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INDEXING WORDS

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SYNOPSIS

Lens protein is a long-lived protein. Therefore, the levels of advanced-stage products of the Maillard reaction in lens proteins are considered to reflect the sum of accumulated glycation over time.

There were significant correlations between the progression of diabetic neuropathy and retinopathy and levels of advanced-stage products accumulated in the lens nucleus prior to surgery.

These results suggest that glycation measured by advanced Maillard product in the lens protein plays an important role in the etiology of diabetic complications.

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INTRODUCTION

Recent studies had demonstrated that glycation, which is a non-enzymatic non-specific binding reaction between proteins and reducing sugars and is termed the Maillard reaction,[2] occurs in various tissues such as crystallins, collagen, myelin and glomerular basement membrane etc. other than hemoglobin (Hb). The early-stage products in the Maillard reaction have been found in proteins such as Hb, plasma protein, nail protein and hair protein, and can be used as clinical indicators of blood glucose control in diabetic patients.[3,5,6]

In the Maillard reaction, early-stage products, after the formation of Schiff bases and the Amadori rearrangement, are converted into advanced products through gradual multiple rearrangements and dehydrations.[9] Advanced-stage products are cross-linked and exhibit fluorescence.[8,9] The accumulation of these products in the tissue has attracted attention in relation to the assessment of the progression of diabetic complications and aging.[1] We measured the advanced-stage products of the Maillard reaction in the lens nucleus of cataracts from diabetic patients and found that levels of advanced-stage products are related to the development of diabetic cataracts.[7] It appears that there is little turnover of lens crystallins. Thus, glycation in the lens nucleus may result in formation and accumulation of advanced-stage products over the course of years. There have been few reports on the relationship between the etiology of diabetic complications and advanced-stage products of the Maillard reacion. We have investigated the relationship between the degree of diabetic complications such as neuropathy and retinopathy and levels of advanced-stage products accumulated prior to surgery in the lens nucleus, which can be regarded as the sum of glycation over time, i.e., an indicator of blood glucose levels over time.

MATERIALS AND METHODS

Thirty-five patients with non-insulin dependent diabetes mellitus (14 males and 21 females between the ages of 44 and 88 yr; mean±S.D. of 67.4±8.5 yr), were included in this study of diabetic retinopathy. The patients were divided the degree of

diabetic retinopathy after the resection of their cataracts, according to three groups, i.e., those without retinopathy, those with background retinopathy, and those with preproliferative and proliferative retinopathy.

Of the thirteen diabetic patients without retinopathy (mean age, 66.1±7.4 yr; duration of diabetes, 8.1±6.6 yr; mean±S.D.), two received injections of insulin, 6 received oral hypoglycemic drugs, and 5 received diet therapy. Of the twelve patients with background retinopathy (mean age, 70.6±3.6 yr; duration of diabetes, 10.9±7.5 yr), 6 received injections of insulin, 5 received oral hypoglycemic drugs, and 1 received diet therapy. Of the ten patients with preproliferative and proliferative retinopathy (mean age, 65.2±12.7 yr; duration of diabetes, 12.5±9.8 yr), 4 received injections of insulin, 5 received oral hypoglycemic drugs, and 1 received diet therapy.

Twenty-three patients with non-insulin-dependent diabetes mellitus (12 males and 11 females between the ages of 52 and 88 yr; mean±S.D. of 68.7±10.2 yr), were entered into the study with diabetic neuropathy. Of these diabetic patients, 9 received injections of insulin, 10 received oral hypoglycemic drugs and 4 received diet therapy. The duration of diabetes (mean±S.D.) was 9.6±7.7 yr.

Peripheral nerve conduction velocity was measured using routine method at a room temperature of 22-25C. The motor nerve conduction velocity (MCV) and the sensory nerve conduction velocity (SCV) were measured in the peroneal nerve and in the sural nerve, respectively.

The surgically isolated cataractous lens was divided into three parts: capsule, cortex, and nucleus. The inner third of the lens was regarded as the nucleus.

About fifty milligrams of the sample were hydrolyzed with 6 N HCL for 30 hrs at 95C and the hydrolysate was evaporated according to the method as described in recent report.[7] The dried sample was subsequently dissolved in distilled water, and subjected to high-performance liquid chromatography (HPLC). Waters HPLC (Tokyo, Japan) and an ODS-120T column (Toyo Soda, Co., Ltd., Tokyo, Japan), 4.6 mm x 25 cm, were used. As a solvent 7 mM H_3PO_4 was employed. The chromatographic conditions were as follows: flow rate, 1 ml/min; detector sensitivity, 0.04 absorbance units of

full scale; UV detector wavelength, 280 nm and 254 nm; excitation wavelength, 370 nm; emission wavelength, 440 nm. If the volume of a sample required to obtain the peak area "S" of tyrosine, which was used as an internal standard in the determination of furosine which is derived from fructose-lysine on acid-hydrolysis, is "V", the volume of a sample for the determination of advanced-stage products is calculated as: (V/S) x 200 (μl). The substance represented by a peak elution in this volume of sample was determined by fluorometry, and the peak area (μV.sec) which was designated peak late No.1 (peak L_1), was regarded as an advanced-stage product value.

The levels of fasting plasma glucose (FPG) and of HbA_{1c} were measured prior to the resection of cataracts.

Serum glucose was measured by the glucose oxidase method, and HbA_{1c} was measured by HPLC using the column chromatography system Auto A_{1c} (Kyoto Daiichi Co., Ltd., Kyoto, Japan).

Statistical analysis was performed by Student's t test.

RESULTS

The peak L_1 levels were 7.0±2.0 x 10^3 μV.sec in the retinopathy-free group, 9.9±2.6 x 10^3 μV.sec in the background retinopathy group, and 13.2±4.0 x 10^3 μV.sec in the preproliferative and proliferative retinopathy group (Fig. 1). The differences among

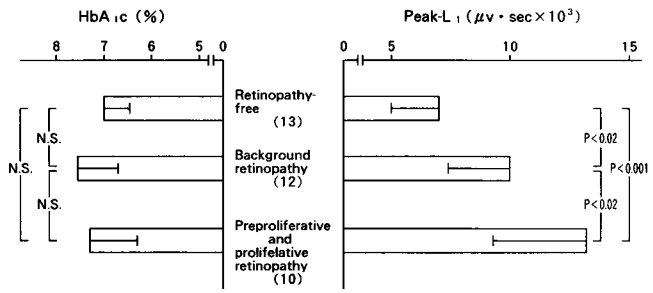

Fig. 1 Relationship between the grade of diabetic retinopathy and the levels of peak L_1 in the nucleus of diabetic cataract and of HbA_{1c} at the time immediately before the surgery. The data represent the mean±S.D. The figures in parentheses indicate the number of cases.

the levels in the three groups were significant. The level of HbA_{1c} immediately prior to surgery tended to be slightly higher in the background and preproliferative and proliferative groups than in the retinopathy-free group. However, there were no statistically significant differences among the three groups.

The level of the peak L_1 in cataractous lens nucleus in diabetic patients correlated significantly with the MCV of peroneal nerve and the SCV of sural nerve (Table 1). However, there were no significant differences between the levels of FPG and HbA_{1c} immediately prior to surgery and the MCV and the SCV, respectively.

Table 1 Correlations between the MCV in peroneal nerve and the SCV in sural nerve and the levels of FPG, HbA_{1c} and advanced Maillard product (peak L_1) in cataractous lens nucleus, respectively.
Parentheses represent the number of cases.

	Coefficient correlation		
	FPG	HbA_{1c}	Peak L_1
MCV	N.S. (22)	N.S. (17)	-0.56 ($P<0.01$) (22)
SCV	N.S. (20)	N.S. (16)	-0.52 ($P<0.02$) (20)

DISCUSSION

The relationship between the blood glucose level and the progression of diabetic complications has been attracting attention. Recently, HbA_{1c} has been measured as an indicator of the long-term blood glucose control. In the present study, although there was no significant difference in mean age among the three groups of patients, the members of the retinopathy-free group tended to have had a slightly shorter duration of illness and had slightly lower levels of HbA_{1c}. However, the differences were not statistically significant. Thus, there was no remarkable differ-

ence in blood glucose levels in the three groups in the present study. Considering that the level of HbA_{1c} reflects the blood glucose level during the previous one to two months or so, it does not reflect the total sum of blood glucose levels over the long period of duration of the diabetes. Since lens protein is a long-lived protein,[4] glycation, and in particular the levels of advanced products of the Maillard reaction in cataracts are considered to reflect the sum of accumulated glycation whose formation is to a great extent dependent on the long-term blood glucose levels.[7] If we assume that glycation is involved in the etiology of diabetic complications,[1] it is possible that levels of advanced products in the lens may to some extent reflect the severity of diabetic complications. On this assumption, we investigated the levels of advanced products in diabetic patients in relationship to the severity of diabetic neuropathy and retinopathy. These results of the present study suggest that glycation plays an important role in the etiology of diabetic complications. Considering the life span of lens protein, if the advanced products of glycation in a cataract are present in the lens protein, then it may be a new indicator, collectable from the living body, of the sum of glycation or the sum of blood glucose levels up to the time of extraction of the cataract.

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