THE PREDOMINANCE OF FLUNISOLIDE IN THE TOPICAL USE OF ANTI-INFLAMMATORY STEROIDS

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Local and systemic anti-inflammatory effects of topically applied flunisolide on hind footpad edema induced by subplantar injection of 1 μg of serotonin in mice were evaluated in comparison with those of three related steroids. The order of the potency for local anti-inflammatory effect observed in the foot injected with the steroids was flunisolide, dexamethasone, betamethasone valerate and beclomethasone dipropionate in decreasing order. In contrast, potency for systemic effect of flunisolide observed on the opposite foot was rather weak compared with its strong local effect. When steroids were administered orally, the order of the anti-inflammatory potency was dexamethasone, beclomethasone dipropionate, flunisolide and betamethasone valerate in decreasing order.

All of the data demonstrate that flunisolide is highly active in topical use, while systemically it is relatively weak, especially by oral administration. Those characteristics of flunisolide could be attributable to its rapid metabolic inactivation in the liver, which suggests a great advantage of its topical use in the clinical medicine.

Keywords — flunisolide, topical corticosteroid therapy, serotonin-induced inflammation, betamethasone valerate, beclomethasone dipropionate, dexamethasone, mouse

INTRODUCTION

Topical corticosteroid therapy is generally thought to have the advantage of maximum local and minimum systemic effects and has been applied with success in the treatment of dermatologic diseases, bronchial asthma and allergic rhinitis.

A synthetic glucocorticoid flunisolide, 6α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione-16,17-acetonide, has been shown to be more than 180 times as potent as hydrocortisone in its systemic anti-inflammatory activity,1) and is reported to be useful, without causing demonstrable adrenal suppression or local toxicity, in the topical treatments of allergic diseases in upper airway in the form of spray or inhalation.2,3

In an attempt to evaluate aptitude of flunisolide for the topical glucocorticoid therapy in comparison with some related steroids, experiments in order to compare local and systemic anti-inflammatory effects of these steroids were carried out by means of mouse footpad edema method using serotonin which is known as a chemical mediator of vascular permeability response in mice and rats.

MATERIALS AND METHODS

Drugs and Chemicals—Flunisolide (Syntex Co., Palo Alto, Calif., U.S.A.), betamethasone valerate (Nihon Shoji Co. Ltd., Osaka), beclomethasone dipropionate (Ko-Ei Kagaku Sangyo Co. Ltd., Tokyo), dexamethasone (Sigma Chemicals Co., St. Louis, Mo., U.S.A.), carboxymethylcellulose sodium (CMC-Na; CelogenR F-3H, Dai-Ichi Kogyo Seiyaku Co. Ltd., Niigata),

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streptomycin sulfate (Kaken Kagaku Co. Ltd., Tokyo), penicillin G potassium (Meiji Seika Co. Ltd., Tokyo), and serotonin creatinine sulfate complex (Tokyo Kasei Kagaku Co. Ltd., Tokyo) were used. All other chemicals were purchased from Wako Pure Chemicals Ltd. (Tokyo).

**Serotonin-induced Edema on the Footpad of Mice**—Male DDY mice (5–7 weeks old: Funabashi Farm, Chiba) were kept before use under well-controlled environment and maintained with tap water and laboratory food (Funabashi Farm) _ad libitum_. Footpad edema was induced with serotonin as previously described.4) Experiments of the footpad edema were carried out under well controlled room temperature of 24±1°C, since development of the footpad edema is sensitive to room temperature.

In the experiments of local administration of anti-inflammatory steroids, which were injected into the footpad as suspensions in a volume of 5 μl per paw, the footpad edema was induced on each hind foot of mice by subplantar injection of

![Graph](image)

**FIG. 1. Dose-response Relationship of Footpad Edema induced with Serotonin in Mice**

Each column represents the mean of 7 animals. Vertical bars show standard error of the mean. Footpad edema is expressed as the increase in footpad thickness measured 12 min after the injection of 5 μl of serotonin solution in the footpad.

![Graph](image)

**FIG. 2. Time Course for the Manifestation of Local and Systemic Anti-inflammatory Effects of locally administered Flunisolide on the Footpad Edema in Mice induced with Serotonin**

Each point represent percent of inhibition calculated from the mean increase in the footpad thickness of control and treated groups. n = 5–6.

In the treated group flunisolide suspension (5 μl) was locally administered in subplantar tissue of a hind foot prior to the serotonin injection, and the vehicle to the opposite foot. In the control group the vehicle was injected in both side of the feet. Inhibitory effect of flunisolide observed in the flunisolide-treated foot was expressed as local effect, and that in the vehicle-treated foot as systemic effect.
5 μl of a solution of serotonin creatinine sulfate in the Tyrode's solution. The pH of the solution was adjusted to 7.4. A microsyringe was used to inject the correct volume of the serotonin solution. Increase in the footpad thickness was measured 12 min after the serotonin injection with a dial gauge calliper (Peacock G, Ozaki Seisakusho Co., Tokyo) calibrated with the graduations of 1/100 millimeter, and expressed in terms of the difference between thickness values measured before and after the serotonin injection. Local anti-edema effect of a steroid was evaluated at the site of its injection, while its systemic anti-edema effect was determined with a footpad of the opposite side in which the vehicle was injected instead of the steroid suspension.

In the experiments of oral administration of anti-inflammatory steroids, serotonin was injected in one hind footpad and the Tyrode's solution was injected in the opposite footpad. Footpad edema was expressed in terms of the difference in the increase of the footpad thickness between serotonin-injected and vehicle-injected feet.

Suspensions of the steroids for local injection were prepared with 0.2% CMC-Na in the Tyrode's solution (pH 7.4) with 0.1 mg/ml of streptomycin and penicillin with the aid of an agate mortar and pestle and a sonicator (Branson, Branson Co., or UR-20P, Tomy Seiko Co. Ltd., Tokyo). Suspensions of the steroids for oral administration were prepared similarly with 0.5% CMC-Na in distilled water in the absence of antibiotics and were administered in a volume of 0.1 ml/10 g body weight.

RESULT
Dose-response Relationship for the Footpad Edema induced with Serotonin
Increase in the footpad thickness was dose-dependent in the dose range of serotonin from 0.3 to 3.0 μg as shown in Fig. 1. The dose of serotonin to yield submaximal response, i.e. 1 μg as the net amount of serotonin, was used throughout the following experiments for quantitative evaluation of anti-edema effects of steroids.

![Graph showing dose-response relationship](image)

**FIG. 3.** Inhibition by Local Injection of Flunisolide of Footpad Edema induced with Serotonin in Mice
Filled and open columns represent respectively the data of flunisolide-treated foot and the opposite foot. The footpad edema was induced with serotonin 4 h after local injection of flunisolide. Definition of local and systemic effects: See Fig. 2.
Each column shows the mean of 7 to 8 animals. Vertical bars show the standard error of the mean.
**Local Anti-Edema Effects of Flunisolide**

**Time Course Study on the Inhibitory Effect of Locally Administered Flunisolide on the Serotonin-induced Footpad Edema**

Time course study on the anti-edema effect of flunisolide was performed in order to determine the optimal time relation between serotonin injection and steroid treatment. In this experiment two dose levels of flunisolide, 0.1 μg and 3.0 μg per paw, were selected based on the results of preliminary experiments.

As summarized in Fig. 2, 0.1 μg per paw of flunisolide was shown to be effective in inhibiting serotonin edema in the flunisolide-treated side over the period of 2–6 h after the steroid treatment, exerting maximum effect at 4 h. Three microgram of flunisolide exerted moderate inhibitory effect on the footpad edema even in the opposite side to the local injection over

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**Fig. 4. Dose-response Relationship of Anti-inflammatory Effects of locally administered Flunisolide and Related Steroids on Footpad Edema induced with Serotonin in Mice**

Filled circles and solid lines represent the local effects and open circles and broken lines show the systemic effects. Definition of local and systemic effects: See Fig. 2. Each point shows percent inhibition calculated from mean increases in the control and treated groups of the footpad thickness. n = 4–8. The lines show the regression lines computed out from the values of percent inhibition. The data for flunisolide are the same as those in Fig. 3.
the same period as in the injected side. The inhibitory effects reached maximum at 4 h and then levelled off. Therefore, in the experiments of local application of the steroids animals were treated with the steroids 4 h prior to the induction of serotonin-footpad edema.

**Dose-response Relationship in the Anti-edema Effects of Locally Administered Steroids**

Flunisolide in the dose range of 0.03 to 30 μg per paw exerted dose-dependent inhibitory effects on the footpad edema (Fig. 3). The local effects observed in the flunisolide-injected side were gradually enhanced in parallel with the increase of the dose from 0.03 up to 3.0 μg per paw. The edema was inhibited by 75.5% at the dose of 3.0 μg per paw. The systemic effects as demonstrated in the opposite footpad injected with the vehicle only, were also exhibited in a dose-related manner over the range of 0.3 to 3.0 μg per paw. The systemic effects were always weaker than the local effects over the full range of the dose examined. The anti-edema effects of three related steroids, betamethasone valerate (0.03—100 μg/paw), beclomethasone dipropionate (0.03—100 μg/paw) and dexamethasone

![Graph](image)

**FIG. 5. Time Course for the Manifestation of Anti-inflammatory Effects of orally administered Flunisolide and Betamethasone Valerate on the Footpad Edema induced with Serotonin**

Each point represents percent inhibition calculated from the mean increase in the footpad thickness of control and treated groups. n= 5—6.

Flunisolide and betamethasone valerate were administered orally at the dose of 10 mg/kg prior to serotonin edema assay.

![Graph](image)

**FIG. 6. Dose-response Relationship of Anti-inflammatory Effects of orally administered Flunisolide (Flu, ●), Betamethasone Valerate (BV, ▲), Beclomethasone Dipropionate (BD, ■), and Dexamethasone (Dex, ▼) on Footpad Edema induced with Serotonin in Mice**

Each point means percent inhibition calculated from mean increases of footpad thickness in control and experimental groups. n= 5—9. Each line shows the regression line computed out from the values of percent inhibition.
(0.16–20 µg/paw), were also examined (Fig. 4). The potency for the local effects was higher than that for the systemic effects in all of those steroids.

**Time Course Study on the Anti-edema Effects of orally administered Flunisolide and Betamethasone Valerate**

Flunisolide at a dose of 10 mg/kg exerted apparent anti-inflammatory effect over the period of 4 to 8 h after its administration with a maximum effect at 6 h (Fig. 5). Betamethasone valerate at a dose of 10 mg/kg showed an inhibitory effect 6 h after its administration (Fig. 5). Therefore, steroids were administered in the following experiments of oral administration 6 h prior to the serotonin injection into the footpad.

**Dose-response Relationship in the Anti-edema Effects of orally administered Steroids**

Oral administration of flunisolide (0.3–30 mg/kg), betamethasone valerate (0.3–30 mg/kg), beclomethasone dipropionate (0.03–10 mg/kg) and dexamethasone (0.1–10 mg/kg) inhibited serotonin footpad-edema in a dose-dependent manner (Fig. 6). Regression lines of the dose response were almost parallel with each other.

**DISCUSSION**

When glucocorticoids are applied locally in the treatment of allergic dermatitis, allergic rhinitis and bronchial asthma, various systemic influences of the steroids could be manifested as untoward side effects. It is important, therefore, to select appropriate species of the steroid in order to minimize systemic effects in the topical glucocorticoid therapy. In the present experiments local therapeutic activity of the steroids was evaluated in terms of anti-inflammatory efficacy observed at the site of the local application. The values of ID₅₀ for the local anti-edema effect, ID₅₀ local, of the four steroids tested were found on the dose-response lines in Fig. 4 and tabulated in the first line in Table I. The data indicate that flunisolide is the most potent local anti-inflammatory steroid among those tested in the present study. The potency of the local effect was in the order of flunisolide > dexamethasone > betamethasone valerate > beclomethasone dipropionate.

Systemic influence of the steroid caused by the topical therapy was evaluated by introducing two kinds of parameters derived from the data of Fig. 4, as shown in the second and the third

<table>
<thead>
<tr>
<th>Table 1. Comparison of Local and Systemic Manifestations of Anti-inflammatory Activity among Flunisolide and Related Steroids</th>
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<tbody>
<tr>
<td>Flunisolide</td>
</tr>
<tr>
<td>ID₅₀ local (µg/paw)</td>
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<tr>
<td>Local effect (%inhibition) at threshold dose for systemic effect</td>
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<tr>
<td>Systemic effect (%inhibition) on local injection at the dose of ID₅₀ local</td>
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</tbody>
</table>

*a) The values of ID₅₀ in the local treatment with the steroids were found from the regression lines on Fig. 4.
*b) Each figure represents the value found from Fig. 4 for anti-edema effect to be manifested at the site of the local injection of the steroid at the threshold dose for the systemic effect which is observed as anti-edema effect at the footpad opposite to the foot injected with the steroid. The threshold dose is defined as the intersection of the horizontal axis with the dose-response line (broken lines in Fig. 4) for the systemic effect.
*c) Figures represent the values found from Fig. 4 for anti-edema effects at the footpad opposite to the injected side when each drug at the dose of ID₅₀ local be administered.
lines in Table I. The second line indicates local anti-edema effects of the steroids at threshold doses for accompanying systemic effect which is evaluated through anti-edema effect in the untreated foot. In other words those values indicate the intensity of the local effects manifested by the steroids at maximum local doses within the dose limit of yielding no accompanying systemic anti-edema effect, suggesting that flunisolide and betamethasone valerate are capable of yielding definite local anti-inflammatory effect without systemic anti-edema effect. The third line in Table I indicates systemic effect to be manifested as anti-edema effect in the footpad opposite to the foot injected with the steroid at the dose of ID\textsubscript{50} local. Those data are also suggesting that flunisolide and betamethasone valerate are more promising for local corticosteroid therapy than beclomethasone dipropionate and dexamethasone.

When glucocorticoids are administered locally by inhalation in the treatment of respiratory diseases such as bronchial asthma, a portion of the drug is swallowed usually in the stomach. This may cause various lines of systemic influence as the side effects. In order to evaluate such a possibility, experiments of oral administration of flunisolide and three related steroids were performed to examine potency of these steroids in the induction of the systemic effect as reflected by inhibitory effect to serotonin-induced footpad edema in mice. From the results of those experiments summarized in Fig. 6, the values of ID\textsubscript{50} oral are found and tabulated in the first line in Table II. Utility of the steroids in the topical therapy should be evaluated by their ability in exerting local effect but also by their inertness in causing systemic effects. Based on the above concept, (ID\textsubscript{50} oral)/(ID\textsubscript{50} local) are calculated and tabulated in the third line in Table II, indicating that flunisolide is the most suitable among four steroids tested.

Turning to the problem of experimental method, the serotonin-induced footpad edema method in mice was first introduced in our laboratory for the study on the mechanism of anti-inflammatory action of glucocorticoids.\textsuperscript{4)} In the present study this method was applied to evaluate therapeutic usefulness in the topical application of the steroids. Local injection of a suspension of the steroids at the site of the induction of serotonin edema in the foot has the advantage to guarantee quantitative administration of the drug compared with other methods such as application of ointments on the skin. In addition, the present method enabled us to evaluate systemic influence of topical glucocorticoid therapy by measuring the same type of response on the foot opposite to the treated side.

Potency of flunisolide in exerting systemic influence has been demonstrated to be comparatively weak especially when administered orally (Table II), while its local anti-inflammatory action is very strong (Table I). Such dissociation

<table>
<thead>
<tr>
<th></th>
<th>Flunisolide</th>
<th>Betamethasone valerate</th>
<th>Beclomethasone dipropionate</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID\textsubscript{50} oral\textsuperscript{a)} (mg/kg)</td>
<td>7.18</td>
<td>15.0</td>
<td>1.71</td>
<td>0.69</td>
</tr>
<tr>
<td>ID\textsubscript{50} local\textsuperscript{b)} (µg/paw)</td>
<td>0.18</td>
<td>2.26</td>
<td>2.89</td>
<td>0.72</td>
</tr>
<tr>
<td>(ID\textsubscript{50} oral)/(ID\textsubscript{50} local)</td>
<td>39.9</td>
<td>6.6</td>
<td>0.6</td>
<td>1.0</td>
</tr>
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\textsuperscript{a)} Values of ID\textsubscript{50} oral were found from the regression lines on Fig. 6.

\textsuperscript{b)} These values were transferred from the first line of Table I.
of systemic and local potency of flunisolide may be explained by high susceptibility of this steroid to metabolic inactivation in mice and primates involving human.\textsuperscript{1,2} Since metabolic degradation of flunisolide in rats is reported to be relatively inactive compared to that in mice and human,\textsuperscript{1,3} mouse is thought to be a suitable species in carrying out the present type of experiments.

REFERENCES


