Antiinflammatory and Antiallergic Activity of *Bidens pilosa* L. var. *radiata* Scherff

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The suspension and the boiling water extract of dried powder from the aerial parts of *Bidens pilosa* L. var. *radiata* Scherff (*Tachiawayukisendangusa*: MMBP) on the Japanese island of Miyako have antiinflammatory and antiallergic properties in experimental diseases. Oral administration of MMBP suspension in carboxy-methyl-cellulose sodium solution inhibited the production of IgE 10 days after immunization with DNP-ascaris in mice. The extract inhibited histamine release from rat peritoneal mast cells induced by compound 48/80 or antigen-antibody reaction. Oral administration of the suspension inhibited dye exudation in rat skin induced by passive cutaneous anaphylaxis. Oral administration of the suspension inhibited dye exudation in rat skin induced by chemical mediators (histamine, substance P, and serotonin). These findings suggest that MMBP may be clinically useful in the prevention of type I allergic disease.

Key words —— *Bidens pilosa*, antiinflammation, antiallergy, histamine

INTRODUCTION

Bidens pilosa L. (*Kosendangusa* in Japanese compositae) is a herbaceous plant widely distributed in Africa, America, China, and Japan. It is used in traditional medicines for inflammation and the treatment of various diseases, including hepatitis and diabetes. *Bidens pilosa* L. var. *radiata* Scherff (*Tachiawayukisendangusa* in Japanese, MMBP) is originally native to tropical America. It was introduced to Miyako Island, in Japan's Okinawa prefecture, where residents harvested and steamed the aerial parts. The dried powder was used as an ingredient in tea such as Kanpo-tea® for traditional drugs. Masuzawa *et al.* reported that MMBP clinically prevented skin ulcers in summer (livedo reticularis with summer ulceration) by improving blood circulation.[1] We investigated the constituents of the dried powder used in the tea to determine its active antioxidant components. The antioxidant activities of the fractions, isolated compounds, and some related compounds were measured and compared with those of Trolox C, a water-soluble tocopheroxyl (vitamin E) analogue, as a standard.[2] The active fractions of the antioxidant activity proved to contain flavonoids, caffeic acid derivatives, and coffee tannins.[3] It is accepted that some antioxidant drugs have an antiallergic effect, and that superoxide generation plays an important role in mast cell activation. The chemical mediators released from mast cells have been centrally implicated in a diverse range of allergic and inflammatory disorders. Flavonoids are known to have antiinflammatory, antiallergic, and antithrombic activities, and these actions can be explained by their antioxidant activity.[4] Their antioxidant mechanisms include the inhibition of enzymes involved in the formation of reactive oxygen species such as xanthine oxidase and NADH oxidase, and the chelation of trace elements (free iron or copper) that are potential enhancers of free-radical generation.[5] Previously it was reported that some Chinese medicines and plants had antiinflammatory and antiallergic activities.[6–9] Moreover, some extracts and flavonoids and caffeic acid were founded to have antiinflammatory and antiallergic activities.[10–13] Therefore MMBP may have antiinflammatory and antiallergic activities. In the present study, we examined the antiinflammatory and antiallergic properties of MMBP using experimental inflammatory and allergic models and examined the mode of action.

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MATERIALS AND METHODS

Animals —— Male Wistar rats (200 g), male ddY mice (18–20 g), and male BALB/c mice (18–20 g) were purchased from Sankyo Japan SLC, Inc., Shizuoka. The animals were then maintained in an air-conditioned room controlled for temperature (24 ± 1°C) and humidity (55 ± 5%), where they were given standard laboratory food, MF Oriental Yeast, Tokyo, Japan) and water *ad libitum*. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Plant Material —— MMBP was cultivated on Miyako Island, Okinawa prefecture, Japan, and the aerial parts were harvested and steamed. The dried powder was provided by Musashino Research Institute for Immunity Co., Ltd. (Tokyo, Japan), and used in this study. For the *in vitro* experiment, the test sample was extracted with water for 24 hr at 100°C and the supernatant was used.

In the *in vivo* experiment (oral treatment), the suspension of MMBP in 0.25% carboxy-methyl-cellulose sodium (CMC-Na) was used. The animals were administered pharmacologic doses of MMBP: 100, 250, and 500 mg/kg estimated to be 10-fold the medicinal dose found in Kanpo-tea®.

Chemicals —— The chemicals used and their sources were as follows: histamine (Sigma Chemical, St. Louis, MO, U.S.A.), serotonin (5-hydroxytryptamine) (Sigma Chemical), substance P (Sigma Chemical), L-α-phosphatidyl-L-serine (Sigma Chemical), DNP-ascaris (LSL Co., Tokyo, Japan), rat-anti-DNP-ascaris antiserum (LSL Co.), ketotifen (Sigma Chemical), cyclophosphamide (Sigma Chemical), and compound 48/80 (Sigma Chemical).

The Production of Antibody (IgE) against DNP-Ascaris in Mice —— To determine the titer of anti-DNP-ascaris-IgE, the BALB/c mice were injected with DNP-ascaris (5 μg) in alum.[14,15] At 5, 10, and 15 days after injection, the IgE titer against DNP-ascaris in the serum was determined using ELISA (Mouse IgE ELISA Kit, Sibayagi, Japan). The cyclophosphamide group was subcutaneously given cyclophosphamide (20 mg/kg) for 5 days prior to the immunization. The mice in the MMBP group were given a suspension of MMBP (100, 250, and 500 mg/kg, p.o.) in 0.25% CMC-Na for 5 days prior to the immunization. The IgE titer, which was in-

duced by DNP-ascaris in the serum, was also determined using ELISA.

Compound 48/80 or DNP-Ascaris-Induced Histamine Release from Isolated Rat Peritoneal Mast Cells —— Briefly, rat peritoneal mast cells were harvested from the abdominal cavity of the male rats and purified by Ficoll density-gradient centrifugation. Physiologic buffered saline [PBS; in mM: NaCl 137, KCl 2.7, CaCl$_2$ 1.8, MgCl$_2$ 1.0, NaH$_2$PO$_4$ 0.4, HEPES 20, D-glucose 5.6, 0.1% (w/v)gelatin, pH 7.4] containing 0.03% heparin was injected into the peritoneal cavity.[12,16–18] The abdominal region was gently massaged for 90 sec, and peritoneal fluid was collected and centrifuged for 1 min at 280 × g at 4°C. After the centrifugation, the precipitant was mixed in 20% Ficoll, and the mixture was loaded onto layers of 40 and 30% Ficoll and then centrifuged for 20 min at 350 × g. Then, the layer with the mast cell pellet was pooled and washed twice in PBS solution. Thereafter, MMBP (2 mg/0.05 ml) or ketotifen (0.05 mg/0.05 ml) dissolved in PBS was added to equal numbers of mast cells (1 × 10^5 cells/ 0.2 ml) and preincubated at 37°C for 10 min before the reaction. Compound 48/80 (0.2 μg/ 0.05 ml) was added to the tubes and incubated at 37°C for 10 min. The reaction was stopped 10 min later by adding cool PBS to the tubes. The tubes were centrifuged for 1 min at 280 × g, and the histamine content in the supernatant was measured using ELISA (SPI Bio, Paris, France). On the other hand, the histamine release from isolated rat peritoneal mast cells was also induced by antibody [anti-DNP-ascaris, rat 48-hr passive cutaneous anaphylaxis (PCA); 1 : 512]. For the stimulus by the antigen (DNP-ascaris), the spontaneous histamine release was examined in rat serum in PBS. Equal numbers of mast cells (2.0 × 10^6 cells/ml) were added to 0.1 ml of anti-DNP-ascaris antiserum and incubated for 1 hr at 37°C. An equal number of mast cells (1 × 10^5 cells/0.2 ml) after the incubation and MMBP (2 mg/0.05 ml) or ketotifen (0.05 mg/0.05 ml) were preincubated for 10 min at 37°C. L-α-phosphatidyl- L-serine (0.01 mg/0.05 ml) and DNP-ascaris (0.02 mg/0.05 ml) in PBS 0.05 ml were added to the tubes and incubated for 10 min at 37°C. The reaction was stopped 10 min later by adding cool PBS. The tubes were centrifuged for 1 min at 280 × g, and the histamine content in the supernatant was measured using ELISA. Spontaneous histamine release was also calculated. Inhibition by MMBP or ketotifen was expressed as a percentage of histamine release compared with total histamine in mast cells before the reaction.

DNP-Ascaris Antiserum-Induced PCA Reaction in Rats —— Rats were passively sensitized on the back skin by an injection of anti-DNP-ascaris antiserum (0.1 ml/site, s.c.).[16,19] At 48 hr after injection, the animals were intravenously injected with a mixture of Evans blue (0.5%) and DNP-ascaris antigen (1 mg/ ml).[20,21] At 30 min after the antigen challenge, the leakage of dye accumulated in the skin was determined colorimetrically at 620 nm. The test rats were divided into four groups. The rats in the control group were administered 0.25% CMC-Na p.o. 1 hr before the injection of antigen and dye ($n = 10$). The rats in the pre-MMBP group were given MMBP (100, 250, and 500 mg/kg, p.o.) with the diet for 10 days before the injection of antigen and dye ($n = 6$). The rats in the MMBP group were administered a suspension of MMBP (500 mg/kg, p.o.) in 0.25% CMC-Na 1 hr before the injection of antigen and dye ($n = 6$–8). The rats in the ketotifen group were administered ketotifen (5 mg/kg, p.o.) 1 hr before the injection of antigen and dye as a positive control ($n = 6$).

Inflammatory Chemical Mediators-Induced Skin Reactions in Rats —— We examined the changes in vascular permeability induced by the inflammatory mediators histamine, substance P, and serotonin.[22] In this inflammatory model, vascular permeability was estimated by dye leakage in the dorsal skin of rats. The animals were intravenously injected with 50 mg/kg of pontamine sky blue 5 min after injection (0.1 ml/site, s.c.) of chemical mediators histamine (10 nmol/site), substance P (1 nmol/site), and serotonin (1 nmol/site) or saline into the back skin. The animals were killed 1 hr after injection, and the skin of each reaction locus was removed to determine whether dye had leaked. The dye in the skin was extracted with 0.6N-phosphate solution : acetone (5 : 13). The dye in the extract was measured colorimetrically at 590 nm.[23] The test rats were divided into four groups. The rats in the control group were administered 0.25% CMC-Na p.o. 1 hr before the injection of dye ($n = 10$). The rats in the pre-MMBP group were given MMBP (500 mg/kg) in the diet for 10 days ($n = 6$). The rats in the MMBP group were administered a suspension of MMBP (100, 250, and 500 mg/kg, p.o.) in 0.25% CMC-Na an hour before the injection of dye ($n = 6$–8). The rats in the ketotifen group were administered ketotifen (5 mg/ kg, p.o.) 1 hr before the injection of dye as a positive control ($n = 6$).

Statistical Analysis —— Data are expressed as means ± S.D. of the number of animals described in the figure legends. Data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test using Stat View software. p Values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

In a previous report, the serum IgE and cytokines (soluble tumor necrosis factor receptor I and regulated upon activation normal T cell expressed and secreted) levels were suppressed by the administration of food flavonoids.[24] In the present study, we examined the effect of oral administration of MMBP suspension in CNC-Na solution on the production of IgE. The level of IgE in serum 10 days after antigen immunization was higher than that at 5 or 15 days (Fig. 1). MMBP dosedependently, in conjunction with cyclophosphamide as a positive control, inhibited the serum IgE level 10 days after immunization (Fig. 2). These results suggest that MMBP and these substances have the potential to regulate the host immune response to type I allergy. Therefore MMBP or flavonoids in MMBP may suppress the production of IgE by improving the helper T cell (Th1-Th2) balance.

Flavonoids are shown to have antiallergic activities, such as the inhibition of histamine release, synthesis of interleukin (IL)-4 and IL-13 and CD40 ligand expression by basophils and mast cells. Polyacetylene glucosides from *Bidens parviflora* W (*Hosobano sendangusa*) inhibit histamine release from rat mast cells induced by compound 48/80 or the antigen-antibody reaction.[25] To determine the antiallergic properties of MMBP, compound 48/80 or the antigenantibody reaction was used to release histamine from rat peritoneal mast cells in this study
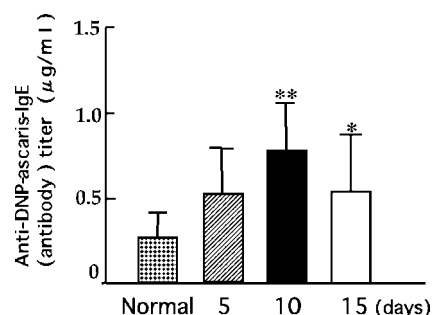

Fig. 1. Titer of Anti-DNP-Ascaris-IgE 5, 10, and 15 Days after Immunization

Each value represents mean ± S.D. of 6 rats. *$p < 0.05$, **$p < 0.01$, significantly different from normal group.
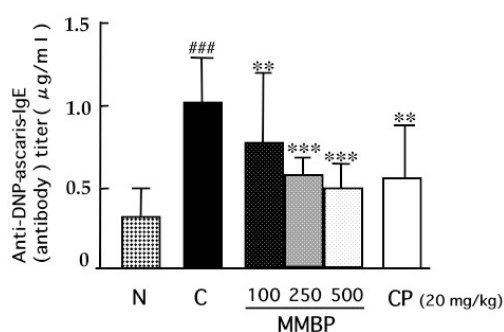
Fig. 2. Effects of MMBP and Cyclophosphamide on Titer of Anti-DNP-Ascaris-IgE 10 Days after Immunization

N: Normal group; C: control group, CMC-Na was given p.o. for 5 days from the injection of antigen (DNP-ascaris, 5 μg); MMBP group: MMBP (100, 250, 500 mg/kg) was given p.o. for 5 days from the injection of antigen; CP: cyclophosphamide group, cyclophosphamide (20 mg/kg) was given p.o. for 5 days from the injection of antigen. Each value represents mean $\pm$ S.D. of 6–9 mice. **$p < 0.01$, ***$p < 0.001$, significantly different from control group, ###$p < 0.001$, significantly different from normal group.
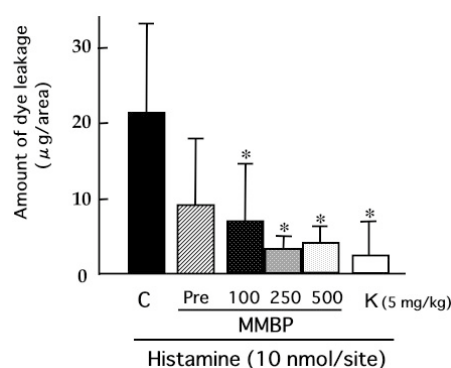

Fig. 4. Effects of MMBP and Ketotifen on Dye Leakage Induced by Histamine (10 nmol)

C: Control group, 0.25% CMC-Na was administered p.o. 1 hr before injection of dye; Pre-MMBP group: MMBP (500 mg/kg) was given with the diet for 10 days; MMBP group: MMBP (100, 250, 500 mg/kg) in 0.25% CMC-Na was administered p.o. 1 hr before injection of dye; K: ketotifen group, ketotifen (5 mg/kg) was administered p.o. 1 hr before injection of dye. Each value represents mean $\pm$ S.D. of 6–10 rats. *$p < 0.05$, significantly different from control group.
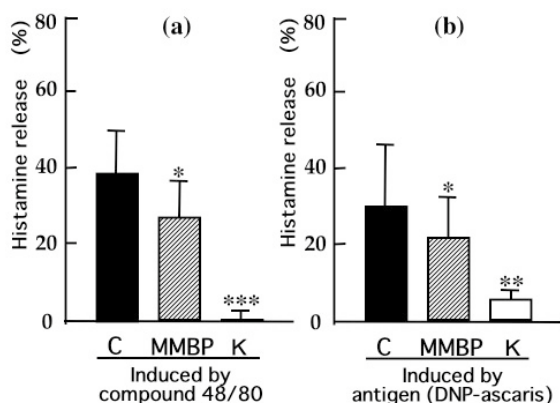

Fig. 3. Effects of MMBP and Ketotifen on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 (0.2 μg/0.05 ml) or Antigen (DNP-Ascaris) (0.02 mg/0.05 ml)

(a) C: Control group; MMBP: MMBP (2 mg/0.05 ml) was added to the mast cells (1×10^5 cells/0.2 ml) in a tube; K: Ketotifen (0.05 mg/0.05 ml) was added to the mast cells (1×10^5 cells/0.2 ml) in a tube; (b) C: Control group; MMBP: MMBP (2 mg/0.05 ml) was added to the mast cells (1×10^5 cells/0.2 ml) in a tube; K: ketotifen (0.05 mg/0.05 ml) was added to the mast cells (1×10^5 cells/0.2 ml) in a tube. Each value (%) represents the mean of 5 experiments (5 rats were used). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, significantly different from each control group.
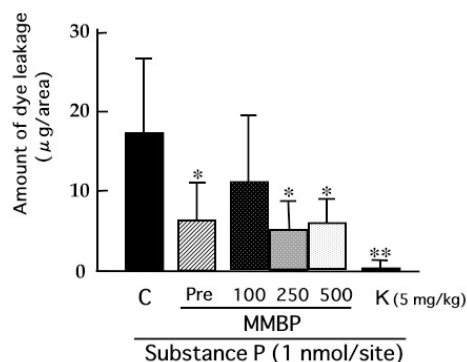

Fig. 5. Effects of MMBP and Ketotifen on Dye Leakage Induced by Substance P (1 nmol)

C: Control group, 0.25% CMC-Na was administered p.o. 1 hr before injection of dye; Pre-MMBP group: MMBP (500 mg/kg) was given with the diet for 10 days; MMBP group: the suspension of MMBP (100, 250, 500 mg /kg) in 0.25% CMC-Na was administered p.o. 1 hr before injection of dye; K: ketotifen group, ketotifen (5 mg/kg) was administered p.o. 1 hr before injection of dye. Each value represents mean $\pm$ S.D. of 6–10 rats. *$p < 0.05$, **$p < 0.01$, significantly different from control group.

(Fig. 3a and 3b). In the experiment, we used the hot-water extract from dry MMBP powder instead of MMBP suspension in CMC solution, because the suspension was impossible to use for the treatment of mast cells. The dried weight of the hot-water extraction from dry MMBP was about 30% that of dry MMBP. However, the amounts of dye leakage with

histamine, substance P, and serotonin were 14.2 $\pm$ 6.9 μg/area, 14.5 $\pm$ 8.9 μg/area, and 15.1 $\pm$ 10.1 μg/area, respectively. These results suggest that the effects of the hot-water extract of MMBP (500 mg/kg) was similar to that of MMBP (500 mg/kg) suspension (compared with the effects of MMBP powder, Figs. 4–6). In this experiment, the addition of compound 48/80 at the concentration of 0.2 μg/0.05 ml induced histamine release of 38.5 $\pm$ 11.0% and the addition of antigen (DNP-ascaris) at the concen-
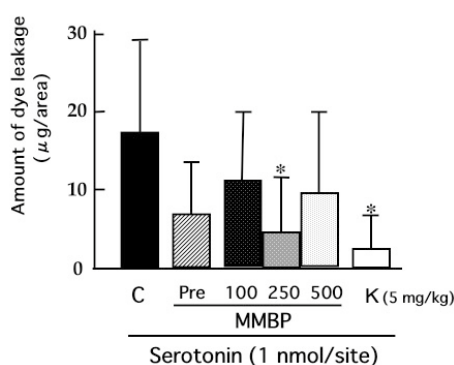
Fig. 6. Effects of MMBP and Ketotifen on Dye Leakage Induced by Serotonin (1 nmol)

C: Control group, 0.25% CMC-Na was administered p.o. 1 hr before injection of dye; Pre-MMBP group: MMBP (500 mg/kg) was given with the diet for 10 days; MMBP group: MMBP (100, 250, 500 mg /kg) in 0.25% CMC-Na was administered p.o. 1 hr before injection of dye; K: ketotifen group, ketotifen (5 mg/kg) was administered p.o. 1 hr before injection of dye. Each value represents mean ± S.D. of 6–10 rats. *$p <$ 0.05, significantly different from control group.
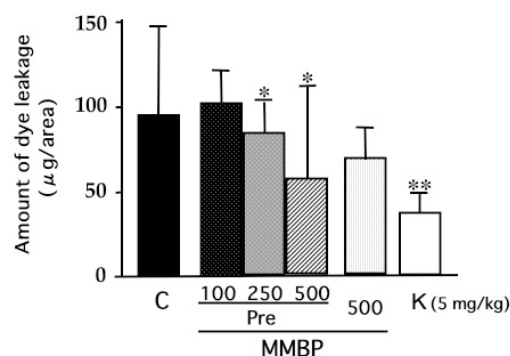

Fig. 7. Effects of MMBP and Ketotifen on Dye Leakage Induced by DNP-Ascaris Antiserum (1 mg/ ml)

C: control group, 0.25%CMC-Na was administered p.o. 1 hr before injection of antigen plus dye; Pre-MMBP group: MMBP (100, 250, 500 mg/kg) was given with the diet for 10 days; MMBP 500 group: the suspension of MMBP (500 mg/kg) in 0.25% CMC-Na was administered p.o. 1 hr before injection of antigen plus dye; K : ketotifen group, ketotifen (5 mg/kg) was administered p.o. 1 hr before injection of antigen plus dye. Each value represents mean ± S.D. of 6–8 rats. *$p <$ 0.05, **$p <$ 0.01, significantly different from control group.

tration of 0.02 mg/0.05 ml induced histamine release of 30.3 ± 16.7%. The spontaneous histamine release was 3.4 ± 0.9% and 19.3 ± 10.1%, respectively. The spontaneous histamine release from rat peritoneal mast cells induced by the antigen was higher than that induced by compound 48/80, because the release occurred after the incubation with rat control serum as the antigen (DNP-ascaris). MMBP inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80 (26.5 ± 9.7%). MMBP also inhibited antigen-induced histamine release from the mast cells (20.1 ± 12.4%). It is possible that ketotifen stabilizes mast cells, acts as an antagonist of the histamine receptor, and inhibits the release of histamine from mast cells. Therefore it seems likely that the antiallergic actions of MMBP inhibited not only the release of histamine from mast cells induced by compound 48/80 but also suppressed the antigen-antibody reaction with mast cells. Oxidative states and freeradical generation induced activation or degradation in mast cells. We speculate that the inhibitory effects on histamine release from mast cells may be attributable to the activity of antioxidant with MMBP. Inoue *et al*. reported that flavonoid glycoside inhibited histamine release induced by compound 48/80 and antigen from mast cells.[12] Recently, Hossen *et al*. have reported that caffeic acid inhibited scratching behavior and vascular permeability induced by compound 48/80 in animal models. In the study, caffeic acid was administered to rats in doses of 200 and 500 mg/

kg, and the administration of only 500 mg/kg was effective.[13] In the present study, MMBP was effective against vascular permeability at a dose of only 200 mg/kg. Therefore it is suggested that the vascular permeability inhibition of MMBP was more effective than that of caffeic acid. Moreover, the antiallergic and antiinflammatory effects of MMBP may depend on its constitution, such as the presence of flavonoids and caffeic acid derivatives. The main components in MMBP are compounds obtained from caffeic acid, flavonoids with glycoside, and compounds obtained from acetylene.[3] In this study, it was considered that the MMBP supernatant had fewer components than the suspension. The MMBP supernatant was the hot water-soluble fraction. MMBP may contain biologically active compounds such as caffeic acid and flavonoid derivatives. Thus, considering the conclusions of previous reports,[12,13] we believe that MMBP has antiallergy effects. On the other hand, it is clear that the compounds obtained from caffeic acid derivatives in MMBP have strong antioxidant activity.[3] It is thus possible that antioxidant activity is involved in the antiallergy effects of MMBP.

Figure 7 shows the effects of MMBP and ketotifen on the exudation of dye in the dorsal skin of rats induced by the PCA reaction. Pretreatment with MMBP and ketotifen by oral administration of the suspension also reduced the exudation of dye in the skin. The effectiveness of MMBP (500 mg/kg) was similar to that of ketotifen in inhibiting the ex-

perimental PCA reaction induced by antigen (DNP-ascaris) and the antibody reaction. Inhibition of the PCA reaction by MMBP pretreatment (250, 500 mg/kg) for 10 days was effective, but that by MMBP (500 mg/kg) for 1 hr was not. This suggests that the antiinflammatory and antiallergic effects of MMBP are attributable not only to its inhibition of the increase in vascular permeability induced by chemical mediators, but also to the inhibition of the antigen-antibody reaction (PCA reaction). The inhibitory antigen-antibody reaction (PCA reaction) of MMBP may be attributable to the repeated administration (MMBP pretreatment for 10 days). In general, natural products are more effective when administered consecutively. Therefore repeated administration enhanced antiinflammatory and antiallergic properties of MMBP.

The reactions induced by intradermal injection of the chemical mediators, histamine, substanceP, and serotonin increase capillary permeability at the local skin site. Figs. 4–6 show the effects of oral administration of MMBP suspension in CMC-Na solution and ketotifen on the exudation of dye in the dorsal skin of rats treated with the chemical mediators. Ketotifen, used as a positive control at the dose of 5 mg/kg, significantly inhibited vascular permeability induced by these chemical mediators. MMBP tended to inhibit the histamine- and substance P-induced dye exudation compared with the control. Significant differences were observed at doses higher than 250 mg/kg (Figs. 4 and 5). On the other hand, MMBP (250 mg/kg) inhibited the serotonin-induced dye exudation without dosedependency (Fig. 6). Repeated administration of MMBP did not enhance its inhibitory effects on the vascular permeability induced by histamine and substance P. Continual repeated administration may be necessary for the antigen-antibody reaction such as the PCA reaction, but a single administration was sufficient to induce local vascular permeability. Therefore we assumed that the inhibitory effects on vascular permeability induced by these chemical mediators were due to a single administration of MMBP. These results indicate that MMBP exerts its antiinflammatory activity by inhibition of IgE production and the release of histamine from mast cells and by permitting local vascular permeability. Previous reports[12,13] indicated that flavonoids and caffeic acid derivatives have free radical-scavenging activity and inhibit histamine release from mast cells, and flavonoids cause inhibition of IgE production. It is suggested that flavonoids and caffeic acid derivatives in MMBP participate in their antiinflammatory or antiallergic activities. This study also suggests that MMBP may be beneficial in regulating type I allergy.

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