood Pharmaceuticals, Buffalo, NY; sun protection-factor 8) 1 h before whole body exposure to 1 MED UVR. Blood samples were obtained 24 and 2 h before the total body exposure to UVR and 1, 2, 3, 7, and 14 days after the exposure. Serum was separated and stored at −20 °C until the time of analysis for vitamin D (7–9).

**Experimental methods**

Serum vitamin D₃ concentrations were determined as previously described (9). Serum samples (3–4 mL) were extracted with methanol-methylene chloride. The lipid extracts then were subjected to preparative chromatography on silica Sep-Pak cartridges (Waters Associates, Milford, MA) for the initial separation of vitamin D from 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and other vitamin D-hydroxylated metabolites (9). The fraction containing vitamin D was further purified by a modified two-stage HPLC procedure (8, 10). The initial step consisted of reverse phase HPLC, in which the samples were chromatographed on a Radial-Pak A column (Waters Associates) and eluted with a solution of 2% water in methanol at a constant flow rate of 2 mL/min. Final purification was performed by straight phase HPLC on a Zorbax-sil column (25 cm × 6.2 mm; DuPont Instruments, Wilmington, DE) with 3% isopropranol in n-hexane as the eluant at a flow rate of 1.5 mL/min. Vitamin D was quantitated by UV absorbance at 254 nm (Waters model 440 UVR detector) (8, 10). The lower limit of detection for this method was 0.5 ng/mL. To test the relative contribution of vitamin D₃ to the total vitamin D concentration, serum samples were analyzed by reverse phase HPLC (8, 9); they were found to contain only vitamin D₃.

**Results**

Comparison of UV absorption spectrum of PABA with the action spectrum for previtamin D₃ photosynthesis in human epidermis revealed marked overlap (Fig. 1). PABA absorbs the radiation between 240 and 400 nm and overlaps the spectrum responsible for the photosynthesis of previtamin D₃ (radiation between 260–315 nm [6]).

Exposure of triplicate samples of human skin to simulated solar radiation resulted in the conversion of 15 ± 2% of the 7-dehydrocholesterol stores in the stratum basale to previtamin D₃ (Fig. 2B). This photochemical reaction was completely blocked by topical application of PABA immediately before exposure to the same amount of simulated solar radiation. Previtamin D₃ was not detected in the stratum basale of any of the PABA-treated skin samples or the skin samples not exposed to simulated solar radiation (Fig. 2, A and C).

PABA had a significant effect on the cutaneous synthesis of vitamin D in vivo. Exposure to 1 MED UVR in subjects receiving PABA vehicle alone produced a marked and significant (P < 0.01) increase in mean
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Fig. 2. Chromatograms of vitamin D₃ precursors in lipid extracts of stratum basale from human skin. Specimen A was untreated skin; specimen B received a topical application of vehicle (ethanol) solution before UV-B irradiation; specimen C received a topical application of PABA in ethanol before UV-B irradiation. PABA blocked the photoisomerization of 7-dehydrocholesterol (7-DHC) to previtamin D₃.

Fig. 3. Mean (±SEM) serum vitamin D₃ concentrations in eight normal subjects. Four subjects (○—○) applied PABA and four applied vehicle (●—●) to the entire skin before exposure to UVB. On day 0, all subjects underwent total body exposure to 1 MED UVR. To convert nanograms of vitamin D per mL to nanomoles per L, multiply by 2.599.

Discussion

These results demonstrate that a single topical application of PABA, the active principle in many sunscreens, interferes significantly with the cutaneous synthesis of vitamin D₃. We found that PABA blocked the photosynthesis of previtamin D₃ in the human epidermis and that the elevation in serum vitamin D₃ concentrations exposed to the equivalent of 1 MED UVR was prevented by a sunscreen containing PABA.

Chronic exposure to sunlight is thought to be an important predisposing factor for skin cancer among Caucasians and has been associated with premature aging of the skin (12). Sunscreens used for preventing sunburning during acute exposures to the sun also are advocated for the prevention of skin cancer recurrences and other chronic effects of solar irradiation (1, 13). However, the high energy UVR responsible for causing erythema, photocycling, and skin cancer is the same radiation responsible for the promotion of vitamin D₃ synthesis in the skin (6).

The effect of topical sunscreening agents to limit or prevent the cutaneous production of vitamin D₃ is probably of little consequence for children and young adults, who obtain adequate vitamin D nutrition from their diet and frequent exposure to sunlight. However, the elderly, who are more prone to developing vitamin D deficiency (14–17), often rely on exposure to the sun as their major if not sole source of vitamin D (18, 19). These individuals could significantly increase their risk of vitamin D deficiency if they consistently apply a sunscreen before going outdoors. To balance the concern about the harmful effects of sunlight with the beneficial effect on vitamin D nutrition, susceptible individuals may omit sunscreening agents and expose their skin only to suberythemal doses of sunlight. In the case of prolonged outdoor activities, a topical sunscreen may be applied immediately.
after the initial exposure.

In conclusion, sunscreens cause a defect in the cutaneous synthesis of vitamin D₃. We suggest that elderly chronic users of these substances should be routinely investigated for vitamin D deficiency.

References

19. MacLaughlin J, Holick MF 1985 Aging decreases the capacity of human skin to produce vitamin D₃. J Clin Invest 76:1536

Travel Awards—VIII International Congress of Endocrinology

The Awards Committee of the International Society of Endocrinology is prepared to make travel grants for the VIII International Congress of Endocrinology, which will be held July 17 to 24, 1988 in Kyoto, Japan. Preference will be given to young endocrinologists and/or to endocrinologists from Third World countries who have demonstrated an interest in endocrine research. These awards are intended for individuals who have no other source of funds to attend the Congress. The awards will be for 80% of the Apex air fare from the awardee’s home to Kyoto.

The deadline for receipt of applications is February 1, 1988. Notification of awards will be March, 1988. Application forms may be obtained from:

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