Montmorillonite-Nasal-Gel, A Prophylactic Measure Against Covid-19

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Abstract

Since December 2019, a novel pneumonia caused by a novel coronavirus (SARS-CoV-2) has spread through the whole world. There are various ways to control the outbreak, such as vaccines, neutralizing antibodies, but prophylactic measures is still one of the important measures to control the outbreak like face masks. Montmorillonite-Nasal-Gel (MNG), a nasal spray gel consisting of a combination of 10% montmorillonite, 2% xylitol and water, has shown the effect to reduce viral infection. We tested the effect of MNG on SARS-CoV-2 infection by using a SARS-CoV-2 S protein pseudovirus system. To evaluate the efficacy of MNG against SARS-CoV-2 infection, SARS-CoV-2 were treated with 50% MNG in BSL-3 laboratory. These experiments appeared that MNG treatment greatly reduced the infection of SARS-CoV-2. As a nasal spray, it should not stimulate the airways. So we did a respiratory stimulating test and found that it has no stimulating effect on the respiratory tract. Montmorillonite is generally considered to have a amount of negative charge on the surface, it can absorb some viruses and bacteria. We assume that montmorillonite can physically adsorb SARS-CoV-2 too. As a personal protective equipment, MNG was approved by National Medical Products Administration (NMPA). MNG can further be used in the protection of all groups.

1. Introduction

The outbreak of coronavirus disease 2019 (Covid-19), which was caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), has been declared a global pandemic in March 2020 by WHO¹. As of October 7, 2021, Covid-19 has affected more than two hundred million people across 213 countries, areas or territories, and left more than four million death worldwide. To date, vaccines are applying through the world. But there are still so many people were infected by the virus and caused thousands of people to death. Moreover, small molecule drugs were expected to treat Covid-19, but only Remdesivir were approved by the FDA to treat Covid-19, and Molnupiravir is the next hopeful drug to treat Covid-19². Virus-neutralizing antibodies were proved effective through clinical trial, such as 47D11, S309^{3,4}. Human immunoglobulin (pH4) for intravenous injection was approved for clinical trials in China on August 30, 2021. However, physical protection is a useful way to fight against the epidemic. Montmorillonite-Nasal-Gel (MNG), a prophylactic preparation against Covid-19, had been approved by NMPA of China, can offer protection from Covid-19 virus.

MNG can adsorb pathogenic microorganisms because of negative charge on its crystal surface. It is a gel made of a mixture of 10% montmorillonite, 2% xylitol and distilled water. The active component of MNG is montmorillonite, came from Chifeng city, Nei Monggol Autonomous Region, China. Montmorillonite were found at Montmorillon, Vienne, France, Nouvelle-Aquitaine. Its formula is $(\text{Na,Ca})_{0.33}(\text{Al,Mg})_2(\text{Si}_4\text{O}_{10})(\text{OH})_2 \cdot n\text{H}_2\text{O}$. The crystal structure of montmorillonite is composed of two layers of silicon-oxygen tetrahedron sheets and aluminum (magnesium)-oxygen (hydroxyl) octahedron sheet which are sandwiched between two layers of silicon-oxygen tetrahedra sheets⁵. The Si^{4+} in the silicon-oxygen tetrahedron is often replaced by Al^{3+} , and the Al^{3+} in the aluminum oxygen octahedron is

replaced by low-valence positive ion, such as Mg^{2+} , Fe^{2+} or other low-valence positive ion resulting in negative charges between the crystal layers (structural layers). In order to maintain electric neutrality, crystal layers are adsorbed positive ions, such as K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Li^+ , H^+ , etc^{5,6}. These ions appear in hydrated state and are inter-exchangeable, so that montmorillonite have characteristics such as ion exchange ability⁷. Because of negative charge on surface, montmorillonite crystal can adsorb positive charge pathogenic microorganisms or certain proteins⁸. Based on the pharmacology of montmorillonite and its unique high adsorption, it is mainly used in the fields of medicine, animal supplements and food additives^{9,10}. The medicinal mechanism of montmorillonite mainly depends on its adsorption and gelation properties. Montmorillonite crystal has a lamellar structure and non-uniform charge distribution, which can anchor or inhibit viruses, germs and toxins in digestive tract¹¹. It can repair and improve the defense function of intestinal mucosa against pathogens through binding with mucosa glycoproteins. Montmorillonite firms colloidal state when added into water¹². Colloidal montmorillonite can bind various pathogens such as bacteria and viruses, inhibiting their growth and infection. As Covid-19 is primarily transmitted via the respiratory route, we assume MNG can be used to prevent Covid-19 transmission. Therefore, we designed an *in vitro* experiment to test its efficacy of anti-SARS-CoV-2. Also we did stimulation test of respiratory tract¹³.

2. Materials And Methods

2.1 Materials

The clinical isolate of SARS-CoV-2 was obtained from Institute of Pathogen Biology Chinese Academy of Medical Sciences and Peking Union Medical College and manipulated in BSL3 containment at Institute of Pathogen Biology Chinese Academy of Medical Sciences and Peking Union Medical College. Thirty male New Zealand rabbits (weighing $2.5\text{ kg} \pm 0.2$) was obtained from Shandong Academy of Agricultural Sciences, China. I promise the experiment was approved by the Animal Ethics Committee of Shandong University of Traditional Chinese Medicine. Ethics Statement No.SDUTAC20200317. I promise all methods were carried out in accordance with relevant guidelines and regulations. I promise all methods are reported in accordance with ARRIVE guidelines. Anesthesia and euthanasia procedures: the rabbit is anesthetized by injecting midazolam (CAS: 59467-64-0), a sedative, through an auricular vein. Then the sodium pentobarbital (CAS:57-33-0) solution was administered at a rate of 0.1 mL/s through an intravenous indignant needle. Experimental animals are raised and tested in an ordinary environment. Temperature: 16-26°C. Maximum daily temperature difference $\leq 4^\circ\text{C}$. Relative humidity: 40%-70%. Minimum number of air changes: ≥ 8 times. Air velocity at cage: $\leq 0.2\text{ m/s}$. Ammonia concentration $\leq 14\text{mg/m}^3$. Noise: $\leq 60\text{ dB}$. Alternation of light and dark:12/12. Experimental cage: floor area 0.3 m^2 , cage height 0.4 m. Pad material has good moisture absorption, less dust, no peculiar smell, no toxicity, no grease, high temperature resistance, high pressure resistance, used after sterilization. Drinking water meets GB 5749 requirements.

2.2 Anti-SARS-CoV-2 in vitro

We first tested the effect of MNG on SARS-CoV-2 infection by using a SARS-CoV-2 S protein pseudovirus system. The lentiviral-based pseudovirus carrying the SARS-CoV-2 S protein (SARS-CoV-2pp) was prepared as previously described¹⁴. The pseudotyped SARS-CoV-2 (ppSARS-CoV-2) carrying a firefly luciferase gene were treated with various concentrations (0%, 5%, 10% and 20%) of MNG at 37°C for 1 h, followed by centrifugation at 3,000*g* for 10 min. Then the supernatant of each group was removed and used to inoculate 293T-ACE2/TMPRSS2 cells. After 48 h incubation, the cells were collected and subjected to luciferase assay.

To better evaluate the efficacy of MNG against SARS-CoV-2 infection, SARS-CoV-2 were treated with 50% MNG in BSL-3 laboratory. After 1 h treatment at 37°C and centrifugation, the supernatants were removed, serially diluted and subjected to infect Vero cells for 2 h. Subsequently, the inoculum were replaced by fresh medium, and cells were incubated at 37°C for 48 h. The viral yield was determined by quantifying the genome copies of SARS-CoV-2 in 1µL culture medium using qRT-PCR as described previously¹⁵.

2.3 Respiratory Stimulation Test

In addition to the study of MNG against SARS-CoV-2 infection, we also did respiratory mucosal stimulation test¹⁶.

Thirty male New Zealand rabbits were randomly divided into normal control group, high-dose group and low-dose group, and the doses administered are shown in Table 1.

Table 1 Dose of drug administration			
	control group (normal saline)	low-dose group	high-dose group
Dose	100µL	50µL	100µL

The animals were executed under overdose anesthesia 2 h after the last administration, and the nasal mucosa and tracheal tissues were fixed with 10% formalin, then paraffin-embedded, sectioned and stained with HE, and the histological changes were observed microscopically¹³.

3. Result

3.1 Anti-SARS-CoV-2 in vitro

We tested the effect of MNG on SARS-CoV-2 infection by using a SARS-CoV-2 S protein pseudovirus system. As shown (Figure 1A), upon treatment with 5%, 10% and 20% MNG, the infection of ppSARS-CoV-2 were drastically decreased. In contrast, MNG treatment didn't interfere with the cell viability (Figure 1B). These data clearly demonstrated that MNG can significantly block SARS-CoV-2 infection.

Figure 1. MNG treatment blocks the infection of pseudotyped SARS-CoV-2. (A) Pseudotyped SARS-CoV-2 were treated with various concentrations of MNG for 1 hr at 37°C. After centrifugation, the infection of pseudotyped SARS-CoV-2 in the supernatants were detected. (B) The cell viabilities were determined upon treatment in parallel with conditions of (A).

In order to evaluate the efficacy of MNG against SARS-CoV-2 infection, we did the experiments of montmorillonite against SARS-CoV-2 *in vitro*. As shown in Table 2, MNG treatment greatly reduced the infection of infectious SARS-CoV-2, emphasizing the potency of MNG as a prophylactic drug to combat the Covid-19 pandemic.

Table 2

The potency of MNG to block infectious SARS-CoV-2 infection

	50% MNG	control	inhibition(%)
original	5632.8 *	14782076.0	99.96
1:4 dilution	58587.9	75618341.0	99.92
1:16 dilution	24790.9	28391468.9	99.91
1:64 dilution	318450.1	27583248.9	98.84
*,The supernatant of MNG exhibited cytotoxicity to Vero cells on this condition.			

3.3 Respiratory Stimulation Test

Microscopic observation showed that the mucosa of the nasal vestibule was well arranged with no interruption, and the ciliated columnar epithelium of the intrinsic nasal mucosa was neatly arranged with clear cilia. The mucosa and submucosal loose connective tissue were not congested and edematous, and no inflammatory cell infiltration or lymphoid hyperplasia was seen. Mucosal and submucosal layers of the trachea and bronchi were clear. The ciliated columnar epithelium was clearly arranged without interruptions. No exudates were seen in the lumen or submucosa, and occasionally exfoliated epithelium or a few lymphocytes (normal) were seen. No significant differences were seen between groups (Figures 2).

Through the mucous membrane stimulation test of New Zealand rabbits, it was found that the gel had no stimulation to the nasal mucosa of the rabbits.

4. Discussion

Montmorillonite becomes a hydrated gel by adding water, which has remarkable adhesion property. It enters the nasal cavity by spraying, and can combine with the mucus on the surface of the upper respiratory tract. It firmly covers the surface of the nasal mucosa and forms a protective barrier film. Therefore, significantly enhances the cohesion and viscoelasticity of the mucus. It effectively blocks the

invasion of viruses into the nasal mucosa cells acting like an "invisible mask". After entering the upper respiratory tract, it can adsorb or fix the viruses, germs and toxins, and then be discharged out of the body with the nasal mucus. As a result, it reduces pathogenic effects. Even if the viruses are discharged with nasal mucus, it will not be transmitted to other people with droplets due to the viscoelasticity of the gel. The surface of montmorillonite crystal appears negative charge⁸. The isomorphic substitution is the origin of the permanent charges that exist on the surface of montmorillonite crystal⁸, so it can adsorb certain viruses and bacteria. We assume that montmorillonite can physically adsorb SARS-CoV-2 either. Specifically, it may interact electrochemically with the cationic residue of spike protein RBD¹⁷. Its adsorption ability is related to the charge density. The gel also inhibits the replication of the virus and prevents the virus from spreading to the lower respiratory tract. The adsorption and immobilization of the virus by montmorillonite are physical effects that does not cause drug resistance. According to theory mentioned above, we assume montmorillonite will be equally effective against mutated coronavirus strains. MNG has been approved by NMPA of China and is available in market. It can be proposed that MNG can greatly reduce the risk of SARS-CoV-2 infection. MNG, in combination with the routine personal protective equipment, can provide further protection to all groups.

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Figures

Figure 1

Relative light unit of each group

Figure 2

Nasal and tracheal tissues of each group

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