

# Potential Beneficial Additive Effect of 1-alpha- Hydroxyvitamin D<sub>3</sub> to Telmisartan on L/NAME Induced Hypertension and Left Ventricular Abnormalities in Rats

#### A Chesis

Submitted for Partial Fulfillment of Master Degree in Pharmacology

### By

#### Nada Kotb Abd Elfatah Kotb

M.B., B.Ch. (2007), Demonstrator in Department of Pharmacology & Therapeutics Faculty of Medicine, Ain Shams University

### Supervised by

#### **Assist. Prof. Ahmed Nour Eldin Hassan**

Assistant Prof. of Pharmacology, Department of Pharmacology & Therapeutics Faculty of Medicine, Ain Shams University

### Dr. Hala Salah Abdel-kawy

Assistant Prof. of Pharmacology, Department of Pharmacology & Therapeutics Faculty of Medicine, Ain Shams University

### Dr. Naglaa Samir Ahmed

Prof. of Pathology, Department of Pathology Faculty of Medicine, Ain Shams University

> Faculty of Medicine Ain Shams University 2013

# بِسْمِ اللَّهِ الرّحَمَٰنِ الرّحيمِ

الِبْتَى اِبِمِهِي عَالِيٍّ فَ عِلَى فَالْحِيِّ [ ... أَنِهُ اِهُوَ عَلَيْ اَلَى اَسِحِرَ نِمَمَلُكِ

الْ يَمْفِرُهِ فِيْ مِنْافِهِ الصَّالِكِيالِ الصَّالِكِيالِ المَّالِمُ الْمِهُ الْمِنْاهِ فَي مُنَامِهُ الْمِنْاهِ فَي مُنْامِّهُ الْمِنْاهِ فَي الْمُنْاءِ فَي الْمُنْاءِ

صدق الله العظيم



First of all, all gratitude is due to God almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.

Really I can hardly find the words to express my gratitude to Ass. Prof. Dr. Ahmed Nour Eldin Hassan Assistant Professor of Pharmacology and Therapeutics, faculty of medicine, Ain Shams University, for her supervision, continuous help, encouragement throughout this work and tremendous effort she has done in the meticulous revision of the whole work. It is a great honor to work under her guidance and supervision.

I would like also to express my sincere appreciation and gratitude to Dr. Hala Salah Abdel Kawy Assistant Professor of Pharmacology and Therapeutics, faculty of medicine, Ain Shams University, for her continuous directions and support throughout the whole work.

Sincere appreciation to Dr. Naglaa Samir Ahmed Professor of pathology, Faculty of Medicine, Ain Shams University for her valuable help in histopathological diagnosis.

Last but not least, I dedicate this work to my family, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.



# **List of Contents**

Subject	Page No.
List of Abbreviations	i
List of Tables	iii
List of Figures	v
Abstract	vii
Introduction	1
Aim of the Work	4
Review of Literature	6
Materials and Methods	22
Results	32
Discussion	72
Summary and Conclusion	85
References	91
Arabic Summary	

#### **List of Abbreviations**

1-alpha-  $(OH) D_3$  : One-alpha- hydroxyvitamin  $D_3$ ACE : Angiotensin converting enzyme

ACEIs : Angiotensin converting enzyme

inhibitors

Ang I : Angiotensin I Ang II : Angiotensin II

ARBs: Angiotensin receptor blockers $AT_1$ : Angiotensin type 1 receptorATIA: Angiotensin receptor type 1A $AT_2$ : Angiotensin type 2 receptor $AT_3$ : Angiotensin type 3 receptors $AT_4$ : Angiotensin type 4 receptor

**BP** : Blood pressure

CBP : CREB binding proteinCKD : Chronic kidney diseasesCRE : cAMP responsive element

**CREB** : cAMP responsive element binding

protein

**CV** : Cardiovascular

CVD : Cardiovascular diseaseDMSO : Dimethylsulphoxide

 $EC_{50}$ : Mean effective concentration 50

ECM : Extracellular matrixEDV : Endothelial vessel

*eGFR* : Estimated glomerular filtration rate

 $E_{max}$ : Maximal contractile response

FGF : Fibroblast growth factor

FGF-23 : Fibroblast growth factor-23

### List of Abbreviations (Cont...)

 $FGFR_1$ : FGF receptor

*H&E* : Hematoxylin-eosin

*i.p* : Intraperitoneal

*L/NAME* : L-nitro arginine methyl ester

LV : Left ventricle

*LVH* : left ventricular hypertrophy

MAPs : Mean arterial pressures

*MMPs* : Matrix metalloproteinases

**PRR** : Prorenin receptor

PTH : Parathyroid hormonePTH : Parathyroid hormone

**RAAS**: Renin angiotensin aldosterone system

**RAS**: Renin-angiotensin system

SHPT : Secondary hyperparathyroidismTACE : TNFalpha converting enzyme

*TIMPs* : Tissue inhibitors of metalloproteinases

*VDR* : Vitamin D receptor

*VDRA* : Vitamin D receptors agonist

*VDRE* : Vitamin D receptor

VitD : Vitamin D

**VSM** : Vascular smooth muscle

VW/BW : Ventricular weight/Bodyweight

**WKY** : Wistar - kyoto

α1-ARS : Alpha 1-adrenergic receptors

# **List of Tables**

Table No.	Title	Page S	No.
<b>Table (1):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in compart to telmisartan in a dose of (10 mg /kg/day) 6 weeks on systolic blood pressure in NAME treated rats	rtan ison for L-	35
<b>Table (2):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in compart to telmisartan in a dose of (10 mg /kg/day) 6 weeks on ventricular weight / body we ratio (mg/g) in L-NAME treated rats	rtan ison for ight	42
<b>Table (3):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in compart to telmisartan in a dose of (10 mg /kg/day) 6 weeks on EC <sub>50</sub> of phenylephrine inducontraction in endothelium intact rat's acrings in L-NAME treated rats	rtan ison for iced ortic	49
<b>Table (4):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa ( $10 \text{mg/kg/day}$ ) or ( $5 \text{mg/kg/day}$ ) in compart to telmisartan in a dose of ( $10 \text{ mg/kg/day}$ ) 6 weeks on $E_{\text{max}}$ of phenylephrine inducontraction in endothelium intact rat's acrings in L-NAME treated rats	rtan ison for iced ortic	50
<b>Table (5):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in compart to telmisartan in a dose of (10 mg /kg/day) 6 weeks on acetylcholine induced relaxation endothelium intact rat's aortic rings in NAME treated rats	rtan ison for n in L-	51

# List of Tables (Cont...)

Table No.	Title	Page No.
<b>Table (6):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in compar to telmisartan in a dose of (10 mg /kg/day) 6 weeks on myocardial damage score in NAME treated rats	nrtan ison ) for 1 L-
<b>Table (7):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in comparto telmisartan in a dose of (10 mg/kg/day) 6 weeks on cardiomyocyte diameter in NAME treated rats	ortan ison ) for 1 L-
<b>Table (8):</b>	Effects of treatment with 1-alpha- (OH) either alone or in combination with telmisa (10mg/kg/day) or (5mg/kg/day) in compar to telmisartan in a dose of (10 mg /kg/day) 6 weeks on aortic media thickness in NAME treated rats	ortan ison ) for 1 L-

# **List of Figures**

Figure N	o. Title Pa <sub>l</sub>	ge No.
Figure (1):	Pathway of vitamin D metabolism and it relationship with PTH and renin-angiotensi system	n
Figure (2):	Cross talk between vitamin D, FGF-23-Klotho and the RAS in healthy subjects and patients with chronic kidney disease	h
<b>Figure (3):</b>	The pathophysiological sequence of vitamin last supplement - FGF-23 excess - cardiovasculas susceptibility in CKD	ar
Figure (4):	Conceptual model of major pathways throug which vitamin D deficiency may lead t cardiovascular disease	0
<b>Figure (5):</b>	The amplifier, tail cuff, pulse transducer an animal restraint cage	
<b>Figure (6):</b>	Typical recording using Lab Chart showing pressure signals	
Figure (7):	Effects of treatment with 1-alpha- (OH) D <sub>3</sub> either alone or in combination with telmisarta (10mg/kg/day) or (5mg/kg/day) in comparison to telmisartan in a dose of (10 mg /kg/day) for weeks on systolic blood pressure in L-NAM treated rats	n co 6 E
Figure (8):	Effects of treatment with 1-alpha- (OH) D <sub>3</sub> either alone or in combination with telmisarta (10mg/kg/day) or (5mg/kg/day) in comparison to telmisartan in a dose of (10 mg/kg/day) for weeks on ventricle weight/ body weight in L NAME treated rats.	n co 6

# List of Figures (Cont...)

Figure No	o. Title	Page No.
Figure (9):	Effects of treatment with 1-alpha- (OH) D <sub>3</sub> alone or in combination with telm (10mg/kg/day) or (5mg/kg/day) in compari telmisartan in a dose of (10 mg /kg/day) for 6 on endothelium intact rat's aortic rings in L-1 treated rats.	isartan son to weeks NAME
<b>Figure (10):</b>	Cumulative response curves for phenyle (10-7 M-10-4M) on rat's aortic rings	
<b>Figure (11):</b>	Endothelial-dependent relaxation induce acetylcholine (10-6 M-0.5×10-2 M) in is perfused rat's aortic rings precontracted phenylephrine (0.5×10-4M-10-6 M)	solated ed by
<b>Figure (12):</b>	Effects of treatment with 1-alpha- (OH) D <sub>3</sub> ealone or in combination with telmis (10mg/kg/day) or (5mg/kg/day) in comparison telmisartan in a dose of (10 mg/kg/day) for 6 where on myocardial damage score in L-NAME treats	artan on to zeeks eated
<b>Figure (13):</b>	Effects of treatment with 1-alpha- (OH) D <sub>3</sub> eith or in combination with telmisartan (10mg/kg (5mg/kg/day) in comparison to telmisartan in a (10 mg /kg/day) for 6 weeks on cardio diameter in L-NAME treated rats	/day) or a dose of myocyte
<b>Figure (14):</b>	Photomicrographs of sections in rat's left ve (H&E)	
<b>Figure (15):</b>	Effects of treatment with 1-alpha- (OH) D <sub>3</sub> alone or in combination with telm (10mg/kg/day) or (5mg/kg/day) in compartelmisartan in a dose of (10 mg/kg/day) for on aortic media thickness in L-NAME treated to	nisartan ison to 5 weeks
<b>Figure</b> (16):	Photomicrographs of sections in rat's thosa orta (H&E)	

#### **ABSTRACT**

BACKGROUND&AIM: Pharmacological therapies based on the renin angiotensin system (RAS) blockade are used extensively for the treatment of hypertension, heart failure, and cardiovascular remodeling, however in spite of their success the prevalence of end-organ damage and residual risk remain still high. Incomplete blockade can occur when treating patients with angiotensin receptor blockers (ARBs). Moreover, high levels of renin and angiotensin I (Ang I) are detectable due to the absence of a negative feedback loop during treatment with ARBs, and this can lead to the direct pathological effects of renin. A lack of vitamin D has been associated with a poor cardiovascular outcome, poor control of blood pressure. The use of vitamin D analogues may provide a survival advantage in dialysis patients and preclinical and clinical data indicate that vitamin D analogues have additional renoprotective effects in addition to RAAS blockade. The present study was designed to examine whether vitamin D can increase the efficacy of telmisartan as regard cardiovascular effects.

**METHOD:** Seven groups of rats were used (7rats each and 11 in L/NAME group). Group (1): is further classified into 2 subgroups; 7 rats each; Control naïve group: received untreated chow and drinking water. Control vehicle group: received 1 ml DMSO. Group (2): is further classified into 5 subgroups; 7 rats each but 11 rats in control group; Control group: Will receive L/NAME 40 mg/kg dissolved in distilled water by gavage. The L/NAME + telmisartan group: receive L/NAME + telmisartan 10 mg/kg by gavage (dissolved in DMSO: 2 mg/ml). The L/NAME + 1-alpha-(OH)D<sub>3</sub>group: receive L/NAME +0.06 ug/kg of 1-alpha-(OH)D<sub>3</sub> intraperitoneal injection (i.p). The L/NAME + telmisartan (10mg/kg) + 1alpha-(OH)D<sub>3</sub>group: receive L/NAME + telmisartan 10 mg/kg + 1-alpha-(OH)D<sub>3</sub>.The L/NAME + telmisartan (5mg/kg) + 1-alpha-(OH)D<sub>3</sub>group: receive L/NAME + telmisartan 5 mg/kg + 1-alpha-(OH)D<sub>3</sub>.All the substances were given for 6 weeks daily except 1-alpha-(OH)D<sub>3</sub> was given every other day. Systolic blood pressure, ventricular weight/body weight (VW/BW), mortality rate, aortic endothelial function and histopathological examination of heart and aorta were measured.

**Results:** L/NAME treated rats induced significant increase in Systolic blood pressure, VW/BW and mortality rate. Also there were significant endothelial dysfunction, cardiomyocyte hypertrophy and thickened aortic media. While telmisartan, 1-alpha-(OH)D<sub>3</sub>, 1-alpha-(OH)D<sub>3</sub> +telmisartan (10mg/kg/d) and

Abstract

1-alpha-(OH) $D_3$  +telmisartan (5mg/kg/d) groups significantly attenuate these changes, but the combination (1-alpha-(OH) $D_3$ +telmisartan (5mg/kg/d)) was the most significant in reducing systolic blood pressure, VW/BW induced by L/NAME treated rats. This combination also improves endothelial dysfunction and histopathological examination of heart and aorta.

**Conclusions:** These results suggest that there is synergestic effect of the combination between 1-alpha- $(OH)D_3$  and telmisartan in reducing cardiac hypertrophy and improving vascular reactivity in aortic rings. The most prominent effect was recorded with combination of 1-alpha  $(OH)D_3$ +telmisartan in subtherapeutic dose.

## Introduction

In the profession is one of the most common worldwide diseases affecting humans. Because of the associated morbidity, mortality and the cost to society, hypertension is an important public health challenge. Hypertension is the most important modifiable risk factor for coronary heart diseases, stroke (the third leading cause of death), congestive heart failure, end-stage renal disease, and peripheral vascular disease. Therefore, health care professionals must not only identify and treat patients with hypertension but also promote a healthy lifestyle and preventive strategies to decrease the prevalence of hypertension in the general population (Albert et al., 2010). Vascular calcifications and left ventricular hypertrophy (LVH) are common findings in hypertensive patient that increase the incidence of cardiac-related deaths (Valdivelso and Ayus, 2008).

Pharmacological therapies based on the renin angiotensin system (RAS) blockade are used extensively for the treatment of hypertension, heart failure, and cardiovascular remodeling, however in spite of their success the prevalence of end-organ damage and residual risk remain still high (*Ocaranza and Jalil, 2012*). However, incomplete blockade can occur when treating patients with angiotensin receptor blockers (ARBs) (*Brewster et al., 2003*). High levels of renin and angiotensin I (Ang I) are detectable due to the absence of a negative feedback loop during

treatment with ARBs, and this can lead to the direct pathological effects of renin (*Hollenberg*, 2010) through binding to its prorenin receptor (PRR) (*Nguyen et al.*, 2002), along with the more classical consequences of Ang I and II production (*Francois et al.*, 2011).

A lack of vitamin D has been associated with a poor cardiovascular outcome, poor control of blood pressure (*Gal-Moscovici and Sprague*, 2010). The use of vitamin D analogues may provide a survival advantage in dialysis patients and preclinical and clinical data indicate that vitamin D analogues have additional renoprotective effects in addition to RAS blockade (*Zhang et al.*, 2010). Therefore, lack of vitamin D should be avoided in CKD patients, as they have a high cardiovascular risk (*Szeto et al.*, 2008).

The first clinical studies suggesting inverse relationship between calcitriol and renin levels were published two decades ago (Burgess et al., 1990) and were recently confirmed in a large cohort study (Tomaschitz et al., 2010). Vitamin D deficiency, defined as calcidiol levels below 15 ng/ml, associates with reduced renal plasma flow responses to infused angiotensin II, suggesting endogenous intrarenal RAS activation in vitamin D deficient subjects (Forman et al., 2010) and intervention with calcitriol decreases plasma renin and angiotensin II levels in hemodialysis patients with secondary hyperparathyroidism (*Park et al.*, 1999).

There has been growing evidence for a role of vitamin D in extraskeletal health, including beneficial effects in the cardiovascular system (*Geleijnse*, 2011). Vitamin D inhibits renin expression and blocks the compensatory induction of renin associated with the use of renin-angiotensin system inhibitors (*Kong et al.*, 2010).

#### **Reasearch hypothesis:**

Inhibiting the action of angiotensin II (Ang II) by ARBs cause a compensatory increase in plasma renin activity due to interruption of the short negative feedback loop by which Ang II inhibits renin release (*Siragy and Carey, 2010*). This study is designed to examine whether vitamin D can increase the efficacy of ARBs and accordingly, the potential of vitamin D-based therapies to reduce dose of ARBs in treating cardiovascular diseases.