Effect of Acetylsalicylic Acid on Angiogenesis Through Assessment of Microvascular Density, VEGF-A, Interleukin-1 and PDGF-B Receptors on Rat Myocardium that Undergo Hypobaric Hypoxia Exposure

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Abstract

Background Myocardial hypoxia in coronary artery disease (CAD) may induce angiogenic response by activating proangiogenic mediators; Vascular Endothelial Growth Factor-A (VEGF-A) and Platelet-derived growth factor (PDGF-B), and proinflammatory mediator Interleukin-1 (Il-1). Acetylsalicylic acid (ASA) was known could reduce tumor growth by inhibiting angiogenesis process.

Objective The study aimed to assess the effect of ASA on the angiogenesis in myocardial tissue which induced by hypobaric hypoxia exposure.

Methods This was an experimental study with double interventions: hypobaric hypoxia exposure and ASA administration. The subjects were divided equally and randomly into four groups: I) control group without any intervention, II) hypobaric hypoxia exposure only, III) hypobaric hypoxia with low dose of ASA (2mg/kgs), and IV) hypobaric hypoxia with high dose of ASA (10 mg/kgs) for 14 days. Hypobaric hypoxia exposure was performed intermittently in a hypobaric chamber. At the end of the study, rats' myocardial walls were extracted and examined for their microvascular density with Hematoxylin-Eosin and anti-CD34 immunostaining. The hearts were also studied for the expression of VEGF-A, Interleukin-1 (Il-1), and PDGF-B receptors by immunohistochemistry, and were assessed by histopathology sections using histoscore.

Results The study subjects were 32 Wistar rats, aged 18-20 weeks weighing 250-350 grams. There were significant differences in microvascular density in both HE (median 4 vs. 2, p=0.021) and anti CD34 stainings (median 3 vs. 1, p=0.028), as well as VEGF A (median 10.5 vs. 3, p=0.002) and Il-1 (median 10.5 vs 3.5, p=0.004) receptors expressions between ASA and without ASA groups. Furthermore, there were differences in microvascular density using anti-CD34 (median 3 vs. 1, p=0.035) and VEGF-A receptor expression (median 6 vs. 2, p=0.022) between low dose and high dose of ASA group.

Conclusion The administration of ASA may decrease angiogenic response of hypobaric hypoxia exposure. High dose of ASA may reduce angiogenesis process stronger than low dose of ASA.

1. Background

Acetylsalicylic acid (ASA) which is the main treatment of CAD, has the potential to inhibit the process of angiogenesis, both at low doses through inhibition of platelet function, and at high doses by direct inhibition in the hypoxic and inflammatory pathways.[1,2] Inhibition of the angiogenesis process by ASA could be evaluated by a decrease in the intensity of proangiogenic factor receptors, Vascular Endothelial Growth Factor-A (VEGF-A) and Platelet-derived growth factor (PDGF-B), as well as proinflammatory cytokine receptors, Interleukin-1 (Il-1) in myocardial tissue, and histopathological microvascular density asessesment as the gold standard. [3,4]

Myocardial hypoxia in coronary artery disease (CAD) or hypobaric hypoxia condition activates cellular and tissue compensatory responses, which ultimately result in protective angiogenesis to maintain

myocardial oxygen supply.[5] The responses to angiogenesis consist of complex mechanism, involving various hypoxia and inflammatory pathways, including a variety of proangiogenic proteins, proinflammatory cells and platelets.[6–9] The Hypoxia Inducible Factor 1-alfa (HIF-1alfa) gene, which is active in hypoxia condition, could control the process of angiogenesis, inflammation, and induces the translation of various proangiogenic proteins, one of which is VEGF-A. VEGF-A activates endothelial cells which then undergo proliferation, migration, and differentiation.[6,9] Alpha granules component in platelets containing VEGF-A and PDGF-B also play roles in the process of angiogenesis. The function of PDGF-B is similar to VEGF-A in stimulating endothelial cells to be active in the process of angiogenesis.[2] Interleukin-1 also induces the release of VEGF-A which originate from T-lymphocytes and monocytes that also plays a role in angiogenesis.[10,11]

There is a gap between the usage of ASA in CAD treatment and the process of angiogenesis which can potentially be interrupted by ASA.[1,4] The benefit of angiogenesis process to form collateral vessels and increase oxygen supply of myocardial tissue; hence angiogenesis process needs to be optimized. Standard treatment of CAD with low doses of ASA, as well as in the treatment of inflammatory diseases by administering high doses of ASA, have the potential effects to inhibit the beneficial angiogenesis process. Study on the effects of ASA on the angiogenesis process in myocardium has not been widely reported. This study has a novelty in the method of evaluating the role of ASA in the angiogenesis process in hypobaric hypoxia myocardium. This study also has a novelty in the method of reviewing ASA inhibition effect on the angiogenesis process in hypobaric hypoxia myocardium. This study applied an experimental animal model with hypobaric hypoxia exposure as the method to induce myocardial hypoxia, which is accompanied with ASA administration for 14 days at certain treatment groups.

1.1 Objective

The objective of this study was to evaluate the effect of ASA, and its dose difference to angiogenesis process through assessment of myocardial microvascular density, VEGF-A, Il-1, and PDGF-B receptors intensity on hypoxia hypobaric Wistar rat model.

2. Methods

2.1 Study design

This was a true experimental study; with double treatment on animal models (in vivo) using male healthy Wistar rats aged 18–20 weeks, weighing 250–350 grams. The animals were obtained from Animal Laboratory, Department of Pharmacology, Faculty of Medicine, Padjadjaran University. The study was performed at the Hypobaric Chamber, Air Force Space and Aviation Medicine Agency "Saryanto" in Jakarta. Animal husbandry was conducted at Department of Pharmacology, while histopathological and immunochemistry examinations at Department of Pathology Anatomy, Faculty of Medicine, Padjadjaran University. Study was conducted in the period of September 2014 until Mei 2015. The inclusion, exclusion and dropout criterias were defined as below:

Inclusion criteria

Rats with ventricle suitable for histopatologic examination with Hematoxylin-Eosin (HE) staining and immunohistochemistry examination with anti CD34.
Rats with ventricle suitable for immunohistochemistry examination to analyze Vascular Endothelial Growth Factor A receptor (VEGF-A R), Interleukin-1 (Il-1 R), and Platelet Derived Growth Factor B receptor (PDGF-B R).

Exclusion criteria

Rats with disability, inactive movement or inactive eating behavior.

Dropout

Death before the study was completed.

2.2 Data collection

All experimental subjects were placed in the Animal Laboratory of Pharmacology Department, Faculty of Medicine, Padjadjaran University, for one week before intervention in order to give environmental adaptation. The experimental rats were weighed at the beginning and the end study. Rats were divided randomly and equally into 4 groups, as follows:

Group I : Control without any treatment

Group II : Receive intermittent hypobaric hypoxia treatment only

Group III : Receive intermittent hypobaric hypoxia treatment and oral administration of

low dose of ASA (2 mg / kg body weight/ day) for 14 days.

Group IV : Receive intermittent hypobaric hypoxia treatment and oral administration of

high anti-inflammatory dose of ASA (10 mg / kg body weight / day) for 14

days.

2.3 Induction of myocardial hypoxia

Hypobaric hypoxia treatment was performed with standard protocol to induce myocardial hypoxia as previously described.[12] Type-I chamber flight profile (modified low-pressure air chamber) was used give hypobaric hypoxia exposure to the subjects (Fig. 1). All rats in the treatment group were placed in the chamber. The pressure was adjusted to an altitude of 35,000 feet for one minute, followed by an altitude of 30,000 feet for 3 minutes, then 25,000 feet for 5 minutes, and finally an altitude of 18000 feet for 30 minutes.

Animals were kept in a designated cage (consisting of 4 cages). All of the cages stored in the well ventilated and lighted room with temperature controlled in the Animal Laboratory of Pharmacology Department, Faculty of Medicine, Padjadjaran University. Food and drink were given *ad libitum*. Acetyl salicylic acid was diluted in water and administered using pipettes.

Euthanasia procedure used standard protocol in the Animal Laboratory, Department of Pharmacology, Faculty of Medicine, Padjadjaran University. The protocol was a combination of intraperitoneal injection of Ketamine at dosage 100 mg/kg rat weight until full anesthetic effect achieved, followed by cervical dislocation. Experienced laboratory personnel performed the euthanasia procedure.

2.4 Histopathological examination

All treatment groups received the same exposure for 4 times, at one-week intervals. At the end of the study (day 28), the rats were euthanized and the rat's heart was immediately taken for histopathological preparations. The paraffin block from each animal's heart was cut with a microtome and divided into 5 histopathological preparations. Histopathological examination performed was microvascular density in myocardium by HE and anti-CD34 staining, as well as VEGF-A, Il-1, PDGF-B receptors antibodies asessment. Calculation of microvascular density with HE staining and anti-CD34 staining were performed by identifying the sagittal capillaries of the sagittally cut blood vessels under a binocular light microscope with 200x magnification. Capillary endothelial density is the average of at least 3 different visual fields. A competent Pathologist blindly performed microvascular density calculation with HE staining and anti-CD34 immunohistochemistry.

2.5 Immunohistochemistry examination

Immunohistochemistry examination uses reagents from Abcam® as follows: Rat monoclonal CD34 primary antibody (Abcam, Cambridge, USA; ab8158), rabbit polyclonal VEGF-A receptor primary antibody (Abcam, Cambridge, USA; ab32152), rabbit polyclonal PDGF-B receptor primary antibody (Abcam, Cambridge, USA; ab32570), rabbit polyclonal Interleukin-1 receptor primary antibody (Abcam, Cambridge, USA; ab9722). Immunohistochemistry examination was performed according to the protocol established by the reagent manufacturer. The interpretation was conduced by scoring system (histoscore) based on the intensity and color distribution on each slide of the preparation. Each slide was assessed based on the average score of intensities (strong: 3, medium: 2, weak: 1) and distributions (> 80%: 4, 50 – 80%: 3, 20 – 50%: 2, < 20%: 1) from three different microscopic fields. The final histoscore results (ranged from 1 to 12) are calculated by multiplying the intensity score with the distribution score.

2.6 Statistical analysis

All examination results were presented descriptively as mean ± standard deviation or median (minimum-maximum), as appropriate. The differences in microvascular density by HE, CD34, histoscore results of VEGF-A receptors, Il-1 receptors and PDGF-B receptors from the four groups of experiment were analyzed using the Independent T-test/Mann-Whitney U test (bivariate analysis) and the ANOVA/Kruskal Wallis test

for multivariate analysis. In this present study, we analyse: 1) the effect of hypoxia (group I vs. group II, III and IV; and group I vs. group II), 2) the effect of ASA (group II vs. group III and IV) and the dose effect of ASA (group III vs. group IV). The significance of statistical test results was based on p-value < 0.05. Data analysis will be performed using IBM SPSS Statistics for Mac, version 20 software (IBM Corp., Armonk, N.Y., USA).

2.7 Ethical statement

This animal experimental study protocol was reviewed and approved by Health Research Ethics Committee of Padjadjaran University, Bandung.

3. Results

This study subjects consisted of 32 Wistar rats aged between 18-20 weeks with initial weight ranging between 250 to 350 grams. Of 32 subjects, a total of 5 rats were dropped out. Where three rats died, one each from groups II, III, and IV at the first hypobaric hypoxia treatment. In the second hypobaric hypoxia treatment, a total of two rats died each from group II and IV. The one-way ANOVA test showed that there was no significant difference of body weight between groups.

Table 1
Comparison of microvascular density with Hematoxylin-Eosin staining at the end of treatment between groups

Number	Treatment group				A	B
	I	II	III	IV	p-value	
1	3	2	3	2	0.037*	0.021*
2	3	2	3	2		
3	3	4	2	2		
4	2	4	2	2		
5	2	4	3	1		
6	1	5	3	2		
7	2		2			
8	3					

Data are presented as the number of microvascular found per field of view.

Statistical analysis using the Kruskal Wallis test.

A = group I compared to groups II, III, IV. B= group II compared with group III and IV.

*p < 0,05 indicates statistically significant.

Table 1 demonstrated a significant microvascular density difference between control group (I) and groups which received hypobaric hypoxia treatment (II, III, and IV) based on HE staining (p = 0.037), particularly an increased number of microvascular was seen in group II. Group III and IV (which received ASA) showed a tendency of microvascular density reduction when compared to group II (without ASA). The Mann-Whitney U test showed no significant differences in microvascular density between group I and group II (p = 0.081), although in general, group II had a higher microvascular density when compared to group I. (Table 1) Furthermore, the Kruskal Wallis analysis showed that there was a significant difference of microvascular density (p = 0.021) between groups who did not receive ASA and those who received ASA.

Table 2
Comparison of microvascular density with anti-CD34 staining at the end of treatment between groups

Number	Treatment group				A	B
	I	II	III	IV	p-value	
1	2	3	5	1	0.024*	0.028*
2	3	5	4	1		
3	3	4	3	3		
4	3	3	2	1		
5	3	2	1	1		
6	2	3	3	2		
7	3		4			
8	3					
Data are presented as the number of microvascular found per field of view.						
Statistical analysis using the Kruskal Wallis test.						
A = group I compared to group II, III, IV. B= group II compared with group III and IV.						
*p < 0,05 indicates statistically significant.						

Table 2 showed the results of microvascular density analysis based on anti-CD34 staining. The Kruskal Wallis test showed a significant difference between control and treatment groups (II, III, IV) (p = 0.024). Statistical analysis using Mann-Whitney U test to compare microvascular density between group I and group II also showed significant results (p = 0.026). There was also a significant difference in the microvascular density (p = 0.028) between groups that received ASA and those which did not received ASA. These results are consistent with the HE staining results, presented in Table 1.

Table 3
Comparison of VEGF-A receptor expression at the end of treatment between groups.

Number	Treatment group				A	B
	I	II	III	IV	p-value	
1	3	12	6	2	0.001*	0.002*
2	6	4	2	2		
3	4	9	4	3		
4	3	12	6	2		
5	3	9	9	3		
6	3	12	6	2		
7	4		3			
8	6					
Histoscore is calculated based on receptor intensity multiplied by% receptor density.						
Statistical analysis using Kruskal Wallis test.						
A = group I compared to group II, III, IV. B = group II compared to group III and IV. * p < 0,05 indicates statistically significant.						

Table 3 presented the results of VEGF-A receptor histoscore in the myocardium between the control group and the treatment groups. Group II, which exposed to hypobaric hypoxia only, had the highest histoscore results. Statistical analysis showed significant differences in the histoscore results (p = 0.001) between the control and treatment groups. The Mann-Whitney U test between group I and group II showed a statistically significant difference in VEGF-A receptor (p = 0.006). The VEGF-A receptor expression increased in group II, then decreased in group III and more decreased in group. Subsequent analysis evaluated the comparison between the groups who received hypobaric hypoxia exposure only and the group which also obtained ASA. There are significant differences (p = 0.002) between groups II and III, IV. These findings showed ASA could reduce the expression of VEGF-A receptors in the rats' myocardium that were exposed to hypobaric hypoxia condition.

Table 4
Comparison of Interleukin-1 receptor expression at the end of treatment between groups

Number	Treatment group				A	B
	I	II	III	IV	p-value	
1	3	12	4	6	0.002*	0.004*
2	3	9	6	2		
3	3	6	4	2		
4	2	12	3	4		
5	6	12	4	2		
6	2	6	6	2		
7	4		9			
8	4					
Histoscore is calculated based on receptor intensity multiplied by % receptor density.						
Statistical analysis using Kruskal Wallis test.						
A = group I compared to group II, III, IV. B = group II compared to group III and IV.						
* p < 0,05 indicates statistically significant.						

The response to hypoxia includes various compensatory mechanisms including activation of the immune response. Hypoxia can increase the expression of Interleukin-1 receptors in myocardium. Table 4 showed that there were significant differences in the expression of Interleukin-1 receptors between groups, which exposed to hypoxia and those without hypoxia (p = 0.002). A statistically significant difference was also found between group I and group II (p = 0.003). Interleukin-1 expression in group II was seen to be highest compared to other groups, and decreased in groups III and IV. This Interleukin-1 receptor expression pattern was similar to the VEGF-A receptor expression pattern in Table 3 where the expression of the Interleukin-1 receptor has the lowest median. The expression of Interleukin-1 receptor and VEGF-A receptor in group II was the highest and after administration of ASA, the receptor expression was seen to decrease.

This study also demonstrated the effect of ASA dose difference on microvascular formation in myocardium. Figure 3 illustrates the difference in microvascular density and multiple receptors expression after hypobaric hypoxia exposure between groups which receiving low doses of ASA compared to those receiving high doses of ASA. The results of anti PDGF-B receptor histoscore demonstrated no difference between control group (group 1) and treatment groups (II, III, IV) (p = 0.809), as well as between group I and group II (p = 0.428). Statistical analysis to assess the effect of ASA to PDGF-B R also did not showed any significancy (p = 0.715).

Figure 3 showed differences in the number of myocardial microvascular density between groups treated with hypobaric hypoxia accompanied with low-dose of ASA and high-dose of ASA. Microvascular density difference based on anti CD34 immunohistochemical staining showed statistically significant (p = 0.035), but did not significant in the standard HE staining examination (p = 0.051). Besides, significant differences were seen at VEGF-A receptors assessment (p = 0.022). The tendency for differences in receptor expression was also seen in the Il-1 receptor although it was not statistically significant (p = 0.051).

Discussion

This present study demonstrated significant differences in microvascular density in the myocardium between groups with hypoxia and those without hypoxia. Similar conditions observed in malignancy. Hypoxia conditions in the core of tumor mass, due to high tumor cells metabolic loads, activate genes that are responsive to hypoxia and ultimately increase the mechanism of angiogenesis. There is a significant increase of microvascular density in uterine malignant tissue compared to the normal myometrial tissue. This increase in microvascular density is also characterized by an increase in VEGF expression in the tumor tissue.[13,14] Tumor cells exposed to cyclic and brief hypoxia exposure can produce higher microvascular density increases compared to normoxic conditions.

Increased expression of VEGF-A receptors due to hypoxia exposure in this study is consistent with previous studies.[15–17] The hypoxia- VEGF-A pathway is shown to be activated through the mediation of HIF-1 activation that was not examined in this study. Another VEGF-A activation pathway is through Il-1 activation. VEGF-A can be significantly increased in vascular smooth muscle cells treated with Il-1 in hypoxic states, showed an association between hypoxia and inflammatory responses.[15] Interleukin-1 can act as a HIF-1 activator in the presence and absence of hypoxia, which subsequently lead to increased expression of VEGF-A. [15,16] Hypoxia increases the production of Il-1 in activated macrophage cells, particulary in atherosclerotic lesions, stimulates migration of other mononuclear cells and progression of atherosclerotic lesions. In that in-vitro study, hypoxia exposure for 48 hours to human macrophage was able to increase Il-1 production compared to normoxia group.[17] The present study demonstrated the expression of VEGF-A and Interleukin-1 differed significantly between the control group and the hypoxia groups, and these results remained significantly different in spite of high doses of ASA administration.

Our study showed significant reduction of microvascular density, VEGF-A, and Il-1 receptors expression among group with ASA and without ASA. This finding is consistent with previous studies.[6,18–20] There is a decrease in VEGF levels in human platelets with the presence of aspirin compared to those without aspirin.[6] Study in cardiovascular field which analyzed the effect of ASA/aspirin to the angiogenesis process in hypoxic myocardium is limited. Most study which analyzed the effect of ASA to the angiogenesis mediators, angiogenesis process and tumor mass growth comes from the oncology field. Shtivelband *et al.* demonstrated a significant and proportional decrease in VEGF levels and cyclooxygenase (COX)-2 levels in ASA groups that showed a correlation between those two factors. The study confirmed the formation of new blood vessels in the matrigel were also fewer in the group receiving

aspirin.[18] The effect of ASA on circulating VEGF levels in the mammary malignancy patients who were undergoing hormonal therapy with tamoxifen and received aspirin was also investigated. The study showed a significant decrease in circulating VEGF levels in the group receiving aspirin.[19] Previous study proves that aspirin can reduce VEGF-A expression and microvascular density in rats with sarcoma. The degree of decreased microvascular density depends on the dose of ASA. Decreased VEGF-A expression also has a correlation with decreased microvascular density.[20] Low dose of ASA decreases the response of angiogenesis which is characterized by a decrease in microvascular density, VEGF-A receptor expression and Il-1 receptor expression. High doses of ASA decrease angiogenesis more strongly than low doses of ASA. Microvascular density, VEGF-A, and Il-1 receptors expression in the myocardium which received high doses of ASA decreased to almost similar levels to the control group.

Contrary to previous studies, activation pathway for angiogenesis through increased expression of PDGF-B receptors and its inhibition by ASA has not been shown to have roles in this study. [21,22] In-vitro study conducted by Li *et al.* showed an increase of PDGF-B expression in bovine pulmonary artery endothelial cells after hypoxia exposure. Exposure to hypoxia in Li's study was performed continuously for two weeks.[21] Study conducted by Freyhaus *et al.* also confirmed an increase in PDGF expression as a result of a decrease in regulation of tyrosine phosphatase, which naturally inhibits PDGF-B. Hypoxia exposure in the study was also continuously using 3% oxygen for 48 hours. The increase in PDGF-B correlated with increase of matrix metalloproteinase activity that play a role in the process of angiogenesis.[22] Different from previous studies with continuous hypoxia exposure, in this study, hypobaric hypoxia exposure was performed intermittently. The intensity of hypoxia might explain the results difference among studies.

Limitations

Hypobaric hypoxia has a systemic effect that is not limited to myocardial tissue. The effect of hypoxia on various organs may produce effects on the myocardial angiogenesis process and can ultimately influence the results of this study. Further research with coronary artery ligation techniques or coronary atherosclerosis models needs to be considered because it is more specific to myocardial hypoxia and resembles the actual condition of CAD. This study does not investigate the angiogenesis process recovery after ASA termination. Further study that examines the effects of ASA termination to the angiogenesis process through measurement of microvascular density, expression of VEGF-A and Il-1 receptors need to be conducted. The degree of hypoxia in subjects during treatment was unknown because no blood oxygen partial pressure and/or oxygen saturation or oxidative stress parameters assessment. Subjects blood gas analysis or oxidative stress parameters during or immediately after hypobaric hypoxia treatment could be collected in further studies. In this study, one pathologist conducted histopathological examinations, thus the analysis of assessment results variability cannot be performed. Furthermore, receptor intensity measurement in this study was a semi-quantitative method, based on subjective assessments by pathologists. The Western Blot technique to measure receptor intensity quantitatively could be considered.

Conclusion

The administration of ASA in groups of rats exposed to hypobaric hypoxia reduces the angiogenesis response, as assessed by decreasing myocardial microvascular density and decreasing the expression of VEGF-A and Interleukin-1 receptors in myocardium. Higher doses of ASA reduce the process of angiogenesis more strongly compared to low doses of ASA.

Abbreviation

ACS	*: Acute Coronary Syndrome*
ANOVA	*: Analysis of Variance*
ASA	*: Acetyl Salicylic Acid*
CAD	*: Coronary Artery Disease*
CD34	*: Cluster Differentiation 34*
COX-2	*: Cyclooxygenase type 2*
ELISA	*: Enzyme-Linked Immunosorbent Assay*
HE	: Hematoxylin Eosin
HIF-1alfa	: *Hypoxia Inducible Factor 1 alfa*
Il-1	*: Interleukin-1*
PDGF B	*: Platelet-Derived Growth Factor B*
VEGF A	*: Vacular Endothelial Growth Factor A*

Appendix

Number of sample calculation

The number of samples of this experimental study was calculated using the Federer formula:

(t-1) (n-1) > 15

t = number of treatments = 4

n = number of rats per treatment group

The minimum number of samples for each experimental group was 6. As much as 20% of the minimum number of samples was added to anticipate the dropout subjects (2 rats of each group), hence total sample needed was 32 rats.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University, Bandung, with ethical approval number 480/UN6.C2.1.2/KEPK/PN/2014. Procurement of the animal was stated in separate ethical & consent statement from Animal Laboratory, Department of Pharmacology, Faculty of Medicine, Padjadjaran University.

Consent for publication

Not applicable

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author JWM.

Competing interests

The authors have no conflicts of interest to declare.

Funding Sources

None

Authors' Contributions

JWM made conception and design of study, implemented the study, analysed and interpreted the study results, drafted the manuscript and revised it. DH, MRS and S contributed to the design of study, analysis, and interpretation of study data. They also gave final approval of the version to be published.

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Figures

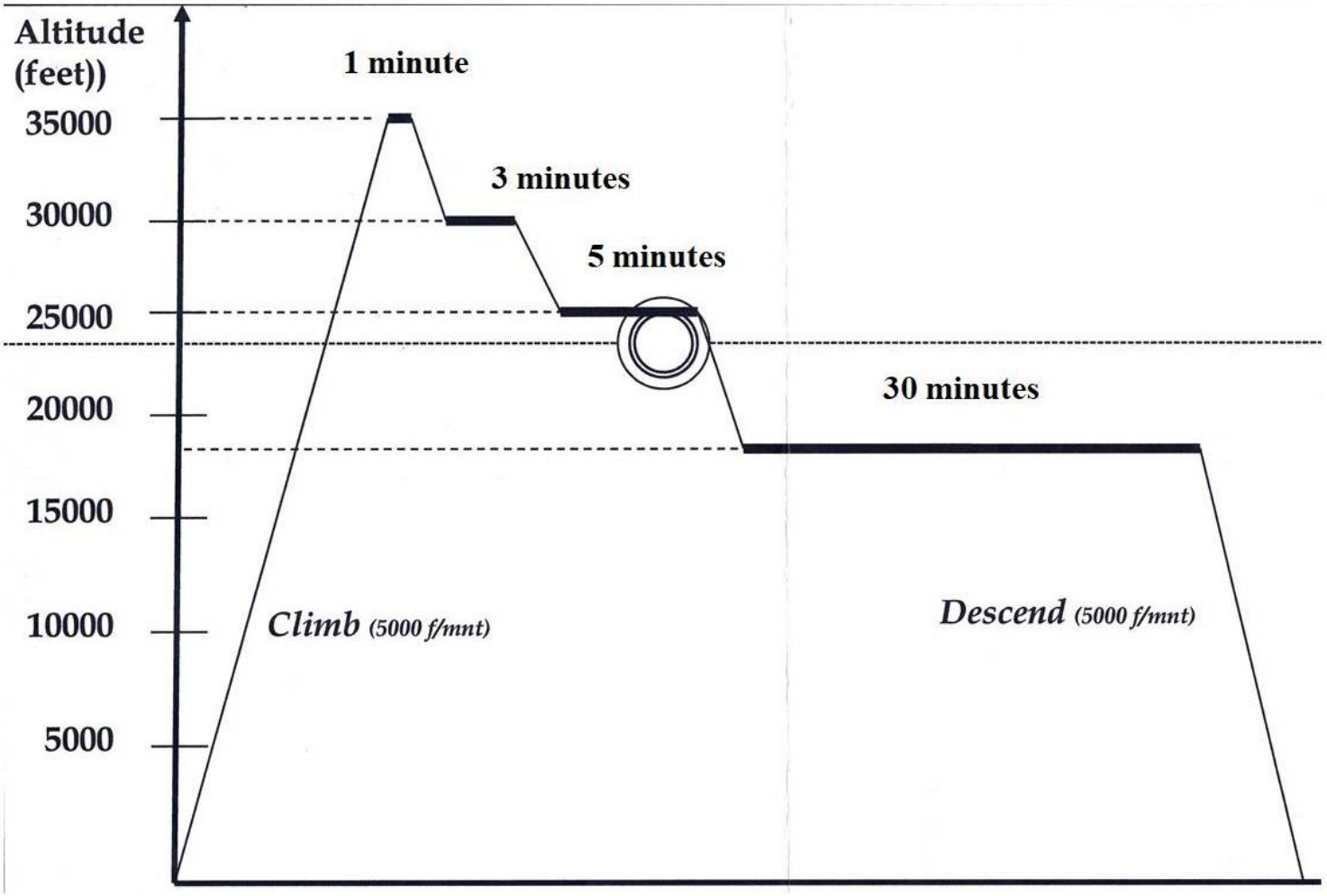
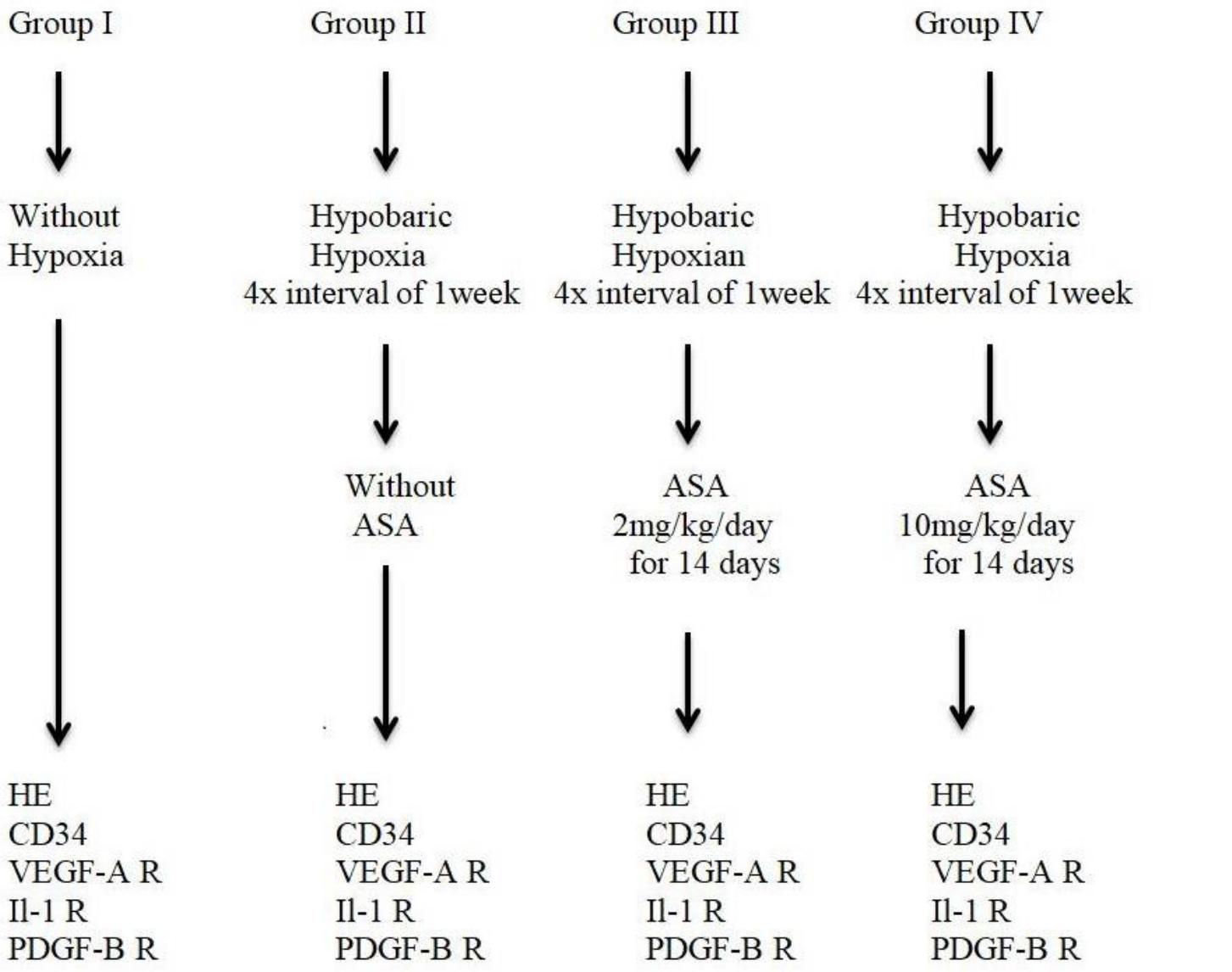


Figure 1

Hypobaric hypoxia treatment protocol12



ASA= Asetylsalisilic acid; HE = Hematoxylin Eosin; VEGF-A R: Vascular Endothelial Growth Factor A receptor, Il-1 R: Interleukin-1 receptor; PDGF-B R: Platelet Derived Growth Factor B receptor

Figure 2

Experimental research pathway

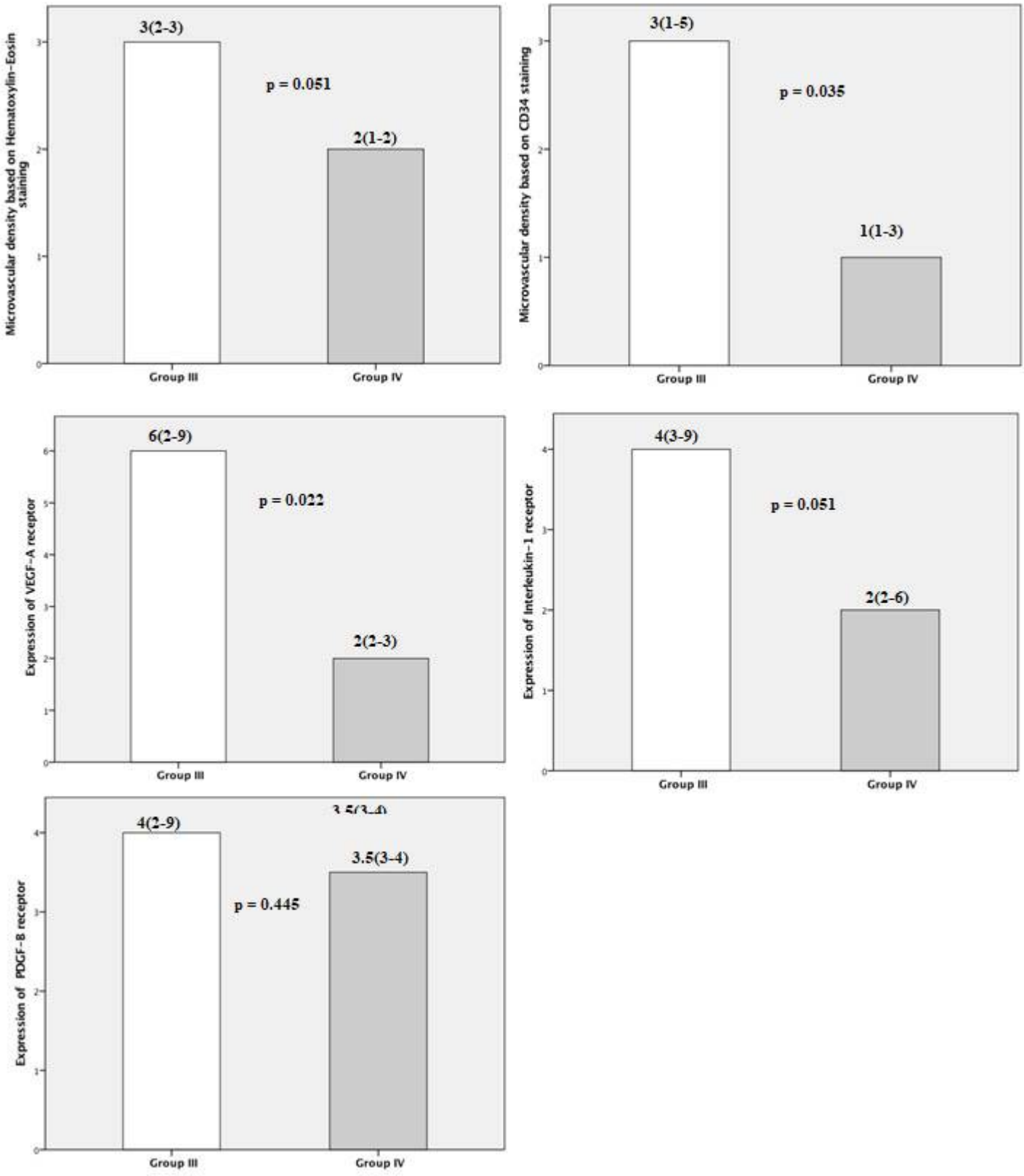


Figure 3

Microvascular density and histoscore for receptors comparisons between low dose and high dose of Acetylsalicylic acid groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ARRIVEChecklistBMCCardRes.docx