Symposium

Correlative Factors of Insulin Resistance in Essential Hypertension

Hongyan TIAN, Aiqun MA, Chongmin LI, Mailing CHENG, Ling BAI, and Huasheng LIU

Essential Hypertension (EH) is correlated with a metabolic disturbance characterized by insulin resistance (IR). In this study, there were observed in 47 subjects with EH and 30 subjects with normal blood pressure. Serum levels of insulin-like growth factor-1 (IGF-1), serum levels of growth hormone (GH), the activity of erythrocyte insulin receptors (EIR), and ATP levels in erythrocytes, the insulin sensitivity index (ISI) was used to study the correlative factors of essential hypertension. 1. Among patients with EH, ISI, GH, and low-affinity insulin binding sites of EIRs (RT2) were found to be in significantly lower amounts, IGF-1 levels and the KD2 of the erythrocyte insulin receptors were noted to be significantly higher. Compared with the control group, there was a marked difference between EH group and the control group. However, no statistical difference was observed between the hypertensive group and the group with normal blood pressure as regards erythrocyte ATP levels, high-affinity insulin binding sites of EIRs (RT1), and the KD1 of EIRs. 2. In the hypertensive group, the ISI was negatively correlated with mean arterial blood pressure (MBP), a family history of hypertension, the body mass index (BMI), the waist-hip ratio (WHR) and IGF-1 levels (r= -0.614**, -0.354**, -0.386**, -0.472**, -0.298**, **p< 0.001, **p< 0.01, *p< 0.05), were positively correlated with RT2 and GH levels (r= 0.301**, 0.275*, **p< 0.01, *p< 0.05). There were no statistically significant differences between ISI and age, sex, smoking history, drinking, RT1, KD1, and ATP levels in erythrocytes. 3. The ISI was used as the dependent variable in multiple linear stepwise regression analysis. MBP (X1), a family history of EH (X2), WHR (X3), GH (X4), IGF-1 (X5), RT2 (X6), and the body mass index (X7) were used as independent variables. X1, X2, X3, X4, X5, X6, and X7 were used in the equations. The results indicate that patients with EH also tend to have IR. We suggest that MBP, a family history of hypertension, BMI, WHR, IGF-1, and RT2 might be independent factors affecting IR in cases of essential hypertension. (*Hypertens Res 2000; 23: 265-270)

Key Words: hypertension, insulin resistance, correlative factors

Introduction

Some cases of essential hypertension (EH) show a metabolic disturbance characterized by insulin resistance (IR), which distributes in a manner of aggregation together with atherosclerosis, coronary heart disease, obesity, and disturbance of blood-lipids (1-5). In recent years, it has been suggested that insulin resistance is a main factor in aggregated distribution (2-4). However, the mechanism of insulin resistance is very complicated. Some factors that may lead to insulin resistance include the existence of insulin antagonists, metabolic factors that affect insulin binding, the activity of insulin receptors, cell glucose metabolism, and any metabolic disturbance (6-9).

There is emerging evidence that the molecular structure...
of insulin-like growth factor-1 (IGF-1) is similar to that of insulin; in addition, receptor expression, and their effects on cardiovascular tissue under normal and pathological conditions are similar (10). Furthermore, studies show that hypertension may in part be due to abnormal effects of IGF-1 (10, 11). Recently, insulin/IGF-1 hybrid receptors were found in vitro, suggesting a contribution of IGF-1 to insulin resistance in women with gestational hypertension (12). The main regulation factor of IGF-1 is growth hormone (GH), which counteracts insulin in the regulation of glucose metabolism. The correlation between IGF-1, growth hormone, and insulin resistance in essential hypertension remains unclear.

Insulin receptors are present in various tissues. Studies have shown that the down regulation of erythrocyte insulin in cases of essential hypertension (13, 14). After the insulin and target cell receptors complex is formed, glucose metabolism inside the cell is regulated and monitored by glucometabolic enzymes. Glucose zymolysis, occurring inside erythrocytes is the only source of ATP in erythrocytes. ATP is the final product of glucose zymolysis.

The underlying mechanism of insulin resistance in cases of essential hypertension remains unclear. The present study used erythrocytes as a model to study correlative factors of insulin resistance in cases of essential hypertension. The aim of study was to detect differences between EH patients and people with normal blood pressure (NBP) constituted the control group (16 males, 14 females). The aged of these subjects ranged form 40-65 years old, mean age 51.02 ± 8.51 year (mean ± SD). The mean BMI was 24.37 ± 2.05 kg/m² (mean ± SD), and mean blood pressure was 119.00 ± 15.24/78.83 ± 8.73 mmHg (mean ± SD). The two groups showed no remarkable differences in age, sex, or body mass index (p > 0.05).

**Materials and Methods**

**Subjects**

This study was approved by the Ethics Committee of Xi'an Medical University. Informed consent was obtained from each subject. Essential Hypertension Group: There were 47 patients in the EH group (25 males and 22 females), all subjects met the diagnostic criteria for hypertension that are specified in the WHO/ISH newsletter of 1993, namely, systolic blood pressure (SBP) was ≥160 mmHg, and/or diastolic blood pressure (DBP) was ≥95 mmHg. The patients suffered form stage I or II essential hypertension. They underwent further examination to exclude such illnesses as secondary hypertension, serious infection, liver and kidney disease, diabetes, and obesity, which was evaluated by body mass index (BMI): males, ×4.39%, females, ×4.79%, BMI values greater than or equal to 120 were excluded (15). The patients were 40-65 years of age, with a mean age of 52.21 ± 6.15 years (mean ± SD). The BMI was 25.43 ± 2.66 kg/m² (mean ± SD), and blood pressure was 160.15 ± 17.00/102.26 ± 13.96 mmHg (mean ± SD). Before the experiments, administration of antihypertensive drugs was discontinued for 2 weeks. The Normal Control Group: Thirty subjects with normal blood pressure (NBP) constituted the control group (16 males, 14 females). The aged of these subjects ranged form 40-65 years old, mean age 51.02 ± 8.51 year (mean ± SD). The mean BMI was 24.37 ± 2.05 kg/m² (mean ± SD), and mean blood pressure was 119.00 ± 15.24/78.83 ± 8.73 mmHg (mean ± SD). The two groups showed no remarkable differences in age, sex, or body mass index (p > 0.05).

**Measurement of Blood Pressure**

Before blood pressure was measured, subjects sat for 20 min in a restful state. Then blood pressure was measured in the right arm with a mercurial sphygmomanometer. The systolic blood pressure was started at an initiating instance of Korotkoff's sound, and the diastolic blood pressure was taken at the fifth instance of Korotkoff's sound. Blood pressure was measured three times for each subject. The interval between repeated measures was 2 min; the mean value was regarded as the expected blood pressure.

**Experimental Methods**

Before the experiment, subjects fasted for 12 h. Ten ml of venous blood was taken, 5 ml of which was processed with heparin to avoid coagulation for the purpose of preparing a red cell suspension. The rest of the blood sample (5 ml) was processed to isolate the serum and was kept at a temperature of −45°. This latter sample was used to measure insulin, growth hormone, and IGF-1. All subjects received 75 g glucose powder, and each subject then underwent a glucose tolerance test (OGTT) and a synchronous insulin release test (InsRT). The following indexes were collimated, and the mean values were calculated.

**Determination of Erythrocyte Insulin Receptors**

The determination of erythrocyte insulin receptors was performed by radioreceptor assay: the Hua Xi Science and Technology Research Institute on Diabetes of China provided the test kit. The red cell suspension was placed in a centrifuge to isolate the red cells. According to the instructions specified in the test kit, RBA data processing software was used to calculated numbers of the high-affinity insulin binding sites (RT₁), the low-affinity binding insulin sites (RT₂), the kinetic dissociation constant of the high-affinity insulin binding sites (KD₁), and the kinetic dissociation constant of low-affinity insulin binding sites (KD₂) in the red cells.
Correlative Factors of IR in EH

Measurement of IGF-1 in Serum
A radio immunoassay (Biosource Co., USA) was conducted and the internal batch variable coefficient was 4.62%.

Measurement of Growth Hormone in Serum
A radio immunoassay (The Northern China Isotope Technology Institute of China provided the experiment kit) was performed and the internal batch variable coefficient was 4.11%.

Measurement of ATP Level in Red Cells
A modified method introduced by Wang (16) was used. Forty ml of blood was drawn form patients in a fasting state and infused into test tubes that had been pre-cooled and that contained 2 ml of 2 mM MgSO4. One ml of the mixture was extracted and bathed in boiling water for 5 min. Then, the solution was placed in a centrifuge for 10 min at 3,000 rmp. The supernatant was kept refrigerated at −45°C for later examination. Under superweak luminescence and with the use of an improved flow system, doubled distilled water was used as the flow carrier. Fluorescein—fluorescein enzyme buffer solution (Sigma, USA) was infused into sample for dark chamber reaction testing. Luminous intensity was recorded immediately and internal batch variable coefficient was 4.59%. The hemoglobin of the mixture was also measured. The unit expressing the amount of ATP present in red cells was expressed as mmol/kg · Hb.

Measurement of Glucose and Insulin in Serum
For glucose and insulin in serum measurements, the glucose-oxidase method, as well as the radio immunoassay (Atomic Energy Isotope Technology Institute of China). The internal variable coefficient was 4.61%, and the variable coefficient was 5.15%.

Measurement of Insulin Sensitivity
The insulin sensitivity index was used to evaluate the insulin sensitivity of each subject. The equation for the insulin sensitivity index (ISI) was 1/(fasting blood sugar multiplied by fasting insulin). Because the distribution of insulin resistance was not normal, the natural logarithm of the ISI was used to render the curve normal (17).

Statistical Analysis
All values were expressed as the mean ± SD, if not specified otherwise. Differences among groups were evaluated by analysis of variance followed by a T-Test. A probability of less than 0.05 was defined as significant. Data were examined by simple correlation. Data were non-parametrically distributed, and the rank correlation coefficients were calculated. Multiple linear regression analysis was performed to examine potential confounders. Coefficients were non-parametrically distributed and were therefore logarithmically transformed before the analysis. After identification of the best subsets in the initial model, parameters were added in a forward stepwise fashion.

Results
OGTT and InsRT
Figure 1 summarizes the finding that EH patients, whether fasting or loaded with glucose, showed glucose and insulin levels in serum that were significantly higher at various time points than those of subjects with normal blood pressure.

Insulin Sensitivity Index
Figure 2 shows that ISI in the EH patients was significantly decreased, as compared with subjects with NBP (p < 0.001).

IGF-1 and Growth Hormone in Blood Serum
EH patients had remarkably higher serum levels of IGF-1

(p < 0.05) and significantly lower serum growth hormone (p < 0.01) than the subjects with NBP (Fig. 3).

Activity of Erythrocyte Insulin Receptors
For EH patients, the number of low-affinity binding sites of the erythrocyte insulin receptors (RT₂) declined (p < 0.01). However, the KD₂ of erythrocyte insulin receptors (p < 0.05) rose significantly compared with the control group. However, the RT₁ and the KD₁ of the erythrocyte insulin receptors of the EH group and the NBP group showed no statistical difference (see the table).

ATP Level in Erythrocytes
Figure 4 shows ATP levels in the erythrocytes; there was no statistical difference (p > 0.05) between the EH group and the NBP group.

Analysis of the Correlative Factors of Insulin Resistance
For EH patients, linear correlation analysis was conducted with ISI as the dependent variable and other multiple factors as independent variables. It was found that ISI negatively correlated with mean arterial blood pressure, a family history of hypertension, body mass index, waist-hip ratio, and serum IGF-1 (r = -0.614\(^a\), -0.374\(^{**}\), -0.386\(^{**}\), -0.472\(^{**}\), -0.298\(^*\), \(^a\) indicating p < 0.001, ** indicating p < 0.01, * indicating p < 0.05). The ISI positively correlated with RT₂ and serum GH (r = 0.301\(^{**}\), 0.275\(^*\), ** indicating p < 0.01, * indicating p < 0.05). For this latter measure, and statistical significance was demonstrated. There was no statistical significance, however, between ISI and age, sex, smoking history, drinking, RT₁, KD₁, and ATP levels in erythrocytes.

Multiple Regression Analysis
According to the results of a simple correlation analysis, a multiple linear regression analysis was applied to the indexes that correlated with the ISI. The analysis and the establishment of the regression equation were conducted using the ISI as the dependent variable; mean arterial blood pressure (X₁), EH familial history (X₂), waist-hip ratio (X₃), GH (X₄), IGF-1 (X₅), RT₂ (X₆), and body mass index (X₇) as independent variables. When a =
Correlative Factors of IR in EH

Taking numerous blood samples and hence the cost is too high for a popularization of the application and thus it cannot be adopted for broad public use. The ISI was calculated based on the reciprocal of the product of the fasting glucose times the fasting insulin level, which is highly correlated with EICT (17); hence, the ISI is widely used in both clinical and research settings.

The mechanism of insulin resistance is very complicated. Several factors may cause IR, namely, high/low insulin secretion, blood insulin antagonists, altered receptor levels in target cells, and eventual physiological effects post-receptor, abnormality anywhere in the insulin cycle can potentially lead to insulin resistance. In this study, the correlative factors were studied as regards receptor activity as well as glucose metabolism in red blood cells, which were used as material for glucose metabolism in general.

The study used a correlation analysis and a multiple regression analysis to discover that insulin resistance was indeed correlated with blood pressure, body mass index, waist-hip ratio, family history of hypertension, IGF-1, and low-affinity insulin receptor sites in red blood cells. In recent years, it has been suggested that such cardiovascular risk factors as obesity, especially visceral obesity, hypertension, and insulin resistance show individualized aggregation. Insulin is the focal unit of the spokes leading to aggregated distribution (4-9). The results of this study confirm this model; furthermore, they suggest that hereditary factors are important factors causing insulin resistance. IGF-1 is also involved in the development of hypertensive insulin resistance. The mechanisms of this disorder require further study.

The insulin receptor protein that traverses the membrane composed of two α sub-units and two β sub-units. Insulin receptors are present in various tissues. In structure, they are homologous and conservative. According to the level of affinity, the insulin receptor is divided into two type, namely, high-affinity receptors and low-affinity receptors. The latter receptor are more numerous and more stable than the former. In addition, low-affinity receptors more accurately reflect the activity of insulin receptors (19). It is easy to obtain red cells. The activity of erythrocyte insulin receptors is consistent with general changes in the body's insulin target cells (19, 20). The present study showed that patients with hypertension demonstrate down-regulation of low-affinity insulin receptors; this finding is consistent with previous (13, 14). The subject have positively correlated with the ISI. The multiple regression analysis indicated that low-affinity binding sites of erythrocyte insulin receptors provided the independent correlative factor. These findings suggest that insulin resistance in cases of hypertension possibly occurs at the receptor level.

Through EICT, Ferrannini found that in the patients with hypertension, total insulin-mediated glucose uptake was reduced and was only involved non-oxidative metabolism of peripheral glucose. It has thus been assumed that the channels for glucose zymolysis are thereby affected; this may in part explain the correlation with the severity of hypertension (21). After the formation of the insulin-target cell receptor complex, glucose metabolism inside the cell was shown to be regulated and monitored by glucometabolic enzymes. Glucose zymolysis in red cells was the only source of ATP in erythrocytes. The final product of glucose zymolysis was ATP. So, ATP levels in red cells can reflect levels of glucose metabolism. This study showed no statistical difference between the ATP level in red blood cells, and no statistical correlation of the ISI in patients with hypertension and subjects with normal blood pressure. These findings suggest that the channels for glucometabolic zymolysis in the red cells of EH patients were not affected.

This study used red blood cells as the model for glucose metabolism. Though the insulin receptor levels were consistent with general changes in the body, however, red blood cells are not insulin-sensitive tissue. Further study is needed as regards the relationship between insulin-sensitive tissue (e.g. skeletal muscle, smooth muscular, and fat), glucose metabolism, and insulin resistance. In addition, further study of the mechanisms of EH, a very complicated pathological process, is necessary.

Acknowledgements

We thank Dr. Yang Hong, Dr. Wang Xue for assisting us in the statistical analysis.

References


