STUDIES ON WF-3681, A NOVEL ALDOSE REDUCTASE INHIBITOR

IV. EFFECT OF FR-62765, A DERIVATIVE OF WF-3681, ON THE DIABETIC NEUROPATHY IN RATS

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Pharmacokinetic properties of WF-3681 and FR-62765 in rats were examined. FR-62765 showed higher peak concentrations in the plasma and higher concentrations in the sciatic nerve than WF-3681 when each compound was administered orally to rats. We therefore evaluated FR-62765 in rats with diabetic neuropathy. As a result, FR-62765 at 32 mg/kg/day administered for 3 weeks to streptozotocin-induced diabetic rats significantly prevented the accumulation of sorbitol in the sciatic nerve and the reduction of motor nerve conduction velocity in the tail. From these results it was concluded that the aldose reductase inhibitor FR-62765 might be a useful drug for diabetic neuropathy.

In diabetes intracellular accumulation of sorbitol formed from excess glucose by the action of aldose reductase, the rate limiting enzyme in the polyol pathway, produces a hyperosmotic effect, which results in cellular swelling and subsequent diabetic complications[1]. In the nerves of patients with diabetes, thus accumulated sorbitol may cause osmotic damage to Schwann cells or endoneurial oedema, concomitantly motor nerve conduction velocity (MNCV) may slow[2].

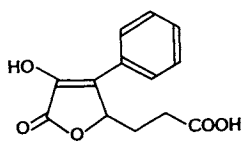
WF-3681, isolated from a cultured filtrate of *Chaetomella raphigera*[3], is an inhibitor of aldose reductase. FR-62765 is a chemically modified analogue of WF-3681 selected after an intensive screening program to find analogues with a stronger *in vivo* activity[4].

In this study, pharmacokinetic profiles of WF-3681 and FR-62765 were compared, and utilizing FR-62765, we attempted to prevent the reduction of MNCV in streptozotocin (STZ)-induced diabetic rats.

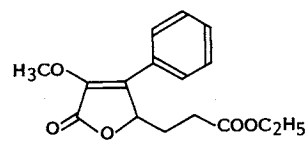
Materials and Methods

Pharmacokinetic Study

Each of the aldose reductase inhibitors (ARIs) suspended in 0.5% methyl cellulose solution was administered orally at a dose of 100 mg/kg to male Sprague-Dawley rats (7-week-old). Blood samples were withdrawn from the tail with heparinized capillaries at 30, 120, 300 and 420 minutes after the administration. Plasma was separated from the blood sample by centrifugation. After the last blood sampling, the rats were killed by exsanguination and the sciatic nerves were removed, washed with saline, and blotted with filter paper. The nerves were homogenized in a glass homogenizer after the addition of



Structure of WF-3681



Structure of FR-62765

methanol. The homogenate was centrifuged at 3,000 × *g* for 20 minutes and the supernatant was separated. ARI concentration in the plasma or in the methanol extract of the sciatic nerve was determined by aldose reductase inhibitory activity. The method for measurement of aldose reductase inhibitory activity *in vitro* was reported previously[3].

Induction of Diabetes and Drug Treatment

Seven-week-old male Sprague-Dawley rats were made diabetic by a single intraperitoneal injection of STZ (75 mg/kg, Sigma Chemical Co., St. Louis, U.S.A.), which had been freshly dissolved in 2 mM citrate buffer (pH 4.5). Seven days after STZ injection, plasma glucose levels of all rats were analyzed by sampling blood from the tail vein. The glucose assay employed was an enzymatic assay based on glucose oxidase and peroxidase (Glucose B-Test, Wako, Osaka, Japan). Rats with plasma glucose levels above 400 mg/deciliter were considered as diabetic. WF-3681 or FR-62765 at a dose of 32 mg/kg suspended in 0.5% methyl cellulose solution was given to these rats orally once a day from day 1 to day 21.

Measurement of MNCV

On days 0, 14, and 21, the MNCV in rats was determined by the method of MIYOSHI and GOTO[5]. Briefly, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). As the measurement of MNCV is very sensitive to temperature, the tail of the rat was immersed in a liquid paraffin bath controlled by a thermostat. The temperature of the rat tail was maintained stable at 37°C during recording, using the needle-type thermodetector (Thermister Thermometer, Model MGA II/III, Shibaura Electric Co., Tokyo, Japan). The body temperature of the animals was maintained by heating with a lamp. The MNCV was determined by stimulating supramaximally the longitudinal nerve of the tail with paired stimulating electrodes. The muscle action potentials were amplified on an oscilloscope (Nippon Koden Co., Tokyo, Japan) and photographically recorded with a Polaroid camera. The MNCV was determined from the photographs as shown in Fig. 1.

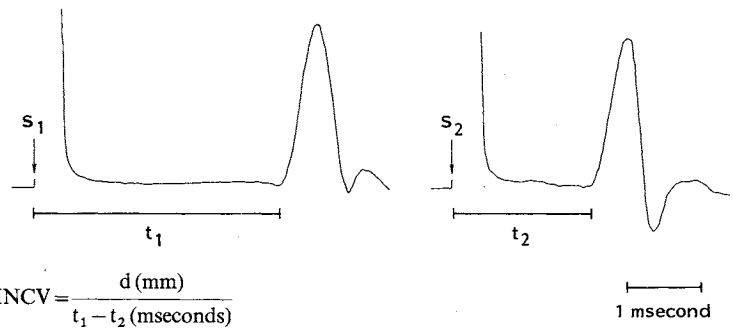
Measurement of Sorbitol Accumulation in the Sciatic Nerve of Diabetic Rats

After the MNCV measurement, the rats were killed by exsanguination and the sciatic nerves were removed, washed with saline, weighed immediately and frozen at −20°C until determination of sorbitol levels. Sorbitol levels in the tissue were measured by the modification of the enzymatic assay of CLEMENTS *et al.* as described previously[4].

Statistical Analysis

Data were expressed as means ± SEM and statistically analysed by Student's t-test and $P<0.05$ was considered significant.

Fig. 1. Muscle action potentials from a control animal produced by supramaximal nerve stimulation at a proximal (S_1), and a distal site (S_2).



d: The distance between stimulating cathodes was measured on the skin. t_1, t_2: latencies of the electrical response of the innervated muscle to proximal (t_1) and distal (t_2) stimulation.

Results

Pharmacokinetic Properties of WF-3681 and FR-62765 in Rats

As shown in Fig. 2, FR-62765 revealed higher plasma levels than WF-3681. The peak concentration of FR-62765 at 60 minutes after dosing was $176.4 \pm 1.8\,\mu g/ml$ and dropped to $57.0 \pm 8.3\,\mu g/ml$ at 420 minutes. At 420 minutes after dosing of FR-62765, 13.1 ± 1.8 ng/mg tissue was detected in the sciatic nerve (Table 1), and this value is 6.5 times higher than that of WF-3681.

Effect of FR-62765 on the MNCV in Tails of Diabetic Rats

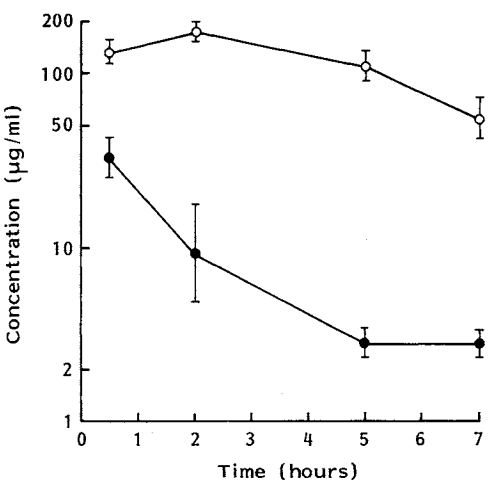
The MNCV of normal, non-treated diabetic and diabetic rats administered with a 32-mg/kg dose of FR-62765 are shown in Table 2. The non-treated diabetic rats showed a significant reduction of MNCV when compared with the normal rats on day 14 and day 21. The diabetic rats dosed with FR-62765 had significantly faster MNCV on both days 14 and 21 when compared to non-treated diabetic rats. There was no significant difference in MNCV between the normal control and FR-62765-treated diabetic rats.

Effect of FR-62765 on the Sorbitol Levels in the Sciatic Nerve of STZ-diabetic Rats

After the MNCV measurement, the sorbitol content in the sciatic nerve of the rats was determined. FR-62765 completely inhibited (98.7% inhibition) the sorbitol accumulation in the sciatic nerve of the diabetic rats without showing any effect on body weight and blood glucose as shown in Tables 3 and 4.

Fig. 2. Plasma concentrations of FR-62765 and WF-3681 in rats after a po dose of 100 mg/kg.

○ FR-62765, ● WF-3681.



Discussion

In the pharmacokinetic study, the concentration of FR-62765 in the sciatic nerve was much higher than that of WF-3681 when measured at 420 minutes after administration of the drugs. The *in vitro* aldose reductase inhibitory activity of FR-62765 was similar to that of WF-3681, the IC_{50} values of FR-62765 and WF-3681 being $0.22\,\mu M$ and $0.25\,\mu M$, respectively[6]. However, our previous report also demonstrated that FR-62765 at a dose of 32 mg/kg significantly prevented the sorbitol

Table 1. ARI concentrations in the sciatic nerve following administration of a 100-mg/kg dose by the po route.

ARI	Tissue ARI level (ng/mg tissue)
WF-3681	2.0 ± 0.18
FR-62765	13.1 ± 1.80

Each value was expressed as mean $\pm$ SE ($n=3$).

Table 2. Effect of FR-62765 on MNCV in STZ diabetic rats.

	MNCV (m/second) (% recovery)		
	Day 0	Day 14	Day 21
Normal control	27.4 ± 1.2	36.8 ± 0.8** (100)	39.5 ± 0.8** (100)
STZ control	28.0 ± 1.1	31.5 ± 0.6 (0)	34.2 ± 1.0 (0)
FR-62765 (32 mg/kg)	27.9 ± 1.1	34.7 ± 0.8** (71)	38.2 ± 0.8* (75.5)

Each value was expressed as means $\pm$ SE ($n=6$).

* $P<0.05$, ** $P<0.01$ versus STZ control.

Table 3. Effect of FR-62765 on the sorbitol accumulation in the sciatic nerves of diabetic rats.

	Sorbitol concentration in the sciatic nerve (nmol/mg wet tissue) (% inhibition)
Normal control	0.30±0.03*** (100.0)
STZ control	2.65±0.13 (0.0)
FR-62765 (32 mg/kg)	0.33±0.06*** (98.7)

Each value was expressed as means±SE ($n=6$).
*** $P<0.001$ versus STZ control.

Table 4. Body weight change and plasma glucose levels.

	Body weight (g)			Plasma glucose (mg/deciliter)		
	Day 0	14	21	Day 0	14	21
Normal control	220±2.9	333±4.4***	383±6.5***	131±5.3	130±2.0	174±4.4
STZ control	222±3.3	243±8.7	226±7.0	589±7.2	579±5.7	577±23.7
FR-62765 (32 mg/kg)	220±2.7	232±7.5	223±6.3	584±9.9	570±3.5	637±22.6

Each value was expressed as means±SE ($n=6$).
*** $P<0.001$.

accumulation in the sciatic nerve of diabetic rats, while WF-3681 at the same dose did not[4]. Pharmacokinetic studies in this paper showed that the concentration of FR-62765 in the plasma and in the sciatic nerve of diabetic rats was much higher than that of WF-3681 when each compound was administered orally. These results prompted us to evaluate FR-62765 in an animal model of diabetic neuropathy. Diabetic neuropathy is a major complication of diabetic mellitus primarily associated with a decrease in MNCV, and is accompanied by structural changes in the nerve which include axonal loss and segmental demyelinations. Oral treatment with FR-62765 at 32 mg/kg for 14 or 21 days significantly prevented the reduction of the MNCV in the tails of diabetic rats. From these observations it was concluded that the aldose reductase inhibitor FR-62765 might be a useful drug for patients with diabetic neuropathy.

References

1) GABBAY, K. H.: The sorbitol pathway and the complications of diabetes. N. Engl. J. Med. 288: 831~836, 1973
2) SIMA, A. A. F.; V. BRIL, V. NATHANIEL, T. A. J. MCEWEN, M. B. BROWN, S. A. LATTIMER & D. A. GREENE: Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with Sorbinil. N. Engl. J. Med. 319: 548~555, 1988
3) NISHIKAWA, M.; Y. TSURUMI, T. NAMIKI, K. YOSHIDA & M. OKUHARA: Studies on WF-3681, a novel aldose reductase inhibitor. I. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 40: 1394~1399, 1987
4) NISHIKAWA, M.; K. YOSHIDA, M. OKAMOTO & M. KOHSAKA: Studies on WF-3681, a novel aldose reductase inhibitor. III. Effects of WF-3681 and its derivatives on sorbitol accumulation in diabetic rats. J. Antibiotics 43: 1186~1188, 1990
5) MIYOSHI, T. & I. GOTO: Serial *in vivo* determinations of nerve conduction velocity in rat tails. Physiological and pathological changes. Electroencephalogr. Clin. Neurophysiol. 35: 125~131, 1973
6) NAMIKI, T.; Y. BABA, Y. SUZUKI, M. NISHIKAWA, K. SAWADA, Y. ITOH, T. OKU, Y. KITAURA & M. HASHIMOTO: Synthesis and aldose reductase-inhibitory activities of structural analogues of WF-3681, a novel aldose reductase inhibitor. Chem. Pharm. Bull. 36: 1404~1414, 1988