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BASIC MEDICINE

Hyperglycemia induces protein nonenzymeatic glycosylation in brain neurons of diabetic rats at early stage★

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Abstract

BACKGROUND: Protein nonenzymatic glycosylation is supposed to be one of mechanisms for chronic complications development in diabetes mellitus, and therefore, might play an important role in the neuronal degeneration.

OBJECTIVE: To study the protein nonenzymatic glycosylation in brain neurons of diabetic rats, and to analyze the pathway of neuronal degeneration at the early stage of hyperglymecia.

DESIGN: Randomized controlled animal experiment.

SETTING: Department of Endocrinology, First hospital Affiliated to General Hospital of Chinese PLA and Beijing Laboratory for Brain Aging, Xuanwu Hospital Affiliated to Capital Medical University.

MATERIALS: Thirty-five male Wistar rats (grade Ⅱ), aged 3 months old, and 11 male purebred Kunming mice (grade Ⅲ) without special pathogen, aged 3 months old, were provided by the Animal Room of Capital Medical University.

METHODS: This experiment was carried out in the Beijing Laboratory for Brain Aging, Xuanwu Hospital Affiliated to Capital Medical University in 1998. The rats in the diabetic model group were intraperitoneally injected into 10 g/L STZ according to 60 mg/kg to establish rat models of diabetes mellitus. The blood glucose and body mass of rats in each group were determined respectively at 1, 2 and 3 months after modeling. The antibodies of advanced glycosylation end products (AGEs) of bovine serum albumin (anti-BSA) were self-prepared: ①The antigen of AGEs-BSA was prepared.②Eleven male Kuming mice (grade Ⅱ) of 3 months old without special pathogen were selected to inoculate AGEs-BSA. ③ The animals were immunized. ④Primary purification and detection of poly-antibodies of AGEs: the AGEs were performed immunohistochemical examination at 1 month after diabetic modeling by ELISA method.

MAIN OUTCOME MEASURES: ① Detection results of blood glucose and body mass of rats in two groups at different time points. ② Determination of polyclonal antibody titer of AGEs-BSA. ③ Changes in immunohistochemical image of AGEs in brain tissue of rats in two groups.

RESULTS: Thirteen rats in the diabetic model group and fifteen rats in the normal model group entered the stage of final analysis. ①Changes of blood glucose and body mass: At 1, 2 and 3 months after modeling, the blood glucose of rats in the diabetic model group were respectively(28.8±2.8), (23.1±5.5), (25.4±5.1) mmol/L, which were significantly higher than those in the normal control group [(6.2±0.9), (6.1±0.8), (6.1±0.7) mmol/L, $P < 0.01$]; At 1, 2 and 3 months after modeling, the body mass of rats in the diabetic model group were respectively (250.1±52.2), (263.8±50.0), (261.5±42.6) g, which were significantly lower than those in the normal control group [(422.6±36.2), (462.6±39.0), (485.0±28.8) g, $P < 0.01$]. ②Determination of antibody titer of immune serum: The mice were treated by AGEs-BSA of different concentrations twice. After that, the titer of AGEs -BSA was determined, and the results of which indicated that a higher absorbance existed at 1∶1 000. ③Determination of antigen concentration: The final titer of antibody in the abdominal dropsy was determined, and the results of which suggested that there was a much higher absorbance in the AGEs-BSA at the concentration of 5–50 mg/L. ④Determination of antibody titer in abdominal dropsy: The antibody titer in abdominal dropsy was detected by ELISA method with antigen at 20 mg/L, which indicated that the maximum absorbance (1.265±0.039) existed at 1∶4 000, and very larger absorbance (0.982±0.067) at 1∶20 000. The polyclonal antibody of AGEs-BSA was successfully prepared. ⑤Immunohistochemical detection results: The immunohistochemical staining of AGEs showed there were positive neurons in the first month in the diabetic model group, whereas it was not significant in the normal

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control group. The positive substances were found mainly in the cytoplasm.
CONCLUSION: Hyperglycemia at the early stage of diabetes mellitus (1 month after modeling) can lead to protein nonenzymeatic glycosylation in brain neurons, and no obvious reactions mentioned above are found in the normal control group. It suggests that the degenerative changes of tissue structure of central nervous system are related with protein nonenzymeatic glycosylation caused by hyperglycemia.
Key Words: hyperglycemia; diabetic mellitus; immunohistochemistry

INTRODUCTION

Protein nonenzymeatic glycosylation is one of basic pathophysiologic changes of chronic complications of diabetes mellitus. It has been much involved in the study of diabetic peripheral neuropathy (PNP). Long lasting hyperglycemia can result in ketoacidosis, hyperosmolar nonketotic diabetic coma, hypoglycemic coma and other acute dysfunctions of central nervous system (CNS). It also causes chronic diabetic encephalopathy [1]. It is mainly related with vessel, metabolism and abnormal neuronal gene expression [2]. Hyperglycemia can not only lead to severe stroke, but also cause mild chronic encephalopathy, which presents simple cognitive dysfunction or mental disorder [3,4]. Animal experiments demonstrate that diabetes mellitus can result in the abnormal changes in cerebral blood flow, metabolism, neurotransmitter and other aspects [1,5]. In this experiment, we used self-made antibodies of advanced glycosylation end products (AGEs) of bovine serum albumin (AGEs-BSA) to observe the effect of early hyperglycemia on nonenzyme glycosylation in rat brain.

MATERIALS AND METHODS

Materials

This experiment was carried out in the Beijing Laboratory for Brain Aging, Xuanwu Hospital Affiliated to Capital Medical University in 1998. Thirty-five male Wistar rats (grade Ⅱ), aged 3 months old, weighing from 210 to 270 g, and 11 male purebred Kunming mice (grade Ⅲ) without special pathogen, aged 3 months old, weighing from 20 to 22 g, were provided by the Animal Room of Capital University of Medical Science [License No.SCXK(Jing) 2005-006]. Streptozocin (STZ, Sigma Company) and rapid blood glucose meter (Boehringer Mannheim, Germany) were used.

Methods

Preparation of animal models of diabetes mellitus: After being fasted for 16 hours, the rats were intraperitoneally injected with 10 g/L STZ solution dissolved by 0.1 mol/L natrium citricum buffer solution (pH 4.6) according to 60 mg/kg. Polydipsia, polyphagia, hyperdiuresis and other symptoms were found within 24 to 48 hours after injection of STZ. Rats with blood glucose ≥ 15 mmol/L indicated successful diabetic models, and were involved in the diabetic model group. The rats were modeled for 3 months successively.

Determination of blood glucose and body mass of rats at different time: Blood glucose and body mass of rats in each group were determined before and at 1, 2 and 3 months after modeling separately. Blood glucose was determined with rapid blood glucose meter.

Preparation of polyclonal antibody of AGEs-BSA:The Wistar rats were randomly divided into 2 groups: diabetic model group (n =20) and normal control group (n =15). The antigen of AGEs-BSA was prepared according to reference [6]. The Kunming mice were divided into 4 groups according to different contents of BSA in AGEs incubated by BSA: BSA 1 mg group (10 μL/mouse, n =3), BSA 2 mg group (20 μL/mouse, n =3), BSA 4 mg group (40 μL/mouse, n =3) and BSA 10 mg group (100 μL/mouse, n =2). The procedure of immunization was carried out according to reference [7]. Preliminary purification of multi-antibodies of AGEs: Abdominal dropsy was centrifuged at 2 500 r/min for 5 minutes. Supernatant fluid was taken, and antibodies were diluted with 1 g/L BSA dilution to remove anti-BSA antibody. AGEs polyclonal antibody was detected by ELISA method as follows: AGEs-BSA and 0.01 mol/L (pH 9.16) buffer bicarbonate were used to prepare into 20 mg/L solution, then which was added into 96-well plate, with 100 μL each well, overnight at 4 ℃. 0.1 mol/L PBST (1 g/L BSA included) was used to dilute antibody at 1∶500, 1∶1 000, 1∶2 000, 1∶4 000, 1∶10 000 and 1∶20 000; Above-mentioned dilution of 100 μL was respectively added into each well, then the 96-well plate was incubated for 30 minutes at 37 ℃, subsequently, rinsed by 0.01 mol/L PBST solution and dried. Tetramethyl benzidine working solution and 0.15 g/L hydrogen dioxide of one drop (50 μL)were added into each well respectively , then 96-well plate was placed away from light for 10 minutes; 1 mol/L sulphuric acid of one drop (50 μL) was added into each well. Absorbance was read with LP400 automatic enzyme reader.

Immunohistochemical detection of hippocampus and cortex of temporal lobe in rat brain tissue: Immunohistochemical staining of hippocampus and cortex of temporal lobe in rat brain tissue was conducted by ABC method 1 month after diabetic modeling as follows: The anesthetized rats were perfused by left ventricle and aortic cannula. Then, the brains of rats were harvested by decapitation. Coronal frozen sections of brain tissues at 40 μm in thickness were sliced. The supernatant sections were rinsed with phosphate buffer solution, incubated for 10 minutes with 0.03 volume fraction to eliminate the activity of endogenous peroxydase and for 30 minutes with 100 g/L goat serum to lighten non-specific staining. The primary antibody AGEs was diluted at 1∶100 and second antibody was diluted at 1∶200. The sections were developed by DAB and observed with a microscope. PBST was used to replace primary antibody as negative control.

Statistical analysis: Data were expressed as Mean±SD. The results of blood glucose and body mass of two groups were performed t test with SPSS 10.0 statistical analysis software.