A COMPARATIVE STUDY OF THE ANTI-ALLERGIC EFFECTS OF DISODIUM BAICALEIN 6-PHOSPHATE (BPS) AND DISODIUM CROMOGLYCATE (DSCG)

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Abstract—A comparative study was carried out on the effects of a soluble derivative of baicalein, disodium baicalein 6-phosphate (BPS) and disodium cromoglycate (DSCG) on the immediate type allergic reactions. BPS not only inhibited reaginic antibody-mediated reactions including antigen-induced mediator release from monkey lung, homologous PCA in rats, and reaginic antibody-mediated degranulation of mast cell, but also non-reaginic antibody-mediated reactions such as mediator release from guinea pig lung sensitized with ovalbumin and that from human lung caused by anti-IgE. The agent, however, did not affect the mediator release from lung of rats sensitized with dinitrophynylated ascaris extract plus Bordetella pertussis. On the other hand, DSCG showed characteristic properties as an inhibitor of reaginic antibody-mediated reaction. It is thus assumed that the functional site of reaginic antibody is well fixed with DSCG at a definite distance between the two-chromone-nuclei while that of IgG is readily fixed with the two molecules of baicalein or BPS.

Baicalein and baicalin are flavones contained in the radix of Scutellaria baicalensis GEORGI (Woogon), which from ancient times has often been used in Chinese medicine as a remedy for allergic diseases and inflammation. We (1–5) have already reported the anti-allergic activity of baicalein and its derivatives, and these substances also have a potent protective action against active and passive anaphylaxis. These agents were also found to inhibit reagin-mediated reaction as well as disodium cromoglycate (DSCG). DSCG does inhibit reagin-mediated reaction with a fairly high specificity (6–12), however, inhibits not at all or only slightly non-reaginic antibody-mediated reactions (7–12) and the mediator release by specific antigen from the leucocytes of allergic patient (13).

From the viewpoint of chemical structures, baicalein is a monochromone derivative while DSCG is a symmetric dichromone derivative, as shown in Fig. 1.

A comparative study was carried out concerning the effects of a soluble derivative of baicalein, disodium baicalein 6-phosphate (BPS) and DSCG on the immediate type allergic reactions with an animal experimental system, and the structure-activity relationship is discussed.

Fig. 1. Chemical structures of baicalein, BPS, and DSCG.
Animals

Hartley guinea pigs of both sexes weighing about 250 g, male and female Wistar rats weighing 150 to 250 g and male monkeys (Macaca irus) weighing 2.5 to 3 kg were used.

Drugs

BPS was a gift from Takeda Chemical Industries LTD, Osaka and DSCG was kindly provided by Fujisawa Pharmaceutical Industries, Osaka, Japan. The other agents used were commercial products.

Antigen-induced release of mediators from lung tissue

Guinea pigs were sensitized with intraperitoneal and intramuscular injections of ovalbumin in a dose of 50 mg each. After 3 to 5 wk, the animals were sacrificed by exsanguination and the lungs were cleared of blood by perfusion with Tyrode's solution via the pulmonary artery. The lungs were then isolated and minced with a tissue chopper. Two hundred milligrams of the minced tissue was suspended in 1.8 ml of Tyrode's solution and warmed for 10 min at 37°C. Since BPS requires more than 20 min incubation time to display the maximal activity in the preparation, the drug solutions were pretreated 30 min prior to incubation with 0.1 ml of 2 x 10^-3 gm/ml ovalbumin for 20 min. The amount of mediator released in the incubation medium was assayed on isolated guinea pig ileum without the treatment of anticholinergic or antihistaminic agents. For further details, see (1, 14).

Immunization of rats was carried out according to the method of Tada and Okumura (15). Splenectomized animals were immunized by injecting 1.0 mg of dinitrophenylated ascaris extract (DNP-As) mixed with 10^10 killed Bordetella pertussis into the four foot pads. Five days later, the animals were given a booster of 0.5 mg DNP-As alone into the back muscles. The 8th day after the 1st immunization, the lungs were isolated and tested for antigen-induced mediator release as above, with a final concentration of 10^-1 gm/ml DNP-As as a challenging antigen. The antibody titer of the serum (rat anti-DNP-As serum) was 1:128 to 1:1024 as estimated by 48 hr homologous passive cutaneous anaphylaxis (PCA). The serum collected from each animal was pooled to serve for PCA and mast cell degranulation studies.

For experiments on monkey lung the method of Goodfriend et al. (16) was employed. The animals were anesthetized with 30 mg/kg of pentobarbital i.v., then exsanguinated. The lungs were isolated by the same method as was used for guinea pigs. One gram of minced lung tissue was passively sensitized by incubation at 37°C for 2 hr with the mixture of 3 ml Tyrode's solution and 2 ml human serum (1:100 of Prausnitz-Küstner titer) sensitive to house dust. The preparation was then washed several times with Tyrode's solution to remove excess antiserum. The sensitized lung tissue was tested for antigen-induced mediator release with a final concentration of 10^-6 gm/ml Dermatophagoides pteronisnus extract as a challenging antigen.

Antihuman IgE-induced release of mediators from human lung

Human lung tissue was obtained at the time of autopsy from a patient who had died of cardiac disease, and who had no clinical signs of an atopic disease. One hundred milli-
grams of minced lung tissue suspended in 0.8 ml Tyrode's solution was warmed at 37°C for 10 min and preincubated with 0.1 ml drug solution for 30 min prior to incubation with 0.1 ml of sheep antihuman IgE (Pharmacia, Uppsala, Sweden) in a final concentration of 10^-6 gm/ml. The amount of mediator released into the medium was assayed on isolated guinea pig ileum by the same method used for guinea pig lung.

Passive cutaneous anaphylaxis (PCA)

Heterologous PCA: Rabbit anti-ovalbumin serum was prepared from rabbits that had been immunized by injecting 10 mg of ovalbumin emulsified with complete Freund's adjuvant intramuscularly 4 times weekly. The serum collected from each animal was pooled 7 to 10 days after the last injection. Heterologous PCA in guinea pigs was carried out according to the method of Ovary (17). The antiserum diluted 16-fold with physiologic saline was injected intradermally in a 0.1 ml dose into 3 sites on the shaved backs of guinea pigs. The same dose of physiologic saline was similarly injected into the other side. After 3 hr, 1.0 ml of 0.5% Evans blue solution containing 5 mg of the antigen was injected intravenously. Thirty minutes later, the animals were sacrificed by exsanguination, and the skins removed. The intensity of PCA was assessed by the method of Harada et al. (18), where the amount of the dye leaked into the skin, the result of PCA, is extracted and estimated colorimetrically.

Homologous PCA: Homologous PCA was carried out according to the method of Tada and Okumura (15). Rat anti-DNP-As serum diluted 40-fold with physiologic saline was injected intradermally in 0.1 ml dose into 3 sites on the shaved backs of rats. Into the opposite side, the same dose of physiologic saline was similarly injected. After 48 hr, the animals were given intravenously 1.0 ml of 0.25% Evans blue solution containing 2 mg of antigen, and were sacrificed 30 min later. Measurement of the dye leakage was carried out as stated above.

Antigen-induced degranulation of mast cell in rat mesenterium

The following two methods were used.

a) The mesenterium of rats immunized with DNP-As plus killed Bordetella pertussis was isolated 8 days after the 1st immunization. One hundred milligrams of small pieces of mesenterium suspended in 0.8 ml Tyrode's solution was warmed at 37°C for 10 min and preincubated with 0.1 ml drug solution for 30 min prior to the incubation with 0.1 ml of DNP-As in a final concentration of 10^-4 gm/ml. The tissue was then fixed with 10% formalin solution containing 0.1% toluidine blue and adjusted to pH 4.0 with acetic acid. The mast cell degranulation as a result of antigen-antibody reaction was measured microscopically.

b) Mesenterium isolated from normal rats was passively sensitized by incubation at 37°C for 1 hr with the mixture of 1 ml Tyrode's solution and 1.5 ml rat anti-DNP-As serum with 1:128 of 48 hr homologous PCA titer. The sensitized mesenterium was washed several times with Tyrode's solution after which the antigen-induced degranulation test was carried out as described above.
RESULTS

Antigen-induced release of mediators from lungs of three species

The amount of antigen-induced release of mediators was calculated by subtracting that of spontaneous release from each value, and the effect of the drug on the mediator release was expressed as percentage to the control. As shown in Table 1, the mediator release from guinea pig lung was decreased in a dose-dependent fashion by $10^{-4}$ to $10^{-3}$ gm/ml of BPS with 41.2 to 56.1% inhibition. This mediator release was hardly affected by DSCG in doses of $10^{-4}$ to $5 \times 10^{-4}$ gm/ml, and was depressed in a dose of $10^{-3}$ gm/ml with 25.5% inhibition. On the other hand, no inhibition was observed with either BPS or DSCG in the mediator release from rat lung. The mediator release from monkey lung, passively sensitized with atopic serum, was depressed by BPS and DSCG in doses of $10^{-6}$ to $10^{-4}$ gm/ml, and the activity of BPS was slightly more potent than that of DSCG.

Antihuman IgE-induced mediator release from human lung

Fig. 2 shows one of the results obtained by assaying the mediator release from human lung. This release was clearly observed with antihuman IgE and was decreased by the pretreatment of $10^{-4}$ gm/ml BPS with $52 \pm 3.75\%$ inhibition of 5 experiments, but was not affected by the same concentration of DSCG.

Heterologous and homologous PCAs

The effects of BPS on heterologous PCA in guinea pigs and on homologous PCA in rats were compared with those of DSCG, where the drugs were given intraperitoneally in a dose of 200 mg/kg 1 hr prior to the antigen treatment. As shown in Table 2, both PCAs were decreased by BPS with an inhibition of about 40%. In contrast, the administration of DSCG had no effect on heterologous PCA, but did decrease homologous PCA to the same degree as with BPS.

Antigen-induced degranulation of mast cells in rat mesenterium

Mast cells in the mesenterium of rats actively sensitized and/or passively sensitized...
Table 1. Antigen-induced mediator release from lungs of 3 species (percent to the control)

| Species | Guinea pig | Rat | Monkey |
|---------|------------|-----|--------|
| Dose (gm/ml) | $10^{-4}$ | $5 \times 10^{-4}$ | $10^{-3}$ | $10^{-4}$ | $10^{-3}$ | $10^{-6}$ | $10^{-6}$ | $10^{-4}$ |
| BPS | 58.8 | 50.7 | 43.9 | 102.0 | 103.4 | 106.8 | 84.6 | 60.8 | 47.9 |
| | $\pm 9.52^\dagger$ | $\pm 13.92^* $ | $\pm 3.85^\dagger$ | $\pm 3.00$ | $\pm 17.25$ | $\pm 3.95$ | $\pm 1.10^\dagger$ | $\pm 1.83^\dagger$ | $\pm 4.52^\dagger$ |
| DSCG | 89.0 | 92.0 | 74.5 | 93.3 | 94.2 | 97.2 | 93.8 | 81.8 | 56.8 |
| | $\pm 9.24$ | $\pm 8.95$ | $\pm 6.75^\dagger$ | $\pm 2.91$ | $\pm 6.85$ | $\pm 1.75$ | $\pm 2.14^*$ | $\pm 2.95^\dagger$ | $\pm 4.12^\dagger$ |

Each value represents the mean ± SE of 4 experiments, except for 3 experiments with rat. *: Statistical significance from the control at p=0.05 and p=0.01 respectively.

Table 2. Heterologous PCA in guinea pigs and homologous PCA in rats

| Substance | Heterologous PCA | Homologous PCA |
|-----------|------------------|----------------|
|           | µg/site | % Inhibition | µg/site | % Inhibition |
| Control   | 20.4±7.67 | — | 8.2±0.14 | — |
| BPS       | 12.2±2.19 | 40.2 | 5.2±0.10 | 36.6 |
| DSCG      | 20.1±4.62 | — | 5.1±0.12 | 37.9 |

Each value represents the mean ± SE of 3 animals.

Table 3. Antigen-induced degranulation of mast cells in the mesenterium of rats

| Sensitized mesenterium | Active | Passive |
|------------------------|--------|---------|
|                        | % Degranulation | % Inhibition | % Degranulation | % Inhibition |
| Control                | 87.0±0.75 (6) | — | 42.0±3.01 (3) |
| BPS                    | 67.1±1.45 (4) | 22.9 | 17.7±4.82 (3) | 57.9 |
| DSCG                   | 84.1±0.88 (6) | — | 22.3±1.33 (3) | 47.0 |

Figures in parentheses indicate number of experiments.
tized in vitro were used for the antigen-induced degranulation study. The number of degranulated mast cells induced with DNP-As as the antigen was calculated by subtracting that of spontaneous degranulation from each control. The degranulation was approximately 90% and 40% in the controls of active and passive sensitizations, respectively, and the effect of drugs on the degranulation was expressed as a percentage to the control. As shown in Table 3, degranulation of mast cells in the actively sensitized mesenterium was inhibited about 20% with $10^{-3}$ gm/ml BPS, but was not affected by DSCG. In contrast, both BPS and DSCG in a dose of $10^{-4}$ gm/ml showed a potent inhibition of the degranulation in the passively sensitized mast cells, the rates being about 40 to 50% of that of the control.

**DISCUSSION**

The anti-allergic agent baicalein and a soluble derivative such as BPS do not inhibit the antigen-antibody combination and are not specific antagonists against chemical mediators released during anaphylaxis (1, 4-5). As with the original compound, the anti-allergic action of BPS may depend on the interference with the activation of SH-dependent enzymes following antigen-antibody reaction and inhibition of the mediator release would necessarily take place.

In the present work, BPS inhibited antigen-induced mediator release from actively sensitized guinea pig lung, while DSCG had a little effect. The antibody responsible for the mediator release in guinea pigs, presumable $7S_{r1}$, appears to be less in the characters of reaginic antibody as this antibody also provokes PCA in rats (19). It has been well established that when rats are immunized with DNP-As plus Bordetella pertussis, an antibody similar to human reagin can be produced (15), and a higher titre of antibody is obtained if splenectomy is done prior to the antigen treatment (20). The antigen-induced mediator release from the lung tissue of the hyperimmunized rats did not decrease with either BPS or DSCG, and degranulation of the mast cells in the mesenterium was only slightly inhibited with BPS. In contrast, a significant decrease was observed with both drugs in the degranulation of mast cells in the mesenterium passively sensitized with anti-DNP-As serum in vitro. If we consider that DSCG does not inhibit the antigen-induced mediator release from the leucocytes of allergic patients (13), and that a certain heat labile and homocytotropic IgG antibody is present in the serum of allergic patients (21-23), the reaction in rats sensitized with DNP-As and Bordetella pertussis may be attributed to other antibodies acting together with the reaginic antibody. A slight inhibition of mast cell degranulation by BPS in the actively sensitized rats suggests that the drug does act on other antibodies as well as on the reaginic antibody. The mediator release from monkey lung tissue sensitized with atopic serum was inhibited by BPS and DSCG to approximately the same degree. Such an inhibition was observed even with a low dose of $10^{-6}$ gm/ml. Thus, both drugs appear to have a similar affinity to the mast cell of monkey lung and human reagin. Patterson et al. (24) suggested that there may be differences in the population of mast cells between the respiratory organs and the skin, since DSCG does not show an inhibition in the mediator release from monkey skin sensitized with human reagin. Therefore, the reactivity of mast
cell to DSCG appears to vary according to the tissue. The mediator release by antihuman IgE from non-atopic human lung was inhibited with the pretreatment of BPS, but was little affected by DSCG. This reaction is presumed to be a reversed type anaphylaxis in the mast cell of lung tissue caused by IgG rather than IgE. Therefore, data obtained on the specific inhibitors of IgE-mediated response such as DSCG, when this reaction system is used may not be valid. BPS not only inhibits reaginic antibody-mediated PCA, but also that by the non-reaginic antibody. In contrast to BPS, DSCG depressed PCA caused by only the reaginic antibody. Thus there appear to be great differences between in vivo and in vitro as well as species in the reaginic antibody-mediated reaction. It should also be considered that drugs elevating cyclic 3', 5'-adenosine monophosphate levels in the cells inhibit the reaginic antibody-mediated reaction (8, 12, 25-27) and that BPS and DSCG depress anaphylactic mediator release independently of the cyclic AMP level (4, 8).

Table 4 summarizes our findings herein that BPS inhibits the reactions caused by both reaginic and non-reaginic antibodies. We have also confirmed that almost all monochromone derivatives such as baicalein show an inhibition of these reactions, though differences in the potency are evident (in preparation). On the other hand, DSCG has characteristic properties as an inhibitor of reaginic antibody-mediated reaction. It may thus be assumed that the functional site of reaginic antibody is well fixed with DSCG at a definite distance of

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**Table 4. Structure-activity relationship of chromone derivatives in the inhibition of immediate type allergic reactions**

| Substance | Antibody-mediated reaction |
|-----------|----------------------------|
|           | Reaginic | Non-reaginic(IgG) |
| [Chemical Structure of BPS] | + | - |
| [Chemical Structure of DSCG] | + | - |

+: inhibited, -: not inhibited

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**Fig. 3.** A postulation of the functional sites of reaginic and non-reaginic antibodies.
approximately 4.80 Å between the two chromone-nuclei, while the site of the non-reaginic antibody, IgG, is shorter or longer than that of reaginic antibody, and is readily fixed with the two molecules of baicalein or BPS and shown in Fig. 3. The partial inhibition of non-reaginic antibody-mediated reaction by a high dose of DSCG, as was seen in mediator release from guinea pig lung, may be due to effectiveness on one side of the functional site with one chromone of the drug. To support this hypothesis, studies are now underway using several related compounds.

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