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A NEW PROSTAGLANDIN E1 ANALOGUE (TFC-612) PREVENTS A DECREASE IN MOTOR NERVE CONDUCTION VELOCITY IN STREPTOZOCIN-DIABETIC RATS

Hitoshi Yasuda, Masanobu Sonobe, Ikuo Hatanaka, Makio Yamashita, Yasufumi Miyamoto, Masahiko Terada, Masanori Amenomori, Ryuichi Kikkawa, Yukio Shigeta, Yukio Motoyama* and Noriyuki Saito**

The Third Department of Medicine,
Shiga University of Medical Science,
Ohtsu, Shiga 520-21, Japan
*New Drug Research Laboratories, Fujisawa Phamaceutical CoLtd.,
Osaka 532, Japan
**Pharmacolog. Section,
Teijin Institute for Bio-Medical Research,
Teijin Limited,Hino, Tokyo 191, Japan

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A new prostaglandin E1 analogue (TFC-612) was orally given to streptozocin-diabetic rats for 4 weeks after the induction of diabetes and its effects on motor nerve conduction velocity were studied. The compound significantly prevented a decrease of the velicity but did not reverse abnormal sorbitol and myo-inositol contents of the sciatic nerve. The results suggest that TFC-612 has a potent effect on diabetic nerve dysfunction via other mechanism than the correction of sorbitol and myo-inositol metabolisms and could be a potential compound for therapy of diabetic polyneuropathy.

Hyperglycemia and associated metabolic derangements of sorbitol and myo-inositol might be involved in the cause of diabetic polyneuropathy (1-3). On the other hand, there have been increasing evidences of vascular implication in the pathogenesis of diabetic mononeuropathy (4) and polyneuropathy (5-13). Nerve blood flow is lower in diabetic than in control rats (10). Endoneurial oxygen tension is decreased in both diabetic rats (10) and patients (13). Morphologically, nerve fiber loss is multifocal and more frequently found in the proximal part of the nerve than expected as the distal symmetric polyneuropathy even at the early stage of the disease, suggesting the contribution of ischemia to the nerve pathology of diabetic polyneuropathy (6,7). An increase in endothelial or basement membrane area is also thought to cause nerve damage by precipitating tissue ischemia

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(5,12). We speculate that microvessel abnormalities intervene between metabolic derangements and nerve fiber damage and are essential for the development of diabetic polyneuropathy at least at the advanced stage of the disease.

On the basis of vascular involvement, the use of prostaglandin E1, which is well known to be a potent vasodilator (14), seems to be reasonable for the treatment of diabetic polyneuropathy. Although anecdotal reports described the clinical effect of the compound on this condition (15), it has not been well established nor reproduced in experimental animals. The present study was performed to ascertain whether prostaglandin E1 is effective for therapy of diabetic polyneuropathy and, if so, whether the effect is associated with sorbitol or myo-inositol metabolism, using a newly developed oral prostaglandin E1 analogue.

Materials and Methods

Animals

Thirty-seven male Sprague-Dawley rats weighing approximately 300 g and 10 weeks old were used for the experiment. Twenty-seven rats were made diabetic by a single injection of streptozocin (45 mg/kg body weight) dissolved in citrate buffer (pH 4.5). In seven diabetic rats, insulin was subcutaneously given by 6-10 Ultralente insulin (Novo) daily for 4 weeks. In 10 diabetic rats, prostaglandin E1 analogue, methyl 6-(((1R,2S,3R)-3-hydroxy-2-((1E,3S,5R)-3-hydroxy-5-methyl-1-nonenyl)-5-oxocyclopentyl)thio) hexanoate (TFC-612) (16,17), dissolved in alcohol and then diluted in saline, was given via gastric tubing at a daily dose of approximately 1ml (0.3 mg/kg) for 4 weeks. Same dose of saline was given in the same manner to other three groups (10 control and 7 insulin-treated and 10 untreated diabetic animals). All animals were maintained in conventional cages with free access to rat chow and water. During the experiment, 1 control rat died of bronchopneumonia and 7 diabetic rats of seemingly metabolic disturbance from sudden onset of diabetes within a week after the injection of streptozocin. After all, 9 control and 20 diabetic rats (6 with insulin treatment, 8 treated with prostaglandin E1 and 6 untreated) survived and served as evaluation.

Electrophysiological examination

At baseline and after 2 and 4 weeks of the experiment, motor nerve conduction velocity was recorded from the left sciatic-posterior tibial nerve conduction system in a temperature-controlled environment under pentobarbital anesthesia. The rectal temperature was maintained at 37 °C with heating lamp and pad. The left hind limb was held in full extension by strapping to facilitate distance measurements. Supramaximal stimuli of 10 HZ were applied to the left sciatic nerve at the sciatic notch and to posterior tibial nerve at the ankle by bipolar electrodes. The muscle action potential were recorded from the first interosseous muscle of the left hindlimb by unipolar pin electrodes. Distal

and proximal latencies were measured from photographs of oscilloscope recordings and velocity was calculated.

Assay for sorbitol and myo-inositol

At the end of the experiment, both sciatic nerves were quickly removed by incision, weighed and homogenized in 8% HClO4 (0.5 ml) and centrifuged at 3000 rev/min for 10 min. The supernatants were neutralized with 2 N KOH and their sorbitol content determined by the enzymatic method of Bergmeyer et al (18). The myo-inositol content was determined by HPTLC technique of Stepanek (19).

Results

Clinical observations:

There were no significant physical and behavioral abnormalities except for soft feces in two rats treated with TFC-612 among all groups. Control and insulin-treated groups showed an increase in body weight during the experiment (control, 302± 7 to 306±34 g; insulin-treated, 309±10 to 312±20 g), whereas a cosiderable decrease was observed in other diabetic groups (TFC-612-treated, 299±6 to 244±18 g; untreated, 299± 7 to 241±15 g). Insulin treatment normalized increased plasma glucose level in diabetic rats, while TFC-612 treatment did not have any effect on glucose level at week 4 (Table 1).

Electrophysiological examination (Table 2):

Motor nerve conduction velocity at baseline was not significantly different among 4 groups. Control and insulin-treated diabetic rats respectively showed 9.0 % and 7.5 % increases in the velocity at week 4. By contrast, untreated diabetic rats showed a 13.9 % reduction whereas only a 0.9 %

Table 1. Sorbitol and myo-inositol contents of sciatic nerve of experimental rats

Treatment	Plasma glucose at Week 4 (mg/dl)	Sorbitol content (n mol/g wet weight)	Myo-inositol content (n mol/g wet weight)
Control rats (n = 9)	149.3 ± 15.5	138.7 ± 42.2	3.7 ± 0.7
Diabetic rats			
untreated (n = 6)	528.0 ± 74.8	508.5 ± 154.7	2.5 ± 0.3
TFC-612 treated (n = 8)	565.5 ± 89.5	583.7 ± 125.5	2.8 ± 0.6
insulin treated (n = 6)	123.0 ± 21.2	146.6 ± 49.2	3.5 ± 0.8

Results expressed as mean ± SD; a, $p<0.05$, b, $p<0.01$, c, $p<0.001$ vs control

Table 2. Motor nerve conduction velocity of experimental rats before and after treatment of TFC-612

Treatment	Motor nerve conduction velocity (m/s)		
	Week 0	Week 2	Week 4
Control rats (n = 9)	51.4 ± 2.6	53.9 ± 3.1	56.5 ± 2.3
Diabetic rats			
untreated (n = 6)	51.9 ± 2.6	46.9 ± 2.3	46.5 ± 3.0
TFC-612 treated (n = 8)	51.5 ±2.4	50.1 ± 3.7	50.5 ± 2.1
insulin treated (n = 6)	49.4 ± 2.3	52.1 ± 3.1	53.1 ± 4.5

Results expressed as mean ± SD; a, $p<0.05$, b, $p<0.01$

reduction was noted in TFC-612-diabetic rats at week 4. Motor nerve conduction velocity at week 4 was significantly different between any two of three groups other than insulin treated one: control (56.5±2.3 m/s) vs untreated (46.5±3.0 m/s), $p < 0.01$; control vs TFC-612-treated (50.5±2.1 m/s), $P < 0.05$; untreated vs TFC-612-treated, $P < 0.05$. Insulin-treated group (53.1±4.5 m/s) significantly differed from untreated diabetic group ($p<0.05$) but not from others. Almost same results were already obtained at week 2.

Sorbitol and myo-inositol content:

As shown in Table 1, the content of sorbitol was significantly increased in both untreated (508.5±154.7 n mol/g wet weight) and TFC-612-treated diabetic groups (583.7±125.5) than in control(138.7±42.2) and insulin-treated groups (146.6± 49.2) at week 4. There was no significant difference in sorbitol content between untreated and TFC-612-treated diabetic animals. The content of myo-inositol showed a significant decrease in untreated(2.5±0.3 n mol/g wet weight) and TFC-612-treated animals (2.8±0.6) compared with control (3.7±0.7) and insulin-treated animals (3.5±0.8). Thus, TFC-612 treatment did not reverse the level of nerve myo-inositol content in diabetic rats.

Discussion

The present study shows that a newly developed prostaglandin E1 analogue (TFC-612) can partially prevent slowing of motor

nerve conduction velocity in the sciatic nerve of streptozocin - diabetic rats. The data support the anecdotal reports which demonstrated the efficacy of prostaglandin E1 by means of intravenous infusion for subjective symtoms and decreased nerve conduction velocity in diabetic patients with neuropathy (15). This preventive effect of prostaglandin E1 against slowing of motor nerve conduction velocity could not be explained by the contents of sorbitol and myo-inositol of the sciatic nerve, because they were not significantly different between PGE1-treated and untreated diabetic animals. Some explanations might be possible for the mechanism of the effect. First, prostaglandin E1 exerts various vasotropic effects including vasodilating (14) and anti-platelet aggregatory actions (20), increasing blood flow (21) and ameriolation of red cell deformability (22) and the clinical efficacy has been reported in patients with peripheral vascular disease (20,21) and ischemic heart disease. The effects might work in the endoneurial microvessel as well, thereby leading to the improvement of nerve function by increasing oxygen delivery. Tuck and Low (10) reported that nerve blood flow was decreased and was accompanied by lowered oxygen tension in streptozocin-diabetic rats with four months' duration of hyperglycemia. Second, direct effects on the nerve might be considered. Prostaglandin E1 has been reported to depolarize sensory nerve terminals (23) and inhibit synaptic transmission in adrenergic and cholinergic nerve terminals (24).

Prostaglandin E1 analogue was orally given in the present study. Originally, prostaglandin E1 was introduced via intraarterial infusion because it is rapidly metabolized during a single passage in lungs. Now that the efficacy has been found even when intravenously infused (15,21), intravenous infusion is widely being used. However, oral administration is more convenient and the duration of efficacy would be expected to be more prolonged after administration.

Taken together, oral dosage of a new prostaglandin E1 analogue, TFC-612, for therapy of diabetic polyneuropathy is very potential and worth considering clinical trial.

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