Hypotensive Effect of a Novel Dihydropyridine with Dual Calcium Channel Blocking and Angiotensin II Antagonistic Properties in a Rat Model
Mohsen Imenshahidi¹, Milad Alipour¹, Farzin Hadizadeh⁴,⁵, Vahideh Sadat Motamed-Shariaty³
¹School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
²Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
³Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

ARTICLE INFO
Article Type: Original Research
Article History:
Received: 25 September 2014
Accepted: 15 March 2014
Keywords:
Angiotensin
Dihydropyridine
Losartan
Blood pressure
Hypertension

ABSTRACT
Background: In our previous work, we synthesized a novel analogue of losartan (Compound A) with dual calcium (Ca²⁺) channel blocking and antagonism of angiotensin II (ANG II) type 1 receptor (AT₁) activity. In this study, the effects of this compound (compound A) on the blood pressure (BP) and the heart rate (HR) of normotensive rats were investigated and compared to losartan, which was used as a positive control. Method: A novel dihydropyridine compound was synthesized by connecting a dihydropyridine nucleus to an imidazole ring. Three doses of compound A (0.25, 0.5 and 1 mg/kg) and three doses of losartan (1, 2 and 4 mg/kg) were administered intravenously to different groups of normotensive male rats, and their effects on mean arterial BP (MABP) and heart rate (HR) were evaluated. Results: All three doses of compound A reduced the MABP in normotensive anaesthetized rats in a dose-dependent manner. The administration of 1 mg/kg of compound A and 4 mg/kg of losartan (the largest doses) caused a reduction of 67.2 ± 2.2 and 69.3 ± 2.9 mmHg in the MABP, respectively. Conclusion: It can be concluded that like losartan, compound A has hypotensive properties. It can also be concluded that compound A has greater potency than losartan.

Introduction
Angiotensin II (ANG II) is an important regulator of the renal microcirculation and exerts major actions on afferent and efferent arterioles. Although it has been clearly demonstrated that ANG II increases intracellular calcium ([Ca²⁺]i) in vascular smooth muscle cells, the sequence of events following activation of ANG II type 1 receptors (AT₁) remains unclear. Typically, the Ca²⁺ response is characterised by a sharp transient rise in intracellular (Ca²⁺)i, followed by a fall towards a sustained plateau above baseline. Although the sustained increases in (Ca²⁺)i are thought to be mediated through Ca²⁺ influx, the early peak response is generally considered to be due to mobilisation of intracellular stores. AT₁ receptor-coupled G proteins activate PLC, which, in turn, activates IP₃ and DAG, leading to the release of Ca²⁺ from the sarcoplasmic reticulum. It has been suggested that increased (Ca²⁺)i activates chloride channels, causing an efflux of chloride and subsequent depolarisation of the cell membrane, leading to opening of voltage-gated Ca²⁺ channels. L-type Ca²⁺ channel blockers prevent constriction of afferent arterioles and also reduce sustained (Ca²⁺)i increases. Losartan (Dup-753) (Fig. 1) is a nonpeptide angiotensin II receptor (type AT₁) antagonist discovered by Duncia et al. in 1990, and its potassium salt (cozaar) has been marketed as an antihypertensive since 1995. To date, many orally available sartans have been developed and are used in the treatment of both hypertension and damage associated with various diseases, such as atherosclerosis and diabetes.

The beneficial properties of new nonpeptide ANG II antagonists, such as losartan, have stimulated the design of many different congeners. All the new nonpeptide ANG II antagonists that have been designed/developed all the new nonpeptide ANG II antagonists that have been designed contain a biphenyl fragment bearing an acidic moiety (i.e. a tetrazole, carboxylic- or sulphonamidocarboxyl group), linked to a heteroaromatic or acyclic group. Almost all chemical manipulations of the fundamental skeleton of sartans have focused on the substitution of the imidazole ring of losartan with different heteroaromatic groups or acyclic structures. In our recently published work, we synthesised a novel analogue of losartan in which a biphenyl fragment was retained and the imidazole nucleus was
connected to a dihydropyridine moiety (figure 1). We showed dual Ca\(^{2+}\) channel blocking and AT\(_1\) antagonist activity for the synthesised compound. In this study, the effects of this compound (compound A) on the blood pressure (BP) of normotensive rats were investigated and compared to losartan as a positive control.

**Materials and methods**

Compound A was prepared as described previously.\(^{19}\) Losartan and KCl were supplied by Sigma, USA. Losartan and compound A were dissolved in a mixture of three parts distilled water to one part dimethyl sulphoxide. This mixture did not affect the BP of the animals. All the drug solutions were prepared on a daily basis. Male Wistar rats weighing 200–250 g were used. The animals were anaesthetised with intraperitoneal ketamine/xylazine (Sigma) (60 mg/kg and 6 mg/kg, respectively). The rat’s body temperature was maintained at 36 ± 1°C with an incandescent lamp placed over the abdomen. The trachea was cannulated, and the animals were artificially ventilated (rate 40 strokes/min, stroke volume 10 ml/kg body weight). The right jugular vein was cannulated for drug administration; the left carotid artery was cannulated with a cannula containing heparinised saline (50 U/mL) and connected to a pressure transducer (MLT844 ADInstruments, Australia) for continuous monitoring of arterial BP. The computerized Power Lab (ADInstruments, v 5.4.2) data acquisition system was used. After surgery, the arterial BP was allowed to stabilise for about 20 min. The mean arterial BP (MABP) and the heart rate (HR) were recorded before administration of graded doses of the drugs. Software was used to calculate the MABP, which was defined as two-thirds of the diastolic pressure plus one-third of the systolic pressure. The effect of three different doses of compound A (0.25, 0.5 and 1 mg/kg), three doses of losartan (1, 2 and 4 mg/kg) and a negative control (all administered intravenously) on the MABP and HR were evaluated in different groups of animals. The interval between the injections was usually 10 min after the MABP had returned to control values. Each dose was injected in a bolus of 0.1 ml.\(^{20}\)

**Statistical analysis**

Changes in the MABP were evaluated as the difference from the basal value. The results are expressed as the mean ± SEM. They were analysed by a one-way analysis of variance (ANOVA), followed by the Tukey–Kramer test. In some cases, when it was appropriate, a Student’s \(t\)-test was used. A value of \(P<0.05\) was considered statistically significant.

**Results and Discussion**

**Effects of compound A on MABP**

As shown in Figure 2, the injection of compound A dose-dependently reduced the MABP (e.g. an injection of 1 mg/kg of the compound A caused a 67.2 ± 2.2 mmHg reduction in the MABP).

**Effect of losartan on MABP**

As shown in Figure 2, the injection of losartan dose-dependently reduced the MABP (e.g. an injection of 4 mg/kg of compound A caused a 69.3±2.9 mmHg reduction in the MABP).

**Effect of the treatments on HR**

Although compound A and losartan resulted in an increase in the HR, the change was not statistically significant (figure 3).

**Discussion**

In our previous in vitro study,\(^{19}\) the potency of compound A was approximately 1000 to 1 compared to...
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that of losartan. In the present study, this ratio was 4 to 1. This difference could be related to enzymatic degradation of the compound in the plasma or liver. Although determination of the mechanism of the hypotensive effect of compound A was not the aim of this study, we can conclude from the decrease in the MABP that it could affect both cardiac and vascular activity. The data in the present study on the HR of the animals support this idea, with no significant reflex tachycardia observed.

In comparison to losartan, tetrazolyl was replaced with an isoester carboxylic acid in compound A. In addition, in compound A, a hydrophobic dihydropyridine moiety was substituted at the 5 position of the imidazole ring. AT1 has Ca2+ channel blocking activity and blood-lowering effects. The enhanced potency of compound A observed herein may be due to tighter bonding to AT1 because of the presence of the hydrophobic dihydropyridine moiety in compound A.

**Conclusion**

The present results show that a dihydropyridine analogue (compound A) reduced the MABP in rats. This effect was immediate upon intravenous administration of compound A. Compared to losartan, compound A seemed more potent in reducing BP, with 1 mg/kg of compound A almost equipotent to 4 mg/kg of losartan. In a previous study, we demonstrated the dual Ca2+ channel blocking and AT1 antagonist activity of this newly synthesized compound.19 The higher potency of compound A may be related to this dual activity, which can induce an additive or synergic effect.

**Acknowledgment**

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences for financial support. This work is part of a Pharm.D M.A. thesis.

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