

INCREASE MIGRATION OF PERIPHERAL BLOOD DERIVED ENDOTHELIAL PROGENITOR CELLS OF STABLE CORONARY ARTERY DISEASE PATIENT WITH ANGIOTENSIN CONVERTING ENZYME INHIBITORS

Hanang Anugrawan Achmad^{1*}, Yudi Her Oktaviano², Djoko Soemantri³

¹Faculty of Dentistry, Airlangga University

²Department Peridontology, Airlangga University

³Department Peridontology, Airlangga University

*Corresponding author: hananganugrawanachmad@gmail.com

ABSTRACT

This research is based on refractory angina pectoris, which remains a problem despite advances in coronary heart disease treatment. Stem cell therapy is still in preclinical research stages to address refractory angina. Endothelial progenitor cells (EPCs) aid in improving endothelium and the growth of new blood vessels. Heart medication has shown to enhance both the quantity and function of EPCs in patients at cardiovascular risk or with heart disease. Previous studies reported that ACE inhibitors (ACEI) have a positive effect on EPCs. Thus, this study analyzes the impact of three different ACE inhibitors on EPC migration in laboratory conditions. Its aim is to ascertain the increase in EPC migration in stable coronary heart disease patients after ACEI administration. The research methodology involves an experimental design with a control group and post-treatment assessment only. Mononuclear cells are isolated from stable coronary heart disease patients' peripheral blood and incubated for 3 days. The EPCs are then divided into captopril, ramipril, lisinopril, and a control group, observed for 48 hours. EPC migration is assessed by counting the cells moving from the upper chamber to the membrane facing the lower chamber using a transwell migration assay after 20 hours, observed with a light microscope and Giemsa staining. Data analysis via ANOVA statistical tests indicates increased EPC migration in the captopril, ramipril, and lisinopril groups compared to the control. Captopril shows the highest effect among the groups, while no significant difference is observed between captopril and lisinopril, as well as between ramipril and lisinopril.

Keywords: Captopril; lisinopril; EPC migration; coronary artery disease; ramipril

INTRODUCTION

Coronary Heart Disease (CHD) is the cause of more than 8.9 million deaths worldwide every year and is the main cause of death for both men and women in 2015. Improved life expectancy for CHD sufferers in several decades can be related to better medical therapy and revascularization management modalities that are increasingly developing in patients with CHD. (Briceno, et al. 2016; Wang, et al. 2016).

Angina is the most common clinical manifestation in patients with stable CHD which describes an imbalance between myocardial supply and demand related to ischemia or hypoxia. Treatment of patients with CHD has developed rapidly but still has problems, namely refractory angina.

Various studies on stem cell therapy have been carried out in Acute Myocardial Infarction (AMI) and chronic ischemic heart failure. The main goal of stem cell therapy in various studies is myocardial muscle regeneration and revascularization which is expected to improve

METHODS

The design of this study was an experimental laboratory study (in vitro study) by administering ACEI to peripheral blood EPCs of stable CHD patients using the “Posttest only control group design” approach or design.

The experimental unit used in this study was venous blood taken from stable CHD patients. Mononuclear cells were isolated from the peripheral blood samples and then cultured in basal medium. The

cardiac contractility and bioelectrical conduction without serious side effects. (Lezaic, et al. 2013; Van der Spoel, et al. 2011).

Endothelial Progenitor Cell (EPC) has a major role in the formation of new blood vessels. Along with the progress of research on EPC in recent years, EPC has become a potential therapeutic target to stimulate angiogenesis, vasculogenesis and improve cardiac performance. (Alba, et al. 2012; Siddique, et al. 2010; Zampetaki, et al. 2008).

Angiotensin Converting Enzyme Inhibitors (ACEI) have been shown to benefit cardiovascular disease thought to be related to EPC function. The number of studies with human subjects examining the effect of ACEI on EPC is still limited. ACEI increased the number, proliferation, migration, adhesion, and formation of EPCs cultured from patients with CHD in in vivo studies. Ramipril increases circulating EPC in patients with acute coronary syndrome as measured by flow cytometry.

experimental unit was then grouped into four groups: group 1 was given captopril, group 2 was given ramipril, group 3 was given lisinopril, and the control group was not given any treatment. A number of EPC cells that had been cultured with the treatment and control groups were placed in the upper cavity of the Transwell Inserts System. The lower cavity of the Transwell Inserts System

is filled with basal medium. Assessment of EPC migration was carried out 20 hours after

treatment.

RESULTS

This study used purposive sampling of patients who met the inclusion and exclusion criteria. Mononuclear cells were separated from other blood elements using Ficoll histopaque. The separated cells were implanted for 3 days in culture media to obtain EPC cells as evidenced by the CD34 marker. EPC cells were counted and implanted with the same number in each treatment and control group for 2 days.

CD34 expression was found in young to more mature EPC cells. Examination was carried out in one of the wells after being cultured in basal medium, washed with PBS and prepared for immunofluorescence examination using CD34, after which it was observed using a fluorescence microscope and CD34 expression was obtained which was marked with a green glow.



Figure 1. CD34 marker expression

Assessment of migrating EPCs using quantification of the number of EPCs migrating towards the membrane facing the lower cavity of

the Transwell Inserts System using a light microscope.

Table 1. Number of EPCs That Migrated

Treatment	Mean ± Standard Intersection	95% CI
Captopril 10 µmol/l	90.000,00 ± 16.837, 458	74.427, 95 – 105.572,05
Ramipril 10 µmol/l	64.285,71 ± 11.824,611	53.349,77 – 75.221,66

Treatment	Mean ± Standard Intersection	95% CI
Lisinopril 10 µmol/l	79.071,43 ± 20.433,691	60.173,42 – 97.969,44
Control	43.714,29 ± 7.216,054	37.040,55 – 50.388,02

The results of calculating the average migration of EPC with Lisinopril 10 µmol/l treatment were $79,071.43 \pm 2,0433.691$ cells. Meanwhile, the average EPC migration in the control group was $43,714.29 \pm 7,216.054$ cells.

EPC migration data in all groups was tested for data normality using the Kolmogorov Smirnov statistical test with the results of all data being normally distributed. Then the data in each group can be followed by analysis using the

ANOVA statistical test which shows a significant difference, namely the p value of 0.000. Then the Least Significant Difference (LSD) test was carried out for all treatment and control groups.

A microscopic view of the number of EPC migrating from the upper cavity to the membrane facing the lower cavity Transwell Inserts System in the captopril, ramipril, lisinopril and control groups is shown in Figure 2.

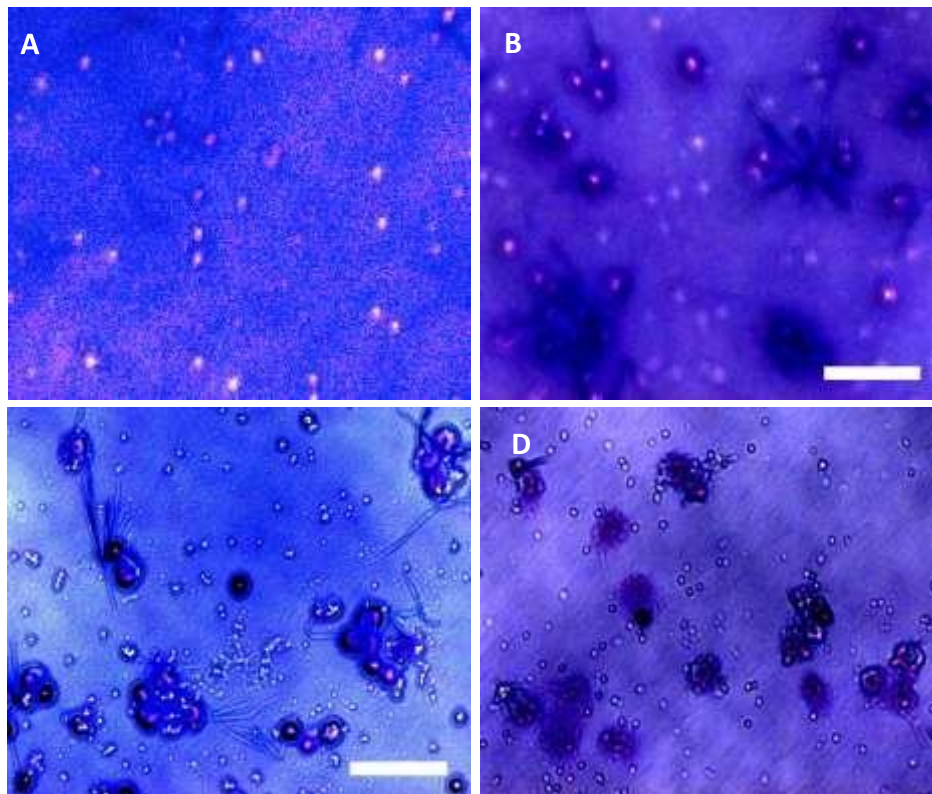


Figure 2. Microscopic appearance of EPC migration in A. control group, B. Captopril 10 µmol/l, C. Ramipril 10 µmol/l, D. Lisinopril 10 µmol/l

DISCUSSION

The ability of EPC migration is an important factor in supporting the process of vasculogenesis and repairing endothelial damage and dysfunction. EPC migration during the process of vasculogenesis is necessary to form the primary vascular plexus. This study analyzed the administration of captopril, ramipril and lisinopril in an effort to increase peripheral blood EPC migration activity in patients with stable Coronary Heart Disease. The results of the EPC migration data analysis showed a significant increase in all types of ACEIs, namely captopril, ramipril and lisinopril compared to the control group.

The increase in EPC migration is influenced by various drug preparations in one class with several mechanisms underlying both the classical angiogenic pathway and outside the pathway. Research by (You et al., 2008) which gave a combination of indapamide and perindopril to rats with limb ischemia was able to improve EPC function and angiogenesis ability.

Angiotensin II exerts a detrimental effect on CD34+ cell proliferation, but other studies have shown that it stimulates VEGFR-2 mRNA resulting in VEGF-induced EPC proliferation. (Imanishi et al., 2005). This study was an In Vitro study where no pulmonary vascular endothelium was found where ACE activity is commonly found to convert angiotensin I to angiotensin II.

Zambidis et al (2008) found expression of ACE (CD 143) on various cell surfaces including differentiated stromal and endothelial cells. ACE

has co-expression with various markers of hematoendothelial cells such as CD34, CD31, KDR/flk-1 which are also owned by EPC. Research by (Kränkel et al., 2008) explained that ACE is also expressed by EPC via quantitative PCR although at low levels. This is based on the research of (Kohlstedt, et al. 2002) which explained that ACE, which is located on the cell membrane, has a cytoplasmic tail containing 3 and 5 serine residues, one of which is in a sequence of 13 amino acids protected by a COOH terminal at the end of the protein.

The kallikrein-kinin system plays a role in tissue revascularization that is experiencing ischemia. Kinin is a substance produced from the breakdown of koninogen by kallikrein which is detected in various tissues.

This study showed that the increase in EPC migration in the captopril group was higher than in ramipril and lisinopril. After being analyzed statistically, a significant difference in improvement was only found between the captopril and ramipril groups. Whereas there was no difference in the increase in EPC migration between the captopril and lisinopril groups and the ramipril and lisinopril groups. (Cacciatore, et al. 2011) studied the increase in the number of circulating EPC in hypertensive patients who were given enalapril and zofenopril and the relationship between the number of circulating EPC and BMI over time.

This study only proves that there is a direct effect of ACEI on increasing EPC migration regardless of all the body's homeostatic

processes. Further research on the ACEI mechanism for EPC migration that assesses parameters on the B22R/PI3K/Akt/Enos axis still needs to be done. In this study it was also carried

CONCLUSION

There was an increase in peripheral blood EPC migration in patients with stable coronary heart disease between the group given captopril, ramipril, and lisinopril and the control group.

There was a difference in increased EPC migration in the peripheral blood of patients with

out in vitro so further research is needed in vivo to confirm the effect of ACEI on patients with stable coronary heart disease related to increased EPC migration.

stable Coronary Heart Disease between the captopril and ramipril groups. Whereas there was no difference in the increase in EPC migration between the captopril and lisinopril groups and the ramipril and lisinopril groups.

BIBLIOGRAPHY

Alba, A. C., Delgado, D. H., Rao, V., Walter, S., Guyatt, G. and Ross, H. J. (2012), "Are endothelial progenitor cells a prognostic factor in patients with heart failure?", *Expert review of cardiovascular therapy*, Vol. 10 No. 2, pp. 167–75.

Briceno, N., Schuster, A., Lumley, M. and Perera, D. (2016), "Ischaemic cardiomyopathy: pathophysiology, assessment and the role of revascularisation", *BMJ Journals Heart*, Vol. 102 No. 5, pp. 397-406.

Cacciatore, F., Bruzzese, G., Vitale, D. F., Liguori, A., de Nigris, F., Fiorito, C., Infante, T., Donatelli, F., Minucci, P. B. and Ignarro, L. J. (2011), "Effects of ACE inhibition on circulating endothelial progenitor cells, vascular damage, and oxidative stress in hypertensive patients", *European journal of clinical pharmacology*, Vol. 67, pp. 877-83.

Imanishi, T., Hano, T. and Nishio, I. (2005), "Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress", *Journal of hypertension*, Vol. 23 No. 1, pp. 97–104.

Kohlstedt, K., Shoghi, F., Müller-Esterl, W., Busse, R. and Fleming, I. (2002), "CK2 phosphorylates the angiotensin-converting enzyme and regulates its retention in the endothelial cell plasma membrane", *Circulation research*, Vol. 91 No. 8, pp. 749–56.

Kränkel, N., Katare, R. G., Siragusa, M., Barcelos, L. S., Campagnolo, P., Mangialardi, G., Fortunato, O., Spinetti, G., Tran, N. and Zacharowski, K. (2008), "Role of kinin B2 receptor signaling in the recruitment of circulating progenitor cells with neovascularization potential", *Circulation research*, Vol. 103 No. 11, pp.

- 1335–43.
- Lezaic, L., Haddad, F., Vrtovec, B. and Wu, J. C. (2013), “Imaging Cardiac stem cell transplantation using radionuclide labeling techniques: clinical applications and future directions”, *Methodist DeBakey cardiovascular journal*, Vol. 9 No. 4, pp. 218-22.
- Siddique, A., Shantsila, E., Lip, G. Y. and Varma. (2010), “Endothelial progenitor cells: what use for the cardiologist?”, *Journal of angiogenesis research*, Vol. 2 No. 6, pp. 1-13.
- Van Der Spoel, T. I., Jansen of Lorkeers, S. J., Agostoni, P., Van Belle, E., Gyöngyösi, M., Sluijter, J. P., Cramer, M. J., Doevendans, P. A. and Chamuleau, S. A. (2011), “Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease”, *Cardiovascular research*, Vol. 91 No. 4, pp. 649–58.
- Wang, H., Naghavi, M., Allen, C., Barber, R. M., Bhutta, Z. A., Carter, A., Casey, D. C., Charlson, F. J., Chen, A. Z. and Coates, M. M. (2016), “Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015”, *The lancet*, Vol. 388 No. 10053, pp. 1459–1544.
- You, D., Cochain, C., Loinard, C., Vilar, J., Mees, B., Duriez, M., Levy, B. I. and Silvestre, J. S. (2008), “Combination of the angiotensin-converting enzyme inhibitor perindopril and the diuretic indapamide activate postnatal vasculogenesis in spontaneously hypertensive rats”, *J Pharmacol Exp Ther*, Vol. 325 No. 3, pp. 766–73.
- Zampetaki, A., Kirton, J. P. and Xu, Q. (2008), “Vascular repair by endothelial progenitor cells”, *Cardiovascular research*, Vol. 78 No. 3, pp. 413–21.