Drugs of the Future 1998, 23(5): 521-529

CCC: 0377-8282/98

Review Article

Benzothiazole aldose reductase inhibitors

Naoki Ashizawa* *and Tomoji Aotsuka*
*Pharmaceutical Res. Labs., Research and Development Div., Grelan Pharmaceut. Co., Ltd., 4-3 Sakaecho 3-chome, Hamura City, Tokyo 205, Japan. *Correspondence*

CONTENTS

Introduction

Chronic diabetes leads to long-term complications which include neuropathy, nephropathy, retinopathy and cataracts. In diabetes mellitus, hyperglycemia causes a marked production of intracellular sorbitol due to aldose reductase (AR) activity (1). AR [EC 1.1.1.21] is the first enzyme of the polyol pathway (2) and catalyzes the reduction of glucose by NADPH to sorbitol, which can in turn be oxidized by the enzyme sorbitol dehydrogenase and by NAD^+ to yield fructose (Fig. 1).

To date, the explanation of involvement of the polyol pathway in the pathogenesis of diabetic complications includes the osmotic stress hypothesis (3), myo-inositol (MI) hypothesis (4), the redox potential hypothesis (5), *etc.* Inhibition of the polyol pathway by AR inhibitors (ARIs) in diabetic animals and man has been shown to ameliorate some of the complications of diabetes (6-9). The mechanisms by which ARIs can reduce diabetic neuropathy are summarized in Figure 2. In the nerve, increase in MI or a decrease in the ratio of NADH/NAD^+ following treatment with ARIs results in the restoration of Na^+/K^+-ATPase activity, a key enzyme for nerve function. Recently, microangiopathy and the resultant nerve hypoxia have received increasing attention as causes of diabetic neuropathy (10, 11). AR and nitric oxide (NO) synthase share NADPH as an obligate cofactor; therefore, enhanced glucose flux through AR is assumed to blunt

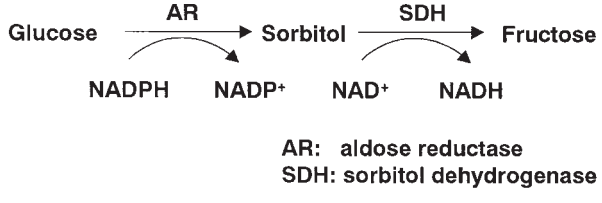


Fig. 1. Scheme of the polyol pathway.

NO synthase activity (12). However, there is growing evidence that alterations in several independent pathways may contribute to impaired endothelium-dependent relaxation of diabetic blood vessels (13). Decreased concentration of 2,3-diphosphoglycerate (2,3-DPG) in red blood cells of diabetic animals, which is assumed to contribute to the nerve hypoxia via the reduction of erythrocyte oxygen unloading, is ameliorated by ARIs. Furthermore, normalization of erythrocyte deformability and platelet aggregation by treatment with ARIs may be involved in the lessening of diabetic complications.

A particular appeal of ARIs is that they may be able to prevent or arrest progression of many of the complications of diabetes without the significantly increased risk for hypoglycemia. Over the past decade, several ARIs of diverse structures have been discovered. The structural classes exhibiting the most potent activity are the carboxylic acids and hydantoins. Some researchers shifted their attention to the potent carboxylic acid ARIs because of the incidence of hypersensitivity side effect with sorbinil, a hydantoin ARI. To date, two carboxylic acid ARIs, *i.e.*, tolrestat and epalrestat, have reached the market (14). Recently, benzothiazole derivatives have received much attention due to their potent AR inhibitory activity both *in vitro* and *in vivo*. In the present review, we describe the structure-activity relationships of benzothiazole ARIs, present their pharmacological efficacy and finally describe their pharmacokinetics and metabolism.

Structure-activity relationships

The first example of a benzothiazole derivative with AR inhibitory activity was reported by scientists at Pfizer. As shown in Table I, a large increase in potency is observed in the oxicam derivative **1b** (R = benzothiazole) when compared with **1a** (R = thiazole). They proposed that there is a hitherto unrecognized binding site on the AR enzyme with strong affinity for benzothiazoles located some distance from the site which binds to acidic groups. Zopolrestat (**2b**) was found to have superior AR inhibitory activity to ponalrestat (**2a**), though both phtharazinone have the same backbone. As indicated by the structural modification of ponalrestat to zopolrestat, replacement of the phenyl ring by the benzothiazole ring maintains or increases AR inhibitory activity. Such interchangeability between the phenyl ring and benzothiazole ring is observed in other backbone structures including quinazolinon-2,4-dione (**3**), 2-oxo-1,4-benzothiazine (**4**) and 2,4,5-trioxoimidazolidine (**5**). The bioisostere of the hydantoin derivative, ARI-509, as well as its benzothiazole congener **6b**, also show potent AR inhibitory activity.

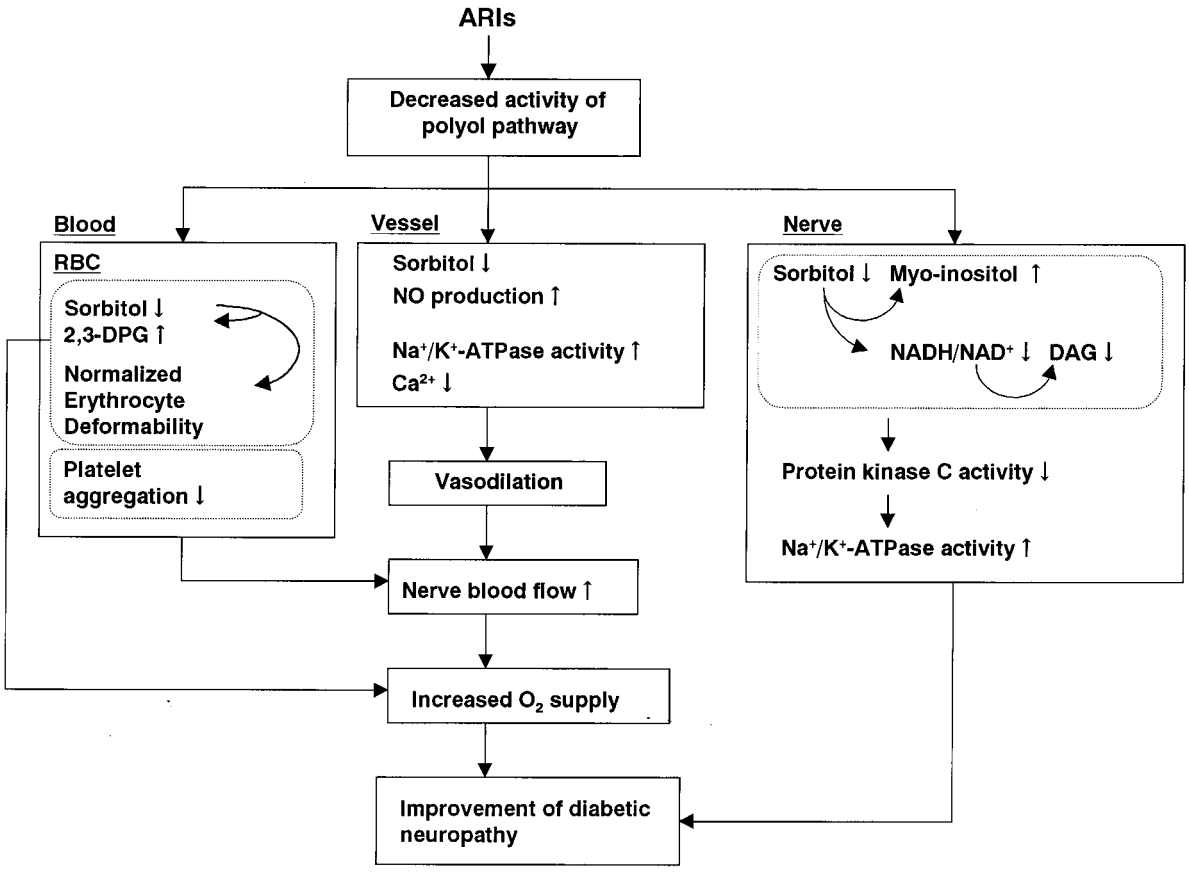


Fig. 2. Hypothesized mechanisms of ARI-induced amelioration of diabetic neuropathy. 2,3-DPG: 2,3-diphosphoglycerate; DAG: diacylglycerol.

Several 4,5,7-trifluorobenzothiazol-2-ylcarboxylic acids (**7**) in which the carboxylic group (P1) was separated from the benzothiazole ring (P2) by 3 to 6 methylene units were synthesized based on the hypothesis that the distance between P1 and P2 crucially influences AR inhibitory activity (Fig. 3). As shown in Table II, the compound with the most potent activity was found to contain five methylene units similar to the existing AR inhibitors such as zopolrestat, zenarestat and SG-210. Modification of the methylene spacer as shown in **7c** resulted in compound **8** (GP-1447) which exhibited increased AR inhibitory activity. It is a remarkable feature that GP-1447 has a simple hydrophobic spacer without amide groups (15).

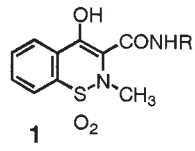
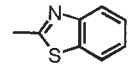
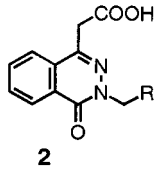
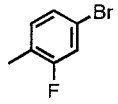
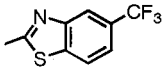
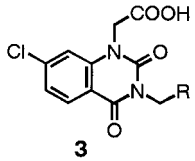
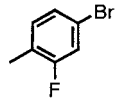
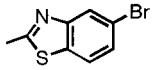
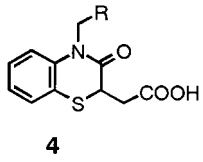
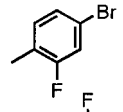
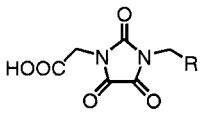
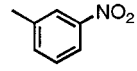
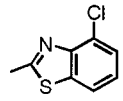
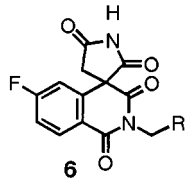
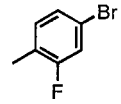
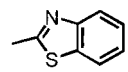
Pharmacological actions

Observation of crystal structure of AR complexed with zopolrestat revealed the inhibitor bound to the active site (24). Using a fluorometric assay, it was further determined that zopolrestat bound to AR complexed with either NADPH or $NADP^+$ (25), and it has been suggested that the mixed inhibition patterns observed in Lineweaver-Burk plots for inhibition by zopolrestat were due to the tight binding of this compound (25). Both SG-210 (26) and GP-1447 (27) exhibited noncompetitive inhibition with respect to DL-glyceraldehyde as substrate. In contrast to epalrestat, inhibition of AR activity by GP-1447 was apparently irreversible as evidenced by the absence of recovery of AR activity after dialysis for 24 h (27).

Effects on aldehyde reductase

Aldehyde reductase (ALR) is closely related (65% identity) to AR (28). ALR is an important enzyme for reduction of many aldehydes, for counteraction and excretion of drugs (29), for reduction of 3-deoxyglucosone, which is an intermediate for advanced glycation end products (AGEs) (30), and for metabolism of methylglyoxal (31). To avoid adverse effects of ARI therapy, AR selectivity is necessarily one of the important considerations (29). Although a number of ARIs have been evaluated for their activities, only a few which inhibit AR selectively have been found. Four ARIs, *i.e.*, zopolrestat, compound **5b**, SG-210 and GP-1447, exhibited almost no inhibition of ALR (Table III). The selective inhibition of AR by these compounds may be due to the benzothiazole rings as lipophilic aromatic groups.

Table I: Aldose reductase inhibition by ARIs possessing various backbone structures.

R			IC_{50} (M)	References
 1	a: 		$>10^{-4}$	16
	b: 		5.5×10^{-6}	16
 2	a: 	Ponalrestat	1.1×10^{-7}	17
	b: 	Zopolrestat	3.1×10^{-9}	16
 3	a: 	Zenarestat	5.7×10^{-9}	18
	b: 		5.0×10^{-9}	19
 4	a: 		6.1×10^{-8}	20
	b: 	SG-210	9.5×10^{-9}	20
 5	a: 	NZ-314	6.2×10^{-8}	21
	b: 		1.2×10^{-8}	22
 6	a: 	ARI-509	1.4×10^{-8}	23
	b: 		1.8×10^{-8}	23

Effects on sorbitol accumulation and myo-inositol depletion

The various pharmacological effects of ARIs are made evident by inhibition of tissue sorbitol accumulation. The ability of benzothiazole ARIs to reverse tissue sorbitol accumulation in rats made diabetic with streptozotocin (STZ) has been reported (16, 26, 27). In this test, ARIs were administered to diabetic rats once daily by oral gavage for 5 days from 7 days after STZ injection. As shown in Table IV, zopolrestat reversed elevated sorbitol accumulations in the sciatic nerve, retina and lens with ED_{50} values of 1.9, 17.6 and 18.4 mg/kg/day, respectively (16). SG-210 was equipotent to zopolrestat in the sciatic nerve, but was about 3 times more potent in the lens (26). GP-1447 was more potent than SG-210 in inhibiting sorbitol accumulation in both the sciatic nerve and lens (27).

In addition to an elevation of nerve sorbitol, experimental diabetes causes a depletion of MI. Treatment with GP-1447 for 2 weeks from 4 weeks after the induction of

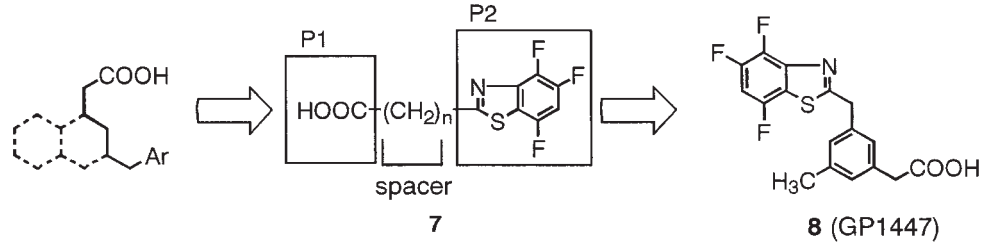


Fig. 3.

Table II: Inhibition of aldose reductase by benzothiazole carboxylic acid.[a]

Compd.	n	% inhibition at 10^{-7}M[b]
7a	3	1
7b	4	35
7c	5	71 (IC_{50} 50 nM)
7d	6	11

[a]See ref. 15; [b]AR inhibitory activity in porcine lens.

diabetes dose-dependently restored the depleted MI content in the sciatic nerve of STZ-induced diabetic rats with an ED_{50} value of 0.47 mg/kg/day (our unpublished data).

When the lenses were incubated for 12 or 16 h in high galactose medium, large amounts of galactitol rapidly accumulated, resulting in noncompetitive inhibition of MI influx (32). Zopolrestat (40 μM) inhibited 80% of polyol formation in the lenses, thereby 80% protection of MI influx (32).

Restoration of motor nerve conduction velocity and effects on neurovascular deficits

The effects of benzothiazole ARIs on motor nerve conduction velocity (MCV) in STZ-induced diabetic rats as a means of assessing efficacy in diabetic neuropathy were reported (26, 27). In this test, ARIs were administered once daily by oral gavage for 2 weeks from 4 weeks after STZ injection. The deteriorated MCV in diabetic rats was dose-dependently ameliorated by treatment with SG-210 (1-30 mg/kg/day) accompanied by reduction in sorbitol content in the sciatic nerve. In that study, ED_{50} values of SG-210 and zopolrestat were determined to be 0.5 and 17.2 mg/kg/day, respectively (26). Treatment with GP-1447 (0.3-3 mg/kg/day) dose-dependently restored the decreased MCV (27).

Since deleterious changes in nerve perfusion are major factors in the etiology of diabetic neuropathy, effects of ARIs on neurovascular deficits have been examined. Reduced sciatic nerve blood flow (SNBF) of STZ-induced diabetic rats as measured by non-contact laser-Doppler flowmetry was partially and dose-dependently recovered after treatment with GP-1447 at doses of 10 and 30 mg/kg/day (33). In this study, there was a correlation between MCV and SNBF, suggesting that vascular factors seem to be involved in the MCV (33). SG-210 was ineffective at a dose of 20 mg/kg/day (our unpublished data). Inhibition of phosphodiesterase (PDE) by GP-1447 (27) may in part contribute to the increase in SNBF. Nonselective PDE inhibition seems to be a

Table III: Inhibition of aldose reductase (AR) and aldehyde reductase (ALR) in vitro *by benzothiazole ARIs.*

ARI	AR IC_{50} (nM)	ALR IC_{50} (μM)	Ratio	Ref.
Zopolrestat	13	50	3800	16, unpublished data
Compound **5b**	12	72	6000	22
SG-210	9.5	>100	>10000	26, unpublished data
GP-1447	9.6	>100	>10000	27

Table IV: Inhibition of sorbitol accumulation in STZ-induced diabetic rats by benzothiazole ARIs.

ARI	Inhibition of sorbitol accumulation ED_{50} (mg/kg/day)				
	Erythrocyte	Sciatic nerve	Lens	Retina	Ref.
Zopolrestat		1.9	18.4	17.6	16
SG-210	< 1	1.9	6.8		26
GP-1447		0.25	1.6	2.9	27

ARIs were administered once daily by oral gavage for 5 days starting 7 days after STZ injection.

common feature of carboxylic acid ARIs, although their IC_{50} values are in the micromolar range (27).

Although some data indicate that vascular factors in the nerve have a great functional significance, SG-210 seems to ameliorate MCV with a relatively small effect on SNBF as described above. SG-210 is reported to increase lowered oxygen tension, which is associated with MCV, at lower doses than required for the same incremental improvement of nerve blood flow in STZ-induced diabetic rats. Erythrocyte oxygen unloading is reduced in diabetes due to changes in 2,3-DPG and glycated hemoglobin. The ameliorating effect of SG-210 on reduced erythrocyte oxygen unloading has been suggested to be involved in the restoration of the oxygen tension in nerve tissue.

There is some evidence of beneficial effects of ARIs on exacerbated endothelium-dependent relaxation induced by high concentration of glucose or diabetes. Treatment with zopolrestat (10 μM) prevented decreased endothelium-dependent relaxation of rabbit aorta seen at elevated concentrations of glucose (44 mM) (34). In alloxan-induced diabetic rabbits, treatment with zopolrestat for 6 weeks at a dose of 150 mg/kg/day in the diet was shown to normalize both elevated red blood cell sorbitol levels and decreased endothelium-dependent relaxation of aorta induced by acetylcholine and adenosine diphosphate (13). Since zopolrestat had no effect on the levels of cyclic GMP or on the increased release of thromboxane A_2 in diabetic aorta, it is suggested that endothelial production of neither NO nor vasoconstrictor prostanoids can be directly implicated in the improvement caused by the drug (13). The result supports the fact that GP-1447-induced restoration of SNBF in STZ-induced diabetic rats was not affected by NO synthase inhibitor (33), although some differences may exist between aortic endothelial cells and peripheral nervous system.

Increased blood viscosity and reduced erythrocyte deformability could potentially contribute to endoneurial hypoxia (35). Treatment with SG-210 or GP-1447 at doses of 15 and 3 mg/kg/day, respectively, prevented the decrease in erythrocyte deformability in STZ-induced diabetic rats (33).

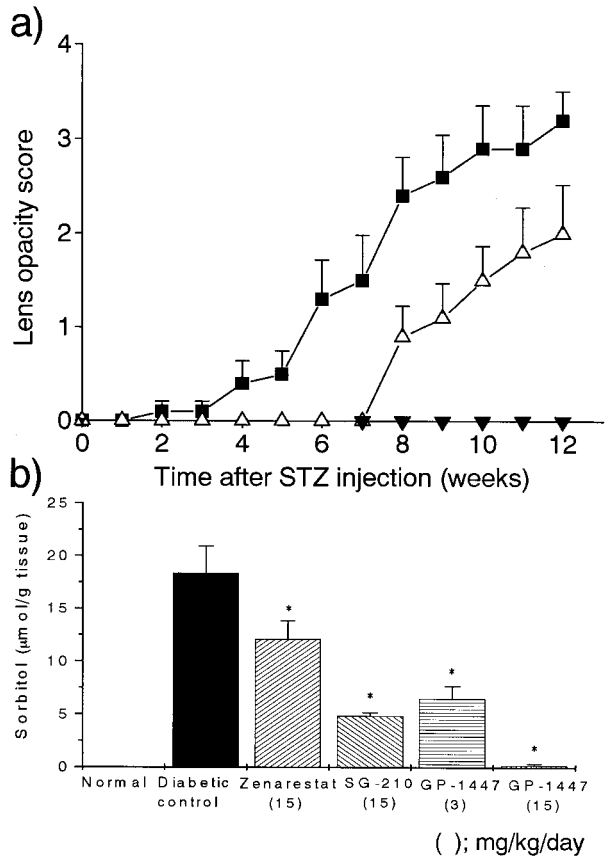


Fig. 4. Preventive effects of various ARIs on cataract formation (a) and sorbitol accumulation in the lenses (b) of STZ-induced diabetic rats. Rats injected with STZ (60 mg/kg i.v.) were treated with vehicle (■) or ARIs at 3 mg/kg/day (GP-1447, ▼) or 15 mg/kg/day (zenarestat, Δ; SG-210, □; GP-1447, ●) for 12 weeks. Lens opacity was determined (score 0 to 4) by using a slit lamp after dilating the pupils (n = 5-10). No cataract formation was observed in normal control rats (○) and diabetic rats treated with GP-1447 at doses of 3 and 15 mg/kg/day or SG-210. $^{*}p$ <0.01, compared with the diabetic control by Dunnett's multiple range test. See ref. 27.

Inhibition of cataract formation

In animal tests, the effects of ARIs on ocular tissue are much less remarkable compared to the effects on nerve tissue. This is presumably because orally administered drugs have to cross the blood-aqueous humor barrier and then reach the lens (16). Following 5 (50 mg/kg) consecutive once-daily oral administrations of zopolrestat, peak concentrations in the sciatic nerve and the lens were 8370 and 843 ng/g, respectively (36). Treatment of STZ-induced diabetic rats with zopolrestat for 6 months, at a dose of 100 mg/kg/day, resulted in preventive effects in the lens including transparency, preserved MI content and ouabain-sensitive Rb influx, an index of Na^+/K^+-ATPase activity (37). As shown in Figure 4, oral treatment with GP-1447 (3 and 15 mg/kg/day) or SG-210 (15 mg/kg/day) reduced the sorbitol level in the lens and consequently prevented the progression of cataract formation in STZ-induced diabetic rats (27). No lens opacity was observed until 12 weeks. In this preventive study, GP-1447 was estimated to be 5 times more potent than SG-210 at inhibiting sorbitol accumulation in the lens (Fig. 4b). Curative effects of ARIs on developed cataracts were also determined. Treatment with GP-1447 at a dose of 15 mg/kg/day for 5 weeks beginning 8 weeks after the induction of diabetes partially reversed the diabetic cataract as evidenced by the lens opacity and histological changes such as swelling of fibers and vacuolation (27).

SG-210 at concentrations of 0.1 and 1 μM prevented opacities and reduced hydration of isolated rat lenses cultured with xylose (38). In rats fed 50% galactose, 10 μg of

SG-210 was locally administered to eye 3 times a day. This topical application of SG-210 lowered increased lens calcium and also prevented proteolysis of lens crystallins and lens opacity induced by feeding of galactose. The inactivated form of calpain in soluble and insoluble protein from lens as measured by immunoblots was decreased in rats fed galactose. SG-210 restored the decreased calpain in the lenses of galactose-fed rats, indicating the inactivation of calpain. It is suggested that proteolysis by calpain is involved as a mechanism in the formation of sugar cataract (38).

Amelioration of worsening of electroretinogram

It is well accepted that the electroretinogram (ERG) specifically indicates retinal function and also provides early warning of retinal abnormalities before ophthalmoscopically visible alterations are detectable in diabetes cases. The b-wave latency of the ERG is known to be related to Müller cell function (39) which is damaged earlier than the retinal blood vessel (40). The delayed peak latencies of oscillatory potentials in the ERG of STZ-induced diabetic rats was significantly restored by treatment with SG-210 for 4 weeks at a dose of 10 mg/kg/day (26). After the final administration of SG-210, the retinal sorbitol content decreased to the normal control level (26).

Effects on nephropathy

The potential of ARIs for normalizing or reducing renal hyperfiltration, one of the major pathogenic factors in diabetic neuropathy, has been shown. In experimental studies measuring whole kidney blood flow, superficial cortical blood flow and single nephron hemodynamics, zopolrestat reduced renal hyperfiltration primarily by protecting against and reversing a decrease in renal microvascular resistance caused by chronic hyperglycemia (41). In STZ-induced diabetic rats, a high dose of zopolrestat (100 mg/kg/day) significantly reduced hyperfiltration (42) and high urinary albumin excretion (42, 43). Zopolrestat was shown to dose-dependently prevent renal hyperperfusion in galactosemic rats (44).

Miscellaneous effects

Altered carotid artery reactivity to ouabain and potassium in alloxan-induced diabetic rabbits was partially restored by treatment with zopolrestat (50 or 150 mg/kg/day) (45).

High glucose conditions have been found to diminish porcine aortic endothelial cell proliferation and increase smooth muscle cell proliferation. Addition of zopolrestat prevented the enhanced proliferation of smooth muscle cells but did not affect high glucose-attenuated endothelial cell proliferation (46). These results indicate that AR is mainly involved in hyperglycemic smooth muscle cell proliferation.

Recently, the protective effects of zopolrestat against ischemic injury in isolated rat heart were reported (47). The increased cytosolic redox state ($NADH/NAD^+$), decreased ATP and phosphocreatine content, as well as left ventricular developed pressure on reperfusion and increased creatinine kinase release in rat heart, were all ameliorated by pretreatment of the heart with zopolrestat (1 μM). It is noteworthy that the protective effects on heart were observed both in spontaneously acute diabetic (BB/W) rats and nondiabetic rats (47). In this study, it is suggested that lowering of $NADH/NAD^+$ ratio by AR inhibition during global ischemia is associated with protection presumably via increased anaerobic glycolysis.

Pharmacokinetics and metabolism

Zopolrestat, SG-210 and GP-1447 were found to be extensively bound to plasma proteins (about 99%), probably due to their carboxyl moiety. After a single i.v. dose of 2 mg/kg zopolrestat to normal rats, the volume of distribution was 0.57 l/kg, total body clearance was 0.84 ml/min/kg, AUC_{0-24} was 35.3 μg.h/ml and half-life was 7.9 h. After a single dose of 50 mg/kg, mean plasma and tissue levels of the compound were higher in normal than in diabetic rats, with levels in the kidney approaching those in plasma in both groups of animals. Plasma half-life in normal rats was slightly longer than that in diabetic rats. Tissue half-lives were similar in both groups and much longer than in plasma. Urinary excretion appeared to be a minor route of elimination, and no glucuronide conjugates were detected. Diabetic rats administered multiple doses of zopolrestat showed no plasma accumulation, whereas concentrations in nerve, kidney and lens increased to varying degrees, reflecting the longer half-lives in these tissues (48).

SG-210 exhibits good oral availability. After a single oral dose of 3 mg/kg to rats, C_{max} of 7.7 μg/ml, AUC of 62.0 μg.h/ml and half-life of 4.6 h were achieved.

GP-1447 exhibits fast elimination from blood. After a single oral dose of 10 mg/kg to dogs, C_{max} of 5.4 μg/ml, AUC of 20.0 μg.h/ml and half-life of 0.58 h were observed (unpublished data).

Pharmacokinetic studies in healthy volunteers administered single oral doses of 50-1200 mg zopolrestat showed linear increases in peak plasma levels, time to peak plasma levels, AUC and urinary elimination. Urinary excretion of the unchanged drug was 40% of dose within 48 h and the mean half-life was estimated to be 18.6 h. Renal clearance was 2.6-5.6 ml/min and appeared to decrease as the dose increased. In subjects administered doses of 800 or 1200 mg/day for 2 weeks, steady state was reached within 7 days. The mean steady-state half-life was 30.3 h. Mean renal clearance was 2.2 ml/min, with approximately 45% of the administered dose being excreted into the urine at steady state. Food intake appeared to have no effect on absorption. These results

indicated the suitability of once-daily administration of zopolrestat in the treatment of diabetic complications (49).

Conclusions

The search for ARIs at Pfizer led to the discovery of the benzothiazole derivative zopolrestat, which is now in phase III clinical trials for the prevention of diabetic neuropathy. The structure-activity relationships have shown that the distance between the carboxylic group and the benzothiazole ring crucially influences AR inhibitory activity as supported by the fact that the carboxylic group of all compounds listed in the present review is separated from the benzothiazole ring by 5 atoms. The long tissue half-life of zopolrestat, which is contrary to that seen in plasma, seems to be involved in the potent activity of this compound *in vivo*. The potent effects of SG-210 and GP-1447 on ocular tissue discriminate them from the other ARIs. To date, GP-1447 is the benzothiazole ARI possessing the most potent AR inhibitory activity *in vivo*.

Zopolrestat, SG-210 and GP-1447 were less toxic than tolrestat in the acute toxicity test in mice (our unpublished data). Benzothiazole ARIs may offer advantages over other ARIs in regard to avoiding adverse effects since these compounds selectively inhibit AR *versus* ALR.

The observed efficacy of benzothiazole ARIs on various diabetic complications can be mediated by the inhibition of sorbitol accumulation in the target tissues. However, the intrinsic sensitivity of a particular tissue to excess polyol pathway flux seems to differ from that of another tissue. Tolrestat completely normalized elevated urinary albumin excretion in 6-month diabetic rats, but reduced the concentration of sorbitol in the renal cortex by only 60% (50). On the other hand, recent data showed that nerve sorbitol correlates in a nonlinear fashion with tissue dysfunction, *i.e.*, a high degree of polyol pathway blockade is necessary to correct neurovascular deficits in experimental diabetes (51). To date, clinical trials using ARIs have identified only modest improvements in neuropathic patients. If strong and sustained inhibition of polyol pathway flux is achieved in a target tissue, substantial improvements in function may occur. It is necessary to examine more effective doses of potent ARIs to assess potential benefits. Since some benzothiazole ARIs exhibit rapid elimination from the blood, high doses of these compounds may be tolerated, leading to a high degree of inhibition of sorbitol accumulation.

Of the various effects of benzothiazole ARIs, it is noteworthy that zopolrestat exhibited a protective effect from ischemic injury in isolated rat heart (47). Normalization of cytosolic redox state is one example of a beneficial effect of ARIs in nondiabetic conditions, in which abnormal redox state is involved.

Further research with appropriately potent and well-tolerated ARIs will help determine how useful ARIs will be for preventing, arresting or treating the long-term complications of diabetes. Since benzothiazole ARIs possess a favorable drug profile, they are promising candidates for the treatment of various diabetic complications.

Acknowledgements

The authors wish to thank Mr. M. Yoshida for preparing this paper and Mr. K. Kumazawa for his thorough research of the chemical literature on benzothiazole ARIs.

References

1. Kador, P.F., Robinson, W.G. Jr., Kinoshita, J.H. *The pharmacology of aldose reductase inhibitors.* Annu Rev Pharmacol Toxicol 1985, 25: 691-714.

2. Sarges, R., Oates, P.J. *Aldose reductase inhibitors: Recent developments.* Prog Drug Res 1993, 40: 99-161.

3. Kinoshita, J.H., Datiles, M.B., Kador, P.F., Robinson, W.G. Jr. In: Diabetes Mellitus, Theory and Practice. Rifkin, H., Porte, D. Jr. (Eds.). Elsevier: New York 1990, 264.

4. Greene, D.A., Lattimer, S.A., Sima, A.A.F. *Pathogenesis and prevention of diabetic neuropathy.* Diab Metab Rev 1988, 4: 201-21.

5. Tilton, R.G., Baier, L.D., Harlow, J.E., Smith, S.R., Ostrow, E., Williamson, J.R. *Diabetes-induced glomerular dysfunction: Links to a more reduced cytosolic ratio of NADH/NAD$^+$.* Kidney Int 1992, 41: 778-88.

6. Greene, D.A., Mackway, A.M. *Decreased myo-inositol content and Na$^+$/K$^+$-ATPase activity in superior cervical ganglion of STZ-diabetic rat and prevention by aldose reductase inhibition.* Diabetes 1986, 35: 1106-8.

7. Tomlinson, D.R., Moriarty, R.J., Mayer, J.H. *Prevention and reversal of defective axonal transport and motor nerve conduction velocity in rats with experimental diabetes by treatment with the aldose reductase inhibitor sorbinil.* Diabetes 1984, 33: 470-6.

8. Raskin, P., Rosenstock, J. *Aldose reductase inhibitors and diabetic complications.* Am J Med 1987, 83: 298-306.

9. Kinoshita, J.H., Nishimura, C. *The involvement of aldose reductase in diabetic complications.* Diabetes Metab Rev 1988, 4: 323-37.

10. Low, P.A. *Recent advances in the pathogenesis of diabetic neuropathy.* Muscle Nerve 1987, 10: 121-8.

11. Yasuda, H., Sonobe, M., Yamashita, M. et al. *Effect of prostaglandin E_1 analogue TFC 612 on diabetic neuropathy in streptozocin-induced diabetic rats: Comparison with aldose reductase inhibitor ONO 2235.* Diabetes 1989, 38: 832-8.

12. Cameron, N.E., Cotter, M.A. *Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: Role of polyol pathway activity.* Diabetologia 1992, 35: 1011-9.

13. Tesfamariam, B., Palacino, J.J., Weisbrod, R.M., Cohen, R.A. *Aldose reductase inhibition restores endothelial cell function in diabetic rabbit aorta.* J Cardiovasc Pharmacol 1993, 21: 205-11.

14. Prous, J.R. *Treatment of diabetic complications.* In: The Year's Drug News - Therapeutic Targets. Prous Science: Barcelona 1994, 275.

15. Aotsuka, T., Abe, N., Fukushima, K., Ashizawa, N., Yoshida, M. *Benzothiazol-2-ylcarboxylic acids with diverse spacers: A novel class of potent, orally active aldose reductase inhibitors.* Bioorg Med Chem Lett 1997, 7: 1677-82.

16. Mylari, B.L., Larson, E.R., Beyer, T.A. et al. *Novel potent aldose reductase inhibitors: 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phtharazineacetic acid (zopolrestat) and congeners.* J Med Chem 1991, 34: 108-22.

17. Stribling, D., Brittain, D.R. In: Approaches in Drug Research. Harms, A.F. (Ed.). Elsevier: Amsterdam 1986, 297-313.

18. Itoh, Y., Ao, S., Notsu, Y., Hashimoto, M. *Novel aldose reductase inhibitors: Synthesis and structure-activity studies of (3-benzyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-1-yl)acetic acids.* Drugs Fut 1992, 17: 31-7.

19. Shimazaki, N., Tanaka, H., Kuroda, A., Hashimoto, S. (Fujisawa Pharm.). *Preparation of quinazolines as aldose reductase inhibitors.* JP 03232885.

20. Aotsuka, T., Hosono, H., Kurihara, T., Nakamura, Y., Matsui, T., Kobayashi, F. *Novel and potent aldose reductase inhibitors: 4-Benzyl- and 4-(benzothiazol-2-ylmethyl)-3,4-dihydro-3-oxo-2H-1,4-benzothiazine-2-acetic acid derivatives.* Chem Pharm Bull 1994, 42: 1264-71.

21. Ishii, A., Kotani, T., Nagaki, Y. et al. *Highly selective aldose reductase inhibitors. I. 3-(Arylalkyl)-2,4,5-trioxoimidazolidine-1-acetic acids.* J Med Chem 1996, 39: 1924-7.

22. Kotani, T., Ishii, A., Nagaki, Y. et al. *Highly selective aldose reductase inhibitors. II. Optimization of the aryl part of 3-(arylmethyl)-2,4,5-trioxoimidazolidine-1-acetic acids.* Chem Pharm Bull 1997, 45: 297-304.

23. Malamas, M.S., Hohman, T.C., Millen, J. *Novel spirosuccinimide aldose reductase inhibitors derived from isoquinoline-1,3-diones: 2-[(4-Bromo-2-fluorophenyl)methyl]-6-fluorospiro[isoquinoline-4-(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone and congeners. 1.* J Med Chem 1994, 37: 2043-58.

24. Wilson, D.K., Tarle, I., Petrash, J.M., Quiocho, F.A. *Refined 1.8 Angstrom structure of human aldose reductase complexed with the potent inhibitor zopolrestat.* Proc Natl Acad Sci USA 1993, 90: 9847-51.

25. Nakano, T., Petrash, J.M. *Kinetic and spectroscopic evidence for active site inhibition of human aldose reductase.* Biochemistry 1996, 35: 11196-202.

26. Matsui, T., Nakamura, Y., Ishikawa, H., Matsuura, A., Kobayashi, F. *Pharmacological profiles of a novel aldose reductase inhibitor, SPR-210, and its effects on streptozotocin-induced diabetic rats.* Jpn J Pharmacol 1994, 64: 115-24.

27. Ashizawa, N., Yoshida, M., Sugiyama, Y. et al. *Effects of a novel potent aldose reductase inhibitor, GP-1447, on aldose reductase activity in vitro and on diabetic neuropathy and cataract formation in rats.* Jpn J Pharmacol 1997, 73: 133-44.

28. Barski, O.A., Gabbay, K.H., Grimshaw, C.E., Bohren, K.M. *Mechanism of human aldehyde reductase: Characterization of the active site pocket.* Biochemistry 1995, 34: 11264-75.

29. Tanimoto, T., Nishiyama, C. *Molecular biology of aldose reductase.* Exp Med 1991, 9: 541-7.

30. Feather, M.S., Flynn, T.G., Munro, K.A., Kubiseski, T.J., Walton, D.J. *Catalysis of reduction of carbohydrate 2-oxoaldehyde (osones) by mammalian aldose reductase and aldehyde reductase.* Biochim Biophys Acta 1995, 1244: 10-6.

31. Ratliff, D.M., Vander Jagt, D.J., Eaton, R.P., Vander Jagt, D.L.. *Increased levels on methylglyoxal-metabolizing enzymes in mononuclear and polymorphonuclear cells from insulin-dependent diabetic patients with diabetic complications: Aldose reductase, glyoxalase I, and glyoxalase II - A clinical research center study.* J Clin Endocrinol Metab 1996, 81: 488-92.

32. Beyer-Mears, A., Diecke, F.P.J., Mistry, K., Cruz, E. *Comparison of the effects of zopolrestat and sorbinil on lens myo-inositol influx.* Pharmacology 1997, 54: 76-83.

33. Yoshida, M., Sugiyama, Y., Akaike, N. et al. *Amelioration of neurovascular deficits in diabetic rats by a novel aldose reductase inhibitor, GP-1447 – Minor contribution of nitric oxide.* Diabetes Res Clin Pract, in press.

34. Tesfamariam, B., Brown, M.L., Cohen, R.A. *Aldose reductase and myo-inositol in endothelial cell dysfunction caused by elevated glucose.* J Pharmacol Exp Ther 1992, 263: 153-7.

35. Simpson, L.O. *Altered blood rheology in the pathogenesis of diabetic and other neuropathies.* Muscle Nerve 1988, 11: 725-44.

36. Smith, D.A., Brown, K., Neale, M.G. *Chromone-2-carboxylic acids: Roles of acidity and lipophilicity in drug disposition.* Drug Metab Rev 1985-86, 16: 365-88.

37. Beyer-Mears, A., Mistry, K., Diecke, F.P.J., Cruz, E. *Zopolrestat prevention of proteinuria, albuminuria and cataractogenesis in diabetes mellitus.* Pharmacology 1996, 52: 292-302.

38. Azuma, M., Inoue, E., Oka, T., Shearer, T.R. *Proteolysis by calpain is an underlying mechanism for formation of sugar cataract in rat lens.* Curr Eye Res 1995, 14: 27-34.

39. Tamai, A., Tanaka, K. *The ERG of the streptozotocin-diabetic albino rat.* Folia Ophthal Jpn 1973, 24: 847-50.

40. Simonsen, S.E. *ERG in diabetics.* In: The Clinical Value of Electroretinography. Francois, J. (Ed.). Karger: Basel 1968, 403-12.

41. Oates, P.J. *Diabetic nephropathy, renal hemodynamics, and aldose reductase inhibitors.* Drug Dev Res 1994, 32: 104-16.

42. Oates, P.J., Ellery, C.A., Goldfarb, S. *Hyperfiltration and albuminuria are dose-dependently reduced in diabetic rats by zopolrestat.* Diabetologia 1993, 36 (Suppl. 1): Abst 848.

43. Oates, P.J., Ellery, C.A. *Aldose reductase inhibitor zopolrestat prevents elevated urinary albumin excretion in diabetic rats.* Diabetes 1992, 41(Suppl. 1): Abst 434.

44. Oates, P.J., Ellery, C.A., Inskeep, P.B., Reed, A.E., Beyer, T.A., Huston, N.J. *Zopolrestat dose-dependently inhibits renal hyperperfusion in galactosemic rats.* Diabetes 1991, 40(Suppl. 1): Abst 524.

45. Tesfamariam, B., Gupta, S., Oates, P.J., Ruderman, N.B., Cohen, R.A. *Reduced Na^+-K^+ pump activity in diabetic rabbit carotid artery: Reversal by aldose reductase inhibition.* Am J Physiol 1993, 265 (Heart Circ Physiol 34): H1189-94.

46. Graier, W.F., Grubenthal, I., Dittrich, P., Wascher, T.C., Kostner, G.M. *Intracellular mechanism of high D-glucose-induced modulation of vascular cell proliferation.* Eur J Pharmacol 1995, 294: 221-9.

47. Ramasamy, R., Oates, P.J., Schaefer, S. *Aldose reductase inhibition protects diabetic and nondiabetic rat hearts from ischemic injury*. Diabetes 1997, 46: 292-300.

48. Inskeep, P.B., Reed, A.E., Ronfeld, R.A. *Pharmacokinetics of zopolrestat, a carboxylic acid aldose reductase inhibitor, in normal and diabetic rats.* Pharm Res 1991, 8: 1511-5.

49. Inskeep, P.B., Ronfeld, R.A., Peterson, M.J., Gerber, N. *Pharmacokinetics of the aldose reductase inhibitor, zopolrestat, in humans.* J Clin Pharmacol 1994, 34: 760-6.

50. McCaleb, M.L., Sredy, J., Millen, J., Ackerman, D.M, Dvornik, D. *Prevention of urinary albumin excretion in 6 month streptozocin-diabetic rats with the aldose reductase inhibitor tolrestat.* J Diabet Complications 1988, 2: 16-8.

51. Cameron, N.E., Cotter, M.A., Dines, K.C., Maxfield, E.K., Carey, F. Mirrlees, D.J. *Aldose reductase inhibition, nerve perfusion, oxygenation and function in streptozotocin-diabetic rats: Dose-response considerations and independence from a myo-inositol mechanism.* Diabetologia 1994, 37: 651-63.