

Keywords:
AT₁-receptor,
stroke

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Accepted for
publication
19th May 2001

JRAAS 2001; 2: 103–06

**Journal of
the Renin-
Angiotensin-
Aldosterone
System**
(Including other
peptidergic systems)

June 2001
Volume 2
Number 2

Non-AT₁-receptor-mediated protective effect of angiotensin against acute ischaemic stroke in the gerbil

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Abstract

Previous studies have shown that angiotensin II (Ang II), by mediating rapid recruitment of collateral circulation, has a protective effect in the setting of acute ischaemia. In an experimental model of acute cerebral ischaemia in the gerbil, Fernandez *et al.* have reported that the mechanism of the protective effect of Ang II is blood pressure (BP)-independent,¹ and that the AT₁-receptor antagonist, losartan, but not the ACE inhibitor (ACE-I), enalapril, decreases mortality following unilateral carotid artery ligation.² The aim of this study was to examine the reproducibility of the respective effects of losartan and enalapril, and to verify that these differential effects are drug class-related. Acute cerebral ischaemia was induced in anaesthetised gerbils by unilateral carotid ligation. The effect of pretreatment with two different ACE-I (enalapril and lisinopril), and two different AT₁-receptor antagonists (losartan and candesartan), administered orally or intravenously, on mortality were compared. Kaplan-Meier survival curves at day three were analysed by a log-rank test. Pretreatment with both enalapril and lisinopril significantly decreased survival at day three compared with controls, while the AT₁-receptor antagonists losartan and candesartan, despite similarly lowering BP, did not increase mortality. Coadministration of losartan and enalapril increased mortality to the same extent as enalapril alone. This study confirms that Ang II contributes to protective mechanisms against acute cerebral ischaemia through non AT₁-receptor-mediated, BP-independent effects.

Introduction

In a series of experimental studies, the group of L. Fernandez in Yale have shown that angiotensin II (Ang II), independently of its hypertensive effect, helps to restore blood flow in ischaemic areas. In the rat, acute ligation of the aorta between the origins of the two renal arteries induced a muscular ischaemic palsy of the hindlimb which was only transient when the ischaemic kidney hypersecreting renin was left in place, but which persisted for over 24 hours when this kidney was removed and hypertension restored by DOC-salt supplementation.³ In the gerbil, a progressive but complete unilateral carotid ligation or an abrupt complete ligation were used to induce ipsilateral brain ischaemia in animals pretreated with an angiotensin-converting enzyme inhibitor (ACE-I) (enalaprilat), or saline as control. In saline-infused animals, there was a significantly lower mortality rate in progressively-ligated animals when compared with abruptly-ligated animals, while

administration of enalaprilat to progressively-ligated animals resulted in mortality rates that were indistinguishable from those of saline-infused abruptly-ligated animals.⁴ The same authors further demonstrated that Ang II infusion increased survival in gerbils subjected to abrupt unilateral carotid ligation.¹ Finally, survival at 48 hours was significantly improved by pretreatment with losartan, but coadministration of enalaprilat abolished the protective effect of losartan.² These results strongly supported the role of the endogenous renin-angiotensin system (RAS) in an adaptive mechanism to acute ischaemia, and suggested:

- that Ang II induces an immediate recruitment of collateral circulation that reduces the severity of focal ischaemia
- that this effect of Ang II is not mediated by the AT₁-receptor.

During the time period when these studies were published, the beneficial effect of ACE inhibition in secondary prevention of cardiac events was progressively established, and ACE-I were shown to provide strong protection against stroke in hypertensive animal models. Thus, little attention was paid to the potential importance of these findings suggesting a protective role of Ang II during acute cerebral ischaemia. The validity of these observations have never been re-evaluated by an independent laboratory. The aim of the present study was to examine the reproducibility of the opposite effects of enalapril and losartan reported by the Yale group in the model of acute cerebral ischaemia in the gerbil, and to verify that this effect is related to their drug class.

Methods

Adult male gerbils (*Meriones unguiculatus*), weighing 60–75 g, were purchased from Janvier (Le Genest St Isle 53940, France) and kept in a controlled-temperature (24°C). They were fed rat chow and tap water was given *ad libitum*.

In a first set of experiments, gerbils were pretreated 2 hours prior to surgery with an oral bolus of either losartan (50 mg/kg), enalapril (10 mg/kg) or the combination of the two, dissolved in 0.5 ml saline, or saline alone by gastric gavage. Surgical procedures were performed under sodium pentobarbital anaesthesia (60 mg/kg of body weight intraperitoneally). After making a midline neck incision, the right common carotid artery was exposed and then ligated with a silk thread. The

skin was sutured and the animals returned to their cages and allowed to recover from anaesthesia. In a subset of animals similarly pretreated, the abdominal aorta was exposed and cannulated for invasive measurement of blood pressure (BP) via an external transducer. BP was measured after 5 minutes, three times at 1 minute intervals, and the canula rinsed with 0.1 ml saline solution after each measurement.

In a second set of experiments, gerbils were anaesthetised and the penian vein was exposed and cannulated. Candesartan cilexetil (1 mg/kg), lisinopril (3 mg/kg), or the vehicle, saline (0.5 ml) was administered intravenously. Unilateral carotid ligation was performed 20–30 minutes following pretreatment. A subset of animals were similarly treated for subsequent invasive measurements of BP.

Results are reported as mean \pm standard error of mean (SEM). Mortality rate in each group of animals was assessed at 24, 48 and 72 hours and a log-rank test was used for statistical analysis of the Kaplan-Meier survival curves over three days using the Statview software (Abacus concept, Inc, Berkeley, CA 1996). Student *t*-test and ANOVA were used for comparison of BP levels.

Results

Effect of losartan and enalapril

Survival rates following carotid ligation in losartan- and enalapril-pretreated gerbils are shown in Figure 1. Survival rates at day three in the control group (treated with saline) was $64.9 \pm 7.8\%$ ($n=37$) and $62.2 \pm 8\%$ in the losartan group ($n=37$); survival was half that in the two groups pretreated with enalapril, either alone ($29.7 \pm 7.5\%$, $n=37$) or in combination with losartan ($32.4 \pm 7.7\%$, $n=37$). Statistical analysis of the Kaplan-Meier survival curves showed that the mortality rate of losartan-pretreated animals was not statistically different from that of the controls, but their mortality was significantly increased by enalapril ($p=0.004$ *vs.* saline and 0.008 *vs.* losartan) and by the combination of enalapril and losartan ($p=0.008$ *vs.* saline and 0.02 *vs.* losartan). The mean BP in saline-pretreated anaesthetised animals was 85.4 ± 8 mmHg, ($n=5$), and was significantly lowered by pretreatment with enalapril (70.0 ± 5.6 mmHg, $n=5$; $p=0.003$) or losartan (68.0 ± 4.7 mmHg, $n=5$; $p=0.004$), but there was no difference between the losartan and enalapril groups. Thus, the deleterious effect of enalapril on survival rate was independent of BP-lowering.

Effect of candesartan and lisinopril

To further examine whether these findings were the result of intrinsic drug class differences between ACE-I and AT-receptor antagonists, or alternatively result from some specific property of losartan or enalapril unrelated to their action on the RAS, the effect of candesartan was compared with that of lisinopril in another set of experiments in animals intravenously pretreated 30 minutes prior to carotid ligation (Figure 2).

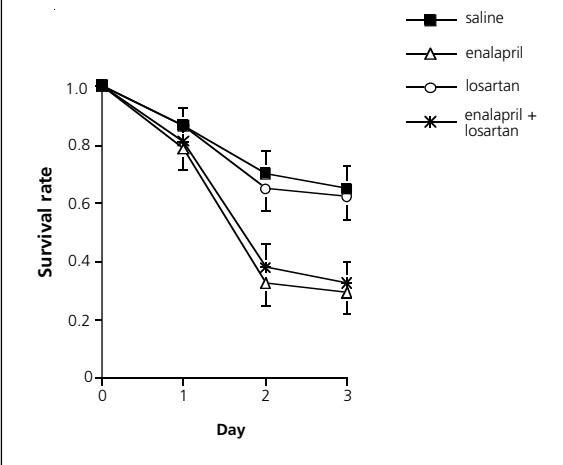
The survival rate at day three was $54.7 \pm 6.8\%$ in the control group ($n=53$) and $61.4 \pm 7.3\%$ in the

Figure 1 Survival after right carotid ligation.

Oral pretreatment 30 minutes before surgery: saline 0.5 ml; $n=37$; enalapril, 10 mg/kg; $n=37$; losartan 50 mg/kg; $n=37$; losartan + enalapril, same doses; $n=37$.

Kaplan-Meier survival analysis, log-rank (Mantel-Cox) test:

- saline *vs.* losartan, ns
- saline *vs.* enalapril, $p=0.004$
- losartan *vs.* enalapril, $p=0.008$
- enalapril *vs.* losartan + enalapril, ns

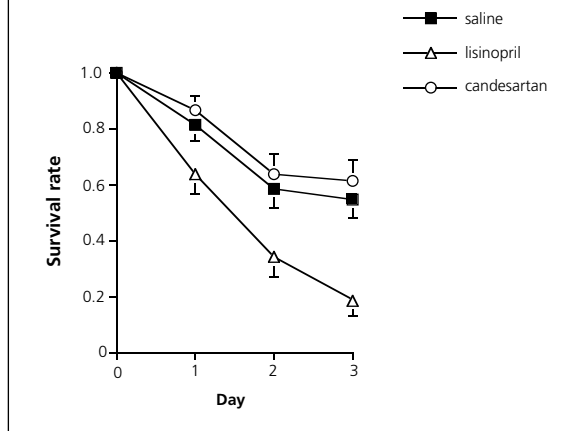


candesartan group ($n=44$); survival was again much lower ($18.2 \pm 5.8\%$) in the group pretreated with the ACE-I, lisinopril ($n=44$). Statistical analysis of the Kaplan-Meier survival curve showed that the mortality rate of candesartan-pretreated animals did not differ from that of the controls, but that mortality was significantly increased by lisinopril ($p=0.0004$ *vs.* saline, and <0.0001 *vs.* candesartan). There was no difference in intra-aortic systolic and diastolic BP (mmHg) between the lisinopril and candesartan groups (lisinopril ($n=3$): $89 \pm 1/66 \pm 2$; candesartan ($n=3$): $86 \pm 1.7/62 \pm 2$), but BP was significantly lower than in saline-pretreated gerbils (controls ($n=5$): $122 \pm 16/96 \pm 14$).

Discussion

Because the gerbil is lacking a complete Willis polygona, unilateral carotid ligation causes ipsilateral brain ischaemia, followed by neurological deficits and a significant death rate within three days.⁵ Gradual, albeit complete, unilateral carotid occlusion produces a less detrimental effect than sudden occlusion, as the progressive occlusion allows adaptive processes to minimise the decrease of blood flow to the ischaemic area. Using this model, Fernandez *et al.* have reported that blockade of the RAS with enalaprilat or saralasin, a non-specific, competitive Ang II antagonist, increased the mortality in progressively-ligated animals to the extent that survival was no longer different from untreated gerbils submitted to abrupt ligation, suggesting that Ang II is involved in the physiological adaptive process of cerebral protection against ischaemia.⁴ They further demonstrated that exogenous infusion of Ang II for 4 hours following abrupt ligation of a carotid artery significantly decreased the mortality rate compared with saline-infused controls, while

Figure 2 Survival rate after right carotid ligation. Intravenous pretreatment with saline (n=53), 3 mg/kg lisinopril (n=44) or 1 mg/kg candesartan (n=44). Kaplan-Meier survival analysis, log-rank (Mantel-Cox) test:
 - saline vs. candesartan, ns
 - saline vs. lisinopril, p=0.0004
 - candesartan vs. lisinopril, p<0.0001



increasing BP to the same extent with metaraminol had no protective effect on mortality.¹ In another study, the effect of pretreatment with enalaprilat and the specific AT₁-receptor antagonist, losartan, were compared in the model of abrupt unilateral carotid ligation.² Pretreatment with losartan significantly increased survival rates, and this beneficial effect was completely blunted when enalaprilat was simultaneously administered. The present study aimed to verify the reproducibility of these findings using different ACE-I and AT₁-receptor antagonists. The main conclusions are that:

- Blockade of Ang II production with enalapril or lisinopril decreases survival following acute cerebral ischaemia compared with placebo, indicating that Ang II is involved in counter-regulatory processes that help to limit the consequence of cerebral ischaemia.
- Blockade of AT₁-receptors with losartan and candesartan had no deleterious effect on survival, indicating that the Ang II-dependent component of the adaptative mechanisms to cerebral ischaemia is not mediated through AT₁-receptors.
- Systemic BP was decreased to the same extent with ACE-I and AT₁-receptor antagonists, implying that the non-AT₁-receptor mediated protective effect of Ang II is not related to its pressor effect.

These conclusions fully corroborate those of Fernandez *et al.*, and confirm that Ang II non-AT₁-receptors are involved in the rapid adaptation to cerebral ischaemia. Characterisation of the endogenous, physiological agents that contribute to preserve neurones against acute cerebral anoxia might open promising new therapeutic approaches for ischaemic strokes. Perhaps even more importantly, understanding the nature and the precise role of non-AT₁-receptors could also help to better define the optimal strategy to prevent acute cerebral ischaemia. As expected from their potent antihy-

pertensive effects and from their anti-inflammatory action on the wall of atherosclerotic vessels, ACE-I have been shown to reduce the risk of stroke in animal models exposed to high BP-dependent cerebrovascular disease^{6,7} and the HOPE study⁸ has recently established their efficiency in reducing stroke risk in secondary prevention in patients with advanced atherosclerosis. However, because of the multiplicity of its active peptides and receptors, the RAS may act as a two-edged sword, and so blocking angiotensin may not be as effective for primary stroke prevention in hypertensive patients.

The first and only large trial comparing ACE-I with conventional treatment in primary prevention in hypertensive patients (CAPPP)⁹ provided indication that, in hypertensive patients, ACE inhibition may be less effective than Ang II-stimulating diuretics in preventing stroke, even though disparities in BP levels between the two groups after randomisation prevent any definitive conclusions from this large trial. A critical review of large morbidity and mortality trials clearly points to the superiority of renin-stimulating diuretics over renin inhibiting beta-blockers in preventing strokes in hypertensive patients, not only at high doses but also at low doses.¹⁰ This discrepancy between the efficacy of diuretics and beta-blockers in clinical stroke protection even led Brown and Brown¹¹ to ask the question: "Does Ang II protect against stroke?" soon after the publication of the MRC trial of 1985.¹² These clinical observations make it plausible that the non-AT₁-receptor-mediated protective effect of Ang II against cerebral ischaemia documented in animal experimental models may have some relevance in humans. The blockade of AT₁-receptors with their specific antagonists, by blunting the negative feedback of Ang II on renin, increases Ang II formation and subsequent stimulation of unopposed non-AT₁-receptors. Since AT₁-receptor antagonists and ACE-I share experimentally similar antihypertensive and anti-atherothrombotic effects, the non-AT₁-receptor-stimulating effect of AT₁ antagonists may offer a cutting edge over ACE-I in populations especially at risk of stroke, such as elderly hypertensives. This stresses the need for clinical trials comparing the effect of AT₁-receptor antagonists and ACE-I on stroke prevention in such a population.

Acknowledgements

The authors thank Takeda (Dr Kozai Y, Dr S Nisse Durgeat) for giving candesartan and financial support as well as the French Society of Hypertension for supporting Dr Mazouz H by a research allocation.

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