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Curative efficacy of penehyclidine combined with edaravone on acute cerebral infarction and their effects on serum TNF-α and NDS score in rats

Y. CHEN[1], Y. ZHAO[2]

[1]Emergency Department, HuaShan Hospital North Fudan University, Shanghai, China
[2]Department of Neurology, Xuhui District Central Hospital, Shanghai, China

Abstract. – OBJECTIVE: To investigate the effects of penehyclidine combined with edaravone on acute cerebral infarction (ACI) in rats.

MATERIALS AND METHODS: A rat model of middle cerebral artery infarction was created. The rats were randomly divided into sham, model and treatment group. After grouping, rats in the treatment groups were treated with edaravone combined with penehyclidine. The rats in the sham and model group were given an equal volume of phosphate-buffered saline (PBS). The therapeutic effects on rats at 3 d and 7 d after treatment were observed, the levels of serum TNF-α, interleukin-6 (IL-6) and high-mobility group box 1 protein (HMGB1) before and after treatment were compared, and the NDS scores were recorded.

RESULTS: After treatment, the effective rate in treatment groups was higher than that in control group. The expression levels of serum TNF-α, HMGB1 and IL-6 in treatment groups showed gradually decreasing trends after treatment, and there were significant differences in the levels before and after treatment ($p<0.05$). At 3 d, the decrease ranges of expression levels of TNF-α, HMGB1, and IL-6 in model and treatment groups were larger than those in control group; there were statistically significant differences in the expression levels between the two groups ($p<0.05$). The NDS score was gradually decreased after treatment, while the activities of daily living (ADL) score were gradually increased after treatment. There were significant differences in the scores between the two groups at each time point ($p<0.05$). There were positive correlations of the expression levels of serum IL-6 and HMGB1 with the expression level of TNF-α (correlation coefficient=0.8731 and 0.9084, $p<0.01$), and there was also a positive correlation between the TNF-α level and the NDS score (correlation coefficient=0.8331, $p<0.01$).

CONCLUSIONS: Penehyclidine combined with edaravone has a better clinical treatment effect on ACI rats, which can significantly reduce the levels of serum TNF-α, IL-6 and HMGB1 and the NDS score, so it is worthy of popularization in clinical application.

Key Words:
Penehyclidine, Edaravone, Acute cerebral infarction, TNF-α, NDS.

Introduction

Acute cerebral infarction (ACI) is also known as ischemic stroke; when the brain blood supply dysfunction occurs, the brain tissues will be in a state of ischemia and hypoxia, and they will even suffer from atrophy and necrosis in severe cases, eventually leading to the brain neurological dysfunction in patients[1]. There are a variety of clinical manifestations of ACI, such as headache, vomiting, alalia and clouding of consciousness[2-4]. In China, there are up to 45 million of patients admitted to hospital for treatment of ACI each year, and its incidence rate shows an increasing trend year by year[5]. The acute onset of most ACI patients was reported to be closely related to the acute inflammatory response involving cytokines and the vascular damage in ischemia reperfusion of the body[6]. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are major substances in inflammatory response, and high-mobility group box 1 protein (HMGB1) is the central molecule that initiates and maintains the inflammatory response. Edaravone has the effect of scavenging free radicals, which can promote the inflammatory absorption and reduce the inflammatory response[7]. Edaravone, a kind of brain protectant, is commonly used in the clinic to treat nerve pathological changes caused by

Corresponding Author: Yi Zhao, MM; e-mail: luttyzhaoX@126.com

cerebral infarction. Penehyclidine hydrochloride, a kind of selective M receptor anticholinergic drug, with strong anticholinergic effects both in the central and peripheral regions, can effectively improve the body's microcirculation function and effectively inhibit the inflammatory response[8]. Penehyclidine hydrochloride can also reduce the expression of proinflammatory cytokines via inhibiting NF-kappa B, thus attenuating the production of TNF-alpha and finally exhibiting its cerebral protection effects. The primary purpose of this study was to investigate the effects of penehyclidine combined with edaravone on ACI and on the serum TNF-α and neurological deficit scale (NDS) score of rats, promoting the clinical popularization and application.

Materials and Methods

Establishment of ACI Model and Grouping

A total of 60 Sprague Dawley rats (180-200 g) were purchased from Vital River Laboratories (Beijing, China). The rat models of the middle cerebral artery infarction were created using the Longa method[9]. The rats showed listlessness, Horner's syndrome on the same side, drooping of the contralateral forelimb, adduction and internal rotation, and spontaneous circling on the affected side for 2 h after the operation, which illustrated that model creation had been successful. If model creation failed, the rat was sacrified. This study was approved by the Animal Ethics Committee of Xuhui District Central Hospital Animal Center.

The experiment was divided into three groups: sham, model, and treatment groups, with 20 rats in each group. In the sham operation group, the vessel was separated but there was no suture occlusion. The rats in the model and treatment groups were operated according to the model creation method.

Treatment Methods

Rats in treatment groups were firstly treated with edaravone, then combined with intravenous injection of 1 mg penehyclidine via the tail vein for 7 consecutive days. The rats in the sham and model group were given an equal volume of phosphate-buffered saline (PBS). The curative effects on both groups were observed at 3 d and 7 d after treatment. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA), 8th Edition, 2010.

Observational Indexes

At 3 d and 7 d after treatment, the therapeutic effects on rats in control and treatment groups were evaluated. The main observational indexes were the curative effects, levels of TNF-α, IL-6 and HMGB1 before treatment and at 3 d and 7 d after treatment; the NDS and activities of daily living (ADL) scores were evaluated. To detect the levels of TNF-α, IL-6 and HMGB1, 7 mL blood were drawn from rats, followed by centrifugal separation, and then stored at -20°C; the serum was detected via double-antibody sandwich enzyme-linked immunosorbent assay.

Judging Criteria for Curative Effect

At 24 h, 3 day, and 7 day after treatment, neurologic deficit scores (NDS) were detected according to the following grading systems. The NDS were quantified by the assessment of ambulation using the hind limbs and by the placing/stepping reflex. Ambulation using lower extremities was graded as follows: 0, normal (symmetrical and coordinated ambulation); 1, toes flat beneath the body when walking but the presence of ataxia; 2, knuckle walking; 3, unable to knuckle-walk but some movement of the lower extremities; 4, no movement of the lower extremities. The placing/stepping reflex was assessed by dragging the dorsum of the hind paw along the edge of a surface; this evoked a coordinating lifting and placing response (ie, stepping), which was graded as follows: 0, normal; 1, weak; 2, none. The NDS were calculated for each rat as the sum of these scores; the maximal score was 8. The assessments were made by 1 observer who was blinded to the treatment groups.

ADL score: It includes ten aspects of activities in daily life, and is divided into four functional levels according to whether they need help from others and the degree of help, namely 0, 5, 10 and 15 points; the total score is 100 points; the higher the score is, the stronger the ADL will be.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for data analysis. Measurement data were presented as mean ± standard deviation. The *t*-test was used for intergroup comparison, and χ^2-test was used for enumeration data; $\alpha=0.05$.

Results

Comparison of Therapeutic Effect Between the two Groups

As shown in Table I, the total effective rate was 84.21% in treatment group and 63.16% in the group. There was a significant difference in the total effective rate between control group and treatment group (χ^2=5.14, p<0.05).

Comparisons of Expression Levels of the three Factors in Serum Before and After Treatment

After treatment, the expression levels of serum TNF-α in treatment group showed gradually decreasing trends compared to model group; the differences were significant before and after treatment ($^*p$<0.05). At 7 d, the decrease range of expression level of TNF-α in observation group was larger than that in model group, and there was a statistically significant difference in the expression level at 7 d after treatment between the two groups ($^\#p$<0.05) (Figure 1).

After treatment, the expression levels of serum IL-6 in treatment group showed gradually decreasing trends compared to model group, and the differences were significant before and after treatment ($^*p$<0.05). At 7 d, the decrease range of expression level of IL-6 in treatment group was larger than that in model group, and there was a statistically significant difference in the expression level at 7 d after treatment between the two groups ($^\#p$<0.05) (Figure 2).

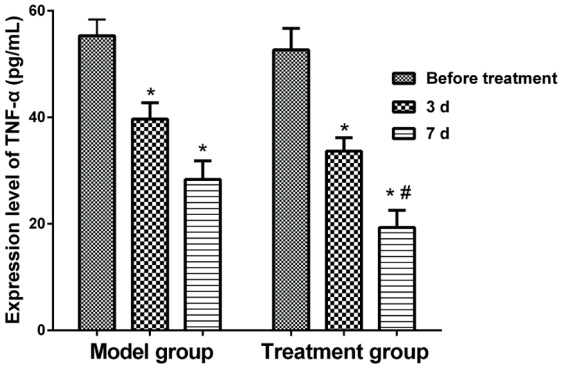

Figure 1. Expression levels of TNF-α before and after treatment.

After treatment, the expression levels of serum HMGB1 in treatment group showed gradually decreasing trends compared to model group, and the differences were significant before and after treatment ($^*p$<0.05). At 3 d and 7 d, the expression levels of serum HMGB1 in model group were significantly higher than those in treatment group, and the differences were statistically significant ($^\%p$<0.05, $^\#p$<0.05) (Figure 3).

Comparisons of NDS and ADL Scores Between the Model and Treatment Group

In the model group, there were no significant differences in NDS and ADL scores of all rats. As shown in Table II, the NDS scores were (13.37±3.05) points and (20.19±2.74) points

Table I. Comparison of therapeutic effect between the two groups (%).

Group	n	Basic cure	Significant progress	Progress	No change	Total effective rate
Control group	19	4 (21.05)	8 (42.10)	4 (21.05)	3 (15.79)	12 (63.16)
Observation group	19	6 (31.58)	10 (52.63)	2 (10.53)	1 (5.26)	16 (84.21)
χ^2		2.03	1.45	1.16	1.92	5.14
p		>0.05	>0.05	>0.05	>0.05	<0.05

Table II. Comparisons of NDS and ADL scores between the model and treatment group.

	NDS score			ADL score		
Group	Before treatment	3 d after treatment	7 d after treatment	Before treatment	3 d after treatment	7 d after treatment
Control group	45.26±3.82	33.64±3.02	20.19±2.74*	32.01±4.35	44.29±7.03	56.84±11.27*
Observation group	44.17±3.75	24.98±2.72$^\#$	13.37±3.05$^{*\%}$	33.16±4.23	52.05±6.96$^\#$	69.48±12.26$^{*\%}$

Note: Compared with that before treatment, $^*p$<0.05; Compared with model group at 3 d after treatment, $^\#p$<0.05; Compared with model group at 7 d after treatment, $^\%$ p<0.05.

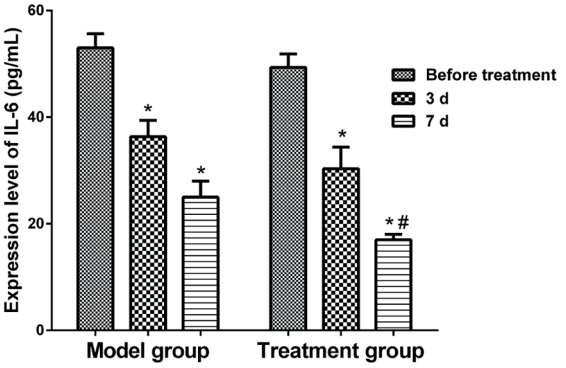

Figure 2. Expression levels of IL-6 before and after treatment.

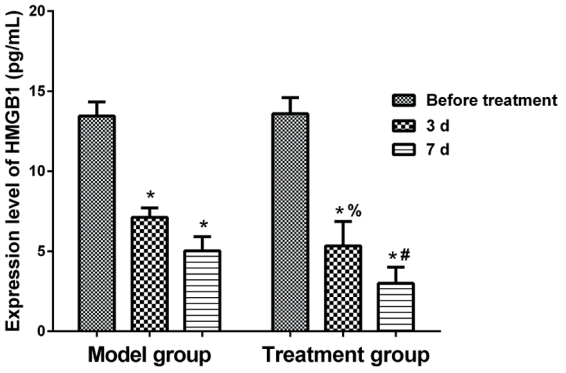

Figure 3. Expression levels of HMGB1 before and after treatment.

at 7 d after treatment, which were significantly lower than those of model group ($^{*}p<0.05$). The ADL scores were (69.48±12.26) points and (56.84±11.27) points, which were significantly higher than those of model group ($^{*}p<0.05$). Overall, the NDS score was gradually decreased after treatment, while the ADL score was gradually increased after treatment. There were significant differences in the scores between the two groups at each time point ($^{\#}p<0.05$, $^{\%}p<0.05$).

Correlation Analysis

There were positive correlations of the expression levels of serum IL-6 and HMGB1 with the expression level of TNF-α (correlation coefficient=0.8731 and 0.9084, $p<0.01$). There was also a positive correlation between the TNF-α level and the NDS score (correlation coefficient=0.8331, $p<0.01$), indicating that the lower the level of TNF-α is and the lower the NDS score is after treatment, the better the treatment effect will be (Figure 4).

Discussion

ACI, as one of the major cardiovascular and cerebrovascular diseases, may cause ischemia and hypoxia in brain tissues, which endanger human health[10]. Ischemic brain injury is mostly accompanied with the inflammatory response mediated by a variety of cytokine, and it is also related to some inflammatory factors in the body[11]. TNF-α and IL-6, as inflammatory mediators, can reflect the degree of inflammatory response in the body[12]. HMGB1, as a kind of DNA-binding protein, can be detected in many tissues and organs in the human body, such as lymphoid tissues[13], brain[14], liver[15] and kidney[16]. It exists in the cytoplasm in brain tissues, and in cytoplast in other tissues[17]. HMGB1 can activate a variety of inflammatory cells outside the cell, and promote the secretion of various types of cytokines involved in the inflammatory response process, playing an important role in the pathophysiological processes of a variety of inflammatory diseases[18].

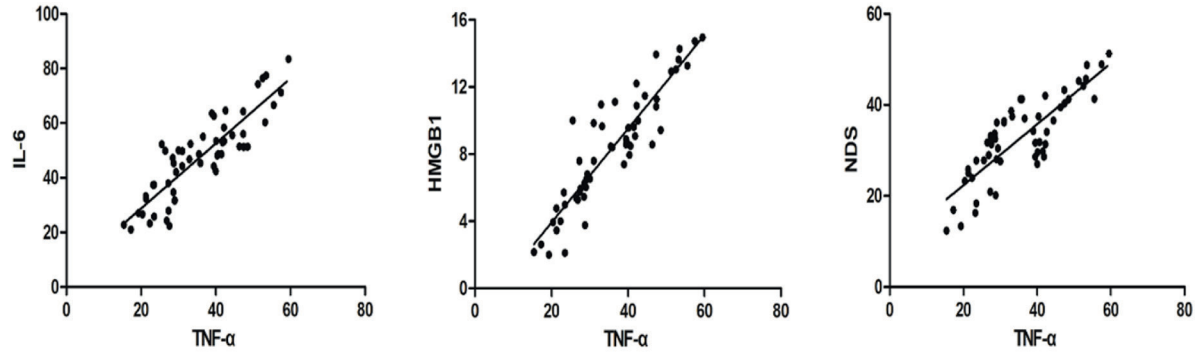

Figure 4. Correlation analysis.

Our results showed that the total effective rate was 84.21% in treatment group and 65.79% in model group, and the difference in the total effective rate was significant between model group and treatment group. After treatment, the expression levels of serum TNF-α, HMGB1 and IL-6 in model group and treatment group showed gradually decreasing trends, and the differences were significant before and after treatment ($p<0.05$). At 7 d, the decrease ranges of expression levels of TNF-α, HMGB1 and IL-6 in treatment group were larger than those in model group, and there were statistically significant differences in the expression levels at 7 d after treatment between the two groups ($p<0.05$), suggesting that the serum TNF-α, HMGB1 and IL-6 may be involved in the secondary inflammatory response of ACI, which can be used as serological indicators to evaluate the degree of neurological deficit. Before treatment with edaravone, there were no significant differences in NDS and ADL scores of all rats. At 7 d after treatment, the NDS scores were (13.37±3.05) points and (20.19±2.74) points, which were significantly lower than those before treatment ($p<0.05$). The ADL scores were (69.48±12.26) points and (56.84±11.27) points, which were significantly higher than those before treatment ($p<0.05$). Overall, the NDS score was gradually decreased after treatment, while the ADL score was gradually increased after treatment. There were significant differences in the scores between the two groups at each time point ($p<0.05$), indicating that after treatment with edaravone, the neurological function of rats in observation group was recovered significantly and the living state was also improved. Moreover, there were positive correlations of the expression levels of IL-6 and HMGB1 and NDS score with the TNF-α level (correlation coefficient=0.8731, 0.9084 and 0.8331, $p<0.01$). The free radicals were produced after ACI, which can induce the inflammatory cascade. Edaravone can produce a strong inhibitory effect on the peroxidation of brain cell membrane and plays a very important role in maintaining the integrity and complete function of brain cell membrane structure[19]. It has been reported that in the rat model of ischemia-reperfusion, after the injection of edaravone via tail vein, the progressions of cerebral edema and cerebral infarction can be alleviated, and the neurological dysfunction can also be partially remitted[20]. Several scholars[21] performed clinical study on hypertensive cerebral infarction using edaravone, and the results showed that the neurological function and ADL score of patients were significantly improved, and the degree of cerebral edema was decreased, suggesting that the application of edaravone can significantly improve the brain neurological function of ACI patients and reduce the disability rate. The molecular mechanism might be that edaravone can eliminate the cytotoxic hydroxyl radicals, thereby inhibiting the cascade of inflammation caused by free radicals and also inhibiting lipid peroxidation[22]. Additionally, animal experiments showed that penehyclidine could significantly reduce the expression of TNF-alpha in the brain tissues of rats with cerebral ischemia reperfusion injury[23]. This might be related to its central anti-acetylcholine effects. TNF- alpha in the neurons at the early stage after reperfusion could be transferred and released by axons, and penehyclidine could reduce the delivery of acetylcholine[24]. However, the specific and detailed mechanisms still need to be further explored.

Conclusions

Penehyclidine combined with edaravone can significantly reduce the levels of serum TNF-α, IL-6 and HMGB1 and the NDS score, improving the survival status of ACI rats; so, it is worthy of popularization in clinical application.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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