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

A randomized controlled study

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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), \(\alpha\)-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, \(\alpha\)-lactic acid, and endotoxin between experimental group and the control group \((P > .05)\). However, significant differences were detected for DAO and \(\alpha\)-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).\textsuperscript{[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.\textsuperscript{[2,3]} Skin purpura and digestive tract symptoms are the most common complaints in patients with this disease.\textsuperscript{[4]} In the past decades, the incidence and recurrence of HSP were gradually increasing, which has invoked great interest of researchers globally.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[3,5,8]} Montmorillonite, a subclass of smectite, is a natural dioctahedral 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...
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 manufacturer’s protocols were followed. The tests were performed on 6-color gradient real-time quantitative PCR machine (Biorad CFX96) and continuous wavelength multifunctional microplate reader (Thermofisher Varioskan Flash, Beijing, China).

2.5. Statistical analysis

Data were analyzed using SPSS software package (version 19.0). Measurements in line with the normal distribution were expressed as mean ± standard deviation (x ± 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

| Clinical symptoms          | Experimental group (n = 28) | Control group (n = 30) |
|----------------------------|----------------------------|------------------------|
| Abdominal pain             | 28                         | 30                     |
| Rash                       | 28                         | 30                     |
| Vomit                      | 7                          | 9                      |
| Bloody stools              | 5                          | 6                      |
| Joint swelling and pain    | 10                         | 8                      |
| Hematuration               | 1                          | 0                      |

| 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             |

The baseline data of study subjects (data are presented as x ± s).
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. 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 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. It not only absorbs water but also toxins, bacteria and virus, in turn alleviates the pathogenic factor adhering to intestinal membrane. 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.

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 (IVlg) and plasma exchange and have been reported for successful treatment of severe HSP. None of patients in the present study received IVlg 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-lactic 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 adults.

### Table 2

|               | DAO, pg/mL | D-lactic acid, µg/L | Endotoxin, ng/L |
|---------------|------------|---------------------|----------------|
| Before        | 34.96 ± 5.90 | 25.96 ± 6.58          | 7.79 ± 3.71    |
| After         | 283.19 ± 31.43 | 239.80 ± 3.32          | 238.81 ± 5.15  |
| Difference    | 1.59 ± 0.56  | 3.42 ± 0.56           | 7.65 ± 3.33    |
| Z             | –3.812      | –3.283               | –1.598         |
| 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 control group; no statistical differences were detected between experimental group and control group (P > .05) before the treatment (first column of each parameter).
2. Statistical differences were detected for DAO and D-lactic acid 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).

### References

[1] Gao et al. Medicine (2018) 97:39 www.md-journal.com
future. Montmorillonite powder has a protective effect on 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 the 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.

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References

[1] Xiaolin G, Yongkun H, Mei L, et al. Study on the changes of gastrointestinal mucosal barrier in children with allergic purpura. Chin J Pract Pediatr 2010;25:286–8.
[2] Hu X, Tai J, Qu Z, et al. A lower proportion of regulatory B cells in patients with Henoch-Schoenlein purpura nephritis. PLoS ONE 2016;11:e0152368.
[3] Bellantoni A, Lo Presti P, Giordano A, et al. A pediatric case of Schoenlein-Henoch purpura with clinical, serologic and electrocardiographic signs of myocardial damage. G Ital Cardiol (Rome) 2013;14:622–5.
[4] Menon P, Singh S, Ahuja N, et al. Gastrointestinal manifestations of Henoch-Schoenlein purpura. Dig Dis Sci 2013;58:42–5.
[5] Solyu A, Oztrak Y, Dogan Y, et al. Screening of cardiac disease in children with Henoch-Schoenlein purpura. Rheumatol Int 2016;36:713–7.
[6] Chen O, Zha XH, Ren P, et al. Henoch Schoenlein purpura in children: clinical analysis of 120 cases. Afr Health Sci 2013;13:94–9.
[7] Nam EJ, Kim GW, Kang JW, et al. Gastrointestinal symptoms in adult patients with Henoch-Schoenlein purpura. Endoscopy 2014;46:981–6.
[8] Sohagia AB, Gunurur SG, Tong TR, et al. Henoch-schoenlein purpura—a case report and review of the literature. Gastroenterol Res Pract 2010;2010:397648.
[9] Thomas S, Mercado JM, DuHaddaway J, et al. Novel colitis immunotherapy targets Bin1 and improves colon cell barrier function. Dig Dis Sci 2016;61:823–32.
[10] Shavrov A, Khartonova AY, Davis EM, et al. A pilot study of confocal laser endomicroscopy to predict barrier dysfunction and relapse in pediatric inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2016;62:873–8.
[11] Jiang YS, Ma YY, Wang ZQ, et al. Therapeutic effects of smecta or smecite powder on rats with paratoxin intoxication. World J Emerg Med 2013;4:144–50.
[12] Buccigrossi V, Russo C, Guarino A, et al. Mechanisms of antidiarrheal effects by diosmectite in human intestinal cells. Gut Pathog 2017;9:23.
[13] C.D. Rudolph, G.E. Lister, L.R. First, et al. Rudolph's Pediatrics 22E. 2010;1381–85.
[14] Jiang XJ, Wang HJ, Cui HF. Combined diosmectite and mesalazine treatment for mild-to-moderate ulcerative colitis: a randomized, placebo-controlled study. Med Sci Monit 2015;21:163–70.
[15] Yao Y, Xu ZY, Chen XP, et al. Effects of experimental liver injury on the intestinal barrier in rats. Zhonghua Gan Zang Bing Za Zhi 2009;17:128–30.
[16] Popoff MR. Multifaceted interactions of bacterial toxins with the gastrointestinal mucosa. Future Microbiol 2011;6:763–97.
[17] Altshuler AE, Kistler EB, Schmid-Schonbein GW. Aundogestin: proteolytic degradation and multiple organ failure in shock. Shock 2016;45:483–9.
[18] OuYang J, Zhang ZH, Zhou YX, et al. Up-regulation of tight-junction proteins by p38 mitogen-activated protein kinase/p53 inhibition leads to a reduction of injury to the intestinal mucosal barrier in severe acute pancreatitis. Pancreas 2016;45:1136–44.
[19] Zheng CD, Duan YQ, Gao JM, et al. Screening for antilipase properties of 37 traditional Chinese medicinal herbs. J Chin Med Assoc 2010;73:119–24.
[20] Dupoint C, Foo JL, Garnier P, et al. Oral diosmectite reduces stool output and diarrhea duration in children with acute watery diarrhea. Clin Gastroenterol Hepatol 2009;7:456–62.
[21] Maisanaba S, Gutierrez-Praena D, Pichardo S, et al. Toxic effects of a modified montmorillonite clay on the human intestinal cell line Caco-2. J Appl Toxicol 2014;34:714–25.
[22] Sobieszczański M, Tubek S, Poplicha D, et al. Henoch-Schönlein purpura (HSP) and high-dose immunoglobulin treatment in patient with familial prostatic adenocarcinoma. Hum Vaccin Immunother 2014;10:358–9.
[23] Cherqaoui B, Chausset A, Stephan JL, et al. Intravenous immunoglobulin for severe gastrointestinal involvement in pediatric Henoch-Schönlein purpura: a French retrospective study. Arch Pediatr 2016, 23:584–590
[24] Shah R, Ramakrishnan M, Vollmar A, et al. Henoch-Schönlein purpura presenting as severe gastrointestinal and renal involvement with mixed outcomes in an adult patient. Cureus 2017;9:e1088.