EFFECTS OF SOME DRUGS ON CAPILLARY PERMEABILITY IN THE ANAPHYLAXIS OF THE MOUSE

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Experimental anaphylaxis has been described in most of the mammals, pigeons and frogs. The guinea pig is known to be particularly susceptible to anaphylaxis and it has been most extensively used in experimental studies on anaphylaxis. It is a fortunate choice since the physiological mechanism of anaphylactic shock in the guinea pig has been proved to be more similar to the manifestations of allergy in man than those of anaphylaxis in other laboratory animals (1). Passive cutaneous anaphylaxis and mortality rate in shock of the guinea pig are used for screening of anti-allergic drugs. Up to this time, several authors have reported the screening methods using the mortality rate in the mouse (2-4), but they estimated the efficacy only by all-or-none criteria, so that these methods seem to be lacking in accuracy. It was reported that anaphylaxis in the rat is associated with a marked increase in intestinal capillary permeability (5). On the other hand, the increase in capillary permeability of the mouse peritoneal cavity has been used for testing anti-inflammatory drugs (6). In this investigation this response is applied for quantitative estimation of the effects of some drugs including anti-inflammatory and anti-allergic drugs on the anaphylaxis of the mouse.

MATERIALS AND METHODS

Groups of eight to ten female ICR-JCL strain mice (obtained from Nihon CLEA, Ltd., Tokyo) weighing 18-27 g were used in most experiments. All animals were fed on a cube diet, allowed unrestricted drinking water, and housed at 22 ± 0.5°C.

Active sensitization: Mice were sensitized by an intraperitoneal injection of a homogenized mixture of 0.05 ml of Freund’s complete adjuvant and 0.05 ml of physiological saline containing 0.25 mg of bovine serum albumin (BSA) (Fraction V, The Armour Laboratories, U.S.A.). All animals used in the following experiments were sensitized, unless described as “non-sensitized”.

Passive sensitization: Normal mice were passively sensitized to BSA by an intravenous injection of 0.1 ml of the same strain mice anti-BSA serum per 10 g body weight. Twenty hours later anaphylactic shock was induced by means of challenging injection of BSA, as mentioned later. Control group received the same treatment except the injection of normal serum instead of the anti-BSA serum.

Experimental procedures: The anaphylactic reaction was induced by an injection
of BSA (50 mg/kg) intravenously or intraperitoneally after competent incubation period of sensitization. Control animals were treated similarly except that they had received no antigen or they were "non-sensitized".

1) The permeability test: Each sensitized animal was given an intravenous injection of Pontamine Sky Blue 6BX (200 mg/kg) in 2% solution; this was followed immediately by an intraperitoneal injection of BSA (50 mg/kg) in 0.5% solution. All dilutions of dye and antigen were prepared with 0.9% saline. After 30 minutes the mice were sacrificed by dislocation of the neck and 5 ml of saline was injected intraperitoneally. The abdomen was gently massaged for one minute. Thereafter the abdominal wall was incised over a funnel. The collected abdominal fluid was filtered through glass wool and centrifuged at 3000 rpm for 15 minutes. In order to clear any turbidity due to protein, 0.1 ml of 0.1 N-sodium hydroxide solution was added to each tube and the absorbance was measured at 570 mp. The amount of dye was expressed as μg/20 g of the mouse.

2) Rectal temperature: Rectal temperature was measured with thermometer (Type 1-63128, Natsume Ltd, Japan) at 15, 30, 60, 90 and 120 minutes after the challenging injection into the sensitized mouse.

3) Desensitization: Desensitization was accomplished by an injection of 100 mg/kg of the antigen subcutaneously to sensitized mice. A mouse that had recovered from severe but not fatal anaphylactic shock was rechallenged with the same antigen in dose of 50 mg/kg 24 hours after the desensitizing injection and the permeability and rectal temperature were measured as stated above.

4) Splenectomy: Splenectomy was performed under ether anesthesia through an incision of the skin in the dorsal left side. One day after the operation, the splenectomized mice were sensitized actively and then were challenged 21 days later with the antigen. The permeability and rectal temperature were measured as stated above.

5) Influences of rabbit anti-mouse lymphocytic serum (7): The spleen of the mouse was mashed by a spatula on a sieve of 200 mesh (0.074 m/m) and separated cells passed through it were suspended in Hank's balanced solution (adjusted at pH 7.6, without pehnol red but with heparin 100 μg/ml). The cell suspension was centrifuged at 1000 g for 5 minutes and the precipitant was resuspended and centrifuged at the same condition repeatedly. Thereafter cell suspensions (10^6 cells/ml) were emulsified in Freund's complete adjuvant and injected into the foot-pads of the male rabbit weighing from 2 to 2.5 kg. The rabbit was exsanguinated 3 weeks after and its serum was decomplemented by heating at 56 C for 30 minutes and kept frozen at −20 C. Normal rabbit serum (NRS) was obtained from an unimmunized rabbit and handled in the same fashion. Mice were sensitized actively with BSA and at the same time they received subcutaneous injection of 0.3 ml of the antiserum. A week later the same dose of the antiserum was injected, and 21 days after the sensitizing injection the shock was induced and measured as mentioned above.

6) Effects of drugs on the permeability in the anaphylaxis of the mouse: We examined the influences of various anti-inflammatory drugs on the permeability, such as aspyrin,
sodium salicylate, indomethacin, sulpyrine, phenylbutazone, oxyphenbutazone, mephenamic acid, flufenamic acid, chloroquine and cortisone acetate. Moreover we used anti-autacoids such as diphenhydramine, tripolidine, promethazine, chlorpromazine, inhibitors of kinin formation such as Trasylol (Bayer) and homochlorcyclizine and miscellaneous drugs such as reserpine and epinephrine. Some constituents of plants which are a saponin fraction of methanol extracts from platycodon root, "platycodin" (8-10), FM 100 extracted from licorice root (11) and saikosides obtained from root of Bupleurum falcatum L. were used in the permeability test. The inhibitory ratio of the drugs in the permeability test was calculated as follows;

$$\text{inhibitory ratio (\%)} = \left( \frac{\text{permeability of control group} - \text{permeability of test group}}{\text{permeability of control group}} \right) \times 100$$

The values following ± sign in tables and the vertical bars in figures are the standard errors of the means. The significance was calculated by using Student's t-test at 5% probability level.

Most drugs were administered orally 30 minutes prior to the challenge but in some cases they were given as cited in Table 5.

RESULTS

Anaphylactic shock after various incubation times of sensitization

The fall of rectal temperature of the mouse in anaphylactic shock was shown from 7 to 21 days after the sensitization but 1 and 3 days after the sensitization there was no change of the rectal temperature. The difference in the leakages of the dye between anaphylactic and control groups was significant (p < 0.05) at 11 and 21 days after the sensitization in comparison with smaller differences on the early days as shown in Fig. 1. Therefore we found that the most competent challenging time was 21 days after the sensitization in the permeability test in this study.

![Rectal temperature and leakage of the dye into the peritoneal cavity of the mouse in anaphylactic shock after various days of sensitization.](image)

Fig. 1. Rectal temperature and leakage of the dye into the peritoneal cavity of the mouse in anaphylactic shock after various days of sensitization. The vertical bars represent S.E.
Specificity of the permeability test and rectal temperature in anaphylactic shock

We used five experimental states; in state I the animals were challenged by the same antigen or BSA after the active sensitization and exhibited "anaphylactic shock", in state II it had no challenge or was treated with bovine gamma-globulin (BGG) instead of BSA after the active sensitization, in state III and IV the animals were treated with BSA and saline, respectively, but they were non-sensitized, and finally in order to examine the influence of adjuvant, in state V the animals were challenged with BSA after the treatment with BSA-free adjuvant treatment. Table 1 shows that the animals in state I which resulted in anaphylactic shock exhibited obvious fall of rectal temperature, but in other states there was no change. In the permeability test, only in state I the increased leakage of the dye was recognized (Table 2).

| Time after challenge (min) | Rectal temperature °C |
|--------------------------|------------------------|
|                          | State I*               | State II  | State III | State IV |
| Preinjection             | 37.2                   | 37.5      | 37.8      | 37.0     |
| 30                       | 33.2                   | 38.1      | 37.8      | 38.0     |
| 60                       | 30.6                   | 37.9      | 37.7      | 37.7     |
| 120                      | 30.9                   | 36.9      | 37.3      | 37.3     |

*On the state I to IV see the text

| State* | Leakage of dye (µg/20g body weight) |
|--------|-------------------------------------|
| State I| 91±12 (9)                           |
| State II| 45±3.2 (10)                         |
| State III| 47±6.1 (8)                          |
| State IV| 27±6.2 (10)                          |
| State V| 56±4.4 (9)                           |

*On the state I to V see the text.

Anaphylactic shock in passive sensitization

The fall of rectal temperature in the sensitized animals was about 5°C 60 minutes after the challenge while little change of the temperature was found in the control group. In the permeability test the leakage of the dye was 76.8 µg in the sensitized group, whereas it was 54.3 µg in the non-sensitized group; the difference was significant (P=0.05). It was 47.1 µg in the non-treated group (Fig. 2).

Effects of desensitization

A severe anaphylactic shock was observed by the injection of the antigen for desensitization and the fall of rectal temperature was about 6.5°C. Thereafter rectal temperature returned to a normal level gradually, and 24 hours later the complete restoration was seen in 8 out of 10 animals. These recovered animals did not indicate any fall of rectal temperature by rechallenging and in the permeability test the desensitized group showed
FIG. 2. Rectal temperature and leakage of the dye into the peritoneal cavity of the mouse in anaphylactic shock in passive sensitization. Non-sensitized animals (•—•) received normal rat serum and challenged with BSA. The vertical bars represent S.E.

FIG. 3. Effects of desensitization on the rectal temperature and leakage of the dye into the peritoneal cavity of the sensitized mouse. First injection of the antigen desensitized the animal which showed no anaphylactic shock with the 2nd challenge (•—••). Sensitized animals (○—○) showed definite anaphylactic shock. Normal rats did not indicate any anaphylactic shock (×••••×). The vertical bars represent S.E.
53 μg in leakage of the dye, whereas normal sensitized one showed 92 μg as seen in Fig. 3 and therefore the inhibitory effect of desensitization was indicated as 42%.

Effects of splenectomy and rabbit anti-mouse lymphocytes serum (RAMLS)

Table 3 shows the changes of rectal temperature in anaphylactic shock by the treatment of splenectomy and RAMLS injection. Some inhibitory effects on the fall of rectal temperature were seen in RAMLS treated mice but not in splenectomized mice. However, mortality rate was completely inhibited in both treatments as compared with that of the control group of ten animals, of which 2 animals died within 60 minutes and 2 animals during 60 to 90 minutes after the challenging. In the RAMLS treated animals a decline of wet weight of spleen was observed, namely, in RAMLS treatment it weighed 125 ± 4 mg per 20 g body weight while 150 ± 13 mg in NRS treated animals. In the permeability test slight reductions of leakages of the dye were observed in both treatments as shown in Table 4.

**TABLE 3. The changes of rectal temperature in anaphylactic shock of the mice splenectomized or treated with RAMLS.**

| Time after challenge (min) | Rectal temperature (°C)   |
|---------------------------|---------------------------|
|                           | a            | b            | c            | d            |
| Preinjection              | 37.8         | 38.3         | 38.6         | 38.2         |
| 15                        | 36.3         | 36.3         | 34.5         | 34.5         |
| 30                        | 33.3         | 33.7         | 32.0         | 32.0         |
| 60                        | 30.9         | 31.9         | 31.3         | 30.3         |
| 90                        | 30.3         | 31.9         | 30.3         | 30.3         |
| 120                       |              | 32.2         | 29.8         | 29.2         |

a. Splenectomized mice were sensitized with BSA and challenged with the antigen 21 days later.
b. Mice received two successive subcutaneous injections of rabbit anti-mouse lymphocytic serum (RAMLS). The shock was induced 21 days after the first injection of BSA and RAMLS.
c. Normal rabbit serum (NRS) was used instead of RAMLS and the shock was induced similarly with RAMLS treatment.
d. Control group was sensitized and challenged with BSA.

**TABLE 4. The changes of leakage of dye in anaphylactic shock of the mice splenectomized or treated with RAMLS.**

| Treatment         | Leakage of dye (μg/20 g body weight) |
|-------------------|--------------------------------------|
| Splenectomy       | 72 ± 8.5 (10)                        |
| Control           | 92 ± 11 (10)                         |
| RAMLS treatment   | 77 ± 7.3 (10)                        |
| NRS treatment     | 105 ± 9.6 (10)                       |

The number in the parenthesis means the number of the animals used. Details are described in Table 3.

Effects of some drugs in the permeability test

As shown in Table 5, aspirin and sodium salicylate showed the inhibitory effect of over 30% in the dosage of 300 mg/kg. Moreover, indomethacin, oxyphenbutazone and
cortisone acetate are also effective. The order of efficacy in four anti-autacoids in the dosage of 10 mg/kg was chlorpromazine > promethazine > diphenhydramine > triploridine. Trasylol which is known as an inhibitor of the enzymes such as kallikrein, showed about 40% of inhibitory effect in the dosage of 100 KIE/kg. Pretreatment of mice with 10 mg/kg of homochlorcyclizine markedly suppressed the leakage of the dye induced by shock. Significant inhibitory effects were seen with 400 mg/kg of platycodin, twice administrations of 5 mg/kg of reserpine, and 0.1 mg/kg of epinephrine.

| Table 5. Effects of various drugs on the capillary permeability in anaphylaxis of mice. |
|-----------------------------------------------|-----------------|-----------------|
| Treatment                          | Dose (mg/kg) | Inhibitory ratio (%) |
|-----------------------------------------------|-----------------|-----------------|
| **Anti-inflammatory drugs**                  |                |                |
| Aspirin                                     | 300            | 33.7*           |
| Na-salicylate                               | 100            | 23.4            |
| »                                            | 300            | 59.3*           |
| Indomethacin                                | 3              | 30.4*           |
| »                                            | 10             | 42.1*           |
| Aminopyrine                                 | 100            | 24.2            |
| Sulpyrine                                   | 100            | 13.2            |
| Phenylbutazone (a)                         | 100            | 28.8*           |
| Oxyphenbutazone (a)                        | 100            | 53.9*           |
| Mefenamic acid                              | 50             | 6.2             |
| Flufenamic acid                             | 50             | 22.8*           |
| Chloroquine                                 | 200            | 2.8             |
| Cortisone acetate (b)                      | 2 × 100        | 37.3*           |
| **Anti-autacoids**                          |                |                |
| Diphenhydramine                             | 10             | 35.5*           |
| Triploridine                                | 10             | 27.4*           |
| Promethazine                                | 10             | 39.7*           |
| Chlorpromazine                              | 3              | 51.7*           |
| »                                            | 10             | 41.7*           |
| Trasylol                                    | 30 (KIE/kg)    | 11.6            |
| »                                            | 100 (KIE/kg)   | 39.2*           |
| Homochlorcyclizine                          | 3              | 24.0*           |
| »                                            | 10             | 37.9*           |
| **Plant products**                          |                |                |
| Platycodin                                  | 400            | 53.1*           |
| Saikosides                                  | 400            | 27.4*           |
| FM 100                                      | 400            | 24.3            |
| Glycerrhetic acid-2K                       | 400            | 21.3            |
| **Miscellaneous drugs**                     |                |                |
| Reserpine (b)                               | 2 × 5          | 81.5*           |
| Epinephrine                                 | 0.1            | 30.5*           |

Most drugs were administered orally 30 minutes prior to the challenge.
(a) Drugs were administered orally 1 hour prior to the challenge.
(b) Drugs were administered orally 2 and 24 hours prior to the challenge.
*Significant at P < 0.05
DISCUSSION

In the present experiments, we found the increased capillary permeability in the peritoneal area by anaphylactic shock in the mouse, suggesting the possibility that the degree of leakage of the dye was considered as the intensity of shock. An increased capillary permeability in the early days after the sensitization as shown in Fig. 1 was considered to be caused by the irritability of Freund's complete adjuvant. This reaction was examined always in comparison with rectal temperature in anaphylaxis since descending of rectal temperature in shock was described in mice by Kind (12) and in guinea pig by Pfeiffer (13) and the phenomenon is well known as one of the symptoms in anaphylactic shock. Anaphylaxis is included in an immediate type of allergy which is characterized by passive transfer with serum from a sensitive animal or individual having a circulating antibody. In this study the result of increasing leakage of dye was seen in anaphylactic shock of the animals sensitized passively with antisera. A mouse that has recovered from a severe but not fatal anaphylactic shock will not react to subsequent injection of the same antigen for several days. This state of desensitization apparently reflects either depletion of antibodies or their saturation with antigen. In this state no changes in the permeability and rectal temperature were observed after the rechallenging as mentioned above. Thus the increasing leakage of dye may be convincingly accepted as one of criteria of anaphylactic shock by means of these immunological experiments. Much has been written about the function of the spleen in various aspects of the phenomenon of anaphylaxis. It has been claimed to be a site of antibody formation and its removal might modify the production of anaphylactic shock (14, 15). Sanyal and West (16) studied the role of the spleen in the guinea pig and the rat and found that in the former the protective action of splenectomy from active anaphylactic shock was noted after a period of sensitization for 10 weeks while no protection was observed against passive anaphylaxis. However, splenectomy failed to modify the development of anaphylactic shock in the rat. Recently Agnew (17) has reported that treatment of rats with a single subcutaneous injection of rabbit anti-rat thymocyte serum resulted in a marked depletion of small lymphocytes in the blood and thoracic duct lymph for approximately 10 days. Most of immunologically competent cells such as small lymphocytes and plasma cells which produce antibody exist in the lymph, spleen or thymus. Hence these techniques of breakdown of lymphocytes induced by antilymphocytic serum or splenectomy are widely used in order to inhibit the production of antibody and to reduce the shock. In our experiments the splenectomy and RAMLS treatment showed the complete inhibition of mortality rate. In the permeability test, however, only a slight inhibition was observed in both treatments. So it was assumed that the spleen in the mouse was not such an important site of antibody formation as it was in the guinea pig.

In the early state of inflammation, the exudation of plasma is induced by the increased capillary permeability and followed by the emigration of cells such as polymorphonuclear leucocytes into the surrounding tissue which suffers from the injury. These symptoms are very similar to such allergic reactions as seen in the papula of allergic patients which
are the exudative changes of the vascular and connective tissue. In the sense, the effects of some antiinflammatory drugs on this anaphylaxis in the mouse was tested.

Most of anti-inflammatory drugs used inhibited this anaphylactic reaction in the mouse except sulpyrine, mafenamic acid and chloroquine. Four plant products were tested and platicodin showed a distinct inhibitory effect on the reaction of the mouse anaphylaxis. Anaphylaxis in the mouse is associated with mast cell degranulation (18). The mast cells of the mouse contain both histamine and serotonin. Serotonin was implicated as a mediator by Fink (19) in the Schultz-Dale reaction of the mouse uterus because it was inhibited with lysergic acid diethylamide. Serotonin has also been implicated as the mediator by in vivo study of Gershon and Ross (20). They demonstrated that serotonin depletion by two different types of reagents, reserpine and methyl-DOPA, suppressed the anaphylactic reaction. On the other hand, Halpern et al. (21) reported that mepyramine and lysergic acid diethylamide, specific potent antagonists of histamine and serotonin respectively, slightly affected the anaphylactic reaction when each of them was injected. However, the anaphylaxis is almost completely abolished by the simultaneous injection of these two antagonists. It is suggested that the anaphylactic shock in the mouse is the result of simultaneous release of both mediators. Furthermore, the anaphylactic reaction is almost suppressed by 2-aminocaproic acid, an inhibitor of kinin forming enzymes, and this suggests that bradykinin must be also considered in shock (4). In the present report, the results of anti-autacoids, trasylol and homochlorcyclizine suggest that these mediators are implicated as the active substances to be released in the mechanism of anaphylaxis in the mouse. We also tested leakage of dye induced by intraperitoneal injection of some mediators as seen in Fig. 4. These results also supported the above consideration.

**Fig. 4.** Leakage of the dye into the peritoneal cavity of the mouse induced by intraperitoneal injection of some possible mediators.

The vertical bars represent S.E.
SUMMARY

Mice were sensitized actively with a homogenized mixture of Freund's complete adjuvant and BSA, and 21 days later they were challenged by intraperitoneal injection of BSA. Consequently, the fall of rectal temperature and increased capillary permeability in the peritoneal area were observed. Similarly, these changes were seen in passive sensitization. As a matter of fact, in desensitized state, no fall of rectal temperature and no increase of leakage of dye were seen. Furthermore, we examined the influence of the splenectomy and rabbit anti-mouse lymphocytic serum on anaphylaxis. The complete suppression of mortality rate in anaphylactic shock resulted from both treatments. On the other hand, a slight reduction in the leakage of dye and a slight inhibition of the change of rectal temperature in anaphylactic shock were observed in both treatments. Thus, we advanced the increased capillary permeability in the peritoneal area as a symptom of the anaphylactic shock in the mouse and applied it to a new screening method for antiallergic and anti-inflammatory drugs. It was shown that some clinically effective drugs such as aspirin, indomethacin and so on showed potent inhibitory effects in the permeability test. In addition, our results suggest that mediators such as serotonin and bradykinin may play an important role in the mechanisms of anaphylaxis in the mouse.

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