Triple VPI CGS 35601 Reduces High Blood Pressure in Low-Renin, High-Salt Dahl Salt-Sensitive Rats

P. Daull,* A. Blouin,† K. Belleville,† M. Beaudoin,† D. Arsenault,* H. Leonard,† P. Sirois,‡ F. Nantel,† A. Y. Jeng,§ and B. Battistini*,†,[1]

*Laval Hospital Research Center, Quebec Heart & Lung Institute, Department of Medicine, Faculty of Medicine, Laval University, Québec, Canada G1V 4G5; †IPS Pharma Inc, Sherbrooke, Canada J1H 5N4; ‡Institute of Pharmacology of Sherbrooke, Department of Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Canada J1H 5N4; and §Cardiovascular Diseases Research, Novartis Institutes for BioMedical Research, East Hanover, New Jersey 07936

We previously reported that CGS 35601, a potent triple inhibitor of angiotensin-converting enzyme, neutral endopeptidase, and endothelin-converting enzyme 1, completely normalized mean arterial blood pressure (MABP) in 36-week-old spontaneously hypertensive rats, a normal renin model. The aim of the present study was to determine the effects of this triple vasopeptidase inhibitor (VPI) on the hemodynamic profile of instrumented, conscious, and unrestrained Dahl salt-sensitive (DSS) rats, a gene-prone, high-salt diet–induced low-renin hypertension model. Male DSS rats (mean weight [±SEM], 385 ± 10 g) were fed a normal diet (Group 1) or a high-salt diet (Groups 2 and 3; 8% NaCl in food) for 6 weeks and then instrumented with a carotid catheter and placed individually in metabolic cages for 30 days. The hemodynamic, hematological, and biochemical profiles were assessed daily. Dose-dependent treatment started after a 7-day stabilization period in Groups 1 and 2 (vehicle dosage, 250 μl/hr) and Group 3 (CGS 35601 dosages of 0.1, 1, and 5 mg/kg/day for 6 days per dose by means of constant intra-arterial infusion), followed by a 5-day washout period. Two additional groups included normotensive Wistar rats (Group 4) and DSS rats that received a double high-salt solid (8% NaCl) and liquid (1% NaCl) diet (Group 5).The MABP in rats receiving CGS 35601 decreased in a dose-dependent fashion toward the baseline level observed in DSS rats receiving a normal diet. The heart rate was unaffected. The hemodynamic profile returned to normal during the washout period. This novel triple VPI is a potent and effective antihypertensive agent with a safe short-term profile that may be of interest for treating hypertension and other cardiovascular diseases. Other hypertensive rat models are being tested. Exp Biol Med 231:830–833, 2006

Key words: angiotensin-converting enzyme; endothelin-converting enzyme; neutral endopeptidase 24.11; vasopeptidase inhibitor CGS 35601

Introduction

More than one-half of hypertensive patients have a salt-sensitive type of hypertension. In this type of essential hypertension, antioxidant levels are decreased, and free radical production is increased (1). The oxidative stress is responsible for severe renal damage, and antioxidant therapy can be helpful (2). Left ventricular hypertrophy (LVH) and diastolic dysfunction (i.e., congestive heart failure [CHF]-D) are early manifestations of cardiovascular organ damage in patients with hypertension. LVH leads to CHF and death if no treatment is performed (3). High sodium intake is responsible for decreased levels of circulating hormones in the renin-angiotensin-aldosterone system (RAAS) and increased blood pressure that lead to cardiac hypertrophy (4). The RAAS is apparently responsible for the development of CHF-D in hypertensive hearts, partly through the endothelin (ET) system (5). Rats with CHF have increased plasma ET-1 levels that can be normalized through treatment with a dual ET-converting enzyme (ECE)/neutral endopeptidase (NEP) inhibitor (6). Several studies have reported that ET type A (ET_A) receptor antagonism improves systolic function and prevents CHF (7).

Vasodilator neurohormones (i.e., natriuretic peptides) that have natriuretic, vasodilatatory, and antiproliferative effects and are able to inhibit the RAAS have been shown to have protective effects on the heart. Therefore, a new class of inhibitors, vasopeptidase inhibitors (VPIs), aimed at simul-

This project was supported by the Heart and Stroke Foundation of Québec (FMCQ), the Fondation de l'Institut de Cardiologie de Québec (FICQ), and unrestricted support from IPS Pharma Inc. B.B. is a Fonds de la Recherche en Santé du Québec-Industry-University Research Scholar.

[1] To whom correspondence should be addressed at Laval Hospital, Laval Hospital Research Center, Quebec Heart & Lung Institute, Pavilion Mallet-Room 2679, 2725 Chemin Ste-Foy, Ste-Foy, Canada, G1V 4G5. E-mail bruno.battistini@med.ulaval.ca

Received October 7, 2005.
Accepted December 7, 2005.

1535-3702/06/2316-0830$15.00

taneously inhibiting angiotensin-converting enzyme (ACE) and NEP, were developed. It was demonstrated that VPIs provide better cardioprotection than ACE or NEP inhibitors alone in mice with CHF induced by myocardial infarction (8). The very promising new drug omapatrilat (OMP), a distinct molecule of this class of drugs, failed in phase III clinical trial and has therefore blunted the interest in VPIs among researchers. Yet VPIs remain very effective antihypertensive drugs (9). Triple inhibition of ACE, NEP, and ECE-1, through the combination of benazepril and an NEP/ECE inhibitor (CGS 26303), was shown to better improve left ventricular hemodynamics than benazepril alone or CGS 26303 alone in rats with CHF (10). Therefore, the use of a triple VPI (11) that simultaneously inhibits ACE, NEP, and ECE-1, with respective IC_{50} values of 22, 2, and 55 n*M*, should be advantageous for the treatment of both hypertension and its related cardiovascular and renal damage.

The aim of the present study was to assess the efficacy of CGS 35601 in Dahl salt-sensitive (DSS) rats, a gene-prone, high-salt diet–induced, low-renin hypertension model, using the integrated rat platform we described previously (12).

Materials and Methods

All procedures were previously approved by the local ethics animal care committee and followed the guidelines on animal welfare established by the Canadian Council on Animal Care.

Anesthesia, Presurgical and Surgical Procedures, and Postoperative Care. Adult male DSS and Wistar rats (14 weeks of age; Charles River, Saint-Constant, Canada) were housed individually in modified metabolic cages (Nalgene, Rochester, NY) under a 12-hr cycle of day and night with unrestricted access to food and drinking water. Wistar rats (Group 4) and control normotensive DSS rats (Group 1) were fed normal rat chow containing 0.3% NaCl (Charles River 5075; Charles River, Saint-Constant, Canada), whereas hypertensive DSS rats (Groups 2 and 3) were fed a high-salt (HS) diet containing 8% NaCl (TD 00516; Harlan Teklad, Madison, WI) during the 6-week before instrumentation. Rats from Group 3 were treated with increasing dosages of CGS 35601 (0.1, 1, and 5 mg/kg/day for 6 days per dose by means of constant intra-arterial infusion) starting 7 days after surgery, followed by a 5-day washout period (Fig. 1a). In addition to a HS solid diet, DSS rats (Group 5) also received 1% NaCl in their drinking water. We intended to administer the HS water for 6 weeks, but because of a high incidence of stroke (which caused higher than normal morbidity and lethality rates), salt was removed from drinking water after just 2 weeks, although administration of the HS solid diet continued. Other subspecies of rats (age-matched, untreated normotensive Sprague-Dawley rats and spontaneously hypertensive rats) were instrumented to assess resting blood pressure by using the same setup (Fig. 1b).

As described elsewhere (12), surgical procedures were performed after administration of inhaled anesthesia (2% isoflurane inhalation; Baxter Corp., Toronto, Canada) in a strictly aseptic environment with sterilized materials. A urethane-coated antithrombogenic vascular catheter (PhysioCath; Data Sciences International, St. Paul, MN) was inserted into the left carotid and tunneled under the skin to the dorsal site of the neck. It was then connected to a low-flow peristaltic pump (Instech-Solomon, Plymouth Meeting, PA) through a stainless steel spring stock protector and swivel via an adjustable counterbalance lever arm (Instech-Solomon). A constant infusion of sterile heparinized (4 U/ml) saline (250 μl/hr) prevented the formation of blood clots within the catheter.

Hemodynamic Profile Determination. The hemodynamic profile was assessed each day at 1300 hrs on calm, resting, unrestrained, and conscious rats. Measurement was made directly through the implanted vascular catheter by using a blood pressure transducer (Harvard Apparatus, Montreal, Canada) connected to a precalibrated computerized system (PowerLab; ADInstrument, Colorado Springs, CO). Heart rate was simultaneously derived from these data and recorded.

Drug and Administration. CGS 35601 (L-tryptophan, *N*-[[1-[[(2*S*)-2-mercapto-4-methyl-1-oxopentyl] amino] cyclopentyl] carbonyl]) was kindly provided by Novartis. It was dissolved at 10 mg/ml in sterile saline:NaOH (0.1 *N*) before its final dilution at 1 mg/ml in saline. The drug solution was then sterilized through a 0.22 μm filter and kept frozen until use. Seven days after surgery, the rats in Group 3 received CGS 35601 intra-arterially through the vascular catheter at dosages of 0.1, 1, 5, mg/kg/day for 6 days at each dosage. A 5-day washout period followed the completion of the regimen.

Statistical Analyses. Results are expressed as mean values ± SEM. Because of the unavailability of some samples and/or data at a given time point, repeated-measures analysis of variance (ANOVA) was inapplicable to compare values obtained at different times. Thus, for comparison within groups, a randomized block design was applied using the following two factors defined for the analysis: the subject effect and the time effect.

Comparison between groups was performed by a three-way ANOVA with a blocking factor representing subjects. Interaction between the time factor and the factor used to compare groups was added to the model. When interaction was statistically significant ($P < 0.05$) for parameters, comparison at different times was analyzed using Student's paired *t* tests. The normality and variance assumptions were met for almost all data. All analyses were conducted using the SAS statistical package (V.6.12; SAS Institute Inc., Cary, NC).

Results

Figure 1b shows the MABP of different chronically instrumented rat subspecies studied with the integrated

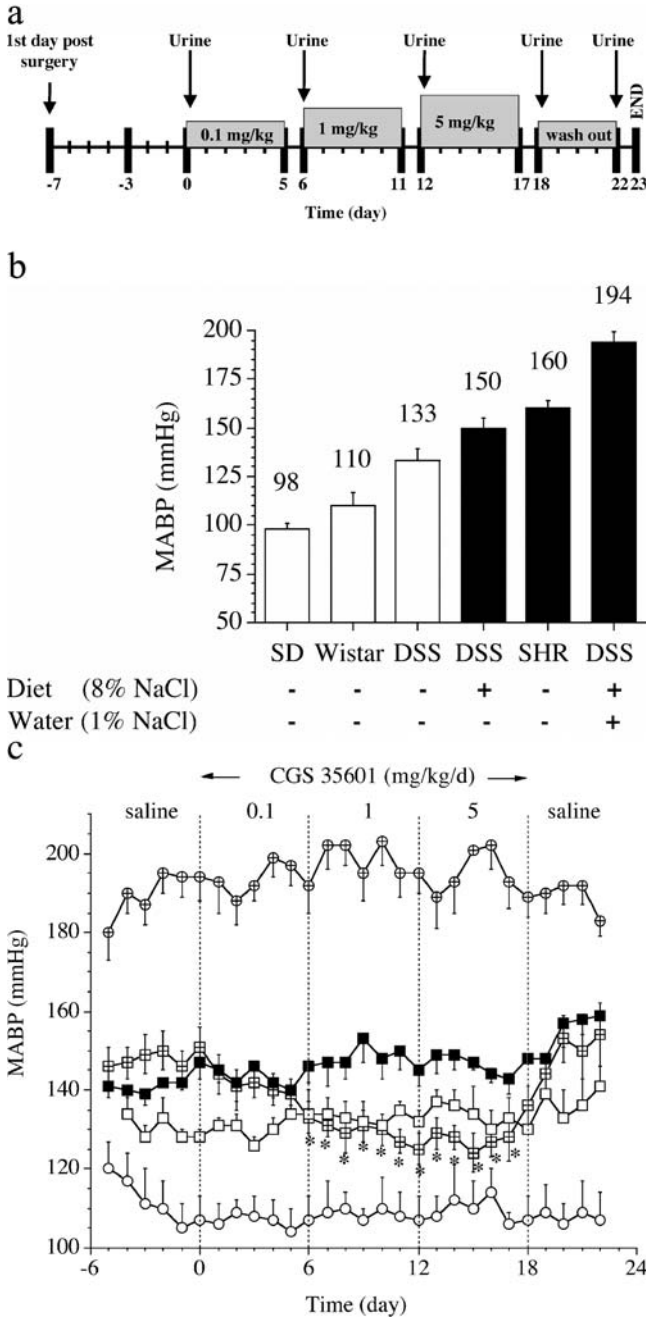

Figure 1. (a) Summary of the experimental protocol used in this chronic set of experiment. (b) Mean arterial blood pressure (MABP, in mm Hg) in resting, instrumented, unrestrained, and conscious Sprague Dawley (SD), Wistar, Dahl–salt sensitive (DSS), and spontaneously hypertensive rats (SHR), following different salt intake. (c) MABP (mm Hg) directly measured through the carotid in chronically instrumented, unrestrained and conscious Group 1 (DSS + vehicle, fed a normal salt diet; □ $n = 7$–8); Group 2 (untreated DSS + vehicle, fed a 8% NaCl solid diet; ■, $n = 8$–12); Group 3 (triple VPI CGS 35601–treated DSS fed an 8% NaCl solid diet; crossed square, $n = 12$–13); Group 4 (Wistar rats + vehicle, ○, $n = 9$); and Group 5 (untreated DSS + vehicle, fed a 8% NaCl solid diet + 1% NaCl in drinking water for 6 weeks; crossed circle, $n = 7$–11). The treatment was started 7 days after surgical implantation of the catheter (t_0) with increasing dosages of CGS 35601 at 0.1, 1, and 5 mg/kg/day by continuous intra-arterial infusion for a total of 18 days (6 days per dose), followed by a 5-day washout period. *, $P < 0.05$–0.001, significantly different between CGS 35601–treated Group 1 and untreated Group 2 over time.

platform (12, 13). It is noteworthy that so-called normotensive rats subspecies do not have the same baseline MABP. DSS rats fed a normal diet (0.3% NaCl) (i.e., normotensive DSS rats) have an increased baseline MABP, compared with Wistar rats, which may, in part, be explained by the low rate at which saline was infused to maintain catheter functionality (0.9% NaCl at 250 μl/hr). It is also important to notice that greater saline intake resulted in a higher degree of hypertension.

CGS 35601 at the lowest dosage tested (0.1 mg/kg/day) decreased the MABP by 3.2% after 6 days (6-day mean, 148 ± 5 to 143 ± 4 mm Hg; $P < 0.05$) (Fig. 1c). A further decrease in MABP was observed at higher dosages CGS 35601. At 1 and 5 mg/kg/day, the MABP decreased by 11.8% (6-day mean, 130 ± 4 mm Hg; $P < 0.01$) and 14.1% (6-day mean, 127 ± 4 mm Hg; $P < 0.001$), respectively, without affecting the heart rate (data not shown). At a CGS 35601 dosage of 5 mg/kg/day, the MABP even decreased below the baseline MABP observed for normotensive DSS rats. After cessation of the treatment, the MABP in Group 3 gradually increased to the baseline value observed in this group before initiation of treatment (Fig. 1c). Triple inhibition with CGS 35601 was also as effective at reducing both the systolic (SBP) and diastolic (DBP) blood pressures (data not shown). However, parameters of renal and hepatic function were not affected (data not shown).

Discussion

Hypertension is at the origin of many pathological conditions and is always implicated in the worsening of such conditions independently of the context. Overexpression of the RAAS and the ET pathway are not only implicated in the increase in blood pressure, as a result of increased angiotensin II (AII) and ET-1 levels, but also through endothelial dysfunction, decreased nitric oxide bioavailability, and increased levels of reactive oxygen species (14). Angiotensin II and ET-1 are proinflammatory molecules. They stimulate the release of proinflammatory cytokines, activate NF-κB, and increase oxidant stress (15, 16). Moreover, AII-related effects are, in part, mediated by ET-1. This supports the notion that a combined therapeutic strategy of inhibiting both AII and ET-1 production would be advantageous.

Dual ACE/NEP inhibitors are very effective antihypertensive drugs. Molecules such as OMP and AVE7688 are not only effective at lowering blood pressure but also exert antifibrotic and anti-inflammatory activities (17, 18). This makes them interesting compounds for the treatment of CHF and nephropathy. However, it is clear that OMP increases renal ET-1 levels in deocorticosterone acetate-salt hypertensive rats (19). This increase in circulating ET-1 levels is deleterious. However, the negative experience encountered with OMP has dramatically reduced the interest of the research community for this class of dual VPIs,

because of the high incidence of angioedema among patients receiving OMP.

New chemical entities that concomitantly inhibit ACE, NEP, and ECE-1 were then developed (11, 20) to take advantage of both the antifibrotic and anti-inflammatory activities observed for dual ACE/NEP inhibitor and the beneficial effects of an ECE-1 inhibitor. It was demonstrated that short-term infusion of CGS 35601 at 5 mg/kg/day over 6 days to spontaneously hypertensive rats reduced the MABP by 40% (from 156 ± 4 to 94 ± 5 mm Hg) (13). In instrumented Zucker diabetic fatty rats, a model of type 2 diabetes, a 5 mg/kg/day dosage of CGS 35601 reduced the MABP by 22%, to 80 ± 4 mm Hg (e.g., below the MABP in vehicle-treated Zucker diabetic fatty rats; Ref. 21).

Our data clearly demonstrate that CGS 35601 is capable of reducing MABP in the DSS rat, which is a low-renin model of hypertension. The highest dosage of CGS 35601 reduced MABP by 14%. The addition of ECE-1 inhibition activity to the dual ACE/NEP inhibition should improve the efficacy and safety profile of the triple VPI strategy. The complete profile of the vasoctive mediators and toxicology of CGS 35601 will be published in detail elsewhere. Therefore, triple VPIs seem to be a promising new class of molecules. However, it has to be demonstrated that angioedema is not a complication in this class of molecules.

1. Manning RD Jr., Meng S, Tian N. Renal and vascular oxidative stress and salt-sensitivity of arterial pressure. Acta Physiol Scand 179:243–250, 2003.
2. Manning RD Jr., Tian N, Meng S. Oxidative stress and antioxidant treatment in hypertension and the associated renal damage. Am J Nephrol 25:311–317, 2005.
3. Diamond JA, Phillips RA. Hypertensive heart disease. Hypertens Res 28:191–202, 2005.
4. Takeda Y. Role of cardiovascular aldosterone in hypertension. Curr Med Chem Cardiovasc Hematol Agents 3:261–266, 2005.
5. Yamamoto K, Masuyama T, Sakata Y, Mano T, Nishikawa N, Kondo H, Akehi N, Kuzuya T, Miwa T, Hori M. Roles of renin-angiotensin and endothelin systems in development of diastolic heart failure in hypertensive hearts. Cardiovasc Res 47:274–283, 2000.
6. Emoto N, Raharjo SB, Isaka D, Masuda S, Adiarto S, Jeng AY, Yokoyama M. Dual ECE/NEP inhibition on cardiac and neurohumoral function during the transition from hypertrophy to heart failure in rats. Hypertension 45:1145–1152, 2005.
7. Yamamoto K, Masuyama T, Sakata Y, Nishikawa N, Mano T, Hori M. Prevention of diastolic heart failure by endothelin type A receptor antagonist through inhibition of ventricular structural remodeling in hypertensive heart. J Hypertens 20:753–761, 2002.
8. Xu J, Carretero OA, Liu YH, Yang F, Shesely EG, Oja-Tebbe N, Yang XP. Dual inhibition of ACE and NEP provides greater cardioprotection in mice with heart failure. J Card Fail 10:83–89, 2004.
9. Taal MW, Nenov VD, Wong W, Satyal SR, Sakharova O, Choi JH, Troy JL, Brenner BM. Vasopeptidase inhibition affords greater renoprotection than angiotensin-converting enzyme inhibition alone. J Am Soc Nephrol 12:2051–2059, 2001.
10. Mellin V, Jeng AY, Monteil C, Renet S, Henry JP, Thuillez C, Mulder P. Triple ACE-ECE-NEP inhibition in heart failure: a comparison with ACE and dual ECE-NEP inhibition. J Cardiovasc Pharmacol 46:390–397, 2005.
11. Trapani AJ, Beil ME, Bruseo CW, Savage P, Firooznia F, Jeng AY. CGS 35601 and its orally active prodrug CGS 37808 as triple inhibitors of endothelin-converting enzyme-1, neutral endopeptidase 24.11, and angiotensin-converting enzyme. J Cardiovasc Pharmacol 44:S211–S215, 2004.
12. Nantel F, Daull P, Duta DN, Blouin A, Berthiaume N, Cayer J, Beaudoin M, Belleville K, Benrezzak O, Sirois P, Battistini B. Integrated in vivo pharmacology using an instrumented, unrestrained and conscious rat platform: application to rat models of diabetes. J Pharmacol Toxicol Methods. 2005 Nov 6 [Epub ahead of print].
13. Daull P, Benrezzak O, Arsenault D, Pheng LH, Blouin A, Cayer J, Beaudoin M, Belleville K, Sirois P, Nantel F, Jeng AY, Battistini B. Triple vasopeptidase inhibition normalizes blood pressure in conscious, unrestrained, and spontaneously hypertensive rats. Am J Hypertens 18: 1606–1613, 2005.
14. Zhou MS, Jaimes EA, Raij L. Atorvastatin prevents end-organ injury in salt-sensitive hypertension: role of eNOS and oxidant stress. Hypertension 44:186–190, 2004.
15. Muller DN, Fiebeler A, Park JK, Dechend R, Luft FC. Angiotensin II and endothelin induce inflammation and thereby promote hypertension-induced end-organ damage. Clin Nephrol 60(Suppl 1):S2–S12, 2003.
16. Das UN. Is angiotensin-II an endogenous pro-inflammatory molecule? Med Sci Monit 11:RA155–RA162, 2005.
17. Pu Q, Amiri F, Gannon P, Schiffrin EL. Dual angiotensin-converting enzyme/neutral endopeptidase inhibition on cardiac and renal fibrosis and inflammation in DOCA-salt hypertensive rats. J Hypertens 23:401–409, 2005.
18. Gross O, Koepke ML, Beirowski B, Schulze-Lohoff E, Segerer S, Weber M. Nephroprotection by antifibrotic and anti-inflammatory effects of the vasopeptidase inhibitor AVE7688. Kidney Int 68:456–463, 2005.
19. Elmarakby AA, Morsing P, Pollock JS, Pollock DM. Omapatrilat increases renal endothelin in deoxycorticosterone acetate-salt hypertensive rats. Vascul Pharmacol 40:253–259, 2003.
20. Inguimbert N, Poras H, Teffo F, Beslot F, Selkti M, Tomas A, Scalbert E, Bennejean C, Renard P, Fournie-Zaluski MC, Roques BP. *N*-[2-(Indan-1-yl)-3-mercapto-propionyl] amino acids as highly potent inhibitors of the three vasopeptidases (NEP, ACE, ECE): in vitro and in vivo activities. Bioorg Med Chem Lett 12:2001–2005, 2002.
21. Daull P, Blouin A, Beaudoin M, Gadbois S, Belleville K, Cayer J, Berthiaume N, Sirois P, Nantel F, Jeng AY, Battistini B. The hemodynamic and metabolic profiles of Zucker diabetic fatty rats treated with a single molecule triple vasopeptidase inhibitor, CGS 35601. Exp Biol Med 231:824–829, 2006.