Original articles

A correlation study of the expression of resistin and glycometabolism in muscle tissue after traumatic brain injury in rats

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【Abstract】Objective: To investigate the expression pattern of resistin (RSTN) in skeletal muscle tissue and its influence on glycometabolism in rats with traumatic brain injury (TBI).

Methods: Seventy-eight SD rats were randomly divided into traumatic group (*n*=36), RSTN group (*n*=36) and sham operation group (*n*=6). Fluid percussion TBI model was developed in traumatic and RSTN groups and the latter received additional 1 mg RSTN antibody treatment for each rat. At respectively 12 h, 24 h, 72 h, 1 w, 2 w, and 4 w after operation, venous blood was collected and the right hind leg skeletal muscle tissue was sampled. We used real-time PCR to determine mRNA expression of RSTN in skeletal muscles, western blot to determine RSTN protein expression and ELISA to assess serum insulin as well as fasting blood glucose (FBG) levels. Calculation of the quantitative insulin sensitivity check index (Q value) was also conducted. The above mentioned indicators and their correction were statistically analyzed.

Results: Compared with sham operation group, the RSTN expression in the skeletal muscle as well as serum insulin and FBG levels revealed significant elevation ($P<0.05$), and reduced Q value ($P<0.05$) in traumatic group. Single factor linear correlation analysis showed a significant negative correlation between RSTN expression and Q values ($P<0.001$) in traumatic group.

Conclusion: The expression of RSTN has been greatly increased in the muscular tissue of TBI rats and it was closely related to the index of glycometabolism. RSTN may play an important role in the process of insulin resistance after TBI.

Key words: ***Brain injuries; Resistin; Insulin resistance; Blood glucose; Insulin sensitivity***

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Traumatic brain injury (TBI) has now become a global public health problem and according to the latest report of World Health Organization, by the year 2020, TBI will become the leading cause of disability and nearly 10 million people will suffer from TBI per year.[1] For a long time, hyperglycemia is believed to increase brain injury mortality and disability rates.[2] Steppan et al[3] found the protein, resistin (RSTN). The current research reports on RSTN are mainly linked with type II diabetes mellitus, obesity, and coronary heart disease. There are few studies reporting the relationship between RSTN and hyperglycemia after TBI. This study aims to assess the muscular RSTN expression in TBI rates and explore its relation with glycometabolism-related parameters, and further to describe the role of RSTN in hyperglycemic rats following TBI. We hope this may provide a new theory for blood glucose research after TBI.

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METHODS

Animals, reagents and instruments

Seventy-eight clean SD male rats weighing 300-350 g were provided by the experimental animal center of Hubei province. RSTN antibodies (sc-16116) were purchased from SANTA Company (USA), ELISA kit for serum RSTN detection from Shanghai Yili Bio-Technology Co., Ltd (China), Trizol from Invitrogen (USA), reverse transcriptase reagent from Fermentas Company (Canada) and PCR kit from TaKaRa (Japan).

Group

All the SD rats were randomly divided into sham operation group (n=6), traumatic group (n=36), and RSTN antibody injection group (hereinafter referred to as RSTN group, n=36). In sham operation group, under the effect of anesthesia, the scalp was exposed and cut through to make a bone window on the skull. In traumatic group, besides this, fluid percussion brain injury model[4] with the pressure of 300 kPa was established. In RSTN group, each rat underwent the same TBI model and then administrated intraperitoneal injection of 1 mg RSTN antibody immediately. At respectively 12 h, 24 h, 72 h, 1 w, 2 w, and 4 w after operation, 6 rats were sacrificed everytime after venous blood collection. The right hind leg skeletal muscle tissue was sampled and stored at a -70 °C refrigerator.

Assessment of RSTN gene (Retn) expression by real-time PCR

The total RNA in muscle tissue was extracted using Tizol technique, followed by spectrophotometry at optical density (OD) 260 nm and 280 nm to determine the RNA concentration (1 OD 260 nm=40 μg RNA). Later RNA was reverse-transcribed to cDNA. The primers were designed using the software Primer 5.0, with their sequences being Actb F: 5'-CACGATGGAGGGGCCGGACTCATC-3'; -Actb R: 5'-TAAAGACCTCTATGCCAACACAGT-3'; Retn F: 5'-CCAGAAGGCACAACCGTCACTA-3'; Retn R: 5'-TCAACCGTCCTCAGGAACCAA-3'. ABI 7900 real-time PCR System was applied, and the reaction conditions were 50°C for 2 min, 95°C for 10 min, 1 cycle; 95°C for 30 s, 60°C for 30 s, 40 cycles. The mRNA expression level of corresponding gene in rats' skeletal muscle tissue was corrected using standard curve method. The ratio of RSTN Retn mRNA to internal reference Actb mRNA (β-actin protein gene) is the relative expression level of Retn.

Assessment of RSTN expression by western blot

Extraction and determination of total protein concentration were conducted according to the kit instructions, including the following procedures: firstly protein denaturation, gluing, gel electrophoresis, transmembrane, sealing with 5% skimmed milk powder for 2 h; secondly incubation with RSTN antibodies (1:500) and GAPDH antibody (1:1 000) overnight at 4°C, washing the membrane using TBS+Tween solution three times; thirdly re-incubation with rabbit anti-sheep IgG HPR secondary antibody (1:20 000) at room temperature, TBS+Tween membrane washing 3 times and 10 min for each; finally adding chemiluminescent substrates and color reagents, followed by exposure, developing and fixing of images in darkroom.

Test of blood glucose, serum insulin and calculation of insulin sensitivity check index

ELISA was used to detect levels of blood glucose and serum insulin in each group, and the kit user manual was referred to for specific test methods. Li et al[5] proposed insulin sensitivity index (ISI) formula: ISI=1/(FPG×FINS) (FPG: fasting plasma glucose concentrations; FINS: fasting insulin concentrations) as the insulin sensitivity parameter. In order to make the data consistent with a normal distribution, natural logarithmic transformation of ISI was done to get the insulin action index (IAI) and the corresponding formula is IAI=-ln(FPG×FINS). Insulin sensitivity, also known as the quantitative insulin sensitivity check index (Q value), is calculated for each test point in each group.

Statistical analysis

SPSS 13.0 software was used to process the data which were expressed as $\bar{x}$±s. Means between groups were compared using single-factor analysis of variance (ANOVA) and correlation analysis was achieved with the use of concomitant variable univariate analysis. P<0.05 was considered statistically significant.

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