Biochemical Pharmacology, Vol. 40, No. 2, pp. 303–307, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

IN VIVO ACTIVITIES OF ALDOSE REDUCTASE INHIBITORS HAVING A 1-(ARYLSULFONYL)HYDANTOIN STRUCTURE

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(*Received* 29 *March* 1989; *accepted* 6 *December* 1989)

Abstract—Two potent aldose reductase inhibitors, 1-[(2,5-dichlorophenyl)sulfonyl]hydantoin (Di-Cl-PSH) and 1-[β-naphthyl)sulfonyl]hydantoin (β-NSH), were tested for usefulness in the treatment of diabetic and galactosemic complications in animal experiments. Both drugs were effective for the treatment of diabetic neuropathy characterized by decreased motor nerve conduction velocity, that is, slowing of tail and sciatic-tibial motor nerve conduction velocities in streptozocin-induced diabetic rats was prevented during 3 weeks by intubating Di-Cl-PSH or β-NSH at 50 mg/kg/day. Lenticular vacuole formation in rats fed a 30% galactose diet was blocked completely for at least 2 weeks by oral administration of Di-Cl-PSH or β-NSH at both 30 and 100 mg/kg/day, whereas all of the eyes of vehicle-treated rats showed vacuole formation by day 4 on the galactose diet. The ED_{50} values of Di-Cl-PSH and β-NSH for inhibition of sorbitol accumulation in the sciatic nerve and lens of streptozocin-induced diabetic rats were also estimated; the values of Di-Cl-PSH and β-NSH were 1.1 and 3.4 mg/kg/day, respectively, for inhibition in the sciatic nerve and 4.8 and 16.0 mg/kg/day, respectively, for that in the lens. This study indicates that Di-Cl-PSH and β-NSH have high potential for future clinical use as aldose reductase inhibitors.

Aldose reductase (EC 1.1.1.21) has been implicated in the pathogenesis of diabetic complications such as cataract, neuropathy, retinopathy, and nephropathy. Many aldose reductase inhibitors have been proven effective in the treatment of these complications in diabetic and galactosemic experimental animals [1, 2]. Clinical trials of aldose reductase inhibitors in humans with diabetes have yielded promising results [3]. Development of more beneficial aldose reductase inhibitors, however, is still necessary.

We previously reported that 1-(arylsulfonyl)hydantoin derivatives have potent aldose reductase inhibitory activities both *in vitro* and *in vivo* [4, 5]. Of such compounds tested, 1-[(2,4,5-trichlorophenyl)sulfonyl]hydantoin (Tri-Cl-PSH†), 1-[(2,5-dichlorophenyl)sulfonyl]hydantoin (Di-Cl-PSH), and 1-[(β-naphthyl)sulfonyl]hydantoin (β-NSH) were found to be the most effective in preventing sorbitol accumulations in the lens and sciatic nerve of streptozocin-induced diabetic rats. They inhibited sorbitol accumulation in the sciatic nerve completely and that in the lens by more than 92%, when given at a dose of 50 mg/kg/day beginning with the early stage of diabetes [5].

The purpose of the present study was to assess whether these compounds are useful for treatment of diabetic and galactosemic complications in animal experiments. The doses causing 50% inhibition of tissue sorbitol accumulation (ED_{50}) were also determined for Di-Cl-PSH and β-NSH in this study.

MATERIALS AND METHODS

Induction of diabetes and administration of drug. Male Wistar strain rats (240–260 g) were made diabetic by a single intraperitoneal injection of streptozocin (50 mg/kg, Sigma Chemical Co., St. Louis, MO, U.S.A.) in 0.01 M citrate buffer (pH 5.1). On day 2 after injection, blood samples from rats fasted for 4 hr were taken by a tail-prick for blood glucose assay. Blood glucose was determined with a commercial kit, Blood Sugar-GOD-Period-Test (Boehringer, Mannheim, F.R.G.), after deproteinization of the blood samples by the method of Somogyi [6]. Rats with blood glucose concentrations from 300 to 600 mg/dL were considered to be diabetic. Beginning with day 3 after injection of streptozocin, diabetic rats were intubated for 21 days with hydantoin compound (50 mg/kg/day) suspended in 5 g/dL gum arabic or with the vehicle (5 g/dL gum arabic) only. The vehicle was also given to a group of normal rats. These diabetic and normal rats were analysed for both motor nerve conduction velocity (MNCV) and tissue polyol contents.

Another series of experiments was performed with streptozocin-induced diabetic rats for determining ED_{50} values of Di-Cl-PSH and β-NSH for inhibition of sorbitol accumulation in the lens and sciatic nerve. Diabetic rats were prepared as described above and divided into nine groups of six rats each. Beginning with day 3 after injection of streptozocin, each group of diabetic rats was intubated for 12 days with Di-Cl-PSH at 0.5, 2, 5, or 20 mg/kg/day and β-NSH at 2, 5, 20, or 50 mg/kg/day. The vehicle (5 g/dL gum arabic) was given to the control group.

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† Abbreviations: Tri-Cl-PSH, 1-[(2,4,5-trichlorophenyl)sulfonyl]hydantoin; Di-Cl-PSH, 1-[(2,5-dichlorophenyl)sulfonyl]hydantoin; β-NSH, 1-[(β-naphthyl)-sulfonyl]hydantoin; and MNCV, motor nerve conduction velocity.

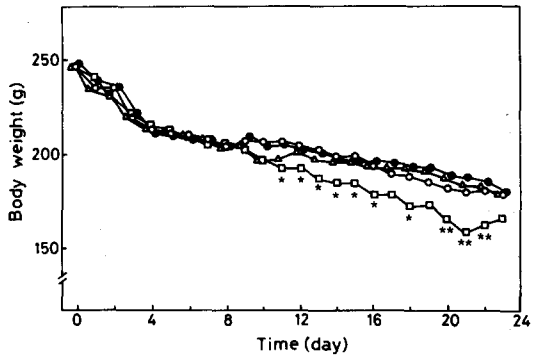

Fig. 1. Effects of Tri-Cl-PSH, Di-Cl-PSH, and β-NSH on body weight of streptozocin-induced diabetic rats. Streptozocin was injected at day 0 and administration of drug (50 mg/kg/day) was continued from day 3 to day 23. Each point indicates the mean body weight of diabetic rats treated with Tri-Cl-PSH (□, N = 5), Di-Cl-PSH (●, N = 8), β-NSH (△, N = 9), or vehicle (○, N = 8). Key: (★) $P < 0.05$ compared with control (vehicle-treated) rats and (★★) $P < 0.01$ compared with control rats.

Measurement of motor nerve conduction velocity. One day after the final administration of hydantoin compound or vehicle, MNCV was measured. One group consisting of both normal and diabetic rats was analysed for the conduction velocity of the tail nerve, and another group, for that of the sciatic-tibial nerve. The measurement of tail MNCV was performed under pentobarbital anesthesia according to the method of Miyoshi [7]. With the tail kept in liquid paraffin at 37°, the tail nerve was stimulated with needle electrodes at two points which were 1 and 6 cm distant from the anus. Supramaximal 0.1 msec stimuli were delivered at 1 Hz. The action potentials were recorded from the segmental muscle located 10 cm distant from the anus with a Nihon Kohden electromyograph MEM-3202 (Nihon Kohden Corp., Tokyo, Japan), and the latencies were measured from a storage oscilloscope. The conduction velocity of the right sciatic-tibial nerve was measured by the method of Yue *et al.* [8].

Measurement of tissue polyols. One day after the final administration of hydantoin compound both in the MNCV experiment and in the ED_{50} experiment, rats were killed by decapitation. Lenses and sciatic nerves were removed and stored at −80° until assayed for polyols. Sorbitol and *myo*-inositol contents in each pair of lenses and sciatic nerves were determined by our method using high-performance liquid chromatography [9]. The limit of detection of the method was about 2 pmol of sorbitol and *myo*-inositol.

Induction of galactose cataract and administration of drug. Male Sprague–Dawley rats (80–100 g) were fed a 30% galactose diet for 14 days. Galactose-fed rats were intubated between 9:00 and 11:00 a.m. with a suspension of Di-Cl-PSH or β-NSH in 5 g/dL gum arabic from day 1 of galactose feeding. The dosage of hydantoin compound was 10, 30 or 100 mg/kg/day. The vehicle (5 g/dL gum arabic) only was given to control galactose-fed rats. Each group consisted of five rats. Appearance of vacuoles at the lens periphery was examined every 2–4 days with a slit-lamp (Kowa SC-6, Kowa Co., Ltd., Nagoya, Japan).

Syntheses of hydantoins. Tri-Cl-PSH, Di-Cl-PSH, and β-NSH were synthesized as described previously [5].

Statistics. The statistical analyses were performed by Student's *t*-test with the level of significance set at $P < 0.05$.

RESULTS

The body weight of streptozocin-induced diabetic rats treated with Tri-Cl-PSH, Di-Cl-PSH, β-NSH, or vehicle only was measured every day throughout the experimental period. Rats treated with Di-Cl-PSH, β-NSH, or vehicle showed almost the same change in body weight, whereas the body weight of rats treated with Tri-Cl-PSH was significantly less than that of vehicle-treated rats on and after day 11 following injection of streptozocin (Fig. 1). As the latter result indicates that Tri-Cl-PSH may have unfavorable effects on diabetic rats, this drug was not used in further experiments for this reason.

Effects of Di-Cl-PSH and β-NSH on tail MNCV are shown in Table 1. The MNCV of vehicle-treated diabetic rats was significantly lower than that of

Table 1. Effects of Di-Cl-PSH and β-NSH on tail and sciatic-tibial motor nerve conduction velocity in streptozocin-induced diabetic rats

Group	Motor nerve conduction velocity (m/sec)	
	Tail nerve	Sciatic-tibial nerve
Normal	33.8 ± 2.6 (10)	52.4 ± 5.3 (5)
Diabetic	27.6 ± 1.9* (8)	41.2 ± 5.0* (6)
Diabetic + Di-Cl-PSH	31.8 ± 4.0†‡ (8)	48.2 ± 2.9†‡ (4)
Diabetic + β-NSH	31.9 ± 3.8†‡ (9)	48.8 ± 1.5‡§ (5)

Diabetic rats were given orally hydantoin compound (50 mg/kg/day) or the vehicle only (5 g/dL gum arabic) for 21 days. Data are the means ± SD with the number of rats given in parentheses.

* $P < 0.001$, compared with normal.

† $P < 0.05$, compared with diabetic.

‡ Not significantly different from normal.

§ $P < 0.01$, compared with diabetic.

Table 2. Effects of Di-Cl-PSH and β-NSH on polyol concentrations in the lens and sciatic nerve of streptozocin-induced diabetic rats

Group (N)	Lens		Sciatic nerve	
	Myo-inositol (μmol/g wet wt)	Sorbitol (μmol/g wet wt)	*Myo*-inositol (μmol/g wet wt)	Sorbitol (μmol/g wet wt)
Normal (5)	0.99 ± 0.18	0.52 ± 0.29	2.98 ± 0.26	0.23 ± 0.03
Diabetic (7)	ND*	37.90 ± 3.40	1.97 ± 0.27†	2.44 ± 0.43
Diabetic + Di-Cl-PSH (6)	0.89 ± 0.12‡	0.86 ± 0.18	2.50 ± 0.45†§	0.08 ± 0.05
Diabetic + β-NSH (7)	0.54 ± 0.13†	3.77 ± 0.78	2.52 ± 0.18‖¶	0.22 ± 0.06

Diabetic rats were intubated for 21 days with hydantoin compound (50 mg/kg/day) or with the vehicle only (5 g/dL gum arabic). Data are the means ± SD with the number of rats given in parentheses.

* Not detectable.

† $P < 0.001$, compared with normal.

‡ Not significantly different from normal.

§ $P < 0.05$, compared with diabetic.

‖ $P < 0.001$, compared with diabetic.

¶ $P < 0.01$, compared with normal.

normal rats. Treatment of diabetic rats with Di-Cl-PSH and β-NSH at 50 mg/kg/day for 3 weeks prevented slowing of the MNCV such that there was no significant difference in MNCV between normal rats and drug-treated rats. The two drugs at the same dosage blocked slowing of the sciatic-tibial MNCV in diabetic rats as well (Table 1). Table 1 indicates that Di-Cl-PSH and β-NSH at 50 mg/kg/day are indistinguishably effective in prevention of slowing of MNCVs of diabetic rats.

Just after the measurement of MNCV, lenses and sciatic nerves of both normal and diabetic rats were removed, and polyol contents in the tissues were measured. As reported by many workers, marked accumulations of sorbitol in both tissues, a significant decrease in nerve *myo*-inositol content, and a complete loss of lens *myo*-inositol were observed in vehicle-treated diabetic rats (Table 2). Di-Cl-PSH and β-NSH at 50 mg/kg/day completely prevented accumulation of sorbitol in the sciatic nerve and restored the nerve *myo*-inositol content to the close-to-normal level. The two drugs were also effective in preventing both sorbitol accumulation and *myo*-inositol depletion in the lens.

Effects of Di-Cl-PSH and β-NSH on lenticular vacuole formation in galactose-fed rats are illustrated in Figs 2 and 3 respectively. By day 4 on the galactose diet, all of the eyes of vehicle-treated galactose-fed rats developed an early-stage cataract which was judged by the appearance of vacuoles at the lens periphery. Two of ten eyes of rats dosed with Di-Cl-PSH at 10 mg/kg/day showed lenticular vacuole formation on day 10, but no vacuole formation was observed during the period of study in rats given the drug at 30 and 100 mg/kg/day (Fig. 2). Similarly, β-NSH at doses of 30 and 100 mg/kg/day, but not at a dose of 10 mg/kg/day, also prevented completely lenticular vacuole formation (Fig. 3).

The effectiveness of Di-Cl-PSH and β-NSH in preventing tissue sorbitol accumulation in diabetic rats is shown in Table 3. The ED_{50} values of both Di-Cl-PSH and β-NSH for inhibition of sorbitol accumulation were about four times higher for the lens than for the sciatic nerve. The table also shows that the

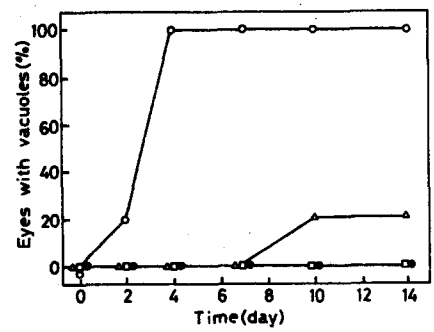

Fig. 2. Effect of Di-Cl-PSH on lenticular vacuole formation in galactose-fed rats. Feeding of a 30% galactose diet and intubation of drug (△, 10 mg/kg/day; □, 30 mg/kg/day; ●, 100 mg/kg/day) or vehicle (○) were continued from day 0 to day 14. Each group consisted of five rats. The appearance of vacuoles at the lens periphery was judged with a slit lamp.

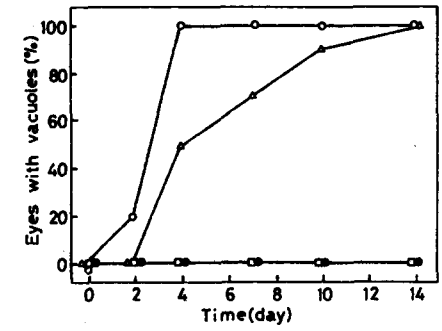

Fig. 3. Effect of β-NSH on lenticular vacuole formation in galactose-fed rats. See legend of Fig. 2 for details.

ED_{50} values of β-NSH were about three times higher than those of Di-Cl-PSH for both tissues.

DISCUSSION

Autopsy revealed no detectable difference in

Table 3. Inhibitory activities of Di-Cl-PSH and β-NSH on sorbitol accumulation in lenses and sciatic nerves of streptozocin-induced diabetic rats

Drug	ED_{50}* (mg/kg/day)	
	Lens	Sciatic nerve
Di-Cl-PSH	4.8	1.1
β-NSH	16.0	3.4

See Materials and Methods for experimental details.
* Dose decreasing sorbitol accumulation by 50%.

appearance of major organs between rats given drug (Tri-Cl-PSH, Di-Cl-PSH, or β-NSH) and control rats. Inquiry into the cause of the decrease in body weight seen by administration of Tri-Cl-PSH must be performed in the future, and detailed toxicological examination of Di-Cl-PSH and β-NSH is also necessary.

Alterations in *myo*-inositol and phosphoinositide metabolism, induced by hyperglycemia and prevented by aldose reductase inhibitors, have been implicated in the decrease of nerve (Na^+,K^+)ATPase activity and also in the subsequent dysfunction of peripheral nerves [10], although the mechanism by which prevention of sorbitol accumulation by aldose reductase inhibitors is linked to blockage of tissue *myo*-inositol depletion is not yet clear. Data obtained in the present study are consistent with this concept; that is, prevention of slowing of both tail and sciatic-tibial MNCVs in streptozocin-induced diabetic rats by administration of Di-Cl-PSH and β-NSH (Table 1) is compatible with the fact that sciatic nerve *myo*-inositol levels in drug-treated rats were significantly higher than those in vehicle-treated rats (Table 2).

Unlike diabetic neuropathy, cataract formation under diabetic and galactosemic states has been explained by the polyol-osmotic hypothesis that cataracts are caused by an elevation of polyols, leading to an increase in lens hydration, which in turn affects lens permeability [11]. The much faster rate of cataract development in galactosemic rats, as distinct from streptozocin-induced diabetic rats, is associated with a faster rate of polyol accumulation in the lens [12]. The faster formation of lenticular vacuoles as well as cataract was the only reason for our use of galactosemic rats in assessing the effects of Di-Cl-PSH and β-NSH on lenticular vacuole formation. Although the galactitol content in the lens of galactosemic rats was not analysed, data in Table 2 on the lens sorbitol content suggest high potencies of both Di-Cl-PSH and β-NSH in prevention of lenticular vacuole formation. That Di-Cl-PSH was more effective than β-NSH in preventing lenticular vacuole formation in galactosemic rats may reflect higher potency of Di-Cl-PSH, as compared with β-NSH, in prevention of sorbitol accumulation in the lens of diabetic rats.

For the purpose of comparing the *in vivo* aldose reductase inhibitory activities of Di-Cl-PSH and β-NSH with those of some other compounds, we determined ED_{50} values of the two hydantoin derivatives for inhibition of tissue sorbitol accumulation. Although there are some differences in experimental protocols (e.g. duration of dosing of drug, method of dosing of drug, and strain of rat) for determining ED_{50} values with streptozocin-induced diabetic rat models among various laboratories, the following deductions can be drawn. The *in vivo* activities of Di-Cl-PSH (ED_{50}, 1.1 mg/kg/day) and β-NSH (ED_{50}, 3.4 mg/kg/day) in the sciatic nerve are comparable to or a little higher than those (ED_{50} values, 5–10 mg/kg/day) of tolrestat [13], ONO-2235 (epalrestat) [14], and ICI 128,436 (statil) [15]. Di-Cl-PSH and β-NSH, however, are not so potent as other hydantoin compounds, sorbinil (ED_{50}, 0.5–1.0 mg/kg/day) [16] and AL-1576 (ED_{50}, 0.04 mg/kg/day) [17]. In the lens, Di-Cl-PSH is more potent than tolrestat, and less active than sorbinil and AL-1576; the ED_{50} values of the four compounds are 4.8, 10–20 [14], 1.0–2.5 [16], and 0.05 mg/kg/day [17] respectively (lens activities of ONO-2235 and statil are not known).

In conclusion, both Di-Cl-PSH and β-NSH prevented slowing of nerve conduction velocity in streptozocin-induced diabetic rats and prolonged effectively cataract formation in galactose-fed rats, warranting the necessity of further investigation focused on the clinical use of the two drugs for the treatment of diabetic complications.

Acknowledgements—We are grateful to Mr. K. Kato, Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., Gotemba, Japan, for carrying out the experiments on galactose cataract. The secretarial assistance of Ms. Kaori Yoshida is gratefully acknowledged. This work was supported by a grant-in-aid for scientific research (No. 60571064) from the Ministry of Education, Science, and Culture of Japan.

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