The Effect of Insulin on Gene Expression of Inducible Nitric Oxide Synthase during Hemorrhagic Shock in Rat

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Abstract. The purpose of this study was to investigate the effect of insulin on gene expression of inducible nitric oxide synthase (iNOS) during hemorrhagic shock. Male Sprague-Dawley rat, weighted from 300 to 400 gram, were used for animal study model. Under anesthesia, cannulation to the femoral vessels through the dissection was done and 2 ml blood per 100 gram was withdrawn for hemorrhagic shock. After 1 hour of shock period, three groups were divided according to resuscitation fluid such as Lactated Ringer’s solution only (LR group), Lactated Ringer’s solution and 20% glucose (D20LR group), and Lactated Ringer’s solution, 20% glucose, and insulin (D20LRI group). Sampling of blood and tissue was performed after observation period for 90 minutes. The measurement of plasma nitric oxide (NO) level and iNOS gene expression in liver tissue was performed. There were no significant differences between plasma NO level of three groups. However, band density of iNOS gene expression were 18350.53 ± 6610.26 in LR group, 9148.33 ± 4081.75 in D20LR group, and 3874.07 ± 2313.40 in D20LRI group respectively with significant differences between three groups (p=0.017). This study showed insulin could suppress the gene expression of iNOS in hemorrhagic shock.

Keywords: hemorrhagic shock, insulin, iNOS

1 Introduction

Nitric Oxide (NO), the important transmitter in the neuronal cell and immune system, is formed in the cell, or going through the cellular membrane, then affect to the near cells. Also, NO is produced by nitric oxide synthase (NOS), and hypoxic state in tissue increases its production [1].

The expression of inducible NOS (iNOS) is remarkable in the condition of various shock, and produce excessive nitric oxide that has correlation with the descent of blood pressure [2]. It is suggested that excessive NO would play a key role to development of refractory hemorrhagic shock, not responsive to fluid resuscitation and various vasopressors [3].

In addition to a decrease in blood glucose, insulin lowers NO in diabetes rat [4], and iNOS expression in smooth muscle cell of vessels [5]. The purpose of this study is to investigate the effect of insulin on iNOS expression in hemorrhagic shock, using rat animal models.
2 Method

2.1 Design and subjects

The subjects were 25 Sprague-Dawley male rats, weighted from 300 to 400 gram. All subjects were divided to three groups according to the resuscitation fluid used in resuscitation period. The control group (LR group) using Lactated Ringer’s solution included 10 rats. The second control group (D20LR group) using Lactated Ringer’s solution and 20% glucose included 5 rats. The experimental group (D20LRI group) included 10 rats, using Lactated Ringer’s solution, 20% glucose, and insulin.

2.2 Procedure

Induction of anesthesia was performed by Gas Anesthesia System using isoflurane, and maintenance of anesthesia was performed with ketamine and xylazine intraperitoneal injection. Under anesthesia, cannulation to the femoral vein and artery through the dissection was done and 2 ml blood per 100 g was withdrawn for hemorrhagic shock. After maintenance of hemorrhagic shock for 1 hour, fluid resuscitation was performed with resuscitation fluids according to the groups. Then, sampling of blood and tissue was performed after observation period for 90 minutes. Measurement of plasma NO level and iNOS gene expression was performed.

2.3 Data Analysis

Data were analyzed through Mann-Whitney and Kruskal-Wallis analysis using SPSS package. The statistical significance threshold was 0.05.

3 Results

Plasma NO levels were 28.21 ± 5.37 µmol/l in LR group, 32.48 ± 5.22 µmol/l in D20LR group, and 26.70 ± 6.52 µmol/l in D20LRI group respectively and there was no significant differences between three groups (p=0.210). D20LRI group showed the lower level of plasma NO than that of LR group, but there was no significance (p=0.597).

Band density of iNOS gene expression in liver tissue were 18350.53 ± 6610.26 in LR group, 9148.33 ± 4081.75 in D20LR group, and 3874.07 ± 2313.40 in D20LRI group respectively with significant differences between three groups (p=0.017). P value between LR group and D20LRI group was 0.02, particularly.
4 Discussion

Insulin has been known simply as metabolic hormone lowering blood glucose. However, other roles including anti-inflammatory action were under the investigation. In addition, insulin had an antioxidant effect, inhibiting expression of NADPH oxidase that produces superoxide radicals.

In hemorrhagic shock of this study, experimental group using insulin showed the reduction of iNOS gene expression, suggesting that insulin could inhibit the progression of irreversible shock. Because insulin is inexpensive and easily used in clinical setting, anti-shock effect of insulin can be expected to improve survival in hemorrhagic shock.

5 Conclusion

This study demonstrated that insulin could suppress the gene expression of iNOS in hemorrhagic shock.

References