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RESEARCH

ROLE OF SIGNAL TRANSDUCTION *ERK1/2* ON THE PROLIFERATION OF *ENDOTHELIAL PROGENITOR CELL* (EPC) OF PATIENTS WITH STABLE ANGINA PECTORIS INDUCED BY GROWTH FACTORS

(Peran Transduksi Sinyal ERK1/2 terhadap Persiapan Proliferasi Endothelial Progenitor Cell (EPC) Pasien Angina Pektoris Stabil yang Diinduksi oleh Faktor Pertumbuhan)

Yudi Her Oktaviono[1,2], Djanggan Sargowo[2,3], Mohammad Aris Widodo[2,4],Yanni Dirgantara[5], Angliana Chouw[5], Ferry Sandra[6,7,8]

ABSTRAK

Sel Progenitor Endotel (EPC) merupakan kelompok sel yang memiliki kekuatan angiogenik yang kemudian dikenal sebagai pilihan pengobatan seluler untuk mengimbas perbaikan lapisan intima pembuluh darah. Berdasarkan beberapa kajian sebelumnya, jumlah EPC di pasien angina pektoris stabil lebih rendah dibandingkan dengan individu yang sehat. Di samping itu, EPC juga dikenal sebagai peramal independen terhadap perjalanan penyakit jantung koroner. Tujuan penelitian ini adalah untuk mengetahui peran transduksi isyarat ERK1/2 terhadap proliferasi EPC yang diambil dari darah tepi pasien angina pektoris stabil dengan imbasan pemberian faktor pertumbuhan. Penelitian ini merupakan kajian percobaan melalui uji laboratoris dengan pendekatan atau rancangan control group time series design. Penelitian dilakukan di Laboratorium Prodia Stem Cell Indonesia di Jakarta pada bulan Januari 2014. Sampel darah tepi diambil dari delapan (8) subjek relawan pasien angina pektoris stabil yang memenuhi patokan kesertaan dan sebagai pembanding digunakan delapan (8) unit darah tepi yang diambil dari orang yang bukan pasien angina pektoris. Metode sel mononuklear (MNC) dari delapan (8) pasien angina pektoris stabil diisolasi selama satu (1) atau tiga (3) hari di medium tertentu dengan atau tanpa penambahan suplemen. EPC yang dihasilkan dan dicat dengan metode pengecatan imunofluoresens untuk mendeteksi CD34, Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) dan CD133. Pemeriksaan proliferasi sel XTT digunakan untuk menilai pertumbuhan EPC setelah kultur antara 1–3 hari, sedangkan perhitungan Colony Forming Unit (CFU) digunakan untuk menilai fungsi EPC kelompok yang terbentuk setelah dikultur antara 1–3 hari. Analisis western blot dilakukan untuk mendeteksi aktifasi ERK1/2. Hasil mengecat imunofluoresens mengukuhkan seluruh petanda membran EPC termasuk CD34, VEGR2 dan CD133. Jumlah rerata EPC yang berdaya hidup di pasien angina pektoris stabil lebih rendah dibandingkan dengan pembandingnya, yaitu masing-masing $5{,}77\times10^3$ dan $23{,}40\times10^3$. Jumlah EPC baik kelompok pasien angina pektoris stabil dan yang pembanding meningkat secara bermakna dengan perangsangan faktor pertumbuhan. Hasil western blot menunjukkan bahwa ERK1 diekspresikan lebih tinggi pasien angina pektoris stabil dibandingkan pembanding. Fosforilasi ERK2 terdeteksi di kelompok pembanding dan menguat secara bermakna seiring waktu dengan perangsangan faktor pertumbuhan. Fosforilasi ini dihambat oleh U0126. Di pasien angina pektoris stabil, fosforilasi ERK2 terdeteksi pada perangsangan faktor pertumbuhan setelah kultur selama tiga (3) hari.

***Kata kunci**: Angina pektoris stabil, EPC, ERK1/2, faktor pertumbuhan*

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ABSTRACT

Endothelial Progenitor Cell (EPC) is a cell with angiogenic potential, the latter is known as an option for cellular-based therapy to induce intimal repair. Based on the former studies, the number of EPC of patients with stable angina pectoris is lower compared with that of healthy individuals. Moreover, EPC is known as an independent predictor of coronary artery disease prognosis. The aim of this study was to know the role of signal transduction ERK1/2 on proliferation of EPC taken from the peripheral blood of patients with stable angina pectoris induced by growth factors. This research is an experimental study through laboratory testing with control group time series design. The study was conducted at the Laboratory of Prodia Stem Cell Indonesia in Jakarta in January 2014. Peripheral blood samples were taken from eight voluntary patients with stable angina pectoris who met the inclusion criteria. As a control other 8 peripheral blood samples were taken from individuals who were not patients with angina pectoris. Using Mononuclear Cell (MNC) method, 8 samples taken from the patients with stable angina pectoris were isolated for one or three days in a particular medium with or without supplement addition. EPC resulted were then stained with immunofluorescence method to detect CD34, Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) and CD133. XTT Cell proliferation assay was used to assess EPC growth after 1-3-day culture, while Colony Forming Unit (CFU) enumeration was used to asses the function of colony of EPC formed after 1–3-day culture. The western blot analysis was used to detect possible activation of ERK1/2. The result of immunofluoresence confirmed all EPC membrane markers including CD34, VEGFR2 and CD133. The baseline average of viable EPC of patients with stable angina pectoris was lower than the controls respectively at $5,77 \times 10^3$ and $23,40 \times 10^3$. The number of EPC of both subjects with stable angina pectoris and control subjectswere significantly increased with growth factor stimulation in a time dependent-manner. The results of western blot showed that higher ERK1 was expressed by subjects with stable angina compared with control subjects. Phosphorylation of ERK2 was detected clearly in control subjects and increased significantly upon growth factor stimulation in a time-dependent manner. The phosphorylation was inhibited by U0126. The subjects with stable angina pectoris, the phosphorylation of ERK2 was detected upon growth factor stimulation in 3-day culture.

Key words: Stable angina pectoris, EPC, ERK1/2, growth factor

INTRODUCTION

Coronary Heart Disease (CHD) is one of the leading causes of death in the world, both in developed and developing countries.[1] The study of society showed that the prevalence of stable angina pectoris as one of the manifestations of CHD, increased sharply along with increasing age in both males and females, between 0.1–1% in females aged 45–54 years and increased to be 10–15% at age 65–74 years. In males aged between 45–54 years, the prevalence was 2–5% and increased to be 10–20% at age 65–74 years. The underlying pathophysiology of CHD is the presence of blood flow disruption of coronary heart due to atherosclerosis triggered mainly by an imbalance of endothelial homeostasis.[1] The findings regarding cell derived from the bone marrow known as endothelial progenitor cell (EPC) that played a role in the repair of endothelial cells and vascular growth have changed the pathogenesis model of this cardiovascular disease.[2]

Treatment to coronary artery may result in serious injury to the endothelial with a risk for long-term endothelial function disorder, thrombogenic reaction and high pressure by balloon inflation can disrupt the balance of EPC function, biological and vascular homeostasis and initiate local inflammatory response.[1,3,4] The research results showed that EPC was essential in quick reendothelialization to restore normal vascular function, lower vascular inflammation, prevent remodeling, angiogenesis and neovascularization of blood vessels. Conversely, inadequate EPC response caused re-endothelialization to delay and persistent inflammation, causing re-stenosis and symptoms of myocardial ischemia.[1,5,6] Therefore, the number of EPC that could be isolated was very small compared with the number required for its treatment, making the efforts to increase the number and function of EPC mainly in CHD patients with clinical symptoms of stable angina pectoris and lower EPC level of blood circulation were still required.

Several studies proved that Vascular Endothelial Growth Factor (VEGF) could induce the differentiation and proliferation of pluripotent adult stem cell into Hematopoeietic Stem Cell (HSC) and cardiac myocyte through phosphorylation of Mitogen-Activated Protein Kinase/Extra Cellular Signal-Regulated Kinase (MAPK/ERK). The in vitro study conducted by Xu *et al*[7] using rat blood showed that VEGF could trigger the differentiation of Multipotent Adult Progenitor Cells (MAPCs) into endothelial cells through signaling pathways of MAPK/ERK1/2.[7–10]

Pathway of MAPK/ERK involves a variety of proteins that can cause cell division and trigger differentiation of Embryonic Stem Cell (ESC). ERK1 and ERK2are members of MAPK and a key to signal transduction pathways that regulate a variety of cellular processes, including proliferation, differentiation and cell survival. Abnormal arrangement of this pathway has been reported for a variety of diseases, including cardiovascular. Although progress has been made in identifying the mechanisms that control cell proliferation mediated by ERK1/2, further research is still required to determine the conditions that allow the activation of ERK1/2 responsible for the proliferation and apoptosis.[11,12]

In connection with the description of the background of the problem, the problems in this study can be formulated as follows: What is the role of signal transduction of ERK1/2 on proliferation of EPC taken from the peripheral blood of patients with stable angina pectoris induced with growth factors? The specific objective of this study was: To determine the expression level and phosphorylation of ERK1/2, as well as the number of initial EPC in experimental unit before and after the induction or addition of growth factor; To determine differences in expression levels and phosphorylation of ERK1/2 and proliferation of EPC between EPC with and without addition of growth factors; To determine differences in expression levels and phosphorylation of ERK1/2 and proliferation of EPC between EPC of peripheral blood of patients with stable angina pectoris and healthy individuals with and without addition of the growth factors; To determine whether the role of signal transduction of ERK1/2 on proliferation of EPC is typical and is a main signal line.

The benefits obtained from this study include: Adding a scientific basis that the effort to increase EPC of blood circulation in patients with stable angina pectoris is possible; Improving knowledge about the role of special cueing lines for signal transduction of ERK1/2 induced by growth factor in promoting proliferation of EPC; As a consideration for the treatment of CHD patients with clinical symptoms of stable angina pectoris who will undergo PCI, which is expected to reduce the incidence of re-stenosis or thrombosis after the treatment.

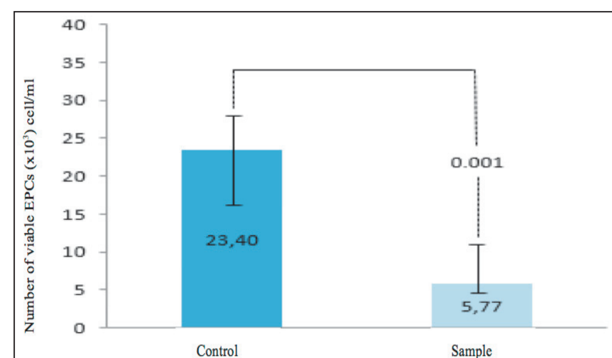
METHODS

This study was a pilot study through laboratory testing with control group time series design. The study was conducted in January 2014 at the Laboratory of Prodia Stem Cell Indonesia in Jakarta. The experimental unit in this study was the EPC of peripheral blood isolated from patients with stable angina pectoris who met the inclusion criteria of the study: male, aged between 50–55 years, patients with stable angina pectoris, the results of assessing coronary angiography indicating lesion with stenosis >50% based on the angiogram, willing to follow the procedures of the study and signing a letter of consent. Patients did not meet the inclusion criteria if they had: a history of stenting, Acute Myocardial Infarction (AMI), Diabetes Mellitus (DM), smoking, acute limb ischemia, history of Coronary Artery Bypass Grafting (CABG). As a control, the study used EPC taken from peripheral blood of healthy individuals who met the inclusion criteria as follows: male, aged between 50–55 years, was not at risk for CHD, results of ECG and treadmill testing were normal, willing to follow the procedures of the study and signing letter of consent.

The sample size was eight (8) for each group, with estimating the possibility of drop-out or damage in the experimental units. The independent variable in this study was treatment in the form of growth factor addition and ERK1/2 inhibition. The dependent variables in this study were the signal expression of ERK1/2, phosphorylation level of ERK1/2 and proliferation of EPC. The peripheral blood samples were taken from eight voluntary subjects who had signed the informed consent. Mononuclear cells (MNC) method of the eight patients with stable angina pectoris was isolated for one or three days in a specific medium with or without the addition of supplement. EPC produced were stained using immunofluorescence staining method to detect CD34, Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) and CD133.

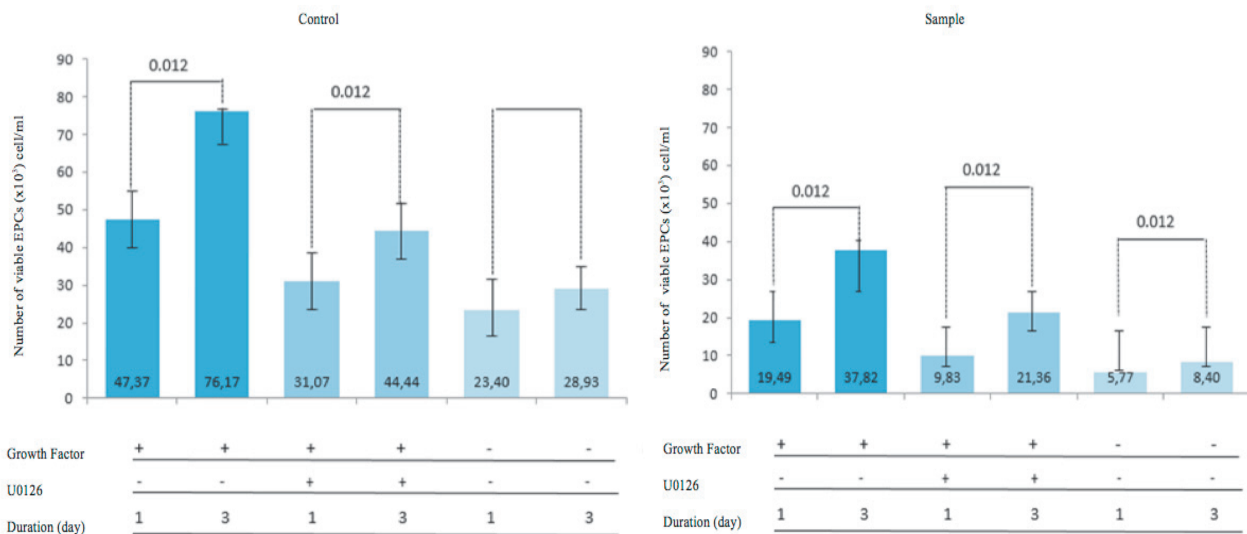
The XTT Cell proliferation assay was used to assess EPC growth after 1-3-day culture, while Colony Forming Unit (CFU) enumeration was used to asses the function of colony of EPC formed after 1-3-day culture. The western blot analysis was used to detect, the possible activation of ERK1/2.The data collected in this study were primary data from the results of the observation to the experimental group and control group in the form of expression of ERK1/2 and proliferation of EPC. The data obtained were then processed using SPSS for Windows 20.0 (IBM Corp., Armonk, NY) and the results were presented in tables and charts. The statistical analysis used three different types of testing, namely the Wilcoxon Mann-Whitney U, Wilcoxon Signed Rank and Spearman.

RESULTS AND DISCUSSION



Source: Primary data

Figure 1. The comparison between the number of control and sample EPC that survived in the experimental group three in one day culture



* Wilcoxon Signed Rank testing

Figure 2. The number of control and sample EPC that survived with or without the growth factors and U0126 after one-day and three-day culture

Table 1. The analysis of differences in the number of control and sample EPC between one-day culture and three-day culture in each experimental group

Variable	Median	p*
Control (n=8)		
Experiment 1	28,801.54	0.012
Experiment 2	10,608.07	0.012
Experiment 3	4,219.66	0.012
Sample (n=8)		
Experiment 1	18,819.87	0.012
Experiment 2	11,679.52	0.012
Experiemnt 3	1,843.39	0.036

Based on this research, the number of control EPC that survived was 23.40×10^3 and the number of EPC taken from patients with stable angina pectoris that survived was 5.77×10^3 on the first day of culture without treatment (growth factors or U0126) using the XTT proliferation method (see Figure 1). This indicated that the baseline number of EPC in healthy individuals was four times greater than the number of EPC in patients with stable angina pectoris.

In Table 1 showed that the control group, having the highest average number of EPC survived on the first day and the third day was in the experimental group with the addition of growth factors (experimental group 1). While the least average number of EPC survived was in the experimental group without treatment (experimental group 3). Similarly, in the group of patients with stable angina pectoris, the highest average number of cell proliferation was in experimental group 1 (group with the addition of growth factors).

Table 2. The analysis of the difference in the number of control and sample EPC that survived in experimental group

Variable	Median	P
Control (n=8)		
1 Day:		
Experiments 1 and 2	15,297.13	0.001
Experiments 1 and 3	23,508.79	0.001
3 Days:		
Experiments 1 and 2	33,490.60	0.001
Experiments 1 and 3	48,090.67	0.001
Sample (n=8)		
1 Day:		
Experiments 1 and 2	15,297.13	0.001
Experiments 1 and 3	23,508.79	0.001
3 Days:		
Experiments 1 and 2	15,296.24	0.001
Experiments 1 and 3	29,254.91	0.001

* Wilcoxon Mann-Whitney U Test

Based on these findings (see Figure 2 and Table 2), it can be analyzed that the baseline number of EPC of control group of healthy individuals was higher than the baseline number of EPC of patients with stable angina pectoris. The addition of U0126 along with growth factors (experiment 2) resulted in the significant decrease in number of EPC of the control group and the sample group.

This study showed opportunity of treatment through adding growth factors. It is expected that the number of EPC in patients with stable angina pectoris can be prepared prior to stent until the baseline number increases and is equal to the baseline number of EPC of healthy individuals, so that the incidence of acute thrombosis and in stent re-stenosis can be

Source: Primary data

Figure 3. Expression of marker of EPC of sample in three-day culture

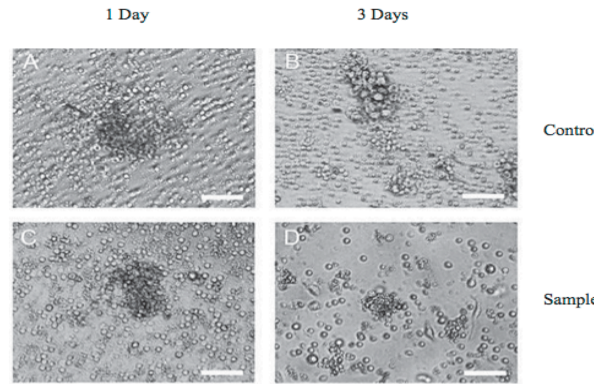
reduced. In general, the number of EPC of patients with stable angina pectoris is lower than that of healthy individuals.

The observation to the marker of EPC was conducted to confirm whether the proliferating cells were actually EPC or not using immunofluorescence. Expression of CD34 was marked by fluorochrome Alexa Fluor 488 (green) and expression of VEGFR2 was marked by fluorochrome phycoerythrin (red), while expression of CD133 was marked by fluorochrome Alexa Fluor 350 (blue) and expression of the combination of the three. Based on this immunofluorescence testing, the results of culturing control and sample EPC showed expression of marker of EPC (see Figure 3).

In one-day culture, it appeared that the initial baseline number of EPC of healthy individuals was higher than the initial baseline number of EPC of patients with stable angina pectoris (see Figure 4 and Figure 5). Based on CFU enumeration, findings which were in line with the assessment of the proliferation of EPC with XTT method was that the CFU of the highest number of control EPC was found in the experimental group 1 (with addition of growth factors on the third day of culture with the average of as many as 273.958). Meanwhile, the experimental group 2 (with addition of growth factors along with U0126) occupied the second place with lower numbers of CFU of EPC than those with addition of growth factors only. Experimental group 3 was the group with the lowest number of EPC compared with group 1 and 2. Similarly, in the sample group, the average number of EPC that survived was found in experimental group 1 (with addition of growth factors) in the third day of culture with the average number of 70,958 followed by the average number of EPC of experimental group 2 (with addition of growth factors along with U0126) as many as 44.250 and the least was the average number of EPC of experimental group 3 (without treatment) as many as 16.125.

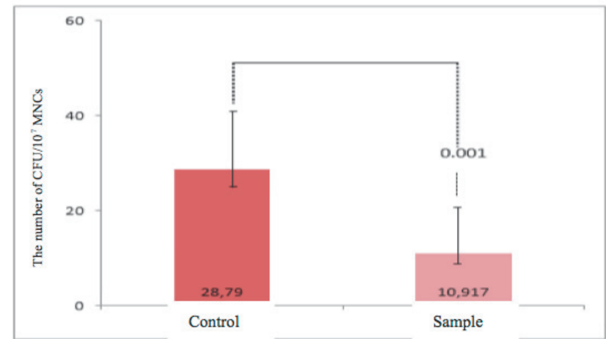
The average number of CFU of EPC of the sample with the addition of growth factors and with or without U0126 until the third day of culture was higher than the average baseline number of CFU (first day of culture) of control group (healthy individuals) without any treatment.

The number of CFU of EPC of sample group 1 on the third day of observation was higher than that on the first day of observation. Based on the data on median obtained through different test results, the number of CFU of each treatment group on the third day increased both the CFU of the control group and the sample



Source: Primary data

Figure 4. CFU of EPC of the control group (A&B) and sample group (C&D) in one-day culture (A&C) and three-day culture (B&D)



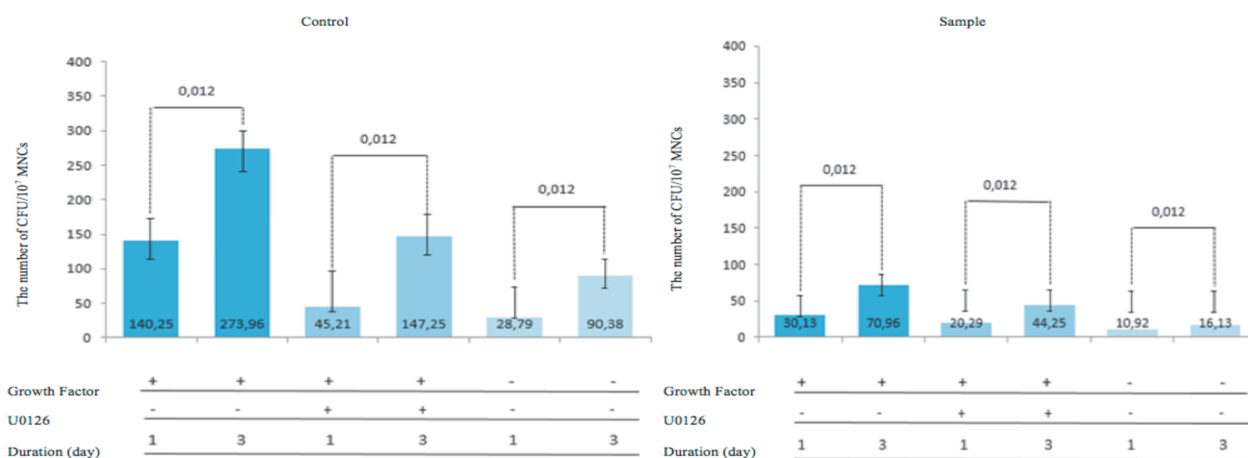
* Wilcoxon Mann-Whitney U Testing

Figure 5. Comparison of the number of CFU of EPC of control group and sample group of experimental group 3 in one-day culture

Table 3. The analysis of the difference in the number of CFU of EPC of control and sample group on the first day and the third day of observation

Variable	Median	p*
Control (n=8)		
Experiment 1	142.51	0.012
Experiment 2	108.17	0.012
Experiment 3	65.33	0.091
Sample (n=8)		
Experiment 1	39.33	0.012
Experiment 2	21.84	0.012
Experiemnt 3	4.33	0.012

* Wilcoxon Signed Rank Test



* Wilcoxon Signed Rank testing

Figure 6. The number of CFU of EPC of control and sample group that survived with or without growth factors and U0126 in one-day and three-day culture

group. The highest median was found in control group of experiment 1. The lowest median on the third day was found in sample group of experiment 3.

The value of p <0.05 was found in the number of CFU of EPC of control group of experimental group 1 and 2 as well as in the number of CFU of EPC of sample group of experimental group 1, 2 and 3. It indicated that there was a significant difference in the CFU of EPC that survived on the first day and the third day (see Table 3).

Table 4. Analysis of the difference in the number of CFU of EPC among experimental groups of control and sample group

Variable	Median	P
Control (n=8)		
1 Day:		
Experiments 1 and 2	86.33	0.001
Experiments 1 and 3	101.49	0.001
3 Days:		
Experiments 1 and 2	120.67	0.001
Experiments 1 and 3	178.67	0.001
Sample (n=8)		
1 Day:		
Experiments 1 and 2	9.17	0.015
Experiments 1 and 3	18.5	0.001
3 Days:		
Experiments 1 and 2	26.66	0.009
Experiments 1 and 3	53.50	0.001

* Wilcoxon Mann Whitney U Test

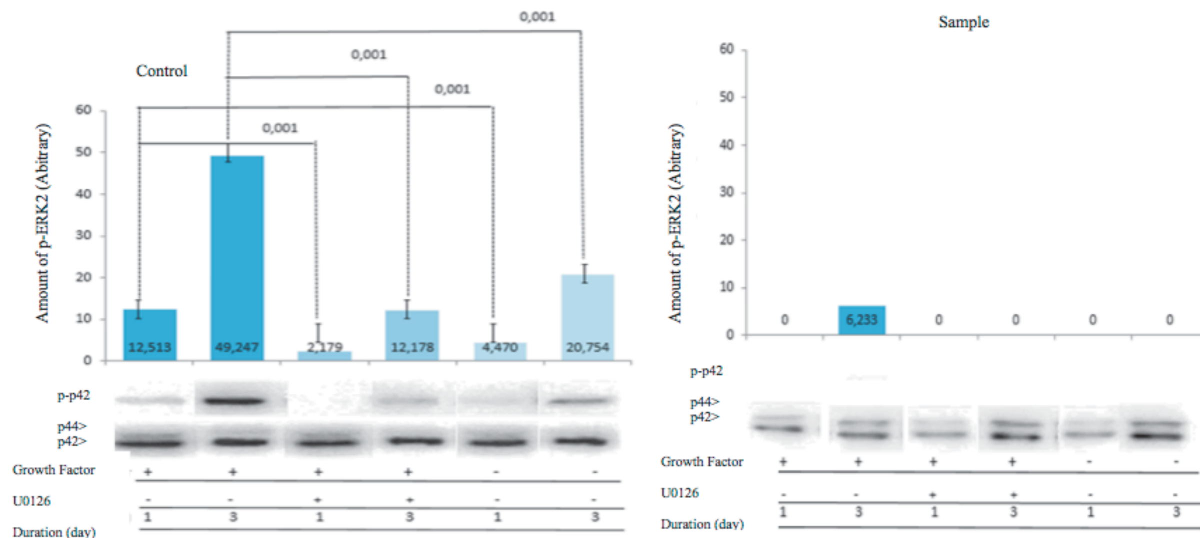
Based on the analysis of differences in the number of CFU of EPC among experimental groups of control and sample group, there were significant differences between the experimental group 1 (with addition of growth factors) both of control and sample group on the first day as well as the third day of observation and the experimental group 2 (with addition of growth factors along with U0126) and the experimental group 3 (group without treatment), with p=0.012. It indicates that there was a significant difference (p<0.05) between the experimental group and the prominent role of growth factors as a component that increased the number of CFU of EPC (see Figure 6 and Table 4).

Table 5. Analysis of the difference in the results of measuring the density of phosphorylated ERK2 tape among the experimental groups

Variable	Median	P
Control (n=8)		
1 Day:		
Experiments 1 and 2	10.61	0.001
Experiments 1 and 3	8.4	0.001
Experiments 2 and 3	2.21	0.001
3 Days:		
Experiments 1 and 2	37.47	0.001
Experiments 1 and 3	28.89	0.001
Experiments 2 and 3	8.58	0.001
Sample (n=8)		
1 Day:		
Experiments 1 and 2	0	1
Experiments 1 and 3	0	1
Experiments 2 and 3	0	1
3 Days:		
Experiments 1 and 2	6.23	0.000
Experiments 1 and 3	6.23	0.000
Experiments 2 and 3	0	1

* Wilcoxon Mann-Whitney U Test

Based on Figure 7, it can be observed that the expression of phosphorylated ERK2 (ERK2 active) in EPC of control group added with growth factors (experimental group 1) was higher than the EPC of control group added with growth factors and U0126 (experimental group 2) and EPC of control group without treatment (experimental group 3) both on the first and third day of culture.



* Wilcoxon Mann-Whitney U testing

Figure 7. Expression of ERK1/2 and phosphorylated ERK1/2 in EPC of control and sample group with or without addition of growth factors and U0126 in one-day culture and three-day culture

Based on the density test to control group (see Table 5), the highest value of tape density was in the experimental group 1 (group receiving supplementation of growth factors on the third day of observation. The lowest value of tape density was in the experimental group 2 (group receiving growth factor and U0126 simultaneously). This indicated that growth factors could stimulate phosphorylation of ERK2 both on the first and third day of observation (with a value of ERK2 phosphorylation on the third day higher than on the first day).

In the experimental group 2 on the third day of observation, the phosphorylation of ERK2 was lower than the ERK2 phosphorylation of experimental group 1 on the third day of observation. This suggested that the boost of the growth factors to phosphorylation of ERK2 on the third day of observation could still compete with the inhibitory impact on phosphorylation of ERK2 by U0126 compared with the first day of observation. In addition, the U0126 inhibition to growth factors was unique, but it was not the only pathway that prevented phosphorylation of ERK2 from happening, so that the decrease in phosphorylation of ERK2 occurred in part.

In the EPC of the samples of stable angina pectoris patients, phosphorylated ERK2 (active ERK2) was only detected in the experimental group 1 on the third day of observation. Furthermore, based on the density test in the sample group, the tape density of phosphorylated ERK2 was only found on the third day of observation in the experimental group 1. In other experimental groups, the phosphorylation of ERK2 was not obtained.

The difference of the above symptoms can be explained as follows: in a healthy individual (control group), the expression of ERK1 and ERK2 was very much different, the expression of ERK2 was dominant. This was evident from the significant difference in thickness of the tape between ERK1 and ERK2 in densitometry analysis (very thick for ERK2 and very thin for ERK1). The findings were contrary to the analysis in patients with stable angina pectoris (sample group) that showed expression of both ERK1 and ERK2. In patients with stable angina pectoris, both the thickness of the tape of expression of ERK1 and ERK2 were seen, with a higher density in the tape of expression of ERK2. With the expression of ERK1 in patients with stable angina pectoris, the expression of ERK2 was inhibited because ERK1 was opposite to ERK2, the same went to the phosphorylation activity. Phosphorylated ERK2 (active ERK2) was only obtained after administration of growth factors for three days. Based on this research, it can be concluded that growth factors were helpful improving phosphorylated ERK2 in patients with stable angina pectoris.

The cascade of ERK1/2 is commonly identified as a growth-promoter. Initially, ERK1 and ERK2 were alleged to play a role in regulating the intracellular signal through phosphorylation of a large number of substrate components both in the cytosol and the nucleus. However, further it could be understood that both have activities that were the opposite. ERK2 was well known to have a positive role in normal cell proliferation and in the proliferation dependent on Ras. The results showed that in healthy individuals, the phosphorylation of ERK2 EPC was greatly supported by the opposite impact, but it would be very low for ERK1

Table 6. The correlation between the characteristics of EPC (number of EPC and CFU-EPC) and the transduction of ERK 1/2 of control and sampel (patients with stable angina pectoralis)

		The number of cell (*p)	CFU EPC (*p)
Transduction	Exp 1	0.0013	0.003
ERK1/2	Exp 2	0.867	0
	Exp 3	0.259	0

* Spearman Test

and stimulation of growth factors. While in patients with stable angina pectoris, the phosphorylation of ERK2 only occurred due to stimulation of growth factors because of the great impact of ERK1 on ERK2. The phosphorylation of ERK2 significantly increased with the supplementation in the form of both growth factors in the control group (on the first day and third day of observation) and samples (on the third day of observation only). This was in line with the results of Xu *et al*[7] in mice, that the growth factor (in this case a VEGF) would only spur the phosphorylation of ERK2 and not ERK1, after an incubation of 30 minutes.

Based on the Spearman correlation test that assessed the correlation between the expression of ERK2 and CFY EPC, significant correlation between signal transduction of ERK2 and the proliferation of EPC in general was obtained (see Table 6). Based on the anaylisis, it can be concluded that the proliferation of EPC was correlated to the increase in the signal transduction due to the addition of growth factors.

CONCLUSIONS AND SUGGESTIONS

The results of the study were as follows: the number of EPC of patients with stable angina EPC was lower than the number of EPC in healthy individuals; the number of EPC in patients with stable angina pectoris could be increased by administering growth factors; There were differences in the expression level and phosphorylation of ERK1/2 between the group of healthy individuals and patients with stable angina pectoris, with a predominance of ERK2 in a group of healthy individuals; phosphorylation of ERK2 in patients with stable angina pectoris could be activated with the administration of growth factors and could be seen on the third day of observation; U0126 as the inhibitor of MEK 1/2 could inhibit the phosphorylation of ERK2 significantly, but could not impede overall. This was possible because there is another path other than the path of ERK1/2; In general, proliferation of EPC in the group induced by growth factors that correlated with the phosphorylation of ERK2.

Suggestions in this study are as follows: the proliferation of special EPC that related to the stimulation of growth factors should be further investigated in-vivo. Further research is required to investigate other pathways other than ERK 1/2 related to the proliferation of EPC; Research on the increasing number of EPC to from time to time is required to see the dynamics of the process; This research can be used as the basis for a clinical trial of EPC provision to patients with stable angina pectoris.

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