Identification of a novel therapeutic target for cerebral ischemia injury: role of BCAAs catabolism in ischemic stroke

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Introduction:

According to the data from Hospital Authority, stroke has been the fourth leading cause of death in Hong Kong[1]. Stroke victims need a series of rehabilitation therapy, including physiotherapy, occupational therapy, and speech therapy, which is a huge medical burden for the government. Reducing the incidence of stroke, identifying effective therapeutic strategies, and improving the recovery from post-stroke injury have been critical and urgent issues for researchers, medical practitioners, and the whole society.

Ischemic stroke, accounting for more than 80% of stroke incidence, is mainly caused by sudden blockage of blood flow from thrombus or embolism in large or small blood vessels in the brain. In the cerebral infarct core, the energy production is immediately impaired due to the deficiency of oxygen and glucose supply, leading to the dysfunction of ATP-dependent ion pumps (e.g. Na+-K+ ATPase) and failure of electrochemical gradients[2]. It further results in the depolarization of neuron cell membrane and Ca2+ influx through opened L-type voltage-gated Ca2+ channel. High concentration of intracellular Ca2+ triggers the release of glutamate, which binds to and activates multiple glutamate receptors (e.g. NMDA, AMPA, Kainate receptor) on post-synaptic neurons, further leading to uptake of Ca2+ and Na+. Elevated intracellular Ca2+ and Na+ induces production of reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, and neuronal inflammation, finally causing neuron apoptosis, necrosis and death[2].

Branched-chain amino acids (BCAAs) are essential amino acids that must be obtained from food. Increasing studies demonstrate that impaired branched chain amino acids (BCAAs) catabolism and high BCAAs accumulation have adverse effects on diabetes, cardiovascular diseases (CVD), and neurological diseases. Although several clinical studies have identified circulating BCAAs levels are altered in patients with ischemic stroke, the results are inconsistent. The exact role of BCAAs catabolism in mediating the ischemic stroke outcome is still unknown.

Objectives:

1.To comprehensively investigate the BCAAs dynamics in the brain and peripheral tissues after ischemic stroke in mice

2. To investigate how ischemia induces the expression of BCKDK in the mouse brain

3. To interrogate whether reversing cerebral BCAAs accumulation could mitigate cerebral ischemia injury via multiple strategies

4. To pin down the underlying mechanisms whereby how defective BCAAs catabolism during

Methods & Materials:

8-week old C57/BL6J mice will be subjected to MCAO surgery for 1h followed with 24h reperfusion. Mice will be administered with BCKDK inhibitor BT2 at 1h post the surgery. The cerebral and serum BCAAs in mice will be measured by HPLC. Infarct volume will be measured by TTC staining. The mRNA levels of key enzymes in BCAAs catabolism will be quantified by qPCR while the protein expression levels will be measured by Western blot.

Research Significance

This study will not only contribute towards the understanding of BCAAs catabolism in mediating the ischemic stroke outcome, but also help to unravel the early pathogenesis of ischemic stroke. Furthermore, our discovery of ischemia-induced BCKDK expression and the downstream molecular events about enhanced glutamate excitotoxicity will provide scientific evidence supporting the development of BCKDK inhibitors as potential therapeutics for cerebral ischemia injury.

1. Cerebral ischemia-reperfusion injury impairs BCAAs catabolism in the brain

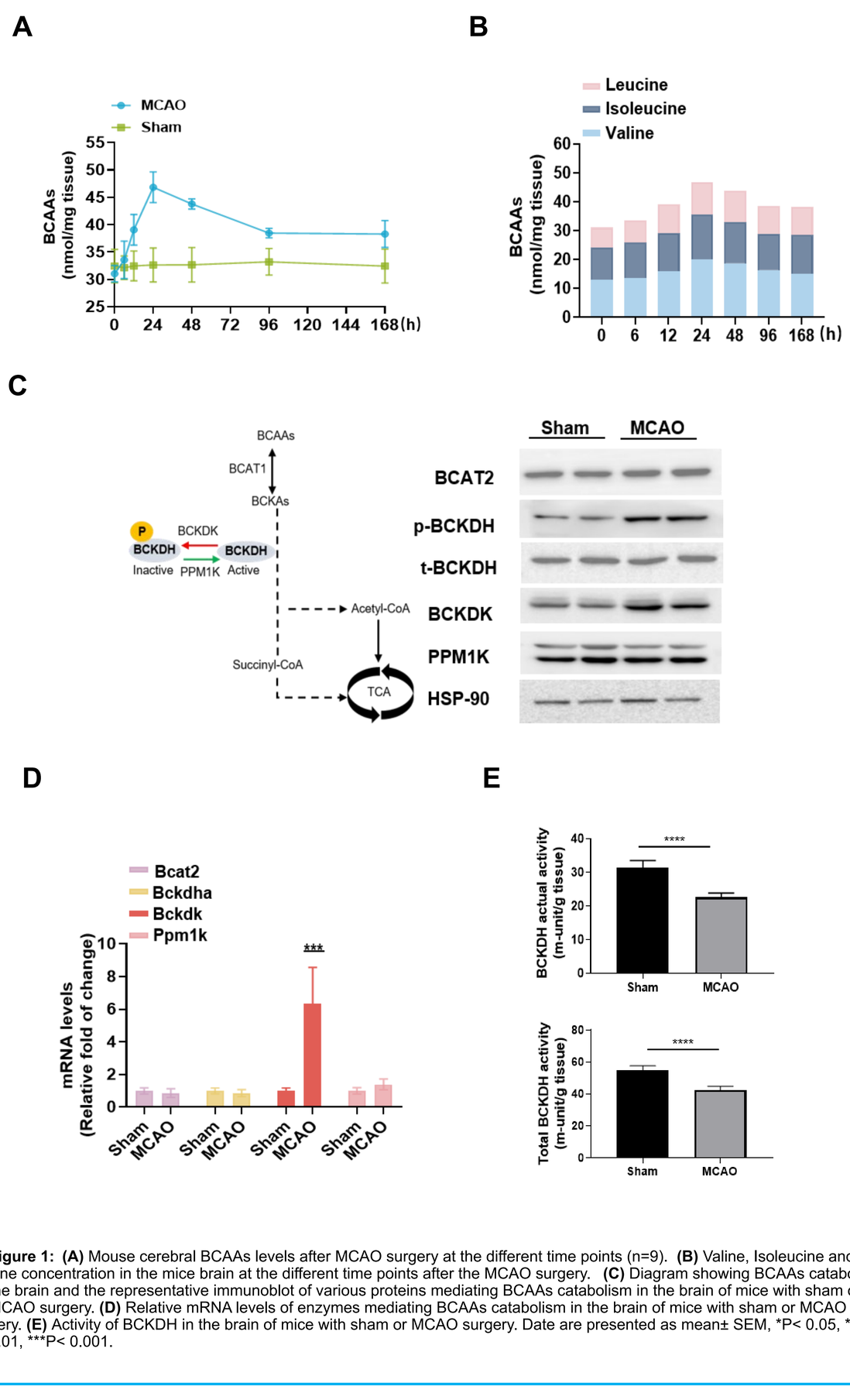

Figure 1: **(A)** Mouse cerebral BCAAs levels after MCAO surgery at the different time points (n=9). **(B)** Valine, Isoleucine and Leucine concentration in the mice brain at the different time points after the MCAO surgery. **(C)** Diagram showing BCAAs catabolism in the brain and the representative immunoblot of various proteins mediating BCAAs catabolism in the brain of mice with sham or MCAO surgery. **(D)** Relative mRNA levels of enzymes mediating BCAAs catabolism in the brain of mice with sham or MCAO surgery. **(E)** Activity of BCKDH in the brain of mice with sham or MCAO surgery. Date are presented as mean± SEM, *P< 0.05, ** P< 0.01, ***P< 0.001.

2. Branched chain keto acid dehydrogenase kinase (BCKDK) is a hypoxia-inducible factor.

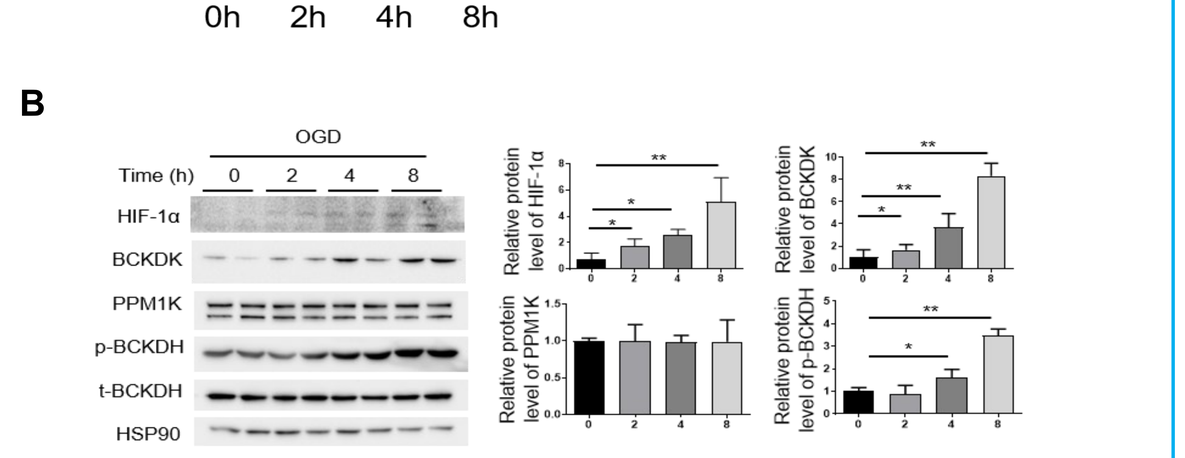

Figure 3: **(A)** Diagram showing the experiment design for the OGD. **(B)** Representative immunoblot of various proteins and the band intensity of each protein relative to their respective control protein or HSP90. The band intensities of p-BCKDH are relative to their control protein t-BCKDH (n=5). Data are presented as mean ± SEM. *P< 0.05, **P< 0,01.

3. Treatment of BCKDK inhibitor BT2 mitigates cerebral ischemia injury.

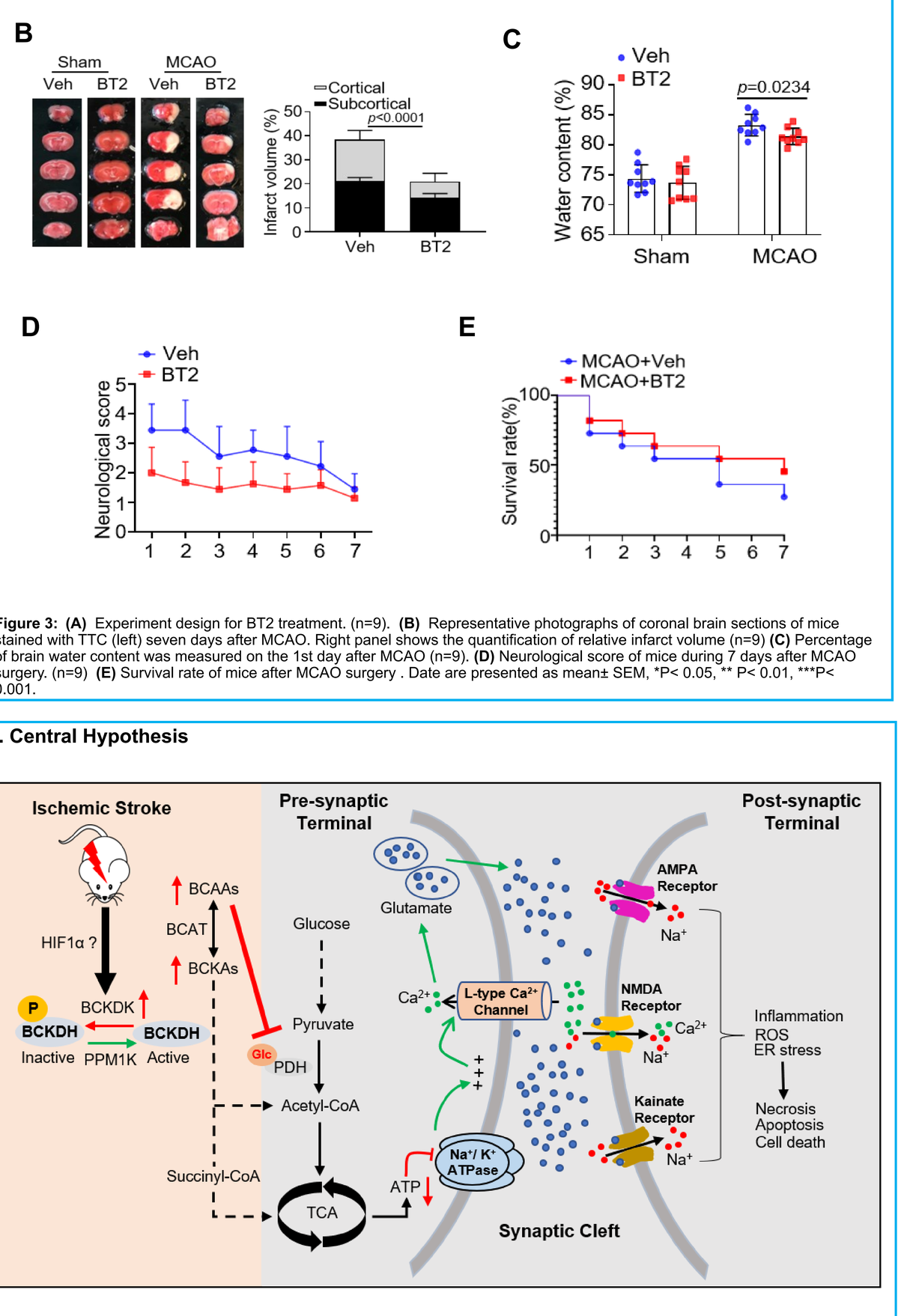

Figure 3: **(A)** Experiment design for BT2 treatment. (n=9). **(B)** Representative photographs of coronal brain sections of mice stained with TTC (left) seven days after MCAO. Right panel shows the quantification of relative infarct volume (n=9) **(C)** Percentage of brain water content was measured on the 1st day after MCAO (n=9). **(D)** Neurological score of mice during 7 days after MCAO surgery. (n=9) **(E)** Survival rate of mice after MCAO surgery . Date are presented as mean± SEM, *P< 0.05, ** P< 0.01, ***P< 0.001.

4. Central Hypothesis

Figure 1. Central hypothesis: Ischemia-induced accumulation of BCAAs controls cerebral injury via enhancing glutamate excitotoxicity. Ischemic stroke upregulates cerebral BCKDK expression, which inactivates the activity of BCKDH, resulting in impaired BCAAs catabolism and accumulation of BCAAs. Elevated BCAAs inactivates the activity of pyruvate dehydrogenase (PDH) and inhibits glucose/pyruvate utilization, leading to reduced ATP production and increased glutamate secretion to the synaptic cleft. Extracellular glutamate binds to and activates glutamate receptors on the post-synaptic terminal, facilitating the influx of Ca2+ and Na+ and inducing cerebral ischemia injury.

Conclusion:

- **Total BCAAs were elevated in the brain tissue at 24h after stroke.**
- **BCKDK was selectively upregulated by ischemic stroke, accompanied with enhanced phosphorylation of BCKDH.**
- **Administration of BCKDK inhibitor BT2 to mice with ischemic stroke substantially reverses downregulated BCKDH activity and alleviates cerebral ischemia injury.**

References:

1. http://www.strokefund.org/eng/aboutus_part1.php.

2. .Belov Kirdajova, D., et al., *Ischemia-Triggered Glutamate Excitotoxicity From the Perspective of Glial Cells. Front Cell Neurosci, 2020.* ***14****: p. 51* **.**