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Hypotensive Effect of a Novel Dihydropyridine with Dual Calcium Channel Blocking and Angiotensin II Antagonistic Properties in a Rat Model

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ABSTRACT

Background: In our previous work, we synthesized a novel analogue of losartan (Compound A) with dual calcium (Ca^{2+}) channel blocking and antagonism of angiotensin II (ANG II) type 1 receptor (AT_1) 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.[1, 3] 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 (AT_1) remains unclear.[2, 4, 6]

Typically, the Ca^{2+} response is characterised by a sharp transient rise in intracellular (Ca^{2+})i, followed by a fall towards a sustained plateau above baseline.[2] Although the sustained increases in (Ca^{2+})i are thought to be mediated through Ca^{2+} influx, the early peak response is generally considered to be due to mobilisation of intracellular stores.[3, 6, 8] AT_1 receptor-coupled G proteins activate PLC, which, in turn, activates IP_3 and DAG, leading to the release of Ca^{2+} from the sarcoplasmic reticulum.[6, 9] It has been suggested that increased (Ca^{2+})i activates chloride channels, causing an efflux of chloride and subsequent depolarisation of the cell membrane, leading to opening of voltage-gated Ca^{2+} channels.[5, 10, 14] L-type Ca^{2+} channel blockers prevent constriction of afferent arterioles and also reduce sustained (Ca^{2+})i increases.[5, 9, 15, 16]

Losartan (Dup-753) (Fig. 1) is a nonpeptide angiotensin II receptor (type AT_1) antagonist discovered by Duncia et al. in 1990, and its potassium salt (cozaar) has been marketed as an antihypertensive since 1995.[17] 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 system by a methylene 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.[18]

In our recently published work,[19] we synthesised a novel analogue of losartan in which a biphenyl fragment was retained and the imidazole nucleus was

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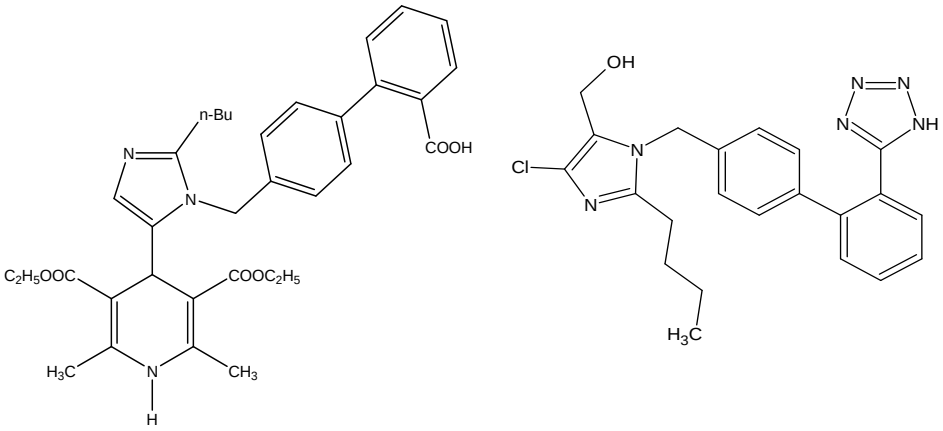
connected to a dihydropyridine moiety (figure 1). We showed dual Ca^{2+} channel blocking and AT_1 antagonist activity for the synthesised compound. In this study, the effects of this compound (compound A) on the blood pressure (BP) of normotensive rats were investigated and compared to losartan as a positive control.



1 2

Figure 1. Structure of compound A(1) and losartan (2)

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

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. AT_1 has Ca^{2+} channel blocking activity and blood-lowering effects. The enhanced potency of compound A observed herein may be due to tighter bonding to AT_1 because of the presence of the hydrophobic dihydropyridine moiety in compound A.

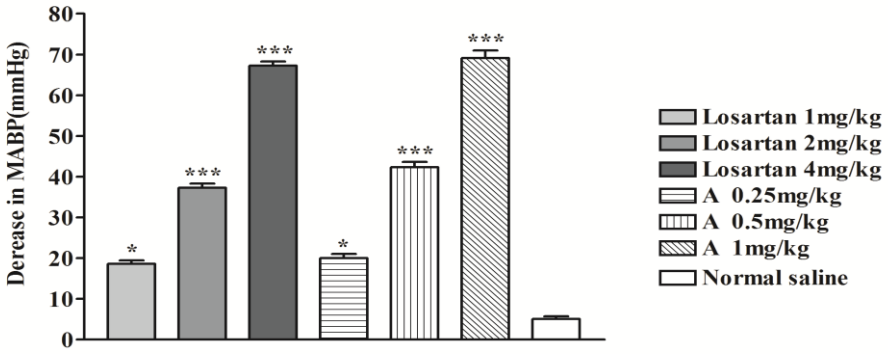


Figure 2. Decrease in mean arterial blood pressure (MABP) in response to various doses of compound A and losartan in rats. The control group received an equivalent volume of vehicle. Each value is the mean+S.E.M. of five experiments. A one-way ANOVA showed a significant difference between all doses of losartan and compound A compared with the vehicle group. ** *P*<0.01, ****P*<0.001.

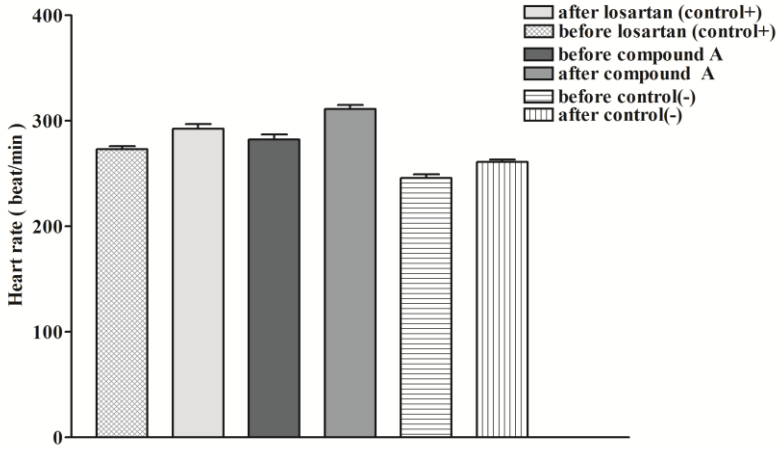


Figure 3. Heart rate (beats per minute) in response to various doses of compound A and losartan in rats. The control group received an equivalent volume of vehicle. Each value is the mean±S.E.M. of five experiments. A *t*-test was used to compare between all the tested groups before and after the injection. There was no significant difference between the groups (*P*>0.05).

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 Ca^{2+} channel blocking and AT_1 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.

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References

1. Arendshorst WJ, Brännström K, Ruan X. Actions of

angiotensin II on the renal microvasculature. *J Am Soc Nephrol* 1999;10:S149.
2. Inscho EW, Mason MJ, Schroeder AC, Deichmann PC, Stiegler KD, Imig JD. Agonist-induced calcium regulation in freshly isolated renal microvascular smooth muscle cells. *J Am Soc Nephrol* 1997;8:569-79.
3. Iversen BM, Arendshorst WJ. ANG II and vasopressin stimulate calcium entry in dispersed smooth muscle cells of preglomerular arterioles. *Am J Physiol Renal Physiol* 1998;274:F498-F508.
4. Conger JD, Falk SA, Robinette JB. Angiotensin II-induced changes in smooth muscle calcium in rat renal arterioles. *J Am Soc Nephrol* 1993;3:1792-803.
5. Ruan X, Arendshorst W. Calcium entry and mobilization signaling pathways in ANG II-induced renal vasoconstriction in vivo. *Am J Physiol Renal Physiol* 1996;270:F398-F405.
6. Salomonsson M, Sorensen C, Arendshorst W, Steendahl J, Holstein-Rathlou NH. Calcium handling in afferent arterioles. *Acta Physiol Scand* 2004;181:421-9.
7. Iversen BM, Arendshorst WJ. AT1 calcium signaling in renal vascular smooth muscle cells. *J Am Soc Nephrol* 1999;10:S84.
8. Loutzenhiser K, Loutzenhiser R. Angiotensin II–Induced Ca2+ Influx in Renal Afferent and Efferent Arterioles Differing Roles of Voltage-Gated and Store-Operated Ca2+ Entry. *Circ Res* 2000;87:551-7.
9. Navar L, Inscho E, Majid S, Imig J, Harrison-Bernard L, Mitchell K. Paracrine regulation of the renal microcirculation. *Physiol Rev* 1996;76:425-536.
10. Carmines PK. Segment-specific effect of chloride channel blockade on rat renal arteriolar contractile responses to angiotensin II. *Am J Hypertens* 1995;8:90-4.
11. Jensen BL, Skott O. Blockade of chloride channels by DIDS stimulates renin release and inhibits contraction of afferent arterioles. *Am J Physiol Renal Physiol* 1996;270:F718-F27.
12. Large WA, Wang Q. Characteristics and physiological role of the Ca (2+)-activated Cl- conductance in smooth muscle. *Am J Physiol Cell Physiol* 1996;271:C435-C54.
13. Steendahl J, Holstein-Rathlou N-H, Sorensen CM, Salomonsson M. Effects of chloride channel blockers on rat renal vascular responses to angiotensin II and norepinephrine. *Am J Physiol Renal Physiol* 2004;286:F323-F30.
14. Takenaka T, Kanno Y, Kitamura Y, Hayashi K, Suzuki H, Saruta T. Role of chloride channels in afferent arteriolar constriction. *Kidney Int* 1996;50:864-72.
15. Carmines PK, Navar LG. Disparate effects of Ca channel blockade on afferent and efferent arteriolar responses to ANG II. *Am J Physiol Renal Physiol* 1989;256:F1015-F20.
16. Takenaka T, Suzuki H, Okada H, Inoue T, Kanno Y, Ozawa Y, et al. Transient receptor potential channels in rat renal microcirculation: actions of angiotensin II. *Kidney Int* 2002;62:558-65.
17. Wang JW, Jia J, Li HM, Wang C. Synthesis of novel isoxazole-contained analogues of Losartan. *Chin Chem Lett* 2000;11:961-2.
18. Rapposelli S, Cuboni S, Digiacomo M, Lapucci A, Trincavelli ML, Tuccinardi T, et al. Synthesis and AT1 affinity evaluation of benzamidophenyl analogs of known AT1 receptor ligands with similar aromatic skeleton. *Arkivoc* 2008;2:268-86.
19. Hadizadeh F, Imen SM, Esmaeili P, Taghiabadi M. Synthesis and effects of novel dihydropyridines as dual calcium channel blocker and angiotensin antagonist on isolated rat aorta. *Iran J Basic Med Sci* 2010;13:195-201.
20. Imenshahidi M, Hosseinzadeh H, Javadpour Y. Hypotensive effect of aqueous saffron extract (Crocus sativus L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats. *Phytother Res* 2010;24:990-4.