Case Report

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The Effect of Eupalinolide B on Amyotrophic Lateral Sclerosis : A Case Report

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ABSTRACT

Objective: To investigate the effect of Eupalinolide B (EB) on amyotrophic lateral sclerosis (ALS). ***Method:*** EB was administered 20 mg/d orally for one month. Muscular tension,muscular strength and neural reflexes etc.before and after experiment were compared. ***Results:*** After EB administration the abnormally lower muscular tension and muscular strength were improved. Ankle clonus disappeared. Amyotrophic lateral sclerosis function rating system revised (ALSFRS-R) stopped worsening. But 10 days after discontinuation of EB, the above improvements disappeared gradually. ***Discussion:*** EB might be effective on ALS and deserves further investigation

Keywords : *Eupalinolide B; amyotrophic lateral sclerosis; sesquiterpenes, Eupatorium Lindleyanum, Chinese herbs.*

INTRODUCTION

Presently recognized possible pathogenesis in ALS includes genic mutation,oxidative stress, loss of neurotrophic factors, glutamate-induced toxicity, inflammation, insufficient protein quality control, accumulation and misfolding of proteins, and mitochondrial dysfunction etc (1-3).Despite various preclinical and clinical studies,no drug with definite effect has been developed up to now. Thus, the development of successful and targeted therapy is challenging and is a major problem faced by scientists to treat ALS (4).

Recently it was reported that EB extracted from Eupatorium lindleyanum (E. lindleyanum) is efficacious for neurodegenerative disease by inhibiting microglia-mediated neuroinflammation in animal experiments (5). To investigate the clinical effect of EB on ALS, EB was administered in a ALS patient .

Case description

The 66-year-old femele patient experienced progressive right upper limb weakness and slowed speech speed in November,2021.One month later, weakness of lower limbs and left upper limb followed. In March 2022, the patient visited General Hospital of People's Liberation Army .Physical examination and neurologic check showed slight interosseus atrophy between thumb and index finger in both hands. Biceps reflex, triceps reflex, knee reflex and ankle reflex graded +++. Ankle clonus and Hofman's sign showed +. Babinski's sign was negative. Mascular tension in four limbs graded ++ according to Ashworth standard. Electromyogram showed neurogenic injury in cervical and lumbar section. Pulmonary function and cerebral spinal fluid test were normal. Based on the above data, the patient was diagnosed clinicaly as ALS.Since then, Riluzole 50 mg twice daily and Butylphthalide soft capsule 0.2 g thrice daily were prescribed. On May 13,2022, she fell backward when standing alone.The disease progressed steadily. The mascular strength of four limbs worsened .Eating and getting up could not be accomplished by herself. Constipation was experienced and sometimes glycerol was used as lubricant. ALSFRS-R went down all the way and scored 17 before experiment.

Experiment

EB was purchased from HerbSubstance Co.Ltd (Chengdu, China). In January 2023, EB dissolved in dimethyl sulfoxide (DMSO) was orally administered 20 mg daily with reference to the reported data about EB (5,6). Riluzole 50 mg twice daily and Butylphthalide soft capsule 0.2 g thrice daily were continued. Before experiment, neurologic check was repeated. Neural reflexes showed the same results as above but ankle clonus seemed stronger. The mascular tension in upper limbs graded+. But it was abnormally lower in lower limbs.She could walk at most 5 meters per day with caregiver's help.

Written consent was signed using an inked thumbprint in lieu of sigature. The experiment was authorized by Ethical Committee of Shanxi Provincial Hospital.

Results

Three days after EB administration , the abnormally lower mascular tension of lower limbs turned to basically normal. At the same time, the mascular strength of lower limbs improved .She could walk under assistance for about 45 meters. Sinteen days after EB administration, ankle clonus disappeared.Biceps reflex, triceps reflex, knee reflex,ankle reflex graded ++.Hofman's sign showed +.Babinski's sign remained negative. Constipation resolved and glycerol has not been used since experiment. ALSFR-R score stopped declining and scored 19 30 days since the start of experiment. As DMSO is irritating and was unpleasant when orally administered, the patient refused to accept further treatment with EB 30 days after the beginning of experiment. About 10 days after discontinuation of EB, muscular tension of lower limbs went back to abnormally low .Muscular strength decreased and ALSFR-R worsened.

Discussion

EB is a member of sesquiterpenes extracted from E. lindleyanum. E. lindleyanum has been used in China and other Asian countries as a traditional medicine for the treatment of cough,fever and tracheitis based on its anti-microbial and anti-inflflammatory activities (7-9).

In cells, some proteins to be degraded are covalently combined by ubiquitin (Ub) through E1, E2, and E3 enzymes (10). Deubiquitinating enzymes (DUBs) can reverse this process by removing Ub moieties (11). Accumulating studies have shown that aberrant DUB function is implicated in multiple human diseases (12-14). Ubiquitin-specific protease 7 (USP7) is an essential member of DUBs in all eukaryotes (15). It has a crucial role in regulating protein stability in multiple biological processes and is of significant value in targeting USPs/DUBs for developing therapeutics (16-17) .

Neuroinflammation which is a important mechanism in progressive neuronal damage is initiated by microglia . Numerous activated microglia are widely found in the brains of preclinical models and patients with neuroinflammation pathology (18).In vivo, pharmacological USP7 inhibition attenuates microglia activation and resultant neuron injury.

Zhang et al (5) investigated the mechanism of USP7 action and found that Cys^{576} is a unique noncatalytic in a HUBL domain within USP7. By selectively modifying Cys^{576} , EB inhibits USP7 to cause a ubiquitination-dependent degradation of Kelch-like ECH-associated protein 1 (Keap1) . Keap1 function loss further results in an Nrf2-dependent transcription activation of anti-neuroinflammation genes in microglia.In animal experiment, pharmacological USP7 inhibition by EB attenuated microglia activation and resultant neuron injury.

Yamashita et al (6) reported that heat shock proteins (HSPs), particularly HSP70, provides resistance to stressors. Treatment of cells with EB activated heat shock factor 1 (HSF1) and thereby induced HSPs70. In addition to its cytoprotective effects against stressor, HSP70 exerts an antiinflammatory action via its inhibitory effect on nuclear factor kappa B (NF-kB) (19-22).In animal experiment reported by Yamashita et al(6) , EB could suppress ultraviolet B radiation induced damage, inflammatory responses in the skin.As inflammation and stressor are important pathological mechanisms in ALS, EB might benifit ALS through the route of HSP70.

Nissl body is a special structure in neuron. It is chiefly comprised ordered structures of alternate lamellae of rough endoplasmic reticulum and arrays of polyribosomes (23). In motor neuron disease (MND), the microstructure of Nissl body has charactristic changes such as enlargement of tigroid,chromatolysis and at last disappearance of Nissl body. In ALS, enlargement of the tigroid seems to occur earlier than central chromatolysis. These pathological changes might result from endoplasmic reticulum stress, disturbed axonal transport or functional compensation of the neuronal deficit (24).

In ALS,sphincter function is commonly preserved .Histological examination of Onuf's nucleus which controls sphincter is basicaly normal. But,in ultrasructure,some sphincteric neurons were atrophic.Whereas in the others, Nissl bodies were reduced in number, showed loss of structural organization or polyribosomal aggregates (23). It indicates that Nissl body,the special apparatus in motor neuron, is vulnurable in ALS.

Zhang et al (5) reported that EB could restore the number of Nissl body in animal ALS model. Because Nissl body is a special apparatus in motor neuron. It indicated that EB migh be of specifity for the treatment of ALS. Based on the reporte effects of EB in animal experiment , especially the restoratio of Nissl body we were inspired to do the present experiment The present experiment demonstrated that after E administration, the formally abnormal lower mascula tension returned to normal and mascular strength increased indicating that the function of lower motor nuron was improved. Later, ankle clonus disappeared indicating that the dysfunction of upper motor neuron was ameliorated. After discontinuatio of EB, the improvements mentioned above disappeared. This further demonstrated the possible effect of EB on ALS. Because EB is insoluble in water , it was disolved in DMSO to improve the absorptivity in the present experiment. But as DMSO is irritating, the administration of EB disolved in DMSO is not acceptable. So, further research on EB such as method of medication etc. are urgently needed to increase its acceptability and to confirm whether it is really safe and effective for ALS.

ALS is a progressive neurodegenerative disease, The surviving motoneurons can partially reinnervate other neuromuscular junctions (25-27).Also evidence from animal models indicates that extensive nerve sprouting and synaptic remodeling occur as a part of the compensatory reinnervation process. But,the reinnervation can only take place when the motor neuron body is mostly intact although the nerve terminals and neuromuscular junctions are damaged (25,26,28). So, we report our case soon after the primary effect of EB was demonstrated hoping to accelate the further research.

Conclusion

EB might be effective for ALS but needs further research.

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Declaration of interest

The authors report no conflict of interest related to the topic of this manuscript.

Author contributions

Huaixiu Wang designed the experiment and wrote the manuscript. Aimei Wang and Fengyun Hu executed the physical examinations and treatment.

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