Early Changes in Dermal Collagen of Mice Exposed to Chronic UVB Irradiation and the Effects of a UVB Sunscreen

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Our previous studies have shown that hairless albino mice receiving chronic UVB irradiation show an increase in type III collagen, in the irradiated skin, after 12–36 weeks treatment. In this study we wished to find the earliest time at which such collagen changes were detectable and also to ascertain whether a topically applied UV-B sunscreen could prevent such changes.

Groups of 10 hairless albino mice were irradiated, dorsally, with a daily dose of 62 mJ/cm² UVB for 3, 6, 9, and 12 weeks. Three parallel groups were used. They received a) no topical treatment, b) sunscreen vehicle alone, and c) sunscreen (containing 2-ethylhexyl 4'-methoxy-cinnamate) applied dorsally at 2 µl/cm². Dorsal and ventral (non-irradiated) skin samples were taken and the types I and III collagen quantified densitometrically after cyanogen bromide digestion and polyacrylamide gel electrophoresis. The ratio of dorsal/ventral type III collagen (D/V III) was determined for each mouse.

The topical treatments caused no change in D/V III in the non-irradiated animals. In the non-topically treated group the irradiated animals showed a considerable increase in D/V III, compared with controls, at 12 weeks (P < 0.001). The group treated with sunscreen vehicle alone also showed a significant increase in D/V III at week 12 (P < 0.01). Both these groups showed a slight elevation in D/V III at week 6. The group treated with sunscreen showed no significant difference in D/V III between irradiated and control animals at any time. J Invest Dermatol 91:590–592, 1988

We have been studying the effect of ultraviolet radiation (UVR) on the collagens in human and mouse skin. In fetal dermis the predominant type of collagen found is type III [1]. During early adult life human skin collagen is formed of 70%–80% type I and 15%–25% type III. In elderly subjects there is an increase (to a variable degree) in type III collagen found in normally unexposed skin (abdomen) [2]. Our studies on samples from elderly subjects have shown a marked increase in type III collagen in sun-exposed sites compared with non-exposed sites in the same individual [3].

The female Skh-1 (hairless, albino) mouse, exposed to UVB (280–315 nm) radiation, has been shown to be a good qualitative model for the histopathology of human solar elastosis [4–6]. We have used these animals for a quantitative study of the dermal collagen changes induced by UVB irradiation. Animals chronically irradiated with UVB showed a significant increase in type III collagen in the exposed skin after 12, 24, 30, and 36 weeks treatment [7].

We have now examined the first 12-week period in more detail to find out how early these collagen changes can be detected and we also studied the effect of a topically applied UVB sunscreen.

MATERIALS AND METHODS

All chemicals were obtained from Sigma (U.K.) except methanol and acetic acid, which were from B.D.H. (U.K.).

Irradiation The irradiation source, dosimetry, and procedures were the same as used in our previous study [7], consisting of a bank of 15 Westinghouse FS20 sunlamps. The output was attenuated and diffused by a wire mesh. The animals were irradiated dorsally about 60 cm below the source for about 13 min. At this distance the irradiance was about 125 µW cm⁻². The dorsal skin of the animals received a dose of 62 mJ cm⁻² per day, 5 d a week. This dose was about half that required to induce mild edema from a single exposure.

Treatment Each experimental group consisted of 10 female Skh-1 hairless, albino mice aged 8–12 weeks.

Non-topically Treated: Four groups were irradiated for 3, 6, 9, and 12 weeks. Five groups were used as controls and kept in identical conditions but sham irradiated for 0, 3, 6, 9, and 12 weeks.

Sunscreen Vehicle Treated: The sunscreen base, a lotion, was applied to the dorsal skin, from head to tail, of all animals in these groups. It was applied at approximately 2 µl cm⁻² about 20 min prior to irradiation or sham irradiation. Four groups were irradiated and four groups were sham irradiated for 3, 6, 9, and 12 weeks.

Sunscreen Treated: Eight groups were sunscreen vehicle treated as above, except that the UVB sunscreen was used. This contained 2-ethylhexyl 4'-methoxycinnamate (Parsole MCX) and had a sun protection factor (SPF) of 8.

Twenty-four hours after the last irradiation each group was killed by cervical dislocation and skin samples of approximately 2 cm² were taken from dorsal and ventral surfaces of each mouse. Samples were frozen at −20°C prior to biochemical analysis.

Analysis of Collagen The analysis of collagen was performed as described previously [7]. Briefly, the dorsal and ventral skin samples were digested with cyanogen bromide based on the method of
Light [8,9]. The resulting peptides were separated by SDS polyacrylamide gel electrophoresis using a Laemmli buffer system [10] and stained with Coomassie Brilliant Blue. The gels were scanned with a densitometer and the peak areas of peptides derived from types I and III collagen used to calculate the percentage of type III collagen in each sample.

Because these animals exhibit a considerable individual variation in skin collagen each animal was used as its own control and the data expressed as a ratio of type III collagen found in the dorsal (irradiated or control) skin over that found in the ventral (non-irradiated) skin-D/V III. A mean ratio was derived for each animal from at least four D/V III values.

To confirm that the peptides measured were derived entirely from collagen some skin samples were treated with a highly purified collagenase prior to digestion with cyanogen bromide. Any peptides difference from the collagen of non-irradiated animals at any of the time points below bands from the gels, demonstrating that the bands measured are derived solely from collagen.

RESULTS

Pre-treatment with collagenase completely removed all the peptide bands from the gels, demonstrating that the bands measured are derived solely from collagen.

The non-irradiated, non-topically treated control animals showed a slight but non-significant decrease in D/V III from week 0 to week 12. Treatment with sunscreen or vehicle had no effect on the collagen of non-irradiated animals at any of the time points (Student’s t test P > 0.2 in all cases), and the results from these three groups at each week were pooled to give the control values used below.

The non-topically treated irradiated mice showed no significant difference in D/V III, compared with controls, at weeks 3, 6, and 9 (P > 0.2 in all cases). However, there was a marked increase from 0.99 ± 0.12 (SD) at week 9 to 1.23 ± 0.15 (SD) at week 12 (Fig 1A). This was significantly higher than the control value at week 12, 0.90 ± 0.11 (SD), (P < 0.001). These animals also showed a slight but insignificant (P > 0.2) elevation in D/V III at week 6.

The animals treated with sunscreen vehicle alone showed a similar pattern to the non-topically treated animals (Fig 1B). At week 12 the irradiated animals showed an increased D/V III of 1.09 ± 0.18 (SD), which was not as marked as the non-topically treated group, but still significantly higher than the controls (P < 0.01). These animals also showed an elevated D/V III at week 6, which was significantly higher than the controls (P < 0.01) in this case.

The animals treated with the UVB sunscreen showed no significant difference (P > 0.2 in all cases) between irradiated and control animals at all times from 3 to 12 weeks (Fig 1C).

DISCUSSION

The analysis of types I and III collagen in the skin has been used to follow dermal changes brought about by aging and UV irradiation. The technique allows quantitative measurement of any changes. After 12-weeks irradiation with UVB the skin of the Skh-1 hairless, albino mouse showed a significant elevation of type III collagen compared to non-irradiated control animals. The increase in D/V III at 12 weeks was somewhat higher than in our previous study, although the variation within each group was similar. The D/V III and group variation of non-irradiated control animals was the same as observed previously. Up to 9 weeks there was no significant difference between control and irradiated groups. One explanation for this long onset time could be the slow metabolism of collagen. Although we do not have data for the Skh-1 mouse, in the human body the average half-life of collagen is about 2.5 years and in rats the half life of mature collagen is about 10-40 weeks [11]. It is possible, therefore, that these collagen changes may be induced by lower doses of UV [e.g., 3 or 6 weeks irradiation may be sufficient] yet take 12 weeks before the changes become manifest. It would be necessary to irradiate animals with a series of different daily doses to ascertain to what extent these collagen changes are time or dose dependent.

It is interesting to note that the onset of these collagen changes coincides with the onset of tumor formation in these animals [7], although in this study only two animals (in the non-topically treated, 12 week irradiated group) showed visible tumors. Whether these phenomena are related is not known.

The increase in type III collagen may be a response to UV-induced dermal damage similar to the type III increase observed in scar tissue [12,13]. Studies using miniature pigs have also shown an increase in dermal type III collagen, measured by immunofluorescence, following UV irradiation [14]. Preliminary studies of the hydroxyproline content of the skin (a marker for collagen content) show that there is a 50%-100% increase in dermal collagen in irradiated skin (expressed as collagen weight per dry weight of whole skin). Irradiation induces skin thickening, and the collagen content per unit area of skin is increased about threefold. This would suggest a general increase in collagen synthesis with higher levels of type III collagen being produced.

We cannot explain the slight elevation in D/V III seen at 6 weeks irradiation in both the non-topically treated and the sunscreen vehicle treated groups. It is possible that this is a response to damage to the immature, soluble collagen, whereas the bulk of collagen, being mature insoluble fibers, may take longer to respond to the UV damage. More detailed studies would be necessary to clarify this situation.

The use of a topically applied UVB sunscreen suppressed the collagen changes observed at 12 weeks (and at 6 weeks). The absorption spectrum of the sunscreen (2-ethylhexyl 4’-methoxycinnamate) closely matches the output spectrum of the FS20 sunlamp source used (Fig 2), so good protection could be expected from a high SPF sunscreen. It has been suggested that, in some cases, heat from the irradiation source could be responsible for some of the changes observed in dermal tissue [15]. In our experiments the complete protection afforded by the UV sunscreen demonstrates that these collagen changes are not brought about by a heat effect. We
promoting repair of such damage [4,5,19). In psoriasis by protection observed. Emollient [18). However, simple spectroscopic studies suggest that the sunscreen vehicle absorbs significantly in the UVB and slightly in the UVA wavelengths, and it is probably this which confers the protection observed. Histologic studies have shown that sunscreens can be highly effective in preventing UV-induced connective tissue damage and in promoting repair of such damage [4,5,19]. Using our biochemical techniques it would be possible to quantitatively evaluate the protection offered by long-term use of sunscreens in vivo.

REFERENCES


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Figure 2. Spectral output of Westinghouse FS20 sunlamps measured by spectroradiometry (——) compared with the spectral absorbance of the UVB sunscreen, 2-ethylhexyl 4’-methoxycinnamate in ethanol (——).