The correlations between insulin administration on hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor-β (VEGF-β) in experimental rat myocardium experienced hyperglycemia with hypoxia

Toni Prasetia¹, Nur Indrawati Lipoeto², Yanwirasti³, Masrul³, Hetti Rusmini³

ABSTRACT

Introduction: Hyperglycemia will lead to the synthesis of endothelium-1, which triggers vasoconstriction, resulting in decreased perfusion and ischemia. This will turn causing hypoxia which can induce hypoxia-inducible factor-1 (HIF-1) to release vascular endothelial growth factor (VEGF). This study aimed to analyze the relationship of HIF-1 expression to VEGF-β in the myocardium of experimental rats experiencing hyperglycemia with hypoxia.

Methods: This research method is purely experimental, all variables are controlled by a post-test only control group design. 30 male Wistar rats aged 3 months with 200–250 g were used. They were divided into two groups using simple random sampling techniques. Before given treatment, blood glucose checks were carried out in both groups. Furthermore, both groups were conditioned to be hyperglycemic and hypoxic for some time. Then, the first group was given insulin while the second group was not given insulin. The expression of HIF-1, VEGF-β was examined using immunohistochemistry. Data analyzed with Spearman test.

Results: The mean blood glucose in the first group was 174 ± 8.80 mg/dl while the second group was 173.67 ± 10.10 mg/dl. The mean value of HIF-1 in the first group was 63.33 ± 24.97% while the second group was 77.33 ± 12.79%. The mean value of VEGF-β in the first group was 37.33 ± 15.80% and in the second group was 13.67 ± 8.76%.

Conclusion: Changes in HIF-1 expression did not have a significant relationship with the expression of VEGF-β, both in control and treatment.

Keywords: Hyperglycemia, hypoxia, HIF-1, VEGF-β, insulin.

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INTRODUCTION

Hyperglycemia is a condition that often occurs today in society due to changes in the behavior of people who consume excessive carbohydrates. Many factors caused hyperglycemia, including insulin resistance, genetic defects that affect insulin action, diseases affecting the pancreas, endocrinopathy, and drug intoxication.

The main target of hyperglycemia is endothelial dysfunction caused by increased production of free radicals by mitochondria. This increase in free radical damages DNA which then activates the enzyme Poly (ADP-ribose) polymerase (PARP). PARP activation will inhibit Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), an enzyme that plays a role in glycolysis. As a result, the glycolysis process is disrupted and seeks upstream pathways in the form of polyol pathways, hexosamine pathways, activation of protein kinase C (PKC), advanced glycosylation end products (AGES).²

Hyperglycemia causes low tissue oxygen tension so that it will induce an increase in hypoxia-inducible factor-1 (HIF-1) expression. This was proven by a study which found that there was an increase of HIF-1 in cells experiencing hypoxia. Furthermore, external administration of HIF-1 could have a protective effect on cells experiencing hypoxia.³

Increased expression of HIF-1 will stimulate the release of target genes such as vascular endothelial growth factor (VEGF). Circulating VEGF in the blood will bind to the VEGF receptor (VEGFR) on the endothelium, causing angiogenesis. A study found a direct relationship between increased HIF-1 and VEGF expression. Other studies suggest that VEGF is increased by hypoxia in vitro. However, in vivo data regarding VEGF regulation in chronic hypoxic disease is still in question.⁴

Insulin is an anabolic hormone that functions to maintain the stability of blood glucose levels. Many studies prove the positive benefits of insulin administration in hyperglycemia. The mechanism of increasing insulin involvement in
the incidence of hyperglycemia is still unknown. This study aimed to evaluate the extent of insulin's influence on apoptosis by looking at the expression of HIF-1 and VEGF; in the myocardium of experimental rats experiencing hyperglycemia with hypoxia.

**METHODS**

This was a pure experimental research since all variables controlled by post-test only control group design. This research was held at the Lampung Veterinary Institute, the Pharmacology Laboratory of the University of Malahayati Lampung, and the Dyatnitisalis Anatomical Pathology Laboratory, Palembang. This research lasted for two years, from January 2021 until September 2023.

**Sampling technique**

The sampling technique is simple random sampling because the population is considered homogeneous. The rats taken for the sample in this study were 30 male rats. Then given standard food with body weight and age that meet the requirements in this study. After sufficient time and body weight, the rats were acclimatized for one week, then divided into two groups randomly.

**Dosage Calculation**

Calculation of the alloxan dose of 120 mg/kg BW, converted in rats with a body weight of 200 g. (200/1000) x 120 mg = 24 mg. Obtained 24 mg/rat 200 gr. administered intraperitoneally (IP) at 10 mg/ml. After that, high-calorie food was given for 7 days.

**Measure of Blood Glucose Levels**

The measurement of blood glucose levels was done by glucometer and blood lancet. The tip of the rat’s tail was disinfected with alcohol cotton and wound it with a blood lancet. The blood then dripped on the glucometer strip. After 10 seconds, the measurement results appeared.

**Interruption Hypoxia Protocol**

Groups 1 and group 2 were subjected to intermittent hypoxic conditions for four hours per day over a period of 3 days (D1-D2-D3). Briefly, the animals were placed in a room with a 12-hour light/dark cycle (08.00-20.00) for the duration of the study. The hypoxic treatment was carried out from 8.00 to 12.00 when the rats were sleeping. The intermittent hypoxic treatment consisted of alternating changes from normoxia (21% O2, 5% CO2 and N2 balance) for 90 seconds and then hypoxia (5% O2, 5% CO2 and N2 balance) to oxygen concentration 30 seconds later in the chamber, measured by the O2 analyzer and changed by the oxygen sensor. The gas exchange setting is connected to a solenoid valve which has been connected to a digital timer to control the gas outlet. Whereas the normal control group was continuously exposed to room air (21% O2) at the same time period. At the same time as hypoxia treatment, group 2 was given subcutaneous NPH insulin 4 units/day. The dose is divided which is given 1 unit at 10.00, 1 unit at 13.00 and the remaining 2 units at 19.00. The goal of insulin therapy is to keep the animal’s glycemia as close to normoglycemia as possible, namely 60-150 mg/dL throughout the day 24 hours. All procedures were carried out in accordance with the Institutional guidelines for the care of laboratory animals and the Guide for the Care and Use of Laboratory Animals published by the Committee for the Update of the Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research, USA, 2011.

**Ethical Requirements**

This research has received Research Ethics Approval from the Research Ethics Commission of the Faculty of Medicine, University of Lampung with Reg No. 118/UN268/DT/2015.

**Statistics analysis**

Data were presented descriptively as mean ± standard deviation (SD) for numerical data. Statistical test between the two groups was carried out by paired t test. The result declared significant if α = 0.05. The correlation between two variables was analyzed with spearman rho tests.

**RESULT**

The characteristics of the research sample presented in table 1. All variables were normally distributed. The relationship between HIF-1 expression to VEGF-β in group-1 mice cardiac myocardium was reported in Table 2. The Spearman’s rho test results between HIF-1 and VEGF-β obtained the p-values of 0.769 and r -0.083. There was no relationship between HIF-1 and VEGF-β.

The relationship between HIF-1 expression to VEGF-β in group-2 mice cardiac myocardium was reported in Table 3. Pearson correlation test between HIF-1 and VEGF-β obtained p-values of 0.490 and r -0.193. There was no relationship between HIF-1 and VEGF-β.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hyperglycemic Rats with Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With insulin</td>
</tr>
<tr>
<td>1 Weight (g), mean ± SD</td>
<td>156.87 ± 8.11</td>
</tr>
<tr>
<td>2 Heart rate, mean ± SD</td>
<td>160.53 ± 15.01</td>
</tr>
<tr>
<td>3 Blood sugar before administration of alloxan IP (mg/dl), mean ± SD</td>
<td>111.80 ± 7.06</td>
</tr>
<tr>
<td>4 Blood sugar after administration of alloxan IP (mg/dl), mean ± SD</td>
<td>174 ± 8.80</td>
</tr>
</tbody>
</table>

**Table 2. Correlations between HIF1 and VEGF-β**

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1</td>
<td>-0.083</td>
<td>0.769</td>
</tr>
</tbody>
</table>

HIF-1: Hypoxia-inducible factor-1

**Table 3. Correlations between HIF1 and VEGF-β in group-2**

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1</td>
<td>-0.193</td>
<td>0.490</td>
</tr>
</tbody>
</table>

HIF-1: Hypoxia-inducible factor-1
DISCUSSION

Studies found that stabilization of HIF-1 would modulate increased VEGF-α in ischemic conditioned rat hippocampal tissue. It was suggested that this process was part of its neuroprotective effect. Transient ischemia in brain tissue also induces HIF-1 and is followed by VEGF-stimulation. This process takes 24-48 hours and returns to basal levels after 7 days. Meanwhile, study by Catrina found that hyperglycemia causes a decrease in the HIF-1 activation response in ischemia. Excessive increase in free radicals will inactivate nitric oxide (NO). Besides that, NO inactivation is also caused by disruption of regulation of endothelial NOS (eNOS), an enzyme that catalyzes the reaction of the amino acid l-arginine to citrulline and NO compounds which are endothelium dependent vasodilators. Reduced NO and activation of PKC will lead to increased synthesis of endothelium-1 (ET-1) which triggers blood vessel vasoconstriction and platelet aggregation thereby accelerating the process of atherosclerosis.

Chronic hyperglycemia also causes changes in coagulation factors and increases platelet aggregation and accelerates endothelial dysfunction. In this condition, vascular tone will decrease so that perfusion decreases and ischemia occurs which results in low oxygen tension. This condition is called hypoxia. In a state of hypoxia, the body will respond. The main response in this condition is the induction of hypoxia inducible factor-1 (HIF-1), which is a transcription factor that is important for the adaptive response of cells to hypoxia.

Hyperglycemia causes low tissue oxygen tension so that it will induce an increase in HIF-1 expression. This was proven by study which found that there was an increase in HIF-1 in cells experiencing hypoxia and external administration of HIF-1 could have a protective effect on cells experiencing hypoxia. Research by Catrina et al. also showed that HIF-1 induction occurred in primary dermal fibroblast cells induced by high glucose. The study also obtained HIF-1 expression in hypoxic and diabetic rat hearts.

Increased expression of HIF-1 will stimulate the release of target genes such as VEGF. Circulating VEGF in the blood will bind to the VEGF receptor (VEGFR) on the endothelium, causing angiogenesis. Studies suggest that VEGF is increased by hypoxia in vitro. However, in vivo data regarding VEGF regulation in chronic hypoxic disease is still in question.

CONCLUSION

There was no correlation between HIF-1 levels and VEGF in cardiac myocardium of experimental rats with hyperglycemia and hypoxia in the control group and group that received insulin.

ETHICAL CLEARANCE

This study was approved the ethical test form health research Ethical Commision, Faculty of Medicine University of Lampung with number 1128/UN26/8/DT/2015.

FUNDING

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CONFLICT OF INTEREST

No conflict of interest exists with regards to this study.

AUTHOR CONTRIBUTION

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study’s results were interpreted upon publication.

REFERENCES


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