



## Artery Research

ISSN (Online): 1876-4401

ISSN (Print): 1872-9312

Journal Home Page: <https://www.atlantis-press.com/journals/artres>

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**To cite this article:** Vohra Kanchan, Krishan Pawan, Varma Sudhir, Kalra Harpreet Singh (2017) Effect of aldosterone antagonists on common carotid artery's intima-media thickness, stiffness indices and flow mediated vasodilatation of brachial artery in CAD patients, Artery Research 18:C, 69–72, DOI: <https://doi.org/10.1016/j.artres.2017.04.005>

**To link to this article:** <https://doi.org/10.1016/j.artres.2017.04.005>

Published online: 3 December 2019



Short communication

# Effect of aldosterone antagonists on common carotid artery's intima-media thickness, stiffness indices and flow mediated vasodilatation of brachial artery in CAD patients



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Received 1 October 2016; accepted 13 April 2017

Available online 5 May 2017

## KEYWORDS

Endothelial dysfunction;  
Carotid artery stiffness  
indices;  
Carotid intima-media  
thickness;  
Flow mediated  
dilatation;  
High sensitivity C-  
reactive protein

## Background

Aldosterone has pro-inflammatory and pro-fibrotic effects in extra-renal tissues including blood vessels that trigger the vascular endothelial dysfunction (ED)<sup>1</sup> and large artery stiffness.<sup>2</sup> The mineralocorticoid receptor (MR) antagonists

have emerged as effective therapies for the management of cardio-vascular disease (CVD) due to their role beyond decreasing the blood pressure,<sup>3</sup> and that to some extent has been attributed through improvement of ED<sup>4</sup> and arterial stiffness<sup>1</sup> via its potent anti-TNF- $\alpha$  action.<sup>5</sup> The European Society of Hypertension/Cardiology guidelines have defined a mean carotid intima-media thickness (cIMT) of over 0.9 mm as a marker of target organ damage and stroke.<sup>6</sup>

## Aim of study

The present study was aimed to determine the effectiveness of MR antagonists in altering the endothelial dysfunction and carotid artery stiffness indices in CAD patients at risk of stroke *i.e.* having mean intima-media thickness of common carotid artery (cIMT) more than 0.9 mm.

## Methods

A randomized, placebo controlled, parallel group, pilot study was conducted and completed, under Department of

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Pharmaceutical Sciences and Drug Research, after due approval from Institutional Ethics Committee of Punjabi University, Patiala (approval number-ICEC/45/2012). Established CAD patients were screened and recruited from the medicine outdoor patient (OPD) clinic at Sadbhavna Medical and Heart institute, Patiala. Patient who met inclusion/exclusion criteria were treated, followed and evaluated as per protocol's requirements. A written informed consent from each patient was obtained prior to recruitment in the study. The study was performed in accordance with the declaration of Helsinki and the code of Good Clinical Practice. Inclusion criteria were: age  $\geq 18$  years; echo-cardiographic evidences of CAD as described by Chang *et al.*, 2009<sup>7</sup>/angiographic-evidences of CAD<sup>8</sup>; serum high sensitivity C-reactive protein (hsCRP) ( $1-10$  mg/dl). All patients had angiographic-evidences of significant CAD defined as a luminal narrowing of  $\geq 50\%$  in at least one major epicardial coronary artery according to American College of Cardiology/American Heart Association (ACC/AHA) guidelines for coronary angiography.<sup>8</sup> Exclusion criteria were: any major surgery/stroke within last 3 months; chronic infections and inflammatory diseases; abnormal renal and liver function; drugs affecting evaluation of arterial function.

CONSORT guidelines were followed for reporting parallel group randomized trials.<sup>9</sup> Based on the guidelines, 1:1 randomization was followed to expose patients equally to drug or placebo using computer assisted random number table. Both patients and investigators involved for vascular ultrasound profiling were blinded to treatment protocol and blinding of treatment assignments was maintained by research staff/monitors of the hospital. Eligible patients were randomized into three groups (group- A, B or C), and were allocated treatment (for 16 weeks) as placebo (PL) tablet, SP (25 mg, OD) or EP (25 mg, OD) tablets to their ongoing therapeutic regimen, respectively.

The sample size was estimated by considering the flow mediated vasodilatation (FMD) as a primary response variable. To find out 20% change ( $\beta = 0.2$ ,  $\alpha = 0.05$ ) in FMD with standard deviation (SD) of 1.60, a sample size of total 10 patients/group was required.<sup>10</sup> Descriptive and inferential statistical analyses were performed with Sigma Stat version 3.5. Results are expressed as mean  $\pm$  SD and number/percentage wherever applicable and considered significant at  $p \leq 0.05$ . Results were compared between three groups using one-way analysis of variance (ANOVA) followed by Tukey's test for normal distributed data and using Kruskal-Wallis ANOVA for non-normal distributed data, respectively. Intra- and inter-observer variability for cardiovascular Doppler profiling was found as 2% and 3.5% for brachial artery and 2.5% and 4% for carotid artery, respectively.

Assessment of endothelial-dependent FMD of the brachial artery was carried out by two cardiologists, who were blinded to the treatment protocol, in accordance with the guidelines detailed by Corretti *et al.*, 2002.<sup>11</sup> FMD was calculated as  $FMD (\%) = 100 \times \{(\text{post-hyperemic diameter} - \text{basal diameter})/\text{basal diameter}\}$ .

Common carotid artery (CCA) intima media thickness (IMT), diastolic (Dd) and systolic (Ds) diameter (D) (using carotid ultrasound) and brachial BP measurements (SBP and

DBP) were used to derive three parameters of arterial stiffness *i.e.* Young's Elastic Modulus (YEM), carotid arterial compliance (CAC) and stiffness index (SI) as described by method of Cipolli *et al.*, 2012.<sup>12</sup> YEM gives an estimate of arterial stiffness that is independent of wall (intima-media) thickness by the formula:  $([\text{systolic blood pressure} - \text{diastolic blood pressure}] \times \text{diastolic diameter})/([\text{systolic diameter} - \text{diastolic diameter}]/\text{IMT})$ . CAC measures the ability of the arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation and was calculated as:  $([\text{systolic diameter} - \text{diastolic diameter}]/\text{diastolic diameter})/(\text{systolic blood pressure} - \text{diastolic blood pressure})$ . SI is considered to be relatively independent of blood pressure and was calculated by the formula:  $(\text{systolic blood pressure}/\text{diastolic blood pressure})/([\text{systolic diameter} - \text{diastolic diameter}]/\text{diastolic diameter})$ .<sup>12</sup>

Overnight fasting blood samples were withdrawn by the laboratory technicians of the hospital; serum and plasma were separated out and stored at  $-40^\circ\text{C}$  until analysis. Serum total cholesterol (TC) and creatinine (commercial biochemical kits – Erba<sup>®</sup> Mannheim, Transasia Biomedical Ltd, India), complete blood count (automated hematology analyzer-model Sysmex XP100), serum sodium and potassium (automated electrolyte analyzer), hsCRP and TNF- $\alpha$  (commercial ELISA Kits) were analysed.

## Results

45 patients were screened, but 12 were excluded because 10 did not meet inclusion/exclusion criteria and 2 did not give their consent. Thus, 33 patients [M/F = 9/2 (group A), 10/1 (B) and 10/1 (C)] were found eligible. The biochemical and clinical characteristics of patients are presented in Table 1. All patients, irrespective of the group, were on a combination regimen of a fixed-dose combination of clopidogrel 75 mg + atorvastatin 10 mg + aspirin 75 mg/rosuvastatin 10 mg, once daily, and either Losartan 2.5 mg/Telmisartan 20 mg/or metoprolol 25 mg, once daily. All patients were overweight (BMI: 25–29 kg/m<sup>2</sup>) and had hypertension, irrespective of the group. None was diabetic. 3 patients in group A, 4 in B and 3 in C also had high TC level, but none met the diagnostic criteria of MetS.

The variation in three treatment groups was not statistically significantly different (all  $p \geq 0.05$ ) at baseline level with respect to all the biochemical and clinical efficacy variables/parameters such as blood pressure, brachial and carotid artery profile parameters, and inflammatory markers. SBP, DBP, hsCRP and TNF- $\alpha$  levels were significantly reduced after SP and EP treatment as compared to placebo, but there was no difference between SP versus EP ( $p \geq 0.05$ ). Both treatments did not affect the metabolic variable, TC. Marker of endothelial dysfunction assessed as FMD of brachial artery were not significantly changed neither with SP/EP nor placebo (all  $p \geq 0.05$ ). There was also no significant effect observed on cIMT and stiffness indices *i.e.* YEM, CAC and SI (all  $p \geq 0.05$ ) after treatment with SP, EP or PL. Serum levels of creatinine, sodium, potassium and CBC were found within normal range after treatment in all groups (Table 1). There were no drop-outs due to any reasons.

**Table 1** Patients' biochemical and clinical profile.

Characteristics	Group A PL	Group B SP (25 mg OD)	Group C EP (25 mg OD)	p	Group A PL	Group B SP	Group C EP	SP vs. PL EP vs. PL EP vs. SP			
								p value			
	<b>At baseline</b>				<b>After 16 week</b>						
Age, years	50.27 ± 5.17	49.09 ± 6.26	49.45 ± 4.52	0.87	—	—	—				
Sex (M/F)	9/2	10/1	10/1	—	—	—	—				
Weight, kg	84.18 ± 2.92	87.09 ± 3.39	86.00 ± 3.22	0.11	84.72 ± 2.32	86.81 ± 3.18	86.09 ± 1.92	0.16			
Height, m	1.70 ± 0.03	1.72 ± 0.04	1.71 ± 0.02	0.34	—	—	—				
BMI, kg/m <sup>2</sup>	29.01 ± 0.88	29.22 ± 0.86	29.12 ± 0.76	0.83	29.21 ± 1.02	29.14 ± 0.92	29.70 ± 0.73	0.98			
SBP, mm Hg	126.83 ± 3.92	124.41 ± 2.18	125.25 ± 2.59	0.18	125.36 ± 3.07	122.54 ± 2.54	121.27 ± 2.05	0.02*	<0.01*	0.27	
DBP, mmHg	83.41 ± 2.99	82.50 ± 2.90	82.75 ± 2.83	0.73	83.00 ± 2.19	80.45 ± 2.06	80.09 ± 1.57	<0.01*	<0.01*	0.66	
TC, mg/dl	185.33 ± 18.44	201.75 ± 17.09	189.83 ± 16.07	0.06	181.54 ± 16.61	192.90 ± 18.59	184.0 ± 18.51	0.30			
hsCRP, mg/l	5.53 ± 0.75	6.00 ± 0.64	5.72 ± 0.65	0.16	5.45 ± 0.95	3.49 ± 0.78	3.68 ± 1.00	<0.01*	<0.01*	0.63	
TNF- $\alpha$ , pg/ml	16.33 ± 2.30	18.00 ± 1.70	17.75 ± 1.75	0.09	15.81 ± 3.34	6.81 ± 2.67	7.09 ± 2.70	<0.01*	<0.01*	0.82	
<b>Brachial artery variables</b>											
BAD, mm	4.59 ± 0.39	4.61 ± 0.34	4.44 ± 0.39	0.52	4.52 ± 0.27	4.51 ± 0.24	4.54 ± 0.23	0.96			
Post H-BAD, mm	4.91 ± 0.38	5.00 ± 0.36	4.80 ± 0.37	0.49	4.92 ± 0.34	4.99 ± 0.25	5.08 ± 0.20	0.40			
Post N-BAD, mm	5.26 ± 0.35	5.34 ± 0.29	5.17 ± 0.35	0.49	5.40 ± 0.30	5.38 ± 0.24	5.36 ± 0.17	0.94			
FMD, %	7.29 ± 1.74	8.29 ± 0.99	8.28 ± 1.99	0.21	10.47 ± 2.53	10.28 ± 2.46	10.94 ± 1.54	0.78			
NMD, %	14.85 ± 2.96	15.93 ± 3.08	16.56 ± 3.50	0.45	18.74 ± 3.13	18.94 ± 2.73	17.95 ± 3.18	0.72			
<b>Carotid artery variables</b>											
IMT, mm	0.90 ± 0.08	0.91 ± 0.08	0.91 ± 0.07	0.84	0.90 ± 0.07	0.91 ± 0.04	0.91 ± 0.05	0.88			
Ds, mm	5.96 ± 0.15	5.90 ± 0.16	5.85 ± 0.19	0.31	5.96 ± 0.15	5.91 ± 0.13	5.85 ± 0.16	0.18			
Dd, mm	5.48 ± 0.18	5.42 ± 0.20	5.34 ± 0.21	0.23	5.48 ± 0.18	5.44 ± 0.15	5.35 ± 0.16	0.15			
YEM, mmHg mm	452.49 ± 85.82	436.82 ± 76.50	415.05 ± 95.62	0.57	451.50 ± 84.81	451.70 ± 104.64	428.39 ± 97.57	0.78			
SI	17.64 ± 2.92	17.39 ± 3.33	16.07 ± 2.91	0.41	17.64 ± 2.90	17.86 ± 4.13	16.66 ± 3.03	0.66			
CAC, %/10 mmHg	2.05 ± 0.35	2.17 ± 0.55	2.31 ± 0.49	0.42	2.00 ± 0.15	2.11 ± 0.43	2.21 ± 0.42	0.60			
<b>Safety variables</b>											
Hemoglobin, Gm	13.92 ± 0.86	13.90 ± 0.82	14.11 ± 0.76	0.75	12.75 ± 0.74	12.77 ± 0.59	13.06 ± 1.12	0.63			
TLC, mm <sup>3</sup>	7818.18 ± 337.00	7990.90 ± 787.97	7081.81 ± 1217.22	0.21	9290.90 ± 548.55	9400.00 ± 329.29	7072.72 ± 1232.95	0.05	0.00*	0.00*	
Creatinine, mg%	0.74 ± 0.12	0.77 ± 0.16	0.78 ± 0.16	0.80	0.65 ± 0.15	0.72 ± 0.17	0.69 ± 0.13	0.49			
Sodium, mEq/l	139.36 ± 2.73	138.00 ± 2.79	140.09 ± 2.58	0.20	137.63 ± 1.85	138.27 ± 1.55	137.72 ± 2.19	0.69			
Potassium, mEq/l	3.76 ± 0.23	3.90 ± 0.21	3.84 ± 0.22	0.37	3.87 ± 0.25	4.13 ± 0.19	3.93 ± 0.23	0.19			

\* $p \leq 0.05$  as statistical significant, M/F: male/female, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, hsCRP: high sensitivity C-reactive protein, TNF- $\alpha$ : tumor necrosis factor alpha, BAD: brachial artery diameter, H: hyperemia, N: nitroglycerine, FMD: flow mediated vasodilatation, NMD: nitroglycerine mediated vasodilatation, IMT: intima-media thickness, Ds: systolic diameter, Dd: diastolic diameter, YEM: Young's elastic modulus, CAC: carotid arterial compliance, SI: stiffness index, TLC: total leucocytes count, PL: placebo; SP: spironolactone; EP: eplerenone.

## Discussion and conclusion

TNF- $\alpha$ , a pro-inflammatory mediator of activation of endothelial cells, results in impaired vasoreactivity by promoting degradation or blocking activation of endothelial nitric oxide synthase (eNOS) enzyme and, by activating apoptotic signaling cascade in endothelial cells.<sup>13</sup> Spironolactone (1–3 mg/kg), based on potent anti-TNF- $\alpha$  action, has proven its effectiveness in improving endothelial dysfunction in metabolic syndrome,<sup>14</sup> rheumatoid arthritis,<sup>5</sup> ankylosing spondylitis<sup>15</sup> and obesity,<sup>16</sup> while there exists scarce published findings for eplerenone. Metabolic syndrome (MetS) patients are considered at more risk for developing stroke and CAD.<sup>17</sup> In a recent work on MetS patients, we had not found any significant effect of neither SP nor EP on insulin resistance and endothelial dysfunction.<sup>10</sup> In present study, that included CAD patients at high risk of stroke (cIMT  $\geq 0.9$  mm), both SP and EP were found effective only in reducing BP and halting inflammatory process to some extent, but not in altering ED, carotid IMT and stiffness indices. Moreover, EP was found no better than SP or vice-versa. Overall, both drugs were found tolerable. Thus, controlling inflammatory cascade.

Present study had several limitations. Carotid artery stiffness indices were derived using brachial BP rather than central aortic BP, which is considered less appropriate due to difference in pulse pressure amplification at central versus brachial artery.<sup>18</sup> Evaluation was carried out only at one dose level in small number of subjects for relatively shorter duration due to lack of funding, time and other institutional constraints. Thus, efficacy at different dose levels must be further explored in large randomized controlled trials of long duration.

## Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Conflict of interest

All authors declare no conflicts of interest.

## Acknowledgment

We sincerely thank Medical and paramedical team of Sadbhavna Medical and Heart Institute for support in execution of the study.

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