WILEY
Online Proofing System Instructions

The Wiley Online Proofing System allows authors and proof reviewers to review PDF proofs, mark corrections, respond to queries, upload replacement figures, and submit these changes directly from the PDF proof from the locally saved file or while viewing it in your web browser.

1. For the best experience reviewing your proof in the Wiley Online Proofing System please ensure you are connected to the internet. This will allow the PDF proof to connect to the central Wiley Online Proofing System server. If you are connected to the Wiley Online Proofing System server you should see the icon with a green check mark above in the yellow banner.


Connected


Disconnected

2. Please review the article proof on the following pages and mark any corrections, changes, and query responses using the Annotation Tools outlined on the next 2 pages.

3. To save your proof corrections, click the "Publish Comments" button appearing above in the yellow banner. Publishing your comments saves your corrections to the Wiley Online Proofing System server. Corrections don't have to be marked in one sitting, you can publish corrections and log back in at a later time to add more before you click the "Complete Proof Review" button below.

4. If you need to supply additional or replacement files bigger than 5 Megabytes (MB) do not attach them directly to the PDF Proof, please click the "Upload Files" button to upload files:

Click Here

5. When your proof review is complete and you are ready to submit corrections to the publisher, please click the "Complete Proof Review" button below:

Click Here

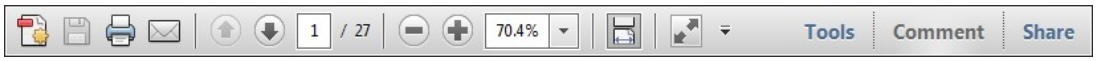
IMPORTANT: Do not click the "Complete Proof Review" button without replying to all author queries found on the last page of your proof. Incomplete proof reviews will cause a delay in publication.

IMPORTANT: Once you click "Complete Proof Review" you will not be able to publish further corrections.

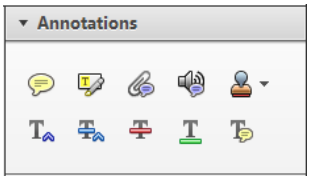
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

WILEY

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

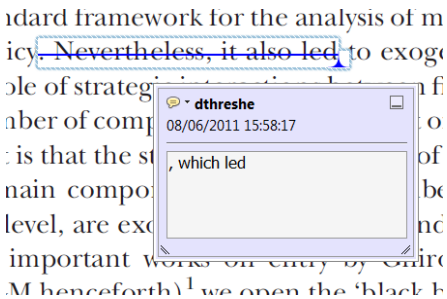


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

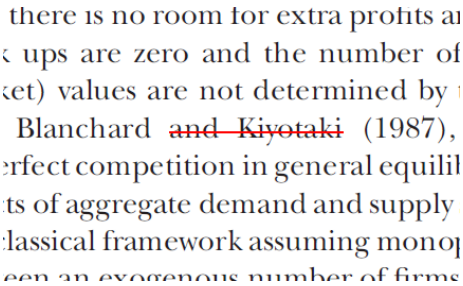


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

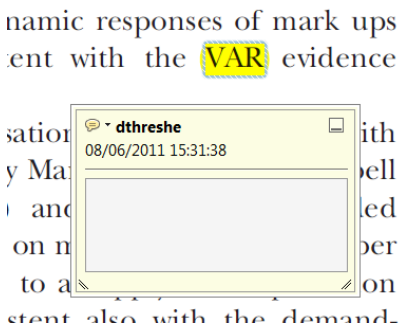


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

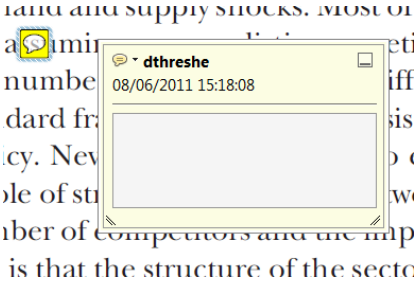


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

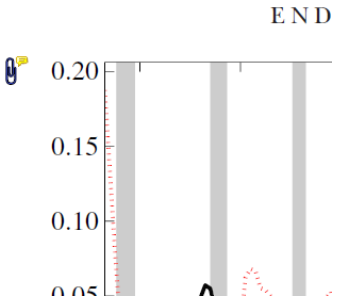


5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

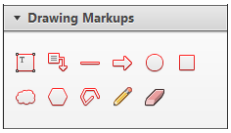
How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



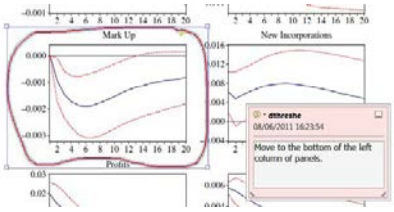
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



Received: 15 March 2016 | Accepted: 19 July 2016

DOI: 10.1111/micc.12298

ORIGINAL ARTICLE

WILEY Microcirculation

Effect of different drug classes on reverse remodeling of intramural coronary arterioles in the spontaneously hypertensive rat

Massimiliano Mancini[1,a] | Angela Scavone[2,a] | Carmem Luiza Sartorio[2] | Rocco Baccaro[2] | Christina Kleinert[2] | Angelina Pernazza[3] | Veronica Buia[2] | Martina Leopizzi[3] | Giulia D'amato[3] | Paolo G. Camici[2]

[1]Istituto "Marco Pasquali" ICOT GIOMI, Latina, Italy

[2]Vita Salute University and Scientific Institute San Raffaele, Milan, Italy

[3]Department of Radiology, Oncology and Pathology, "Sapienza" University, Rome, Italy

Correspondence
Paolo G. Camici, Vita Salute University San Raffaele, Via Olgettina 58, 20132 Milan, Italy.
Email: camici.paolo@hsr.it

Abstract

Background: Symptoms and signs of myocardial ischemia in the absence of obstructive coronary disease are common in hypertensive patients. This can be explained by CMD due to adverse remodeling of coronary arterioles which have also been reported in the SHR.

Objective: The aim of this study was to compare the effects of ramipril, perindopril, candesartan, atenolol, amlodipine, indapamide, and HMR1766 on CMD in the SHR.

Methods: Eight groups of 24-week-old SHR were treated for 8 weeks. BP was measured invasively at the end of the treatment. After sacrifice, hearts were mounted on a Langendorff apparatus for the measurement of hyperemic CF. Hearts were then processed for histomorphometric analysis.

Results: All compounds, except HMR1766, induced a significant reduction in BP. Perindopril and candesartan increased hyperemic CF, whereas the other compounds had no significant effect. Perindopril, ramipril, atenolol, indapamide, and HMR1766 induced significant reverse arteriolar remodeling, whereas candesartan and amlodipine did not.

Conclusions: The effect of antihypertensive treatment on CMD is not only dependent on BP reduction. Compounds with comparable antihypertensive efficacy may exert different effects on CF and induce different degrees of reverse arteriolar remodeling.

KEYWORDS

antihypertensive drugs, coronary blood flow, coronary microcirculation, hypertension, SHR, vascular remodeling

1 | INTRODUCTION

Arterial hypertension is an established major risk factor for IHD.[1–5] Patients with hypertension often complain of anginal symptoms and display electrocardiographic changes suggestive of myocardial ischemia. Accordingly, studies in hypertensive patients with or without LVH have CFR that is often reduced even in the absence of obstructive CAD at angiography.[6,7]

The latter findings can be explained, at least in part, by functional and structural abnormalities at the level of the microcirculation that are believed to be the substrate of CMD.[6] CMD can result from functional mechanisms, including impaired dilatation or increased constriction of coronary resistance vessels, as well as from adverse remodeling of intramural coronary arterioles consisting of smooth

[a]These authors equally contributed to the work.

Journal Name MICC | Manuscript No. 12298 | WILEY | No. of pages: 9 | Dispatch: 30-7-2016 | PE: Sujitha. G | CE: Priya C

muscle hypertrophy, variable degrees of intimal thickening and increased collagen deposition in the tunica media and the perivascular environment.[8–11] Similar arteriolar changes have been demonstrated in the SHR, indicating that this is a good animal model for studying CMD and vascular remodeling.[10] Previous work from our laboratory has demonstrated an inverse linear relation between arteriolar medial area and hyperemic coronary flow in the SHR.[12]

ACE inhibitors have been shown to partially reverse coronary small artery and arteriolar remodeling in hypertensive patients[13,14] and also in the SHR.[15–19] Recent work from our group has expanded these results by showing that ACE inhibitors can reverse CMD and CF both in patients and the SHR.[12]

The aim of this study was to assess the effect on coronary microvascular remodeling and hyperemic CF in the SHR model of the most commonly used drugs for the treatment of hypertension comprehending ACE inhibitors ramipril and perindopril, angiotensin receptor blocker candesartan, beta-blocker atenolol, sulfonamide diuretic indapamide, and dihydropyridine calcium channel blocker amlodipine, and a novel experimental sGC activator (HMR-1766).

2 | MATERIALS AND METHODS

Adult male SHR (350–400 g body weight) and WKY rats were obtained from Charles River Laboratories (Calco, IT). Animals were housed under controlled temperature (22°C) and lighting (12/12-hour light/dark cycle) with free access to food and water. All experiments were performed according to the institutional guidelines which comply with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. After a stabilization period of 7 days, SHRs were randomly assigned to the following groups: placebo (n=13), ramipril (n=11, 10 mg/kg/day, Servier), perindopril (n=10, 1.2 mg/kg/day, Servier), candesartan (n=8, 3 mg/kg/day, Sigma Aldrich), atenolol (n=7, 50 mg/kg/day, Sigma Aldrich), indapamide (n=5, 1.5 mg/kg/day, Servier), amlodipine (n=6, 5 mg/kg/day, Servier), HMR-1766 (n=10, 30 mg/kg/twice a day, Sanofi), age-matched WKY rats served as normotensive controls (n=5). All drugs were administered by oral gavage for 8 weeks. The dosage of each compound was chosen to mimic previous human studies according to the previously published protocols[12,20–27] and company guidelines.

2.1 | Systolic blood pressure measurement

At the end of the treatment animals were terminally anesthetized (inhaled isoflurane 3% in O_2) and placed on a heating pad to maintain the body temperature at 37°C. SBP was similarly measured in all groups by cannulating the carotid artery with a Millar MIKRO-TIP catheter (SPR-320, 2F) connected to an amplifier and Powerlab system (AD Instruments).

2.2 | Assessment of coronary flow and resistance

For ex vivo assessment of CF and resistance, Langendorff perfusion of the heart was performed as described previously.[10] Briefly, after completion of SBP measurement, hearts from heparinized animals (1000 U/kg, i.p.) were rapidly excised and placed in ice-cold buffer. Retrograde perfusion of the coronary arteries was established through a cannula inserted into the aortic root while the heart was allowed to beat spontaneously. The perfusate consisted of modified Krebs-Henseleit buffer (mmol/L: 118 NaCl, 4.7 KCl, 1.66 $MgSO_4$, 25 $NaHCO_3$, 1.8 $CaCl_2$, 1.18 KH_2PO_4, and 5.5 glucose; pH 7.4) equilibrated with a 5% CO_2-95% O_2 gas mixture and warmed (37°C) in a nonrecirculating system. Hearts were perfused at a constant pressure of 100 mm Hg, monitored via an integrated force transducer connected to an amplifier and Powerlab system (AD Instruments, UK). CF was continuously monitored and recorded using a Doppler Flow Probe (Transonic Systems Inc., USA) connected to a Powerlab amplifier. After an equilibration period of 30 minutes, hearts were exposed to two minutes of global ischemia followed by reperfusion and reactive peak hyperemic CF was measured.

2.3 | Histology and histomorphometrical analysis

Following Langendorff perfusion, hearts were weighed and prepared for histological and histomorphometrical analyses by cutting them into three to four short-axis sections and fixating the two mid-sections in 10% neutral buffered formalin solution (Sigma Aldrich). After fixation, sections were processed for paraffin embedding and multiple 4-µm thick sections from each block were deparaffinized, rehydrated, and stained with hematoxylin-eosin for microscopic evaluation of vessel morphology. Stained slides were evaluated under light microscopy by a dedicated pathologist and high-resolution images of all cross-section intramural arterioles (vessel diameter ≤ 200 µm) were acquired at 20× magnification. Images were analyzed using ImageJ 1.48v software (National Institute of Health, Bethesda, MD).[28] LA and total VA were directly measured and the following parameters were derived: medial area (VA–LA), lumen area to vessel area ratio (LA/VA), lumen diameter [$\sqrt{(LA/3.14)}$]×2 and vessel diameter [$\sqrt{(V/3.14)}$]×2.

For the evaluation of fibrosis, picrosirius red-stained sections were prepared according to the manufacturer's instructions (Bio Optica Spa Milano, Italy). The presence, type, and extent of fibrosis were determined via collagen fraction staining and thresholding automated analysis using ImageJ.[28] To assess interstitial fibrosis, 10 images from each rat were acquired at 10× magnification and measured avoiding areas of scarring. Perivascular fibrosis was measured as a percent of the vessel area with automated thresholding for collagen staining in five vessels acquired at 40× magnifications from each animal.

2.4 | Statistical analysis

All data are expressed as mean±SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Dunnett's post hoc test. A $P<.05$ was considered statistically significant.

3 | RESULTS

3.1 | Differences between SHR and WKY

SHR treated with placebo had higher SBP (Fig. 1D, SHR placebo: 214.7±4.4 vs WKY: 126.2±2.3 mm Hg; *P*<.001) and reduced hyperemic CF (Fig. 2, SHR placebo: 11.9±0.6 vs WKY: 20.2±1.91 mL/min/g; *P*<.001) compared to WKY rats.

SBP values in the SHR placebo rats were comparable to those reported in a previous study from our group, which studied animals with similar age and applied the same protocol for BP measurement.[12] Furthermore, heart weight to body weight ratio (SHR placebo: 4.95±0.10 vs WKY: 3.53±0.07; *P*<.001; Fig. 1C) and arteriolar medial area (SHR placebo: 5333±271.9 vs WKY: 1800.0±169.3 μm^2; *P*<.001; Fig. 3A) were significantly higher in SHR than in WKY.

At variance with all other treatments that had no effect, only indapamide induced a significant reduction in body weight compared to SHR placebo (332±24 vs 380±3.7, respectively, *P*<.001; Fig. 1A). We also noted a significant decrease in HW (Fig. 1B) and HW/BW ratio (Fig. 1C) that was observed after treatment with ramipril, perindopril, candesartan, and atenolol. At the doses used, all compounds, except HMR1766, induced a significant reduction in SBP compared to SHR placebo group (Fig. 1D). Hyperemic CF increased significantly in SHR treated with perindopril and candesartan (Fig. 2).

3.2 | Arteriolar remodeling and interstitial and perivascular fibrosis

As shown in Fig. 3A, all compounds used, except candesartan and amlodipine, induced a significant reduction in medial area. A very significant increase in the lumen to vessel area ratio was observed with perindopril and to a lesser extent with atenolol and indapamide (Fig. 3B). Lumen diameter did not show any significant change among treatment groups, while whole vessel diameter showed a significant reduction in all treatment groups except for amlodipine (Fig. 3C and D).

Furthermore, a comparison of interstitial fibrosis between SHR placebo and other animal groups is shown in Fig. 4. Compared to SHR placebo, a reduction in interstitial fibrosis was observed with all treatment groups except for indapamide and amlodipine.

As shown in the Fig. 5, atenolol was the only treatment without a significant reduction in perivascular fibrosis, while the groups treated with candesartan, indapamide, and amlodipine reduced this parameter,

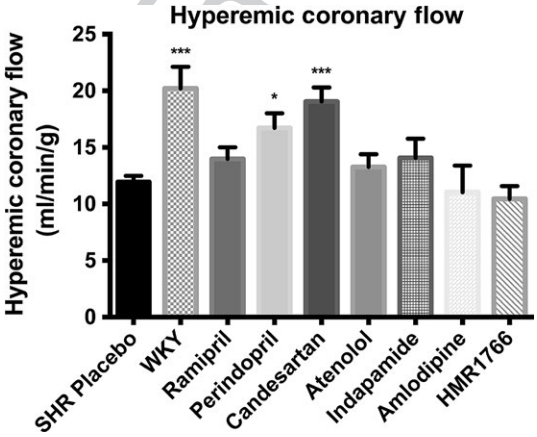


FIGURE 2 Changes in hyperemic CF, after two minutes of global ischemia, in treated SHR groups and WKY compared to SHR placebo. *P<.05; **P<.01; ***P<.001; vs SHR placebo

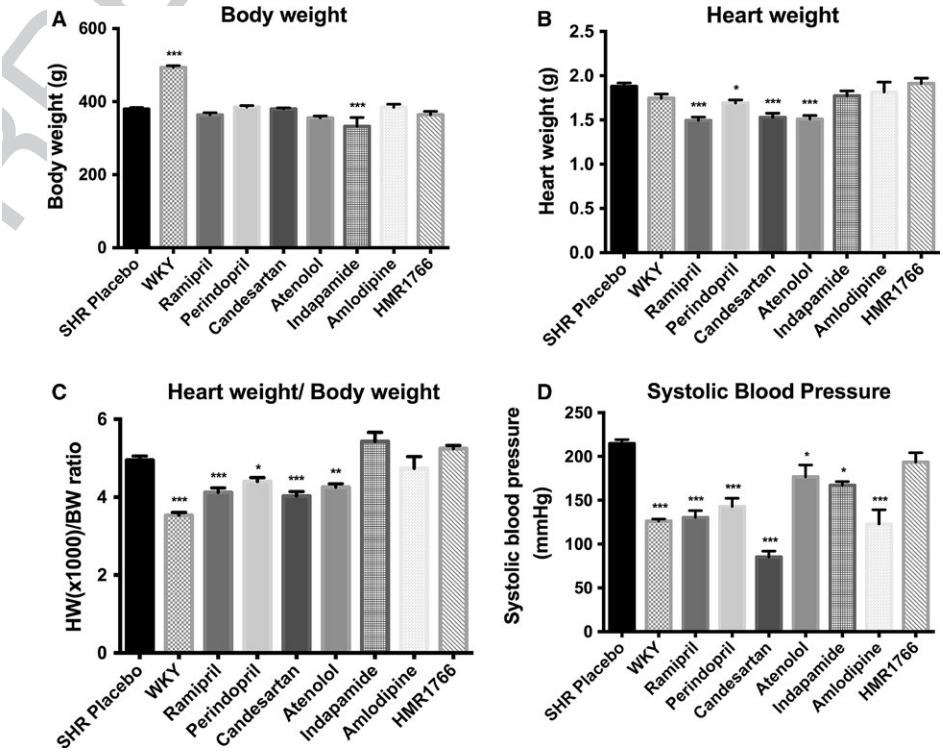


FIGURE 1 Effects of drugs on main physiological parameters in treated SHR groups and WKY compared to SHR placebo. Changes in body weight (A), heart weight (B), heart weight to body weight ratio (C), and SBP (D) after 8 weeks of drug treatment. *P<.05; **P<.01; ***P<.001 vs SHR placebo

COLOUR

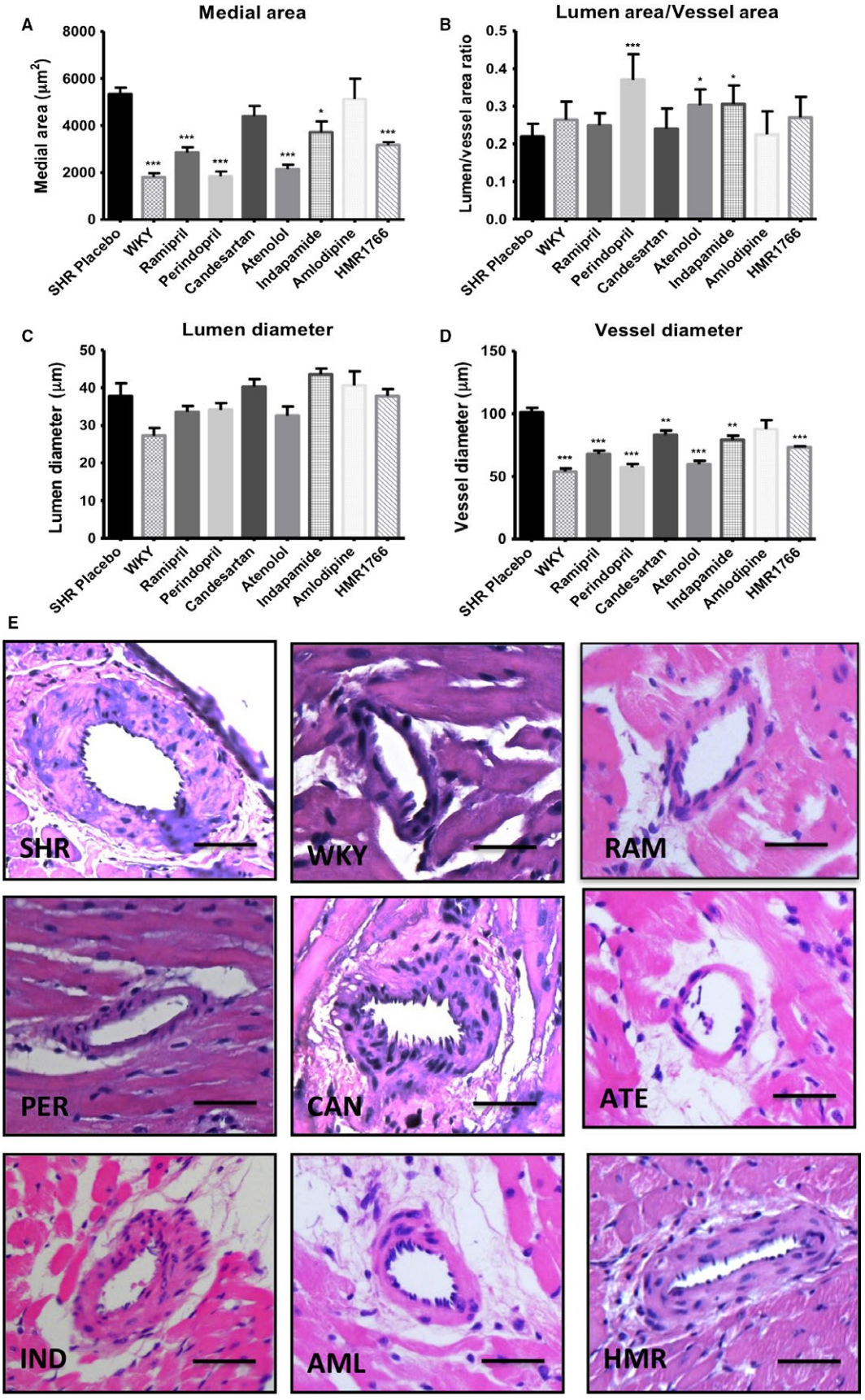


FIGURE 3 Histomorphometric analysis. Changes of medial area (A), lumen to vessel area ratio (B), lumen (**C**), and vessel (D) diameter of drug-treated SHR compared to control animals. Representative hematoxylin and eosin images of each group (E). **P*<.05; ***P*<.01; ****P*<.001 vs SHR placebo. Calibration bar: 50 μm

COLOUR

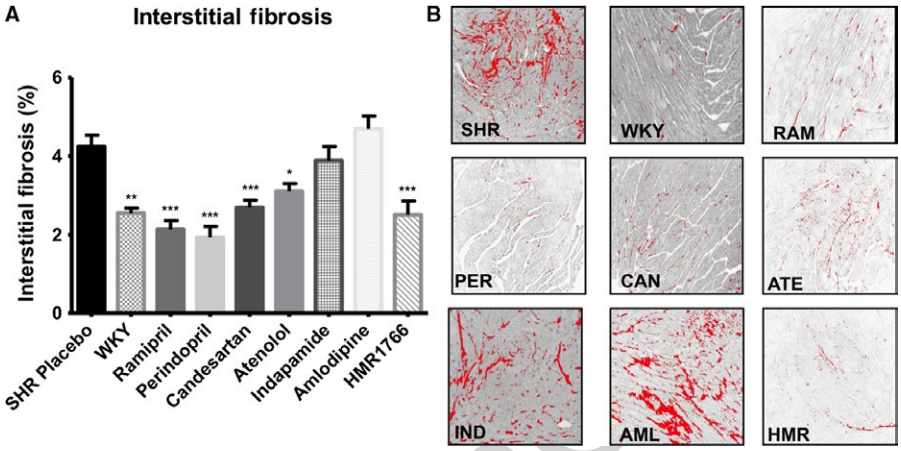


FIGURE 4 Interstitial fibrosis. Percent changes in interstitial fibrosis (collagen fraction) after drug treatment (A) and representative images of each group, as indicated (B). *P<.05; **P<.01; ***P<.001 vs SHR placebo

COLOUR

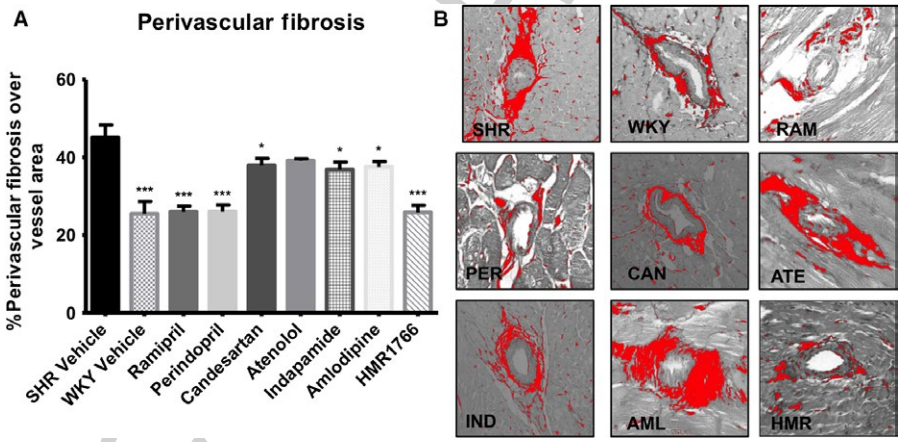


FIGURE 5 Perivascular fibrosis. Percent quantification of perivascular fibrosis over vessel area after drug treatments (A) and representative images of each group, as indicated (B). *P<.05; ***P<.001 vs SHR placebo

but to a lesser extent compared to those treated with ramipril, perindopril, and HMR-1766.

The results of all pharmacologic treatments on physiologic and anatomic parameters are summarized in Table 1.

3.3 | Relationship between arteriolar structure and hyperemic CF

The relationship between arteriolar medial area and hyperemic CF for all the study groups is shown in Fig. 6. Although all drugs used, except candesartan and amlodipine, induced significant reverse arteriolar remodeling compared to placebo, only perindopril and candesartan induced a significant increase in hyperemic CF.

4 | DISCUSSION

In this study, we provide novel evidence indicating that although the majority of the drugs used induced a reduction of SBP in the SHR, their effect on arteriolar structure and CF was variable and partly independent from their ability to reduce SBP. Only perindopril achieved a reduction of arteriolar medial area that was paralleled by a significant increase in hyperemic CF. The data on perindopril are consistent with previous work from our group showing that 6 months treatment with this agent, in combination with indapamide, improved myocardial blood flow, measured with PET, in hypertensive patients. In an ancillary study, the same drug combination improved CF and reversed arteriolar remodeling in SHR.[12] Furthermore, it has been previously shown that perindopril increases lumen/vessel ratio and improves CFR in SHR.[29–31] The other ACE inhibitor used in our study, ramipril, induced a significant reduction in medial thickness, but had no significant effect on hyperemic CF. In agreement with our results, Kaneko et al. demonstrated that 3 weeks of treatment with ramipril did not improve CF or CFR in SHR.[32] The disparity in the effects of these ACE inhibitors could be due to differences in intrinsic biochemical properties and pharmacological mechanisms of ramipril and perindopril,[11] and with particular regards on the ability of the latter to improve endothelial dysfunction.[33,34] Consistent with previous reports, ACE inhibitors induced a reduction in both interstitial and perivascular fibrosis,[35,36] which could be explained by both the inhibition of RAAS system and new alternative pathways, for example, Ac-SDKP[37,38] or Plzf angiotensin coreceptor modulation.[39]

The ARB candesartan induced an increase in CF without any significant reduction in arteriolar medial area. However, this drug reduced significantly interstitial and perivascular fibrosis. There are no previous data in the literature on the effect of this drug on remodeling of coronary arterioles. Several studies carried out on gluteal subcutaneous small arteries of hypertensive patients have shown that ARB

TABLE 1 Overview of results.

	WKY	Ramipril	Perindopril	Candesartan	Atenolol	Indapamide	Amlodipine	HMR1766
BW (g)	***	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.
HW (g)	n.s.	***	*	***	***	n.s.	n.s.	n.s.
HW/BW	***	***	*	****	**	n.s.	n.s.	n.s.
SBP (mm Hg)	***	****	***	***	*	*	***	n.s.
HCF (mL/min/g)	***	n.s.	*	***	n.s.	n.s.	n.s.	n.s.
MA (μm^2)	***	***	***	n.s.	***	*	n.s.	***
LA/VA	n.s.	n.s.	***	n.s.	*	*	n.s.	n.s.
LD (μm)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
VD (μm)	***	***	***	**	***	**	n.s.	***
IF (%)	**	***	***	***	*	n.s.	n.s.	***
PF (%)	***	***	***	*	n.s.	*	*	***

Summary of differences between WKY and treated groups vs SHR placebo.

BW, body weight; HW, heart weight, SBP, systolic blood pressure; HCF, hyperemic coronary flow; MA, media area; LA, lumen area; VA, vessel area; LD, lumen diameter; VD, vessel diameter; IF, interstitial fibrosis; PF, perivascular fibrosis.
*P<.05; **P<.01; ***P<.001 vs SHR placebo; n.s., not significant.

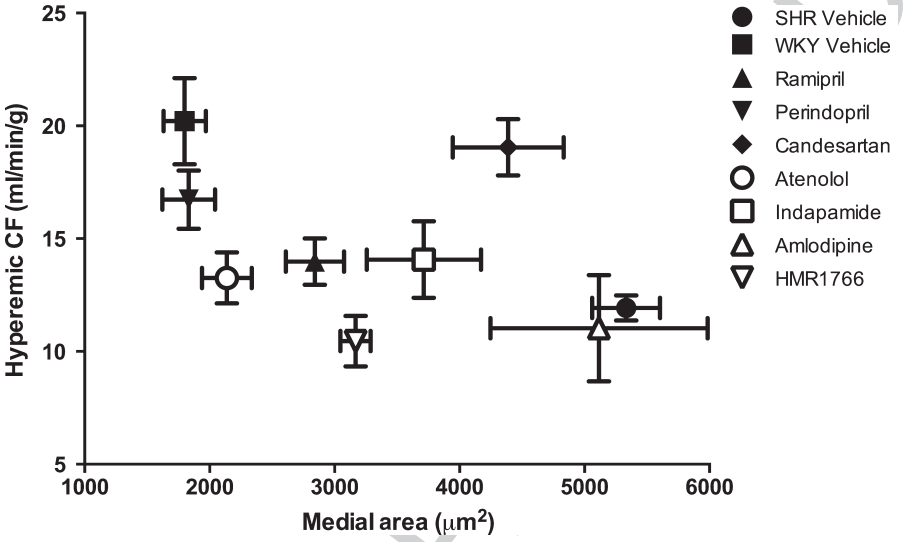


FIGURE 6 Relationship between hyperemic CF and medial area in all groups

could normalize the structure of these vessels.[40] Tomas and colleagues showed that 3 months of treatment with candesartan in hypertensive patients improved CFR without significant effects on carotid intima-media thickness.[41] Bottcher et al., however, have shown that peripheral perfusion responses to transient forearm ischemia do not correlate with hyperemic coronary blood flow measured with PET.[42] This lack of correlation indicates that the mechanisms of microvascular control in peripheral and coronary vascular beds are different and data from one district cannot be necessarily extrapolated to the other. It is also noteworthy that the effect of candesartan on microvascular function is partly due to amelioration of endothelial dysfunction which could explain the lack of reverse arteriolar remodeling despite the observed increase in hyperemic CF.[11]

In this study, the beta-blocker atenolol induced a significant reduction in interstitial fibrosis and arteriolar medial area without improving hyperemic CF. Consistent with our results, Buus et al. showed that 1 year treatment with atenolol had no effect on CFR measured with PET in hypertensive patients.[43] The role of atenolol on SBP and CF is controversial and this could be due to a variety of causes. The discrete reduction of SBP observed in the SHR treated with atenolol could be either caused by an increased peripheral resistance or circulating norepinephrine.[44] Moreover, atenolol, at the dose used in this study, could cause blockade of β2-adrenoceptors that mediate vasodilatation and unmasking of α_2-adrenoceptors with resultant vasoconstriction/reduced vasodilatation of resistance vessels.[45] These mechanisms could explain the lack of increase in hyperemic CF despite reverse remodeling of the arterioles. The role of α_2-adrenoceptors unmasking could be particularly important in the SHR which is characterized by excessive activity of the sympathetic nervous system and relative NO deficiency.[46]

In our study, indapamide significantly reduced arteriolar remodeling and perivascular fibrosis, but had no effect on CF. Previous work with indapamide on SHR cerebral arterioles showed that low dose (1 mg/kg/day) of the drug normalized cross-sectional area of the vessel wall, but failed to decrease external diameter. These findings suggest that although indapamide treatment may not reduce eutrophic inward remodeling, it may attenuate hypertrophic inward remodeling.[11,47]

Moreover, our results show that the calcium channel blocker amlodipine did not show any effect on CF. This is consistent with several previous studies carried out in hypertensive patients showing that treatment with this drug did not improve CFR measured by magnetic resonance imaging or PET.[48,49] Furthermore, we demonstrated that amlodipine did not induce a reduction in arteriolar medial area, but conversely reduced perivascular fibrosis.

Finally, we analyzed the effects of the experimental compound HMR1766, an sGC activator. This enzyme is the target of NO and mediates the formation of cyclic guanosine monophosphate (cGMP), the second messenger involved in the vasodilator response to NO. Production of NO is known to be reduced in several cardiovascular diseases including hypertension and heart failure. It has been demonstrated that sGC activators could have a beneficial effect in cardiac hypertrophy associated with cardiovascular disease and heart failure.[50,51] A recent study by Fraccarollo et al. has shown that HMR1766 reduced LV diastolic filling pressure and pulmonary edema, improved LV contractile function and diastolic stiffness without lowering blood pressure showing inhibited human cardiac fibroblast differentiation and extracellular matrix protein production.[52] Moreover, Beyer et al. showed that stimulation of sGC with BAY 41-2272 inhibits fibrosis.[53] Consistent with these observations, we found a reduction in interstitial and perivascular fibrosis after treatment with HMR1766. Furthermore, in two different animal models of pulmonary hypertension, Dumitrascu et al. have shown that both the sGC stimulator BAY 41-2272 and the sGC activator BAY 58-2667 could reverse pulmonary microvascular remodeling which is similar to that observed in coronary arterioles in the SHR.[54] Consistent with these results, we demonstrate that HMR1766 does not have any significant effect on SBP, but induces significant reverse remodeling of coronary arterioles as well as reduces interstitial and perivascular fibrosis.

An important limitation of our study is the lack of perfusion fixing at constant pressure that might have influenced the assessment of morphometrical parameters.

5 | PERSPECTIVES

During the past decade coronary microvascular dysfunction has been identified as an important additional mechanism of myocardial ischemia. Coronary microvascular dysfunction, in isolation or in combination with classic atherosclerotic disease of the epicardial arteries, has emerged as a new therapeutic clinical target.

The results of the present investigation provide evidence of different antiremodeling properties of drugs with similar blood pressure lowering effects. Clinically, this information might guide the physician in the choice of the most appropriate compound for those hypertensive patients with symptoms and signs of myocardial ischemia despite angiographically normal coronary arteries in whom angina might be due to microvascular dysfunction.[6]

FUNDING INFORMATION

This work was supported by the Ministero della salute - ricerca finalizzata 2011-2012 [NET-2011-02347173].

CONFLICT OF INTEREST

Paolo G. Camici is a Consultant for Servier International.

ABBREVIATIONS

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BP, blood pressure; BW, body weight; CAD, coronary artery disease; CF, coronary flow; CFR, coronary flow reserve; CMD, coronary microvascular dysfunction; CMD, coronary microvascular dysfunction; HW, heart weight; IHD, ischemic heart disease; LA, lumen area; LVH, left ventricular hypertrophy; NO, nitric oxide; PET, positron emission tomography; SBP, systolic blood pressure; SEM, standard error of the mean; sGC, soluble guanylate cyclase; SHR, spontaneously hypertensive rat; VA, vessel area; WKY, Wistar kyoto.

REFERENCES

1. Drazner MH. The progression of hypertensive heart disease. *Circulation*. 2011;123:327–334.
2. Hermann M, Flammer A, Luscher TF. Nitric oxide in hypertension. *J Clin Hypertens*. 2006;8:17–29.
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365:217–223.
4. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903–1913.
5. Murphy BP, Stanton T, Dunn FG. Hypertension and myocardial ischemia. *Med Clin North Am*. 2009;93:681–695.
6. Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med*. 2007;356:830–840.
7. Schwartzkopff B, Motz W, Frenzel H, Vogt M, Knauer S, Strauer BE. Structural and functional alterations of the intramyocardial coronary arterioles in patients with arterial hypertension. *Circulation*. 1993;88:993–1003.
8. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. *Hypertension*. 1999;33:998–1001.
9. Feihl F, Liaudet L, Levy BI, Waeber B. Hypertension and microvascular remodelling. *Cardiovasc Res*. 2008;78:274–285.
10. Mancini M, Petretto E, Kleinert C et al. Mapping genetic determinants of coronary microvascular remodeling in the spontaneously hypertensive rat. *Basic Res Cardiol*. 2013;108:316.
11. Mulvany MJ. Vascular remodelling of resistance vessels: can we define this? *Cardiovasc Res*. 1999;41:9–13.
12. Neglia D, Fommei E, Varela-Carver A et al. Perindopril and indapamide reverse coronary microvascular remodelling and improve flow in arterial hypertension. *J Hypertens*. 2011;29:364–372.

13. Heeneman S, Sluimer JC, Daemen MJ. Angiotensin-converting enzyme and vascular remodeling. *Circ Res*. 2007;101:441–454.
14. Schiffrin EL, Deng LY. Comparison of effects of angiotensin I-converting enzyme inhibition and beta-blockade for 2 years on function of small arteries from hypertensive patients. *Hypertension*. 1995;25:699–703.
15. Dohi Y, Criscione L, Pfeiffer K, Luscher TF. Angiotensin blockade or calcium antagonists improve endothelial dysfunction in hypertension: studies in perfused mesenteric resistance arteries. *J Cardiovasc Pharmacol*. 1994;24:372–379.
16. Li JS, Schiffrin EL. Effect of calcium channel blockade or angiotensin-converting enzyme inhibition on structure of coronary, renal, and other small arteries in spontaneously hypertensive rats. *J Cardiovasc Pharmacol*. 1996;28:68–74.
17. Rizzoni D, Castellano M, Porteri E et al. Effects of low and high doses of fosinopril on the structure and function of resistance arteries. *Hypertension*. 1995;26:118–123.
18. Sharifi AM, Li JS, Endemann D, Schiffrin EL. Effects of enalapril and amlodipine on small-artery structure and composition, and on endothelial dysfunction in spontaneously hypertensive rats. *J Hypertens*. 1998;16:457–466.
19. Shaw LM, George PR, Oldham AA, Heagerty AM. A comparison of the effect of angiotensin converting enzyme inhibition and angiotensin II receptor antagonism on the structural changes associated with hypertension in rat small arteries. *J Hypertens*. 1995;13:1135–1143.
20. Isabelle M, Simonet S, Ragonnet C et al. Chronic reduction of nitric oxide level in adult spontaneously hypertensive rats induces aortic stiffness similar to old spontaneously hypertensive rats. *J Vasc Res*. 2012;49:309–318.
21. Kim S, Zhan Y, Izumi Y, Iwao H. Cardiovascular effects of combination of perindopril, candesartan, and amlodipine in hypertensive rats. *Hypertension*. 2000;35:769–774.
22. Ma F, Lin F, Chen C et al. Indapamide lowers blood pressure by increasing production of epoxyeicosatrienoic acids in the kidney. *Mol Pharmacol*. 2013;84:286–295.
23. Malacco E, Omboni S, Mallion JM, Volpe M; Group ES. Antihypertensive efficacy of olmesartan medoxomil and ramipril in elderly patients with mild to moderate hypertension grouped according to renal function status: a retrospective analysis. *High Blood Press Cardiovasc Prev*. 2012;19:213–222.
24. Nagai M, Horikoshi K, Izumi T et al. Cardioprotective action of perindopril versus candesartan in renovascular hypertensive rats. *Cardiovasc Drugs Ther*. 2004;18:353–362.
25. Oigman W, Gomes MA, Pereira-Barretto AC et al. Efficacy and safety of two ramipril and hydrochlorothiazide fixed-dose combination formulations in adults with stage 1 or stage 2 arterial hypertension evaluated by using ABPM. *Clin Ther*. 2013;35:702–710.
26. Shi Q, Abusarah J, Baroudi G, Fernandes JC, Fahmi H, Benderdour M. Ramipril attenuates lipid peroxidation and cardiac fibrosis in an experimental model of rheumatoid arthritis. *Arthritis Res Ther*. 2012;14:R223.
27. Zhou X, Shang D, Zhang T, Li L, Zhou T, Lu W. Modeling of angiotensin II-angiotensin-(1-7) counterbalance in disease progression in spontaneously hypertensive rats treated with/without perindopril. *Pharmacol Res*. 2012;66:177–184.
28. Rasband WS. ImageJ. U.S. National Institutes of Health, Bethsda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2012.
29. Brilla CG, Janicki JS, Weber KT. Cardioreparative effects of lisinopril in rats with genetic hypertension and left ventricular hypertrophy. *Circulation*. 1991;83:1771–1779.
30. Clozel JP, Kuhn H, Hefti F. Effects of chronic ACE inhibition on cardiac hypertrophy and coronary vascular reserve in spontaneously hypertensive rats with developed hypertension. *J Hypertens*. 1989;7:267–275.
31. Rakusan K, Cicutti N, Maurin A, Guez D, Schiavi P. The effect of treatment with low dose ACE inhibitor and/or diuretic on coronary microvasculature in stroke-prone spontaneously hypertensive rats. *Microvasc Res*. 2000;59:243–254.
32. Kaneko K, Susic D, Nunez E, Frohlich ED. ACE inhibition reduces left ventricular mass independent of pressure without affecting coronary flow and flow reserve in spontaneously hypertensive rats. *Am J Med Sci*. 1997;314:21–27.
33. Ceconi C, Francolini G, Bastianon D, Gitti GL, Comini L, Ferrari R. Differences in the effect of angiotensin-converting enzyme inhibitors on the rate of endothelial cell apoptosis: in vitro and in vivo studies. *Cardiovasc Drugs Ther*. 2007;21:423–429.
34. Joannides R, Bellien J, Thurlure C, Iacob M, Abeel M, Thuillez C. Fixed combination of perindopril and indapamide at low dose improves endothelial function in essential hypertensive patients after acute administration. *Am J Hypertens*. 2008;21:679–684.
35. Young MJ, Funder JW. The renin-angiotensin-aldosterone system in experimental mineralocorticoid-salt-induced cardiac fibrosis. *Am J Physiol*. 1996;271:E883–E888.
36. Zimmermann R, Kastens J, Linz W, Wiemer G, Scholkens BA, Schaper J. Effect of long-term ACE inhibition on myocardial tissue in hypertensive stroke-prone rats. *J Mol Cell Cardiol*. 1999;31:1447–1456.
37. Castoldi G, di Gioia CR, Bombardi C et al. Prevention of myocardial fibrosis by N-acetyl-seryl-aspartyl-lysyl-proline in diabetic rats. *Clin Sci*. 2010;118:211–220.
38. Peng H, Carretero OA, Vuljaj N et al. Angiotensin-converting enzyme inhibitors: a new mechanism of action. *Circulation*. 2005;112:2436–2445.
39. Liska F, Mancini M, Krupkova M et al. Plzf as a candidate gene predisposing the spontaneously hypertensive rat to hypertension, left ventricular hypertrophy, and interstitial fibrosis. *Am J Hypertens* 2014; 27: 99–106.
40. Schiffrin EL. Vascular remodeling in hypertension: mechanisms and treatment. *Hypertension*. 2012;59:367–374.
41. Tomas JP, Moya JL, Barrios V et al. Effect of candesartan on coronary flow reserve in patients with systemic hypertension. *J Hypertens*. 2006;24:2109–2114.
42. Bottcher M, Madsen MM, Refsgaard J et al. Peripheral flow response to transient arterial forearm occlusion does not reflect myocardial perfusion reserve. *Circulation*. 2001;103:1109–1114.
43. Buus NH, Bottcher M, Jorgensen CG et al. Myocardial perfusion during long-term angiotensin-converting enzyme inhibition or beta-blockade in patients with essential hypertension. *Hypertension*. 2004;44:465–470.
44. Schiffrin EL. Remodeling of resistance arteries in essential hypertension and effects of antihypertensive treatment. *Am J Hypertens*. 2004;17:1192–1200.
45. Heusch G, Baumgart D, Camici P et al. Alpha-adrenergic coronary vasoconstriction and myocardial ischemia in humans. *Circulation*. 2000;101:689–694.
46. Pinterova M, Karen P, Kunes J, Zicha J. Role of nifedipine-sensitive sympathetic vasoconstriction in maintenance of high blood pressure in spontaneously hypertensive rats: effect of Gi-protein inactivation by pertussis toxin. *J Hypertens*. 2010;28:969–978.
47. Chillon JM, Baumbach GL. Effects of indapamide, a thiazide-like diuretic, on structure of cerebral arterioles in hypertensive rats. *Hypertension*. 2004;43:1092–1097.
48. Naya M, Tsukamoto T, Morita K et al. Olmesartan, but not amlodipine, improves endothelium-dependent coronary dilation in hypertensive patients. *J Am Coll Cardiol*. 2007;50:1144–1149.
49. Toyama T, Sato C, Koyama K et al. Olmesartan improves coronary flow reserve of hypertensive patients using coronary magnetic resonance imaging compared with amlodipine. *Cardiology*. 2012;122:230–236.
50. Costell MH, Ancellin N, Bernard RE et al. Comparison of soluble guanylate cyclase stimulators and activators in models of cardiovascular disease associated with oxidative stress. *Front Pharmacol*. 2012;3:128.
51. Mitrovic V, Jovanovic A, Lehinant S. Soluble guanylate cyclase modulators in heart failure. *Curr Heart Fail Rep*. 2011;8:38–44.

52. Fraccarollo D, Galuppo P, Motschenbacher S, Ruetten H, Schafer A, Bauersachs J. Soluble guanylyl cyclase activation improves progressive cardiac remodeling and failure after myocardial infarction. Cardioprotection over ACE inhibition. *Basic Res Cardiol*. 2014;109:421.
53. Beyer C, Zenzmaier C, Palumbo-Zerr K et al. Stimulation of the soluble guanylate cyclase (sGC) inhibits fibrosis by blocking non-canonical TGFbeta signalling. *Ann Rheum Dis*. 2015;74:1408–1416.
54. Dumitrascu R, Weissmann N, Ghofrani HA et al. Activation of soluble guanylate cyclase reverses experimental pulmonary hypertension and vascular remodeling. *Circulation*. 2006;113:286–295.

How to cite this article: Mancini, M., Scavone, A., Sartorio, C. L., Baccaro, R. , Kleinert, C., Pernazza, A., Buia, V., Leopizzi, M., D'amati, G. and Camici, P. G. (2016) Effect of different drug classes on reverse remodeling of intramural coronary arterioles in the spontaneously hypertensive rat. Microcirculation, 0:1–9.doi:10.1111/micc.12298

Author Query Form

Journal: MICC

Article: 12298

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

Many thanks for your assistance.

Query reference	Query	Remarks
1	**AUTHOR: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.**	
2	**AUTHOR: In the sentence 'The effect of ... on BP reduction.', please check the usage 'not only', do you mean '...not dependent on...; please rewrite or confirm that the sentence is correct.**	
3	**AUTHOR: To facilitate sequential numbering, reference numbers have been reordered. Please check.**	
4	**AUTHOR: Please give address information for Servier: town, state (if applicable), and country.**	
5	**AUTHOR: Please give address information for Sigma Aldrich: town, state (if applicable), and country.**	
6	**AUTHOR: Please give address information for Sanofi: town, state (if applicable), and country.**	
7	**AUTHOR: Please give address information for AD Instruments: town, state (if applicable), and country.**	
8	**AUTHOR: Please give address information for Transonic Systems Inc: town, state (if applicable).**	
9	**AUTHOR: Please not that "but amlodipine" has been changed to "except for amlodipine" in the sentence "Lumen diameter did not ... treatment groups but amlodipine." Is this correct?**	
10	**AUTHOR: References [32] and [33] are identical. Hence, reference [33] is deleted and rest of the references is renumbered. Please check.**