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Effect of Montmorillonite powder on intestinal mucosal barrier in children with abdominal Henoch–Schonlein purpura

A randomized controlled study

Xiaolin Gao, MD[a], Ruixue Miao, MM[a], Yuhong Tao, MD[a], Xiuying Chen, BA[a], Chaomin Wan, MD[a,*], Ruizhen Jia, MM[b,*]

Abstract

Background: Our previous studies found that intestinal barrier function has been changed in children with abdominal Henoch–Schonlein purpura (HSP). Montmorillonite has been shown to be protective for digestive tract mucosa.

Objective: The present study aimed to investigate whether Montmorillonite powder could improve the intestinal mucosal barrier function in children with abdominal HSP.

Methods: Using a randomized controlled study design, we compared plasma levels of diamine oxidase (DAO), D-lactate, and endotoxin in children with abdominal HSP before and after Montmorillonite powder treatment.

Results: Among 28 patients in experimental group and 30 in control group, there was no significant difference in age, sex, height, weight, and course of disease between 2 groups ($P > .05$). Before treatment, there was no statistical difference in DAO, D-lactic acid, and endotoxin between experimental group and the control group ($P > .05$). However, significant differences were detected for DAO and D-lactate after treatment in comparison to before treatment in the Montmorillonite experimental group ($P < .05$). Such differences were not found in the control group ($P > .05$).

Conclusion: Montmorillonite powder is effective in the treatment of HSP via maintaining intestinal mucosal barrier function.

Abbreviations: DAO = diamine oxidase, ELISA = enzyme-linked immunosorbent assay, HSP = Henoch–Schonlein purpura, IVIg = intravenous immunoglobulin, ROS = reactive oxygen species.

Keywords: abdominal Henoch–Schonlein purpura, children, intestinal mucosal barrier, randomized controlled study

1. Introduction

In our previous studies, it has been found that intestinal barrier function has been changed in children with Henoch–Schonlein purpura (HSP).[1] HSP is one of the most common small vasculitis in childhood, with clinical manifestations including nonthrombocytopenic purpura, arthritis or arthralgia, abdominal pain, gastrointestinal bleeding and so on.[2,3] Skin purpura and digestive tract symptoms are the most common complains in patients with this disease.[4] In the past decades, the incidence and recurrence of HSP were gradually increasing, which has invoked great interest of researchers globally.[2–5] In recent years, a large number of studies have shown that the intestinal tract of children with HSP has varying degrees of congestion, edema, erosion, ulcers, bleeding, and other morphological changes.[6–8]

Intestinal tract not only serve as an organ for digestion and absorption, but also has important barrier function. The latter consists of 4 parts: mechanical barrier, biological barrier, immune barrier, and chemical barrier, all of which play protective roles in the body and prevent the invasion of intestinal harmful substances into the host. Under the action of noxious stimulation, the barrier function of intestinal tract could be comprised and lead to disruption of normal physiology.[9,10]

The current clinical management of abdominal HSP mainly focuses on antiallergy, activation of blood circulation to decrease blood stasis and reduction of symptoms. To date, there is no an ideal medicine to improve the performance of the intestinal tract.[3,5,8] Montmorillonite, a subclass of smectite, is a natural dioctahedron phyllosilicate that has 2 tetrahedral sheets of silica sandwiching a central octahedral sheet of alumina. It has been shown that Montmorillonite had protective effect for digestive tract mucosa via multiple mechanisms. First, Montmorillonite is

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[a] Department of Pediatrics, West China University Second Hospital, Sichuan University, [b] Open Laboratory, West China Institute for Women's and Children's Health, Chengdu, Sichuan Province, China.

[*] Correspondence: Chaomin Wan, Department of Pediatrics, Western Women's and Children's Research Institute, Number 20, 3rd Section, People's South Road, Chengdu, Sichuan Province 610041, China (e-mail: wanchaomin@scu.edu.cn); Ruizhen Jia, Open Laboratory, Western Women's and Children's Research Institute, Number 20, 3rd Section, People's South Road, Chengdu, Sichuan Province 610041, China (e-mail: 1002113878@qq.com).

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capable of binding to mucin, in turn enhances the mucus barrier function and maintains the normal physiology of the digestive tract. In addition, Montmorillonite is thought to reduce the sensitivity of the colon and to promote the recovery and regeneration of the epithelial cells in the digestive tract. Furthermore, it can activate the coagulation factors, VII and VIII, and meditate a local hemostatic function in digestive tract. Lastly, Montmorillonite has minimal side effect and no impact in stool color and unlikely covers other pathological conditions. Currently, Montmorillonite powder is widely used in the treatment of diarrhea, ulcer, and gastrointestinal hemorrhage.[11–13] However, the effect of Montmorillonite on the function of intestinal mucosal barrier in children with HSP have not been well studied. Our recent studies demonstrated that the damage of intestinal mucosal barrier is present in children with HSP.[8] In the current randomized controlled study, we applied Montmorillonite powder in children with abdominal HSP and evaluated its effect on intestinal mucosal barrier function in these patients.

2. Materials and methods

2.1. Subjects

From September 2015 to August 2016, children with abdominal HSP were consecutively enrolled upon diagnosis in Huaxi Second Hospital of Sichuan University. Informed consents have been signed and given by the subjects and/or guardians. This study was approved by the ethics committee of Huaxi Second Hospital, Sichuan University, and registered at China Clinical Trial Registration Center (Registration No: chiCTR-IPR-16008658).

2.2. Inclusion and exclusion criteria

The patients who met all of the following inclusion criteria were recruited into this study: first time diagnosis of abdominal HSP[14] at Huaxi Second Hospital; the age of children upon diagnosis was <18 years old; willing to participate in the study and giving the consent; no other significant complications of heart, kidney, and other organ diseases. Patients were excluded if they were treated with similar drugs of Montmorillonite powder during the same period; or suffered from shock due to gastrointestinal bleeding.

2.3. Design

Using a random number table, the subjects were randomly assigned to the experimental and control groups. The patients in experimental group received routine treatment plus Montmorillonite powder (Ipsen Ltd, Tianjin, China 3 g/time, 2 times a day, which was discontinued when digestive tract symptoms relieved within 3–5 days). The control group received only routine treatment, which were primarily to manage the symptoms. According to the specific symptoms, patients might receive one or combined medications, including Claritin for allergy, Dipyridamole/Salvia for Vasculitis,[1,4,8] Hydrocortisone for joint swelling and abdominal pain, and antibiotics for infection. The basic data of all subjects were continuously recorded. Three milliliters fasting venous blood was collected before treatment and after treatment (the 3rd day after remission of digestive symptoms). Blood samples were placed in sterile and pyrogen-free test tube with anticoagulant (heparin) and subjected to centrifugation. Plasma samples were collected and stored at −20°C refrigerator for further testing.

Patients were followed up during scheduled clinical visits. Subjects who missed the visits were contacted by telephone and mail. Patients who were lost in the follow-up period were regarded as no change in intestinal mucosal barrier function and ineffective treatment and excluded.

2.4. Experimental methods

All plasma samples were tested for the intestinal barrier function using three indexes: diamine oxidase (DAO), D-lactic acid, and endotoxin using human DAO ELISA kit (F00616), human D-lactate ELISA kit (F7036-A), human endotoxin ELISA kit (F2768-A), respectively. All kits were purchased from Beijing YanBiXin Technology Co. Ltd, Beijing, China and the manufacture's protocols were followed. The tests were performed on 6-color gradient real-time quantitative PCR machine (Biord CFX96) and continuous wavelength multifunctional microplate reader (Thermofisher Varioskan Flash, Beijing, China).

2.5. Statistical analysis

Data were analyzed using SPSS software package (vision 19.0). Measurements in line with the normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$). The comparison of 2 sample mean of complete randomized design was conducted using t test. The rank sum test was used when the condition is not satisfied. The difference was considered significant when $P < .05$.

3. Results

In the present study, 28 patients were included in the experimental group, and 30 in the control group. All patients fulfilled the scheduled follow-up visits. Baseline characteristics of studied subjects are summarized in Table 1. No significant differences in age, sex, height, weight, and course of disease were detected between the 2 groups ($P > .05$).

Plasma DAO (pg/mL), D-lactic acid (μg/L), and endotoxin (ng/L) in patients with abdominal HSP before and after the treatment of Montmorillonite powder were compared using rank-sum test. The data and statistical analysis results are shown in Table 2. Briefly, significant differences of DAO and D-lactate were detected before and after treatment of Montmorillonite powder for abdominal HSP ($P < .05$). Our data collectively support that

Table 1

The baseline data of study subjects (data are presented as $\bar{x} \pm s$).

	Experimental group (n = 28)	Control group (n = 30)
Male, n	17	18
Female, n	11	12
Age	8.37 ± 0.14	8.16 ± 0.67
Height, m	1.28 ± 0.21	1.26 ± 0.75
Weight, kg	25.21 ± 1.85	24.61 ± 1.33
Course of disease, d	14.1 ± 0.48	13.4 ± 2.12
Clinical symptoms (n)		
Abdominal pain	28	30
Rash	28	30
Vomit	7	9
Bloody stools	5	6
Joint swelling and pain	10	8
Hematuresis	1	0

Table 2

Plasma DAO, D-lactic acid, and endotoxin in patients with abdominal HSP before and after the treatment of Montmorillonite powder ($\bar{x} \pm s$)*.

	DAO, pg/mL			D-lactic acid, μg/L			Endotoxin, ng/L		
	Before	After	Difference*	Before	After	Difference*	Before	After	Difference*
Experimental	34.96 ± 5.90	25.96 ± 6.58	7.79 ± 3.71	283.19 ± 31.43	236.16 ± 35.7	47.03 ± 3.06	76.63 ± 12.28	73.01 ± 14.71	3.62 ± 4.42
Control	39.05 ± 0.49	37.79 ± 1.59	1.25 ± 0.58	239.80 ± 3.32	238.81 ± 5.15	3.42 ± 0.56	68.95 ± 12.66	65.41 ± 7.65	5.13 ± 3.33
Z		−3.812			−3.283			−1.898	
P		.00			.01			.058	

DAO = diamine oxidase.

* (1) The basal levels of DAO, D-lactic acid, and endotoxin before treatment were similar in experimental group and the control group; no statistical differences in DAO, D-lactic acid, and endotoxin were detected between experimental group and the control group ($P > .05$) before the treatment (first column of each parameter). (2) Statistical differences were detected for DAO and D-lactate after treatment in comparison to their basal levels before treatment in the Montmorillonite experimental group ($P < .05$). Such differences were not found in the control group ($P > .05$).

Montmorillonite powder is effective in the treatment of HSP via improving intestinal mucosal barrier function.

4. Discussion

The appropriate indexes for the evaluation of intestinal mucosal barrier function are controversial, it is generally accepted that the increase of plasma DAO, D-lactic acid, and endotoxin level reflects the decrease in mucosal barrier function.[8,15–18] DOA is a highly active intracellular enzyme present in humans and mammals intestinal villus epithelial cells, with the highest activity in Jejunoileum. The presence of DAO in the upper villi of the small intestinal mucosa reflects the sound integrity of structure and function of the small intestine. The increase of DOA in plasma usually implies the disruption of the intestinal barrier. D-lactic acid is a metabolic product of gastrointestinal bacteria. When intestinal mucosa cells are injured by due to pathological reasons, the tight junctions between cells may be damaged and cause the increase in intestinal permeability. As a result, a large amount of D-lactic acid enters the blood circulation. Since there is no rapid metabolizing enzyme system for D-lactic acid in mammals, the increased level of D-lactic acid in the blood reflects the intestinal mucosal damage and permeability change. Endotoxin is the component of lipopolysaccharide in cell wall of gram negative bacteria. When intestinal mucosal barrier function is comprised, the bacteria or endotoxin enter host blood circulation and maintain for a period of time. Therefore, the increased level of plasma endotoxin also reflects the change of intestinal permeability.

Via measuring plasma levels of DAO, D-lactic acid, and endotoxin, we evaluated the intestinal mucosa barrier function in abdominal HSP patients before and after Montmorillonite powder as well as in comparisons with abdominal HSP patients who only receive routine treatment. Our data showed while plasma DAO and D-lactic acid were controlled by Montmorillonite, suggesting a protective effect on intestinal barrier function and permeability in children with HSP. However, since the endotoxin level did not change significantly, it was suggested that Montmorillonite could not prevent migration of intestinal endotoxin.

Previous studies demonstrated that Montmorillonite powder is effective in relieving gastrointestinal tract poisoning by absorbing toxin and reducing pathologic damage. Regarding the molecular mechanism, it was thought to be relevant to Montmorillonite's structure. Montmorillonite is a type of aluminosilicate consisting of a double aluminum and magnesium silicate, which shows the layer structure and heterogeneity charge distribution. Such unique structure provides a remarkable binding capacity and adsorption activity.[19] It not only absorbs water but also toxins, bacteria and virus, in turn alleviates the pathogenic factor adhering to intestinal membrane.[20] In addition to the absorption ability, Montmorillonite powder covers the mucosa and binds mucous glycoprotein and subsequently strengthens the mucosal barrier function and prevents pathogenic factors from entering blood circulation. For example, Montmorillonite powder was shown to prevent absorption of paraquat dichloride, subsequently reduce reactive oxygen species (ROS) production, elevate glutathione, and reduce cytotoxicity.[11,21]

The current routine management of HSP includes limiting the exposure to antigen, promoting blood circulation, as well as the antiinfection if necessary. In certain complicated cases, additional treatment may be needed to control urgent or prominent symptoms such as intestinal bleeding, joint swallowing and pain, and hematuresis. In addition to the routine treatment, children with abdominal HSP patients in the present study also received hemostatic medication, proton pump inhibitors, and antacids. Patients who showed the manifestations of joint and renal complication also received glucocorticoids. Intravenous immunoglobulin (IVIg) and plasma exchange and have been reported for successful treatment of severe HSP.[22–24] None of patients in the present study received IVIg treatment. Data from this study demonstrated that Montmorillonite powder is effective in the treatment of abdominal HSP and may be a new medication for the management of abdominal HSP.

From the present study, we found that DAO and D-lactate levels in the Montmorillonite experimental group ($P < .05$) were significantly decreased after treatment in comparison to their basal levels before treatment. In contrast, such differences were not found in the control group ($P > .05$). As indicated, in this study, routine treatment medications have no effect on DAO, D-lactic acid, and endotoxin that are indices of intestinal barrier function but Montmorillonite powder significantly decreased 2 indices (DAO and D-lactic acid) in patients with HSP. In the meantime, this study has three limitations. First, the sample size is relatively small (n = 28 in the experimental group and n = 30 in the control group). The limited statistical strength may explain why one parameter (Endotoxin) for the barrier functions was not significantly different between experimental and control groups. Second, the present study is focused on whether Montmorillonite has an effect on the function of intestinal mucosal barrier. Our data support the effectiveness of Montmorillonite, but it remains unknown how Montmorillonite exerts its effect in the intestinal tract. Lastly, the patient population in the present study are children, it remains to study whether Montmorillonite powder is

effective in the treatment of adult HSP. We are very interested in conducting an investigations with a larger size of samples to elucidate the molecular mechanisms by Montmorillonite improve the barrier functions.

5. Conclusion

In the present study, we studied the function changes of intestinal mucosal barrier in children with HSP. Our results indicate that Montmorillonite powder has a protective effect on intestinal barrier function and is beneficial for children with abdominal HSP. The mechanisms remain to be further studied in the future.

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Author contributions

Xiaolin Gao conceptualized and designed the study and drafted the initial manuscript. Yuhong Tao was in charge of data collection and analysis. Ruixue Miao and Xiuying Chen were responsible for collecting specimens and carrying out part of the experiments. Ruizhen Jia was responsible for preserving specimens and carrying out part of the experiments. Chaomin Wan and Ruizhen Jia coordinated and supervised the entire study from specimen collection to manuscript revision. The abovementioned authors carried out the initial analyses and reviewed and revised the manuscript. All authors approved the final manuscript.

Conceptualization: Xiaolin Gao.
Data curation: Ruixue Miao.
Formal analysis: Yuhong Tao.
Funding acquisition: Xiaolin Gao.
Investigation: Xiaolin Gao, Ruixue Miao, Xiuying Chen.
Methodology: Xiaolin Gao, Xiuying Chen, Ruizhen Jia.
Project administration: Xiaolin Gao, Yuhong Tao, Ruizhen Jia.
Supervision: Chaomin Wan, Ruizhen Jia.
Validation: Xiaolin Gao, Yuhong Tao, Chaomin Wan, Ruizhen Jia.
Visualization: Chaomin Wan.
Writing – original draft: Xiaolin Gao.
Writing – review & editing: Xiaolin Gao, Chaomin Wan, Ruizhen Jia.

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