Potential Beneficial Additive Effect of 1-alpha- Hydroxyvitamin D_3 to Telmisartan on L/NAME Induced Hypertension and Left Ventricular Abnormalities in Rats

A Thesis

Submitted for Partial Fulfillment of Master Degree
in Pharmacology

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2013

بِسْمِ اللّهِ الرّحمَنِ الرّحيمِ

[...رَبِّ أَوزِعنِي أَن أَشكُرَ نِعمَتَكَ
الَّتِي أَنْعَمْتَ عَلَيَّ و عَلى والِدَيَّ
و أَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ و أَدْخِلْنِي
بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ]

صدق الله العظيم

Acknowledgement

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.

Nada Kotb Abd elfatah Kotb

List of Contents

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 receptor
AT1A	: 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 protein
CKD	: Chronic kidney diseases
CRE	: cAMP responsive element
CREB	: cAMP responsive element binding protein
CV	: Cardiovascular
CVD	: Cardiovascular disease
DMSO	: Dimethylsulphoxide
EC_{50}	: Mean effective concentration 50
ECM	: Extracellular matrix
EDV	: 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 hormone
PTH	: Parathyroid hormone
RAAS	: Renin angiotensin aldosterone system
RAS	: Renin-angiotensin system
SHPT	: Secondary hyperparathyroidism
TACE	: 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

List of Tables *(Cont...)*

List of Figures

List of Figures *(Cont...)*

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_3group: receive L/NAME +0.06 ug/kg of 1-alpha-(OH)D_3 intraperitoneal injection (i.p)**.**The L/NAME + telmisartan (10mg/kg) + 1-alpha-(OH)D_3group: receive L/NAME + telmisartan 10 mg/kg + 1-alpha-(OH)D_3.The L/NAME + telmisartan (5mg/kg) + 1-alpha-(OH)D_3group: receive L/NAME + telmisartan 5 mg/kg + 1-alpha-(OH)D_3.All the substances were given for 6 weeks daily except 1-alpha-(OH)D_3 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_3, 1-alpha-(OH)D_3 +telmisartan (10mg/kg/d) and

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

Hypertension 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 an 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.