

# Effect of prednisone on spontaneous canine asthma

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*This study was designed to investigate the effect of corticosteroids in spontaneous ragweed-sensitive asthmatic dogs. Two animals were treated with prednisone, 40 mg. daily for 3 weeks, and with gradually tapered doses for 6 weeks. The animals were challenged with an aerosol or ragweed extract (RW) before and at intervals during and after drug therapy, and their skin reactivity to the antigen was determined at the same time. The Prausnitz-Küstner (PK) titer and the amount of serum necessary to passively transfer ragweed anaphylaxis to nonatopic dogs were also determined at the same intervals. Treatment of the atopic dogs with corticosteroids completely prevented the ragweed-induced asthma during and for up to 3 months after cessation of therapy. Although the skin reactivity of the atopic dogs was depressed, the PK titer of the sera did not change appreciably during therapy. Increased amounts of serum, however, were necessary to passively sensitize nonatopic dogs for ragweed anaphylaxis for up to 7 months after cessation of drug therapy. The results of the study indicate that corticosteroids can abolish active and passive allergic responses in dogs for as long as 7 months after cessation of the drug, but the mechanism of action is not clear.*

The beneficial clinical effect of prednisone on asthma is well known. Although its mechanism of action remains unclear, we were afforded an opportunity to study the drug's effect on asthma using spontaneously ragweed-sensitive asthmatic dogs. Physiologic and immunologic responses of the animals following ragweed challenge were measured before, during, and after corticosteroid therapy.

## MATERIALS AND METHODS

### Antigens

Ragweed extract (RW) consisted of equal parts of giant and short ragweed in buffered saline. Following protein nitrogen determination by the usual method, the RW was stored in the refrigerator in a concentration of 10,000 protein nitrogen units (PNU) per milliliter. Serial dilutions for testing were made on the same day that they were used.

### Experimental animals

Two adult female dogs (8.5 Kg. and 14.5 Kg., respectively) sensitive to ragweed were used for this study. Sensitivity to ragweed was demonstrated by the presence of immediate

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Supported by Training Grant AI 316 from The National Institute of Allergy and Infectious Disease.

Received for publication April 9, 1973.

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TABLE I. Direct skin tests and passive transfer studies

|             | Dog 1   |                   | Dog 2  |                  |
|-------------|---------|-------------------|--------|------------------|
|             | Direct* | Passive transfer† | Direct | Passive transfer |
| Control     | 1       | 1:2048            | 1      | 1:512            |
| Drug period |         |                   |        |                  |
| 40 mg.‡     | 100     | 1:256             | 100    | 1:128            |
| 20 mg.      | 100     | 1:256             | 100    | 1:256            |
| 10 mg.      | 100     | 1:256             | 100    | 1:512            |
| 5 mg.       | 100     | 1:1024            | 100    | 1:256            |
| Post-drug   |         |                   |        |                  |
| 2 weeks     | 1       | N.D.              | 1      | N.D.             |
| 4 weeks     | 1       | 1:1024            | 1      | 1:512            |

\*Direct skin tests recorded as the least amount of ragweed (in PNU) required to elicit a positive skin test.

†Passive transfer titers are expressed as the highest dilution of serum eliciting a positive test following challenge with antigen.

‡Amount of prednisone administered per day.

N.D.: not done.

wheel-and-flare reactions following intradermal challenge with RW and the induction of acute asthma in these dogs after insufflation with RW in a closed chamber, as described previously.<sup>1</sup> In addition, serum from these dogs was capable of transferring the immediate wheel-and-flare reactions to the skin of nonatopic dogs, subsequently challenged by local or intravenous injection of RW. Systemic anaphylaxis could be demonstrated in nonsensitive dogs challenged intravenously with ragweed 24 hours after they were passively sensitized by the intravenous injection of 0.25 to 0.5 ml. per kilogram body weight of serum from the experimental animals. Recipient dogs were small dogs (8 to 12 Kg.) to conserve the large amounts of serum required.

### Experimental design

Both dogs received oral prednisone for a total of 9 weeks as follows: 40 mg. per day for 3 weeks, 20 mg. per day for 2 weeks, 10 mg. per day for 2 weeks, and 5 mg. per day for 2 weeks. Studies were done prior to the onset of drug therapy, during each dosage period, and every 2 to 5 weeks for 28 weeks after discontinuance of the drug.

Two nonatopic dogs were given prednisone in a dosage of 40 mg. per day for 4 weeks and were passively sensitized with atopic sera.

### Direct skin tests

Each atopic dog was tested every 2 weeks during the drug treatment and every 2 to 5 weeks after discontinuance of the drug. Testing was performed by the intradermal technique using RW in the concentration of 1,000, 100, 10, 1, and 0.1 PNU per milliliter, after which Evans blue dye was given intravenously, as described previously.<sup>1</sup> The reactions were considered positive if a definite bluing response occurred within 20 minutes after injection of antigen.

### Passive transfer studies

*Serum studies.* Sera were collected at the same time intervals from both atopic dogs and stored at 4° C. until used. The sera were tested within 2 days of collection. The skin-sensitizing antibody titer of each serum was determined by the passive transfer technique, utilizing dogs previously determined to be nonsensitive to RW. Twofold serial dilutions of 0.02 ml. of serum were injected intradermally in duplicate in each of 2 dogs. The passively sensitized sites were challenged 24 hours later by the intradermal injection of 0.03 ml. of RW in a concentration of 1,000 PNU per milliliter, followed by the intravenous administration of Evans blue dye. Control sites consisted of serum from nonatopic dogs. The titer of each serum was determined to be the weakest dilution giving a positive test. Dogs were used for passive transfer only once.

**TABLE II.** Passive anaphylaxis—anaphylaxis induced in nonsensitive dogs by passive transfer of varying amounts of sera from the atopic dogs.

|                                  | Dog 1 | Dog 2 |
|----------------------------------|-------|-------|
| Control                          |       |       |
| 0.25 ml.*                        | 1/3†  | 3/6   |
| 0.5 ml.                          | 20/20 | 8/8   |
| Drug period (mg. prednisone/day) |       |       |
| 40 mg.                           |       |       |
| 1.5 ml.                          | 1/5   | 0/6   |
| 2.0 ml.                          | 1/3   | 0/4   |
| 2.5 ml.                          | 0/2   | 0/2   |
| 3.5 ml.                          | 2/2   | 2/2   |
| 20 mg.                           |       |       |
| 1.5 ml.                          | 0/4   | 0/4   |
| 3.5 ml.                          | 2/2   | 2/2   |
| 10 mg.                           |       |       |
| 1.5 ml.                          | 0/3   | 0/4   |
| 3.0 ml.                          | 0/2   | 0/2   |
| 3.5 ml.                          | 2/2   | 2/2   |
| 5 mg.                            |       |       |
| 0.5 ml.                          | 0/2   | 0/2   |
| 1.5 ml.                          | 1/5   | 1/5   |
| 2.5 ml.                          | 2/4   | 2/3   |
| Post-drug                        |       |       |
| 2 weeks                          |       |       |
| 1.5 ml.                          | 0/3   | 0/3   |
| 4-6 weeks                        |       |       |
| 1.5 ml.                          | 0/2   | 0/2   |
| 9 weeks                          |       |       |
| 0.5 ml.                          | 0/2   | 0/2   |
| 13 weeks                         |       |       |
| 0.5 ml.                          | 0/1   | 0/1   |
| 16 weeks                         |       |       |
| 0.5 ml.                          | 0/2   | 0/2   |
| 1.0 ml.                          | 1/2   | 1/1   |
| 20 weeks                         |       |       |
| 0.5 ml.                          | 1/1   | 0/1   |
| 1.0 ml.                          | 0/1   | N.D.  |
| 23 weeks                         |       |       |
| 0.5 ml.                          | 0/2   | 0/2   |
| 1.0 ml.                          | 0/2   | 0/2   |
| 28 weeks                         |       |       |
| 0.5 ml.                          | 0/2   | 0/2   |

\*Amount of serum infused into nonatopic dogs (in ml./kg. body weight).

†Dogs exhibiting anaphylaxis/number of dogs challenged.

N.D.: not done.

**TABLE III.** Inhalation challenge—results and time in minutes exposed for each dog during periods of study

|                        | Dog 1        | Dog 2        |
|------------------------|--------------|--------------|
|                        | No. min.     | No. min.     |
| Control period         | Asthma 5     | Asthma 5     |
| Corticosteroid therapy | No asthma 60 | No asthma 60 |
| Post-drug period       |              |              |
| 2-12 weeks             | No asthma 60 | No asthma 60 |
| 13 weeks               | Asthma 60    | No asthma 60 |
| 16 weeks               | Asthma 30    | Asthma 45    |
| 20 weeks               | Asthma 17    | Asthma 30    |
| 23 weeks               | Asthma 10    | Asthma 15    |
| 28 weeks               | Asthma 10    | Asthma 15    |
| 52 weeks               | Asthma 5     | Asthma 5     |

### **Passive systemic anaphylaxis**

Sera collected at the same intervals from the sensitive dogs were administered intravenously in duplicate or triplicate to nonsensitive dogs in amounts ranging from 0.25 to 3.5 ml. per kilogram body weight. The 2 nonatopic dogs who received prednisone for 4 weeks were given 0.5 ml. per kilogram of atopic dog serum collected before therapy. The passively sensitized dogs were challenged 24 hours later with RW, given intravenously in a dosage of 5,000 PNU per kilogram body weight. They were observed for the next hour for evidence of anaphylaxis manifested by lethargy, vomiting, hematemesis, diarrhea, and tenesmus. Lethargy and vomiting along with diarrhea or tenesmus were considered an anaphylactic reaction. Recipient dogs were used only once for passive transfer because of the possibility of sensitization to the donor sera.

### **Inhalation challenge**

The naturally sensitive dogs were placed in a closed chamber at intervals, and RW was nebulized in a concentration of 10,000 PNU per milliliter for periods of time ranging from 5 to 60 minutes. The dogs were removed from the chamber and observed for evidence of asthma manifested by wheezing and obvious dyspnea. When asthma was moderately severe, the animals were given 0.3 ml. of 0.1% epinephrine subcutaneously.

## **RESULTS**

### **Direct skin tests**

The results of the studies are summarized in Tables I, II, and III. During the control period both atopic dogs demonstrated a positive skin test to RW in a concentration of 1 PNU per milliliter, and control sites with nonatopic sera were negative. During drug therapy the positive tests could be demonstrated only with a concentration of 100 PNU per milliliter. Within 2 weeks after discontinuation of the corticosteroids, the skin sensitivity returned to the control level and remained there for the remainder of the study.

### **Passive transfer studies**

In the case of Dog 1, there appeared to be a decrease in PK titer for 40 mg. prednisone, however, in Dog 2 this was less evident. The effect persisted throughout the drug period and returned to pretreatment levels when measured 4 weeks after cessation of therapy. All control sites were negative.

### **Passive systemic anaphylaxis**

During the control period, all passively sensitized dogs developed anaphylaxis following RW injection when given 0.5 ml. per kilogram of serum from the untreated atopic dogs. During the drug periods and for the entire post-drug period, larger doses of sera were necessary for the same amount of antigen to induce anaphylaxis. The prednisone-treated and passively sensitized nonatopic dogs also failed to demonstrate anaphylaxis after challenge.

### **Inhalation challenge**

Both atopic dogs had moderate asthma requiring epinephrine after 5 minutes of exposure during the control period, but no asthma was observed in either dog after 60 minutes of exposure during the corticosteroid period and for a period of up to 13 weeks after discontinuance of the drug. Both dogs gradually in-

creased in degree of ragweed sensitivity, and at the conclusion of the study they were again quite responsive to challenge. One year after the study revealed that they had returned to pre-drug therapy levels. White blood counts and hematocrits of these dogs remained at a normal level throughout the study. There were no weight changes noted throughout the study.

## DISCUSSION

Although the exact mechanism of action of corticosteroids in asthma is still unclear, a number of mechanisms of action of the drug have been postulated by Aviado.<sup>2</sup> These include: (1) nonspecific anti-inflammatory effect, (2) inhibition of antibody formation, (3) inhibition of the storage or formation of histamine, (4) interference with the parasympathetic bronchoconstriction, and (5) direct smooth muscle relaxation. If the metabolism of prednisone in dogs is similar to the short half-life demonstrated in man,<sup>3</sup> it would be difficult to explain the persistence of the inhibition of asthma demonstrated in these dogs. However, little is known about corticosteroid metabolism in dogs. If an orally administered drug is metabolized slowly, small but persistent amounts may be sufficient to augment the catecholamines, especially epinephrine, which would protect against the immunologically induced bronchospasm. There are indications that adrenal steroids potentiate the circulatory response to vasoconstrictors, particularly catecholamines.<sup>4</sup> The suppression of the direct skin tests and passive transfer titers was transient and not complete. Dougherty<sup>5</sup> in 1954 reported that adrenocortical hormones generally do not inhibit the release of histamine resulting from antigen-antibody union, though they may suppress the tissue response to histamine. On the other hand, Middleton and Radcliffe<sup>6</sup> demonstrated that high doses of hydrocortisone partially inhibit histamine release from whole leukocyte suspension in ragweed-sensitive subjects. If this can be correlated with skin reactivity then it may explain the decrease in skin and serum reactivity observed in the present study. The wheal-and-flare reaction measured 1, 2, 8, and 24 hours after intradermal injection of antigen was not inhibited by the prior administration of 40 mg. of prednisone given to atopic patients, according to Galant and associates.<sup>7</sup> In human atopic disease, the seasonal rise in IgE following ragweed exposure is inhibited by corticosteroids, as shown by Henderson, Larson, and Gleich.<sup>8</sup> If this has a suppressive effect on IgE production, there may be a lag in its reaccumulation, which is manifested by a persistent inhibition of asthma. Clinically, we see asthmatics who seem to do well for a prolonged period of time after a short course of corticosteroids. However, we usually ascribe this to a lack of precipitating or antigenic factors. Possibly a similar phenomenon occurs in man, as has been demonstrated in the present canine study.

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