Research Article

Aldehyde Dehydrogenase Isoform 1 Predicts a Poor Prognosis of Acute Cerebral Infarction

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Abstract

To investigate the prognostic potential of serum aldehyde dehydrogenase isoform 1 (ALDH1) level in acute cerebral infarction, and the molecular mechanism in mediating neurological deficits, a total of 120 acute cerebral infarction cases within 72 h of onset were retrospectively analyzed. Serum ALDH1 level in them was detected by qRT-PCR. Receiver operating characteristic (ROC) and Kaplan–Meier curves were depicted for assessing the diagnostic and prognostic potentials of ALDH1 in acute cerebral infarction, respectively. An in vivo acute cerebral infarction model in rats was established by performing MCAO, followed by evaluation of neurological deficits using mNSS and detection of relative levels of ALDH1, Smad2, Smad4, and p21 in rat brain tissues. Pearson’s correlation test was carried out to verify the correlation between ALDH1 and mNSS and relative levels of Smad2, Smad4, and p21. Serum ALDH1 level increased in acute cerebral infarction patients. A high level of ALDH1 predicted a poor prognosis of acute cerebral infarction patients. In addition, ALDH1 was sensitive and specific in distinguishing acute cerebral infarction cases, presenting a certain diagnostic potential. mNSS was remarkably higher in acute cerebral infarction rats than that of controls. Compared with sham operation group, relative levels of ALDH1, Smad2, and Smad4 were higher in brain tissues of modeling rats, whilst p21 level was lower. ALDH1 level in brain tissues of modeling rats was positively correlated to mNSS, and mRNA levels of Smad2 and Smad4, but negatively correlated to p21 level. Serum ALDH1 level is a promising prognostic and diagnostic factor of acute cerebral infarction, which is correlated to 90-day mortality. Increased level of ALDH1 in the brain of cerebral infarction rats is closely linked to neurological function, which is associated with the small mothers against decapentaplegic (Smad) signaling and p21.

1. Introduction

Cerebrovascular diseases have become the number one killer in China [1]. Owing to the high incidence, mortality, and recurrence, cerebrovascular diseases have brought a heavy burden to the society and families. Ischemic cerebral infarction cases account for about 70–80% of strokes, which is caused by the interruption of local cerebral blood perfusion due to cerebrovascular occlusion. Cerebral ischemia and hypoxia further result in ischemic necrosis of corresponding brain regions [2]. Neurological deficits and repair mechanisms are dynamically processed right after cerebral infarction, in which angiogenesis, destruction of the blood-brain barrier, and inflammatory response are closely interacted with each other [3]. They synergistically affect the survivals of neurons, nerve synapse repair, and regeneration, which are important for the prognosis of cerebral infarction patients.

A fast and accurate diagnosis of cerebral infarction contributes to timely treatment and satisfactory clinical outcomes. At present, CT and MRI are widely used for the diagnosis of cerebral infarction, although they have disadvantages of complicated procedures, expensive medical cost, and the demand for moving patients. It is necessary to search for noninvasive serum biomarkers that are effective in diagnosing and predicting the progression of cerebral infarction [4, 5].

ALDH1 is a member of the acetaldehyde dehydrogenase gene superfamily, which mainly catalyzes the irreversible oxidation of retinal to retinoic acid. It participates in tissue...
2. Materials and Methods

2.1. Subjects. Seventy acute cerebral infarction patients treated in Wuhan Hospital of Traditional Chinese Medicine were recruited. Inclusion criteria were as follows: (1) cerebral infarction was diagnosed based on the Report of the WHO Task Force on Stroke and other Cerebrovascular Disorders [11]; (2) cerebral infarction was confirmed by cranial CT or MRI; (3) onset of cerebral infarction ≤72 h; (4) age >18 years. Exclusion criteria were as follows: (1) intracranial hemorrhage; (2) a history of infection within 2 weeks before onset; (3) a history of malignant tumors; (4) a recent medication of hormones or immunosuppressants. Modified Rankin scale (mRS) was used for evaluating the recovery of neurological function after stroke (>2, poor prognosis; ≤2, good prognosis) [12]. Survival and adverse events were recorded. Recruited patients were followed up for 90 days. Seventy healthy volunteers receiving physical examinations in the same period, who were confirmed without stroke by cranial MRI, were included as controls. This experiment was approved by the Medical Ethics Committee of Wuhan Hospital of Traditional Chinese Medicine. Signed written informed consents were obtained from the participants before this study.

2.2. Blood Sample Collection and Processing. Venous blood (4 mL) was collected from every participant in the morning after overnight fast and placed in a 5 mL Eppendorf (EP) tube. The blood sample was centrifuged at 2500 g/min for 12 min, and the serum was isolated, labeled, and stored at −80°C.

2.3. MCAO Model. This study was approved by the Animal Ethics Committee of Wuhan Hospital of Traditional Chinese Medicine Animal Center. Sixty male Sprague-Dawley (SD) rats in the clean level weighing 230–250 g were housed in a temperature-controlled room (21 ± 2°C) on a 12:12 h light/dark cycle (lights on at 06:00). All rats had free access to water and food. Rats were randomly assigned to cerebral infarction group (n = 20), sham operation group (n = 20), and control group (n = 20). An in vivo acute cerebral infarction model in rats was established by performing middle cerebral artery occlusion (MCAO) [13]. Briefly, rats were anesthetized and placed in a supine position. After creating a midline incision on the neck, the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were isolated. The distal end of ECA was ligated, and artery clamps were placed at the distal end of ICA and the proximal end of CCA. ECA ligation was then cut, and the artery clamp at ICA was withdrawn. A nylon suture was inserted in ECA through the opening of ECA ligation until blocking the middle cerebral artery blood flow. The suture was fixed, which was withdrawn 2 h later. Rats in the sham operation group were operated on by artery isolation, and then, the incision was sutured layer by layer. The quality of the MCAO model was evaluated by the Zea Longa score on a 5-point scale as follows: 0, absence of neurological deficits; 1, unable to fully lift the opposite forepaw; 2, circling to the opposite side; 3, falling to the opposite side; 4, unable to spontaneously walk and loss of consciousness. After anesthesia awareness, rats with 1–3 Zea Longa scores were considered as qualified modeling animals. After evaluation of neurological deficits, rats were anesthetized and sacrificed for collecting brain tissues.

2.4. Evaluation of Neurological Deficits. Neurological deficits of postoperative rats were evaluated using the modified neurological severity score (mNSS) at indicated time points, including tests of balance, walking, abnormal movement, sensory, reflex, and raising by tail [14]. With a range of 1–18 grades, neurological deficits were categorized as mild (1–6 grades), moderate (7–12 grades), and severe level (13–18 grades).

2.5. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), total RNAs were extracted from tissues and reversely transcribed to complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT (TaKaRa, Tokyo, Japan). qRT-PCR was carried out using SYBR Green Kit (TaKaRa, Tokyo, Japan) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference. Primer sequences were shown in Table 1.

2.6. Statistical Analysis. Statistical product and service solutions (SPSS) 20.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Data were expressed as \( \bar{x} \pm s \). Differences between groups were compared by the t-test, and those among groups were compared by one-way ANOVA, followed by the SNK-q test. Prognostic and diagnostic potentials were assessed by Kaplan–Meier and ROC methods, respectively. Pearson’s correlation test was applied for assessing the correlation between ALDH1 and other indexes. \( P < 0.05 \) was considered as statistically significant.

3. Results

3.1. Clinical Data of Subjects. In case group, there were 38 and 32 male and female patients, respectively, with an average age of 66.13 ± 2.69 years. Thirty-five males and thirty-five females were included in the control group with an average age of 65.9 ± 2.81 years. No significant differences in age and sex were identified between groups nor as history of hypertension, diabetes, dyslipidemia, coronary heart disease, and atrial fibrillation (\( P > 0.05 \), Table 2). It is concluded that
baseline characteristics were comparable between the case and control groups.

3.2. Serum ALDH1 Level Increased in Acute Cerebral Infarction Patients. Compared with that of healthy volunteers, serum ALDH1 level was markedly higher in acute cerebral infarction patients ($P < 0.05$, Figure 1(a)), suggesting that the abnormally expressed serum ALDH1 level may be involved in the onset of cerebral infarction. ROC curves demonstrated that the area under curve (AUC) of ALDH1 in distinguishing acute cerebral infarction was 0.7912, with the sensitivity and specificity of 70% and 75.71% at the cut-off value of 1.5, respectively ($P < 0.001$, Figure 1(b)). It is proven that ALDH1 was a promising diagnostic marker in acute cerebral infarction. Moreover, 70 recruited patients were categorized to high-level group ($n = 39$) and low-level group ($n = 31$) by the median serum ALDH1 level. A worse survival was identified in high-level group, indicating that an increased serum ALDH1 level was unfavorable to the prognosis of cerebral infarction ($HR = 1.521, P = 0.0065$, Figure 1(c)).

3.3. Neurological Deficits of MCAO Rats. To investigate the function of ALDH1 in acute cerebral infarction, MCAO model was performed, and 2 rats in cerebral infarction group died. Eighteen survivor rats presented neurological deficits, manifesting as circling to the right and unable to fully lift the right forepaw, suggesting the successful modeling of cerebral infarction. Rats in other two groups were all survived. mNSS was statistically significant among three groups (10.41 ± 2.15, 0.40 ± 0.09, and 0.45 ± 0.06 in cerebral infarction, sham operation, and control group, respectively) ($F = 430.296, P < 0.001$). However, no significant difference in mNSS was detected between sham operation and control groups ($P > 0.05$, Table 3).

### Table 1: Primer sequences.

| Gene           | Primer sequence          |
|----------------|--------------------------|
| ALDH1 (Homo)   | Forward: 5' - GCCAGGTAGAAGGAGATAAGGAGG -3'  |
|                | Reverse: 5' - TATAATAGTGCCGCCCCTCTCGGAAG -3'  |
| ALDH1 (Rattus) | Forward: 5' - GCCCTGGAGACGATGGATAC -3'  |
|                | Reverse: 5' - TCCATCTGCAAGTCCAAGTC -3'  |
| Smad2 (Rattus) | Forward: 5' - TGGTGAGTGATGTGTTGTCAT -3'  |
|                | Reverse: 5' - GAACATAGACATACACCTGAAGGCTTTTT -3'  |
| Smad4 (Rattus) | Forward: 5' - ACTATGGCTCGGTGCGAGAA -3'  |
|                | Reverse: 5' - GAGAACCTCGGGAGCCTTTC -3'  |
| p21 (Rattus)   | Forward: 5' - TGCTAGCGTGTTGCTGTC -3'  |
|                | Reverse: 5' - TGATGCTGGAGCTGTTAACAG -3'  |
| GAPDH (Homo)   | Forward: 5' - ATGGGAAGGTGGAAGG -3'  |
|                | Reverse: 5' - TTACTCCTTGAGGCCC -3'  |
| GAPDH (Rattus) | Forward: 5' - ATGGGAAGGTGGAAGG -3'  |
|                | Reverse: 5' - TTACTCCTTGAGGCCC -3'  |

### Table 2: Baseline clinical data between case and control groups.

| Variable                  | Control group ($n = 70$) | Case group ($n = 70$) | t/$\chi^2$ | P  |
|---------------------------|--------------------------|-----------------------|------------|----|
| Age (years)               | 65.9 ± 2.81              | 66.13 ± 2.69          | 1.437      | 0.153|
| Sex (n)                   |                          |                       |            |    |
| Male                      | 35                       | 38                    | 0.258      | 0.735|
| Female                    | 35                       | 32                    |            |    |
| Hypertension (n)          |                          |                       |            |    |
| I.A.                      | 23                       | 28                    | 0.771      | 0.483|
| N.A.                      | 47                       | 42                    |            |    |
| Diabetes (n)              |                          |                       |            |    |
| I.A.                      | 14                       | 18                    | 0.648      | 0.421|
| N.A.                      | 56                       | 52                    |            |    |
| Dyslipidemia (n)          |                          |                       |            |    |
| I.A.                      | 28                       | 31                    | 0.264      | 0.732|
| N.A.                      | 42                       | 39                    |            |    |
| Coronary heart disease (n)|                          |                       |            |    |
| I.A.                      | 7                        | 13                    | 2.1        | 0.147|
| N.A.                      | 63                       | 57                    |            |    |
| Atrial fibrillation (n)   |                          |                       |            |    |
| I.A.                      | 4                        | 6                     | 0.431      | 0.512|
| N.A.                      | 66                       | 64                    |            |    |

I.A. is applicable; N.A. not applicable.
3.4. Relative Levels of ALDH1, Smad2, Smad4, and p21 in Brain Tissues of MCAO Rats. Compared with those of sham operation and control groups, mRNA levels of ALDH1, Smad2, and Smad4 were markedly higher in cerebral infarction group, whilst p21 level was lower ($P < 0.05$). mRNA expression levels were similar between sham operation and control groups ($P > 0.05$, Figure 2).

3.5. Correlation between ALDH1 and mNSS, Smad2, Smad4, and p21. Interestingly, ALDH1 level in rat brain was positively correlated to mNSS of MCAO rats ($r = 0.6182$, Figure 3(a)) and relative levels of Smad2 and Smad4 ($r = 0.7709$ and 0.5335, $P < 0.05$, Figures 3(b) and 3(c)), which was negatively correlated to p21 level ($r = 0.8207$, $P < 0.001$, Figure 3(d)).

4. Discussion

With the aging population and improvement of life quality, cerebrovascular diseases, alongside cardiac diseases, and malignant tumors have become the top three causes of death. In China, at least 1.5 million people die of stroke annually, ranking 25% of all causes of death [15, 16]. Cerebral infarction is a common subtype of stroke that severely threatens human health and increases medical cost [15, 16].

ALDH1 is a zinc-containing enzyme located in the cytoplasm. It is responsible for catalyzing the oxidation of acetaldehyde to acetic acid. ALDH1 is expressed in hematopoietic and neural stem cells and is involved in cellular metabolism and stem cell differentiation. ALDH1 is a vital mediator of self-renewal and tumorigenesis of breast cancer cells [17]. In cervical cancer cells, highly expressed ALDH1 triggers proliferative, colony formation, and migratory capacities, serving as an independent risk factor for poor prognosis [18, 19]. Zheng and Zheng [20] suggested that cervical cancer cells overexpressing ALDH1 are featured by strong capacities of differentiation, self-renewal, and carcinogenesis. However, in vivo and in vitro cultured cervical cancer cells with a low abundance of ALDH1 lack these malignant phenotypes.

The novelty of the present study was that it is the first attempt for us to investigate the effects of ALDH1 on acute cerebral infarction and to explore the potential underlying molecular mechanism. Our results showed that serum
ALDH1 level markedly increased in acute cerebral infarction patients. Its diagnostic and prognostic potentials in cerebral infarction were further verified. Through retrospectively analyzing follow-up data of recruited acute cerebral infarction patients, ALDH1 was found to be linked with 90-day all-cause mortality. Therefore, we believed that serum ALDH1 level was of significance in predicting adverse events and prognosis of acute cerebral infarction.

The Smad family directly participates in signal transduction of the transforming growth factor-beta (TGF-β) superfamily, which is critically important in regulating intracellular homeostasis and cell functions [21, 22]. A body injury triggers TGF-β release from cells, which binds cell surface receptors to induce a positive feedback to phosphorylate other TGF-β receptors. The Smad signaling is then activated by phosphorylated TGF-β receptors and further accelerates phosphorylation of intracellular Smad2 and Smad3, leading to a spatial conformational change [23, 24]. A heterotrimer complex formed by cross-linking with Smad4 is then translocated into nuclei, where mediates target gene transcription and induces inflammatory response, oxidative stress, and cell apoptosis [25]. Cyclin-dependent kinase inhibitor 1A (p21) is one of the targets of Smad4, which is associated with cell senescence, death, and apoptosis [26, 27]. In brain tissues of rats with acute cerebral infarction, we found that Smad2 and Smad4 were upregulated, and p21 was downregulated. ALDH1 level in brain tissues was positively correlated to mNSS, and relative levels of Smad2 and Smad4, but negatively correlated to p21 level. It is indicated that the influence of ALDH1 on neurological dysfunction caused by acute cerebral infarction may be attributed to the Smad signaling and its target gene p21.

5. Conclusions

Taken together, we have proven the clinical significance of serum ALDH1 level in predicting the onset of acute cerebral infarction and short-term poor prognosis. ALDH1 was closely linked to neurological dysfunction following cerebral infarction with the involvement of the Smad signaling and p21. Detection of serum ALDH1 level is simple and non-invasive, which is a promising method to be applied in monitoring cerebral infarction.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.
Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Jing Yang and Jie Duan contributed equally to this work.

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