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Original article

Protective effects of MCR-1329, a dual α_1 and angII receptor antagonist, in mineralocorticoid-induced hypertension

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ABSTRACT

Background: With the prototypical structures of losartan and prazosin as the axis of our research, MCR-1329 emerged as a potential designed multiple ligand from a series of compounds designed to possess dual antagonistic activity on the α_1 and AT_1 receptor. After confirming the activity of MCR-1329 in *in vitro* and acute *in vivo* models, the present study was undertaken to determine the efficacy of MCR-1329 in a mammalian test system.

Methods: A rat model of deoxycorticosterone acetate (DOCA)-salt induced renal hypertension following unilateral nephrectomy was utilized to determine the effect of MCR-1329. For mechanistic evaluations, MCR-1329 was evaluated on rat aortic strips *in vitro* and on rat aortic smooth muscle cells to determine the role of MCR-1329 on phosphoinositide 3 kinase (PI_3K) signaling.

Results: Results of the study showed that MCR-1329 prevents development of arterial hypertension. It was also observed that MCR-1329 upheld the intimal structures of major arteries like the thoracic aorta. Acetylcholine (Ach)-mediated relaxation remained intact in arteries from MCR-1329 treated animals. It was observed that MCR-1329 partially prevents Thr-308 phosphorylation of Akt following ligand-mediated receptor stimulation in vascular smooth muscle cells. Addition of LY294002 to the reaction medium caused a near-complete inhibition of Akt-phosphorylation. This suggested that MCR-1329 elicits its antihypertensive role by blocking activation of receptor-mediated PI_3K–Akt downstream signaling as well as through preservation of arterial integrity.

Conclusions: MCR-1329 has the potential to be evaluated further for clinical development as a potential antihypertensive agent with multiple mechanisms of action.

Introduction

Lifestyle modifications and dietary approaches to stop hypertension (DASH) [1] are seldom effective in controlling blood pressure in hypertensive populations owing to non-compliance. Several agencies have drafted guidelines for the use of drugs in the management of hypertension and these guidelines are reinforced by data from landmark trials like the ALLHAT, UKPDS, INVEST and the HOPE trial among others [2–5]. Practising physicians have adopted the use of multidrug therapy [6] since the past couple of decades to manage hypertension with multifactorial molecular etiologies.

Cross-talks between angII and α_1 adrenoceptors have been studied in the past [7]. It has been demonstrated that increased sensitivity to angII-mediated vasoconstriction may be attributed, in part, to potentiation of α_1 adrenoceptor-mediated vasoconstriction by angII [8]. Vittorio and colleagues retrospectively reviewed the interactions observed between the adrenergic system and renin angiotensin aldosterone system (RAAS) in major clinical trials like Val-HeFT and CHARM-added [9], suggesting that α_1 adrenoceptor and AT_1 receptor cross talk occurs at two levels: at the molecular receptor and the second messenger levels.

Different researchers have already shown the role of $PI_3K\gamma$ in increased smooth muscle contractility mediated through angII [10]. Thus, $PI_3K\gamma$ mediates the downstream signaling effects of the AT_1 receptors, specifically with vasoconstriction as the end-result. Akt stands as a link between $PI_3K\gamma$ and L-type calcium channels (LTCC) in resistance arteries, regulating the increased myogenic tone upon stimulation [11]. Patel and co-workers have shown that even in the absence of angII, pressure exerted on the arterial walls may activate AT_1R and lead to stimulation of $PI_3K\gamma$ through $\beta\gamma$

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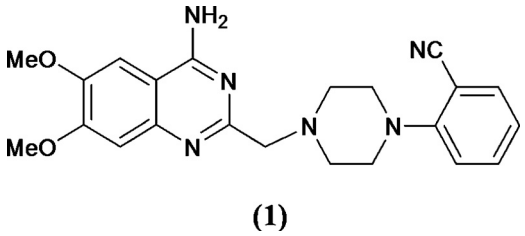

(1)

Fig. 1. Chemical structure of MCR-1329.

signaling [12]. Inhibition of kinase-dependent signaling was found to markedly impair the myogenic tone in isolated vessels [10,13]. Inhibition of $PI_3K\gamma$ signaling impairs accessory subunit ($Ca_v\beta2a$) phosphorylation and thus decreases the probability of opening of LTCC [14]. Yamboliev and colleagues were able to show that norepinephrine leads to an increase in phosphorylated PI_3K and Akt upon adrenergic stimulation [15]. Budzyn and colleagues also reported that wortmannin, a PI_3K inhibitor, attenuates contractile responses to phenylephrine in arterial preparations [16].

The present study was undertaken with the purpose of studying the effect of MCR-1329 (Fig. 1) *in vivo* in an animal model of hypertension, along with its role in affecting the PI_3K–Akt pathway. MCR-1329 belongs to a series of 6,7-dimethoxyquinazolines based on the structural modifications involving prazosin and losartan [17,18]. MCR-1329 and compounds of the related series were designed to show balanced modulation of α_1 and angII receptors, and thus regulate cross-talks. We have previously shown that MCR-1329 affects the contractile process of large blood vessels by acting as a competitive antagonist to phenylephrine and angII [17]. In this paper, we have tried to evaluate the effect of MCR-1329 in a rodent model of deoxycorticosterone acetate (DOCA)-salt induced hypertension.

Materials and methods

Materials

MCR-1329 was synthesized by the Pharmaceutical Chemistry Lab of Faculty of Pharmacy, The M.S. University of Baroda. Rat aortic smooth muscle cell line, A10 clone, was procured from National Centre for Cell Sciences (NCCS), Pune, India. Phenylephrine hydrochloride, angiotensin II, prazosin, acetylcholine chloride, DOCA and bovine serum albumin were procured from Sigma–Aldrich, St. Louis, MO, USA. Losartan potassium was a kind gift from Torrent Pharmaceuticals Ltd., Gujarat, India. Dulbecco's minimum essential medium (DMEM, High Glucose), trypsin: ethylenediamine tetraacetic acid (EDTA) solution, fetal bovine serum (Australian donor herd origin), antibiotic–antimycotic solution, tissue culture flasks (T-25 and T-75), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and lyophilized phosphate buffered saline (PBS, pH 7.2) were procured from HiMedia, Mumbai, India. 1° antibody directed against Thr-308 phosphorylated site of Akt (rabbit origin; Cat# sc-16646-R) was procured from Santa Cruz Biotechnology, Inc., USA and 2° Alexa Fluor 594 conjugated goat anti-rabbit antibody was purchased from Life Technologies, USA. Heparin, ketamine, diazepam and tramadol were procured locally. Diagnostic kits for clinical chemistry were purchased form Crest Biosystems, India.

Animals

Animals were procured from licensed animal breeders. Animals were housed in an air-conditioned room (23 ± 2 °C, 50–65% RH) in polycarbonate cages with paddy husk (Shree Dutt Agro Pvt. Ltd., Vadodara) as bedding with 12 hr light–12 hr dark cycles. They had free access to pelleted diet (Pranav Agro Foods Pvt. Ltd., Pune, India) and tap water unless mentioned otherwise. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) *vide* protocol no. MSU/PHARM/IAEC/2013/01. All experimental procedures were carried out in accordance to the guidelines provided by CPCSEA (committee for the purpose of control and supervision of experiments on animals), Ministry of Environment, Forests and Climate Change, Govt. of India, New Delhi, India.

Study design

Adult male Wistar rats (20–24 weeks; 250–350 g) were used for the study. Left unilateral nephrectomy (UNX) was performed in all the animals and accordingly left kidney was surgically removed under anesthesia (ketamine 100 mg/kg, *ip*; diazepam 5 mg/kg, *im*). After surgery, the animals were allowed to recover for two weeks. Animals were trained for recording tail-cuff pressures during the recovery period. Animals in the negative control group were not subjected to unilateral nephrectomy. At the end of the recovery period, animals were randomized into the following groups as shown in Table 1.

All treatments were administered daily (*po*) for four weeks. Non-invasive tail-cuff systolic pressures were measured every week. On the penultimate day of the study, animals were placed in metabolic cages for collection of urine over a 24-h period. At the terminal day of the study, tail-cuff pressures were recorded in all animals followed by invasive recording of arterial blood pressure. Blood samples were withdrawn before sacrificing the animals, after which kidneys were removed for evaluating extracellular matrix deposition and aorta were isolated for studying endothelial dysfunction.

Evaluation of parameters

Systolic blood pressure was measured by invasive and non-invasive techniques as described previously [17]. Blood samples

Table 1
Grouping of animals.

Group no.	Group	Description	Treatment	Number of animals
1	Negative control	Vehicle treatment (no surgery)	0.5% Sodium CMC	6
2	Positive control	UNX + DOCA/salt	0.5% Sodium CMC	6
3	UNX control	UNX only	0.5% Sodium CMC	6
4	Treatment group	UNX + DOCA/salt + MCR-1329	MCR-1329 (10 mg/kg) in 0.5% sodium CMC	6
5	Standard group	UNX + DOCA/salt + prazosin + losartan	Prazosin (5 mg/kg) and losartan (5 mg/kg) in 0.5% sodium CMC	6

At the end of the recovery period, animals in Groups 2, 4 and 5 were switched to 1% NaCl and 0.2% KCl in drinking water and were given injections of DOCA in olive oil (25 mg/kg, *sc*, twice a week) up to four weeks.