Montmorillonite ameliorates hyperthyroidism of rats and mice attributed to its adsorptive effect

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Abstract

The present study aims to evaluate the adsorbing effect of montmorillonite on thyroid hormone in the entero-hepatic circulation. The concentration of thyroid hormone in the serum of hyperthyroidism model rats and in solution was measured by radioimmunoassay and ultraviolet spectrometry, respectively. The body weight, temperature, and consumption of food and water were observed in hyperthyroidism model rats. Furthermore, hypoxia tolerance, sodium-pentobarbital-induced sleep time, spontaneous activities were measured on hyperthyroidism model mice after being treated with montmorillonite. Results showed that montmorillonite adsorbed thyroxin (T4) and triiodothyronine (T3) in vitro. Montmorillonite at dosage of 1.0 g/kg and 0.3 g/kg decreased thyroid hormone levels on hyperthyroidism model rats; Montmorillonite (2.0 g/kg and 0.6 g/kg) prolonged the sleep time, improved the hypoxia tolerant capacity and reduced the spontaneous activities of the hyperthyroidism model mice. These results suggest montmorillonite has anti-hyperthyroidism effect attributed to its adsorptive effect.

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1. Introduction

Entero-hepatic circulation of thyroid hormone involves transfer of hormone to intestines in bile from the liver and reabsorption of some intestinal hormone, via portal blood back to liver (DiStefano et al., 1992). The rate of entero-hepatic circulation of thyroid hormone is about 30% in normal rats. Thyrotoxic states are characterized by an increased entero-hepatic circulation of thyroid hormones, as well as an increased urinary and faecal excretion of both conjugated and free thyroid hormones (Bergman et al., 1996; Nguyen et al., 1993). We suppose that the level of thyroid hormone should be lower when adsorptive substances are used to sequester thyroid hormone of entero-hepatic circulation in intestine (Mercado et al., 1996).

Montmorillonite is a layered silicate with the property of adsorbing organic substances either on its external surfaces or within its interlaminal space, by the interaction or substitution of the exchange cations presented in these spaces (Ramos and Hernández, 1996). It is well known that smectite produced in France is a popular drug for children’s diarrhea. And now montmorillonite is widely used as feedstuff additive and drug carrier (Lin et al., 2002). Also it is found to be a catalyst of polymerization of amino acid adenylates because of its adsorption on amino acid. However, to date, no study has been conducted to test its effect for hyperthyroidism. The study was thus conducted to evaluate its adsorption effectiveness on thyroid hormone in vitro and in vivo.

2. Materials and methods

2.1. Reagents

Montmorillonite was provided by Hainan Derun Motmorillonite Factory. Thyroxin (T4) and triiodothyronine (T3) were purchased from Sigma Chemical Co., St. Louis. Sodium levothyroxin was from Shenzhen Zuolian Pharmacy Co. Ltd.
Thyroid tablets was bought from Yanzhou Shengbao Pharmacy Co. Ltd. Assay kits for total T₃ and total T₄ were from Chinese Atomic Energy Academy of Science.

2.2. Animals

ICR mice weighing 18–22 g and male Sprague–Dawley rats weighing 180–220 g were purchased from Animal Center of Xi'an Jiaotong University School of Medicine. All animals were handled according to the guidelines provided by the Animal Care and Use Committee at Shaanxi Province. The animals were housed in groups, maintained at 21–24 °C on a 12-h light and 12-h dark cycle, with free access to water and standard rat food.

2.3. Adsorption experiments in vitro

One percent of sodium dodecyl sulphate (SDS) stock solution containing T₃ or T₄ (50 µg/ml) was prepared, and was diluted to the solutions containing 10 µg/ml. Scan was made by ultraviolet–visible spectrophotometer from 200 nm to 400 nm with 1% SDS as the control solution. A maximal absorption wavelength of 230 nm was obtained.

Working solutions containing 40, 30, 20, 10, 8, 6, 4, 2 µg/ml T₃ or T₄ were prepared with 1% SDS. Their absorption was measured in 230 nm, and the standard curves were obtained. T₃: \( A = 0.0617C - 0.0022 \) \( (r = 0.9998) \); T₄: \( A = 0.0691C + 0.030 \) \( (r = 0.9997) \).

T₃ and T₄ (10 µg/ml) were put into two 100 ml flasks, respectively. Montmorillonite 300 mg was added into each flask which was put in the 37 °C bath and shaken frequently. Suspension (5 ml) was taken out from each flask after 5, 15, 30, 60, and 120 min, respectively. The suspension was filtered and the adsorption rate (percentage) was calculated from the following formula:

\[
\text{Adsorption rate(percentage)} = \frac{C_0 - C_1}{C_0} \times 100\%
\]

\( C_0 \) is the concentration of T₄ and T₃ before montmorillonite, \( C_1 \) is the remainder concentration after being adsorbed.

2.4. Hyperthyroidism model of rats

2.4.1. Anti-hyperthyroidism effect at different time

Rats were orally administrated with thyroid tablets 90 mg/kg/day for 3 days, the serum concentration of total T₃ and total T₄ was measured. Then the animals were divided into two groups according to total T₃ and total T₄ level: model group and montmorillonite group. Thyroid tablets of 90 mg/kg was given to rats in the early morning daily. Montmorillonite of 300 mg/kg was orally administrated 3 h after taking thyroid tablets in montmorillonite group. Model group was given equivalent volume of distilled water. Rat blood was taken by cutting their tails at the 1st, 2nd, 3rd, 5th, 7th day after giving montmorillonite, and serum was separated by centrifuge, and total T₃ and total T₄ level was determined.

2.4.2. Anti-hyperthyroidism effect at different doses

Rats were divided into six groups, each of which contained 10 rats and different treatments were given as following: normal control group (distilled water); model group (thyroid tablets); tapazol group (thyroid tablets+tapazol 15 mg/kg); montmorillonite 1.0 g/kg group (thyroid tablets+montmorillonite 1.0 g/kg); montmorillonite 0.3 g/kg group (thyroid tablets+montmorillonite 0.3 g/kg); montmorillonite 0.1 g/kg group (thyroid tablets+montmorillonite 0.1 g/kg). 90 mg/kg of thyroid tablets was orally administrated once daily for 7 days. Montmorillonite or tapazol was orally administrated 3 h after thyroid tablets (90 mg/kg/day for 7 days, i.g.). The body weight, anorectal temperature, and food and water consumption of the rats were measured after the last drug was taken. Then rats’ blood was taken from cutting tails, the serum concentration of total T₃ and total T₄ was measured.

2.4.3. Measurement of total T₃ and total T₄ in serum

The concentration of total T₃ and total T₄ was determined with a highly sensitive, specific radioimmunoassay by a FM-2000γ-counter. Radioimmunoassay kits of total T₃ and total T₄ were used according to the manufacture’s instructions (Chinese Atomic Energy Academy of Science, Beijing, China). The serum samples and a series of standard T₃ and T₄ solutions were added to tubes in which 125I-T₃ or 125I-T₄ was subsequently added. Then they were mixed with the anti-T₃ (T₄) microsphere suspension and kept in 37 °C for 15 min. All the tubes were centrifuged for 20 min afterwards, then the radioactive counts of their supernatant were recorded. Therefore the standard curves were obtained and the concentration of total T₃ and total T₄ in serum could be calculated.

2.5. Hyperthyroidism model of mice

Mice were divided into 6 groups as: control; hyperthyroidism model; tapazol 15 mg/kg; montmorillonite 2.0 g/kg; montmorillonite 0.6 g/kg; montmorillonite 0.2 g/kg. The mice received sodium-levothyroxin (30 µg/kg/day) intraperitoneally for 7 days except the control mice. At the same time, montmorillonite or tapazol was orally administrated. On the 8th day, the mice were put into the YLS-2A multifunctional spontaneous activity counter and spontaneous activity was recorded. The sleep time of the mice was recorded after being injected sodium-phentobarbital (35 mg/kg, i.p.). The hypoxia-resisting time was recorded on the mice, each of which was put in a 250 ml jar containing 15 g of sodium lime and were airproofed with vaseline.
2.6. Statistical analysis

The data are expressed as mean±S.E.M. The one-way analysis of variance (ANOVA) followed by Dunnett’s test was applied for comparisons for more than two groups. And 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 significant.

3. Results

3.1. Adsorptive effect of montmorillonite in vitro

The adsorption rate–time curve showed that montmorillonite adsorbed thyroid hormone rapidly. The adsorption reached equilibrium in 30 min (Fig. 1). The maximal adsorption percentage of T3 and T4 were 58.6% and 52.1%, respectively. Table 1 showed the adsorbing effect of montmorillonite (0.3 mg/ml) on T3 and T4. When the concentration of T3 were 2, 10, and 50 μg/ml, the concentration of T3 adsorbed by montmorillonite were 1.76, 5.80 and 7.16 μg/ml, respectively; and adsorption percentage were 88.0%, 58.0%, and 14.3%, respectively. When the concentration of T4 were 2, 10, and 50 μg/ml, the concentration of T4 adsorbed by montmorillonite were 1.85, 5.25 and 7.74 μg/ml, respectively; and adsorption percentage were 92.5%, 52.5%, and 15.5%, respectively. Table 2 showed the adsorbing effect of different concentration of montmorillonite on 10 μg/ml T4 and T3. When the concentration of montmorillonite were 0.3, 3.0, and 30.0 mg/ml of T3 were 2, 10, and 50 μg/ml, the concentration of T4 adsorbed by montmorillonite were 1.85, 5.25 and 7.74 μg/ml, respectively; and adsorption percentage were 92.5%, 52.5%, and 15.5%, respectively. Table 2 showed the adsorbing effect of different concentration of montmorillonite on 10 μg/ml T4 and T3. When the concentration of montmorillonite were 0.3, 3.0, and 30.0 mg/ml.
ml, the adsorption percentage of montmorillonite on T4 were 16.2%, 53.8% and 66.0%, respectively; and the adsorption percentage on T3 were 42.6%, 56.4% and 69.0%, respectively.

3.2. Effect of montmorillonite on hyperthyroidism model rats

The concentration–time curves of total T3 and total T4 in serum showed that levels of total T3 and total T4 in serum were increased after thyroid tablets were administrated. The levels of total T3 and total T4 in montmorillonite group became lower from the fifth day on rats administrated with montmorillonite than the model rats (P<0.05 and P<0.01). (Fig. 2)

The effect of montmorillonite on the serum concentration of thyroid hormone in hyperthyroidism model rats showed that montmorillonite decreased both total T3 and total T4 levels obviously (Fig. 3). Montmorillonite (1.0 g/kg and 0.3 g/kg) decreased the total T3 and total T4 levels of model group which was higher than normal group.

Fig. 4 showed that effect of montmorillonite on food and water consumption of hyperthyroidism model rats prepared by administrating thyroid tablets (90 mg/kg/day, i.g.) for 7 days. The data represents the mean±S.E.M for 10 rats. * indicates significant difference from model group, P<0.05.

3.3. Effect of montmorillonite on hyperthyroidism model mice

Fig. 5 showed that montmorillonite decreased spontaneous activity and prolonged sodium-pentobarbital-induced sleep time of hyperthyroidism model mice induced by sodium-levothyroxin 30 μg/kg intraperitoneally for 7 days. The count values of spontaneous activity became lower with montmorillonite 2.0 g/kg and 0.6 g/kg. Montmorillonite at 2.0 g/kg, 0.6 g/kg and 0.2 g/kg groups prolonged the sleep time of model group (P<0.05) which was shorter than normal control group (P<0.01).

Fig. 6 showed the effect of montmorillonite on hypoxia tolerance capacity of hyperthyroidism model mice induced by...
sodium-levothyroxin 30 μg/kg intraperitoneally for 7 days. Montmorillonite (2.0 g/kg and 0.6 g/kg) prolonged the hypoxia tolerance time compared to the model group (P<0.05).

4. Discussion

The patients with thyrotoxicosis often show symptoms resulting from excess circulation thyroid hormone (Solomon et al., 1993). Thyroid hormone is conjugated with glucuronides and sulphates in liver. The conjugated products are excreted in the bile and entered the intestine, where bacteria can degrade conjugated hormone to free T₃ and T₄. Free hormones are reabsorbed in intestine. An enterohepatic circulation forms as a result (DiStefano et al., 1993). About 1/3 of thyroid hormone participates in the enterohepatic circulation. The ratio is obviously increased in hyperthyroidism. It has been suggested that serum total T₃ and total T₄ level can decrease if thyroid hormone entering intestine from enterohepatic circulation is sequestered by sorbents, which may be beneficial to hyperthyroidism (Shakir et al., 1993; Surks and Sievert, 1995).

The study in vitro showed that montmorillonite could adsorb thyroid hormone. In the in vivo experiment, the level of serum total T₃ and total T₄ in hyperthyroidism model group was much higher than the control group. In addition, the consumption of water and food, body weight and anorectal temperature in hyperthyroidism model group was increased compared to the control group, demonstrating that the model was successful. Similarly, the model mice which were injected with levothyroxin became analepsia, and their oxygen consumption increased, too.

Montmorillonite significantly decreased total T₃ and total T₄ levels and water consumption of hyperthyroidism model rats. Montmorillonite also decreased spontaneous activity and prolonged hypoxia tolerance time and sodium-pentobarbital-induced sleep time of hyperthyroidism model mice. These suggest montmorillonite has an effect on anti-hyperthyroidism, which was attributed to its adsorptive effect. Montmorillonite not only adsorbs free T₃ and T₄, blocking the enterohepatic circulation of thyroid hormone, but also adsorbs intestine bacteria which is necessary for the degradation of conjugated hormone to free T₃ and T₄, decreasing free T₃ and T₄ level in intestine. What’s more, montmorillonite has the ability to cover the surface of the intestine and prevents the reabsorption of the hormone. These may involve in the anti-hyperthyroidism effect of montmorillonite.

Montmorillonite doesn’t enter the body circulation, so its side effect will be much slighter compared to other anti-hyperthyroidism drugs such as tapazol and propylthiouracil which would induce granulocyte reduction and immunity deficiency (Qiu, 2002). Therefore, it is lucky for patients who are subtly to have leukocyte reduction after taking the medicine of tapazol and propylthiouracil. Also, it may be used as an adjunctive therapy, that is, to combine with tapazol or other drugs.

Cholestyramine, an ionic exchange resin, can interfere with the absorption of ingested thyroid hormone, too. It has showed that combination therapy with propylthiouracil, propranolol and cholestyramine in the treatment of Graves’ hyperthyroidism achieves a euthyroid state more rapidly and completely than that with only methimazol and propranolol (Tsai et al., 2005). Its anti-hyperthyroidism effect was also attributed to its adsorptive effect. 50 mg of cholestyramine can bind approximately 3 mg of T₄ (Harmon and Seifert, 1991), which blocks reabsorption of thyroid hormone in enterohepatic circulation. Constipation and abdominal discomfort are the primary side effects of hole-styramine treatment. Heart burn, nausea and transient increase in serum aminotransferase level occur, too.

Honestly, the adsorptive effect of montmorillonite is also physical, not special. So other active substances or nutrients in intestine may be adsorbed if it is used for a long time (Kozak and Domka, 2004). So, it is necessary to study some important substances like vitamins and iron and so on, to find out if montmorillonite absorbs them and how much the adsorption will be.

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References


