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Pharmacological characterization of cyclosporine A-induced kaolin intake in rats

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Abstract

Kaolin intake behavior of rats is known to be one of the useful animal models to evaluate the emetic and antiemetic actions of drugs. The present study was aimed at elucidating the pharmacological characterization of cyclosporine A (CsA)-induced kaolin intake in rats. Subchronic treatment (once a day for 3 days) with CsA produced a dose- and time-dependent increase in kaolin intake. Scopolamine (muscarinic antagonist), mepyramine (selective histamine H_1 antagonist) and diphenhydramine (H_1 and muscarinic antagonist) but neither domperidone (dopamine D_2 antagonist) nor ondansetron (serotonin 5-HT_3 antagonist) significantly inhibited CsA-induced kaolin intake. These findings suggest that an activation of central muscarinic and H_1 receptor is closely associated with CsA-induced kaolin intake in rats. Use of scopolamine and/or diphenhydramine may be possible regimens to alleviate and avoid nausea and vomiting in patients with CsA therapy.

Keywords: Cyclosporine; Kaolin intake; Nausea; Vomiting; Antiemetic agents; Rats

1. Introduction

Cyclosporine A (CsA), an immunosuppressant, is widely used to prevent allograft rejection in solid organ transplantation and in fatal graft-versus-host disease after bone marrow transplantation (Kahan, 1992; Kahan, 1989; Powles et al., 1980). Multicenter, randomized trials in the USA and Europe were conducted to compare the efficacy and safety of tacrolimus- versus CsA-based immunosuppressive regimens for organ transplantated patients (A Report of The European Tacrolimus Multicenter Renal Study Group, 1997; Pirsch et al., 1997; The U.S. Multicenter FK506 Liver Study Group, 1994). In these trials, adverse events including impaired renal function, cardiovascular disorders, gastrointestinal disorders and neurological complications were found to occur with a relatively high frequency (20–40%) in both groups (A Report of The European Tacrolimus Multicenter Renal Study Group, 1997; Pirsch et al., 1997; The U.S. Multicenter FK506 Liver Study Group, 1994). CsA injured brain capillary endothelial cells and inhibited the function and expression of P-glycoprotein, a multidrug efflux pump (Kochi et al., 1999; Kochi et al., 2000). We recently reported that CsA increased permeability of the brain endothelial cells (Dohgu et al., 2000). These findings suggest that CsA could pass through the blood–brain barrier by producing dysfunction of the P-glycoprotein, a multidrug efflux pump, and hyperpermeability of the brain capillary endothelial cells (Dohgu et al., 2000; Kochi et al., 1999; Kochi et al., 2000). We previously reported that CsA produced convulsions by inhibiting neural activity of γ-aminobutyric acid (GABA) and binding properties of $GABA_A$ receptor (Shuto et al., 1999). A facilitatory action of CsA and tacrolimus on the stimulation-evoked nitric oxide production in glial cells was also attributable to the occurrence of neurotoxicity (Ikesue et al., 2000).

Among symptoms referable to the gastrointestinal system including nausea, vomiting, dyspepsia, diarrhea and constipation in the renal and liver transplant recipi-

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ents with CsA therapy, nausea and vomiting occurs in about 40% and 20% of patients, respectively (Pirsch et al., 1997; The U.S. Multicenter FK506 Liver Study Group, 1994). There is no study on the mechanism of emetic action of the immunosuppressant and a protective regimen against this adverse effect. Mitchell et al. found that poison or motion induced the intake of nonnutritive substances such as kaolin in rats, this behavior is called pica, suggesting that pica is an illness-response behavior analogous to nausea and/or vomiting in other species (Mitchell et al., 1976, 1977). Based on pharmacological evidence using clinically effective antiemetic drugs (Takeda et al., 1993), this kaolin intake behavior of rats is considered to be one of the useful animal models including drug-induced vomiting in dogs, cats and ferrets to investigate antiemetic and emetic actions of drugs (Takeda et al., 1993). The present study was, therefore, aimed at elucidating the pharmacological characterization of CsA-induced kaolin intake in rats using antiemetic agents (dopamine D_2 antagonists, serotonin 5-HT_3 antagonists, histamine H_1 antagonists and muscarinic antagonists) (Brunton, 1996; Mitchelson, 1992).

2. Methods

2.1. Animals

Male Wistar rats aged 8 weeks and weighing 250–320 g (Kyudo, Saga, Japan) were used. They were housed individually in a cage ($21.5 \times 32 \times 14$ cm) under a 12-h light/dark schedule (the lights on 07:00 h) at a temperature of 22 ± 2 °C and were given water and food (standard chow; Oriental Yeast, Chiba, Japan) *ad libitum*. This study was reviewed by the ethics committee regarding animal experiments at the Faculty of Medicine, Kyushu University and was performed according to the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and the law (No. 105) and notification (No. 6) of the Japanese Government.

2.2. Drugs

Cyclosporine (Sandimmun injection; Novartis Pharma, Tokyo, Japan) was diluted with the vehicle solution consisting of 13% polyoxyethylene castor oil, 7% ethanol and 80% saline (the mixture as a five-fold dilution of vehicle of the Sandimmun injection). Domperidone (a gift from Kyowa Hakko, Tokyo, Japan) was dissolved in saline containing 0.01% Tween 20 (Wako, Osaka, Japan). Ondansetron (Zofran injection; GlaxoSmithKline, Tokyo, Japan) was diluted with saline. Scopolamine hydrobromide, scopolamine n-butylbromide (butylscopolamine), mepyramine maleate and diphenhydramine hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in saline.

2.3. Kaolin

Kaolin was prepared according to the method of Mitchell et al. (1976) with minor modifications. Kaolin (hydrated aluminum silicate; Wako) was mixed with 3% gum arabic (Kanto Chemical, Tokyo, Japan) in distilled water to form a thick paste. The mixture was tubed and partially dried in a dryer. Then this was extruded from a tube, cut into a column of the same size as that for food pellets ($1.33\ cm^2 \times 3$ cm) and again dried completely in a dryer.

2.4. Experimental procedure

Kaolin pellets were placed on the stainless steel grid cover of the cage for one week before the experiment to allow the animals to adapt to its presence. To measure the kaolin consumption during a 22-h period at the start of 19:00 h, the remaining kaolin on the grid cover and kaolin spilled in the cage were collected and weighed to the first decimal level of g at 17:00 h every day. The amount of food intake was measured in the same manner as kaolin intake. Then the new kaolin and food pellets were placed on the grid cover at 19:00 h.

The rats showing kaolin intake less than 1.0 g/day for the last 2 days during one week-adaptation were used for drug tests. The animals were injected intraperitoneally in a volume of 0.5 ml/100 g body weight with vehicle or CsA (20 and 50 mg/kg) 30 min before placement of the new pellets for 3 consecutive days. Domperidone (1 mg/kg ip), ondansetron (2 mg/kg ip), scopolamine (0.5 and 1 mg/kg ip), butylscopolamine (2 mg/kg ip), mepyramine (10 and 20 mg/kg ip) and diphenhydramine (20 mg/kg ip) were injected 10 min before each administration of CsA (50 mg/kg ip) once a day for 3 days. The amounts of kaolin and food intake during a 22-h period at the start of 19:00 h were measured before and 1, 2 and 3 days after the first injection of vehicle or CsA.

Effect of subchronic treatment (once a day for 3 days) with mepyramine (20 mg/kg ip) alone was examined on spontaneous motor activity of each rat in the isolated cage ($700\ cm^2$) by monitoring locomotor activity of rats using a motor activity recording system composed of infrared sensors interfaced with a computer (Activity Sensor, Model NS-AS01, Neuroscience, Tokyo, Japan). The counts of locomotor activity were monitored during a 22-h period at the start of 19:00 h for 4 consecutive days before and 1, 2 and 3 days after the first injection of mepyramine.

2.5. Statistical analysis

Values are expressed as means ± S.E.M. Statistical analysis was carried out by a one-way analysis of variance (ANOVA) to test for the overall significance of effects with subsequent individual comparisons using the Bonferroni/Dunn test. Differences were regarded as statistically significant at $P < .05$.

3. Results

3.1. Effects of CsA on kaolin intake in rats

Subchronic treatment (once a day for 3 days) with intraperitoneal injection of CsA at doses of 20 and 50 mg/kg produced dose-dependent increase in kaolin intake in rats. The time course of CsA-induced kaolin intake is shown in Fig. 1A. CsA 20 mg/kg produced a significant increase in kaolin intake after the 3rd injection. When rats were injected with CsA 50 mg/kg, the time-dependent increases in kaolin intake become significant after the 2nd and 3rd injection. The total amounts of kaolin intake during a 3 day period in rats treated with vehicle, CsA 20 mg/kg and CsA 50 mg/kg once a day for 3 days were 0.5 ± 0.2, 2.2 ± 0.6 and 5.3 ± 0.9 g, respectively (Fig. 1B). CsA dose-dependently increased the total kaolin intake over 3 days.

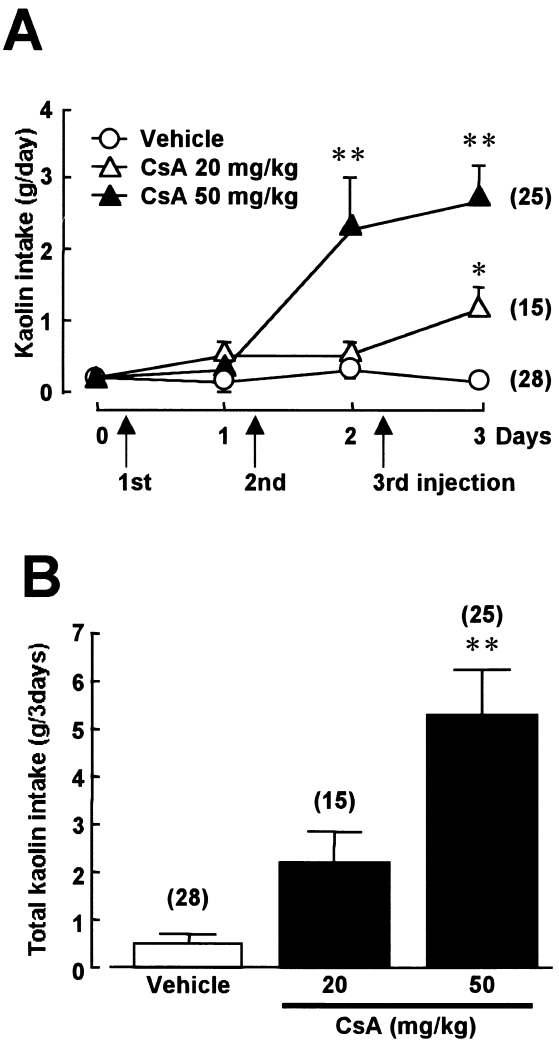

Fig. 1. Time course (A) and cumulative amounts (B) of kaolin intake induced by subchronic treatment with vehicle or CsA (20 and 50 mg/kg ip) once a day for 3 days in rats. Values represent means ± S.E.M. for the number of rats shown in the parentheses on the right side of each symbol (A) and the top of each column. ** $P < .01$, * $P < .05$ vs. vehicle-treated group.

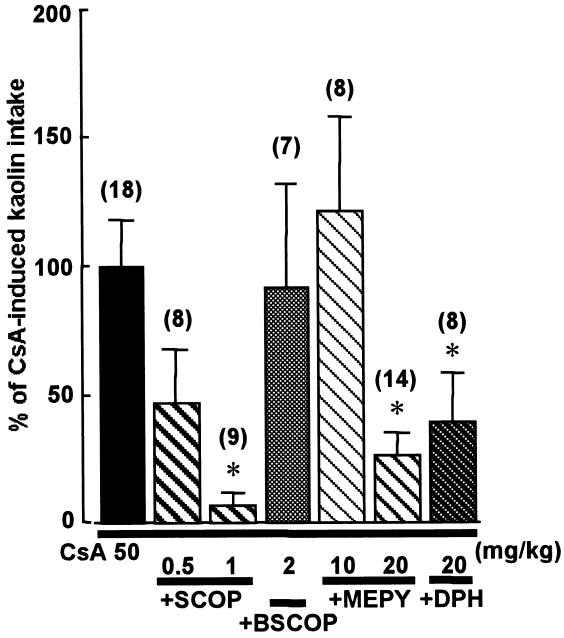

Fig. 2. Effects of scopolamine (SCOP), butylscopolamine (BSCOP), mepyramine (MEPY) and diphenhydramine (DPH) on kaolin intake induced by CsA (50 mg/kg ip, 1 × 3 days) in rats. Scopolamine (0.5 and 1 mg/kg ip), butylscopolamine (2 mg/kg ip), mepyramine (10 and 20 mg/kg ip) and diphenhydramine (20 mg/kg ip) were injected 10 min before each administration of CsA (50 mg/kg ip) once a day for 3 days. Data are expressed as percent of CsA-induced kaolin intake (5.4 ± 1.0 g over 3 days). Values represent means ± S.E.M. for the number of rats shown in the parenthesis on the top of each column. * $P < .05$ vs. the corresponding CsA alone-treated group.

Subchronic treatment with CsA 20 and 50 mg/kg caused a significant reduction in food intake and body weight gain, when compared with vehicle. The total food intake for vehicle, CsA 20 mg/kg and CsA 50 mg/kg were 65.4 ± 1.6, 46.2 ± 1.8 ($P < .01$) and 29.2 ± 2.0 ($P < .01$) g over 3 days, respectively. Body weight gain for 3 days after the first injection of vehicle, CsA 20 mg/kg and CsA 50 mg/kg was 17.8 ± 1.1, −4.0 ± 2.1 ($P < .01$) and −8.2 ± 2.4 ($P < .01$) g over 3 days, respectively.

3.2. Effects of antiemetic drugs on CsA-induced kaolin intake

Fig. 2 shows the effects of scopolamine, butylscopolamine, mepyramine and diphenhydramine on CsA-induced kaolin intake. The total kaolin intake induced by subchronic treatment (1 × 3 days) with CsA 50 mg/kg was 5.4 ± 1.0 g over 3 days. Scopolamine (0.5 and 1 mg/kg) dose-dependently decreased CsA (50 mg/kg)-induced kaolin intake by 53.3 ± 21.1% and 92.8 ± 4.0%, respectively. While butylscopolamine 2 mg/kg failed to inhibit kaolin intake induced by CsA. Mepyramine at a dose of 20 mg/kg but not 10 mg/kg significantly decreased CsA (50 mg/kg)-induced

Table 1
Effects of domperidone (1 mg/kg ip) and ondansetron (2 mg/kg ip) on kaolin intake induced by subchronic treatment with CsA (50 mg/kg ip) once a day for 3 days in rats

CsA 50 mg/kg (% of control)		
Control	+ Domperidone, 1 mg/kg	+ Ondansetron, 2 mg/kg
100.0 ± 44.5	64.9 ± 35.6	72.6 ± 34.5

Domperidone (1 mg/kg ip) and ondansetron (2 mg/kg ip) were injected 10 min before each administration of CsA (50 mg/kg ip) once a day for 3 days. Data are expressed as percentage of the control group treated with CsA alone. Values represent means ± S.E.M. of seven rats.

kaolin intake by 74.1 ± 8.9%. The total kaolin intake induced by subchronic treatment (1 × 3 days) with CsA 50 mg/kg was 4.8 ± 2.1 g over 3 days (control) (Table 1). Domperidone 1 mg/kg and ondansetron 2 mg/kg did not affect CsA (50 mg/kg)-increased kaolin intake (Table 1).

The total food intake for CsA 50 mg/kg alone and CsA-combined treatment with scopolamine 1 mg/kg, mepyramine 20 mg/kg and diphenhydramine 20 mg/kg was 30.8 ± 2.5, 41.3 ± 3.6, 39.4 ± 2.2 and 39.4 ± 4.4 g over 3 days, respectively. The body weight gains for 3 days after the first injection with CsA 50 mg/kg alone and CsA-combined treatment with scopolamine 1mg/kg, mepyramine 20 mg/kg and diphenhydramine 20 mg/kg were − 6.6 ± 2.7, − 1.2 ± 3.6, 0.6 ± 2.8 and − 5.1 ± 3.0 g over 3 days, respectively.

The basal locomotor activity in rats before the first injection was 11957 ± 1265 counts during a 22-h period. After the 1st, 2nd and 3rd injection of mepyramine 20 mg/kg alone, motor activities became 99.9 ± 4.5%, 96.7 ± 2.7% and 99.8 ± 4.1% of basal ($n = 5$), respectively, showing no significant changes when compared with the basal. The total food intake in rats treated with vehicle and mepyramine 20 mg/kg was 58.1 ± 1.5 ($n = 7$) and 61.9 ± 3.5 ($n = 5$) g over 3 days, respectively. The body weight gain for 3 days after the first injection of vehicle and mepyramine 20 mg/kg were 17.5 ± 1.0 ($n = 7$) and 18.6 ± 2.3 ($n = 5$) g/3 days, respectively. Subchronic treatment (1 × 3 days) with mepyramine 20 mg/kg by itself showed no effects on food intake and body weight gain.

4. Discussion

The present study demonstrated that CsA induced a significant and dose-dependent kaolin intake in rats, this phenomenon was inhibited by scopolamine and mepyramine but neither domperidone nor ondansetron. Then, we investigated the effects of various antiemetic drugs on CsA-induced kaolin intake in rats to pharmacologically characterize this pica-related behavior. Scopolamine at doses of 0.5–1 mg/kg shows an antagonistic action on muscarinic receptor in rats (Anglade et al., 1999). These doses of scopolamine inhibited CsA-induced kaolin intake by 90% but not butylscopolamine at a dose up to 2 mg/kg, a peripheral muscarinic antagonist (Brown and Taylor, 1996). These findings suggest that an activation of muscarinic receptor in the brain rather than the periphery is involved in CsA-induced kaolin intake in rats. Mepyramine 20 mg/kg, a selective H_1 receptor antagonist, significantly inhibited CsA-induced kaolin intake by 80%. The 20 mg/kg of mepyramine alone, although this dose used in the present study was relatively high, did not influence spontaneous motor activity, food intake and body weight gain in rats, suggesting the possible involvement of histaminergic mechanisms in mediating CsA-induced kaolin intake. Diphenhydramine at a dose of 20 mg/kg, which shows an antagonistic action on H_1 receptor and muscarinic receptor, significantly decreased CsA-induced kaolin intake by 60%. This dose of diphenhydramine significantly reduced the double rotation-induced kaolin intake in rats (Morita et al., 1988b), an animal model of motion sickness (Morita et al., 1988a). These findings suggest the notion that CsA produces the emetic action by activating the central histaminergic and cholinergic systems. The possibility that the peripheral mechanisms are involved, at least in part, in the emetic action of CsA can not be excluded. CsA-induced kaolin intake was not changed by domperidone 1 mg/kg (D_2 receptor antagonist) and ondansetron 2 mg/kg (5-HT_3 receptor antagonist). These agents specifically inhibited apomorphine- and cisplatin-induced kaolin intake in rats, respectively (Takeda et al., 1993). The central dopaminergic and serotoninergic system are, therefore, unlikely to contribute to CsA-induced kaolin intake. Scopolamine, mepyramine and diphenhydramine protected against CsA-induced kaolin intake but this protective action was not well associated with the improvement of CsA-reduced food intake and body weight gain. The neuroanatomical and neurochemical mechanisms in the brain regulating nausea and vomiting are separated from those for appetite or energy metabolism, although a close talk between these mechanisms exists in the hypothalamic level (Grunberg and Hesketh, 1993; Kalra et al., 1999; Mitchelson, 1992). Therefore, the separate sites and/or different neurochemical mechanisms for antiemetic and anorexic action of CsA may be attributable to these controversial findings.

In conclusion, we present here the first evidence that CsA induces kaolin intake in rats, a behavior known to simulate nausea and vomiting in humans (Takeda et al., 1993). The present findings suggest that an activation of muscarinic and H_1 receptor is closely associated with CsA-induced kaolin intake in rats. Use of scopolamine and/or diphenhydramine may be possible regimens to alleviate and avoid nausea and vomiting in patients with CsA therapy.

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