Rapid communication

High levels of erythrocyte aldose reductase and diabetic retinopathy in NIDDM patients

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Summary Erythrocyte aldose reductase was determined in 90 NIDDM patients by a two-site ELISA using recombinant human aldose reductase. The level of aldose reductase did not correlate with age, duration of diabetes, fasting blood glucose and HbA_{1c} of the patients. Among 38 patients with diabetes for more than 10 years, aldose reductase in those with retinopathy (including non-proliferative and proliferative) was significantly higher than in those without, while no dif-

ference in the means of the average HbA_{1c}, maximum and minimum blood pressure levels was observed between the two groups. The results indicate that the level of aldose reductase in the erythrocyte of diabetic patients is associated with the presence of retinopathy. [Diabetologia (1994) 37: 328–330]

Key words Aldose reductase, diabetic retinopathy, erythrocyte.

Aldose reductase (EC 1.1.1.21) is a member of the NADPH-dependent aldo-keto reductase family which catalyses the reduction of glucose to the corresponding sugar alcohol, sorbitol. Based on a wealth of experimental data, the key role of this enzyme in the development of various diabetic complications such as neuropathy [1, 2], retinopathy [3, 4] and cataracts [5] was postulated. There has been, however, little study on the association between susceptibility to these complications and the level of tissue aldose reductase itself in human subjects.

We recently developed a specific two-site ELISA based on purified recombinant human aldose reductase expressed in a baculovirus system [6, 7]. Unlike the assay of enzyme activity which requires prior isolation of the enzyme from the coexisting reductase family

with overlapping substrate specificity, this immunoassay method enabled direct measurement of aldose reductase in human tissues including erythrocytes. Determination of erythrocyte aldose reductase in healthy individuals indicated a variable level of the enzyme expression in a human population [7]. This led us to undertake the investigation on erythrocyte aldose reductase in diabetic patients with special reference to the presence of complications.

Subjects and methods

Patients: Blood was drawn from 90 NIDDM patients after informed consent was obtained. The study was performed in accordance with the principles of the Declaration of Helsinki. The patients were attending the out-patient clinic at the Diabetic Center, Tokyo Women's Medical College. The presence of background or proliferative retinopathy was assessed by fluorescein angiography. Determinations of the enzyme level were made without previous knowledge of the origin of the sample.

Immunoassay of erythrocyte aldose reductase

Erythrocyte fraction was prepared from heparinized whole blood as previously described [7]. Following the addition of an equal volume of 20 mmol/l phosphate buffer (pH 7.0), erythro-

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus.

Table 1. Clinical features of the NIDDM patients and correlation coefficients with erythrocyte aldose reductase

	Sex (male/female)	Age (years)	Duration ^a (years)	Fasting blood glucose (mmol/l)	HbA _{1c} (%)
Total (<i>n</i> = 90)	54/36	55 ± 12	9.5 ± 7.6	9.1 ± 2.9	8.7 ± 1.8
<i>r</i>		-0.01 (NS)	0.02 (NS)	-0.08 (NS)	-0.07 (NS)

Values are mean ± SD. ^a Duration after clinical diagnosis. HbA_{1c}, average of HbA_{1c} levels during the past 2 years. *r*, Pearson's correlation coefficient. The mean ± SD of erythrocyte aldose reductase in these patients was 4.46 ± 1.37 ng/mg Hb

cytes were haemolysed by two cycles of freezing and thawing using a dry ice acetone bath. The level of aldose reductase was determined in the supernatant fraction of the haemolysate obtained by centrifugation at 15,000 × *g* for 15 min. To assess the inter-assay variation, a frozen stored aliquot of the same haemolysate preparation was run in each assay as internal quality control. Haemoglobin concentration in the sample was measured by the haemoglobinocyanide method [8].

Statistical analysis

For the comparison of two means, Student's *t*-test was used and association was evaluated by Pearson correlation (TTest and CORR procedures in PC-SAS, Release 6.0.3, SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

The clinical features of the diabetic patients studied are summarized in Table 1. Between men and women no difference in the mean enzyme level was observed (data not shown). No statistically significant correlation

was observed between the enzyme level and age, duration of diabetes, fasting blood glucose and the average HbA_{1c} level of the patients. The mean ± SD of erythrocyte aldose reductase in these patients (*n* = 90) was 4.46 ± 1.37 ng/mg Hb. When the enzyme level of the patients was compared with that of 33 healthy individuals, 4.75 ± 1.21 ng/mg Hb [7], no significant difference was demonstrated between the two groups.

Among 38 patients with diabetes for more than 10 years, the level of aldose reductase was significantly higher in those with retinopathy than in those without any complications (Fig. 1). Among 11 patients with diabetes for more than 20 years, the level of aldose reductase was associated with the severity of retinopathy (data not shown). On the other hand, the mean ± SEM of the average HbA_{1c} levels in the individual patients recorded during the most recent 2-year period were 8.4 ± 0.4% (*n* = 15) for those without complications and 8.5 ± 0.3% (*n* = 23) for those with retinopathy. Between the two groups no statistical difference was observed in the mean value of SD of HbA_{1c} levels in the individual patients. Furthermore, there was no statistical difference in the means of the average maximum and minimum blood pressure recorded during the most recent 2-year period between the two groups (data not shown). These data imply that glycaemic control as well as blood pressure level of the patients in the two groups were equivalent at least during the observation period.

The results therefore indicate that the level of aldose reductase in erythrocytes of diabetic patients is associated with the presence of retinopathy. In a previous study the activity of glyceraldehyde reductase (aldose-reductase-like enzyme) isolated from the erythrocyte was described to be higher in diabetic patients with retinopathy [9]. Recently Hamada et al. [10] reported on the activity of aldose reductase isolated from erythrocytes of IDDM patients. They found the activity in the fractionated enzyme preparation to be significantly higher in the patients with complications than in those without. Although an entirely distinct strategy for directly measuring the enzyme level was employed, our results were in line with the former findings where the activity was evaluated in an enzyme preparation recovered from erythrocytes of diabetic patients. It thus appears that our present investigation defined the activity determined in the earlier studies as that of aldose reductase itself, and not other members of aldo-keto reductase family.

Fig. 1. Erythrocyte aldose reductase in the patients who were diabetic for more than 10 years. The mean levels of aldose reductase (the mean ± SEM: 3.68 ± 0.23 ng/mg Hb in patients without retinopathy; 4.89 ± 0.38 in patients with retinopathy) are shown by horizontal bars, which are significantly different (*p* < 0.025) by Student's *t*-test. ○, patients without retinopathy (*n* = 15); △, patients with retinopathy (non-proliferative, preproliferative, or proliferative, *n* = 23)

It still remains to be established, however, whether the high erythrocyte aldose reductase level is a causal factor of retinopathy or simply its result. To that end, monitoring the enzyme level in diabetic patients during the course of the disease appears to be necessary. The high sensitivity and specificity of our assay method circumvent the previous laborious work used to isolate aldose reductase, which will reduce the amount of blood samples and cost required. Our method may thus become a useful tool with which to tackle this critical question in diabetic patients. The data on the enzyme level will also aid the optimization of administration of aldose reductase inhibitors when exploring their efficacy in prevention or intervention of the progress of diabetic complications.

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