Increase of Plasma Sialic Acid upon UV-B Irradiation in Mouse

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In many inflammatory conditions including burns, the plasma sialic acid level rises as an acute responder. Sunburn is a kind of burn. In this study, sunburn was experimentally caused in mice by UV-B irradiation and their plasma sialic acid levels were measured. The levels increased, and reached the maximum 3 d after irradiation. This level was maintained for about 2 d, then it returned to normal within about one week. The increase in the level correlated with the UV dose and the severity of inflammation resulting in sunburn. This assay system was applied to assess the virtue of UV-cut cream in experimental sunburn.

Keywords sialic acid; UV-B; mouse plasma; experimental sunburn; acute responder; UV-cut

Sialic acid, which is a component of glycoproteins and glycolipids, is widely distributed in man and animals. Plasma sialic acid is derived mainly from α1-acid glycoprotein which is an acute phase protein. The sialic acid level in plasma increases in many inflammatory conditions including infectious diseases,1–3 rheumatoid arthritis and collagen diseases,4,5 liver and heart diseases,4,6,7 burns and external wounds, including surgery.1,6,7 Since sunburn by solar ultraviolet (UV) rays is a type of burn, it has been assumed that plasma sialic acid levels would also increase under this condition. However, as there are no reports which have proven this assumption, we measured the levels of plasma sialic acid in a mouse model of UV-B induced sunburn.

MATERIALS AND METHODS

Mice Female DDY mice purchased from Japan SLC (Shizuoka Laboratory Animal Farm, Hamamatsu, Japan) were used at 8 weeks of age.

UV Irradiation The dorsal fur of the mice was removed with hair clippers and remaining hair was removed with depilatory cream. The next day, the mice were irradiated by UV-B lamp (Toshiba FL20S·E, peak emission at 314 nm, Toshiba Electric Co., Ltd, Tokyo, Japan) at a 40 cm distance corresponding to 0.065 mW/cm² for 60 min. The UV dose was measured with UV radiometer, UV 103 (Macum Photometrics, Ltd., Livingston, Scotland).

Plasma Samples Mice plasma was collected from the heart or the suborbital sinus at various times after irradiation.

Determination of Sialic Acid Plasma sialic acid levels were determined using the commercially available kit, Determiner SA (Kyowa Medex, Tokyo). The assay procedure in the manual was followed. The assay is based on two processes. The first is successive hydrolytic digestion with neuraminidase, N-acetyl neuraminic acid aldolase and pyruvic acid oxidase. The second is the production of a blue color by the final product (hydrogen peroxide) with 4-aminoantipyrine, sodium N-(2-hydroxy-3-sulphopropyl)-3,5-dimethoxy-aniline and peroxidase. The sialic acid content was calculated from the ratio to the reference value of the standard sample in the kit. All results were expressed as the mean of data obtained from five mice.

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UV-Cut Experiment About 0.3 g of UV-cut cream, Sundown SPF30 (Johnson & Johnson Consumer Products, Skillman, N.J., U.S.A.) was spread on the bare skin and the mice were UV-irradiated at a 40 cm distance for 45 min. As a reference, hand cream without UV-cut components (Nivea-Kao Co., Ltd., Tokyo) was used.

Statistical Analysis The 95% confidence limit, standard deviation (S.D.) or regression was calculated from mean values. Differences between experimental groups were determined by the Student t-test.

RESULTS

When mice were UV-irradiated for 60 min, the plasma sialic acid level increased from 2 d and reached the highest level 3 d after irradiation. The high level was maintained for 2 d, after which the level rapidly decreased to normal (Fig. 1). The peak level was about 1.5-fold higher than the normal level and significantly differed from that of the control (p<0.01). The irradiated skin showed the most severe inflammation 3 d after irradiation, when the sialic acid level was the highest. All mice were kept alive to observe any clinical changes in the irradiated skin. In

Fig. 1. Changes in the Sialic Acid Levels in Mouse Plasma Induced by UV Irradiation

Six groups of mice were irradiated for 60 min (corresponding to 230 mJ/cm²) and plasma was collected daily. The means of sialic acid levels (circles) and standard deviations (bars) of data from five animals are shown. Significantly different level from day 0 was shown by * (p<0.01) and ** (p<0.05).

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parallel with the decrease in the sialic acid level, the inflammation healed, forming a large eschar within 7 d. According to these results, we determined the plasma sialic acid levels on the 3rd day post-irradiation in subsequent experiments. The levels increased in parallel with the period of irradiation (Table 1). When the mice were irradiated for 15 min, corresponding to a total of 58 ml/cm², sialic acid concentration increased to a level that significantly differed from that of non-irradiated mice ($p < 0.05$). Like the severity of sunburn in these mice, the severity of the eschar appearing on the 7th day also depended on the irradiation period (data not shown).

The correlation between the extent of sunburn and the increase in the sialic acid level was determined more precisely. The dorsal area from which the hair was almost completely removed was covered with a piece of wide packaging tape with a window of 0, 1.3, 2.5, 5.0 or 10.0 cm². After irradiation for 60 min the tape was removed and the amount of sialic acid in the plasma was measured on the 3rd day. From the mean value of each group, the regression was calculated. The regression line of the plasma sialic acid level was linear according to the window’s size through which mice received various total doses of UV (Fig. 2). Finally, sunburn traces in the shape of each window were seen on these mice on the 7th day of irradiation (data not shown).

To evaluate the practical application of this assay system, we performed a sun-screen test for protection from sunburn with a commercial UV-cut cream. The UV-cut cream dramatically blocked sunburn and, as expected, the plasma sialic acid level was not increased. On the other hand, sialic acid of mice applied with hand cream as a reference increased to the level of control mice resulting in sunburn (Table 2).

**DISCUSSION**

In this study, we confirmed our working hypothesis that sunburn caused by UV rays raises the plasma sialic acid level as reported for other inflammatory conditions. The sialic acid levels were linked to the severity of the inflammation. Therefore, the increase in the plasma sialic acid level might be an indirect effect via skin inflammation, namely sunburn rather than a direct effect of UV phototoxicity.

The sialic acid increase in the plasma corresponded to the UV dose as shown by the regression line. In the window experiment, the deviation of each mean value from the regression line was minimum. The excellent linearity of the regression line means that physical UV dose received in mice can be calculated from sialic acid level. On the basis of this excellent correlation between the sialic acid level and UV dose, we believe that measuring the plasma sialic acid may be a simple method with which to assess a quantitatively physiological UV effect such as sunburn. In fact, this idea was verified by the UV-cut experiment in which the virtue of UV-cut cream was possible to represent as quantitatively measurable value.

Plasma proteins in mice have rarely been studied and analytical reagents for the plasma components such as monoclonal antibody are not available. Therefore, in this study, we could not clarify the exact nature of the increase in sialic acid levels in mouse plasma. In a preliminary study, we found that the sialo-compound increased in the plasma was bound-type and not free sialic acid. Furthermore, it was not associated with lipids according to the results of buoyant density centrifugation (data not shown). Some sialo-glycoproteins have been identified among human plasma proteins, such as $\alpha_1$-acid glycoprotein, transferrin, ceruloplasmin, $\alpha_2$-macroglobulin, and haptoglobin. All of them are acute phase responders,
among which $\alpha_1$-acid glycoprotein is the most abundant. Perhaps this may also be true in mouse plasma. We are now studying which protein(s) increases in the mouse plasma.

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REFERENCES


