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Abstract

The relationship between the effect of aldose reductase inhibitors (ARIs) on the activation of the polyol pathway and on diabetic neuropathy has not been fully established. To address this issue, we investigated the effect of epalrestat (150 mg/day), an ARI, on erythrocyte sorbitol levels as an index of polyol activation and on nerve function test results in 43 patients with diabetic peripheral polyneuropathy. After 6 months of epalrestat administration, erythrocyte sorbitol levels did not decrease in patients as a whole. However, a decrease in erythrocyte sorbitol levels during epalrestat administration was significantly correlated with baseline erythrocyte sorbitol levels (ρ = -.47, P < .01): the higher the level at baseline, the greater the decrease after epalrestat treatment. Moreover, the mean sorbitol level during epalrestat treatment was associated with the beneficial effect of epalrestat on vibration sensitivity as measured with a C-128 tuning fork (ρ = -.66, P < .01) and/or a pallesthesiometer TM-31A (ρ = .53, P < .05). On the other hand, erythrocyte sorbitol levels did not reflect the prognosis of nerve conduction velocity. These findings at least partly suggest a causal relationship between polyol activation and the development of diabetic neuropathy. ARI treatment may be clinically useful in the control of polyol activation, especially in patients with excessive accumulation of sorbitol.

Keywords: aldose reductase inhibitor, diabetic neuropathy, epalrestat, erythrocyte sorbitol level

1. Introduction

The polyol pathway is thought to play a major metabolic role in the development of diabetic neuropathy (Greene & Sima, 1992; Sima & Sugimoto, 1999; Zochodne, 1999; Vinik, Pittenger, McNitt, & Stansberry, 2000). Persistent hyperglycemia increases the polyol pathway activity in conjunction with accumulation of sorbitol and fructose in nerves, and this is accompanied by a reduction in *myo*-inositol uptake and inhibition of Na^+/K^+-ATPase, resulting in Na^+ retention, edema, myelin swelling, axoglial disjunction, and nerve degeneration (Greene, Lattimer, & Sima, 1987; Vinik, Pittenger, McNitt, & Stansberry, 2000). Moreover, an increase in the activity of aldose reductase, an enzyme that catalyzes the reduction of glucose to sorbitol in the first step of the polyol pathway, may compete with nitric oxide synthase for NADPH, resulting in a reduction in nitric oxide. This reduction results in reduced nerve blood flow and thus to nerve ischemia and nerve dysfunction (Tilton et al., 1993; Stevens, 1995). Therefore, the inhibition of aldose reductase can be expected to improve and/or prevent diabetic neuropathy.

Over the past two decades, many clinical trials of aldose reductase inhibitors (ARIs) have been performed. However, these trials have not fully proven the efficacy of ARIs for the treatment of diabetic neuropathy (Pfeifer, Schumer, & Gelber, 1997). One reason may be that few studies on humans have demonstrated any clear relationship between the effect of ARIs on diabetic neuropathy and on the activation of the polyol pathway. It is possible that ARI treatment is effective for diabetic patients only when the drug sufficiently suppresses the activity of the polyol pathway. To address this issue, we investigated the effect of epalrestat, an ARI, on

erythrocyte sorbitol levels as an index of polyol activation (Malone, Knox, Benford, & Tedesco, 1980; Hamada et al., 1995) as well as on nerve function test results of patients with diabetic peripheral polyneuropathy.

2. Patients and methods

2.1. Patients

Adult patients with diabetic peripheral polyneuropathy were recruited from 5 institutions specializing in diabetes in the Hokuriku area of Japan. These patients had been following fixed therapeutic regimens for their diabetes for the preceding 3 months with their blood glucose levels being kept steady. Diabetic polyneuropathy was diagnosed according to the criteria proposed by Dyck (1988). Candidates were excluded if they had other major diseases such as cancer, liver cirrhosis, and severe renal failure, or if they were pregnant. Informed consent was obtained from all subjects.

A total of 49 patients were recruited for this study. During the study period, 5 patients were lost to follow up, and one patient discontinued epalrestat treatment due to vomiting. The background of the 43 patients who completed the study is given in Table 1. The mean duration of diabetes was 13.6 years, and 93% of the patients were under medical treatment with oral hypoglycemic agents or insulin. Retinopathy was diagnosed in 65% and nephropathy in 44%. Before epalrestat administration, 91% of the patients had at least one subjective symptom of

peripheral polyneuropathy, with numbness being the most common symptom (88% of the patients).

2.2. Study protocol

Epalrestat was administered to subjects for at least 6 months. The dosage was 150 mg/day: one 50-mg Kinedak tablet (Ono Pharmaceutical Co., Osaka, Japan) was orally administered three times daily before each meal. No drugs which might have a possible effect on neuropathy, including vitamin B12, peripheral vasodilators, analgesics, anti-convulsants, and Chinese herbal remedies, were administered at any time during the study period.

Vibration sensitivity was measured with a C-128 tuning fork and/or a pallesthesiometer TM-31A at the medial malleolus. Motor and sensory nerve conduction velocities (MNCV and SNCV) were determined at the median nerve. All nerve function tests were performed under the same conditions before epalrestat administration, and after 6 months of drug administration.

Blood samples for the measurements of erythrocyte sorbitol and hemoglobin A_{1c} (HbA_{1c}) levels were obtained the morning after an overnight fast, before and after epalrestat administration. Erythrocyte sorbitol levels were determined with an enzymatic assay as previously described (Malone, Knox, Benford, & Tedesco, 1980), and expressed as nmol/g hemoglobin (Hb).

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2.3. Statistical analyses

Data are presented as means ± S.D. The Wilcoxon's signed-rank test was used to compare the data before and after epalrestat administration. Correlations were assessed with the aid of Spearman's rank order correlation coefficient. Statistical significance was defined as $P < .05$. All analyses were performed with the computer program StatView, version 5.0 (SAS Institute, Cary, NC).

3. Results

At baseline, erythrocyte sorbitol levels tended to correlate with HbA_{1c} levels ($\rho = .32$, $P = .056$). However, this correlation completely disappeared after 6 months of epalrestat administration ($\rho = .02$, $P = .90$), indicating that treatment with epalrestat affected erythrocyte sorbitol levels. Interestingly, the higher the erythrocyte sorbitol level at baseline, the greater the decrease after epalrestat administration (Fig. 1; $\rho = -.47$, $P = .002$). Although HbA_{1c} levels slightly decreased from 7.4% to 7.2% after epalrestat administration, erythrocyte sorbitol levels did not significantly change in patients as a whole (Table 2). In addition, epalrestat administration did not affect any nerve function except for vibration sensitivity as measured with a TM-31A. All function test results, however, improved in approximately half of the patients (data not shown). Thus, with respect to sorbitol levels and nerve function, both responders and non-responders to epalrestat may have been present.

We next examined whether the effect of epalrestat on erythrocyte sorbitol levels might reflect changes in nerve function during epalrestat treatment. Table 3 shows the correlation of erythrocyte sorbitol level with percentage change in nerve function test results. Erythrocyte sorbitol levels at baseline significantly correlated with percentage change in vibration sensitivity test results as measured with a C-128. Additionally, sorbitol levels after treatment tended to correlate with percentage changes in C-128 and TM-31A. Furthermore, the mean sorbitol level during treatment significantly correlated with percentage changes in C-128 and TM-31A. With regard to HbA_{1c} levels, a similar significant correlation with vibration test results was not found (Table 4).

On the other hand, nerve conduction velocities did not correlate with these erythrocyte sorbitol levels at all, whereas SNCV correlated with HbA_{1c} levels after epalrestat administration. Thus, erythrocyte sorbitol levels measured during epalerestat treatment might partly reflect changes in nerve function.

4. Discussion

In the present study, 6-month treatment with epalrestat did not decrease erythrocyte sorbitol levels in patients as a whole. However, a decrease in erythrocyte sorbitol levels during epalrestat administration significantly correlated with initial baseline erythrocyte sorbitol levels. Moreover, the mean sorbitol level during epalrestat treatment was associated with the prognosis of vibration sensitivity. To the best of our knowledge, this is the first study to demonstrate a correlation between erythrocyte sorbitol levels and changes in nerve function tests during ARI administration.

Nakayama et al. (2001) have reported that 24 weeks of epalrestat treatment ameliorated papillary light reflex and F-wave latency, but not nerve conduction velocity, in patients with mild diabetic neuropathy. On the other hand, ARI, zenarestat, has shown to reduce both erythrocyte and nerve sorbitol contents in a dose-dependent manner and that this sorbitol suppression was accompanied by significant improvements in the slowing down of nerve conduction velocity and the loss of small myelinated nerve fibers in patients with diabetic polyneuropathy (Greene, Arezzo, & Brown, 1999). Therefore, the effects of zenarestat, not only on erythrocyte sorbitol levels, but also on nerve function, are seemingly greater than those of epalrestat found in Nakayama's and our studies. However, this difference in treatment effect may be caused by several factors, including the duration of ARI treatment, the level of glycemic control, and the severity of neuropathy in the participants. In the zenarestat study, the effects on nerve function were evaluated after 52 weeks of zenarestat administration. Twenty-four weeks of epalrestat treatment might be too

short to investigate the effect on nerve conduction velocity. Moreover, HbA_{1c} levels at baseline were less than 7.0% in 50% of our subjects, whereas patients with good glycemic control were excluded in the zenarestat study. Furthermore, the other factors may have greater effects, because a clinical study (Brown et al., 2004) has demonstrated that the changes of nerve function test results during 12 months were independent of baseline HbA_{1c} stratification in a large diabetic peripheral neuropathy population. It has been speculated that the ability to improve or preserve nerve function may be limited by disease severity (Hotta et al., 2001). The zenarestat study targeted only patients with mild or moderate neuropathy and excluded patients with advanced nerve damage. Subjects with relatively severe neuropathy were included in the present study, resulting in a high percentage of patients with retinopathy and/or nephropathy. Thus, regarding erythrocyte sorbitol levels and nerve function, both ARI responders and non-responders appear to be present. Asano et al. (2004) have reported that the daily administration of another ARI fidarestat suppressed erythrocyte sorbitol levels in diabetic patients much more potently than treatment with epalrestat. Further studies are needed to evaluate the differences of treatment effects among ARIs.

In placebo-controlled double-blind studies (Goto, Hotta, Shigeta, Sakamoto, & Kikkawa, 1995; Hotta et al., 2001), ARI treatment has been reported to improve not only nerve conduction velocity but also various subjective symptoms of diabetic neuropathy. Although the present study did not employ a controlled design, numbness was improved in 78% of the patients during the study period, a finding that is compatible with results reported in a previous controlled study with epalrestat (Goto, Hotta, Shigeta, Sakamoto, & Kikkawa, 1995). Because nerve function test

results are known to bear no relationship to the severity of symptoms (Greene et al., 1981), it is probable that ARI treatment can improve subjective symptoms without any effect on nerve function. Further placebo-controlled studies are needed to clarify whether erythrocyte sorbitol levels are associated with the efficacy of ARIs in improving subjective symptoms of diabetic neuropathy.

In conclusion, mean erythrocyte sorbitol levels during epalerestat treatment could reflect the prognosis of vibration sensitivity in patients with diabetic peripheral polyneuropathy. Our findings support the theory that increased activity of the polyol pathway plays a role in the development of diabetic neuropathy. ARI treatment may be clinically useful in the control of polyol activation, at least in patients with excessive sorbitol accumulation.

Appendix

Kanazawa University Multicenter Diabetes Study Group includes the authors and the following physicians: T. Swada, E. Takazakura, H. Hayakawa, K. Kawai, Y. Kato, S. Muramoto, T. Ikeda, Y. Noto, K. Asayama, H. Miyakoshi, S. Uchida, N. Hirai, K. Osawa, Y. Bando, Y. Ieki, T. Hayakawa, G. Okabe, H. Takitani, M. Taniguchi, and H. Yamashita.

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Figure Legend

Figure 1

Correlation between baseline level and change in erythrocyte sorbitol level during epalrestat treatment. The higher the erythrocyte sorbitol level was at baseline, the greater the decrease after 6 months of epalrestat administration (ρ = -.47, P = .002).

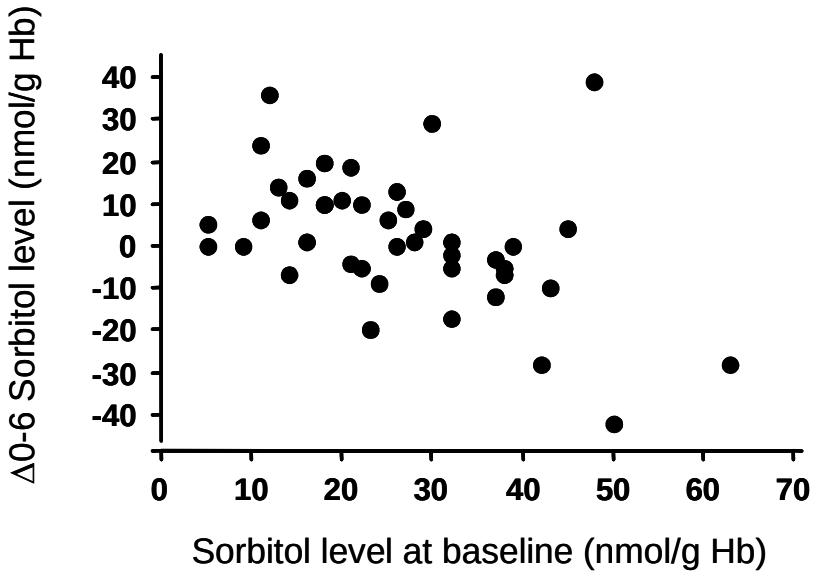
Δ0-6 Sorbitol level (nmol/g Hb)
40
30
20
10
0
-10
-20
-30
-40
0
10
20
30
40
50
60
70
Sorbitol level at baseline (nmol/g Hb)

Fig. 1

Table 1

Background of subjects

Total number	43
Sex	
Men	19 (44)
Women	24 (56)
Age (years)	64.8 ± 9.7
Type of diabetes	
Type 1	2 (5)
Type 2	41 (95)
Duration of diabetes (years)	13.6 ± 7.2
Treatment	
Diet	3 (7)
Oral hypoglycemic agents	18 (42)
Insulin	22 (51)
Retinopathy	28 (65)
Nephropathy	19 (44)

Data are expressed as n (%), or means ± S.D.

Table 2

Changes in erythrocyte sorbitol and HbA1c levels, and nerve function tests

	n	Baseline	6 months later
Erythrocyte sorbitol level (nmol/g Hb)	43	26.3 ± 12.9	28.5 ± 15.3
HbA_{1c} (%)	37	7.4 ± 1.1	7.2 ± 1.1*
C-128 (s)	24	7.1 ± 3.0	7.5 ± 2.3
TM-31A (μm)	19	52.4 ± 36.4	33.1 ± 17.0*
MNCV (m/s)	34	49.2 ± 6.3	49.5 ± 6.8
SNCV (m/s)	19	49.0 ± 8.0	45.2 ± 13.2

Data are means ± S.D. *P <.05 vs. baseline.

Table 3

Correlation of erythrocyte sorbitol level with percentage change in the nerve function tests before and after 6 months of epalrestat administration

	Nerve function tests	ρ	*P*
Baseline	C-128	-.62	< .01
	TM-31A	.36	.13
	MNCV	-.03	.88
	SNCV	.02	.95
6 months later	C-128	-.39	.06
	TM-31A	.41	.08
	MNCV	.25	.15
	SNCV	-.16	.51
Mean	C-128	-.66	< .01
	TM-31A	.53	< .05
	MNCV	.12	.51
	SNCV	-.11	.65

Table 4

Correlation of HbA_{1c} level with percentage change in the nerve function tests before and after 6 months of epalrestat administration

	Nerve function tests	ρ	*P*
Baseline	C-128	-.43	.06
	TM-31A	.46	.06
	MNCV	.20	.30
	SNCV	-.29	.30
6 months later	C-128	.13	.56
	TM-31A	.04	.87
	MNCV	-.02	.93
	SNCV	-.65	< .05
Mean	C-128	-.21	.37
	TM-31A	.26	.29
	MNCV	.12	.52
	SNCV	-.49	.08