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Research article

Rapamycin decreased blood-brain barrier permeability in control but not in diabetic rats in early cerebral ischemia

Oak Z. Chi [a,*], Geza K. Kiss [a], Scott J. Mellender [a], Xia Liu [a], Harvey R. Weiss [b]

[a] Department of Anesthesiology, Rutgers Robert Wood Johnson Medical School, 125 Paterson Street, Suite 3100, New Brunswick, NJ 08901, USA
[b] Department of Neuroscience and Cell Biology, Rutgers Robert Wood Johnson Medical School, 675 Hoes Lane West, Piscataway, NJ 08854, USA

HIGHLIGHTS

- In early cerebral ischemia, BBB disruption was greater in diabetes than control.
- The K_i of ^{14}C-AIB showed a higher baseline BBB permeability in early diabetes.
- Rapamycin lowered BBB disruption in the control but not in the diabetic rats.
- The mTOR pathway may not be important in altering BBB permeability in diabetes.

ARTICLE INFO

ABSTRACT

Diabetes causes functional and structural changes in blood-brain barrier (BBB). The mammalian target of rapamycin (mTOR) has been associated with glucose metabolism, diabetes, and altering BBB permeability. Since there is only a narrow therapeutic window (3 h) for stroke victims, it is important to investigate BBB disruption in the early stage of cerebral ischemia. We compared the degree of BBB disruption in diabetic and in control rats at two hours of reperfusion after one hour of middle cerebral artery (MCA) occlusion with or without inhibition of mTOR. Two weeks after streptozotocin ip to induce diabetes, MCA occlusion was performed. In half of the rats, an mTOR inhibitor, rapamycin was given for 2 days before MCA occlusion. After one hour of MCA occlusion and two hours of the reperfusion, the transfer coefficient (K_i) of ^{14}C-α-aminoisobutyric acid was determined to quantify degree of BBB disruption. Ischemia-reperfusion increased the K_i in the control animals. Streptozotocin increased the K_i in the ischemic-reperfused (IR-C, +22%) as well as in the contralateral cortex (CC, +40%). Rapamycin decreased the K_i in the IR-C (-32%) as well as in the CC (-26%) in the control rats. However, rapamycin did not affect K_i in the IR-C or in the CC in the diabetic rats. Our data demonstrated a greater BBB disruption in diabetes in the ischemic as well as non-ischemic cortex even in the early stage of cerebral ischemia-reperfusion and that acute administration of rapamycin did not significantly affect BBB permeability in diabetes. From our quantitative analysis of BBB disruption, the vulnerability of BBB in diabetes has been emphasized in the early stage of cerebral ischemia-reperfusion and a less important role of the mTOR pathway is suggested in altering BBB permeability in diabetes.

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1. Introduction

Diabetes has been associated with altered function of blood-brain barrier (BBB) and damage to the integrity of its structure. BBB damage plays a significant role in diabetes associated central nervous system disorders such as Alzheimer's disease, cerebral ischemia and enhancement of secondary brain injury [1–3]. Stroke also causes BBB disruption. Maintaining functional and structural integrity of the BBB during cerebral ischemia may be associated with a reduction in neuronal damage [4,5]. Because of narrow therapeutic window of stroke, it is important to investigate the degree of BBB disruption within three hours of cerebral ischemia-reperfusion [6].

In cerebral ischemia, multiple signaling pathways, factors and chemicals are involved in disrupting the BBB. Recently it has been shown that mammalian target of rapamycin (mTOR) is associated with altering BBB permeability and neuronal survival in the early

stage of cerebral ischemia-reperfusion [7,8]. mTOR inhibitors have been used as anticancer agents and immunosuppressants. One of the main problems in using mTOR inhibitors was that they alter glucose metabolism and frequently induce diabetes [9].

The effects of mTOR pathway on BBB function in diabetes are not clear. We hypothesized that inhibition of mTOR pathway before cerebral ischemia-reperfusion would attenuate BBB disruption in diabetic rats. To test this hypothesis we compared BBB disruption quantitatively using ^{14}C-α-aminoisobutyric acid (^{14}C-AIB, molecular weight 104 Da) and ^{3}H-dextran (molecular weight 70,000 Da) in the control and diabetic rats after one hour of ischemia and two hours of reperfusion. We determined BBB permeability at this time point since stroke has a very narrow therapeutic time window (3 h) for better outcome [6].

2. Materials and methods

2.1. Animal preparation

We followed the US Public Health Service Guidelines and the Guide for the Care of Laboratory Animals (DHHS Publication No. 85-23, revised 1996) in this research. We also obtained approval from our Institutional Animal Care and Use Committee.

Thirty-two male Fischer 344 rats weighing 220–250 g were used. They were randomly divided into four groups, 8 rats in each group: (1) control, (2) rapamycin, (3) streptozotocin, and (4) streptozotocin + rapamycin. For the streptozotocin and streptozotocin + rapamycin group, to induce diabetes, streptozotocin 60 mg/kg in citric acid buffer (0.09 mol, pH 4.5) was administered ip 2 weeks before middle cerebral artery (MCA) occlusion. For the control and rapamycin group, vehicle was injected instead of streptozotocin. On 13th and 14th day, 20 mg/kg of rapamycin (LC Laboratories, Woburn, MA) dissolved in normal saline and DMSO was injected ip once a day for two days for the rapamycin and streptozotocin + rapamycin group. For the control and streptozotocin group, the vehicle was injected instead of rapamycin. All rats were fasted for sixteen hours on the day before MCA occlusion. On 15th day all rats were ventilated through a tracheal tube with 2% isoflurane in an air-oxygen mixture for MCA occlusion. The isoflurane concentration was maintained at 1.4% after MCA occlusion. A femoral arterial catheter was inserted to connect to Statham P23Db pressure transducer and an Iworx data acquisition system to monitor heart rate and blood pressure, and to obtain blood samples for analysis of hemoglobin, blood gases and pH using a Radiometer blood gas analyzer (ABL80). A venous catheter was used to administer radioactive tracer and normal saline. For blood glucose determination a Contour Device (Bayer Healthcare, IN, USA) was used. Body temperature was monitored with a servo-controlled rectal thermistor probe. It was maintained at 37 °C ±0.5 with a heating lamp. As a representative pericranial temperature, temporalis muscle temperature was monitored using a thermocouple probe (Omega Engineering, Inc., Stamford, CT), and it averaged 37 °C.

2.2. Transient MCA occlusion

To study cerebral ischemia-reperfusion, we performed transient occlusion of a MCA using an intraluminal thread. Through a midline ventral cervical incision, the common carotid artery was exposed and was carefully separated from the adjacent nerve. A 4.0 monofilament thread with silicone covered tip was inserted into the stump of the external carotid artery and advanced approximately 1.7 cm into the internal carotid artery until resistance was met. The filament was kept in place for 60 min to block MCA. Then it was removed to allow reperfusion and the external carotid artery was closed. All measurements were performed

after 120 min of reperfusion. BBB permeability parameters were determined in the ischemic-reperfused cortex (IR-C), contralateral cortex (CC), ipsilateral hippocampus (IH), contralateral hippocampus (CH), cerebellum (CBLL), and pons.

2.3. Determination of BBB permeability

After one hour of MCA occlusion and two hours of reperfusion, to determine BBB permeability, 20 μCi of ^{14}C-α-aminoisobutyric acid (^{14}C-AIB) (molecular weight 104 Da, Amersham, Arlington Heights, Illinois) was rapidly injected intravenously and flushed with 0.5 mL of normal saline. Blood samples were collected from the femoral arterial catheter at 20 s intervals for the first 2 min and then, every min for the next 8 min. Five min after injecting ^{14}C-AIB, 20 μCi of ^{3}H-dextran (molecular weight 70,000 Da, Amersham, Arlington Heights, IL) was injected iv and flushed with 0.5 mL of normal saline. After collecting the ten min arterial blood sample, the animals were decapitated and their brains were quickly frozen in liquid nitrogen. The following brain regions were dissected: IR-C, CC, IH, CH, CBLL, and pons. Brain samples were solubilized in Soluene^{TM} (Packard, Downers Grove, IL). Arterial blood samples were centrifuged and the plasma was separated. Plasma and brain samples were counted on a liquid scintillation counter that was equipped for dual label counting. Quench curves were prepared using carbon tetrachloride. All samples were automatically corrected for quenching. The blood-brain transfer coefficient for ^{14}C AIB was determined assuming a unidirectional transfer of ^{14}C AIB over a 10 min period of the experiment using the following equation as described previously [8,10]:

$$K_i = \frac{Am - (Vp \times CT)}{\int_o^T Cp(t)dt}$$

where Am is the amount of ^{14}C AIB radioactivity in the tissue per gram and Vp is the volume of plasma retained in the tissue. It is determined from the ^{3}H-dextran data and the following equation: Vp = A′m/C′p where A′m is the amount of ^{3}H-dextran radioactivity in the tissue per gram and C′p is the concentration of ^{3}H-dextran in the plasma at the time of decapitation. Cp(t) is the arterial concentration of ^{14}C AIB over time t and CT is the arterial plasma concentration of ^{14}C AIB at the time of decapitation. In the equation used to determine K_i, Vp × CT is a correction term that accounts for the label ^{14}C retained in the vascular compartment of the tissue, Am.

2.4. Statistical analysis

A two-way analysis of variance was performed using the general linear model (PROG GLM) from the SAS Institute (Cary, NC) to assess the differences in K_i, volume of dextran distribution and vital signs between the experimental groups and among the various examined regions. The statistical significance of differences was determined using the Tukey test. All data were expressed as mean ± standard deviation and the significance was defined as $p < 0.05$.

3. Results

3.1. Hemodynamics and blood gases

Hemodynamic variables and blood gases of the experimental groups prior to measuring BBB permeability parameters are presented in Table 1. Blood pressures were similar among the experimental groups but heart rate was significantly higher in the streptozotocin treated groups when compared with the control and rapamycin groups that were not treated with streptozotocin. Blood gases and hemoglobin were similar among the experi-