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Effects of an Aldose Reductase Inhibitor, 1-[(*p*-Bromophenyl)-sulfonyl]hydantoin, on Cataract Formation and Tissue Polyol Levels in Galactosemic Rats

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1-[(*p*-Bromophenyl)sulfonyl]hydantoin (*p*-Br-PSH), an inhibitor of aldose reductase, was intubated once daily at a dose of 25 or 50 mg/kg into rats fed a 50% galactose diet. Cataract formation was decreased in rats dosed with *p*-Br-PSH at 50 mg/kg from the first day on galactose diet relative to untreated galactosemic rats (control rats). In the groups pretreated with *p*-Br-PSH for 10 d before the start of galactose diet feeding, subsequent treatment with the drug markedly delayed cataract formation. Treatment with *p*-Br-PSH at 50 mg/kg from the first day (day 0) on galactose diet produced a statistically significant though small (<11%) decrease in the lenticular galactitol level at day 5 and 9. These results suggest that the delay of cataract formation by treatment with *p*-Br-PSH is caused not only by the decrease in osmotic pressure resulting from reduction in the lenticular galactitol level but also by other unknown mechanisms.

Treatment with *p*-Br-PSH at 50 mg/kg reduced galactitol accumulation by about 50% at day 5 and 9 in the sciatic nerve and by 26% at day 26 in the retina. The sciatic nerve *myo*-inositol level in control rats was decreased by 42% at day 9 compared with that of normal diet fed rats. The galactosemic rats treated with *p*-Br-PSH at 25 and 50 mg/kg showed increases (16 and 34%, respectively) in the sciatic nerve *myo*-inositol level. The importance of these findings for the treatment of diabetic complications is discussed.

Keywords——aldose reductase inhibitor; galactosemia; hydantoin derivative; galactitol; cataract; *myo*-inositol

In the presence of high sugar levels (*e.g.* in diabetes and galactosemia), aldose reductase converts the sugars to sugar alcohols. These sugar alcohols are not rapidly metabolized, nor do they penetrate cell membranes easily. Thus, once formed under these conditions, they accumulate to very high levels in cells, leading to hypertonicity and consequent tissue changes such as cataract formation. Treatment of diabetic or galactosemic rats with potent aldose reductase inhibitors decreases the accumulation of sugar alcohols, which in turn appears to prevent the formation of cataracts in lenses,[1-3] the decrease of peripheral nerve conduction velocity,[3-5] and the basement membrane thickening of retinal capillaries.[6] Some aldose reductase inhibitors have been reported to improve peripheral neuropathy in diabetic patients.[7,8] It is still hoped to develop new type(s) of aldose reductase inhibitor for the treatment of diabetic complications.

We previously reported that 1-[(*p*-bromophenyl)sulfonyl] hydantoin (*p*-Br-PSH) exhibits potent inhibition of rat and bovine lens aldose reductases with IC_{50} of 7×10^{-7} and 3.7×10^{-7} M, respectively,[9] and also has a hypoglycemic effect in rabbits.[10] In this study, we examined the effects of this compound upon the development of cataract formation in the lens and the contents of polyols (galactitol and *myo*-inositol) in the lens, sciatic nerve and retina of rats fed a 50% galactose diet.

Materials and Methods

Materials——*p*-Br-PSH was chemically synthesized as reported previously.[9] A pre-packed glass column of Silicone XE-60 (5% w/w) on Uniport HPS for gas liquid chromatography was purchased from Gasukuro Kogyo, Inc. (Tokyo, Japan). A kit for blood glucose determination (GOD-Perid test) was obtained from Boehringer Mannheim Inc. (Mannheim, Germany). Trifluoroacetic anhydride and mannitol were from Nakarai Chemicals, Ltd. (Kyoto, Japan). Galactitol was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). *myo*-Inositol was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals and Treatment——Female Sprague-Dawley rats (45—60 g) were fed a normal diet of laboratory chow or a 50% galactose diet containing 2.5% glycerol for the purpose of preventing scattering of the galactose powder.

Galactose-fed rats were intubated once daily with a suspension (0.01 ml/g body weight) composed of *p*-Br-PSH (2.5 or 5.0 mg/ml), carboxymethyl cellulose sodium salt (CMC-Na) (3.0 mg/ml) and $NaHCO_3$ (8.0 mg/ml) or with a placebo, a solution containing CMC-Na (3.0 mg/ml) and $NaHCO_3$ (8.0 mg/ml). Thus, the dosage of *p*-Br-PSH was either 25 or 50 mg/kg/d.

Assessment of Cataract Formation——The cataract formation in galactose-fed rats was observed with the naked eye and is shown as the percentage of eyes with nucleus cataracts.

Determination of Blood Glucose——Blood samples from the tail vein were deproteinized with a combination of $Ba(OH)_2$ and $ZnSO_4$ solutions by the method of Somogyi,[11] and analyzed for glucose with a commercial kit (GOD-Perid test).

Polyol Analysis——Pairs of lenses, sciatic nerves or retinas were removed after decapitation, homogenized in 1.5 ml of purified water containing 0.1 μmol of mannitol as an internal standard, and heated in a boiling-water bath for 15 min for deproteinization. After cooling for 30 min in ice, the homogenate was centrifuged at 20000 $\times g$ for 60 min and the supernatant was again centrifuged at 20000 $\times g$ for 40 min. An aliquot (0.75 ml) of the supernatant obtained after the second centrifugation was lyophilized and then subjected to reaction with a mixture of 20 μl of trifluoroacetic anhydride and 20 μl of ethyl acetate at 60 °C for 15 min. An aliquot (2 μl) of the reaction mixture was injected into a column of Silicone XE-60 (5% w/w) on Uniport HPS maintained at 160 °C in a gas chromatograph (model GC-5A, Shimadzu Seisakusho Ltd., Kyoto, Japan) equipped with a flame ionization detector. The detector temperature was 185 °C and the flow rate of nitrogen carrier gas was 30 ml/min. Galactitol and *myo*-inositol were identified by their retention times and quantitated against the internal standard by analysis of peak heights.

Results

Effect on Cataract Formation and Body Weight

The incidence of cataract formation was decreased in rats dosed with *p*-Br-PSH at 50 mg/kg from the first day (day 0) on galactose diet relative to control rats (placebo-dosed rats) (Fig. 1). While nucleus cataracts were observed in all of the control rats on day 15, only 10% of rats treated with *p*-Br-PSH (50 mg/kg) suffered from nucleus cataracts at that time.

In the groups pretreated with *p*-Br-PSH for 10 d before the start of galactose diet feeding, subsequent treatment with the drug markedly delayed cataract formation (Fig. 2). By day 20 on galactose diet, 100% of the eyes of control rats had developed nucleus cataracts. On the other hand, no nucleus cataracts were observed until day 18 and 26 in rats receiving 25 and 50 mg/kg *p*-Br-PSH, respectively.

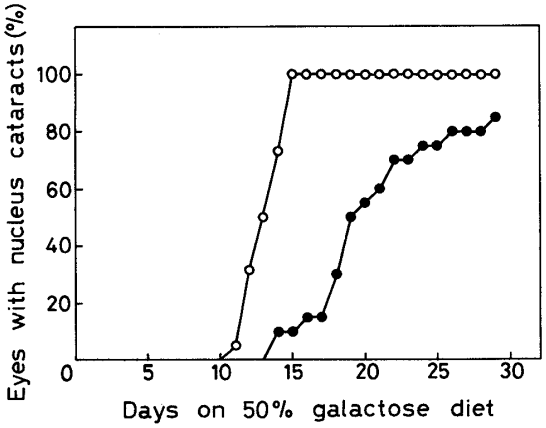

Fig. 1. Effect of Treatment with *p*-Br-PSH on Formation of Nucleus Cataracts in Galactosemic Rats

Treatment with *p*-Br-PSH at 50 mg/kg/d was started from the first day (day 0) on a 50% galactose diet. There were 10 rats, *i.e.* 20 eyes tested, in each group. —○—, control rats; —●—, *p*-Br-PSH treated rats.

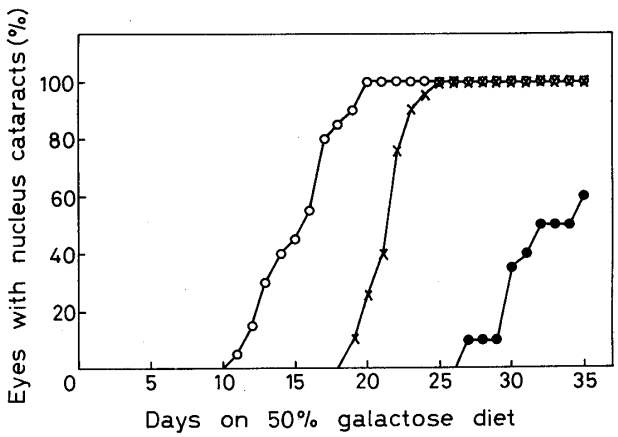

Fig. 2. Effect of Pretreatment and Subsequent Treatment with *p*-Br-PSH on Formation of Nucleus Cataracts in Galactosemic Rats

Rats were dosed with *p*-Br-PSH at 25 and 50 mg/kg/d during pretreatment (for 10 d on normal diet) and subsequent treatment (from day 0 to 35 on a 50% galactose diet). There were 10 rats, *i.e.* 20 eyes tested, in each group. —○—, control rats; —×—, rats dosed with *p*-Br-PSH at 25 mg/kg/d; —●—, rats dosed with *p*-Br-PSH at 50 mg/kg/d.

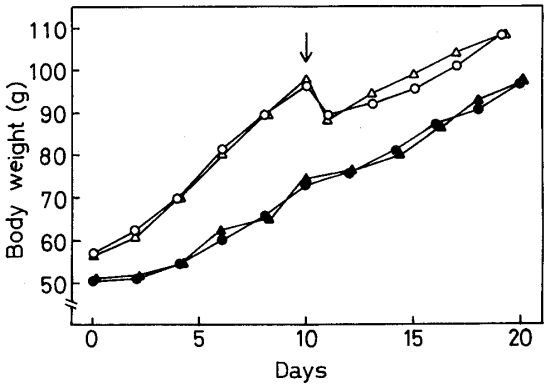

Fig. 3. Effect of Treatment with *p*-Br-PSH on the Growth of Rats

In the pretreatment experiment, rats were dosed with *p*-Br-PSH at 50 mg/kg/d during pretreatment (from day 0 to 9 on normal diet) and subsequent treatment (from day 10 to 19 on a 50% galactose diet). The start of a 50% galactose diet is shown by an arrow. —○—, control rats; —△—, *p*-Br-PSH dosed rats.

In the non-pretreatment experiment, rats were fed a 50% galactose diet and also dosed with *p*-Br-PSH at 50 mg/kg/d from day 0. —●—, control rats; —▲—, *p*-Br-PSH dosed rats.

In the pretreatment experiment, although the body weight of rats decreased transiently when normal diet was changed to galactose diet, the growth curves of drug-treated rats and control rats were almost the same throughout the course of the experiment (Fig. 3). In the non-pretreatment experiment as well, there was essentially no difference in growth rate between drug-treated rats and control rats.

Effect on Galactitol and *myo*-Inositol Levels in Tissues

Galactitol levels in the lens, sciatic nerve, and retina of rats, to which *p*-Br-PSH or placebo had been given from the first day (day 0) on galactose diet, are shown in Table I. No detectable level of galactitol was observed in those tissues of normal diet-fed rats (data not shown). In the lens of control rats the galactitol level was 71.9 ± 3.8 and 93.6 ± 8.4 μmol/g wet wt. at day 5 and 9 on galactose diet, respectively. Treatment with *p*-Br-PSH at 50 mg/kg reduced significantly, though not markedly, the lenticular galactitol levels at both days, whereas no significant decrease was observed in rats treated with *p*-Br-PSH at 25 mg/kg. The galactitol levels in the sciatic nerve of control rats were 17.1 ± 2.5 and 25.0 ± 3.0 μmol/g wet wt. at day 5 and 9, respectively. In rats receiving *p*-Br-PSH (25 and 50 mg/kg) in addition to galactose diet, galactitol accumulation was reduced by 36 and 50%, respectively, at day 5. A similar degree of reduction was also observed at day 9. The retina of control rats accumulated galactitol at a level of 6.8 ± 0.8 μmol/g wet wt. at day 26 on galactose diet. Statistically significant though small decreases in galactitol levels were observed in the drug-treated rats (5.4 ± 0.3 and 5.0 ± 0.4 μmol/g wet wt. at doses of 25 and 50 mg/kg, respectively).

myo-Inositol levels in the sciatic nerve of normal rats and galactosemic rats are shown in Table II. Treatment with *p*-Br-PSH or placebo was begun from the first day (day 0) on galactose diet. No significant difference in the sciatic nerve *myo*-inositol level was observed between normal and control rats at day 5. At day 9, however, control rats had decreased their sciatic nerve *myo*-inositol contents by 42% compared with normal rats (3.2 ± 0.3 *vs.* 5.5 ± 0.7 μmol/g wet wt.). The sciatic nerve *myo*-inositol levels of rats treated with *p*-Br-PSH at

TABLE I. Effect of *p*-Br-PSH on Galactitol Levels in Tissues of Galactose-Fed Rats

	Galactitol content (μmol/g wet wt.)[a]					
	Lens			Sciatic nerve		Retina
	Day 5	Day 9	Day 18	Day 5	Day 9	Day 26
Control	71.9±3.8	93.6± 8.4	12.8±3.5	17.1±2.5	25.0±3.0	6.8±1.0
p-Br-PSH (25 mg/kg)[b]	68.8±3.6	91.0±14.0	26.0±5.0[e]	11.0±1.9[d]	16.9±1.1[e]	5.4±0.6[d]
p-Br-PSH (50 mg/kg)[b]	65.9±3.5[d]	83.6± 7.5[c]	32.6±9.0[e]	8.6±1.1[e]	13.4±1.2[e]	5.0±0.8[d]

a) Values are means±S.D. for 10—11 determinations. *b*) *p*-Br-PSH was given from the first day (day 0) on a 50% galactose diet. *c*) $p<0.05$ compared to control. *d*) $p<0.01$ compared to control. *e*) $p<0.001$ compared to control.

TABLE II. Effect of *p*-Br-PSH on *myo*-Inositol Level in the Sciatic Nerve of Galactose-Fed Rats

	myo-Inositol content (μmol/g wet wt.)[a]	
	Day 5	Day 9
Normal	4.8±0.7	5.5±0.7
Control	4.2±0.6	3.2±0.3[c]
p-Br-PSH (25 mg/kg)[b]	4.1±0.7	3.7±0.1[d]
p-Br-PSH (50 mg/kg)[b]	4.3±0.6	4.3±0.2[e]

a) Values are means±S.D. for 10—11 determinations. *b*) *p*-Br-PSH was given from the first day (day 0) on a 50% galactose diet. *c*) $p<0.001$ compared to normal. *d*) $p<0.01$ compared to control. *e*) $p<0.001$ compared to control.

25 and 50 mg/kg were increased by 16 and 34%, respectively, compared with that of control rats.

Discussion

The effectiveness of *p*-Br-PSH in delaying cataract formation in galactosemic rats was clearly shown in the present study (Figs. 1 and 2). *p*-Br-PSH was more effective in rats pretreated with the drug for 10 d before the start of galactose diet in addition to drug treatment relative to rats treated with the drug from the first day on galactose diet without pretreatment. A possible explanation for this effect of pretreatment is that *p*-Br-PSH accumulates in the lens at a concentration sufficient for exerting its effect, *i.e.*, inhibition of aldose reductase, prior to galactose diet feeding. Alternatively, the difference in effectiveness may be due to the difference in the body weight of animals between the two experiments. The mean body weight of rats used in the pretreatment experiment was 55 g at the start of pretreatment and 98 g at the first day (day 0) on galactose diet, while that of rats used in the other experiment was 50 g at day 0 on galactose diet (Fig. 3).

A slower development of cataract formation in the control rats of the pretreatment experiment (Fig. 2) relative to the non-pretreatment experiment (Fig. 1) is compatible with reports[12,13] that galactose cataract matures more slowly in the adult rat than in the young rat.

The lenticular galactitol level of untreated galactosemic rats was 71.9 μmol/g wet wt. at day 5 on galactose diet, increased to a peak of 93.6 μmol/g wet wt. at day 9, and then decreased dramatically to 12.8 μmol/g wet wt. at day 18 (Table I); these values are consistent with the results reported by Hu *et al.*[14] The rapid decrease of the galactitol level after day 9 on galactose diet may be interpreted as indicating that the galactitol accumulated in the lenticular

cells began to leak out of the cells because the swelling of cells caused by hypertonicity resulted in the disruption of cell membranes. Higher galactitol levels in the lens of drug-treated rats relative to control rats at day 18 may be explainable in terms of delayed disruption of cell membranes resulting from delayed accumulation of galactitol. Since *p*-Br-PSH at 50 mg/kg reduced the galactitol accumulation by only less than 11% at day 5 and 9, it seems unlikely that the delay of cataract formation by treatment with *p*-Br-PSH is caused solely by the decrease in osmotic pressure resulting from reduction in the lenticular galactitol level. The precise mechanism of the effect of *p*-Br-PSH in delaying cataract formation remains to be clarified.

The effect of *p*-Br-PSH in decreasing galactitol accumulation was evident in the sciatic nerve relative to the lens. This result suggests that *p*-Br-PSH may be more effective in the peripheral nerves than in the lens for the treatment of diabetic complications.

The osmotic mechanism for diabetic retinopathy based on the polyol pathway activity is conceivable since aldose reductase is reported to be restricted to the mural cells of the retinal capillaries[15] and to the Müller cells.[16] However, evidence that aldose reductase is involved in diabetic retinopathy has not yet been obtained.[17] Treatment with *p*-Br-PSH produced no marked decrease in the retinal galactitol concentration in this study.

The decrease of *myo*-inositol content in peripheral nerves has been suggested to be related to the defect of nerve functions in experimental diabetes,[18-20] although the mechanism underlying this putative involvement has not yet been clarified. A defect in nerve conduction velocity has also been observed in galactose-fed rats.[21] In addition, Finegold *et al.*[22] have suggested that increased polyol pathway activity is a likely factor of the fall in the nerve *myo*-inositol content in experimental diabetes and also that polyol pathway inhibition increases the nerve *myo*-inositol content. Therefore, we intended to see whether the change in the galactitol level is associated with that in the *myo*-inositol level in the sciatic nerve. In the present study, however, treatment with *p*-Br-PSH increased the *myo*-inositol level in the sciatic nerve at day 9 on galactose diet, but not at day 5, whereas the drug treatment decreased the sciatic nerve galactitol level at both times. Absence of association between the polyol pathway activity and the nerve *myo*-inositol level observed at day 5 may be attributable to the use of galactosemia, but not experimental diabetes, in this study.

p-Br-PSH lowered the blood glucose concentration of normal rabbits by about 20% at 3 h after administration at 50 mg/kg.[10] In contrast, no decrease in blood glucose concentration was observed in normal rats during 5 h after dosing at 50 mg/kg (data not shown). However, treatment of galactosemic rats with *p*-Br-PSH for 27 d from the first day on galactose diet produced a small yet statistically significant decrease (17%) in blood glucose concentration (data not shown). The difference in hypoglycemic activity of *p*-Br-PSH between rabbits and rats may be due to a species difference.

The absence of any difference in growth rate between drug-treated rats and control rats (Fig. 3) suggests that *p*-Br-PSH does not cause any change in body weight as a side effect.

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