JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1499–1504

Received May 19, 2009
Accepted August 23, 2009
DOI 10.1211/jpp/61.11.0009
ISSN 0022-3573

Research Paper

Montmorillonite adsorbs uric acid and increases the excretion of uric acid from the intestinal tract in mice

Zhao Ma[a,b], Li-hui Long[a], Jing Liu[a] and Yong-xiao Cao[a]

[a]Department of Pharmacology, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi and
[b]The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, P. R. China

Abstract

Objectives The aim was to evaluate the adsorbing effect of montmorillonite on uric acid, promoting diffusion of uric acid from blood to intestine, preventing absorption of uric acid in intestine and reducing uric acid level in serum.

Methods The adsorbing effect of montmorillonite on uric acid was observed *in vitro.* The intestine and blood vessel of rats were circularly perfused with intestinal perfusate and vascular perfusate, respectively. A model of hyperuricaemia in mice was prepared by intraperitoneal injection of hypoxanthine and potassium oteracil. The concentration of uric acid was determined by the method of urate oxidase and peroxide enzyme.

Key findings The results showed that different concentrations of montmorillonite could adsorb uric acid in a concentration-dependent manner. The adsorbing effect was fast. The adsorptive rate was high in acid solution and was low in alkaline solution. When blood vessels were circularly perfused by vascular perfusate containing uric acid, the concentration of uric acid in vascular perfusate was decreased and the concentration of uric acid in intestinal perfusate was increased, suggesting that uric acid in blood vessels diffused into the intestine. When the intestine was perfused with intestinal perfusate containing uric acid, the uric acid concentration in vascular perfusate was increased, but the uric acid concentration of intestinal perfusate was decreased, suggesting that uric acid was absorbed in the intestine. The uric acid concentrations of intestinal perfusate and vascular perfusate in montmorillonite 0.5 and 1.0 g/kg groups were lower than the control group. Concentrations of uric acid in serum and urine in the montmorillonite 1 and 2 g/kg groups were lower compared with mice in the hyperuricaemic group.

Conclusions The results suggested that montmorillonite adsorbed uric acid and promoted diffusion of uric acid from blood vessels to intestine, prevented absorption of uric acid in intestine and decreased uric acid level in serum.

Keywords hyperuricaemia; montmorillonite; perfusion; uric acid

Introduction

Montmorillonite (MMT) has two silica–oxygen tetrahedral sheets sandwiching an aluminium or magnesium octahedral sheet, which has a stratified structure and heterogeneic electric charge distribution. In addition, MMT is hydrophilic due to the presence of ionic bonds and the ability to form hydrogen bonds with water.[1–3] Thus, MMT is a layer silicate with a strong adsorbing property and is extensively used in industry, agriculture, and as a medical treatment for conditions such as hyperthyroidism, chronic renal failure, peptic ulcer, gastritis, colonitis and diarrhoea.[4–11] It can adsorb heavy metals such as nickel, rubidium, sulfur, stibium, aluminium, barium, lead, cadmium, calcium; bacteria and viruses such as *Vibrio cholerae*, bacterium flexuosus, *Staphylococcus* etc.; agents such as quinolones, moroxydine, halomine, cholic acid, nitrobenzene, glutaric dialdehyde, polyacrylamide, phenol, aniline, phenobarbital, wintermin, methylsulfonic acid sodium dodecylbenzene sulfonate, hydroxamic siderophore ferrioxamine B, cetyltrimethylammonium and cetylpyridinium.[11–13]

In humans a lack of urate oxidase (uricase) results in uric acid being the end product of purine metabolism. The balance of serum uric acid is decided by absorption, production, decomposition and excretion of purine. The reason for hyperuricaemia is an increase of uricogenesis and/or reduction of uric acid excretion. Medicines reducing the concentration of uric acid in serum are divided into two types: those inhibiting uricopoiesis and those

Correspondence: Yong-xiao Cao, Department of Pharmacology Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi, 710061 P. R. China.
E-mail: yxy@xjtu.edu.cn

increasing the excretion of uric acid. Promoting excretion of uric acid is mainly through inhibiting renal proximal tubule absorption. However, in most patients with hyperuricaemia renal function is insufficient. Thus it is not ideal to increase excretion of uric acid from the kidneys. The gastrointestinal tract is also an important way to excrete uric acid, and the gastrointestinal mucosa possesses the characteristics of a semi-permeable membrane. Recently, we found that MMT adsorbed urea and creatinine and accelerated excretion of urea and creatinine from the intestine.[14,15] Approximately one-third of uric acid in blood is secreted into the intestinal tract, in which some uric acid can be reabsorbed. We presumed that MMT could adsorb uric acid. We have evaluated the adsorbing effects of MMT, promoting the diffusion of uric acid from blood vessels to intestine and preventing absorption of uric acid in the intestine.

Materials and Methods

Reagents

MMT was provided by Hai'nan Xiansheng Medicine Science & Technology Co. Ltd, China. Uric acid and hypoxanthine were purchased from Sigma Chemical Co. Ltd, St Louis, MO, USA. A reagent box for uric acid was purchased from Nanking Jiancheng Bioengineering Institute, China. Trypsinase was purchased from Shaanxi Huamei Co. Ltd, China. Potassium oxonate, a urate oxidase inhibitor, was purchased from Shandong Zhongketaidou Chemical Co. Ltd, China. Benzbromarone was purchased from Yichang Changjiang Pharmaceutical Co. Ltd, China.

Animals

Sprague–Dawley rats (200–250 g) and ICR mice (18–22 g) were purchased from the Animal Center of Xi'an Jiaotong University College of Medicine, China. All animals were handled according to the guidelines provided by the Animal Care and Use Committee at Shaanxi Province. The experimental protocols for using the animals had been reviewed and approved by the animal ethics committee at Xi'an Jiaotong University. The room temperature was 26 ± 3ºC. The rats were anaesthetized by pentobarbital sodium 50 mg/kg.

Perfusates

The simulated intestinal fluid used was KH_2PO_4 13.6 g/l, and the pH was regulated to 6.8. The fluid was mixed with a solution containing trypsinase 20 g. The intestinal perfusate was composed of the following: NaCl 6.90 g/l, KCl 0.35 g/l, $MgSO_4$ 0.30 g/l, $NaHCO_3$ 2.10 g/l, KH_2PO_4 0.16 g/l, $CaCl_2$ 0.28 g/l, and glucose 2.18 g/l.[10,16] Vascular perfusate was prepared by adding 15 g/l gelatin into the intestinal perfusate. Perfusate containing uric acid 140 mg/l was prepared by adding uric acid into intestinal perfusate or vascular perfusate.

Adsorption experiments *in vitro*

Uric acid (120 mg) was dissolved into 1 L simulated intestinal fluid. The uric acid solution (4 ml) was added to five flasks containing MMT 0, 5, 20, 80, or 320 mg, respectively. Adsorptive percent rate (χ) and adsorption rate per gram (χ') were calculated from the following formulae:

$$\chi = ((C_0 - C_1)/C_0) \times 100\% \quad (1)$$

$$\chi' = (C_0 - C_1)/C\tilde{m} \quad (2)$$

where C_0, C_1 and Cm are the concentration of uric acid before MMT, the concentration remaining after adsorption, and weight of MMT, respectively.

The adsorbing effect of MMT (20 g/l) on different concentrations of uric acid, the adsorption–time relationship and the influence of pH on adsorption of MMT were determined, respectively, by the same method.

Circular perfusion

The anaesthetized rats were laparotomized. The superior mesenteric artery and portal vein were exposed and intubated as entrance and exit, respectively. The superior mesenteric artery, portal vein, reservoir fluid bottle and peristaltic pump were connected. The blood vessel was circularly perfused at a flow rate of 2 ml/min (37ºC). In the same way, the upper end of the duodenum and the lower end of the colon were intubated as entrance and exit, respectively.[17–19]

To investigate the diffusion of uric acid from blood vessels to intestine, the blood vessels were circularly perfused with vascular perfusate containing uric acid and the intestine was circularly perfused with intestinal perfusate without uric acid. When absorption of uric acid was investigated, the intestine was circularly perfused with intestinal perfusate containing uric acid and the blood vessel was circularly perfused with vascular perfusate without uric acid. Suspensions of MMT were prepared in 1% carmellose Na^+ solution and administered into the intestine when the effect of MMT on diffusion and absorption of uric acid was studied. Vascular and intestinal perfusates (0.5 ml) were collected from the exit at 0.25, 0.5, 1, 2, 3 and 4 h, respectively.

Hyperuricaemia model in mice

Mice were randomly divided into six groups as follows: group 1, control; group 2, hyperuricaemia model; groups 3–5, administered MMT 0.5, 1.0 and 2.0 g/kg, respectively; group 6, administered benzbromarone 8 mg/kg. With the exception of control group 1 mice, which were injected with normal saline, groups 2–6 were intraperitoneally injected with hypoxanthine 300 mg/kg and potassium oxonate 300 mg/kg once a day for two days to prepare the hyperuricaemia model.[20,21] MMT was administered orally and benzbromarone was intraperitoneally injected twice daily after modelling. At 2, 4 and 8 h after administration of MMT or benzbromarone, blood was drawn from the ophthalmic venous plexus and centrifuged for 5 min to obtain the serum. The urine samples were collected 8 h after administration of MMT or benzbromarone. Uric acid in serum and urine was determined by the method of urate oxidase and peroxide enzyme.

Statistical analysis

The data are expressed as mean ± SEM. The one-way analysis of variance followed by Dunnett's test was applied for comparisons of more than two groups. The unpaired *t*-test was

used when two sets of data were compared. A two-tailed test with a P value less than 0.05 was considered as significant.

Results

Adsorbing effect of different concentrations of MMT on uric acid

The adsorbing effect of different concentrations of MMT on 120 mg/l uric acid showed that MMT could adsorb uric acid in a concentration-dependent manner. The amount of uric acid remaining became less as the concentration of MMT increased. The adsorption percentages of MMT at 1.25, 5, 20, 80 and 320 g/l were 20.0, 27.5, 60.4, 90.0 and 92.9%, respectively. The amount of uric acid adsorbed per unit weight of MMT was 18.7, 6.4, 3.5, 1.3 and 0.3 mg/g, respectively.

Adsorption of MMT on different concentrations of uric acid

The adsorption of the same concentration (20 g/l) of MMT on different concentrations of uric acid was studied. When the concentration of uric acid was 14.6, 28.4, 55.1 or 105.4 mg/l, the concentration of uric acid adsorbed by MMT was 10.2, 19.3, 37.9 and 73.1 mg/l, respectively; and adsorption percentages were 70.0, 68.0, 68.9 and 69.3%, respectively.

Adsorptive time–effect relationship

The adsorbent time–effect curve of MMT on uric acid showed that the adsorbing effect of MMT was fast. The adsorption rate was 60% at 5 min and it was close to the maximum at 60 min, and reached the maximum at 90 min.

Adsorption at different pH

Figure 1 shows that the adsorption of MMT varied with pH. The adsorption of MMT increased as pH decreased. The adsorptive rate was high in an acid environment (pH 2~6) while it was low in an alkaline environment (pH 8~10).

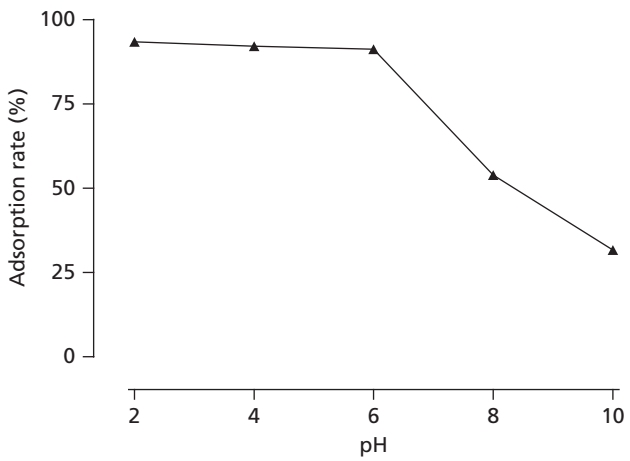

Figure 1 The adsorption rate–pH curve of montmorillonite on uric acid. Data are means ± SEM, $n = 6$.

Effect of MMT on diffusion of uric acid from blood vessel to intestine

The blood vessel and the intestine were circularly perfused by vascular perfusate containing uric acid 140 mg/l and intestinal perfusate without uric acid, respectively. In the control group, the concentration of uric acid in the intestinal perfusate was gradually increased as time increased. After perfusion for 15 min, the uric acid concentration in vascular perfusate reached the maximum. As time increased, the concentration of uric acid in vascular perfusate gradually decreased, but the concentration of uric acid in intestinal perfusate gradually increased. A tendency to a balance was reached in the intestinal and vascular perfusates after 4-h perfusion. The results suggested that uric acid in blood vessels diffused into the intestinal tract. MMT 0.25 g/kg administered to the intestine accelerated the decrease of the uric acid concentration in vascular perfusate and reduced the increase of uric acid concentration in intestinal perfusate. MMT 0.5 and 1.0 g/kg decreased the uric acid concentration in intestinal perfusate after perfusion for 2 and 1 h, respectively. Over the same time, the decrease of uric acid concentration in vascular perfusate was faster in MMT 0.5 and 1.0 g/kg groups than in the MMT 0.25 g/kg group (Figure 2). The results suggested that MMT promoted diffusion of uric acid from blood vessels to intestinal tract.

Effect of MMT on absorption of uric acid in intestine

The intestine and blood vessel were circularly perfused with intestinal perfusate containing uric acid 140 mg/l and vascular perfusate without uric acid, respectively. In the control group, uric acid concentration of vascular perfusate increased gradually, but uric acid concentration of intestinal perfusate decreased gradually. As time progressed, uric acid concentrations in vascular perfusate and intestinal perfusate approached equilibrium, suggesting that uric acid was absorbed in the intestine. When MMT 0.25 g/kg was administered in intestine, the decrease of uric acid concentration in intestinal perfusate was faster than the control group, and the increase of uric acid level in vascular perfusate slowed down. MMT 0.5 and 1.0 g/kg decreased the uric acid concentrations in intestinal perfusate and vascular perfusate, accelerated the decrease of uric acid concentration in intestinal perfusate, and inhibited the increase of uric acid concentration (Figure 3). The results suggested that MMT prevented the absorption of uric acid in intestine.

Effect of MMT on a mouse hyperuricaemia model

Hyperuricaemic mice were prepared by injecting hypoxanthine and potassium oxonate intraperitoneally. Table 1 shows the effect of MMT on the level of serum uric acid in hyperuricaemic mice. The serum uric acid concentration of the model group mice was higher than the control group, suggesting that the model was successful. MMT 0.5 g/kg showed a tendency of decreasing the uric acid level and MMT 1.0 and 2.0 g/kg decreased the serum uric acid level at 2, 4 and 8 h compared with the model group. The same was observed for benzbromarone. Moreover, the serum uric acid level in the

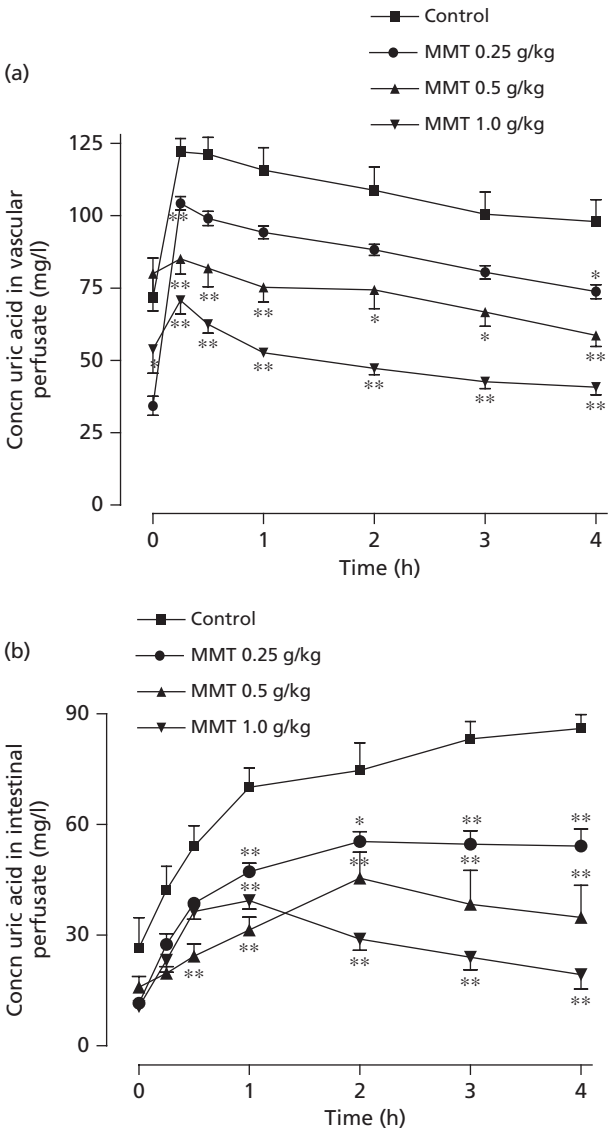

Figure 2 Effect of montmorillonite on the diffusion of uric acid from blood vessel to intestine. The blood vessel and the intestine of rats were circularly perfused by vascular perfusate containing uric acid 140 mg/l and intestinal perfusate without uric acid, respectively. Suspension of montmorillonite (MMT) was administered into the intestinal tract. (a) Concentration–time curves of uric acid in vascular perfusate. (b) Concentration–time curves of uric acid in intestinal perfusate. Data are means ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control.

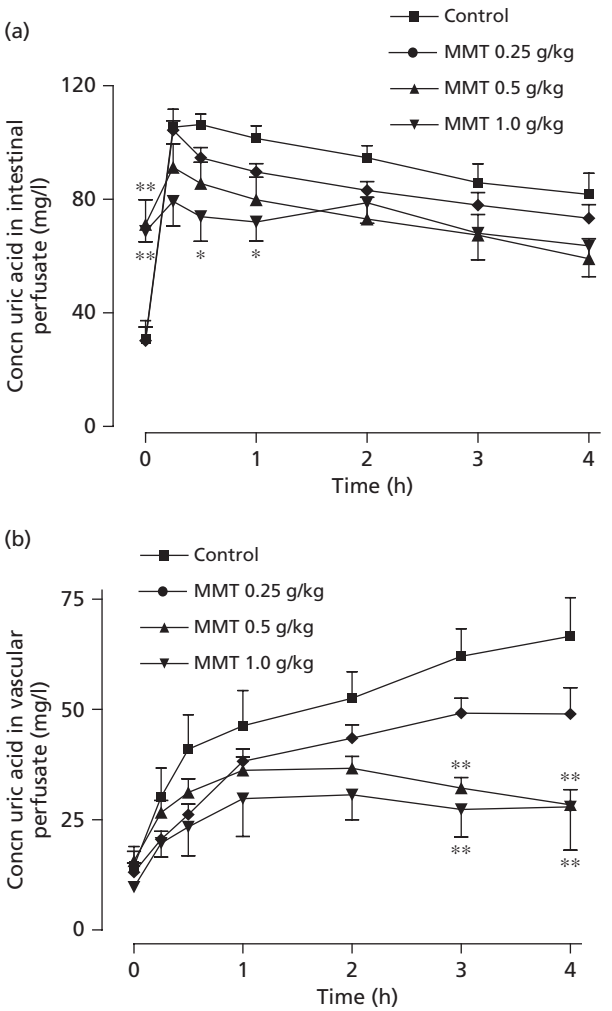

Figure 3 Effect of montmorillonite on absorption of uric acid in intestine. The intestine and blood vessel of rats were circularly perfused with intestinal perfusate containing uric acid 140 mg/l and vascular perfusate without uric acid, respectively. Suspension of montmorillonite (MMT) was administered into the intestinal tract. (a) Concentration–time curves of uric acid in intestinal perfusate. (b) Concentration–time curves of uric acid in vascular perfusate. Data are means ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control.

MMT 2.0 g/kg group was lower than that of the MMT 1.0 g/kg group, suggesting that the effect of MMT was in a dose-dependent manner.

The uric acid concentration and total uric acid of urine in the model group were higher than in the control, showing that the mice with hyperuricaemia increased the excretion of uric acid through the kidney. MMT 1.0 g/kg decreased the concentration of uric acid in urine and had a tendency of decreasing total uric acid of urine. MMT 2.0 g/kg decreased uric acid concentration and total uric acid in urine compared with model group (Figure 4). Benzbromarone increased uric acid concentration and total uric acid in urine compared with the model group.

Discussion

Hyperuricaemia is a disorder of purine metabolism or renal excretion of uric acid. It is related to the clustering of a number of cardiovascular risk factors.[22–24] Uric acid is the final product of DNA and nucleotide metabolism. A person produces approximately 750 mg uric acid and excretes 500–1000 mg uric acid each day. The concentration of uric acid in the extracellular liquid depends on the balance between the speed of uricogenesis and excretion. Hyperuricaemia is caused by an abnormally high production or abnormally low excretion of serum uric acid. When uric acid in serum is

Table 1 Montmorillonite decreased the uric acid level in serum of hyperuricaemic mice

Time (h)	Concentration of uric acid in serum (mg/l)					
	Control	Model	Montmorillonite (g/kg)			Benzbromarone
			0.5	1.0	2.0	
2	19.1 ± 2.27**	79.6 ± 4.74	74.7 ± 4.77	64.3 ± 4.23*	51.4 ± 2.44**	40.9 ± 2.98**
4	19.2 ± 2.20**	95.2 ± 3.56	85.8 ± 4.97	72.3 ± 4.45**	59.2 ± 3.37**	46.9 ± 3.89**
8	16.7 ± 2.12**	86.5 ± 3.01	80.2 ± 3.22	64.0 ± 4.54**	51.0 ± 3.53**	45.6 ± 3.57**

Hyperuricaemia was induced by hypoxanthine and potassium oxonate. Data are means ± SEM, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with model.

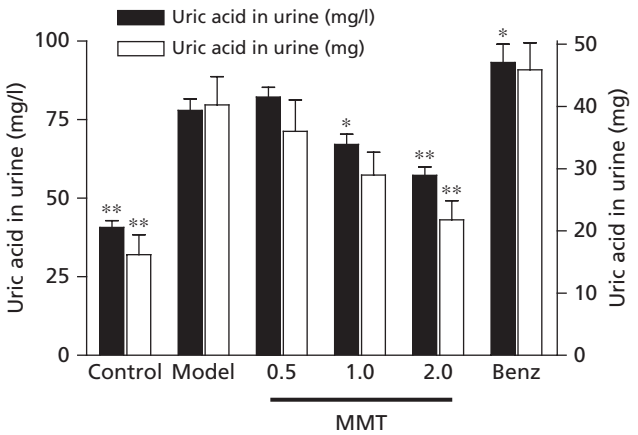

Figure 4 The effect of montmorillonite on the concentration and the amount of uric acid in urine of hyperuricaemic mice. Hyperuricaemia was induced by hypoxanthine and potassium oxonate. Benz, benzbromarone; MMT, montmorillonite. Data are means ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with model group.

higher than the saturation concentration, uric acid salt deposition can be formed in the organism, which leads to gout.[21,25] In the normal human body most uric acid is discharged as free uric acid salt, of which approximately two-thirds is from urine and one-third from the intestine.[26] For patients with insufficient renal function, intestinal excretion becomes an important second-line defence. Patients with gout displaying a renal excretion disorder account for 90%. As a result, intestinal excretion of uric acid increases significantly.

MMT possesses the characteristic of adsorption. This study has proved our assumption that MMT could adsorb uric acid, promote uric acid diffusion from blood vessels to intestine and prevent uric acid absorption in the intestine. In the simulated intestinal solution, MMT could adsorb uric acid potently and fast. The pH value affected the adsorption rate noticeably. The adsorptive rate was high in acid solution while it was low in alkaline solution. Uric acid, as a weak acid, can be dissociated in the alkaline environment to form a negatively-charged ion state. MMT possesses a characteristic of cationic exchange. Therefore, MMT could adsorb much less uric acid in an alkaline environment than in an acid environment. In pH 6~8, which is the range in the intestinal tract, the adsorptive rates varied from 50 to 90%. Therefore, MMT could work well in the intestine.

In the perfusion model, uric acid diffused from blood vessel to intestine and was absorbed in the intestine. MMT could promote the diffusion of uric acid from blood vessel to intestine and prevent the absorption of uric acid in intestine. This is because MMT adsorbed uric acid in intestine, reduced the concentration of uric acid in intestine, and increased the concentration gradient of uric acid between blood vessel and intestine.

Uric acid concentration increased in hyperuricaemic mice. The mice increased the excretion of uric acid through kidney. As a result, the concentration of uric acid in urea increased. MMT increased the excretion of uric acid in intestine so that the concentration of uric acid in serum was decreased. The concentration and total content of uric acid in urine decreased as the serum level of uric acid varied. Benzbromarone can promote the excretion of uric acid through kidney. Therefore, the concentration of uric acid in urine was high and was low in serum. This study has shown that MMT can reduce serum uric acid level. Furthermore, MMT has a huge surface area and can cover extensively the mucous membrane of the digestive tract. MMT does not enter the blood circulation. It and its absorbate, uric acid, can be excreted from the digestive tract. Animal experiments did not show any obvious long-term or short-term toxicity of MMT, suggesting that MMT is safe.[14,27,28] MMT could be beneficial to patients with hyperuricaemia and it may not produce general adverse effects. It will hopefully become a new effective way to prevent and treat hyperuricaemia. However, the adsorption of MMT is nonselective, so it might adsorb some nutrients. Further study is needed.

Conclusions

MMT adsorbed uric acid, promoted diffusion of uric acid from blood vessel to intestine, prevented absorption of uric acid in intestine, and decreased uric acid level in serum and urine of acute hyperuricaemia model mice.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was supported by the National University Student Innovative Experimental Project of China.

References

1. Bhattacharyya KG *et al*. Adsorption of a few heavy metals on natural and modified kaolinite and montmorillonite: a review. *Adv Colloid Interfac* 2008; 140: 114–131.
2. Greesh N *et al*. Adsorption of 2-acrylamido-2-methyl-1-propanesulfonic acid (amps) and related compounds onto montmorillonite clay. *J Colloid Interface Sci* 2008; 319: 2–11.
3. Tyan HL *et al*. Effect of reactivity of organics-modified montmorillonite on the thermal and mechanical properties of montmorillonite/polyimide nanocomposites. *Chem Mater* 2001; 13: 222–226.
4. Parolo ME *et al*. Tetracycline adsorption on montmorillonite: Ph and ionic strength effects. *Appl Clay Sci* 2007; 4: 1–8.
5. Cai Y *et al*. Montmorillonite ameliorates hyperthyroidism of rats and mice attributed to its adsorptive effect. *Eur J Pharmacol* 2006; 551: 156–161.
6. Elmore AR. Final report on the safety assessment of aluminum silicate, calcium silicate, magnesium aluminum silicate, magnesium silicate, magnesium trisilicate, sodium magnesium silicate, zirconium silicate, attapulgite, bentonite, fuller's earth, hectorite, kaolin, lithium magnesium silicate, lithium magnesium sodium silicate, montmorillonite, pyrophyllite, and zeolite. *Int J Toxicol* 2003; 22: 37–102.
7. Ramos Girona AJ, Hernandez Giménez E. [*In vitro* adsorption of aflatoxins by means of sorbent compounds: montmorillonite]. *Rev Iberoam Micol* 1997; 14: 72–77 [in Spanish].
8. Xu BQ *et al*. Experiment study on reaction of smectite with mud acid and fluorboric acid. *Chem Engineering Oil Gas* 2007; 36: 328–330.
9. Wang XQ *et al*. Nanometer montmorillonite instead of zinc oxide influences the performance trait of weanling pigs. *Hubei J Anim Vet Sci* 2007; 3: 7–9.
10. Borges AC *et al*. Recovery of impaired K^+ channels in mesenteric arteries from spontaneously hypertensive rats by prolonged treatment with cholecalciferol. *Br J Pharmacol* 1999; 127: 772–778.
11. Yang K *et al*. Sorption of sodium dodecylbenzene sulfonate by montmorillonite. *Environ Pollut* 2007; 145: 571–576.
12. Praus P *et al*. Study of cetyltrimethylammonium and cetylpyridinium adsorption on montmorillonite. *J Colloid Interface Sci* 2006; 304: 29–36.
13. Siebner-Freibach H *et al*. Thermospectroscopic study of the adsorption mechanism of the hydroxamic siderophore ferrioxamine b by calcium montmorillonite. *J Agric Food Chem* 2006; 54: 1399–1408.
14. Zhang YT *et al*. Montmorillonite adsorbs creatinine and accelerates creatinine excretion from the intestine. *J Pharm Pharmacol* 2009; 61: 459–464.
15. Long LH *et al*. Montmorillonite adsorbs urea and accelerates urea excretion from the intestine. *Appl Clay Sci* 2009: doi: 10.1016/j.clay.2009.1007.1010.
16. Lin FH *et al*. A study of purified montmorillonite intercalated with 5-fluorouracil as drug carrier. *Biomaterials* 2002; 23: 1981–1987.
17. Brunsden AM *et al*. Mesenteric afferent nerves are sensitive to vascular perfusion in a novel preparation of rat ileum *in vitro*. *Am J Physiol* 2002; 283: G656–665.
18. Kugler AR *et al*. Disposition of quinapril and quinaprilat in the isolated perfused rat kidney. *J Pharmacokinet Biopharm* 1995; 23: 287–305.
19. Taft DR. The isolated perfused rat kidney model: a useful tool for drug discovery and development. *Curr Drug Discov Technol* 2004; 1: 97–111.
20. Boonfueng T *et al*. The impact of Mn oxide coatings on Zn distribution. *J Colloid Interface Sci* 2006; 298: 615–623.
21. Chen GL *et al*. Studies on mouse hyperuricemia model. *Chin Pharmaco Bull* 2001; 17: 350–352.
22. Nakagawa T *et al*. The conundrum of hyperuricemia, metabolic syndrome, and renal disease. *Intern Emerg Med* 2008; 3: 313–318.
23. Puig JG *et al*. Hyperuricemia, gout and the metabolic syndrome. *Curr Opin Rheumatol* 2008; 20: 187–191.
24. Ribeiro RC *et al*. Recombinant urate oxidase for prevention of hyperuricemia and tumor lysis syndrome in lymphoid malignancies. *Clin Lymphoma* 2003; 3: 225–232.
25. Song WC *et al*. Influence of hyperuricemia and uric acid nephropathy in rats administrated hushentongfengjiaonang. *Chin Pharmaco Bull* 2004; 20: 342–346.
26. Wu Y *et al*. Single-phase titania nanocrystallites and nanofibers from titanium tetrachloride in acetone and other ketones. *Inorg Chem* 2007; 46: 5093–5099.
27. Wiles M *et al*. Toxicological evaluation and metal bioavailability in pregnant rats following exposure to clay minerals in the diet. *J Toxicol Environ Health A* 2004; 67: 863–874.
28. Afriyie-Gyawu E *et al*. Chronic toxicological evaluation of dietary novasil clay in Sprague-Dawley rats. *Food Addit Contam* 2005; 22: 259–269.