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Research Paper

Montmorillonite adsorbs creatinine and accelerates creatinine excretion from the intestine

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Abstract

Objectives This study aims to evaluate the sorption by montmorillonite of creatinine and the accelerating effect of montmorillonite on creatinine excretion from the intestine.

Methods The sorption of montmorillonite was observed *in vitro.* Also, rat intestinal tract and blood vessels were perfused circularly with perfusate with or without creatinine, respectively, to study the promotion of creatinine diffusion from the blood vessel to the intestine and the inhibition of creatinine absorption in the intestinal tract. The effect of decreasing the serum concentration of creatinine was studied in an acute hypercreatininaemia mouse model. The concentration of creatinine was determined by the basic picric acid method.

Key findings Montmorillonite adsorbed creatinine markedly in the simulated intestinal solution in a concentration-dependent manner. The sorption–time curve of montmorillonite with creatinine showed that the sorption was fast. The adsorption rate reached a maximum in 10 min. The pH of the solution influenced the sorption, the rate of which was higher at a low pH than at a high pH. Creatinine could diffuse from the blood vessel to the intestine and was reabsorbed in the intestine. Montmorillonite promoted the diffusion and inhibited the absorption. Montmorillonite decreased the serum creatinine level of hypercreatininaemia mice prepared by injecting creatinine intraperitoneally.

Conclusions Montmorillonite adsorbs creatinine and accelerates its excretion from the intestine.

Keywords adsorption; creatinine; hypercreatininaemia; montmorillonite

Introduction

Montmorillonite, an aluminosilicate clay, has a 2 : 1 layer structure. Each layer has a small net negative charge because of the isomorphous substitution of ions in the framework. This charge imbalance is offset by interlayer hydrated cations. These interlaminar cations can be exchanged with other metal cations. It is well known that montmorillonite has a great adsorption capacity, which is attributed to its large specific surface area and high cation exchange capacity.[1,2] Montmorillonite can adsorb organic substances, bacteria,[1] heavy metals,[3] etc. either on its external surfaces or within its interlaminar spaces by interaction with, or substitution of, the exchange cations present in these spaces. The high adsorbability of montmorillonite is due to its large surface area and high swelling capacity. Moreover, the addition of montmorillonite to the diet has not shown any health risk for humans and animals.[4] Montmorillonite is extensively used in industry, agriculture and medical treatment.[5–7] Previously we demonstrated that montmorillonite can adsorb thyroid hormone.[8] Therefore, the application of montmorillonite as an adsorbent for the remediation of toxicity is of great interest. Creatinine is a creatine metabolic substance; the level of serum creatinine can be increased as a result of the insufficiency of its excretion.[9] The major objective in this paper is to evaluate the ability of montmorillonite to adsorb creatinine from aqueous solution and to investigate its effect on accelerating the excretion of creatinine from the intestine.

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Materials and Methods

Reagents

Montmorillonite was provided by Hai'nan Xiansheng Medicine Science & Technology Co. Ltd (Haokou, China). Creatinine was purchased from Chemical Co. Ltd of China Chem

(Shanghai, China). The reagent box of creatinine was purchased from Nanking Jiancheng Bioengineering Institute (Nanking, China). The suspension of 10%, 5% and 2.5% montmorillonite was prepared with 1.0% sodium carboxymethyl cellulose (CMC-Na^+) as solvent.

The intestinal perfusate composition (g/l) was: NaCl 6.90, KCl 0.35, $MgSO_4$ 0.30, $NaHCO_3$ 2.10, KH_2PO_4 0.16, $CaCl_2$ 0.28 and glucose 2.18. The vascular perfusate was prepared by adding 15 g/l gelatin to the intestinal perfusate. Intestinal or vascular perfusate containing creatinine 80 mg/l was also prepared.

Animals

Sprague-Dawley rats, 180–220 g, and ICR mice, 18–22 g, were purchased from the Animal Center of Xi'an Jiaotong University College of Medicine. The rats and mice 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.

Apparatus

The UV-1601 ultraviolet–visible spectrophotometer was produced by Shimadzu Corporation (Kyoto, Japan). The TGL-16G table-centrifuge was produced by Anting Scientific Instrument Factory (Shanghai, China). The AE240S-electronic analytical balance was made by Metler-Toledo Group (Zurich, Switzerland).

Adsorption experiments *in vitro*

Simulated intestinal fluid

KH_2PO_4 (6.8 g) was added to distilled water (500 ml) and the pH was adjusted to 6.8 with 0.1 M NaOH solution. Then the fluid was mixed with a solution containing pancreatic enzyme 10 g. The mixed solution was diluted to 1 L with distilled water.

The adsorbing effect of montmorillonite on creatinine in vitro

Creatinine was weighed and dissolved in a flask containing 100 ml simulated intestinal fluid. The creatinine solution (4 ml) was put into 5-ml flasks containing montmorillonite 0, 5, 20, 80, 320 or 1280 mg. The flasks were shaken and the adsorption of the filtrate was measured after 2 h. A scan was made by ultraviolet–visible spectrophotometer (690 nm) with distilled water. The adsorption rate and adsorption rate of montmorillonite (per gram) were calculated from the following formulas:

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

$$\chi' = (C_0 - C_1)C_m \quad (2)$$

where C_0, C_1 and C_m are the concentration of creatinine before montmorillonite, the concentration remaining after adsorption and weight of montmorillonite, respectively. The adsorbing effect of montmorillonite (20 g/l) on different concentrations of creatinine (226, 113, 56, 23 or 11 mg/l), the adsorbtion–time relationship and the influence of pH on adsorption of montmorillonite were determined, respectively, based on the same method.

Circulating perfusion of blood vessel and intestine in rats

After being fasted for 18 h, rats were anaesthetised with pentobarbital sodium 50 mg/kg and the abdominal cavity was cut along the linea alba. The superior mesenteric artery and janitrix were intubated as entrance and exit of blood vessel circulating perfusion, respectively. The entrance and export were connected by a peristaltic pump and a bottle storing the perfusate. The blood vessel circuit was perfused with vascular perfusate (37°C) at a flow rate of 2 ml/min for 5 min by the peristaltic pump. At the same time, the beginning of the duodenum and the end of the colon were intubated as entrance and exit of intestinal circulating perfusion. The intestinal tract was washed with physiological saline (37°C). Then, the intestinal circuit was prepared by connecting the entrance, exit and the bottle storing the intestine perfusate with a peristaltic pump at a flow rate of 1.5 ml/min. A 0.5-ml sample of the perfusate of the blood vessel circuit or the intestinal circuit was collected from their exit points at different times, and kept at 4°C.

To study the diffusion of creatinine from the blood vessel to the intestinal tract, the blood vessel and the intestinal tract were perfused circularly with 100 ml vascular perfusate containing 80 mg/l creatinine and 50 ml of intestinal perfusate without creatinine, respectively. To test creatinine absorption in the intestinal tract, the blood vessel and the intestine were perfused by vascular perfusate without creatinine and intestinal perfusate containing 80 mg/l creatinine, respectively. Montmorillonite (1.0, 0.5 and 0.25 g/kg) was administered to the intestine to evaluate its effect on diffusion and absorption.

Acute hypercreatininaemia mouse model

The fasting mice were divided into seven groups, each of which contained 10 mice, and different treatments were given as follows: normal group (distilled water); model group (creatinine 0.55 g/kg); montmorillonite groups (creatinine 0.55 g/kg + montmorillonite 0.25, 0.5, 1 or 2 g/kg).

The mice were intraperitoneally injected with creatinine suspension 0.55 g/kg, except that the normal group was administered with normal saline. Montmorillonite was administered orally to mice. The blood was collected by removing the eyeballs 2.5 h after creatinine administration, and the serum concentration of creatinine was determined.

Statistical analysis

All data are expressed as mean ± SEM. Unpaired Student's *t*-test was used to compare two sets of data and one-way analysis of variance for comparisons of more than two data sets. $P < 0.05$ was considered to be significant.

Results

Sorption effect of different concentrations of montmorillonite on creatinine

Figure 1 shows the adsorbing effect of different concentrations of montmorillonite on 226 mg/l creatinine. After being adsorbed by montmorillonite, the concentration of creatinine

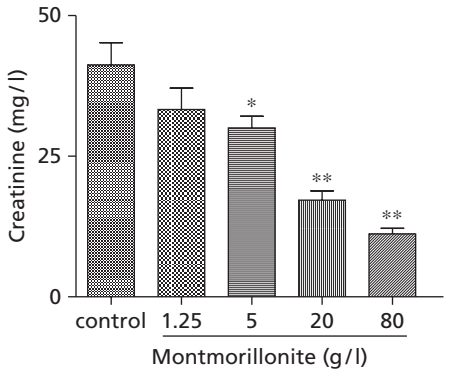

Figure 1 Adsorbing effect of different concentration of montmorillonite on creatinine in simulated intestinal solution. Data are means ± SEM, $n = 5$. *$P < 0.05$, **$P < 0.01$ vs control.

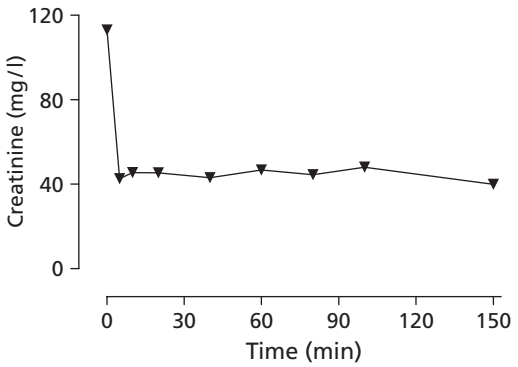

Figure 3 Adsorptive–time curve showing effect of montmorillonite (20 g/l) on creatinine (113 mg/l) *in vitro*.

was decreased with an increase in the concentration of montmorillonite.

Sorption effect of montmorillonite on different concentrations of creatinine

Figure 2 shows the sorption effect of the same concentration (20 g/l) of montmorillonite on different concentrations (226, 113, 56, 23, 11 mg/l) of creatinine. The adsorption capacity was increased with an increase in creatinine concentration. The sorption ratio was in the range 38.1–61.8%.

Sorption–time relationship

The sorption–time relationship of 20 g/l montmorillonite with 113 mg/l creatinine was studied. The adsorption was rapid (Figure 3). The adsorption rate by montmorillonite of creatinine reached its maximum value at 10 min with small fluctuations subsequently.

Influence of pH on adsorption

Montmorillonite possesses ion exchange capability. Therefore, the solution pH may affect its sorption. The results in Figure 4 shows that the adsorbing effect of montmorillonite was more powerful at lower pH than at higher pH. The adsorbing rates were 100%, 98%, 88%, 79%, 28% and 10% at pH 2, 4, 6, 8 and 10, respectively.

Accelerating the diffusion of creatinine from the blood vessel to the intestine

When the blood vessel was circularly perfused with vascular perfusate containing creatinine and the intestinal tract was circularly perfused with intestinal perfusate without creatinine, the concentration of creatinine in the vascular perfusate increased to its maximum in 15 min then decreased slowly. The concentration of creatinine in the intestinal perfusate increased gradually (Figure 5a). After perfusion for 4 h, the concentration of creatinine in the intestinal and vascular perfusates reached a balance and the concentration was close to 50 mg/l. The results suggested that creatinine could diffuse from the blood vessel to the intestine. After montmorillonite was administered to the intestinal tract, the decrease in creatinine concentration in the vascular perfusate in the montmorillonite 1.0 g/kg group was more obvious than that in the control group (Figure 5b). The concentration of creatinine in the intestinal perfusate in the montmorillonite 0.5 and 1.0 g/kg groups initially decreased, followed by an

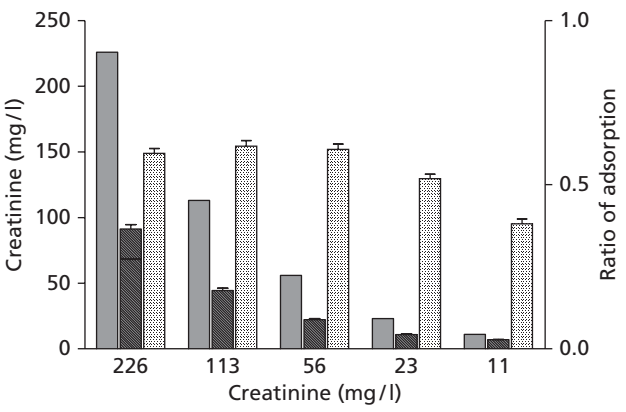

Figure 2 Adsorbing effect of montmorillonite (20 g/l) on different concentrations of creatinine *in vitro*. 1st column in each group of 3, before adsorption; 2nd column, after adsorption; 3rd column, ratio of adsorption. Data are means ± SEM, $n = 5$.

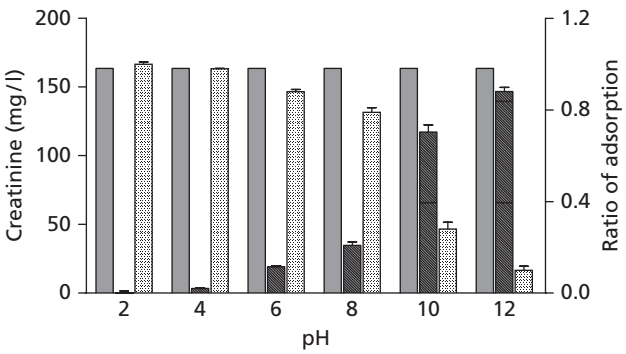

Figure 4 Influence of pH on montmorillonite adsorption of creatinine *in vitro*. 1st column in each group of 3, before adsorption; 2nd column, after adsorption; 3rd column, ratio of adsorption. Data are means ± SEM, $n = 5$.

increase (Figure 5c). After circular perfusion for 4 h, the creatinine concentrations in the montmorillonite 1.0 g/kg group in the intestinal and vascular perfusates were 25.9 ± 4.6 mg/l and 39.9 ± 3.9 mg/l and were decreased by 51.5% and 30.8%, respectively, compared with the control group. The results showed that montmorillonite facilitated the diffusion of creatinine from the blood vessel to the intestine.

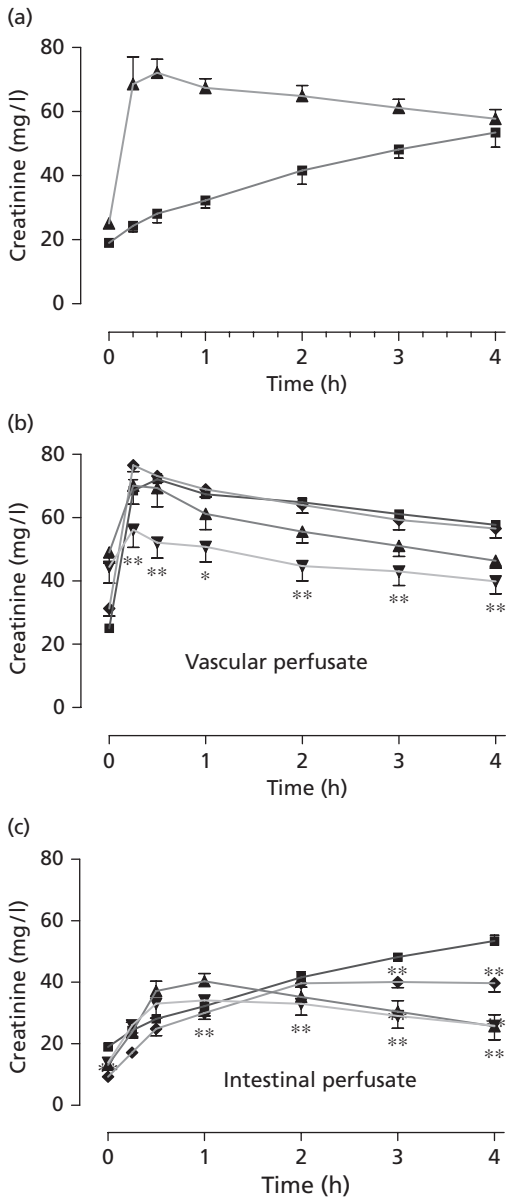

Figure 5 Diffusion of creatinine from blood to intestine in rats (a) and the acceleration of this diffusion by montmorillonite (b, c). The blood vessel and intestinal tract were perfused circularly with vascular perfusate containing 80 mg/l creatinine. After being administered with montmorillonite, the intestinal tract was perfused circularly with intestinal perfusate without creatinine. Data are means ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control. (a) ▲, vascular perfusate; ■, intestinal perfusate. (b, c) ■, control; ♦, montmorillonite 0.25 g/kg; ▲, montmorillonite 0.5 g/kg; ▼, montmorillonite 1.0 g/kg.

Montmorillonite prevents absorption of creatinine in the intestine

When the blood vessel was perfused with vascular perfusate without creatinine and the intestinal tract was perfused with intestinal perfusate containing creatinine, the concentration

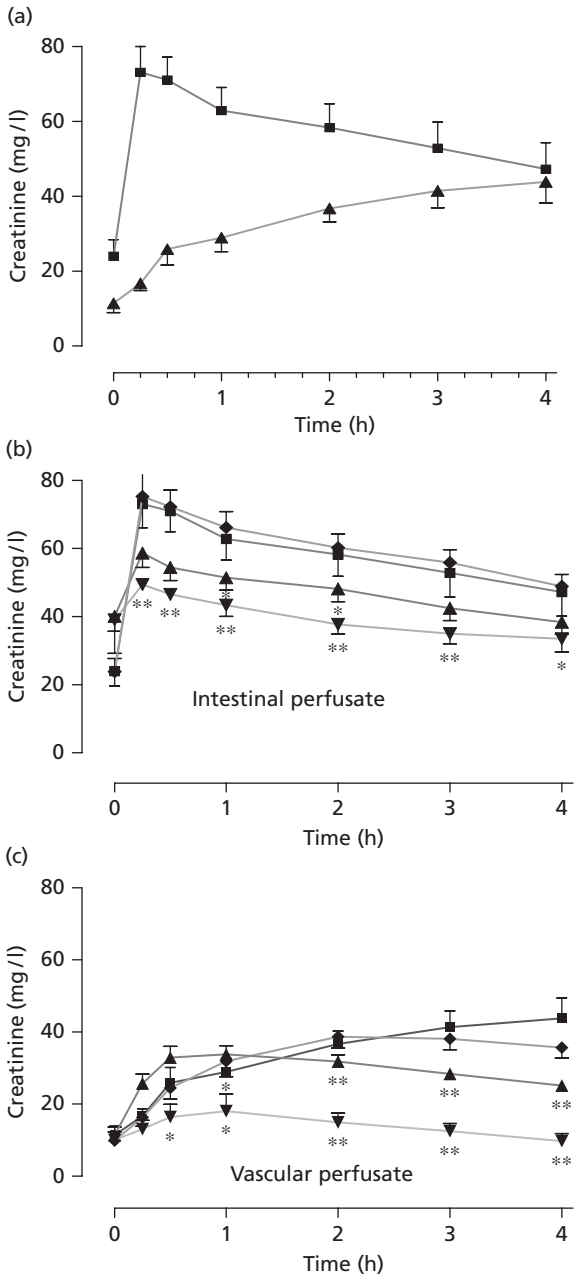

Figure 6 The creatinine is absorbed from the intestine of rats (a) and montmorillonite inhibits this absorption (b, c). After being administered with montmorillonite, the intestinal tract was perfused circularly with intestinal perfusate containing 80 mg/l creatinine. At the same time, the blood vessel was perfused circularly with vascular perfusate without creatinine. Data are means ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control. (a) ▲, vascular perfusate; ■, intestinal perfusate. (b) ♦, control; ■, montmorillonite 0.25 g/kg; ▲, montmorillonite 0.5 g/kg; ▼, montmorillonite 1.0 g/kg. (c) ■, control; ♦, montmorillonite 0.25 g/kg; ▲, montmorillonite 0.5 g/kg; ▼, montmorillonite 1.0 g/kg.

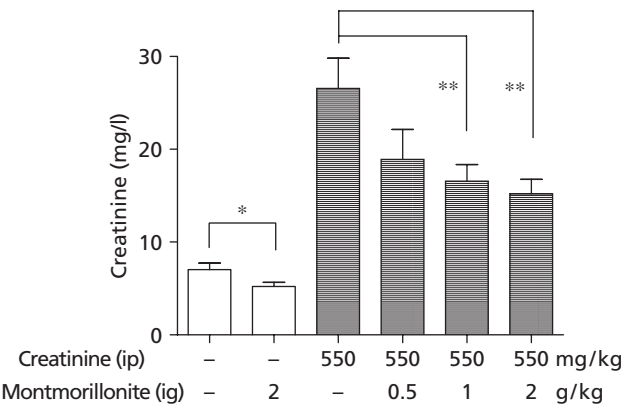

Figure 7 Effect of montmorillonite on serum creatinine levels in the mouse model of acute hypercreatininaemia. Data are means ± SEM, n = 7–10. *P < 0.05, **P < 0.01. ig, intragastric; ip, intraperitoneal.

of creatinine in the intestinal perfusate increased to its maximum in 15 min after perfusion, then decreased gradually. The concentration of creatinine in the vascular perfusate increased continuously (Figure 6a). After perfusion for 4 h, the concentration of creatinine in both the vascular perfusate and the intestinal perfusate reached a balance, and was close to 50 mg/l. The result suggested that creatinine in the intestinal tract could be absorbed into the blood.

When montmorillonite was administered to the intestine, the creatinine concentration in the intestinal perfusate in the montmorillonite groups was lower than in the control group at different times (Figure 6b). The creatinine levels in the montmorillonite 0.5 and 1.0 g/kg groups at different time were lower than those in the control group (Figure 6c). The results showed that montmorillonite reduced the absorption of creatinine in the intestine.

Effect of montmorillonite on the level of serum creatinine in mice

The acute hypercreatininaemia mouse model was prepared by injecting creatinine intraperitoneally. The serum creatinine concentration of the model group was 3.8 times that of the normal group (Figure 7). The levels of serum creatinine in the montmorillonite 0.5, 1.0 and 2.0 g/kg groups were significantly lower than that of the model group. Also, montmorillonite 2.0 g/kg could decrease the blood creatinine concentration of normal mice. The results showed that montmorillonite decreased the level of serum creatinine in both the hypercreatininaemia mouse model and in normal mice.

Discussion

Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, an energy storage compound in muscle. The creatine in muscle is mainly produced through a reversible dehydration reaction and is slowly released into the blood. Creatinine is mainly excreted via urine and the concentration of blood creatinine is mainly related to the glomerular filtration rate, which is 125 ml/min in a healthy person. Creatinine is soluble in water and distributes throughout the body water, equilibrating between various fluid regions with the help of Na^+/Cl^- transporters on cell membranes. It also has been detected in sweat, peritoneal fluid, synovial fluid, bronchoalveolar lavage fluid and aqueous and vitreous humor.[10]

The intestinal mucosa is a semi-permeable membrane allowing the passage of a number of materials including creatinine. Research in the 1950s and 1960s discovered small quantities of creatinine in the vomitus and faeces of uraemic human beings. An approach to intestinal dialysis was investigated in a normal person and in a patient with chronic uraemia having a renal creatinine clearance of 10 ml/min.[11] Both subjects drank 1–1.5 l of non-absorbable solutions of polyethylene glycol or mannitol. The uraemic patient's intestinal clearance was 6–10.4 ml/min for creatinine, 4 ml/min for uric acid and 10.7–15.4 ml/min for phosphate, which compares favourably with those undergoing 12-h weekly haemodialysis.

Huang *et al.*[12] investigated the clearance of creatinine through the gastrointestinal tract of rabbits. After intravenous administration of creatinine, intestinal dialysis was carried out. The results showed that the creatinine concentration in the dialysate was 266 mmol/l, the creatinine concentration ratio of dialysate and blood was 21.8% and the creatinine clearance in the intestine was 0.29 ml/min. Our study showed a similar result. When the blood vessel and intestinal tract were perfused circularly by vascular perfusate containing creatinine and intestinal perfusate without creatinine, respectively, the concentration of creatinine in the vascular perfusate decreased gradually, and the concentration of creatinine in the intestinal perfusate increased gradually, suggesting that creatinine can diffuse from the blood vessel to the intestine. When the intestinal tract was perfused with intestinal perfusate containing creatinine and the blood vessel was perfused with vascular perfusate without creatinine, the creatinine level in the intestinal perfusate decreased gradually followed by a fast increase, and the creatinine level in the vascular perfusate increased continuously, suggesting that creatinine can be absorbed in the intestine.

In renal failure, creatinine excretion from the kidney is reduced, and excretion from the intestine is increased. Treatment of uraemia with intestinal dialysis results in uraemic toxins being excreted from the intestine. Yang reviewed the outlook of uraemic toxin removal from the gastrointestinal tract.[13] In the uraemic state, creatinine clearance from the intestinal mucosa increases markedly. A patient with uraemia can remove an average of 2.9 g creatinine daily via the intestine, which is far more than the normal urine removal. We assumed that sorbents absorb enteric uraemic toxins, effectively blocking absorption of the toxins in the intestine. The sorbent and the toxins are then eliminated from the body with the stool. There is evidence to support this.[12] When an adsorbent, coated aldehyde oxystarch, was adminstered to dialysate, the creatinine concentration ratio of dialysate in blood was increased to 27.58%, and the clearance of creatinine in the intestine was increased to 0.44 ml/min. Moreover, ethanol, in combination with intestinal sorbents or alone, could allow intestinal dialysis to remove considerably more creatinine in rats and goats.[14]

Montmorillonite is a good adsorbent and provides protection against the toxicity of various natural and synthetic chemical products and heavy metals because of the existence

of several types of active site, such as Bronsted and Lewis acid sites and ion-exchange sites.[4,15] It is composed of units made up of two silica tetrahedral sheets with a central alumina octahedral sheet. The tetrahedral and octahedral sheets combine in such a way that the tips of the tetrahedra of each silica sheet and one of the hydroxyl layers of the octahedral sheet form a common layer.[3] In montmorillonite, adsorption can occur both at the edge sites and at the planar (internal) sites of the clay mineral.[16] The edge hydroxyl groups are particularly active in various types of interaction. Montmorillonite reduces the bioavailability of toxins in the gastrointestinal tract.[17,18] Recently, we found that montmorillonite can adsorb uric acid in simulated intestinal solution and reduce the level of uric acid in an acute hyperuricaemia mouse model.[9] Our current study showed that montmorillonite adsorbed creatinine in simulated intestinal solution in a concentration-dependent manner. The adsorptive effect of the same concentration of montmorillonite (20 g/l) on different concentrations of creatinine (11–226 mg/l) was inversely proportional to the concentration of creatinine. The residual creatinine is proportional to the concentration of creatinine. However, the sorption ratio at 226, 113 and 56 mg/l creatinine was constant, around 60%, suggesting that the sorption by montmorillonite of creatinine was non-specific and not very powerful. The sorption was fast, reaching its maximum value at 10 min. The sorption of creatinine was influenced by the pH of the aqueous medium and the sorption was more powerful at lower pH than at higher pH. The reason may be that pH affects the active sites of montmorillonite and the ionisation of creatinine. In this study, montmorillonite was found to facilitate the diffusion of creatinine from blood vessel to intestine, prevent the absorption of creatinine in the intestinal tract and decrease the level of serum creatinine in a mouse model of acute hypercreatininaemia. The clear reason for this is that montmorillonite adsorbs creatinine in the intestine, decreases the concentration of creatinine in the intestinal tract, increases the concentration gradient between serum and intestinal juice and accelerates the diffusion from blood vessels to the intestinal tract.

Montmorillonite does not enter the blood circulation.[19,20] After being administered for 6 h, montmorillonite and its adsorbate are discharged from the intestinal tract. The addition of montmorillonite clay to the diet does not show any health risks for humans. The addition of montmorillonite clay (5 or 20 g/kg diet) does not show any toxic effect on biochemical, haematological or immunological parameters of mice, chickens or pregnant rats,[4,15,18,21] suggesting that montmorillonite is safe.

Conclusions

Montmorillonite adsorbs creatinine and accelerates creatinine excretion from the intestine. It acts as a good adsorbent of creatinine due to its low cost, high abundance, easy manipulation and harmlessness to the environment.

Declarations

Conflict of interest

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

Funding

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