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Original Research Article

Protective effect of the combination of Azilsartan Medoxomil and Chlorthalidone on doxorubicin-induced cardiomyopathy and nephropathy in wistar rats

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Abstract

Background: Hypertension is the leading cause of mortality for cardiovascular and renal diseases. Azilsartan medoxomil (AZL) available in fixed dose combination with chlorthalidone (CHL), is approved for patients with inadequate response to monotherapy with antihypertensives. Preclinical studies suggest pleiotropic effects of AZL. However, no studies have directly examined cardio-renal benefits of this combination. We investigated the effects of AZL/CHL combination on doxorubicin-induced cardiomyopathy and nephropathy in rats.

Aim & Objective: To induce cardiomyopathy and nephropathy in wistar rats by administering six equal intraperitoneal (I.p) injections (2.5 mg/kg) of doxorubicin over a period of 2 weeks (alternate days). and to investigate the effects of a combination of azilsartan medoxomil and chlorthalidone on doxorubicin induced cardiomyopathy and nephropathy in rats.

Materials and Methods: Doxorubicin (DOX) was administered to Wistar rats at a dose of 2.5mg/kg, i.p. in six injections for a period of two weeks. The effect of AZL 3mg/kg/d and its combination with CHL 2.5mg/kg/d, p.o. for 3 weeks, was studied on serum LDH, albumin, creatinine and lipid profile as well as myocardial and renal TBARS and GSH. The histology of cardiac and renal tissue was assessed by H.& E. staining. The combination was compared with that of AZL+Ramipril (RAM) 10mg/kg/d on DOX-induced cardiomyopathy and nephropathy.

Results: AZL/CHL and AZL/RAM significantly decreased the LDH, triglycerides, total & LDL cholesterol, creatinine and TBARS while increased the levels of albumin, HDL cholesterol, and GSH. DOX caused loss of myofibrils and vacuolization in the cardiac muscle, and hyaline casts with degenerative changes in the renal tubules. Whereas AZL/CHL revealed normal/less damaged myofibrils with no vacuolization, AZL/RAM revealed less damaged myofibrils with vacuolization. AZL/CHL showed normal glomerulus and tubular tissue while AZL/RAM revealed less damaged glomerulus and dilatation of tubular tissue.

Conclusion: The AZL/CHL combination was better than AZL/RAM and AZL alone in preventing doxorubicin-induced cardiomyopathy and nephropathy in rats.

Keywords: Doxorubicin (DOX), Azilsartan (AZL), Chlorthalidone (CHL), Hypertension, Chlorthalidone (CTDN)

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1. Introduction

The prevalence of uncontrolled hypertension remains unacceptable despite efforts to increase awareness, treatment, and control of blood pressure (BP). Population-based surveys indicate that only 66% of patients treated for hypertension achieve BP control of less than 140/90 mmHg.[1] Most hypertensive patients require two or more medications to adequately control BP.[2-5]

In December 2011, the US Food and Drug Administration (FDA) approved a new fixed-dose combination antihypertensive medication, for patients with an inadequate response to monotherapy or those in whom multiple drugs are required to achieve BP control. The new product combines an angiotensin receptor blocker (ARB), azilsartan medoxomil and a thiazide-type diuretic, chlorthalidone. Azilsartan medoxomil (AZL) is the only ARB approved in a fixed-dose tablet with chlorthalidone (CHL); all other ARB combinations include hydrochlorothiazide (HCTZ) as the diuretic component. Data from in vitro studies show that AZL has superior AT^1 binding affinity and slower dissociation than Olmesartan, telmisartan, valsartan, and irbesartan[10] and is characterized by a superior ability to control 24-hour systolic blood pressure (BP). Preclinical studies indicate that AZL may also have potentially beneficial effects on cellular mechanisms of cardiometabolic disease and insulin-sensitizing activity that could involve more than just blockade of AT^1 receptors

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and/or reduction in BP. However, the clinical relevance of these additional actions is unknown.[11] Takagi et al. report on the antithrombotic, antiproliferative, and potentially antifibrotic effects of AZL and its beneficial effects on insulin sensitivity and glucose metabolism.[12]

CHL is a thiazide-type diuretic that differs chemically from traditional thiazides (e.g. HCTZ) by its lack of a benzothiadiazine molecular structure. CHL is approximately 1.5-2 times as potent as HCTZ at equivalent doses and has a longer duration of action than HCTZ.[13] CHL seems to have pleiotropic effects beyond BP reduction and achieves significantly lower total & LDL cholesterol compared with HCTZ. A systematic review of randomized control trials revealed that CHL reduced cardiovascular events by 21% relative to HCTZ.[14]

The combination of AZL and CHL has demonstrated safety and efficacy in lowering BP in hypertensive patients to a greater degree than olmesartan medoxomil/hydrochlorothiazide and AZL/HCTZ. As a fixed-dose combination tablet, it offers several clinical advantages. However, no studies have directly examined cardiovascular morbidity and mortality benefits associated with this combination. Moreover, in randomized controlled trials although AZL /CHL reduced blood pressure to a greater extent than comparators; minor transient increases in serum creatinine without significant effects on potassium homeostasis were reported.[6,15]

2. Materials and Methods

2.1. Drugs and chemicals

Azilsartan medoxomil was obtained from Takeda Pharmaceuticals. Chlorthalidone was obtained from IPCA Ltd. Ramipril was obtained from Dr. Reddy's Laboratories. Doxorubicin was obtained from Dabur India Ltd. The kits for estimation of Total cholesterol, Triglycerides, LDL Cholesterol, HDL Cholesterol, Lactate Dehydrogenase, Albumin, and Creatinine were obtained from Span Diagnostics, Surat, India.

3. Animals and Experimental Design

The study was carried out on adult Male Wistar rats (250-300gm g) obtained from the Central Animal House Facility of Jamia Hamdard. The protocol was approved by the Institutional Animal Ethics Committee of Jamia Hamdard (IAEC/JH-1190). Animals were housed in polypropylene cages and allowed to acclimatize for one week with 12 h light/ dark cycle under controlled room temperature (23 ± 2°C) and relative humidity (60 ± 5%) and had free access to a standard commercial pellet diet (Nav Maharashtra Chakan Oil Mills Ltd, India).

3.1. Treatment schedule

Group's treatment

1. Control: 0.1 mL/100 g of normal saline i.p. (in six equal injections over a period of 2 weeks)
2. Doxorubicin (DOX) (Toxic control): 2.5 mg/kg, six equal i.p. injections, over a period of 2 weeks (alternate days)
3. Azilsartan (AZL)+Chlorthalidone (CHL)+ Doxorubicin (DOX)3mg/kg/day; p.o. in 0.5% carboxymethylcellulose for 3 weeks + 2.5mg/kg/day in water for 3 weeks +2.5 mg/kg, six equal i.p. injections over a period of 2 weeks (1st and 2nd weeks)
4. Azilsartan (AZL)+ Doxorubicin (DOX)3mg/kg/day; p.o in 0.5% carboxymethylcellulose for 3 weeks +2.5mg/kg, six equal i.p. injections over a period of 2 weeks (1st and 2nd weeks)
5. Azilsartan (AZL)+Ramipril (RAM)+Doxorubicin (DOX) 3mg/kg/day; p.o. in 0.5% carboxymethylcellulose for 3 weeks + 10 mg/kg/day in water +2.5mg/kg, six equal i.p. injection over a period of 2 weeks (1st and 2nd weeks)
6. Azilsartan (AZL) + Chlorthalidone (CHL) per se 3mg/kg/day; p.o. in 0.5% carboxymethylcellulose for 3 weeks + 2.5mg/kg/day in water; p.o. for 3 weeks.

The animals were weighed 24 h after the last dose, blood was collected from the retrobulbar plexus under light ether anesthesia and the serum was prepared for biochemical estimations of LDH, Lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides), albumin and creatinine. Animals were then euthanized using CO_2 and hearts and kidneys were removed, washed with the normal saline, and estimated for thiobarbituric acid reactive substance (TBARS), glutathione (GSH), and protein as parameters for lipid peroxidation. A section of cardiac and renal tissue was preserved in 10% formalin for subsequent H. & E. staining for histopathology.

3.2. Biochemical estimations in serum

3.2.1. LDH

The serum LDH activity was measured by kinetic method using a U.V spectrophotometer by measuring the oxidation of lactate to pyruvate which is accompanied by a simultaneous reduction of NAD+ to NADH. The LDH activity in serum is proportional to the increase in absorbance due to the reduction of NAD+ at 340 nm.

3.2.2. Lipid profile

The serum levels of total cholesterol, HDL cholesterol and triglycerides were estimated using colorimetric enzymatic assays as per the instructions of the manufacturers.[16] The Friedewald's equation was used for calculating LDL cholesterol: LDL Cholesterol = Total Cholesterol- (HDL Cholesterol+ triglycerides/5)

3.2.3. Albumin and creatinine

The albumin and creatinine were estimated by colorimetric assays using the Bromocresol Green Method and Jaffe's reaction respectively.[17,18]

4. Biochemical Estimation in Cardiac and Renal Tissue

4.1. TBARS

Lipid peroxidation was estimated by measuring the levels of TBARS that were measured as nanomoles of malondialdehyde in cardiac tissues by the method of Ohkawa et al. The pink chromogen produced by the reaction of the thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation, was estimated at 540 nm.[19]

4.2. GSH

GSH was estimated by the Ellman method, i.e., 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), is reduced by –SH groups to form one mole of 2-nitro-5-mercaptobenzoic acid per mole of –SH. The nitro mercaptobenzoic acid anion has an intense yellow color that was determined spectrophotometrically at 412nm. The tissue glutathione was expressed as µg/mg of protein and the proteins were estimated by the method of Lowry et al. which is based on the principle that protein reacts with Folin Ciocalteau reagent to yield a colored complex, the absorbance of which is determined spectrophotometrically at 750 nm.[20]

Histology: Histologic analysis required cross-sectional slices of heart and kidney tissue preserved in formalin, embedded in paraffin. Hematoxylin and eosin dye were used to examine histological changes in these sections. Under the microscope, pictures were captured.

2.8. Statistical analysis

Data were expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Tukey's test. In all the tests, values were considered statistically significant when $P < 0.05$

5. Results

5.1. General observation

The overall appearance of all animal groupings was noted throughout the research. All animals lost weight after 3 weeks of doxorubicin therapy. All groups treated with doxorubicin and its combination with azilsartan medoxomil and chlorthalidone or ramipril had scruffy fur, red exudate around the eyes, diarrhea, and sicker, weaker, and sluggish animals. However, 16% mortality was observed in each of the groups receiving, a combination of azilsartan medoxomil and chlorthalidone and combination of azilsartan and ramipril along with doxorubicin.

5.2. Effect of azilsartan medoxomil and combination of azilsartan medoxomil with chlorthalidone on Serum Lactate dehydrogenase (LDH)

Serum LDH level was significantly increased ($p<0.001$) in the doxorubicin (DOX) treated group (group-2) as compared to the control (group-1). (As shown in **Table 1**). Treatment with AZL + CHL + DOX (group 3) decreased LDH ($p<0.05$) compared with toxic (group - 2). LDH levels significantly decreased ($p<0.001$) in those receiving AZL+ DOX (group-4) compared to (group-2). Treatment with AZL + RAM+DOXO (group-5) significant decrease in LDH ($p<0.01$) compared with toxic (group - 2). Thus, it was found that doxorubicin increased serum LDH and treatment decreased serum LDH.

Table 1: Effect of azilsartan medoxomil and combination of azilsartan medoxomil with chlorthalidone on Serum Lactate dehydrogenase (LDH)

Groups	Treatment	LDH(IU/L)
1.	Control	39.24± 2.42
2.	DOX (Toxic)	152.83±3.01***
3.	AZL+ CHL + DOX	137.34±2.50 *
4.	AZL+ DOX	124.85±1.14***
5.	AZL+RAM+DOX	133.12± 4.04**
6.	AZL+CHL *per se*	44.90±2.97

All values are expressed as means ± SEM.
***P<0.001(2 vs 1, 4 vs 2)
**P<0.01(5 vs 2)
*P<0.05(3 vs 2)

5.3. Effect of azilsartan medoxomil and combination with Chlorthalidone on doxorubicin-induced altered lipid profile

Administration of doxorubicin-induced significant alteration in the markers of lipid profile which was evident by the increased level of TC, TG, LDL, and reduced level of HDL ($P < 0.001$). Treatment with AZL + CHL was found to reverse the level of TC, TG, and LDL. But, in treatment with AZL + CHL, the level was increased. As shown below **Table 2**.

Table 2: Effect of azilsartan medoxomil and combination of azilsartan medoxomil and Chlorthalidone on serum Total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL) levels, and Low-Density Lipoprotein (LDL).

Groups	Treatment	Total cholesterol (TC) (mg/dl)	Triglycerides (mg/dl) (TG)	HDL Cholesterol (mg/dl) (HDL)	LDL Cholesterol (mg/dl) (LDL)
1.	Control	95.20±5.92	78.89±3.73	46.81±1.82	41.19±2.10
2.	DOX (Toxic)	226.41±19.29***	289.10±2.86***	24.37±1.09***	141.82±2.37***
3.	AZL+CHL+DOX	150.33±10.29***	147.50±1.11***	52.75±1.81***	47.77±2.1***
4.	AZL+DOX	140.29±8.02***	136.75±3.93***	55.33±2.62***	42.82±1.24***

5.	AZL+RAM+ DOX	144.92± 8.24***	147.60± 1.17***	50.74±1 .12***	68.95±2. 5***
6.	AZL+ CHL per se	89.74±3 .06	146.54± 2.55	44.44±1 .01	46.70±3. 75

All values are expressed as mean ±S.E.M
***P<0.001(2 vs 1, 3 vs 2, 4 vs 2, 5 vs 2)

5.4. Effect of azilsartan medoxomil and combination with Chlorthalidone on doxorubicin-induced altered serum Albumin and creatinine.

Administration of doxorubicin-induced significant alteration in the markers of decreased serum Albumin and increased creatinine ($P < 0.001$). Treatment with drugs was found to reverse serum Albumin which is increased albumin and decreased creatinine. Treatment drugs showed increased albumin and decreased creatinine. As shown in the below **Table 3**.

Table 3: Effect of azilsartan medoxomil and combination of azilsartan medoxomil and chlorthalidone on serum albumin and creatinine level.

Groups	Treatment	Albumin (g/dl)	Creatinine (mg/dl)
1.	Control	11.35±1.62	0.46±0.03
2.	DOX(Toxic)	2.83±1.25***	3.20±0.27***
3.	AZL+ CHL + DOX	8.78±1.03**	0.65±0.13***
4.	AZIL+DOX	9.50±0.39**	0.84±0.12***
5.	AZL+ RAM+ DOX	9.38±0.77**	0.92±0.23***
6.	AZL+ CHL *per se*	10.0±0.22	1.04±0.23

All values are expressed as mean ±S.E.M
***P<0.001(2 vs 1, 3 vs 2, 4 vs 2, 5 vs 2)
**P<0.01(3 vs 2, 4 vs 2, 5 vs 2)

5.5. Effect of azilsartan medoxomil and combination with chlorthalidone on doxorubicin-induced altered cardiac TBARS and GSH.

Administration of doxorubicin-induced significant alteration in the markers of a significant increase in cardiac TBARS and a significant decrease in GSH level ($P < 0.001$). Treatment with the drug was found to reverse serum TBARS (decreased TBARS) and increased GSH levels. Azilsartan medoxomil and chlorthalidone per se did not significantly reduce cardiac TBARS. GSH levels were not significantly different in azilsartan medoxomil and chlorthalidone per se groups.

Table 4: Effects of azilsartan medoxomil and combination of azilsartan medoxomil and chlorthalidone on cardiac TBARS and glutathione levels.

Groups	Treatment	TBARS (nmol of MDA/mg of protein)	GSH (µg/mg protein)
1.	Control	1.76±0.26	7.57±0.78
2.	DOX (Toxic)	5.76±0.46***	1.08±0.3***
3.	AZL + CHL+ DOX	2.85±0.35***	6.37±0.70***
4.	AZL+ DOX	3.25±0.71**	5.45±0.80**
5.	AZL + RAM + DOX	2.46±0.35***	6.58±0.95***
6.	AZL+ CHL *per se*	1.46±0.14	5.36±0.85

All value are expressed as mean± SEM
***P<0.001(2 vs 1, 3 vs 2, 5vs 2)
** P<0.01(4 vs 2)

5.6. Effect of azilsartan medoxomil and combination with chlorthalidone on doxorubicin-induced altered renal TBARS and GSH.

Administration of doxorubicin-induced significant alteration in the markers of a significant increase in renal TBARS and a significant decrease in GSH level ($P < 0.001$). Treatment with the drug was found to reverse serum TBARS (decreased TBARS) and increased GSH levels. Azilsartan medoxomil and chlorthalidone per se did not significantly reduce renal TBARS. GSH levels were not significantly different in azilsartan medoxomil and chlorthalidone per se groups.

Table 5: Effect of azilsartan medoxomil and combination of azilsartan medoxomil and chlorthalidone on renal TBARS and glutathione levels.

Groups	Treatment	TBARS (nmoles of MDA /mg protein)	GSH (µg/mg protein)
1.	Control	1.11±0.29	6.41±0.61
2.	DOXO (Toxic)	5.39±1.1***	1.35±0.36***
3.	AZL+CHL+DOXO	1.51±0.32***	5.19±0.75**
4.	AZL+ DOXO	1.29±0.18***	4.61±0.65*
5.	AZL+RAM+DOXO	1.72±0.29***	5.31±0.87**
6.	AZL+CHL *per se*	1.53 ±0.36	6.63±0.69

All value is expressed as mean ± SEM
***P<0.001(2 vs 1, 3 vs 2, 5 vs 2)
** P < 0.01(3 vs 2, 5 vs 2)
* P < 0.05 (4 vs 2)

5.7. Effect of Azilsartan medoxomil and chlorthalidone on doxorubicin -induced histopathological damage

Administration of doxorubicin caused significant cardiac and renal damage which was estimated by H and E staining. Treatment with azilsartan medoxomil and in combination with Chlorthalidone showed protection of the cardiac and renal tissue. Per se group showed normal arrangements of myofibril without any vacuolization or shrinkage of cardiac and renal tissue.

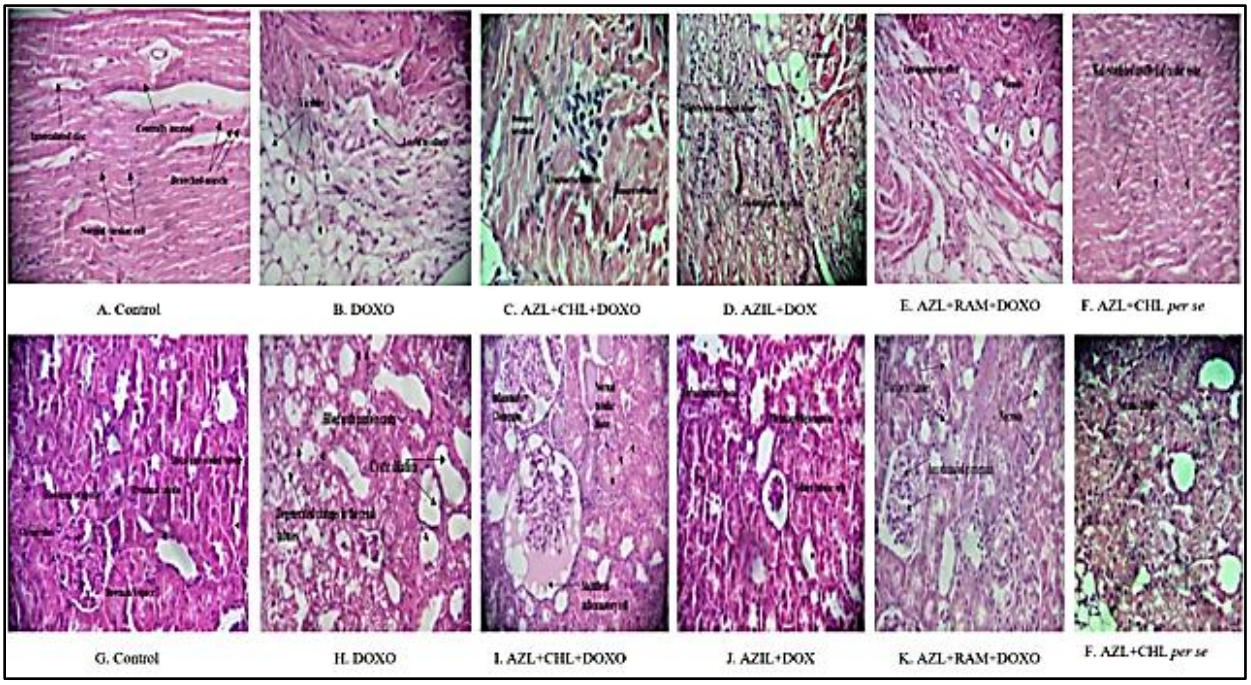

Figure: 1 (Showing A-F: cardiac tissue, G-F: renal tissue) the histological damage caused by doxorubicin and the protective effects of Azilsartan medoxomil and chlorthalidone. The doxorubicin treated grouped showed cardiac and renal degeneration. AZL+CHL+DOXO, AZL+RAM+DOXO, and AZL+CHL treated groups showed more significant and protective effects.

6. Discussion

Multifactorial hypertension causes pathophysiologic alterations in target organs throughout time. Preliminary investigations combining ACE inhibitors with calcium channel blockers, diuretics, and ARBs reduced left ventricular hypertrophy and improved renal function.[14] However, no clinical studies have examined cardiovascular outcomes for azilsartan medoxomil/chlorthalidone combo treatment. This research examined the effects of azilsartan medoxomil and chlorthalidone on doxorubicin-induced cardiomyopathy and nephropathy. Several animal models have shown that doxorubicin cardiomyopathy resembles congestive heart failure in humans.[15] This study showed that doxorubicin-induced cardiomyopathy and nephropathy increased serum LDH, TBARS, and decreased GSH and also altered lipid profile i.e., TG, TC, LDL, HDL. Treatment with Azilsartan medoxomil and chlorthalidone showed decreased TBARS and increased GSH, decreased TG, TC, LDL, and increased HDL, and also increased albumin and decreased creatinine. Histological aberration is one of the important criteria for cardiac and renal damage. When we administered doxorubicin, we found noticeable damage which was evident by significant cellular degeneration and vacuolation. Administration of Azilsartan medoxomil and chlorthalidone effectively reversed the damage toward normal and exhibited protective effects.

7. Conclusion

According to biochemical and histological investigation, Wistar rats given doxorubicin in six equal intraperitoneal doses of 2.5 mg/kg over two weeks developed cardiomyopathy and nephropathy. AZL alone, with CHL significant effect on doxorubicin-induced cardiomyopathy and nephropathy, according to biochemical estimates. The histological study showed that AZL with CHL prevented doxorubicin-induced cardiomyopathy and nephropathy. The AZL+CHL combination was better than AZL+RAM and AZL alone in preventing doxorubicin-induced cardiomyopathy and nephropathy in rats.

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9. Conflicts of Interest

The authors declare no conflict of interests.

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References

1. Centers for Disease Control and Prevention (CDC). Vital signs: awareness and treatment of uncontrolled hypertension among adults-United States, 2003-2010. *MMWR Morb Mortal Wkly Rep*. 2012; 61:703-9.
2. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme, inhibitor or calcium channel blocker vs diuretic: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT).

JAMA. 2002;288(23):2981-97. https://doi:10.1001/jama.288.23.2981
3. Dahl of B, Devereux RB, Kjeldsen SE. Cardiovascular morbidity and mortality in the Losartan Intervention for Endpoint reduction in hypertension
4. Study (LIFE): a randomized trial against atenolol. *Lancet.* 2002; 359:995-1003. https://doi:10.1016/S0140-6736(02)08089-3
5. Pepine CJ, Handberg EM, Cooper-DeHoff RM. A calcium antagonist vs a non-calcium antagonist hypertension treatment strategy for patients with coronary artery disease. The International Verapamil-Trandolapril Study (INVEST): a randomized controlled trial. *JAMA.* 2003;290(21):2805-16. doi:10.1001/jama.290.21.2805
6. Dahlof B, Sever PS, Poulter NR. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcome Trial-Blood Pressure Lowering ARM (ASCOT-BPLA): a multicenter randomized controlled trial. *Lancet*. 2005; 366:895-906
7. Pierini D, Andersion KV. Azilsartan medoxomil/chlorthalidone: A Fixed dose Combination Antihypertensive Ann Pharmacother 2013; 47(5):694-703. https://doi:10.1345/aph.1R618
8. Venkata C, Ram S. Angiotensin receptor blockers: current status and prospects. *Am J Med*. 2008;121:656-63 https://doi: 10.1345/aph.1R618.
9. Hernandez-Hernandez R, Sosa-Canache B, Velasco M. Angiotensin II receptor antagonists' role in arterial hypertension. *J Hum Hypertens* 2002; 16: S93-9. https:/doi:10.1038/sj.jhh.1001352
10. Zaiken K, Cheng JW. Azilsartan medoxomil: a new angiotensin receptor blocker. *Clin Ther.* 2011; 33: 1577-89. https://doi:10.1016/j.clinthera.2011.10.007
11. Ojima M, Igata H, Tanaka M. In vitro antagonistic properties of a new angiotensin type 1 receptor blocker, azilsartan, in receptor binding and function studies. *J Pharmacol Exp. Ther.* 2011; 336:801-8. https://doi:10.1124/jpet.110.176636
12. Kurtz TW, Kajiya T. Differential pharmacology and benefit/risk of azilsartan compared to other sartans. Vasc Health Risk Manag. 2012; 8:133-43.
13. Takagi H, Mizuno Y, Niwa M, Goto S, Umemoto T for the ALICE (All-Literature Investigation of Cardiovascular Evidence) Group. A meta-analysis of randomized controlled trials of azilsartan therapy for blood pressure reduction. *Hypertens Res.* 2014; 37(5):432–7. https://doi:10.1038/hr.2013.142
14. Carter B, Ernst M, Cohen J. Hydrochlorothiazide versus chlorthalidone: evidence supporting their interchangeability. *Hypertension.* 2004; 43:4-9. https://doi:10.1161/01. HYP.0000103632.19915.0E
15. Roush GC, Holford TR, Guddati AK. Chlorthalidone compared with hydrochlorothiazide in reducing cardiovascular events: systematic review and network meta-analyses. *Hypertension.* 2012; 59:1110-7. https://doi.org/10.1161/Hypertensionaha.112.191106
16. Kipnes MS, Alison H, Safety, Tolerability, and Efficacy of Azilsartan Medoxomil with or Without Chlorthalidone During and After 8 Months of Treatment for Hypertension. *J Clin Hypertens (Greenwich).* 2015; 17:183–92. https://doi:10.1111/jch.12474
17. De Hoff JL, Davidson LM, Kritchevsky D. An enzymatic assay for determining free and total cholesterol in tissue. *Clin Chem*. 1978;24(3):433-5.
18. Webster D. The immediate reaction between bromocresol green and serum as a measure of albumin content. *Clin Chem*. 1977;23(4):663-5.
19. Slot C. Plasma creatinine determination a new and specific Jaffe reaction method. Scandinavian *J linical and labor Invest*. 1965;17(4):381-7. https://doi:10.3109/0036551650 9077065
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Ana Biochem*. 1979;95(2):351-8. https://doi:10.1016/0003-2697(79)90738-3
21. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem.* 1968; 25(1):192-205. https;//doi:10.1016/0003-2697(68)90092-4.

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