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Intestinal probiotics in relieving clinical symptoms of severe hand, foot, and mouth disease and potential mechanism analysis

F. ZHU[1], Z. JIANG[2], H.-W. LI[2]

[1]Department of Emergency, Xuzhou Children's Hospital affiliated to Xuzhou Medical University, Xuzhou, Jiangsu Province, China
[2]PICU, Xuzhou Children's Hospital Affiliated to Xuzhou Medical University, Xuzhou, Jiangsu Province, China

Abstract. – OBJECTIVE: In this prospective cohort study, the efficacy and action mechanism of an intestinal probiotic formulation, Golden Bifid, in severe hand, foot, and mouth disease (HFMD) were determined in 63 consecutively admitted patients successfully treated in the Pediatrics Emergency Department of our hospital.

PATIENTS AND METHODS: All patients had a persistent fever; 43 patients had rashes on hands, feet, crissum, and hips; and 45 patients had neurological signs and symptoms. Patients were treated with standard supportive therapy along with ventilator-assisted respiration combined with bedside hemofiltration for removal of circulatory toxins and to achieve acid-base equilibrium and electrolyte stability. Golden Bifid was orally administered for 2 weeks, and vaccination was performed after patients were in stable condition. Additional supportive nursing care was also provided. Patients were categorized into the effective treatment (n = 40) and ineffective treatment (n = 23) groups.

RESULTS: Serum levels of proinflammatory factors [interleukin (IL)-1β, IL-6, tumor necrosis factor-α, and interferon-γ] were significantly decreased and those of anti-inflammatory factors (IL-13, IL-4, and IL-10) were significantly increased after treatment in the effective treatment group. In contrast, in the ineffective treatment group, serum proinflammatory factor levels were significantly increased and serum anti-inflammatory factor levels were significantly decreased. Between-group difference was significant. After treatment, serum D-lactic acid, diamine oxidase, and endotoxin levels were significantly decreased in the effective group and significantly increased in the ineffective group by intra- and inter-group comparisons.

CONCLUSIONS: Intestinal probiotics were effective in relieving clinical symptoms of severe HFMD, maintaining intestinal immunity and anti-inflammatory responses, and enhancing intestinal barrier function, with better safety and efficacy, which should be further evaluated for more extensive clinical applications.

Key Words:
Intestinal probiotics, Hand disease, Foot disease, Mouth disease, Immunity, Inflammation, Serum D-lactic acid dehydrogenase, Diamine oxidase, Endotoxin.

Abbreviations

HFMD = hand, foot, and mouth disease; IL = interleukin; tumor necrosis factor-α = TNF-α; interferon = IFN; CA16 = coxsackie A16; EV71 = enterovirus 71; ELISA = enzyme-linked immunosorbent assay; D-LDH: D-lactic acid dehydrogenase; DAO = diamine oxidase; CR = complete response; PR = partial response.

Introduction

Hand, foot, and mouth disease (HFMD) is a common communicable disease that can be caused by more than 20 enteroviruses, mainly coxsackie A16 (CA16) and enterovirus 71 (EV71). Children under the age of 5, particularly those under the age of 2, are at high risk. The morbidity due to HFMD has been increasing in recent years. HFMD is now the number one in morbidity among C-class infectious diseases[1]. Because enteroviruses are typically highly infectious, propagate fast, utilize numerous transmission routes, and are associated with recessive infection, HFMD can lead to an outbreak within a large area[2]. Children usually present with mild maculopapular rash and herpetic eruptions in hands, feet, mouth, and hips accompanied with fever during an attack. Typically, HFMD is self-limiting and resolves within 2 weeks. In severe cases,

Corresponding Author: Huawei Li, PICU; e-mail: liweihua1975@126.com

however, it may lead to complications including respiratory failure, pulmonary edema, sterile meningoencephalitis, myocarditis, and even death[3]. Enteroviruses invading the gastrointestinal tract can multiply, primarily in intestinal mucosa, and continuously release enterotoxins into the bloodstream, which can spread throughout the body via circulation[4]. There are no specific vaccines or drugs for the prevention and treatment of HFMD[5]. This study was conducted to determine whether Golden Bifid, an intestinal probiotic formulation, was effective as an adjuvant therapy in children with severe HFMD disease.

Patients and Methods

Patients

A total of 63 patients that were consecutively admitted with severe HFMD and were successfully treated in the Pediatric Emergency Department of our hospital between January 2013 and January 2016 were included in this study. All patients met the clinical diagnostic criteria of HFMD set by the Ministry of Public Health of China in 2008 and had persistent fever[6]. Of these patients, rashes in hands, feet, crissum, and hips were found in 43 patients, whereas 45 patients had neurological signs and symptoms including depression, anxiety, and somnolence. Herpes and oral ulcers, dyspnea, hypertension, compromised circulatory function in limbs, and pulmonary edema were found in 39, 15, 14, 8, and 7 patients, respectively. Patients were divided into the effective treatment (n = 40) and ineffective treatment (n = 23) groups based on treatment outcomes. There were 26 boys and 14 girls in the effective treatment group, with an average age of 3.5 ± 1.4 years (range, 1-6 years). The time of disease onset was 2-6 days (average, 3.7 ± 1.5 days). Conversely, there were 14 boys and 9 girls in the ineffective treatment group, with an average age of 3.8 ± 1.6 years (range, 1.5-6.5 years). The time of disease onset was 2.5-6.5 days (average, 3.5 ± 1.7 days). Baseline characteristics of patients were comparable between the two groups.

Methods

All patients in the present work were treated with antiviral and anti-inflammatory drugs. In addition, intracranial pressure was controlled by mannitol *via* a venous pump. Where necessary, mild hypothermia therapy was used, and stable circulation and adrenocortical hormonal levels were achieved with standard medical procedures. Circulating toxins were removed by the combination of ventilator-assisted respiration and bedside hemofiltration to achieve and maintain acid-base equilibrium and electrolyte stability. Dosage of oral administration of Golden Bifid therapy was as follows: two tablets tid for patients under the age of 3 years and three tablets tid for patients over the age of 3 years. Tablets were grounded and dissolved in warm milk for oral administration. Vaccinations were performed after the stabilization of patients' clinical condition. Treatment was supported by effective nursing using isolation, oral and skin care, and temperature control. Specifically, patients were disinfected and immediately isolated after admission to prevent spread, and vital signs were closely monitored. Furthermore, oral cavities of patients were cleaned by sterile saline solution daily, and those with severe oral ulcers were administered 1% iodine glycerol after rinsing with 3% hydrogen peroxide to improve healing of oral mucosa. Finally, skin and body temperature were closely monitored. Bedding was kept clean and dry, and nails were clipped to avoid secondary infection due to scratching of lesions. High fever was controlled with routine approaches including physical cooling.

Observational Indices and Evaluation Methods

Serum levels of proinflammatory factors, including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)-γ and those of anti-inflammatory factors including IL-13, IL-4, and IL-10, were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, 3-5 ml venous blood samples collected from fasting patients were centrifuged at 2000 g for 30 min, and supernatants were collected and stored at -20°C until analysis using ELISA kits from Sigma-Aldrich (St. Louis, MO, USA), according to the manufacturer's instructions. For the comparison of functional parameters of gut barrier before and after treatment, D-lactic acid dehydrogenase (D-LDH), diamine oxidase (DAO), and endotoxin levels were determined by spectrophotometric measurement, o-dianisidine method, and limulus ameobocyte chromogenic assay, respectively. All kits were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as per manufacturer's instructions.

Treatment outcomes were evaluated according to the following definitions: (1) complete response (CR) defined as complete resolution of skin lesions and absence of new rashes, accompanied

with complete resolution of all complications such as fever, oral ulcer, dyspnea, and pulmonary edema; (2) partial response (PR) defined as reduction of more than 50% of rashes and improvement or alleviation of all symptoms; (3) no response defined as the persistence of rashes and failure of resolution of all symptoms or persistence of serious clinical condition.

Statistical Analysis

SPSS® version 20.0 software (SPSS Inc., Armonk, NY, USA) was used for statistical analyses. Quantitative data were presented as means ± standard deviation. Independent sample *t*-test was used for comparisons among groups and paired *t*-test was used for intra-group comparisons. Enumeration data were presented as numbers or percentages, and χ^2-test was used for comparison among groups. Differences with *p*-values < 0.05 were considered statistically significant.

Results

Comparison of Serum Levels of Inflammatory Factors

Serum levels of the proinflammatory factors (IL-1β, IL-6, TNF-α, and IFN-γ) and those of the anti-inflammatory factors (IL-13, IL-4, and IL-10) before treatment were not significantly different between the groups ($p > 0.05$, Table I). However, after treatment, serum levels of these proinflammatory factors were significantly decreased, and those of the anti-inflammatory factors were significantly increased in the effective treatment group ($p < 0.05$ for all, Table I). In contrast, in the ineffective treatment group, serum levels of all evaluated pro-inflammatory factors were significantly increased, whereas those of the anti-inflammatory factors were significantly decreased ($p < 0.05$ for both, Table I). The differences between the two groups were statistically significant ($p < 0.05$, Table I).

Comparison of Serum Indicators of Intestinal Barrier Function

Serum D-LDH, DAO, and endotoxin levels before treatment between the two groups were not significantly different ($p > 0.05$, Table II). These indices, however, were significantly decreased in the effective treatment group and significantly increased in the ineffective treatment group following treatment by both intra-group and inter-group comparisons ($p < 0.05$ for all, Table II).

Discussion

Alternative occurrence between type EV71 and type CA16 HFMD is a main type. EV71, a neurotropic virus, can easily cause nervous system lesions and complications. Probiotics are active microorganisms administered as dietary supplements with various benefits to the human body. Endogenous probiotics consist of *Lactobacillus*, *Clostridium butyricum*, *Lactobacillus acidophilus*, *and Bifidobacterium*[7,8], which are naturally found in intestines and may improve microecological balance[9]. Probiotics may inhibit

Table I. Comparison of serum levels of inflammatory factors among patients with different treatment outcomes.

Serum factors		Effective treatment	Ineffective treatment	t	p
IL-1β (ng/mL)	Before treatment	156.4 ± 32.6	157.8 ± 35.5	0.069	0.924
	After treatment	133.4 ± 25.7	179.2 ± 43.5	5.326	0.032
TNF-α (ng/mL)	Before treatment	26.8 ± 7.2	25.4 ± 7.6	0.125	0.836
	After treatment	12.3 ± 5.2	44.7 ± 16.8	5.624	0.030
IL-6 (ng/mL)	Before treatment	45.6 ± 7.7	43.7 ± 8.2	0.326	0.724
	After treatment	37.6 ± 6.2	65.3 ± 9.6	5.127	0.035
IFN-γ (μmol/L)	Before treatment	15.5 ± 3.3	15.3 ± 3.5	0.215	0.766
	After treatment	14.5 ± 2.6	18.7 ± 3.4	4.968	0.037
IL-13 (ng/mL)	Before treatment	16.7 ± 5.2	16.55.3	0.426	0.695
	After treatment	21.8 ± 4.5	13.5 ± 3.4	5.102	0.035
IL-4 (ng/mL)	Before treatment	246.3 ± 64.9	234.7 ± 75.8	0.328	0.532
	After treatment	312.6 ± 97.8	172.5 ± 53.4	5.562	0.027
IL-10 (ng/mL)	Before treatment	62.3 ± 16.8	61.5 ± 17.9	0.364	0.512
	After treatment	73.4 ± 19.7	51.6 ± 18.2	5.128	0.030

IL: interleukin; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon gamma.

Table II. Comparison of serum indicators of intestinal barrier function among patients with different treatment outcomes.

Group		Effective treatment	Ineffective treatment	t	p
D-LDH (mg/L)	Before treatment	10.5 ± 3.5	11.2 ± 3.9	0.068	0.945
	After treatment	5.6 ± 2.4	16.8 ± 3.6	5.632	0.029
DAO (U/mL)	Before treatment	4.2 ± 1.3	4.3 ± 1.4	0.125	0.867
	After treatment	3.5 ± 1.2	5.7 ± 1.6	5.124	0.032
Endotoxin (EU/ml)	Before treatment	0.6 ± 0.2	0.6 ± 0.2	0.000	1.000
	After treatment	0.5 ± 0.2	0.7 ± 0.2	4.859	0.035

D-LDH: D-lactic acid dehydrogenase; DAO: diamine oxidase; EU: Endotoxin Unit.

the growth of pathogenic bacteria such as *Aeromonas hydrophila*, *Vibrio cholerae*, *Shigella flexneri*, *Salmonella typhi*, *Campylobacter jejuni,* and *Staphylococcus aureus*[10,11] while promoting the growth of beneficial species including *Lactobacillus acidophilus, Bifidobacterium,* and *Streptococci* to reduce the production of harmful amines and ammonia[12].

The findings of our investigation demonstrated that oral administration of Golden Bifid might reduce the symptoms of severe HFMD. The major ingredients of Golden Bifid, also referred to as *Bifidobacterium lactobacillus* triple viable bacterial tablet, are *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Streptococcus thermophiles*, and probacterial factors that promote the growth and reproduction in human intestine to replenish intestinal probiotics[13]. They were shown to suppress the activation of four serum proinflammatory factors, IL-1β, TNF-α, IL-6 and IFN-γ by effectively decreasing their expression levels to relieve inflammation. For example, probiotics were demonstrated to modulate vascular permeability and resolve pulmonary edema by inhibiting TNF-α and IFN-γ[14]. Probiotics also mediate the levels of anti-inflammatory factors IL-13, IL-4, and IL-10. Specifically, increases in IL-10 levels may reduce IL-10-mediated sympathetic nerve stimulation, thrombocytopenia, increased pulmonary vessel permeability that can precipitate pulmonary edema. Thus, reductions in serum levels of these anti-inflammatory factors can facilitate the resolution of meningitis, brainstem encephalitis, and pulmonary edema[15].

Endogenous probiotics, via receptor-mediated regulatory mechanisms, can promote the secretion of intestinal mucoproteins and enhance intercellular connections within the intestinal epithelial layer, thereby strengthening mucosal barrier for effective filtering of macromolecular substances such as toxic, bacterial, and chemical factors[16]. Intestinal epithelial permeability leads to the generation of high levels of D-lactic acid by aggressive bacteria that enter into the circulation through intestinal mucosa in conditions of compromised intestinal mucosal barrier[17]. Increased plasma levels of both will result in increased endotoxin levels[18]. Supplementation with endogenous probiotics induces the production of certain organic acids including lactic acid and acetic acid, thereby reducing intestinal pH and absorption of aggressive bacteria, normalizing intestinal permeability, and recovering intestinal barrier function[19].

The findings of the present study showed that serum levels of proinflammatory factors IL-1β, TNF-α, IL-6, and IFN-γ were significantly decreased, whereas those of anti-inflammatory factors IL-13, IL-4, and IL-10 were significantly increased following treatment in patients with effective treatment, compared to those in the ineffective treatment group. In contrast, serum proinflammatory factor levels were significantly increased and anti-inflammatory factor levels were significantly decreased in the ineffective group. Furthermore, serum D-LDH, DAO, and endotoxin levels were significantly decreased after treatment in the effective group and significantly increased after treatment in the ineffective group; additional analysis showed that these changes were significant between the groups.

Conclusions

Golden Bifid was effective in alleviation of the clinical symptoms of severe HFMD, maintenance of intestinal immunity and anti-inflammatory response, and improvement of intestinal barrier function, suggesting that intestinal probiotics were safe and effective in severe HFMD treatment, which should be further evaluated for more extensive clinical applications.

Conflict of interest

The authors declare no conflicts of interest.

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