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Pharmacological and pharmacokinetic study of olmesartan medoxomil in animal diabetic retinopathy models

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Abstract

A close relationship between the renin–angiotensin system and the pathophysiology of diabetic retinopathy has been suggested, several angiotensin II type 1 receptor (angiotensin AT1 receptor) antagonists being effective in animal models. Therefore, we examined the efficacy of an angiotensin AT1 receptor antagonist, olmesartan medoxomil (CS-866), in animal retinopathy models.

In diabetic stroke-prone spontaneously hypertensive (SHRSP) rats, 4-week treatment with CS-866 prevented the elongation of oscillatory potential peaks dose-dependently which almost normalized at 3 mg/kg/day. Next, in oxygen-induced retinopathy mice, CS-866 at 1 mg/kg significantly prevented the retinal neovascularization. In these animal models, plasma concentrations of CS-866 were comparable to the in vitro IC_{50} value of the angiotensin AT1 receptor.

In summary, our data demonstrated that CS-866 was effective in early and late stage retinopathy models through the inhibition of the angiotensin AT1 receptor. These findings suggest the possibility of CS-866 as a therapeutic agent for diabetic retinopathy.

Keywords: Angiotensin; Diabetic retinopathy; Electroretinogram; Olmesartan medoxomil; Oxygen induced retinopathy

1. Introduction

Diabetic retinopathy is one of the major diabetic complications and a leading cause of adult blindness in developed countries. Diabetic retinopathy is believed to occur in almost all of type 1 (Klein et al., 1984) and more than 60% of type 2 diabetic patients after 20 years of diabetes (American Diabetes Association, 2000). The progression of the disease is usually sequential and is divided into non-proliferative and proliferative retinopathy. In early stage diabetic retinopathy, non-proliferative diabetic retinopathy, hyperglycemia initiates metabolic abnormalities leading to retinal vessel hyperpermeability, decrease in retinal blood flow and some neuronal impairment in the retina. In late stage diabetic retinopathy, proliferative diabetic retinopathy, capillary occlusion causes retinal hypoxia and initiates neovascularization. Newly formed vessels are vulnerable and the rupture of those vessels sometimes leads to blindness. In this way, diabetic retinopathy seriously reduces patients' quality of life, however, medical treatment for diabetic retinopathy except for tight glycemic control (The Diabetes Control and Complications Trial Research Group, 1993) is restricted to surgical operations (Early Treatment Diabetic Retinopathy Study Research Group, 1991; The Diabetic Retinopathy Vitrectomy Study Research Group, 1988). Recently available pharmacotherapy with disease-modifying drugs would be a better option for the medical treatment of diabetic retinopathy.

Recent studies have shown that the renin–angiotensin system plays an important role in the pathophysiology of

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diabetic retinopathy. In an in vitro study, angiotensin II was shown to stimulate the expression of vascular endothelial growth factor (VEGF), a key factor in the pathogenesis of diabetic retinopathy, and its receptor in retinal cell cultures (Otani et al., 1998, 2000). In an animal study, Gilbert et al. (2000) demonstrated that treatment of diabetic rats with an angiotensin-converting enzyme inhibitor reduced the upregulated VEGF mRNA level and the increase in retinal permeability. Moreover, evidence from clinical studies strongly suggests the beneficial effects of renin–angiotensin system inhibition in patients with diabetic retinopathy. Parving et al. (1989) reported that an angiotensin-converting enzyme inhibitor alone or in combination with diuretics, inhibits retinal vascular permeability to fluorescein in hypertensive type 1 diabetic patients with background retinopathy. In the EUCLID (EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus) study (Chaturvedi et al., 1998), treatment with lisinopril significantly reduced the development of retinopathy in non-hypertensive type 1 diabetic patients. In the UK Prospective Diabetic Study (UK Prospective Diabetes Study Group, 1998), tight blood pressure control with captopril reduced the progression of diabetic retinopathy in hypertensive patients with type II diabetes.

Olmesartan medoxomil (5-methyl-2-oxo-1,3-dioxolen-4-yl) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl] phenyl}methylimidazol-5-carboxylate), CS-866, is a potent and selective angiotensin AT1 receptor blocker (Mizuno et al., 1995) which has recently been approved for the treatment of hypertension in the United States, Japan and European countries. The drug contains a medoxomil ester moiety and is cleaved by an endogenous esterase to release active metabolite (RNH-6270). Clinical trials in hypertensive patients (Neutel et al., 2002) revealed a long lasting action and a good tolerance of the drug without significant adverse effects at a dose of 80 mg. A once-daily regimen is effective and doses of up to 40 mg/body/day are approved. Olmesartan is also reported to be effective in animal models of atherosclerosis, liver disorders and diabetic nephropathy (Koike, 2001; Kurikawa et al., 2003; Mizuno et al., 2002).

To confirm the relationship between the renin–angiotensin system and diabetic retinopathy and to evaluate the potential of CS-866 for the treatment of diabetic retinopathy, we conducted in vivo pharmacological and pharmacokinetic experiments on early retinal electrophysiological change in diabetic rats and on hypoxia-induced neovascularization in mice.

2. Materials and methods

2.1. Materials

Streptozotocin, Tris and neutral buffered formalin were purchased from Sigma Chemical Co. (St. Louis, MO). Citric acid, trisodium citrate, methylcellulose 50 cP, carboxymethyl cellulose sodium salt, magnesium chloride, maleate and adenosine diphosphate (ADP) were obtained from Wako Pure Chemicals (Osaka, Japan). Lead nitrate and ammonium sulfide were purchased from Kanto Chemical, Co., Inc. (Tokyo, Japan). Ketamine hydrochloride was obtained from Sankyo Yell Yakuhin Co., Ltd. (Tokyo, Japan). Xylazine was obtained from Bayer Ltd. (Tokyo, Japan). Heparin sodium injection was obtained from Takeda Chemical Industries, Ltd. (Osaka, Japan). CS-866, RNH-6270, RNH-6272 and TCV-116 were synthesized in our company (Sankyo Co., Ltd., Tokyo, Japan). Other reagents and solvents used were of analytical grade or high performance liquid chromatography (HPLC) grade.

2.2. Electroretinogram study

2.2.1. Animals

All experiments were carried out in accordance with the Animal Experimentation Guidelines of Sankyo Co., Ltd. Male spontaneously hypertensive-stroke prone rats (SHRSP)/Izm (6 weeks of age) were purchased from SLC Japan (Yokohama, Japan) and used at 7 weeks of age after 1 week of quarantine and acclimatization. Rats were given water and a standard laboratory diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum.

2.2.2. Induction and determination of diabetes

Diabetes was induced in rats by a single intravenous injection of streptozotocin freshly made up in 2 mM citric acid buffer (pH 4.5) at a dose of 40 mg/kg. Age-matched control animals were given buffer only (non-diabetic group). At 6 weeks after the streptozotocin injection, diabetes was defined as a glucose level of greater or equal to 250 mg/dl in tail vein blood. Diabetic rats were divided into four groups so that each group showed a similar mean value of plasma glucose and body weight (diabetic control group and 0.3, 1 and 3 mg/kg CS-866 treated groups). The non-diabetic group and diabetic control group were given vehicle (0.5% methylcellulose) and the three treated groups were administered the specified dose of CS-866. The vehicle or the drug was administered orally once daily for 4 weeks from the day of group assignment.

2.2.3. Measurement of blood glucose and hemoglobin A_{1C} (HbA_{1C})

Blood glucose and HbA_{1C} were determined 2 days before the group assignment, 2 and 4 weeks after the dose initiation. Blood samples were collected from the tail vein and the plasma glucose levels were measured by a hexokinase-glyceraldehyde 6 phosphate dehydrogenase method. HbA_{1C} was determined by a latex agglutination method. Both parameters were determined using an AU400 chemistry analyzer (Olympus Optical Company Ltd., Tokyo, Japan).

2.2.4. Measurement of cardiovascular parameters

Blood pressure (systolic, diastolic and mean blood pressure) and heart rate were measured before treatment (baseline), 2 and 4 weeks after the dose initiation by a tail–cuff method using an automated invasive blood-pressure meter (BP-98A, Softron Co., Ltd., Tokyo, Japan).

2.2.5. Electroretinogram measurement

An electroretinogram study was performed at 4 weeks after the dose initiation. After more than 1 h of adaptation to darkness, rats were anesthetized with intramuscular administration of 50 mg/kg ketamine hydrochloride and 2 mg/kg xylazine. Pupils were dilated with tropicamide-phenylephrin (Mydrin-P®) and a contact lens-type bipolar electrode (Kyoto Contact Lens Laboratory, Kyoto, Japan) was placed on the cornea of the left eye. A xenon lamp for stimulation was placed 20 cm above the eye. Electroretinograms induced by the light stimuli (0.3 J, 1 Hz) were recorded using a Neuropack (MEB-7102, Nihon Kohden Corporation, Tokyo, Japan; time constant: 0.03 s, high cut: 1000 Hz,) and the latencies of the oscillatory potential peaks (O1, O2 and O3) were determined from the electroretinograms.

2.3. Oxygen-induced retinopathy study

2.3.1. Animals

One-week-old C57BL/6 mice with their mothers were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and Charles River Japan Inc. (Kanagawa, Japan).

Mice were given water and a standard laboratory diet (FR-2; Funabashi Farm Co., Ltd., Chiba, Japan) ad libitum and maintained on a 12-h light–dark cycle.

2.3.2. Oxygen-induced retinopathy

The neovascularization in mouse retina was induced as described previously (Smith et al., 1994). Animals were exposed to hyperoxic conditions (70–75% oxygen) for 5 days followed by another 5 days of normal oxygen conditions. Oxygen concentration was monitored with an OM25ME oxygen analyzer (Maxtec Inc., Salt Lake City, UT, USA). After the hyperoxygen exposure, pups were randomly assigned to each treatment group (day 0). Treatment was then carried out for 5 days from day 0 to day 4.

CS-866, TCV-116 (angiotensin AT1 receptor antagonist) or vehicle (0.5% carboxymethylcellulose solution) was orally administered once daily. Doses ranged from 1 to 30 mg/kg. Drugs or vehicle was applied at a volume of 4 ml/kg using a microsyringe (Hamilton Co., Reno, NV, USA) connected with a polyethylene tube (PE20, Becton Dickinson and Company, NJ, USA).

2.3.3. Visualization of the vasculature

On day 5, animals were sacrificed by cervical dislocation. The right eyes of each mouse were enucleated and fixed in 10% neutral buffered formalin at approximately 2–8 °C overnight.

After the fixation, the retina was surgically removed and divided into four quadrants of approximately equal size. The retina was further processed for ADPase staining as described previously (Lutty and McLeod, 1992). Briefly, the retina was washed in distilled water 5 times, and then incubated at 37 °C for 30 min in a 0.2 M Tris–maleate buffer (pH 7.2) containing 0.1% lead nitrate, 0.12% magnesium chloride and 0.1% ADP. Finally, the retina was rinsed 5 times in distilled water, and developed with 2% ammonium sulfide.

2.3.4. Determination of retinal neovascularization

The degree of neovascularization was evaluated in a blind manner by an examiner who was not informed of the treatment regimen. The retinal neovascularization was determined by a grading method described previously (Zhang et al., 2000). Briefly, each retinal quadrant was further divided into three equal parts (clock hours) by visual estimation and each clock hour was scored positive when abnormal vascular tufts were found. The number of positive clock hours in the retina was defined as the neovascularization score and then each retina was given a score of between 0 and 12 clock hours.

2.4. Statistical analyses

Statistical analyses for pharmacological experiments were performed using the SAS® System Release 8.2 (SAS Institute Inc., Tokyo, Japan). The equality of variance between two groups was initially evaluated by the F-test. When the equality was significantly rejected ($P<0.05$), mean differences were evaluated by the Welch test, whereas the Student t-test was performed for other cases. For multiple comparison, the Dunnett test was performed. A value of $P<0.05$ was considered statistically significant.

2.5. Pharmacokinetic analysis in diabetic SHRSP rats and oxygen-induced retinopathy mice

The pharmacokinetic study was conducted using diabetic SHRSP rats and oxygen-induced retinopathy mice developed according to the same method described above. CS-866 suspension in 0.5% carboxymethylcellulose was orally administered to rats at the dose of 3 mg/kg and mice at the dose of 1, 3 or 10 mg/kg. After collecting blood, the plasma concentrations of RNH-6270, the active metabolite of CS-866, were determined by a fluorescence-HPLC method using RNH-6272 (an analog of RNH-6270) as internal standard. Briefly, the mixture of the plasma sample and RNH-6272 solution was applied onto a preactivated mini-column (Bond Elut PH, Varian Technologies Japan Ltd, Tokyo, Japan), and eluted with methanol after washing. The eluate was evaporated and redissolved with the mobile phase for HPLC. After centrifugation, the supernatant was subjected to fluorescence-HPLC (LC-10A system, Shimadzu Corporation, Kyoto, Japan) using a column switch-

ing technique. Shim-pack WAX-1 (4.0 × 50 mm, Shimadzu Corporation, Kyoto, Japan) and YMC-pack ODS-A A-312 (6.0 × 150 mm, YMC Co., Ltd., Kyoto, Japan) were used for entrapment and analysis, respectively. Mobile phase 1 (acetonitrile/0.05 M phosphate buffer (pH 7.0), 13/87) and Mobile phase 2 (acetonitrile/0.05 M ammonium acetate, 28/72, adjusted with acetic acid to pH 3.75) were used, and the column-switching time was set at 5 min. Analytes were detected at wavelengths of 260 nm (excitation) and 370 nm (emission).

The plasma unbound fraction was estimated by an ultrafiltration method using [^{14}C]-RNH-6270, and plasma unbound concentrations of RNH-6270 were calculated by multiplying the total plasma concentrations by the unbound ratio (the ratio of the unbound drug concentration to the total drug concentration).

3. Results

3.1. Electroretinogram study

3.1.1. Changes in body weight, plasma glucose concentration and HbA_{1C} level

The changes in body weight, plasma glucose concentration and HbA_{1C} levels in normal and streptozotocin-induced diabetic animals are summarized in Table 1. At 6 weeks after the streptozotocin treatment, the body weight was significantly lower in diabetic rats compared with non-diabetic animals. CS-866 treatment (0.3, 1 or 3 mg/kg) did not affect the body weight throughout the experimental period.

Plasma glucose concentrations and HbA_{1C} levels in the diabetic groups were significantly higher in diabetic animals compared with the non-diabetic group at all time points. Neither parameter was affected by 4-week treatment with CS-866 (0.3, 1 or 3 mg/kg).

Table 1
Body weight, plasma glucose concentrations and HbA_{1C} levels in non-diabetic and diabetic SHRSP rats treated with or without CS-866

Parameter	ND	DC	CS0.3	CS1	CS3
Body weight (g)					
0W	296 ± 5	234 ± 7**	235 ± 7	236 ± 10	234 ± 6
2W	282 ± 9	237 ± 6**	243 ± 8	242 ± 10	240 ± 7
4W	291 ± 8	291 ± 8**	241 ± 6	249 ± 8	248 ± 10
Blood glucose (mg/dl)					
0W	139 ± 7	509 ± 23‡	499 ± 19	506 ± 27	501 ± 21
2W	134 ± 7	520 ± 21‡	517 ± 19	524 ± 23	539 ± 23
4W	124 ± 4	490 ± 34‡	510 ± 24	479 ± 23	503 ± 21
HbA_{1C} (%)					
0W	2.9 ± 0.0	7.9 ± 0.2‡	8.0 ± 0.2	7.7 ± 0.2	7.8 ± 0.2
2W	2.8 ± 0.1	8.2 ± 0.2‡	8.4 ± 0.2	8.0 ± 0.1	8.1 ± 0.2
4W	2.5 ± 0.1	8.4 ± 0.4‡	8.5 ± 0.3	8.3 ± 0.2	8.5 ± 0.2

ND: non-diabetic group treated for 4 weeks with vehicle. DC: 10-week diabetic group treated for 4 weeks with vehicle. CS0.3, CS1 and CS3: 10-week diabetic group treated for 4 weeks with CS-866 (0.3, 1 and 3 mg/kg/day, respectively). Results are expressed as the mean ± S.E.M. (n = 10).
** P < 0.01 vs. ND (by the Student t- test).
‡ P < 0.01 vs. ND (by the Welch test).

Table 2
Blood pressure and heart rate in non-diabetic and diabetic SHRSP rats treated with or without CS-866

Parameter	ND	DC	CS0.3	CS1	CS3
Systolic blood pressure (mm Hg)					
0W	226 ± 4	200 ± 4[a]	201 ± 3	200 ± 3	201 ± 5
2W	236 ± 5	194 ± 3[b]	187 ± 5	189 ± 4	189 ± 3
4W	237 ± 4	197 ± 4[a]	189 ± 4	183 ± 4	180 ± 5[c]
Diastolic blood pressure (mm Hg)					
0W	177 ± 6	145 ± 6[a]	150 ± 4	146 ± 5	150 ± 5
2W	195 ± 9	136 ± 4[b]	139 ± 9	129 ± 7	136 ± 4
4W	183 ± 7	136 ± 9[a]	135 ± 6	127 ± 6	126 ± 7
Mean blood pressure (mm Hg)					
0W	193 ± 6	163 ± 5[a]	167 ± 3	164 ± 4	167 ± 5
2W	208 ± 8	155 ± 3[b]	155 ± 8	149 ± 6	153 ± 4
4W	201 ± 6	156 ± 7[a]	153 ± 5	146 ± 5	144 ± 6
Heart rate (beats/min)					
0W	352 ± 11	303 ± 8[a]	302 ± 5	292 ± 5	298 ± 6
2W	412 ± 12	301 ± 9[a]	320 ± 11	309 ± 7	318 ± 7
4W	370 ± 11	305 ± 10[a]	303 ± 8	296 ± 8	305 ± 6

ND: non-diabetic group treated for 4 weeks with vehicle, DC: 10-weeks diabetic group treated for 4 weeks with vehicle. CS03, CS1 and CS3: 10-week diabetic group treated for 4 weeks with CS-866 (0.3, 1 and 3 mg/kg/day, respectively). Results are expressed as the mean ± S.E.M. (n = 10).
[a] P < 0.01 vs. ND (by the Student t-test).
[b] P < 0.01 vs. ND (by the Welch test).
[c] P < 0.05 vs. DC (by the Dunnett test).

3.1.2. Changes in cardiovascular parameters

The changes in cardiovascular parameters (heart rate, systolic, diastolic and mean blood pressure) are summarized in Table 2. These parameters were significantly decreased in diabetic groups compared with the non-diabetic group during the experimental period (Table 2). At 4 weeks after the treatment, significant reduction in systolic blood pressure was observed in the CS-866 3 mg/kg-treated group compared with the diabetic control group. For other parameters, there were no significant differences between the diabetic control and CS-866-treated groups.

3.1.3. Electroretinography

The latencies of the oscillatory potential peaks (O1, O2 and O3) in the diabetic control group were significantly elongated compared with the non-diabetic group at 10 weeks after the streptozotocin injection (Fig. 1). Treatment with CS-866 dose-dependently prevented this elongation and 3 mg/kg CS-866 treatment completely arrested this elongation at the O2 and O3 peak latencies.

3.1.4. Pharmacokinetic study in diabetic SHRSP rats

The time courses of the plasma concentration of RNH-6270 in diabetic SHRSP rats are shown in Fig. 2, and the pharmacokinetic parameters are summarized in Table 3.

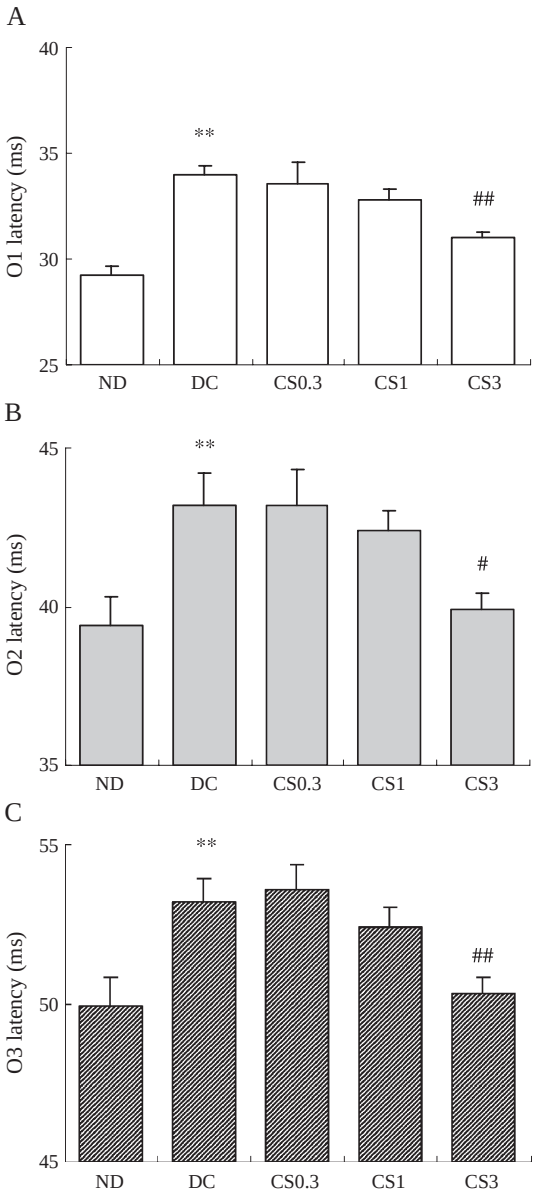

Fig. 1. Effect of CS-866 on the latency of oscillatory potential peaks (A: O1; B: O2 and C: O3) in non-diabetic and diabetic SHRSP rats. ND: non-diabetic group treated for 4 weeks with vehicle. DC: 10-week diabetic group treated for 4 weeks with vehicle. CS03, CS1 and CS3: 10-week diabetic group treated for 4 weeks with CS-866 (0.3, 1 and 3 mg/kg/day, respectively). Results are expressed as the mean ± S.E.M. ($n = 10$). $^{**}P < 0.01$ vs. ND (by the Student t-test), $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. DC (by the Dunnett test).

Data are not shown, but the pharmacokinetic parameters in the streptozotocin-induced diabetic rats were comparable to those in the non-diabetic rats indicating that diabetic conditions did not severely affect the pharmacokinetic profile of RNH-6270. In order to consider the PK/PD relationships, the unbound C_{max} was calculated by multiplying the C_{max} value (total concentrations) by the plasma unbound ratio (0.014), and the value determined as 1.3 ng/ml. The unbound ratio of RNH-6270 in rat plasma (0.986) was similar to that in human plasma (0.012).

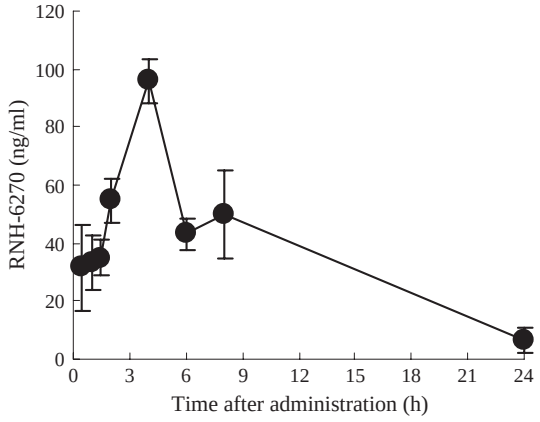

Fig. 2. Pharmacokinetic study of RNH-6270 in diabetic SHRSP rats. The plasma concentrations of the active metabolite (RNH-6270) after oral administration of CS-866 were determined as described in the Materials and methods. Results are expressed as the mean ± S.E.M. ($n = 4$).

3.2. Oxygen-induced retinopathy

3.2.1. Pharmacological study in mouse oxygen-induced retinopathy model

We investigated the effects of angiotensin AT1 receptor blockers on the retinal neovascularization in a mouse oxygen-induced retinopathy model. As shown in Fig. 3A and B, hypoxic conditions caused retinal neovascularization in the mouse pups. CS-866 at oral doses of 1, 3, 10 and 30 mg/kg significantly and dose-dependently reduced the hypoxia-induced neovascularization. Another angiotensin AT1 receptor antagonist, TCV-116 (Fig. 3B), also inhibited the neovascularization in a dose-dependent manner. CS-866 and TCV-116 induced significant inhibition at doses of 1 and 10 mg/kg, respectively.

3.2.2. Pharmacokinetic study

The total and plasma unbound concentrations of RNH-6270 2 and 24 h after oral administration of CS-866 at doses of 1, 3 or 10 mg/kg to oxygen-treated mouse pups are shown in Table 4. The plasma concentrations at 2 and 24 h post-dosing increased proportionally to the dose. The unbound ratio of RNH-6270 in mouse plasma was 0.0192, and the plasma unbound concentration of the active metabolites at 2 h post-dosing was calculated to be 6.06 ng/ml for 1 mg/kg, 18.0 ng/ml for 3 mg/kg and

Table 3
Pharmacokinetic parameters of RNH-6270 in plasma after oral administration of CS-866 at a dose of 3 mg/kg to diabetic SHRSP rats

Parameter	Mean ± S.E.M.
$AUC_{0-24\ h}$ (ng h/ml)	656 ± 236
C_{max} (ng/ml)	95.9 ± 7.4
T_{max} (h)	4.0 ± 0.0
$t_{1/2}$ (h)	5.7 ± 2.1

Results are expressed as the mean ± S.E.M. ($n = 4$).

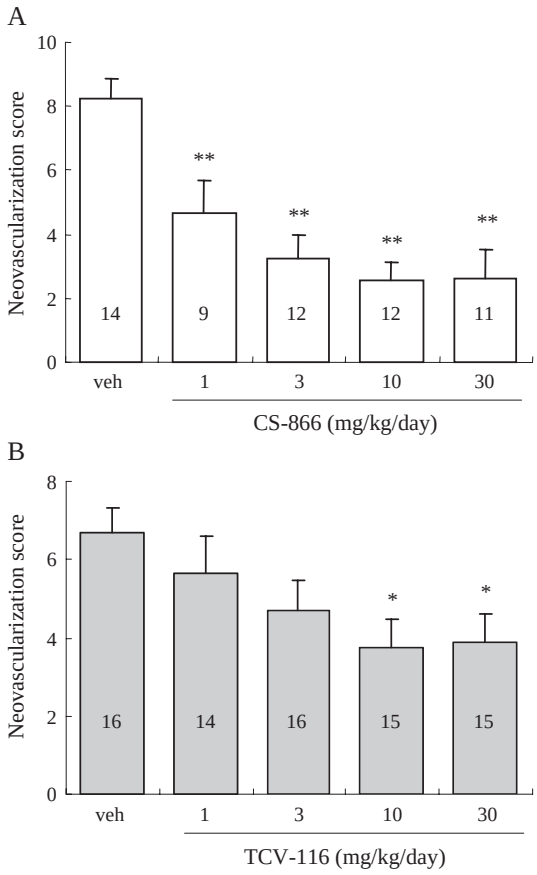

Fig. 3. Effects of angiotensin AT1 receptor antagonists on hypoxia-induced neovascularization in mouse pups. Hypoxia-induced retinal neovascularization was produced as described in the Materials and methods. Mouse pups were treated with vehicle (veh) or indicated doses of (A) CS-866 or (B) TCV-116 for 5 days. The number inside each bar indicates group size. Results are expressed as the mean ± S.E.M. $*P<0.05$, $**P<0.01$ vs. vehicle (by the Dunnett test).

72.9 ng/ml for 10 mg/kg CS-866 administration (mean values).

4. Discussion

Diabetic SHRSP rats are reported to be suitable models for the evaluation of potential anti-diabetic retinopathy drugs because they have vulnerable retinal vessels similar to those in patients with diabetic retinopathy and the retinopathy develops at high incidence and in a short period after onset of diabetes (Nagisa et al., 2001).

The oscillatory potentials are usually elongated by hyperglycemia (Yonemura et al., 1962) and thus used for diagnosis of retinal dysfunction in retinopathy patients. We evaluated the effect of CS-866 on early stage diabetic retinopathy using the SHRSP model by measuring the latencies of oscillatory potentials in electroretinograms.

CS-866 dose-dependently corrected the delayed latencies of oscillatory potentials in the diabetic SHRSP rats. This result was consistent with a previous report using an angiotensin AT1 receptor antagonist (TCV-116) in diabetic SHRSP rats (Nagisa et al., 2001).

Systolic blood pressure was slightly but significantly suppressed by oral administration of CS-866 at a dose of 3 mg/kg 24 h after administration. It is speculated that the reduction in systolic blood pressure would be more significant at the earlier phase after administration because the plasma concentration of the drug would be higher. Hypertension is known to be one of the risk factors for diabetic retinopathy and reducing blood pressure is shown to be effective in the treatment of diabetic retinopathy (UK Prospective Diabetes Study Group, 1998). However, the lowered blood pressure would not be the reason for the amelioration of oscillatory potential latencies by CS-866. A previous clinical study reported that angiotensin-converting enzyme inhibitors prevented abnormalities in the electroretinogram, despite other hypotensive agents such as beta-blockers or calcium antagonists showing no effect (Cosenzi et al., 1999), suggesting that the retinal local angiotensin AT1 receptor, but not high systolic blood pressure, plays an important role in the pathophysiology of diabetic retinopathy. In agreement with this hypothesis, our results showed that diabetes significantly lowered systolic blood pressure and induced electroretinogram change in SHRSP rats. Furthermore, the diabetic SHRSP rats treated with CS-866 were still hypertensive (systolic blood pressure: 180 mm Hg) at 24 h after dosing, whereas delayed oscillatory potential latencies were almost normalized. Although the mechanism by which the renin–angiotensin system inhibitors ameliorate electroretinogram abnormalities is still unclear, our results using CS-866 as an angiotensin AT1 receptor antagonist supported the important role of the local renin–angiotensin system in the pathophysiology of early phase diabetic retinopathy and suggest that CS-866 may have potential in treatment of early stage diabetic retinopathy. The precise mechanism of CS-866 action is unclear but the advanced glycation end product (AGE) pathway might be involved because AGE-dependent slowing in ERG oscillatory potentials in diabetic animals (Segawa et al., 1998) and CS-866-dependent reduction in AGE formation (Nangaku et al., 2003) have been reported.

In addition to the pharmacological experiment, we conducted a parallel pharmacokinetic experiment in an SHRSP rat model. We measured plasma concentrations of the active metabolite of CS-866 (RNH-6270) in non-diabetic and diabetic SHRSP rats and found that the C_{max}

Table 4
Plasma concentrations of RNH-6270 after oral administration of CS-866 at doses of 1, 3 and 10 mg/kg to oxygen-treated mice

Dose (mg/ml)	Plasma RNH-6270 conc. (ng/ml)	
	2 h post-dose	24 h post-dose
1	319 ± 116	36.7 ± 6.3
3	948 ± 95	134 ± 29
10	3840 ± 229	426 ± 125

Results are expressed as the mean ± S.E.M. ($n=4$).

of the protein-unbound drug (1.3 ng/ml recalculated as a protein unbound value) was comparable to the in vitro IC_{50} value of RNH-6270 (3.4 ng/ml; Mizuno et al., 1995). Therefore, the present pharmacokinetic data supported the notion that the ameliorative effect of oral administration of CS-866 on electroretinogram abnormalities is via inhibition of the angiotensin AT1 receptor. The ameliorative effects of angiotensin AT1 receptor blockers on diabetic retinopathy have already been documented, but the present study is the first published report that discusses the anti-retinopathy effect of angiotensin AT1 receptor antagonists using pharmacokinetic data.

We evaluated the potential of CS-866 in late stage diabetic retinopathy, where extensive retinal neovascularization is observed. An oxygen-induced retinopathy model was chosen for the evaluation of retinal neovascularization because commonly used diabetic animal models usually do not reached the proliferative stage. In this model, neovascularization has been ameliorated by candidate drugs for diabetic retinopathy treatment such as anti-VEGF agents (Ozaki et al., 2000; Aiello et al., 1995) and renin–angiotensin system blockers (Tadesse et al., 2001; Lonchampt et al., 2001). Therefore, we considered that this animal model would be suitable for evaluating compounds for potential treatment of diabetic retinopathy, especially of proliferative diabetic retinopathy. In the present study, orally administered CS-866 ameliorated the neovascularization dose-dependently and significant inhibition was observed at all doses tested, 1 mg/kg, 3 mg/kg and 10 mg/kg. This result suggested the effectiveness of CS-866 in the treatment of proliferative diabetic retinopathy. The preventive effect of the drug would be due to angiotensin AT1 receptor inhibition because another angiotensin AT1 receptor antagonist (TCV-116) also exhibited the dose-dependent inhibition.

To support the results from the pharmacological experiment, a pharmacokinetic experiment was performed also in this oxygen-induced retinopathy model. We measured the plasma drug concentrations at 2 and 24 h after drug administration after an oral dose of 1 mg/kg. The plasma unbound RNH-6270 concentration at 2 h after drug administration (1 mg/kg) was 6.1 ng/ml. As shown in the electroretinogram study, the plasma unbound RNH-6270 concentration was comparable to the IC_{50} value of the drug in this mouse model. These pharmacokinetic data confirmed that CS-866 prevented the oxygen-induced angiogenesis through the inhibition of the angiotensin AT1 receptor.

The pharmacokinetic data in these two animal models also imply that the plasma concentrations of the drug in the animal models were within the range observed in clinical studies. In a previous clinical study of CS-866 for antihypertension therapy, the C_{max} and the area under the curve after an oral administration of 40 mg (the highest dose clinically used) were 693 ± 78 and 4229 ± 500 ng/ml, respectively (Schwocho and Masonson, 2001). The C_{max} of unbound RNH-6270 in clinical situation was estimated to be 8.3 ng/ml by multiplying the C_{max} by the free/bound ratio (0.012), which is higher than that obtained in animal experiments. Although the difference in species should be carefully considered, it is possible that occurrence of abnormalities in the electroretinogram and neovascularization in diabetic retinopathy is suppressed by an administration of a clinically available dose of CS-866.

In conclusion, we obtained evidence that CS-866 was effective in early ERG impairment in diabetic rats and hypoxia-induced neovascularization. These results confirmed the close relationship between the renin–angiotensin system and the pathophysiology of diabetic retinopathy. Pharmacokinetic data supported the evidence that CS-866 ameliorated these abnormalities through the inhibition of the angiotensin AT1 receptor. CS-866 would provide effective drug therapy for treatment of diabetic retinopathy. The precise mechanism of action and pharmacokinetic properties in diabetic patients should be elucidated hereafter.

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