Journal of the American Society of Hypertension 1(6) (2007) 423–432

Original Article

Telmisartan ramipril combination therapy reduces strokes and improves cardiac and renal protection in stroke prone spontaneously hypertensive rats

Ying Zhou, MD, Fangmin Yu, PharmD, Ada R. Ene, MD, and Daniel F. Catanzaro, PhD*

Weill Cornell Medical College, New York, New York, USA

Manuscript received April 20, 2007 and accepted August 13, 2007

Abstract

Clinical and animal experimental studies suggest that combination therapy using angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors provides superior blood pressure (BP) lowering and target organ protection than either agent alone. We tested combination therapy with telmisartan and ramipril in lowering BP and protecting against stroke and target-organ damage in salt-fed stroke prone spontaneously hypertensive rats. Twenty-five rats were assigned to each of five groups: control (C), telmisartan (T), ramipril (R), and telmisartan + ramipril at full (TR) and at half-dose (½TR). Full dose telmisartan was 1 mg/kg/day and ramipril .4 mg/kg/day. Rats were fed a stroke prone diet for 8 weeks starting at age 7.5 weeks. Eighty-three percent C and 56% R showed behavioral signs of stroke. There were no strokes in other groups. BP was lower than control in all groups and lowest in TR. Urinary protein excretion, renal damage scores, and left ventricle cardiac collagen areas were lower than controls in all telmisartan treatment groups and lowest in TR. Telmisartan was superior to ramipril in preventing strokes, and telmisartan/ramipril combination therapy provided better BP control and greater cardio-renal protection than telmisartan alone.

Keywords: Combination therapy; angiotensin receptor blocker; angiotensin-converting enzyme inhibitor; spontaneously hypertensive stroke prone rats; target organ damage.

Introduction

Renin-angiotensin system (RAS) blockade with angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) have been shown both to lower blood pressure (BP) and to protect against damage to target organs including the brain, kidneys, and heart.[1–6] However, ACE inhibitors may not fully suppress angiotensin II (Ang II) formation,[7] while ARBs alone may not provide the additional benefits of ACE inhibition, such as reducing breakdown of bradykinin, Ac-SDKP, and substance P.[8] Accordingly, a number of clinical studies have shown that combination therapy with an ACE inhibitor and an ARB is more effective than monotherapy.[9–11] Such combination therapy has also been shown to reduce albuminuria in diabetic and nondiabetic patients,[10–16] may be protective against cardiovascular events in patients with congestive heart failure and diastolic dysfunction,[17,18] and may reduce aortic arterial stiffness in patients will essential hypertension.[19] Although RAS blockade lowers the incidence of stroke in both animals and in humans,[1–3,5,6,20] the effects of dual blockade on stroke have not been addressed. Improved BP lowering effects of ARB/ACE inhibitor combination therapy have also been demonstrated in animal studies.[21–26] However, none examined the effects of dual inhibition on the brain and other target organs.

In the present study, we sought to determine the effects of fixed dose telmisartan/ramipril combination therapy (TR) against stroke and damage to heart and kidneys in spontaneously hypertensive stroke prone rats (SHRSP); compared to treatment with either agent alone, TR treatment at half-dose, and untreated controls. The primary endpoint was behavioral signs of stroke upon which animals were euthanized. Systolic blood pressure (SBP), urine protein excretion, and plasma renin concentration were measured before treatment, and at the middle and end of the 8-week study.

This work was supported by a Grant from Boehringer Ingelheim Pharma GmbH & Co. KG.

Conflict of interest: none.

*Corresponding author: Daniel F. Catanzaro, Weill Cornell Medical College, Division of Cardiovascular Pathophysiology, 1300 York Avenue, Box 118, New York, New York 10065. Tel: 212-746-6335.

E-mail: dfcatanz@med.cornell.edu

1933-1711/07/$ – see front matter
doi:10.1016/j.jash.2007.08.002

Renal and cardiac damage were assessed histologically after euthanasia.

Methods

Animals and Treatment Protocols

All procedures involving animals were approved by the Weill Cornell Medical College Institutional Animal Care and Use Committee (IACUC). SHRSP were obtained from Charles River Laboratories (Wilmington, MA) at 6.5 weeks age and acclimated to the laboratory setting for 1 week before commencing the stroke prone diet (Ziegler Brothers, Gardeners, PA 522801 containing 4% NaCl, plus .9% NaCl in the drinking water). Telmisartan was provided by Boehringer Ingelheim Pharmaceutical, Inc. (Hopkinton, MA).

Rats were randomly assigned to one of five treatment groups (25 rats per group): control (C), telmisartan (T), ramipril (R), telmisartan + ramipril (TR), and a group that received half the dose of each agent in the combination dose (½TR). Drugs were administered in the drinking water to deliver telmisartan 1 mg/kg/day or ramipril .4 mg/kg/day. The TR group was administered the full dose of each drug while the ½TR group received half the dose of each drug. Drug concentrations in the drinking water were adjusted to maintain a constant dosing based on the rats' water consumption and body weight, which were measured weekly. Animals were monitored twice daily, and any animal showing behavioral signs of stroke (circling, inactivity, hyperactivity, and paralysis) were euthanized.

Physiological Measurements

BP was measured at baseline and then every 2 weeks by tail-cuff sphygmomanometery using a Visitech BP2000, Apex, NC platform. At baseline, 4 and 8 weeks, blood was collected from the tail vein for plasma renin concentration (PRC) determination, and the rats were placed in metabolic cages for urine collection to determine urine protein concentration.

Biochemical and Histological Measurements

Plasma renin concentration was measured by radioimmunoassay of Ang I in plasma incubated in the presence of excess angiotensinogen. Urinary protein was measured using a modified Bradford microplate assay.[27] Tissues were weighed, fixed in paraformaldehyde, embedded in paraffin, sectioned, and stained with either picrosirius red or Masson's stain. Kidney sections were scored for glomerular damage according to the following scale: 0 = no injury; 1 = mesangial expansion/thickening of the basement membrane; 2 = mild-moderate segmental hyalinosis/sclerosis; 3 = diffuse hyalinosis/sclerosis involving >50% of the tuft; 4 = diffuse glomerulosclerosis with total tuft obliteration and collapse.[27] Tubulointerstitial injury was graded as: 0 = no injury; 1 = lesions <25% area; 2 = lesions 25% to 50% area; 3 = lesions 51% to 90% area; 4 = lesions >90% area.[27] Collagen area of the heart was determined by polarization microscopy of picrosirus red stained mid-coronal sections.[27] Histological analyses were performed on tissues from 10 to 12 animals from each group. All histological analyses were performed on sections in randomized order by an operator blinded to the treatment grouping.

Statistics

Data were analyzed using the Student *t* test. For multiple comparisons, *P* values were adjusted using the Bonferroni method. Survival data were analyzed using the Kaplan-Meier method and differences in survival curves assessed using a log rank test. A value of $P < .05$ was considered significant for all analyses.

Results

Body weight increased over the course of the study in all treatment groups for the first 4 weeks and then began to diverge (Figure 1). Among the control group, body weights began to decline after 4 weeks and by 8 weeks were 10% lower on average. Among R and ½TR groups, weight gain slowed after 6 weeks and then declined slightly by 8 weeks. In the T and TR groups, body weights continued to increase until the end of the study. Body weights in the ½TR and R groups declined slightly during the last 2 weeks of treatment. At the end of the study, all animals receiving treatment had body weights significantly greater than C except for R.

SBP was higher than baseline in all animals after 2 weeks exposure to the high-salt diet, irrespective of treatment (Figure 2). However, even at this early stage, SBP among groups had diverged to a rank order similar to that at the end of the study, although the spread was much narrower at 2 weeks (<20 mm Hg) than at 8 weeks (>30 mm Hg). After 8 weeks, SBP was highest in the control group and lower in all treatment groups in descending order R, ½TR, T, and TR. T alone yielded significantly lower SBP than R alone, and the ½TR group had an intermediate pressure. However, TR had the lowest SBP of any group at all treatment points, and was significantly lower than T alone. At 8 weeks, all differences in SBP between groups were significant except for ½TR and T.

Animals were monitored daily for behavioral signs of stroke, and animals with signs of stroke were euthanized. Stokes occurred in 83% of control animals and 56% of animals in the R group (Figure 3). The incidence of stroke in these two groups was significantly higher than controls, and rate of strokes differed significantly between these two groups. One animal in the R group died at day 3 from non-neurological causes. All other animals were stroke free and survived until the end of the study.

Urinary protein excretion, measured as an index of renal