Ischemic stroke (IS) is one of the most common types of stroke. It is an acute cerebrovascular disease that can seriously harm human health. IS is characterized by poor prognosis and easy coexistence with other chronic diseases, bringing severe psychological and economic burden to patients around the world [1]. The core treatment of IS is to unlog blocked blood vessels, but even after the unlog is completed, there are still risks like cerebral ischemia-reperfusion (CIR) injury [2]. The brain pathological mechanism of the CIR injury process is complex, usually involving energy metabolism disorder, inflammatory response, oxidative stress injury, cell necrosis and apoptosis, excitatory toxicity, and other processes [3]. The coexistence of brain injury and recovery during reperfusion makes the
treatment of IS more difficult [4]. Therefore, it is important to study the mechanism of anti-CIR injury for the treatment of IS to improve the quality of life of patients with cerebral ischemia and reduce the mortality of the disease.

After an acute stroke, the cascade of brain remodeling is activated, and there are vital regional differences in the survival, migration, and maturation of brain cells, which have different effects on subsequent functions [5]. New neuronal precursors in the subventricular zone migrate to the cortex after stroke and are closely related to metabolic regulation [6]. New neurons in the subgranular zone of the hippocampus with abnormal function or morphology after stroke are associated with hippocampal dependence defects, affecting cognitive functions such as memory and pattern separation [7]. It has also been shown that neurovascular coupling and oxygenation are lower in the hippocampus than in the cortex under pathological conditions such as stroke. It is suggested that the hippocampus is more susceptible to hypoxic injury in the presence of neurovascular coupling damage [8]. The pathological features of the hippocampus and cortex are different and correlated during ischemic injury. The balance of excitatory and inhibitory pathways in the hippocampus is disrupted after middle cerebral artery distal occlusion (MCAO), when the oscillatory activity of the hippocampal EEG curve is more pronounced and the brain connections between the cortex and the hippocampus are disrupted. Impairment is closely related to changes in cognitive function after stroke [9]. By performing MCAO in the somatosensory cortex and recording the functional dynamics between the cortex and hippocampus, increased cortical activity was found to modulate hippocampal activity [10].

Shuxuetong injection is a standardized product extracted from traditional Chinese medicine Shuizhi (Hirudo) and Dilong (Pheretima). Each ampoule SXT (2 mL) is extracted from 0.5 g Shuizhi and 0.5 g Dilong. TCM believes that blood stasis block is the key to the onset of IS, and SXT has the effects of promoting blood circulation and removing stasis and activating channels (Huo Xue Hua Yu & Tong Jing Huo Luo), which has been widely used in the treatment of cardiovascular and cerebrovascular diseases, with precise curative effect and high safety for stroke diseases [11, 12]. SXT has a protective effect on brain microvascular endothelial cells after oxygen-glucose deprivation reperfusion (OGD/R), which is related to its effects on platelet aggregation, antithrombosis, reduction of mitochondrial superoxide production, inhibition of inflammation, and inhibition of vascular endothelial cells growth factors [13]. The main active components of SXT include hirudin and lumbrakinase. Hirudin has anticoagulant, antithrombotic, antifibrin, antiapoptosis, and other pharmacological effects [14]. Lumbrakinase has antibacterial and anti-inflammatory effects and promotes the repair of the nervous system [15]. Researchers analyzed and predicted bioactive peptides in SXT and screened out YLKT peptides that had protective effects on OGD/R injury of astrocytes [16].

Over time, metabolomics has evolved from an exploratory tool for qualitative studies to a more mature and complete biochemical technique for quantitative studies [17]. The identification of different metabolomic profiles helps to identify and improve clinical treatment strategies for diseases [18]. The brain has a high level and specific metabolic activity that is sensitive to ischemic injury [19]. Therefore, metabolomic studies of IS have a certain research base. Wang et al. described the metabolic characteristics of each subtype of IS, and the differential metabolites in patients with large atherosclerosis and small arterial occlusions were mainly related to glycerophospholipid metabolism and histidine metabolism [20]. The metabolomic analysis of edaravone on CIR injury in mice by Ma et al. screened cysteine sulfonate decarboxylase as a possible therapeutic target [21]. Other researchers have subdivided them by gender, identifying 49 metabolites that are significantly altered in female patients with acute IS, mainly glycerophosphocholine, and 39 metabolic pathways that are significantly altered in male patients with acute IS, including 5-α-Androstane-3-α,17-β-diol and threonate [22].

In the early stage of this project, the pharmacological effects of SXT injection on cerebral ischemia-reperfusion model rats were evaluated from the perspectives of transcriptomics and proteomics. In order to further study the pharmacological effects, this study aims to explore SXT’s role in the process of the injection against CIR injury and its influence based on different parts of the brain metabolism, to explore the different parts of the brain function and metabolic material basis.

2. Materials and Methods

2.1. Animals. In total, 88 SPF male SD rats aged 7–9 weeks with an average body weight of 240–270 g were purchased from Beijing Weitong Lihua Co., Ltd. The certificate number of the experimental rats was SCXK (Beijing) 2019-0010. All animals were fed under the conditions of temperature (22 ± 2)°C, relative humidity (50 ± 10) % and a 12-hour dark/light cycle. All the procedures in the experiment were approved by the Medical Ethics Committee of China Academy of Chinese Medical Sciences.

2.2. Drugs and Reagents. Shuxuetong injection freeze-dried powder (Manufacturer: Youbo Pharmaceutical Co., Ltd., No.20042201, freeze-dried powder content: 22.38 mg mL⁻¹), Edaravone Injection (manufacturer: Nanjing Xiansheng Dongyuan Pharmaceutical Co., Ltd., No. 80–151211), TTC (2,3,5-Triphenylte-Trazoliumchloride, Factory: McLean, No. C1221739), 4% paraformaldehyde phosphate buffer (Factory: Solarboi, No. P1110), methanol (chromatopure, factory: McLean), formic acid (chromatographic pure, manufacturer: McLean), acetonitrile (chromatographic pure, manufacturer: McLean), and rat MCAO nylon plug (thread diameter: 0.26 mm, tip diameter: (0.36 ± 0.02) mm, manufacturer: Beijing Shadong Company, No. 2636-100).

2.3. Surgery and Drug Administration. The cerebral ischemia-reperfusion model of rats was prepared after 3 days of adaptive feeding. According to Zea Longa method, a little improvement was made [23, 24]. t-MCAO was performed...
on rats, with specific operations as follows: we weigh and anesthetize the rats, make an incision at the middle part of the neck, and separate the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). The distal ECA was then ligated. The CCA and ICA was temporarily clamped. Prepare at the intersection of ECA and ICA. At the ECA cut, insert the marked thread from ECA and pull back to insert the thread in the ICA direction about 18∼19 mm. At this point, ICA’s temporary clamp has been opened. After fixation of the bolus, the ECA was burned and timed. The surgical incision was sutured and disinfected, and the animals were placed at a constant temperature of 37°C. After 15 min of ischemia, the groups were administered by tail vein injection. After 2 h of ischemia, the insertion line was removed and the temporarily clamped CCA was opened to ensure the establishment of reperfusion. For the sham-operated group, the steps were the same, except that the line was not inserted. The experimental animals were randomly divided into six groups: sham group (sham), model group (model), SXT high-dose group, medium-dose group, and low-dose group (SXT-H, 1.08 mg·kg⁻¹; SXT-M, 0.54 mg·kg⁻¹; SXT-L, 0.27 mg·kg⁻¹), positive drug group (edaravone injection, ED, 1.35 mL·kg⁻¹). Saline was given in the sham-operated and model groups.

2.4. Neurological Function Score. Six hours after modeling, the neural function of experimental rats was evaluated in the form of scoring, and Logna scoring method was adopted [25]. The rats were divided into five grades according to their behaviors: 0 point, no symptoms of nerve damage; 1 point, left front paw cannot be fully extended; 2 points, when walking, the rats turned to the left side (paralyzed side); 3 points, when walking, the rat body tipped to the left side (paralyzed side); 4 points, cannot walk spontaneously, conscious loss.

2.5. The Volume of Cerebral Infarction Measured by TTC. After neurological function evaluation, the rats were euthanized, and their brains were taken for staining with TTC [26]. The extent of cerebral infarction was calculated as a percentage of the total volume of the infarcted portion. Taking the position 3 mm away from the forehead as the starting point, slices were carried out in the order from front to back, and the thickness of a single slice was about 2 mm. The slices were stained with 2% TTC buffer solution and placed in a constant temperature light-proof box at 37°C for 10 min during the dyeing process. Uninfected brain tissue appears rose-red, while infarcts appear white. After staining, 4% paraformaldehyde buffer was used for fixation. After 30 minutes of fixation, the slices were sequentially arranged in the correct order and then electronically scanned and recorded. Image J was used to determine the area of different color brain regions, and the infarct rate was calculated according to the area:

Infarction rate = \( \frac{V_c - V_i}{V_c} \times 100\% \),

\[ V_c = d \times \sum A_c (Ac - Single \ slice \ area), \]

\[ V_i = d \times \sum A_i (Ai - Red \ area \ of \ single \ brain \ slice). \] (1)

2.6. Sample Pretreatment. The rats were decapitated, and their brains were taken out and dissected on ice. The cortex and hippocampus of the affected side were taken out and frozen at −80°C for later use. The cortex and hippocampus of 3 groups were extracted with 1 mL methanol as the extraction solvent. The cortex and hippocampus were homogenized, and the cortex and hippocampus were ground for 45 s and 60 s, respectively, with a 70 Hz high-throughput tissue grinder. The tissue grinding machine was purchased from Ningbo Xinzhi Biotechnology Co., Ltd. The model is ScientZ-192. Methanol 800 µL was added to 200 µL cortex abrasive solution, which was vortexically mixed for 1 min, then centrifuged at 12 000 r·min⁻¹ for 5 min at 4°C, and 5 µL supernatant was injected. The centrifuge (GT16-3) was purchased from Beijing Times Belli Centrifuge Co., Ltd. The abrasive solution of the hippocampus was centrifuged at 12 000 r·min⁻¹ at 4°C for 5 min, and 5 µL supernatant was used for sample injection.

2.7. UPLC-Q/TOF-MS Analysis. The separation was performed by UPLC (1290 binary liquid chromatograph, Agilent Technologies, USA) and screened by ESI-MS (Targeted MS/MS mode). The LC system consisted of Agilent ZORBAX Eclipse XDB-C18 (RRHD) 1.8 µm column and an American Phenomenex guard column. The mobile phase consisted of solvent A (0.1% formic acid-water) and solvent B (0.1% formic acid-acetonitrile), gradient elution (0-1 min, 95% A; 1-6 min, 95–70% A; 6–20 min, 70–5% A), and the mobile phase velocity was 0.5 mL·min⁻¹. Column temperature is 45°C and sample manager temperature is 4°C. The mass spectrometry was performed by Q/TOF-MS with a dual Agilent jet (AJS) ESI source. The scanning mass-charge ratio ranges from 50 to 1500, and the scanning rate is 1.00 spectra·s⁻¹. The capillary voltage is 4000 V and 3500 V (positive and negative mode, respectively), and the voltage is 175 V. Atomizer pressure is 35 psi, gas temperature 325°C, and continuous gas flow 5 L·min⁻¹. Instrument mode is set to an extended dynamic range. Quality control (QC) samples from normal rat cortex or hippocampus were performed at the beginning of the sequence to avoid small changes in chromatographic signal strength.

2.8. Data Analysis. SPSS 25.0 software (SPSS, Chicago, USA) was used to perform the Kruskal–Wallis test for neurological function scores. Under the condition of normal distribution and homogeneity of variance, a one-way ANOVA test was used for cerebral infarction volume data. The results of the cerebral infarction area were presented by means ± standard
error of the mean (SEM). $P < 0.05$ indicates that the difference was statistically significant. Raw data are collected and calibrated using the MassHunter Workstation (B0.06.00, Agilent) based on the $M/\!Z$ value and retention time of the ion signal. The results of ion ESI$^-$ and ESI$^+$ were imported into SIMCA-P (11.5) for multivariate analysis. Principal component analysis (PCA) is used as an unsupervised method to visualize data and identify outliers. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used for supervised regression modeling of datasets. Screen for metabolites with significant differences between the model group and the sham group and between the dosed group and the model group. Metabolites with $P < 0.05$ were used as differential metabolites. Metabolic pathway analysis was performed using MetaboAnalyst 5.0 and the KEGG database.

3. Results

3.1. Pharmacodynamics of SXT Injection against Cerebral Ischemia-Reperfusion. After modeling, the rats in the model group showed neurological impairment and cerebral infarction. Compared with the model group, the neurological function evaluation and cerebral infarction volume of the high-dose, medium-dose, and low-dose SXT groups and the ED group were significantly different and showed a quantitative effect relationship. The higher the concentration of SXT, the more significant the therapeutic effect, as shown in Figure 1 and Table S1.

3.2. Metabolomics Results of Cortex. PCA, OPLS-DA, and S-plot[M2] results of cortical tissues are shown in Figure 2. Results of cortical positive and negative ions are in Table S2. Each dot in the figure represents a sample of cortical tissue, and the distribution of each group represents the metabolic distribution of the sample. PCA analysis showed ($R^2 = 0.682$, $Q^2 = 0.413$) that the separation trend of metabolites in the three groups was obvious. The distribution of metabolites in each group was different, and the distribution within the group was relatively concentrated. The distribution of the administered group was close to that of the sham-operated group. OPLS-DA analysis showed that the cortical tissue samples in the model and sham-operated groups were distributed on both sides of the space, and the distribution of samples within the group was relatively concentrated. The distribution of the administered group was close to that of the sham-operated group. OPLS-DA analysis showed that the cortical tissue samples in the model and sham-operated groups were distributed on both sides of the space, and the distribution of samples within the group was relatively concentrated, indicating that the metabolites of the posterior cortex were significantly changed. Modeling and the changes were closely related to the pathogenesis. In S-plot[M2] figure, the further the distance from the origin, the more likely the reactive ions are to become differential metabolites between groups.

3.3. Metabolomics Results of Hippocampus. PCA, OPLS-DA, and S-plot[M2] results of hippocampal tissue are shown in Figure 3. Results of hippocampal positive and negative ions are shown in Table S3. PCA analysis showed ($R^2 = 0.674$, $Q^2 = 0.204$) that compared with the sham operation group, the model group had a significant separation trend, and the
distribution of the administration group and the sham operation group were closer, revealing the therapeutic effect of the drug from the perspective of metabolic distribution. The OPLS-DA results showed that 6h after IR, the samples of the model and sham-operated groups were symmetrically distributed on both sides of the space, and the distribution of samples within the groups was relatively concentrated indicating that hippocampal metabolites changed significantly by modeling. In the S-plot plot, the farther away from the origin they are, the more likely the reactive ions are to be differential metabolites between groups.

3.4. Differential Metabolite Results. The differential metabolites among different groups are shown in Table 1. The differential metabolites obtained by screening were all metabolites with significant differences between the model group and the sham operation group and between the administration group and the model group. All the differential metabolites obtained by screening belong to ESI− ion mode, and all have the performance of callback; that is, drug administration can reverse the injury of the model group to a certain extent, making the expression of the drug administration group more similar to that of the sham group. A total of 13 different metabolites were screened from the cortex, including amino acids (e.g., glutamine, dihydroorotic acid, and threonic acid), alkaloids (e.g., humanetine and yohimbic acid), and other acid products (including benzenesulfonic acid, glycric acid, and hydroxy acid) which were the main products. Five kinds of differential metabolites were screened from the hippocampus, mainly amino acids (glutamic acid) and benzene-related derivatives (benzoquinone, benzophenone, and benzoic acid).

3.5. Metabolic Pathway Enrichment Results. Metabolic pathway enrichment of differential metabolites was carried out through the KEGG database, and the enriched pathways are shown in Figures 4 and 5. There were four metabolic pathways with significant differences in the cortex: D-glutamine and D-glutamate metabolism, glyoxylate and dicarboxylate metabolism, pyrimidine metabolism, and nitrogen metabolism. Different metabolite concentrations in the metabolic pathways of the hippocampus and eight pathways with significant differences, respectively, are D-glutamine and D-glutamate metabolism, arginine biosynthesis, alanine, aspartate, and glutamate metabolism, nitrogen metabolism, butanoate metabolism, histidine metabolism, citrate cycle (TCA cycle), and pyruvate metabolism.

![Figure 2](image-url)
Figure 3: Metabolic analysis of hippocampus samples at 6 h after cerebral ischemia. (a) PCA-X image of hippocampal samples at 6 h after cerebral ischemia. (b) OPLS-DA of hippocampal samples at 6 h after cerebral ischemia. (c) S-plot [M2] of hippocampal samples at 6 h after cerebral ischemia.

Table 1: Differential metabolites of Model group, Sham group, and SXT-M group.

| Part      | No. | Metabolite name          | Retention time (min) | Measured mean mass (Da) | Chemical formula | Normalized abundance (×10^5) |
|-----------|-----|--------------------------|----------------------|-------------------------|------------------|-----------------------------|
|           |     |                          |                      |                         |                  | Sham | Model | SXT-M |
| Cortex    | 1   | Orthanilic acid          | 0.85                 | 172.0213                | C₆H₇NO₃S       | 1.15 | 1.92   | 1.62  |
|           | 2   | Glutamine                | 0.87                 | 145.0610                | C₃H₅N₂O₃       | 21.50| 30.72  | 24.54  |
|           | 3   | Humantenine              | 17.73                | 353.1976                | C₂₁H₂₆N₂O₃     | 4.93 | 3.17   | 5.11  |
|           | 4   | Dodecylbenzenesulfonic acid | 24.89               | 325.1818                | C₁₈H₁₈O₃S      | 53.40| 64.05  | 51.95  |
|           | 5   | Dodecyl sulfate          | 24.40                | 265.1460                | C₁₃H₂₆O₂S      | 113.22| 129.05 | 106.75 |
|           | 6   | Yohimbic acid            | 25.14                | 339.1974                | C₂₀H₂₀N₂O₃     | 42.16| 49.02  | 40.19  |
|           | 7   | S-sulforaphene           | 1.38                 | 174.0010                | C₆H₉NOS₂       | 3.48 | 4.78   | 4.00   |
|           | 8   | 3-Hydroxypropanoic acid  | 1.31                 | 89.0240                 | C₃H₆O₃         | 137.90| 171.41 | 141.70 |
|           | 9   | Dihydroorotic acid       | 0.28                 | 157.0108                | C₅H₁₀O₄        | 3.26 | 3.54   | 3.06   |
|           | 10  | Glyceric acid            | 0.76                 | 105.0166                | C₃H₅O₄         | 2.49 | 2.69   | 2.39   |
|           | 11  | Embelin                  | 24.86                | 293.1771                | C₁₉H₂₈O₄       | 81.56| 93.37  | 77.97  |
|           | 12  | Etidronate               | 23.68                | 204.9879                | C₂H₆O₇P₂       | 1.62 | 1.86   | 1.45   |
|           | 13  | Threonic acid            | 23.14                | 135.0102                | C₄H₇O₃         | 1.45 | 1.59   | 1.45   |

| Hippocampus | 1   | Fumaric acid             | 1.29                 | 115.0030                | C₄H₄O₄         | 0.32 | 0.28   | 0.32   |
|            | 2   | Embelin                  | 13.24                | 293.1733                | C₁₇H₂₀O₄       | 12.01| 6.18   | 9.10   |
|            | 3   | Glutamic acid            | 14.55                | 146.0348                | C₅H₇NO₄        | 5.40 | 3.69   | 5.25   |
|            | 4   | Butylparaben             | 12.04                | 193.0854                | C₁₁H₁₈O₄       | 3.26 | 2.04   | 2.90   |
|            | 5   | 2,4-Dihydroxybenzenophene | 0.84                 | 213.0473                | C₁₃H₁₈O₃       | 4.11 | 6.39   | 5.01   |

The model group was compared with the sham group, ▲▲▲P < 0.001. The SXT-M group was compared with the model group: *P < 0.05, **P < 0.01, and ***P < 0.001.
4. Discussion

There are complex pathological mechanisms in the process of CIR injury. It is helpful and significant to extend the study of its mechanism to different regions and scopes to clarify the transmission process of the disease. This study explored the effects of SXT injection on the distribution of metabolites in different parts of the brain of rats with cerebral ischemia-reperfusion in order to extend the relevant mechanism and target from the metabolites and lay a material foundation for further clarifying the pathological mechanism and site difference of CIR injury.

In order to determine the appropriate dose and effect of the dosing group, we performed a pharmacodynamic evaluation of SXT injection before metabolic analysis. The results showed a significant therapeutic effect in the high-
medium-, and low-dose groups of SXT. However, in combination with other studies in the group, the risk of bleeding was higher in the SXT high-dose group, provided that both the high-dose and medium-dose groups had significant therapeutic effects [27]. Therefore, the SXT medium-dose group (0.54 mg/kg) was selected for the follow-up metabolomics experimental study. The results showed that the disorders of brain metabolism in the model group were closely related to the metabolic synthesis of various amino acids, carbohydrates, nucleotides, and energy. SXT injection can regulate a variety of metabolites, correct the high and low levels of some metabolites, improve metabolic disorders in many aspects, and exert pharmacological effects on the treatment of cerebral ischemia-reperfusion from multiple perspectives, which is consistent with the overall concept.

SXT can restore and regulate the metabolic disorders of the cortex and hippocampus caused by CIR, in which D-glutamine and D-glutamate metabolism and nitrogen metabolism are the same differential metabolic pathways of the cortex and hippocampus. The effects of SXT on these two pathways are mainly manifested as inhibition of cortical glutamine and promotion of hippocampal glutamate. Glutamate is the most abundant excitatory neurotransmitter in the brain, most of which is present in cortical synapses. It is involved in learning and memory in the brain and is closely related to the pathological process of stroke disease [28]. In total, 50–60% of the neurotransmitter glutamate is derived from the glutamate-glutamate cycle between neurons and astrocytes. Glutamate is converted to glutamine by glutamate synthase in astrocytes, and glutamine transporter proteins on the plasma membrane of astrocytes and neurons mediate the transfer of glutamine from astrocytes to neurons. Glutamine is converted to glutamate in neurons by glutaminase, which is loaded into synaptic vesicles for further release [29, 30]. Ischemic stroke severely destroys the synaptic network in the hippocampus. Transient ischemic occlusion is responsible for about 30% of synaptic closure in the hippocampus, and glutamate receptor expression in the ischemic hippocampus decreases. The transport of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) is inhibited [31]. Glutamine was significantly increased in the model group, possibly due to the increase in the reactivity of astrocytes in response to excitatory injury. Under ischemic conditions, the continuous increase of glutamine can further aggravate the osmotic swelling of astrocytes and neurons, thus aggravating the metabolic disorders in the brain [32]. Therefore, the effect of SXT may be related to the protection of neurons in the cortex, inhibition of the excessive activation of astrocytes in the cortex, and promotion of glutamic acid conversion and uptake in the hippocampus, thereby reducing brain cell swelling and neurological dysfunction after CIR in rats.

The effects of SXT on metabolic pathways in different parts of CIR are both different and correlated. Glyoxylate and dicarboxylate metabolism and pyrimidine metabolism are the unique metabolic pathways enriched by cortical differential metabolites. The effects of SXT are mainly shown as inhibition of glyceric acid and inhibition of dihydroorotic acid. The specific pathways enriched in the hippocampus include arginine biosynthesis, alanine, aspartate, and glutamate metabolism, butanoate metabolism, histidine metabolism, citrate cycle (TCA cycle), and pyruvate metabolism. The effect of SXT is mainly manifested as the promotion of fumaric acid. Differential metabolic pathways can be mainly attributed to carbohydrate metabolic pathway, nucleotide metabolic pathway, and amino acid metabolic pathway. Glyceric acid is derived from the oxidation of glyceral, and the metabolic pathways of glyoxylate and dicarboxylate metabolism are closely associated with the TCA cycle. The chain cycle of glyoxylate oxidative decarboxylation can be used as the pro-tometabolic cycles analogue of the TCA cycle, converting glyoxylate into CO₂ and producing aspartic acid in the presence of ammonia [33]. Dihydroorotic acid is an amino acid compound and can be oxidized to orotic acid under the action of dihydroorotate dehydrogenases (DHODHs), which plays an important role in pyrimidine metabolism pathway and energy metabolism [34]. DHODH is associated with oxidative phosphorylation process through its electron receptor coenzyme Q (CoQ). Oxidative phosphorylation damage of cells will lead to the decrease of CoQ, resulting in the inhibition of DHODH function and the increase of ROS production [35]. Fumaric acid is a precursor of L-malate in the TCA cycle and is formed by the oxidation of succinic acid by succinate dehydrogenase (SDH). Selective accumulation of succinic acid in the TCA cycle is a common metabolic feature of tissue ischemia and is closely associated with the generation of mitochondrial ROS during reperfusion. The ischemic accumulation of succinic acid is caused by the reversal of SDH, which reduces the amount of fumaric acid and intensification of CIR injury [36].

Nitric oxide (NO), a small molecule involved in normal blood flow, vasodilation, and neuronal and glial activity, plays a neuroprotective and neurotoxic role in different stages of cerebral ischemia. It is a key factor in pathological development after stroke [37]. NO is produced by the oxidation of arginine by nitric oxide synthase (NOS) or the reduction of nitrite by various reductases [38]. There are three subtypes of nitric oxide synthase (NOS) involved in NO synthesis: neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and inductive nitric oxide synthase (iNOS). ENOS and nNOS are known as structural nitric oxide synthases (cNOS) and depend on Ca²⁺ and calmodulin complexes [39]. In ischemic conditions, the content of superoxide (O₂⁻) and other reactive oxygen molecules (ROS) increases, and NO reacts with O₂⁻ to generate active nitrogen (RNS), including highly toxic peroxynitrite (ONOO⁻), and other nitrogen oxide components such as NO₂⁻ and NO₃⁻, leading to a cascade redox reaction that leads to neurotoxicity [40]. Changes in NO induced by ischemia are closely related to the activity of NOS subtypes. In the early stage of ischemia, the release of NO produced by eNOS surges, which may protect neurons from death by inducing vasodilation and inhibiting microvascular aggregation, but in the late stage of stroke, excessive NO produced by nNOS or iNOS can lead to cell apoptosis and neuron death [41]. Therefore, selective nNOS or iNOS inhibitors and eNOS agonists may have positive therapeutic significance for ischemic stroke.
In the metabolomic study of the mouse IS model, arginine biosynthesis and alanine, aspartate, and glutamate metabolism have been implicated in the development of disease [42]. During the acute phase of IS, arginine is significantly elevated and may exert a protective effect by improving cerebral microcirculation in the prethrombotic environment induced by complement activation [43]. Arginine administration is associated with clinical and biochemical improvement of stroke-like episodes in patients with mitochondrial disease. During stroke-like episodes in patients with mitochondrial disease, transient changes and fading were observed in brain magnetic resonance imaging (MRI) within 1 week of arginine administration [44]. Bone bridge protein peptides containing arginine, alanine, and aspartate motifs significantly reduced brain infarct volume in the MCAO model [45]. Mitaki et al. showed that histidine-rich glycoprotein was significantly upregulated in future IS patients, which also provided a possible biomarker for predicting or targeting treatment of IS [46]. In addition to therapeutic and predictive effects, some amino acids are often used to modify therapeutic factors to improve therapeutic and targeting effects. Arginine was applied to modify polyanidoamine dendrimer, reduce the cytotoxicity of the compound, and improve its gene delivery ability in animal models of stroke [47]. Unmodified stem cell-derived extracellular vehicles (EVs) are poorly targeted and cannot be transported to ischemic sites. When their domain fuses arginine-glycine-aspartic acid to form the RGD-EV complex, their targeting of ischemic brain lesions is significantly improved and can be used as a potential therapeutic agent for IS [48]. Fatty acid-binding protein inhibitor linked with butanoic acid molecule has a neuroprotective effect on CIR injury in mice and has the potential as a neuroprotective drug [49].

The ischemia-reperfusion injury that occurs after cerebral infarction is usually treated by lowering cranial pressure and dehydration [50]. For the ischemia-reperfusion injury of the limb, embolization and revascularization are performed after arterial embolization of the lower or upper extremity, and ischemia-reperfusion injury occurs after surgery, and osteofascial syndrome occurs in severe cases, and osteofascial syndrome dissection and decompression is performed if necessary, and VSD decompression and drainage is now commonly used in clinical practice, and when muscle swelling caused by ischemia-reperfusion injury is completely relieved, second-stage suturing can be performed [51–53]. During the reuse of VSD drainage for ischemia-reperfusion injury, it is important to prevent infection and closely observe the ischemic status of the limb, which may cause limb necrosis in severe cases [54]. In Western medicine, statins are effective in stabilizing atherosclerotic plaques and inhibiting inflammatory responses and are commonly used as clinical lipid-regulating drugs, and sparing blood combined with Western medicine for transient ischemic attacks can significantly improve the therapeutic effect and reduce the risk of cerebral infarction in patients [55, 56].

5. Conclusions

In this study, CIR rats were selected as models to analyze the metabolomics of the cortex and hippocampus of the rats after SXT injection, aiming to reveal the characteristics of metabolic molecules and pathways and to understand the key process of SXT repairing different parts of the brain. We demonstrate the feasibility of using metabolomics to analyze regional characteristics of CIR and explore the mechanisms involved. Our data provided not only 13 cortical differential metabolites (glutamine, dihydroorotic acid, glyceric acid, etc.) and 5 hippocampal differential metabolites (glutamic acid, fumaric acid, etc.) but also 4 metabolic pathways that were significantly altered in the cortex and 8 metabolic pathways that were significantly altered in the hippocampus. These findings provide evidence for the prevention and treatment of ischemic stroke by SXT injection and lay a foundation for the follow-up study on the mechanism of cerebral ischemia-reperfusion. However, there are limitations to the experiment, our experimental sample is small, and more clinical trials are needed to find different effects, including dose changes.

Data Availability

The data used to support the findings of this study are included within the supplementary information files.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors’ Contributions

Tingyue Jiang and Jiakang Jiao contributed equally to this work.

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Supplementary Materials

Supplementary materials are the original data used to support the results of this study. Table S1: Pharmacodynamics of SXT injection against cerebral ischemia-reperfusion. Table S2: Positive and negative ions in the cortex. Table S3: Positive and negative ions in the hippocampus. (Supplementary Materials)

References

[1] J. Zhang, S. Song, Y. Zhao, G. Ma, Y. Jin, and Z.-J. Zheng, “Economic burden of comorbid chronic conditions among survivors of stroke in China: 10-year longitudinal study,” BMC Health Services Research, vol. 21, no. 1, p. 978, 2021.

[2] R. Xu, L. Wang, L. Sun, and J. Dong, “Neuroprotective effect of magnesium supplementation on cerebral ischemic diseases,” Life Sciences, vol. 272, Article ID 119257, 2021.
[3] X. Min, L. Zhao, Y. Shi et al., "Gomisin J attenuates cerebral ischemia/reperfusion injury by inducing anti-apoptotic, anti-inflammatory, and antioxidant effects in rats," Bioengineered, vol. 13, no. 3, pp. 6908–6918, 2022.

[4] P. Huang, H. Wan, C. Shao, C. Li, L. Zhang, and Y. He, "Recent advances in Chinese herbal medicine for cerebral ischemic reperfusion injury," Frontiers in Pharmacology, vol. 12, Article ID 688596, 2022.

[5] M. Ceanga, M. Dahab, O. W. Witte, and S. Keiner, "Adult neurogenesis and stroke: a tale of two neurogenic niches," Frontiers in Neuroscience, vol. 15, Article ID 700297, 2021.

[6] H. Li, S. Li, C. Ren et al., "Hypoxic postconditioning promotes neurogenesis by modulating the metabolism of neural stem cells after cerebral ischemia," Experimental Neurology, vol. 347, Article ID 113871, 2022.

[7] M. I. Cuartero, A. Garcia-Culebras, C. Torres-López et al., "Post-stroke neurogenesis: friend or foe?" Frontiers in Cell and Developmental Biology, vol. 9, Article ID 657846, 2021.

[8] H. Zhang, R. J. Roman, and F. Fan, "Hippocampus is more susceptible to hypoxic injury: has the rosseta stone of regional variation in neurovascular coupling been deciphered?" Genome Science, vol. 44, no. 1, pp. 1–14, 2022.

[9] J.-W. He, G. Rabiller, Y. Nishijima et al., "Experimental cortical stroke induces aberrant increase of sharp-wave-associated ripples in the hippocampus and disrupts cortico-hippocampal communication," Journal of Cerebral Blood Flow and Metabolism, vol. 40, no. 9, pp. 1778–1796, 2020.

[10] Z. Ip, G. Rabiller, J. W. He et al., "Cortical stroke affects activity and stability of theta/delta states in remote hippocampal regions," in Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, pp. 5225–5228, Berlin, Germany, July 2019.

[11] J. Y. Zhao, X. L. Wang, X. F. Wang, R. R. Yuan, and Y. K. Zhang, "Meta-analysis and GRADE evaluation of shuxuetong injection in treatment of stroke in progressive," China Journal of Chinese Materia Medica, vol. 47, no. 3, pp. 807–818, 2022.

[12] H.-Q. Gu, X.-W. Xie, J. Jing et al., "Shuxuetong for prevention of recurrence in acute cerebrovascular events with embolism (SPACE) trial: rationale and design," Stroke and Vascular Neurology, vol. 5, no. 3, pp. 311–314, 2020.

[13] Z. Y. Sun, F. I. Wang, H. Guo et al., "Shuxuetong injection protects cerebral microvascular endothelial cells against oxygen-glucose deprivation reperfusion," Neural Regeneration Research, vol. 14, no. 5, pp. 783–793, 2019.

[14] R. C. Jun, F. X. Xiao, Q. Z. Hui et al., "Pharmacological activities and mechanisms of hirudin and its derivatives - a review," Frontiers in Pharmacology, vol. 12, Article ID 660757, 2021.

[15] W. Dong, T. Zhishu, L. Qian, W. Xuejing, and L. Hugang, "Research progress on the extraction of active substances of earthworm and its effect on trauma repair," Journal of Chinese Medicinal Materials, vol. 8, pp. 1997–2001, 2021.

[16] X. Jiao, Y. Xing, H. Wang et al., "A strategy based on gene sequencing and molecular docking for analysis and prediction of bioactive peptides in Shuxuetong injection," Biophysical Chemistry, vol. 282, Article ID 106749, 2022.

[17] J. C. Alarcon-Barrera, S. Kostidis, A. Ondo-Mendez, and M. Giera, "Recent advances in metabolomics analysis for early drug development," Drug Discovery Today, vol. 27, 2022.

[18] V. Gonzalez-Covarrubias, E. Martinez-Martinez, and L. Del Bosque-Plata, "The potential of metabolomics in biomedical applications," Metabolites, vol. 12, no. 2, p. 194, 2022.

[19] M. Jelinek, M. Jurajda, and K. Duris, "Oxidative stress in the brain: basic concepts and treatment strategies in stroke," Antioxidants, vol. 10, no. 12, p. 1886, 2021.

[20] X. Wang, L. Zhang, W. Sun et al., "Changes of metabolites in acute ischemic stroke and its subtypes," Frontiers in Neuroscience, vol. 14, Article ID 580929, 2021.

[21] H. F. Ma, F. Zheng, L. J. Su et al., "Metabolomic profiling of brain protective effect of edaravone on cerebral ischemia-reperfusion injury in mice," Frontiers in Pharmacology, vol. 13, Article ID 814942, 2022.

[22] N. Poupore, R. Chosed, S. Arce, R. Rainer, R. L. Goodwin, and T. I. Nathaniel, "Metabolomic profiles of men and women ischemic stroke patients," Diagnostics, vol. 11, no. 10, p. 1786, 2021.

[23] H. Liang, N. Matei, D. W. McBride et al., "Activation of TGR5 protects blood brain barrier via the BRCA1/Sirt1 pathway after middle cerebral artery occlusion in rats," Journal of Biomedical Science, vol. 27, no. 1, p. 61, 2020.

[24] J. Zhang, Z. Li, W. Liu, W. Zeng, C. Duan, and X. He, "Effects of bone marrow mesenchymal stem cells transplantation on the recovery of neurological functions and the expression of Nogo-A, NgR, Rhoa, and ROCK in rats with experimentally-induced convalescent cerebral ischemia," Annals of Translational Medicine, vol. 8, no. 6, p. 390, 2020.

[25] H. Liang, C. Xu, S. Hu et al., "Repetitive transcranial magnetic stimulation improves neuropathy and oxidative stress levels in rats with experimental cerebral infarction through the Nrf2 signaling pathway," Evidence-based Complementary and Alternative Medicine: eCAM, vol. 2021, Article ID 3908677, 8 pages, 2021.

[26] Y. H. Shi, X. L. Zhang, P. J. Ying et al., "Neuroprotective effect of astragaloside IV on cerebral ischemia/reperfusion injury rats through sirt1/mptp pathway," Frontiers in Pharmacology, vol. 12, p. 427, 2021.

[27] X. Liu, Q. Wang, Y. Cui, X. Li, and H. Yang, "In-depth transcriptomic and proteomic analyses of the hippocampus and cortex in a rat model after cerebral ischemic injury and repair by Shuxuetong (SXT) injection," Journal of Ethnomedicine and Pharmacology, vol. 249, Article ID 112362, 2020.

[28] T. W. Sedlak, B. D. Paul, G. M. Parker et al., "The glutathione cycle shapes synaptic glutamate activity," Proceedings of the National Academy of Sciences, vol. 116, no. 7, pp. 2701–2706, 2019.

[29] M. Hayashi, "Structure-function relationship of transporters in the glutamate-glutamine cycle of the central nervous system," International Journal of Molecular Sciences, vol. 19, no. 4, p. 1177, 2018.

[30] L. L. Luo, Y. F. Li, H. M. Shan et al., "L-glutamine protects mouse brain from ischemic injury via up-regulating heat shock protein 70," CNS Neuroscience and Therapeutics, vol. 25, no. 9, pp. 1030–1041, 2019.

[31] F. A. Shah, T. Li, L. T. A. Kury et al., "Pathological comparisons of the hippocampal changes in the transient and permanent middle cerebral artery occlusion rat models," Frontiers in Neurology, vol. 10, p. 1178, 2019.

[32] Q. Huang, C. Li, N. Xia et al., "Neurochemical changes in unilateral cerebral hemisphere during the subacute stage of focal cerebral ischemia-reperfusion in rats: an ex vivo 1H magnetic resonance spectroscopy study," Brain Research, vol. 1684, pp. 67–74, 2018.

[33] G. Springsteen, J. R. Yerabolu, J. Nelson, C. J. Rhea, and R. Krishnamurthy, "Linked cycles of oxidative decarboxylation of glyoxylate as protometabolic analogs of the citric acid cycle," Nature Communications, vol. 9, no. 1, p. 91, 2018.
[34] M. S. Filipe, N. R. Patricia, and M. P. Manuela, “Investigating the amino acid sequences of membrane bound dihydrouroatine oxidoreductases (DHOQOs): structural and functional implications,” *Biochimica et Biophysica Acta (BBA) Bioenergetics*, vol. 1862, no. 1, Article ID 148321, 2021.

[35] S. Boukalova, S. Hubackova, M. Milosevic, Z. Ezrova, J. Neuil, and J. Bohlenda, “Dihydroorotate dehydrogenase in oxidative phosphorylation and cancer,” *Biochimica et Biophysica Acta, Molecular Basis of Disease*, vol. 1866, no. 6, Article ID 165759, 2020.

[36] E. T. Chouchani, V. R. Pell, E. Gaude et al., “Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS,” *Nature*, vol. 515, no. 7527, pp. 431–435, 2014.

[37] J. M. Wieronska, P. Cieslik, and L. Kalinowski, “Nitric oxide-dependent pathways as critical factors in the consequences and recovery after brain ischemic hypoxia,” *Biomolecules*, vol. 11, no. 8, p. 1097, 2021.

[38] R. M. J. Palmer, D. D. Rees, D. S. Ashton, and S. Moncada, “L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation,” *Biochemical and Biophysical Research Communications*, vol. 153, no. 3, pp. 1251–1256, 1988.

[39] Y. F. He, M. M. Haque, D. J. Stuehr, and H. P. Lu, “Conformational states and fluctuations in endothelial nitric oxide synthase under calmodulin regulation,” *Biophysical Journal*, vol. 120, no. 23, pp. 5196–5206, 2021.

[40] V. I. Lushchak and O. V. Lushchak, “Interplay between reactive oxygen and nitrogen species in living organisms,” *Chemico-Biological Interactions*, vol. 349, Article ID 109680, 2021.

[41] A. Ally, I. Powell, M. M. Ally, K. Chaitoff, and S. M. Nauli, "Role of neuronal nitric oxide synthase on cardiovascular functions in physiological and pathophysiological states," *Nitric Oxide*, vol. 102, pp. 52–73, 2020.

[42] J. Jia, H. Zhang, X. Liang et al., “Application of metabolomics to the discovery of biomarkers for ischemic stroke in the murine model: a comparison with the clinical results,” *Molecular Neurobiology*, vol. 58, no. 2, pp. 641S–6426, 2021.

[43] T. Molnar, D. Csuka, G. Pusl, L. Nagy, P. Garred, and Z. Illes, “Associations between serum L-arginine and ficolins in the early phase of acute ischemic stroke - a pilot study,” *Journal of Stroke and Cerebrovascular Diseases*, vol. 29, no. 8, Article ID 104951, 2020.

[44] M. Almannai and H. A. W. El, “Nitric oxide deficiency in mitochondrial disorders: the utility of arginine and citrulline,” *Frontiers in Molecular Neuroscience*, vol. 14, p. 146, 2021.

[45] D. Davaanyam, I. D. Kim, and J. K. Lee, “Intranasal delivery of RGD-containing osteopontin heptamer peptide confers neuroprotection in the ischemic brain and augments microglia M2 polarization,” *International Journal of Molecular Sciences*, vol. 22, no. 18, p. 9999, 2021.

[46] S. Mitaki, Y. Wada, A. M. Sheikh, S. Yamaguchi, and A. Nagai, “Proteomic analysis of extracellular vesicles enriched serum associated with future ischemic stroke,” *Scientific Reports*, vol. 11, no. 1, Article ID 24024, 2021.

[47] Y. K. Lee, J. Lee, M. Kim, G. Y. Kim, J. S. Choi, and M. Lee, “Brain gene delivery using histidine and arginine-modified dendrimers for ischemic stroke therapy,” *Journal of Controlled Release*, vol. 330, pp. 907–919, 2021.

[48] T. Tian, L. Cao, C. He et al., “Targeted delivery of neural progenitor cell–derived extracellular vesicles for anti-inflammation after cerebral ischemia,” *Theranostics*, vol. 11, no. 13, pp. 6507–6521, 2021.

[49] Q. Guo, I. Kawahata, T. Degawa et al., “Fatty acid-binding proteins aggravate cerebral ischemia–reperfusion injury in mice,” *Biomedicines*, vol. 9, no. 5, p. 529, 2021.

[50] E. Jättler, P. D. Schellinger, A. Aschoff, K. Zweckberger, A. Unterberg, and W. Hacke, “Clinical review: therapy for refractory intracranial hypertension in ischaemic stroke,” *Critical Care*, vol. 11, no. 5, p. 231, 2007.

[51] U. Sayar, T. Ozer, and I. Mataraci, “Forearm compartment syndrome caused by reperfusion injury,” *Case Reports in Vascular Medicine*, vol. 2014, Article ID 931410, 3 pages, 2014.

[52] W. W. Xu, Y. Y. Zhang, J. Su et al., “Ischemia reperfusion injury after gradual versus rapid flow restoration for middle cerebral artery occlusion rats,” *Scientific Reports*, vol. 8, no. 1, p. 1638, 2018.

[53] J. Pechar and M. M. Lyons, “Acute compartment syndrome of the lower leg: a review,” *The Journal for Nurse Practitioners*, vol. 12, no. 4, pp. 265–270, 2016.

[54] J. H. Lee, Y. H. Park, H. J. Byon, H. S. Kim, C. S. Kim, and J. T. Kim, “Effect of remote ischaemic preconditioning on ischaemic-reperfusion injury in pulmonary hypertensive infants receiving ventricular septal defect repair,” *British Journal of Anaesthesia*, vol. 108, no. 2, pp. 223–228, 2012.

[55] S. Peng, L. W. Xu, X. Y. Che et al., “Atorvastatin inhibits inflammatory response, attenuates lipid deposition, and improves the stability of vulnerable atherosclerotic plaques by modulating autophagy,” *Frontiers in Pharmacology*, vol. 9, p. 438, 2018.

[56] S. Khare, “Risk factors of transient ischemic attack: an overview,” *Journal of Midlife Health*, vol. 7, no. 1, pp. 2–7, 2016.