RESEARCH ARTICLE

Enhancement of EPC migration by high-dose lisinopril is superior compared to captopril and ramipril [version 1; peer review: 2 approved]

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Abstract

Background: Angiotensin-converting enzyme (ACE) inhibitors have been shown to promote endothelial progenitor cell (EPC) function. However, the efficacies of different ACE inhibitors in improving the migratory capabilities of ECPs in coronary artery disease (CAD) patients is unclear. This study compared the effectiveness of captopril, lisinopril, and ramipril toward the migration capability of impaired EPCs from CAD patients.
Methods: We isolated peripheral blood mononuclear cells (PBMCs), separated EPCs from PBMCs, and divided them into an untreated group (control) and treated groups of captopril, lisinopril, and ramipril at doses of 1mM, 10mM, and 100mM. EPC migration was evaluated using the Boyden chamber assay. Analysis of variance (ANOVA) was performed using SPSS 25.0.
Results: This study showed that treatment with captopril, lisinopril, and ramipril starting at the lowest dose (1 mM) increased EPC migration (65,250 ± 6,750 cells; 60,750± 5,030 cells; and 49,500 ± 8,400 cells, respectively) compared to control (43,714 ± 7,216 cells). Increased migration of EPCs was observed by increasing the treatment dose to 10 mM with captopril, lisinopril, and ramipril (90,000 ± 16,837 cells; 79,071 ± 2,043 cells; and 64,285 ± 11,824 cells, respectively). The highest EPC migration was shown for lisinopril 100 mM (150,750 ± 16,380 cells), compared to captopril and ramipril at the same dose (105,750 ± 8112 cells and 86,625 ± 5,845 cells, respectively).
Conclusions: Captopril, ramipril, and lisinopril were shown to increase

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EPC migration in a dose-dependent manner. Low-dose (1 mM) and medium-dose (10 mM) captopril had a larger effect on ECP migration than lisinopril and ramipril. Meanwhile, high-dose lisinopril (100mM) had the highest migration effect, suggesting it may be preferable for promoting EPC migration in CAD patients.

Keywords
ACE Inhibitors, Coronary artery disease, Endothelial progenitor cells, Migration

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Introduction

Endothelial dysfunction and impaired endothelial regeneration are thought to play an important role in the pathogenesis of arteriosclerosis in coronary arterial disease (CAD)[1]. Endothelial regeneration is not only fulfilled by resident endothelial cells but also repaired by endothelial progenitor cells (ECPs) originating from the bone marrow[2]. ECPs are premature circulating cells, a specific subtype of hematopoietic stem cells that differentiate into endothelial cells *in situ* and promote neovascularization[3,4]. Several studies have shown that in patients with CAD, there is a significant decrease in the number and migratory function of circulating EPCs, which leads to impaired neovascularization of ischemic tissue[5,6]. Low EPC counts can predict severe endothelial dysfunction, cardiovascular events, and deaths from cardiovascular causes[7,8]. It is suggested that intracellular damage and impaired redox balance in EPCs due to oxidative stress are the predisposes of imbalance in vascular pathology[9,10].

Angiotensin-converting enzyme (ACE) inhibitors are widely used in cardiovascular disease and have been shown to be associated with beneficial effects on EPCs in several *in vitro* and clinical studies[11–14]. An animal study on mice with increased left ventricular pressure showed that ramipril increases the number and improves EPC migration[12]. A clinical study in hypertensive patients showed that enalapril and zofenopril reduce EPC levels and prevent vascular damage and carotid intima-media thickening[13]. In a small clinical trial, administration of ramipril for four weeks in patients with stable CAD augments and increases the functional activity of EPCs, including migration, adhesion, and *in vitro* capacity of vasculogenesis[15].

However, no studies have investigated the role of ACE inhibitors of captopril and lisinopril in relation to the EPCs. In addition, the comparison between different types of ACE inhibitors toward the impaired migration function of EPCs remains to be investigated. We aimed to evaluate the effects of captopril, lisinopril, and ramipril on EPCs migration from CAD patients.

Methods

Ethical statement

Our study protocol was approved by the Institutional Ethics Committee of Dr. Soetomo General Hospital (945/KEPK/II/2019). Informed consent for peripheral blood sampling procedures and participation in research studies was obtained from all patients before the blood was drawn. We have omitted all data that could reveal the identity of the patients.

Study population

In the present study, we used peripheral blood samples from the same participants and performed similar methodology to that described in our previous study[16]. From June 2018 to December 2018, we studied a total of eight patients with stable CAD who underwent coronary angiography. Only patients with the left main coronary artery stenosis of more than 50% or stenosis in other coronary arteries more than 70% were recruited. To prevent the effects of myocardial ischemia on ECP kinetics, we excluded patients with a history of new-onset acute myocardial infarction. In addition, patients with anemia, diabetes, a history of percutaneous coronary intervention, or coronary artery bypass grafting were not included in the study. Physical examination was performed to determine body mass index (BMI) and to assess the vital signs. We also examined the lipid profile and performed echocardiography to assess left ventricular function. The characteristics of the study population are summarized in Table 1.

Preparation of blood samples and mononuclear cell isolation

We collected 40 ml peripheral blood samples from the median cubital vein following WHO guidelines on drawing blood[17]. From freshly drawn heparinized blood, we isolated peripheral blood mononuclear cells (PBMCs) using Ficoll Histopaque 1077 (Sigma-Aldrich, USA). Briefly, peripheral blood was diluted 1:1 with phosphate buffer saline (PBS) + 2% fetal bovine serum (FBS) to a total volume of 30–35 ml. It was then carefully layered into 20 ml of Ficoll Histopaque 1077 (Sigma-Aldrich, USA) in a 50 ml conical tube. Subsequently, the tube was put into a centrifuge at 300xg for 30 minutes. The PBMC layer was obtained in the form of a buffy coat layer. Using a sterile plastic pipette, the PBMCs were carefully taken and put into another 50 ml conical tube. Furthermore, PBMC was added with PBS + 2% FBS in a ratio of 1: 1, then stirred until homogeneous and centrifuged at 300xg for 7 minutes. This step was repeated with the supernatant removed, 15 ml of PBS + 2% FBS was added to the precipitate formed at the bottom of the tube and centrifuged at 300xg for 7 minutes. Finally, the supernatant was removed, and the sediment was dissolved with a basal medium. Cells were concentrated up to 5×10^6 cells/ml.

Table 1. The characteristics of the study population.

Variable	Mean ± SD
Age (years)	54.50 ± 4.31
BMI (kg/m2)	25.39 ± 2.13
Heart rate (times/minute)	86 ± 8.68
Systolic blood pressure (mmHg)	137.50 ± 24.35
Diastolic blood pressure (mmHg)	80 ± 7.56
Triglyceride (mg/dl)	97 ± 11.64
LDL (mg/dl)	145 ± 61.11
HDL (mg/dl)	35 ± 7.64
Total cholesterol (mg/dl)	200.50 ± 74.75
Left ventricle ejection fraction (%)	53.5 ± 4.11

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

Isolation and culture of endothelial progenitor cells

To separate EPCs from PBMCs, we used standard protocols[18]. Briefly, PBMCs isolated from blood samples in a concentration of 5×10^6 cells/mL were collected in Stemline II Hematopoietic Stem Cell Expansion Medium (Sigma-Aldrich, USA) supplemented with endothelial basal medium (EBM) containing 40 ng/ml of vascular endothelial growth factor (VEGF) and 15% FBS. Then PBMCs were seeded in the six-well plate with fibronectin coating. The cultures were maintained with a humidified atmosphere at 37°C and 5% carbon dioxide. Forty-eight hours after seeding, we separated the medium liquid ontaining the non-adherent cells from the adherent cells attached to the bottom of the plate. All the medium liquid containing the non-adherent cells was collected into one tube, centrifuged with a spin at 300xg for 7 minutes, and the supernatant was discarded. The precipitate formed was dissolved with basal medium and supplement with a concentration of 1×10^6 cells/ml. We confirmed the cells as EPCs through immunofluorescence tests using fluorescein isothiocyanate (FITC) mouse anti-human CD34 monoclonal antibody (cat. no. 343604; Biolegend, USA).

Treatment groups

Isolated EPCs were divided into three treatment groups and one negative control group. The treatment groups were divided into 1) captopril 1 μM, 10 μM, and 100 μM; 2) lisinopril 1 μM, 10 μM, and 100 μM; and 3) ramipril 1 μM, 10 μM, and 100 μM. The cultures were maintained with a humidified atmosphere at 37°C and 5% carbon dioxide for 48 hours. Each data point represented the mean value of quadruplicate cultures.

Migration assay

We used the Boyden chamber migration assay to measure ECP migration. Briefly, migration chambers with 8μm pore-size filters were placed in 24-well plates. Using 1 mmol/L EDTA in PBS, isolated EPCs were detached and then centrifuged at 400xg for ten minutes. EPCs were seeded in the upper chamber (5×10^5/ml in serum-free medium), and the lower compartment of the Boyden chamber was filled with endothelial basal medium. After 24 hours of incubation at 37°C, we scraped off non-migratory cells on the upper chamber with cotton swabs. The migration chamber was put into a new basal medium and added with 500μL of trypsin + EDTA 0.5% solution. After 10 minutes of incubation, we verified using a light microscope to ensure more than 90% of adhering cells were released from the lower surface of the migration chambers. For quantification, the cells were harvested and stained with trypan blue/Giemsa. Migrated ECPs were counted using an TC20 automated cell counter (Bio-Rad, USA).

Statistical analyses

Continuous data were presented as mean ± SD. Multiple experimental group analysis of total migrated EPCs was performed using analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant. All statistical analysis was completed using SPSS version 25.0 for Windows.

Results

CD34 expression and migration capability of endothelial progenitor cells

CD34 is a positive marker for EPCs, and CD34 expression was found in the early to mature culture of EPCs. CD34 expression was characterized by the presence of green luminescence using a fluorescence microscope, indicating the presence of EPCs, as shown in Figure 1[19]. The migration capability of EPCs was evaluated by calculating the number of cells that moved from the upper chamber to the membrane facing the lower chamber with Giemsa staining (Figure 2).

ACE inhibitors increased endothelial progenitor cells migration

The number of EPC migrations in the captopril-treated group at different doses (65,250 ± 6,750 cells at 1 mM; 90,000 ± 16,837 cells at 10mM; and 105,750 ± 8112 cells at 100 mM) was significantly higher than the control group (43,714 ± 7,216 cells) ($p < 0.05$) (Figure 3). The number of EPC migrations in the lisinopril-treated group at different doses (60,750 ± 5,030 cells at 1 mM; 79,071 ± 2,043 cells at 10mM; and 150,750 ± 16,380 cells at 100 mM) was significantly higher than the control group (43,714 ± 7,216 cells) ($p < 0.05$) (Figure 4). The number of EPC migrations in the ramipril-treated group at different doses (49,500 ± 8,400 cells at 1 mM; 64,285 ± 11,824 cells at 10mM; and 86,625 ± 5,845 cells at 100 mM) was significantly higher than the control group (43,714 ± 7,216 cells) ($p < 0.05$) (Figure 5).

The increase in the migration of EPCs was consistent with the increase in the dose of ACE inhibitor. Captopril at doses of 1 mM and 10 mM had a higher migration effect than lisinopril and ramipril at the same doses ($p < 0.05$). Meanwhile,

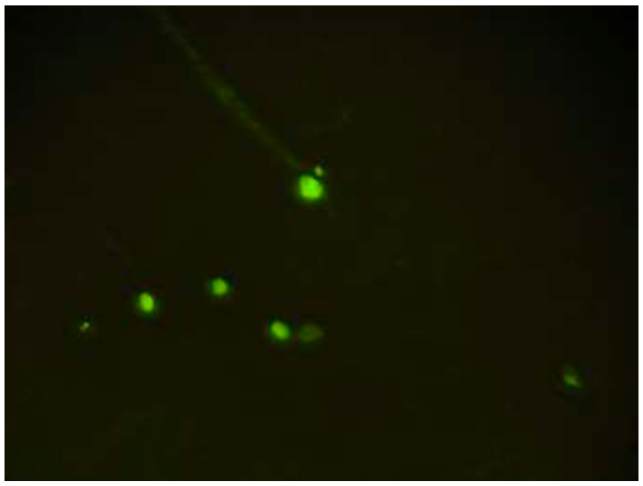

Figure 1. CD34 expression was characterized by the presence of green luminescence in endothelial progenitor cell culture.

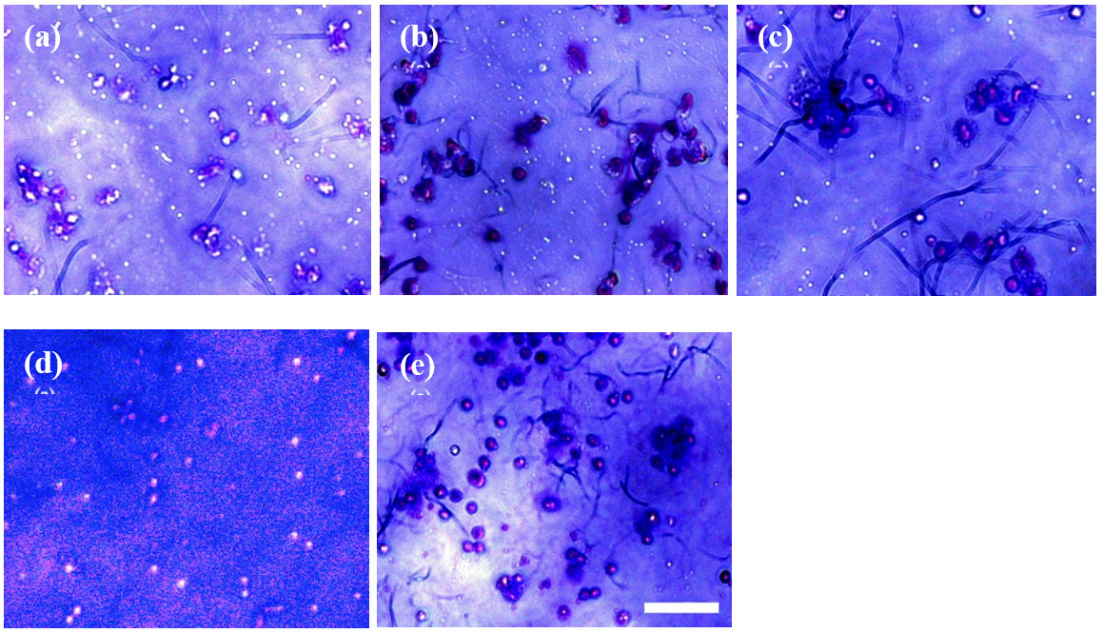

Figure 2. Light-inverted microscope view of endothelial progenitor cells under 48 h-treatment of (**a**) 100 mM captopril, (**b**) 100 mM lisinopril, (**c**) 100 mM ramipril, (**d**) negative control (medium only), and (**e**) positive control (100 ng/mL VEGF). White bar represents 100μM.

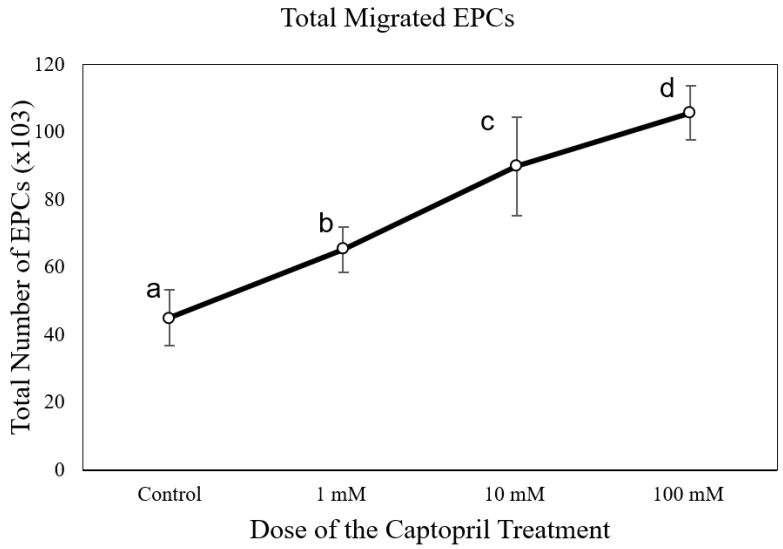

Figure 3. Total migrated endothelial progenitor cells (EPCs) on increasing dose of captopril treatment. Total migrated cells are expressed as mean ± SD (n = 4). Different annotations [a,b,c,d] denounce significant difference in ANOVA test ($p<0.05$).

lisinopril at a dose of 100mM had the highest migration effect ($p < 0.05$) (Figure 6).

Discussion

In this present study, we demonstrated that captopril, lisinopril, and ramipril therapy in EPC cultures from CAD patients was associated with improved migration of EPCs. This study showed that ACE inhibitor treatment increases EPCs migration in a dose-dependent manner. At the doses of 1 mM and 10 mM, there was no significant difference in EPCs migration between captopril and lisinopril. However, both of them exceeded the results of ramipril at the same dose. Meanwhile, lisinopril at the dose of 100 mM had a superior outcome compared to captopril and ramipril at the same dose.

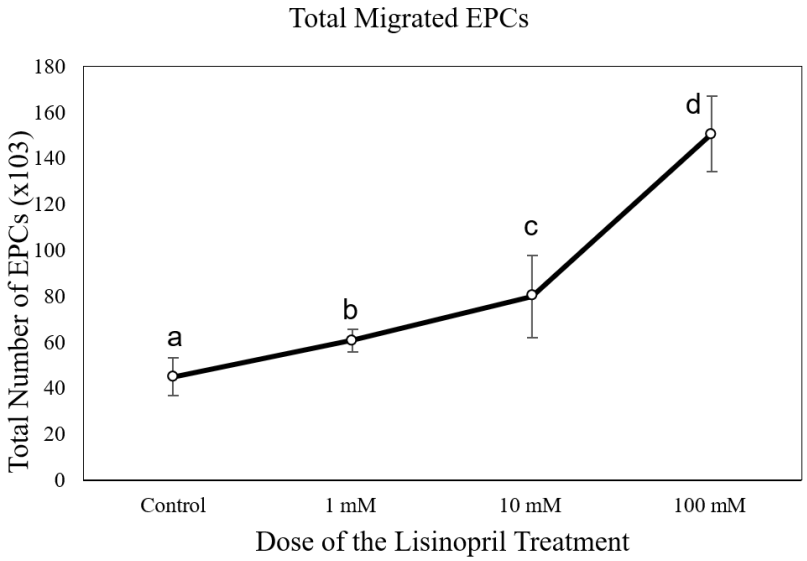

Figure 4. Total migrated endothelial progenitor cells (EPCs) on increasing dose of lisinopril treatment. Total migrated cells are expressed as mean ± SD (n = 4). Different annotations [a,b,c,d] denounce significant difference in ANOVA test ($p<0.05$).

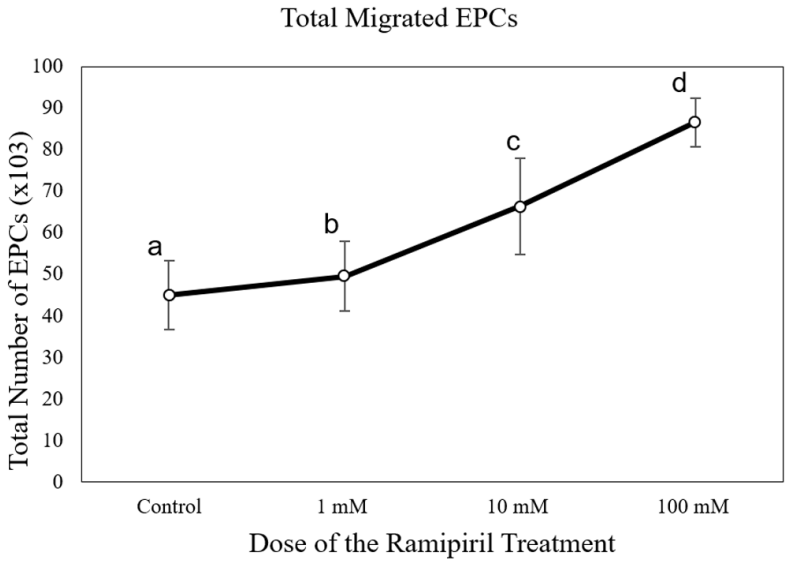

Figure 5. Total migrated endothelial progenitor cells (EPCs) on increasing dose of ramipiril treatment. Total migrated cells are expressed as mean ± SD (n = 4). Different annotations [a,b,c,d] denounce significant difference in ANOVA test ($p<0.05$).

Circulating EPCs are derived from hematopoietic stem cells produced in the bone marrow, which can repair endothelial dysfunction through endogenous mechanisms. In patients with CAD, the number and migration capacity of ECPs are decreased, and thus they are unable to maintain adequate endothelial stability[6,20–22]. During ischemic conditions, EPCs are known to play an essential role in reendothelization and neovascularization. Animal and clinical studies have shown that EPCs contribute up to 25% of newly formed vascular endothelial cells after ischemic conditions[23,24].

Several pharmacological agents have reported the beneficial effects on EPCs, such as HMG-CoA reductase inhibitors/statin[25,26], one of which has been demonstrated by our previous study[16], peroxisome proliferator-activated receptor (PPAR) agonists[27], dihydropyridine calcium channel blocker[28], and angiotensin

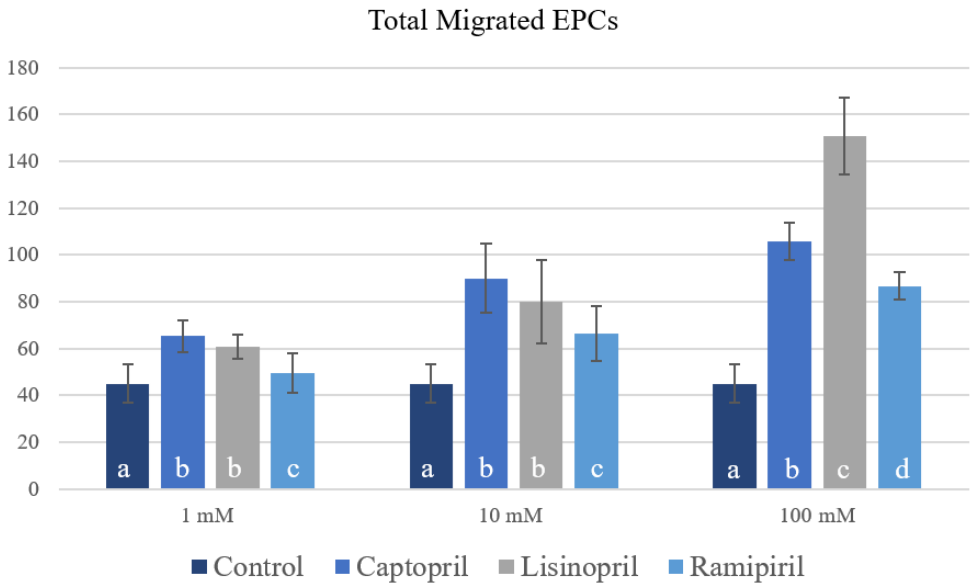

Figure 6. Total migrated endothelial progenitor cells (ECPs) on increasing dose of captopril, lisinopril and ramipiril. Total migrated cells are expressed as mean ± SD (n = 4). Different annotations [a,b,c,d] denounce significant difference in ANOVA test ($p<0.05$).

II receptor antagonists (ARB)[29]. Antioxidative agents with anti-inflammatory properties, such as ginsenoside, salvianolic acids, berberine, Ginkgo biloba, resveratrol, and puerarin, also have been found to increase the number or functional activity of EPCs[30]. ACE inhibitors, which are widely used in cardiovascular therapy, such as for hypertension and congestive heart failure, may have a potential role in restoring the role of EPCs in repair, healing, and neovascularization[11,31]. Several studies have demonstrated the role of ACE inhibitors in increasing the number and function of EPCs in patients with hypertension and stable CAD[13,15]. Each of the ACE inhibitors has a different chemical functional group, which may explain the varying effects of different ACE inhibitor types in several studies, either *in vitro* or *in vivo*. Sulfhydryl-containing ACE inhibitors are known to be the most effective compared to other types of ACE inhibitors[31–34]. Captopril has one sulfhydryl group, and zofenopril has two sulfhydryl groups to coordinate the zinc ion of the active side, whereas lisinopril, ramipril, and enalapril do not have sulfhydryl groups[35–38]. Sulfhydryl-containing ACE inhibitors can reduce oxidative stress and stimulate nitric oxide (NO) activity in human endothelial cells[39] and patients with primary hypertension[40]. *In vitro* studies have shown that zofenopril is more effective compared to enalapril in preventing foam cell formation and thereby slowing atherosclerosis. In addition, zofenopril can also reduce reactive oxygen species and increase NO production in the endothelium[37,41–44]

The finding that ACE inhibition therapy augmented the number of circulating EPCs in patients with CAD, and also enhanced EPCs functional activity, may provide a novel strategy to improve neovascularization and reendothelialization after ischemia, thereby providing a therapeutic concept to improve EPC numbers and functions in patients with CAD.

Conclusion

Captopril, ramipril, and lisinopril were shown to increase EPC migration in a dose-dependent manner. Low-dose (1 mM) and medium-dose (10 mM) captopril had a larger effect on ECP migration than lisinopril and ramipril. Meanwhile, high-dose lisinopril (100mM) had the highest migration effect, suggesting it may be preferable for promoting EPC migration in CAD patients.

Data availability

Underlying data

Figshare: Dataset for Enhancement of EPC migration by high-dose lisinopril is superior compared to captopril and ramipril. https://doi.org/10.6084/m9.figshare.13130303.v2[19]

This project contains the following underlying data:

- Transwell_Migration_Assay_Dataset.xlsx
- Image Repository.zip (original, unedited microscopy images in JPG format)
- Clinical and demographic data of study population.docx

References

1. Ross R: **Atherosclerosis--an inflammatory disease.** *N Engl J Med.* 1999; **340**(2): 115–126.
PubMed Abstract | Publisher Full Text

2. Endtmann C, Ebrahimian T, Czech T, *et al.*: **Angiotensin II impairs endothelial progenitor cell number and function *in vitro* and *in vivo*: implications for vascular regeneration.** *Hypertension.* 2011; **58**(3): 394–403.
PubMed Abstract | Publisher Full Text

3. Asahara T, Masuda H, Takahashi T, *et al.*: **Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization.** *Circ Res.* 1999; **85**(3): 221–228.
PubMed Abstract | Publisher Full Text

4. Hu Y, Foteinos G, Xiao Q, *et al.*: **Rapid endothelial turnover in atherosclerosis-prone areas coincides with stem cell repair in apoe-deficient mice.** *Atherosclerosis.* 2008; **199**(2): 467.
Publisher Full Text

5. Oktaviono YH, Sargowo D, Widodo MA, *et al.*: **Proliferation of Peripheral Blood-derived Endothelial Progenitor Cells from Stable Angina Subjects.** *Indones Biomed J.* 2014; **6**(2): 91.
Publisher Full Text

6. Vasa M, Fichtlscherer S, Aicher A, *et al.*: **Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease.** *Circ Res.* 2001; **89**(1): E1–7.
PubMed Abstract | Publisher Full Text

7. Werner N, Wassmann S, Ahlers P, *et al.*: **Endothelial progenitor cells correlate with endothelial function in patients with coronary artery disease.** *Basic Res Cardiol.* 2007; **102**(6): 565–571.
PubMed Abstract | Publisher Full Text

8. Werner N, Kosiol S, Schiegl T, *et al.*: **Circulating endothelial progenitor cells and cardiovascular outcomes.** *N Engl J Med.* 2005; **353**(10): 999–1007.
PubMed Abstract | Publisher Full Text

9. Loomans CJM, De Koning EJP, Staal FJT, *et al.*: **Endothelial progenitor cell dysfunction in type 1 diabetes: another consequence of oxidative stress?** *Antioxid Redox Signal.* 2005; **7**(11–12): 1468–1475.
PubMed Abstract | Publisher Full Text

10. Rehman J, Li J, Orschell CM, *et al.*: **Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors.** *Circulation.* 2003; **107**(8): 1164–1169.
PubMed Abstract | Publisher Full Text

11. You D, Cochain C, Loinard C, *et al.*: **Combination of the angiotensin-converting enzyme inhibitor perindopril and the diuretic indapamide activate postnatal vasculogenesis in spontaneously hypertensive rats.** *J Pharmacol Exp Ther.* 2008; **325**(3): 766–773.
PubMed Abstract | Publisher Full Text

12. Müller P, Kazakov A, Jagoda P, *et al.*: **ACE inhibition promotes upregulation of endothelial progenitor cells and neoangiogenesis in cardiac pressure overload.** *Cardiovasc Res.* 2009; **83**(1): 106–114.
PubMed Abstract | Publisher Full Text

13. Cacciatore F, Bruzzese G, Vitale DF, *et al.*: **Effects of ACE inhibition on circulating endothelial progenitor cells, vascular damage, and oxidative stress in hypertensive patients.** *Eur J Clin Pharmacol.* 2011; **67**(9): 877–883.
PubMed Abstract | Publisher Full Text

14. Wang CH, Verma S, Hsieh IC, *et al.*: **Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system.** *J Mol Cell Cardiol.* 2006; **41**(1): 34–43.
PubMed Abstract | Publisher Full Text

15. Min TQ, Zhu CJ, Xiang WX, *et al.*: **Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease.** *Cardiovasc Drugs Ther.* 2004; **18**(3): 203–209.
PubMed Abstract | Publisher Full Text

16. Oktaviono YH, Al Farabi MJ, Meuthia F, *et al.*: **Rosuvastatin is Superior Compared to Simvastatin and Atorvastatin to Induce Endothelial Progenitor Cells Migration.** *J Clin Diagnostic Res.* 2019; **13**(5): OC05–OC08.
Publisher Full Text

17. World Health Organization (WHO): **WHO guidelines on drawing blood: best practices in phlebotomy.** World Health Organization. 2010.
Reference Source

18. Bueno-Betí C, Novella S, Lázaro-Franco M, *et al.*: **An affordable method to obtain cultured endothelial cells from peripheral blood.** *J Cell Mol Med.* 2013; **17**(11): 1475–1483.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Al Farabi MJ: **Dataset for Enhancement of EPC migration by high-dose lisinopril is superior compared to captopril and ramipril.** *figshare.* Dataset. 2020.
http://www.doi.org/10.6084/m9.figshare.13130303.v2

20. Fadini GP, Coracina A, Baesso I, *et al.*: **Peripheral blood CD34$^+$KDR$^+$ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population.** *Stroke.* 2006; **37**(9): 2277–2282.
PubMed Abstract | Publisher Full Text

21. Liguori A, Fiorito C, Balestrieri ML, *et al.*: **Functional impairment of hematopoietic progenitor cells in patients with coronary heart disease.** *Eur J Haematol.* 2008; **80**(3): 258–264.
PubMed Abstract | Publisher Full Text

22. Briguori C, Testa U, Riccioni R, *et al.*: **Correlations between progression of coronary artery disease and circulating endothelial progenitor cells.** *FASEB J.* 2010; **24**(6): 1981–1988.
PubMed Abstract | Publisher Full Text

23. Murayama T, Tepper OM, Silver M, *et al.*: **Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization *in vivo*.** *Exp Hematol.* 2002; **30**(8): 967–972.
PubMed Abstract | Publisher Full Text

24. Suzuki T, Nishida M, Futami S, *et al.*: **Neoendothelialization after peripheral blood stem cell transplantation in humans: a case report of a Tokaimura nuclear accident victim.** *Cardiovasc Res.* 2003; **58**(2): 487–492.
PubMed Abstract | Publisher Full Text

25. Llevadot J, Murasawa S, Kureishi Y, *et al.*: **HMG-CoA reductase inhibitor mobilizes bone marrow--derived endothelial progenitor cells.** *J Clin Invest.* 2001; **108**(3): 399–405.
PubMed Abstract | Publisher Full Text | Free Full Text

26. Landmesser U, Engberding N, Bahlmann FH, *et al.*: **Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase.** *Circulation.* 2004; **110**(14): 1933–1939.
PubMed Abstract | Publisher Full Text

27. Gensch C, Clever YP, Werner C, *et al.*: **The PPAR-gamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells.** *Atherosclerosis.* 2007; **192**(1): 67–74.
PubMed Abstract | Publisher Full Text

28. Passacquale G, Desideri G, Croce G, *et al.*: **Nifedipine improves the migratory ability of circulating endothelial progenitor cells depending on manganese superoxide dismutase upregulation.** *J Hypertens.* 2008; **26**(4): 737–746.
PubMed Abstract | Publisher Full Text

29. Bahlmann FH, de Groot K, Mueller O, *et al.*: **Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists.** *Hypertension.* 2005; **45**(4): 526–529.
PubMed Abstract | Publisher Full Text

30. Lin CP, Lin FY, Huang PH, *et al.*: **Endothelial progenitor cell dysfunction in cardiovascular diseases: role of reactive oxygen species and inflammation.** *Biomed Res Int.* 2013; **2013**: 845037.
PubMed Abstract | Publisher Full Text | Free Full Text

31. Napoli C, Cicala C, D'Armiento FP, *et al.*: **Beneficial effects of ACE-inhibition with zofenopril on plaque formation and low-density lipoprotein oxidation in watanabe heritable hyperlipidemic rabbits.** *Gen Pharmacol.* 1999; **33**(6): 467–477.
PubMed Abstract | Publisher Full Text

32. Candido R, Jandeleit-Dahm KA, Cao Z, *et al.*: **Prevention of accelerated atherosclerosis by angiotensin-converting enzyme inhibition in diabetic apolipoprotein E-deficient mice.** *Circulation.* 2002; **106**(2): 246–253.
PubMed Abstract | Publisher Full Text

33. Sun YP, Zhu BQ, Browne AE, *et al.*: **Comparative effects of ACE inhibitors and an angiotensin receptor blocker on atherosclerosis and vascular function.** *J Cardiovasc Pharmacol Ther.* 2001; **6**(2): 175–181.
PubMed Abstract | Publisher Full Text

34. Chobanian AV, Haudenschild CC, Nickerson C, *et al.*: **Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit.** *Hypertension.* 1990; **15**(3): 327–331.
PubMed Abstract | Publisher Full Text

35. Unger T: **The role of the renin-angiotensin system in the development of cardiovascular disease.** *Am J Cardiol.* 2002; **89**(2A): 3A–9A; discussion 10A.
PubMed Abstract | Publisher Full Text

36. Kowala MC, Grove RI, Aberg G: **Inhibitors of angiotensin converting enzyme decrease early atherosclerosis in hyperlipidemic hamsters. Fosinopril reduces plasma cholesterol and captopril inhibits macrophage-foam cell accumulation independently of blood pressure and plasma lipids.** *Atherosclerosis.* 1994; **108**(1): 61–72.
PubMed Abstract | Publisher Full Text

37. Buikema H, Monnink SH, Tio RA, *et al.*: **Comparison of zofenopril and lisinopril to study the role of the sulfhydryl-group in improvement of endothelial dysfunction with ACE-inhibitors in experimental heart failure.** *Br J Pharmacol.* 2000; **130**(8): 1999–2007.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Predel HG, Düsing R, Bäcker A, *et al.*: **Combined treatment of severe essential hypertension with the new angiotensin converting enzyme inhibitor ramipril.** *Am J Cardiol.* 1987; **59**(10): 143D–148D.
PubMed Abstract | Publisher Full Text

39. Jacoby DS, Rader DJ: **Renin-angiotensin system and atherothrombotic disease: from genes to treatment.** *Arch Intern Med.* 2003; **163**(10): 1155–64.
PubMed Abstract | Publisher Full Text

40. Napoli C, Bruzzese G, Ignarro LJ, *et al.*: **Long-term treatment with sulfhydryl angiotensin-converting enzyme inhibition reduces carotid intima-media thickening and improves the nitric oxide/oxidative stress pathways in newly diagnosed patients with mild to moderate primary hypertension.** *Am Heart J.* 2008; **156**(6): 1154.e1–8.
PubMed Abstract | Publisher Full Text

41. Cominacini L, Pasini A, Garbin U, *et al.*: **Zofenopril inhibits the expression of adhesion molecules on endothelial cells by reducing reactive oxygen species.** *Am J Hypertens.* 2002; **15**(10 Pt 1): 891–895.
PubMed Abstract | Publisher Full Text

42. Evangelista S, Manzini S: **Antioxidant and cardioprotective properties of the sulphydryl angiotensin-converting enzyme inhibitor zofenopril.** *J Int Med Res.* 2005; **33**(1): 42–54.
PubMed Abstract | Publisher Full Text

43. de Nigris F, D'Armiento FP, Somma P, *et al.*: **Chronic treatment with sulfhydryl angiotensin-converting enzyme inhibitors reduce susceptibility of plasma LDL to *in vitro* oxidation, formation of oxidation-specific epitopes in the arterial wall, and atherogenesis in apolipoprotein E knockout mice.** *Int J Cardiol.* 2001; **81**(2–3): 107–115; discusssion 115-6.
PubMed Abstract | Publisher Full Text

44. Scribner AW, Loscalzo J, Napoli C: **The effect of angiotensin-converting enzyme inhibition on endothelial function and oxidant stress.** *Eur J Pharmacol.* 2003; **482**(1–3): 95–99.
PubMed Abstract | Publisher Full Text

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Version 1

Reviewer Report 13 October 2021

https://doi.org/10.5256/f1000research.29142.r96122

✔ **Dyana Sarvasti**
Department of Internal Medicine, Faculty of Medicine, Widya Mandala Catholic University, Surabaya, Indonesia

This study explores the role of ACE inhibitors (captopril, lisinopril, and ramipril) in improving the migratory capabilities of EPCs in coronary artery disease (CAD). Overall, the research published by this author is very interesting. However, several things need to be added to this research report so that readers can understand it better. For example, in the abstract, it is necessary to add the characteristics of the patients included in this study so that the reader can understand the study's outline.

In the research method, it is also necessary to explain whether or not the patients included in this study are already on ACE inhibitor therapy. Was the number of patients included in the study (eight patients) sufficient to conclude? What about the effect of other therapies (not ACE inhibitors) that the patient had taken before being included in the study? How to “clean up” the various confounders in the study? Some of these questions may need to be added and explained in this research report.

In addition, there are some inconsistencies in the writing of abbreviations and mistypes that need to be corrected. Here are some points related to this:

- “*However, the efficacies of different ACE inhibitors in improving the migratory capabilities of ECPs in coronary artery disease (CAD) patients **is** unclear*.” The singular verb “**is”** does not appear to agree with the plural subject **"patients"** or **"the efficacies"**.

- *“Keywords: ACE Inhibitors, **C**oronary artery disease, **E**ndothelial progenitor cells, **M**igration*.” It should be in lowercase.

- The description of the **abbreviation CAD** differs between the description in the abstract and the introduction.

- The author is inconsistent in writing the abbreviation for endothelial progenitor cells.

Sometimes it says "**EPC"**, but in other parts, it says "**ECP"**.

- "*It is suggested that intracellular damage and impaired redox balance in EPCs due to oxidative stress **are the predisposes of** imbalance in vascular pathology*." The phrase "**are the predisposes of"** maybe wordy. Consider changing to "**predisposes to**".

- "*Forty-eight hours after seeding, we separated the medium liquid **ontaining** the non-adherent cells from.....*". It should be "**containing**".

- "*The precipitate formed was dissolved with basal medium and **supplement** with.....*". It should be "**supplemented**".

- The author is inconsistent in writing the unit dose of the drug. In the abstract, it says "**mM"**, but in other parts, it says "**μM"**.

- "*Migrated EPCs were counted using **an** TC20 automated cell counter (Bio-Rad, USA)*". It should be "**a**".

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cardiovascular prevention and rehabilitation.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 17 May 2021

https://doi.org/10.5256/f1000research.29142.r84488

Mehdi Hassanpour
Department of Biochemistry and Clinical Laboratories, Tabriz University of Medical Sciences, Tabriz, Iran

The manuscript entitled "***Enhancement of EPC migration by high-dose lisinopril is superior compared to captopril and ramipril***" seems an interesting topic in the field of cardiac and vessel cell biology. However, there are some points that need revision before indexing.

Reviewer comments:

1. The topic is interesting and the aim of the study is commendable, but this article has some typos errors, abbreviations, and grammatical issues and needs editing.

2. The ***EPC*** should be in complete form in the title of the article.

3. As a result of EPC confirmation through immunofluorescence tests, what percentage of cultured cells were CD34 positive?

4. The underlying molecular mechanism and involved signaling pathways of Lisinopril on EPC migration were neglected and should be discussed.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical Biochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.