

Aldose Reductase Inhibition Ameliorates Pupillary Light Reflex and F-Wave Latency in Patients With Mild Diabetic Neuropathy

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OBJECTIVE — The present study was conducted to investigate the effect of an aldose reductase inhibitor, epalrestat, on autonomic and somatic neuropathy at an early stage in type 2 diabetic patients by assessing the pupillary light reflex and minimum latency of the F-wave.

RESEARCH DESIGN AND METHODS — A total of 30 diabetic patients with subclinical or mild diabetic neuropathy were randomly allocated to a control group ($n = 15$) and epalrestat (150 mg/day) group ($n = 15$). After 24 weeks, the pupillary light reflex test, cardiovascular autonomic function tests, and nerve conduction study were performed.

RESULTS — The beneficial effect of epalrestat on the pupillary light reflex was observed in the minimum diameter after light stimuli ($P = 0.044$), constriction ratio ($P = 0.014$), and maximum velocity of constriction ($P = 0.008$). Among cardiovascular autonomic nerve functions, the ratio of the longest expiratory R-R interval to the shortest inspiratory R-R interval during deep breathing was significantly improved by epalrestat ($P = 0.037$). Minimum latencies of F-wave of median and tibial motor nerves were significantly shortened by epalrestat ($P = 0.002$ and $P = 0.001$, respectively); however, no significant effects were observed in motor or sensory nerve conduction velocity.

CONCLUSIONS — These observations suggest that epalrestat may have therapeutic value at the early stage of diabetic neuropathy and that the pupillary light reflex and minimum latency of F-wave may be useful indicators of diabetic neuropathy.

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D iabetic neuropathy is generally classified into somatic and autonomic neuropathy. Although somatic neuropathy can be characterized by symptoms such as numbness, paresthesia, and

abnormal sensation, the symptoms of autonomic neuropathy do not appear until the advanced stage.

Diabetic autonomic neuropathy causes functional disorders of many or-

gans, such as cardiovascular, gastrointestinal, genitourinary, metabolic, and pupillary dysfunctions. Among these, cardiovascular autonomic neuropathy may increase the risk of sudden death and affects the mortality of diabetic patients (1,2). Therefore, the diagnosis and treatment of autonomic neuropathy at an early stage is important for the management of diabetic patients.

It is well known that diabetic autonomic neuropathy develops within a short duration of diabetes even when somatic neuropathy is not apparent (3,4). Furthermore, the abnormalities in pupillary functions can be detected earlier than those in cardiovascular autonomic functions and are considered the earliest signs of diabetic autonomic neuropathy (5–10). Therefore, the pupillary light reflex test has been used for evaluating diabetic autonomic neuropathy.

It has been demonstrated that strict glycemic control can decrease the incidence of diabetic complications, including autonomic neuropathy (11). However, it is impossible to completely prevent the development of diabetic complications by glycemic control alone, except by performing a pancreas transplantation. Therefore, different therapeutic agents have been under development based on the pathogenic hypotheses of diabetic complications (12). Among those agents, the efficacy of aldose reductase inhibitors (ARIs) on diabetic neuropathy has been most extensively investigated (13). However, many studies failed to demonstrate the usefulness of ARIs for the treatment of diabetic neuropathy. Those studies, however, included patients with established neuropathy and were performed with nerve function tests that are less sensitive, which may explain to some extent the negative results. Therefore, clinical studies of the effects of ARIs on diabetic neuropathy should be conducted with patients without clinically overt neuropathy.

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Abbreviations: AC, the maximum acceleration of constriction (mm/s^2); ANOVA, analysis of variance; ARI, aldose reductase inhibitor; CR, constriction ratio [$\text{CR} = (\text{D1} - \text{D2})/\text{D1}$]; D1, initial diameter before light stimulus (mm); D2, minimum diameter after light stimulus (mm); E/I ratio, ratio of the longest expiratory R-R interval to the shortest inspiratory R-R interval; NCV, nerve conduction velocity; QTc, corrected QT time; T1, latency to constriction (ms); T2, time to half of the maximum constriction (ms); T3, time to the maximum constriction (ms); T5, recovery time (time to 63% redilatation) (ms); VC, the maximum velocity of constriction (mm/s); VD, the maximum velocity of dilatation (mm/s).

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

thy or those with mild neuropathy, and with sensitive nerve function tests.

The present study was conducted to investigate the effect of an ARI, epalrestat (14,15), on autonomic nerve functions in patients with mild diabetic neuropathy by using the pupillary light reflex test. The effect on somatic nerve functions was also examined by measuring the minimum latency of the F-wave, which is a sensitive and reliable parameter of nerve conduction (16–19).

RESEARCH DESIGN AND METHODS

Subjects

The study subjects were recruited from the patients who consecutively visit the diabetes clinic of Nagoya University Hospital. A total of 30 diabetic patients who have mild or no symptoms (e.g., numbness, paresthesia, or abnormal sensation in the distal end of lower extremities) of diabetic neuropathy with mild deficits in vibration perception threshold or tendon reflexes were enrolled in the present study. Patients with severe symptoms (e.g., pain, continuous paresthesia, or lack of sensation), peripheral vascular disease, or any other causes of peripheral neuropathy, including severe liver and renal dysfunction, malignant diseases, hypothyroidism, or excessive alcoholic intake, were excluded. We also excluded patients with diseases interfering with cardiovascular reflexes (e.g., ischemic heart disease, heart failure, or valvular heart disease), those with past histories of ocular operations or diseases affecting pupillary reflexes (e.g., preproliferative or proliferative diabetic retinopathy, iris disorders, glaucoma, uveitis, or central nervous system diseases), and those receiving cardiac glycosides, anticholinergics, sympathomimetics, β -blockers, or other agents affecting the heart rate variability or ophthalmic agents affecting the pupillary light reflexes. All patients had been stabilized in terms of their glycemic control for 3 months before the study.

Study design

A randomized controlled open study was conducted over 24 weeks. After obtaining informed consent, the baseline neurological function was established, and the patients were randomly allocated to either the control or epalrestat (150 mg/day) group. Neurological assessments includ-

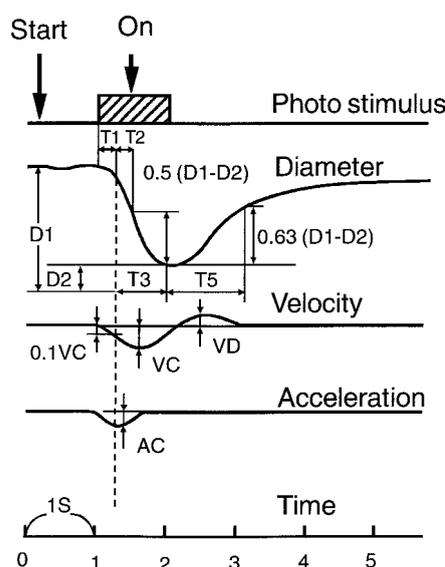


Figure 1—Schema of pupillography.

ing neurological examination, nerve conduction tests, pupillary light reflex test, and cardiovascular autonomic function tests were performed by investigators who were blinded to experimental group assignment before entry into the trial and at the end of the trial. During the study, the patients continued their antidiabetic therapy, such as diet, exercise, oral hypoglycemic agents, or insulin, and no attempt was made to alter the therapy or the level of glycemic control.

Pupillary light reflex

The pupillary light reflex test was performed on both eyes using a portable infrared video-pupillography system (Irisorder model C-2514; Hamamatsu Photonics, Hamamatsu, Japan), which consists of a goggle-shaped measurement portion with a charged coupled device camera and a control portion with a video monitor and microcomputer with software controlling the light stimulus and data analysis. After setting the goggles closely on the patient's face and fully covering the patient's eyes, 15-min dark adaptation was allowed. Schema of the pupillography and different parameters of pupillary light reflex test are shown in Fig. 1. The measured parameters of the pupillary light reflex test are as follows: D1, initial diameter before light stimulus (mm); D2, minimum diameter after light stimulus (mm); CR, constriction ratio [$CR = (D1 - D2)/D1$]; T1, latency to constriction (ms); T2, time to half of the max-

imum constriction (ms); T3, time to the maximum constriction (ms); T5, recovery time (time to 63% redilatation) (ms); VC, the maximum velocity of constriction (mm/s); VD, the maximum velocity of dilatation (mm/s); and AC, the maximum acceleration of constriction (mm/s^2).

Cardiovascular autonomic nerve functions

After 5 min rest on a bed in the supine position, an electrocardiograph (Auto-cardiner model FCP-4129; Fukuda Electronics, Tokyo) was used to measure the R-R interval of 100 heart beats and calculate automatically the corrected QT time (QTc) and resting heart rate variation. The ratio of the longest expiratory R-R interval to the shortest inspiratory R-R interval (E/I ratio) during deep breathing at a rate of six times per minute was calculated. Postural changes of blood pressure were obtained by measuring the blood pressure in the resting supine position on a bed and after standing up.

Somatic nerve functions

The nerve conduction study was performed in the left upper and lower extremities with an electromyograph system (Viking Four; Nicolet, Madison, WI). Motor nerve conduction velocity (NCV), minimum latency of F-wave in median and tibial nerves corrected for height, and sensory NCV in median and sural nerves were measured. Vibration perception thresholds were determined on the medial malleolus in the left lower extremities using a C64 tuning fork (20), which can quantify the severity of deficits in vibratory sensation (score 8: normal; score 1: severest).

Glycemic control

To assess glycemic control, stable HbA_{1c} levels were measured every 4 weeks.

Statistical analysis

Results are expressed as means \pm SEM. Statistical analyses were conducted with the Statview program (Abacus Concepts, Berkeley, CA). The differences between the two groups were assessed by analysis of variance (ANOVA). Paired two-tailed Student's *t* tests were performed for comparison of the differences between baseline and follow-up measures within groups. A putative treatment effect was evaluated with the two-tailed test, which compared the differences between base-

Table 1—Clinical characteristics of the patients

	Epalrestat	Control
<i>n</i>	15	13
Sex (M/F)	10/5	10/3
Age (years)	60.9 ± 1.8	62.2 ± 1.8
Duration of diabetes (years)	14.1 ± 1.6	11.2 ± 2.3
HbA _{1c} (%)	7.71 ± 0.22	7.39 ± 0.31
Therapy		
Diet alone	2	1
Oral hypoglycemic agent	7	7
Insulin	6	5
Symptomatic somatic neuropathy	6	5

Data are *n* or means ± SEM.

line and follow-up values observed in both groups. ANOVA for repeated measures was also used. Significance was defined as $P < 0.05$.

RESULTS— At the start of this study, 30 patients were enrolled in the study, and 15 patients each were allocated to either the control group or epalrestat group. Two male patients in the control group were withdrawn after entering the study because of emigration or inappropriate medication. Thus, 15 patients in the epalrestat group and 13 patients in the control group completed the study.

Clinical characteristics

The clinical characteristics of the two groups were well matched (Table 1), and no significant differences were observed with regard to sex, age, duration of diabetes, or therapy for diabetes. Although six patients in the epalrestat group and five patients in the control group had very mild symptoms of somatic neuropathy,

they had no severe symptoms or any symptoms of autonomic neuropathy (e.g., orthostatic dizziness or gastroparesis). Other patients (eight in the control group and nine in the epalrestat group) had no symptoms of somatic or autonomic neuropathy. Thus, from the viewpoint of the subjective symptoms, no differences were observed in the severity of diabetic neuropathy between the two groups.

Glycemic control

There were no significant differences in the HbA_{1c} levels at the beginning of this study between the two groups (control: 7.39 ± 0.31%; epalrestat: 7.71 ± 0.02%, $P = 0.453$) (Table 1). During the study period, HbA_{1c} levels similar to those at the beginning of the study were maintained and were 7.39 ± 0.29 and 7.69 ± 0.27% in the control and epalrestat group, respectively, at the end of the study ($P = 0.404$).

Pupillary light reflex

There were no significant differences in any parameters of the light reflex test between the two groups at baseline. No changes in D1 or D2 between the beginning and the end of the study were observed in the control group. In the epalrestat group, on the other hand, D2 was decreased at the end of the study compared with that at the beginning, and the epalrestat group demonstrated a significant decrease in D2 compared with the control group ($P = 0.044$) (Table 2). CR was decreased and increased in the control and epalrestat group, respectively, at the end of the study, and the epalrestat group showed a significant increase in CR compared with the control group ($P = 0.014$). The follow-up values of T1, T2, T3, and T5 were not significantly different from those of the baseline either in the control or epalrestat group. Although VC, VD, and AC were slightly worsened and improved in the control and epalrestat group, respectively, a significant amelioration by epalrestat was observed only in VC ($P = 0.008$) but not in VD or AC.

Cardiovascular autonomic nerve functions

No significant differences in any parameter of cardiovascular autonomic nerve functions at the beginning of the study were observed between the two groups. Treatment with epalrestat had no significant effects on the resting heart rate variation, QTc at rest, or postural change in systolic blood pressure but significantly increased the E/I ratio during deep breathing ($P = 0.037$) (Table 3).

Table 2—Effect of epalrestat on the pupillary light reflex test

Parameter (normal value)	Epalrestat		Control		<i>P</i>
	Before	After	Before	After	
D1 (mm) (6.42 ± 0.12)	5.08 ± 0.13	5.01 ± 0.11	4.74 ± 0.24	4.70 ± 0.25	0.852
D2 (mm) (4.22 ± 0.09)	3.67 ± 0.11	3.48 ± 0.08	3.42 ± 0.15	3.44 ± 0.17	0.044
CR (0.334 ± 0.007)	0.275 ± 0.016	0.294 ± 0.015	0.253 ± 0.023	0.244 ± 0.025	0.014
T1 (ms) (251 ± 3)	259 ± 5	252 ± 6	266 ± 11	267 ± 10	0.307
T2 (ms) (321 ± 7)	240 ± 7	233 ± 10	260 ± 27	262 ± 27	0.337
T3 (ms) (1,086 ± 21)	918 ± 37	897 ± 41	836 ± 48	824 ± 52	0.824
T5 (ms) (1,610 ± 54)	1,354 ± 44	1,346 ± 50	1,331 ± 119	1,367 ± 102	0.738
VC (mm/s) (4.54 ± 0.10)	4.00 ± 0.19	4.22 ± 0.19	3.64 ± 0.30	3.49 ± 0.34	0.008
VD (mm/s) (2.15 ± 0.09)	1.65 ± 0.06	1.70 ± 0.07	1.70 ± 0.07	1.56 ± 0.09	0.056
AC (mm/s ²) (61.5 ± 2.2)	55.5 ± 1.9	57.9 ± 2.43	54.92 ± 1.91	51.92 ± 2.84	0.145

Data are means ± SEM. *P* values concern the comparison of the differences in the test results before and after the treatment between each treatment group. Normal values given are from Nagoya University Hospital.

Table 3—Effect of epalrestat on cardiovascular autonomic function tests

Parameter	Epalrestat		Control		P
	Before	After	Before	After	
Heart rate variation (%)	2.10 ± 0.28	2.66 ± 0.44	2.35 ± 0.48	2.16 ± 0.62	0.249
Deep breathing E/I ratio	1.20 ± 0.05	1.30 ± 0.06	1.27 ± 0.06	1.17 ± 0.05	0.037
QTc (s)	0.412 ± 0.007	0.414 ± 0.008	0.419 ± 0.008	0.418 ± 0.018	0.792
Postural changes in systolic blood pressure (mmHg)	12.5 ± 4.7	4.5 ± 3.6	9.7 ± 2.7	9.8 ± 2.1	0.052

Data are means ± SEM. P values concern the comparison of the differences in the test results before and after the treatment between each treatment group.

Somatic nerve functions

The motor and sensory NCVs were near the lower limits of normal ranges and showed no differences either between the two groups at the beginning of the study or in changes within each group. On the other hand, minimum latencies of F-wave of median and tibial motor nerve were significantly shortened in the epalrestat group ($P = 0.020$ and $P = 0.011$, respectively) (Table 4). The vibration perception thresholds of both groups were decreased at the beginning of the study with no differences between the control and epalrestat groups. Although there were no significant changes during the study in both groups, the epalrestat group demonstrated a trend toward improvement.

CONCLUSIONS— In the present study, type 2 diabetic patients without clinically overt diabetic neuropathy or with mild diabetic neuropathy were treated with an ARI, epalrestat, for 24 weeks. Epalrestat was demonstrated to be effective in several parameters of autonomic nerve functions such as D1, CR, and VC in the pupillary light reflex test and the E/I ratio during deep breathing as a marker of cardiovascular autonomic

functions. A beneficial effect on somatic nerve functions was also observed in the minimum latency of F-wave but not in NCV.

The basis of this study was that polyol pathway hyperactivity plays an important role in the development of diabetic neuropathy (12,13,21). Different hypotheses for the pathogenesis of diabetic complications besides increased polyol pathway activity have been proposed, including altered protein kinase C activity, increased oxidative stress, and an acceleration of nonenzymatic glycation (12). As far as diabetic neuropathy is concerned, the role of polyol pathway hyperactivity has been most extensively investigated using different ARIs (22). The beneficial effect of ARIs on the development of diabetic neuropathy has been reported in diabetic animals (23–26), and many clinical trials of ARIs on autonomic as well as somatic neuropathy have been performed, as summarized by Pfeifer et al. (27). However, the efficacy of ARIs on human diabetic neuropathy has not been clearly established. In those trials, NCV and/or cardiovascular autonomic function tests, some of which are not sensitive or reproducible, were used to assess the nerve functions, and the

patients with established severe diabetic neuropathy whose morphologic abnormalities were irreversible were included among the study subjects. In addition, the study period of some trials was not long enough to detect the amelioration or progression of nerve dysfunction in ARI-treated or -untreated patients, respectively. These factors could account for the results of some of the previous clinical trials. It has therefore been recommended that clinical trials of ARIs be conducted with patients of mild diabetic neuropathy during a long study period and that nerve functions be assessed by sensitive and reproducible tests (27).

Among a variety of autonomic nerve function tests, the pupillary light reflex test was the main focus in this study. Although the pupillary light reflex test has been considered to be able to detect abnormalities in autonomic nerve functions at an earlier stage than other tests (5–10) and has been used for evaluating diabetic autonomic neuropathy, only a few (28,29) of the previous trials of ARIs used this test, in which only VD and T1 among the different parameters were measured, and no beneficial effects of ARIs as observed in the present study were found.

Table 4—Effect of epalrestat on somatic nerve functions

	Epalrestat		Control		P
	Before	After	Before	After	
NCV					
Median motor NCV (m/s)	52.2 ± 2.0	51.7 ± 2.0	54.0 ± 1.9	55.1 ± 1.8	0.634
Tibial motor NCV (m/s)	42.7 ± 1.7	42.8 ± 0.7	39.0 ± 1.1	38.9 ± 1.1	0.896
Median sensory NCV (m/s)	57.0 ± 2.4	58.5 ± 1.4	59.9 ± 2.3	60.1 ± 2.1	0.477
Sural sensory NCV (m/s)	46.0 ± 1.0	49.7 ± 2.7	46.7 ± 2.3	44.3 ± 2.0	0.082
Minimum latency of F-wave					
Median motor nerve (ms)	25.8 ± 1.0	23.1 ± 1.1	22.9 ± 0.7	23.1 ± 0.8	0.020
Tibial motor nerve (ms)	43.8 ± 1.0	41.1 ± 1.5	43.6 ± 0.9	44.8 ± 1.2	0.011
Vibration perception threshold	4.8 ± 0.3	5.2 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	0.055

Data are means ± SEM. P values concern the comparison of the differences in the test results before and after the treatment between each treatment group.

Because the severity of neuropathy in those studies was similar to that in our study, D2, CR, or VC, which were improved by epalrestat in the present study, should have been measured.

Although the pupillary function is regulated by the sympathetic and parasympathetic nervous systems in a complicated manner (30–32), pupillary constriction, in general, is regulated by parasympathetic nerves and dilatation by sympathetic nerves (5,6,9). Therefore, D2, CR, VC, T1, T2, and T3 represent indexes of parasympathetic nerve functions, and D1, T5, and VD represent indexes of sympathetic nerve functions. In the present study, a beneficial effect of epalrestat on the pupillary light reflex was shown only in D2, CR, and VC, which means that epalrestat improved parasympathetic neuropathy but not sympathetic neuropathy. This may also mean that the severity of parasympathetic nerve dysfunction is milder than that of sympathetic nerve dysfunction. Although it remains controversial whether the primary abnormalities develop in parasympathetic or sympathetic nerves of diabetic patients (6,32,33), our results may support the notion that the sympathetic nervous system may be affected earlier or at least to a greater extent than the parasympathetic nervous system in the development of diabetic autonomic neuropathy.

The effect of epalrestat on somatic neuropathy was also evaluated in this study, and the minimum latency of F-wave was ameliorated, but motor and sensory NCVs were not. Most of the clinical trials of ARIs have used motor or sensory NCV to assess the somatic nerve functions, and a few trials (34,35) were conducted with assessments of both NCV and minimum latency of F-wave, both of which were improved by an ARI. This is inconsistent with our results, which may be due to the differences in the severity of somatic neuropathy of the study subjects in the two studies. Our study included diabetic patients with asymptomatic neuropathy, and the mean values of NCV were within the normal range. In contrast, the study by Fagius et al. (34) included symptomatic neuropathy alone, and NCV was delayed compared with that in our study. Therefore, the somatic neuropathy in the present study might have been very mild, which may account for the lack of improvement of somatic nerve functions in the NCV, which is a less sensitive and

reproducible measure than the minimum latency of F-wave (11–13).

In conclusion, the present study demonstrated the beneficial effect of an ARI, epalrestat, on autonomic and somatic neuropathy at an early stage in diabetic patients by assessing the pupillary light reflex test and minimum latency of F-wave in spite of the relatively short study period of 24 weeks. In conducting prevention or intervention studies on diabetic neuropathy, it would be very important to determine the stage of diabetic neuropathy and the most appropriate measures to use for evaluating nerve functions. The efficacy of an ARI on nerve conduction and morphometry in diabetic patients was recently reported by Greene et al. (36). Further studies on mild diabetic neuropathy by using sensitive measures such as the pupillary light reflex test and minimum latency of F-wave would likely confirm the therapeutic value of ARIs.

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