Induction of Plasminogen Activator by UV Light in Mouse Keratinocyte-Derived Cell Line, Pam 212

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Key Words

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It has been known that in vivo UV irradiation induced marked elevation of tissue plasmin (PL) activity [1,2]. The mechanism of this PL induction, however, remains unclear. The purpose of this study was to determine the effects of UV light on plasminogen activator (PA) production by keratinocytes (KC).

Pam 212 cells were cultured in RPMI 1640 supplemented with 10% FCS, and washed 3 times with PBS before UV exposure. Cells were irradiated by fluorescent UVB lamps (Toshiba FL-20SE 30) or an irradiation monochromator (CRM-FM, Japan Spectroscopic Co., Tokyo). Cells were then fed with fresh medium with or without FCS, and cultured for an additional 24 h. Conditioned medium was assayed for PL or PA activity. PL activity was measured using a synthetic fluorogenic peptide substrate, Boc-Val-Leu-Lys-MCA [3]. In PA assay, culture medium was incubated with plasminogen (PLG) for 45 min at 37 °C, and PA activity was calculated from the resultant PL activity.

Without UV exposure, the conditioned medium contained significantly higher PL activity than fresh medium. When cells were pretreated with sublethal doses (100–300 J/m²) of UVB, PL activity increased in a dose-dependent fashion up to 250% of nonirradiated control. In contrast, lethal doses suppressed PL activity. A time course study demonstrated the initial enhancement to occur within 24 h after UV exposure. PL activity seen in culture medium appeared to be derived from exogenous PLG contained in FCS, because removal of PLG from FCS by lysine-Sepharose affinity chromatography completely diminished the PL elevation.

Without UV exposure, a significant amount (178 mIU/ml) of PA production was detected in the conditioned medium. The action spectrum (AcSp) for PA production was studied by irradiating 25 J/m² of monochromatic light from 250 to 380 nm. AcSp ranged from 250 to 320 with a peak at 260–270 nm. Irradiation with 25 J/m² at 270 nm induced an increase 34 times this activity. Addition of cycloheximide to culture medium completely inhibited PA secretion.

Although UV irradiation has been reported to enhance PA production by fibroblasts [4], it remained unclear whether UV has similar effects on KC as well. By using Pam 212 cells, we found UV-induced synthesis of PA by KC. It was suggested that UV exposure enhanced PA production by epidermal KC in situ and that this process might play an important role in UV-induced modulation of the skin.

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