Changes in Serum Levels of Sialoglycoproteins in Mice Exposed to UV-B Radiation

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In the acute phase reaction, hepatocytes in the liver are activated and increase the plasma levels of acute phase reactants. Our previous study has shown that plasma sialic acid, an acute phase reactant, was increased following exposure of mice to UV-B radiation. Plasma sialic acid is derived from many plasma components. To clarify the type of plasma sialic acid that is increased by exposure to UV-B radiation, we performed two-dimensional electrophoresis and staining for sialic acid. Consequently, the increases in haptoglobin and hemopexin were marked and 90% or more of the increased sialic acid was derived from these two glycoproteins after exposure to UV radiation. The increase in α_1 -acid glycoprotein levels was slight and did not contribute to the total increase in plasma sialic acid after exposure to UV radiation. Plasma levels of several proteins including antichymotrypsin (ACT), were reduced following exposure to UV radiation. The discrepancy between our results and published ones regarding ACT levels are discussed in terms of the type of cytokine.

Key words UV-B; acute phase reactant; sialoglycoprotein; haptoglobin; hemopexin; sialic acid staining

It is well known that during various types of inflammation the acute phase protein concentration in blood increases. However, little is known about acute phase responses following sunburn due to UV irradiation. Urbanski *et al.* reported that UV irradiation of humans results in increased circulating IL-6 levels, causes fever and induces complement reactive protein (CRP) as an acute responder.¹⁾ However, they did not examine other acute phase reactants. Recently, we reported that UV-B irradiation of mice increases total plasma sialic acid.²⁾ Since plasma sialic acid is an acute phase reactant, we also confirmed that UV radiation is an acute phase stimulator. Plasma sialic acid originates from sialoglycoproteins or glycolipids in blood. Representative sialoglycoproteins in plasma include α_1 -acid glycoprotein (α_1 -AGP), haptoglobin, hemopexin, and α_1 -antichymotrypsin (α_1 -ACT) which are also known as acute phase reactants.^{3–10)} In our previous study, however, we did not analyze the characteristics of the plasma sialic acid increased by UV irradiation.

Up to now, many studies on the acute phase response have been limited to measurement of one or two acute phase proteins. To understand the changes in the entire spectrum of acute phase plasma proteins, Baumann *et al.* developed a specific approach.¹¹⁾ In their study, two-dimensional electrophoresis was introduced for the purpose of analyzing the acute phase proteins excreted by primary hepatocytes from mice with inflammation evoked by a chemical substance (turpentine).

In this study, in order to determine the characteristics of the increased sialic acid produced by UV-B irradiation, the acute phase proteins in plasma samples from sunburned mice were analyzed with a unique approach and the type of plasma sialic acid increased following UV-B irradiation was determined.

MATERIALS AND METHODS

Mice Female ddY mice (SLC, Hamamatsu) were used at the age of 8 weeks. Four mice were used in each group, unless otherwise stated.

UV Irradiation and Plasma Samples According to our

previous report,²⁾ dorsal hair was clipped and the remaining hair was removed with a depilatory cream. On the next day, the skin was irradiated once with a UV-B lamp (Toshiba FL20SE, peak emission at 314 nm) at 230 mJ/cm² corresponding to 7–10 minimal erythema dose (MED) in human skin and increasing the plasma sialic acid about 1.5 to 2.0 fold.²⁾ Mice were bled on the 3rd day after UV irradiation.

Centrifugation of Serum 140 mg potassium bromide was added to 0.4 ml serum ($d=1.21$) and the serum was sandwiched between high density sodium bromide-saline ($d=1.346$) and 0.9% saline in a polycarbonate centrifugation tube. After centrifugation at 300000 *g* for 5 h, the sandwiched solution was collected in 5-drop fractions from the bottom of the tube. Each fraction was assayed for density and sialic acid.

Determination of Sialic Acid Plasma levels of sialic acid were determined using a commercially available kit, Determiner SA (Kyowa Medex, Tokyo).

Two-Dimensional Electrophoresis The method of O'Farrell (1975)¹²⁾ was used with a slight modification. Urea-solubilized serum was fractionated by isoelectric focusing on polyacrylamide gel with 2% ampholine (LKB, 1.6% with a pH of 5–7, 0.4% with a pH of 3.5–10). Five microliters of the urea-solubilized serum was placed on the gel in a capillary tube (115 mm long, 2.5 mm in diameter) and the gel was electrophoresed for 30 min at 200 V followed by 2 h at 700 V. The pH gradient was confirmed by using pI markers (Oriental Yeast Co. Ltd., Osaka) in the same electrophoresis run. First-dimension gels were equilibrated with SDS-sample buffer [4% SDS, 62.5 mM-Tris-HCl (pH6.8), 5% β -mercaptoethanol, 10% glycerol] and loaded on to 10% flat SDS-polyacrylamide gel for the second dimension electrophoresis.

Staining and Identification of Sialoglycoprotein Spots The protein electrophoresed in the flat gel was blotted on to a nitrocellulose membrane. The membrane was treated with biotin-labelled lectin, MAM (*Maackia amurensis*; Honen Corporation, Tokyo) which was specific for sialic acid and then treated with avidin-horse radish peroxidase (HRP). For color production of HRP, a double staining method was employed using two substrates, chloro-naphthol and diaminobenzidine.

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The marked spots were identified by referring to Baumann's 1983 report.¹¹⁾

Quantitative Assay of Protein Spots The density of four representative spots was determined by laser scanning densitometry with a Personal Scanning Imager (Molecular Dynamics Co., Sunnyvale, CA). The perimeter of the spot was marked on the television monitor and the density signal of the circled spot was integrated and digitized as the volume.

RESULTS

In a preliminary experiment, we centrifuged serum to see if increased plasma sialic acid was derived from glycolipid or glycoprotein. If the sialic acid in question is derived from glycolipid, the increased sialic acid should be present in the

Fig. 1. Sedimentation Pattern of Plasma Sialic Acid following High Speed Centrifugation

The serum samples were weighted with potassium bromide and centrifuged as described in Materials and Methods. After centrifugation, the serum was separated into 10 fractions and assayed for density (closed triangle) and sialic acid content. Open circle, UV-irradiated mouse serum; closed circle, normal mouse serum.

top fractions. If the sialic acid originates from glycoprotein, it should be present in the bottom fractions. The centrifugation pattern of the plasma is shown in Fig. 1. A slight increase in sialic acid in the plasma from UV-irradiated mice was noted in the top fractions. However, a marked increase in sialic acid was seen in fractions about one-third from the bottom where the plasma proteins were concentrated. Since it was found that the sialic acid increased by UV irradiation originated from protein(s), we performed further studies on sialoglycoproteins following UV irradiation.

Before electrophoresis, the sialic acid in serum samples was determined. The plasma sialic acid in UV-irradiated mice was 2.3-fold higher than that in normal mice (141.6 and 61.8 mg per 100 ml). After two-dimensional electrophoresis of these sera, peroxidase-staining of each protein spot was performed by the specific binding of sialic acid-specific lectin as described in Materials and Methods. The results for each spot were compared with those of the two-dimensional electrophoresis described by Baumann *et al.*¹¹⁾ The haptoglobin spot of the serum from normal mice increased from trace amounts to a dense spot after exposure to UV irradiation (Fig. 2). Similarly, hemopexin, of which the original amount in unexposed mice was relatively high, further increased. Alpha-1 acid glycoprotein (α_1 -AGP) also increased but the density remained low. The transferrin spot was not clearly identified in either pattern, with or without UV irradiation. Other spots showing an increase in density following UV irradiation were unidentified because they differed from the pattern of spots reported by Baumann *et al.* On the other hand, several spots including that of anti-chymotrypsin (ACT) were reduced.

In addition, four representative spots in this figure were quantitatively assayed (Table 1). The density of haptoglobin in serum of UV-irradiated mice was 118-fold higher than that in normal serum. The increase in hemopexin following UV irradiation was not so large (about 3.5-fold) but the final density was high. On the other hand, the level of α_1 -AGP was increased following UV irradiation (about 10.5-fold), but the increase in density was not very large, being one order lower than that of haptoglobin and hemopexin.

Fig. 2. Sialoglycoprotein Pattern of Individual Mouse Serum following Two-Dimensional Electrophoresis

Serum from representative mice was selected, one in which the sialic acid level was normal and one in which there was a typical increase due to UV irradiation; these were examined by the procedures for two-dimensional electrophoresis and protein spot staining described in Materials and Methods. Arrows in the figure show proteins identified by cross-reference to the map constructed by Baumann *et al.* in 1983.¹¹⁾ A. Normal mouse serum; B. UV-irradiated mouse serum. Abbreviations are as follows: α_1 -AGP, α_1 -acid glycoprotein; β -HG, β -haptoglobin; HPX, hemopexin; ACT, antichymotrypsin.

Table 1. Quantitative Assay of Protein Spots in Fig. 2

Protein	Serum sample	Density* (Volume)	Fold increase	Increased density
α_1 -AGP	Normal	35.13		
	UV irradiated	368.69	10.5	333.56
Haptoglobin	Normal	19.34		
	UV irradiated	2282.97	118.0	2263.63
Hemopexin	Normal	820.98		
	UV irradiated	2924.39	3.6	2103.41
α Anti-chymotrypsin	Normal	379.74		
	UV irradiated	19.86	1/19.1	—

* Total density in the circled spot was digitized as volume.

DISCUSSION

The biological significance of the acute phase response is not well understood. Nevertheless, the reaction may have an important physiological role as the acute phase proteins are highly conserved during evolution, and normal levels of these proteins are maintained even during severe malnutrition.¹³⁾ Of the acute phase proteins, the functions of plasma sialoglycoprotein have been studied for many years. Haptoglobin and hemopexin are involved in the metabolism of biological iron to prevent loss of iron from the body. An increase in both proteins in the blood might contribute to the prevention of hemolytic anemia.^{14–16)} Recently, α_1 -AGP has attracted attention because it can modulate some drug concentrations in the blood.^{17–20)}

α_1 -AGP is the most typical sialo-glycoprotein, with sialic acid accounting for 12% of its molecular weight. However, we found that the concentration in mouse serum was very low and its increase following UV irradiation was relatively small. On the other hand, the increases in haptoglobin and hemopexin were marked in the acute phase reaction after UV irradiation. Haptoglobin, a small amount of which is present in normal serum, was increased following UV irradiation to 100-fold or more the level in plasma. Hemopexin, the normal level of which is relatively high, also increased to the same level as haptoglobin. Thus, the increase in plasma sialic acid following UV irradiation was attributed to increased synthesis of haptoglobin and hemopexin but not α_1 -AGP. These findings differ from that of a study in which rats were exposed to chemical agents or bacterial endotoxins, and α_1 -AGP became most abundant in the liver.²¹⁾

At present, only two plasma proteins, albumin and transferrin, are known to be negative responders in the acute phase reaction.²²⁾ In this study, we found that concentrations of some plasma sialo-proteins including ACT decreased after UV irradiation. There are several reports that increased ACT is associated with cancer,²³⁾ Alzheimer's disease²⁴⁾ and acute phase reactions such as surgical trauma.²⁵⁾ However, no decrease of ACT in the acute phase has been reported. Baumann and Gaudie divided the acute phase reactants into two groups according to the involved cytokine, such as IL-1 or IL-6.²⁶⁾ They reported that α_1 -ACT increased via IL-6. Our finding that the ACT level was reduced following UV irradiation is noteworthy since this differs from their observation²⁶⁾ because ACT should increase via IL-6 and UV irradiation induces IL-6 in humans and mice.^{27–32)} These findings suggest that the acute phase response via sunburn following UV irra-

diation is modulated by a complicated cytokine network. Finally, a different acute phase reaction from the chemical stimulation reported by Baumann and Gaudie²⁶⁾ may be evoked in mice exposed to UV radiation.

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