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Solubility and Distribution of Dexamethasone Acetate in Oil-in-Water Creams and Its Release from the Creams[1)]

SHIGEO TANAKA,*,[a] YASUJI TAKASHIMA,[a] HIROSHI MURAYAMA,[a] and SEISHI TSUCHIYA[b]

Research Laboratory, Taisho Pharmaceutical Co., Ltd.,[a] 1–403 Yoshino-cho, Omiya, Saitama 330, Japan and Tokyo College of Pharmacy,[b] 1432 Horinouchi, Hachioji, Tokyo 190–03, Japan

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Oil-in-water creams having various capacities to dissolve dexamethasone acetate (DA) were prepared by changing the concentration of polysorbate 80. The solubility of DA in the creams and the distribution of DA in each phase of the creams were examined to see how they influenced DA release from the creams. The solubility of DA in the creams was obtained by calculation from the solubility of DA in each component of the cream. These results on DA release were compared with data on the human vasoconstrictor activity of these creams.

In creams of low DA concentration, DA was solubilized, and the DA release rate decreased as the concentration of polysorbate 80 increased, being inversely proportional to the solubility of DA in the cream. On the other hand, in creams of high DA concentration, DA was present partially in the soild state, and the DA release rate increased as the concentration of polysorbate 80 increased. The release rate was dependent on the ratio of solubilized DA in the cream.

However, vasoconstrictor activity was dependent on the concentration of free DA in the aqueous phase of the cream.

Keywords——dexamethasone acetate; oil-in-water cream; release rate; solubility; suspension; distribution; vasoconstrictor activity

It is generally recognized that the efficacy of topical pharmaceuticals may be influenced by the formulation and the state of the drug in the base. Topical pharmaceuticals may therefore need to have designed formulations.[2)]

Frequently, ointments and creams have been prepared by suspending drugs in the bases, depending on the solubility or the drug stability of the drugs concerned. Theoretical treatment of the drug release rate from ointments containing drugs in suspension[3)] and simulation techniques for analyzing the diffusion of drugs and the dissolution rate of solid drugs in ointments[4)] have been reported. However, in suspension-type ointments, it was reported that crystalline conversion and the growth of drug crystals in the ointment reduced the amount of drug released from the ointment.[5)] Malone *et al.*[6)] demonstrated that fluclorone acetonide entirely solubilized in ointments and creams showed superior drug release and vasoconstrictor activity as compared with the drug in suspension. Another study dealing with FAPG®, which contains fluocinonide, was reported.[7)]

The relationship between the distribution of fluocinolone acetonide solubilized in oil-in-water creams and its release from the creams has been reported in a previous paper.[8)] In this study, dexamethasone acetate (DA), which has a lower solubility than fluocinolone acetonide, was chosen as a model drug, and the ratio of solubilized DA in the creams and the drug release rate were investigated. The results were compared with those on vasoconstrictor activity, which has been reported to parallel the clinical efficacy.[9)]

Experimental

Material——The DA used was of pharmacopoeial grade (USP XX), as were the other components of the creams

(JP X). Other materials used were of reagent grade.

Preparation of Creams——The formulae of the tested creams are listed in Table I. The cream base was prepared by adding purified water (heated to 70 °C) to the oil components and surfactant at the same temperature. The mixture was cooled to room temperature with stirring. DA crystals (1—5 μm) were incorporated in a part of the base and this was added to the residual base through a 200 mesh sieve. The cream obtained was mixed thoroughly, and used after storage for 2 weeks in an incubator at 30 °C.

Solubility of DA in the Cream Components——Excess DA crystals were added to a mixture of isopropyl myristate and light mineral oil (20 : 15), purified water, or an aqueous solution of polysorbate 80, and the mixture was shaken for 48 h in an incubator at 30 °C. The sample was filtered with a Toyo 5C filter (Toyo Filter Co., Ltd., Tokyo). The concentration of DA in the filtrate was measured by high-performance liquid chromatography (HPLC). In the cases of purified water and the aqueous solution of polysorbate 80, the filtrates were directly injected into the HPLC. In the case of the mixture of isopropyl myristate and light mineral oil, DA was extracted with methanol, and 10 μl of the methanol layer was injected into the HPLC. Since the solubility of DA in stearyl alcohol could not be measured by the above method, it was estimated from the partition coefficient between water and stearyl alcohol. A mixture of 10 ml of DA aqueous solution (10 μg/ml) and 10 ml (8.3 g) of stearyl alcohol was warmed to 70 °C, and shaken vigorously for 10 min, before being put in an incubator at 30 °C and shaken for 24 h. The mixture was filtered and the filtrate obtained was injected into the HPLC. The partition coefficient of DA between water and stearyl alcohol was calculated from the change in the DA concentration in the aqueous phase. The solubility of DA in stearyl alcohol was therefore estimated as the product of the partition coefficient and the solubility of DA in water.

Distribution of DA in Creams——The aqueous and oil phases of the cream were separated by the ultracentrifugation method described in a previous paper.[8] DA in the separated oil phase was extracted with methanol. The DA concentration in the methanol was measured by HPLC. Since the separated aqueous phase contained DA crystals and micelles of polysorbate 80, the aqueous pahse was filtered with a Millipore filter (0.45 μm) and then the filtrate was ultrafiltered as described in a previous paper.[8] The DA concentration in the ultrafiltrate was measured by directly injecting it into the HPLC.

DA Release from Cream——The technique employed was the same as that described in a previous paper.[8] The DA concentration in the acceptor solution was measured by HPLC after extraction with chloroform and concentration of the extract. The quantity of DA release (μg) from the cream was plotted against the square root of time, and the release rate ($\mu g/\sqrt{h}$) was calculated from the slope of the straight line obtained.

Conditions of HPLC——The amount of DA in each solution was determined by HPLC using a Hitachi 633 liquid chromatograph with a 15 cm × 4.0 mm i.d. stainless steel column packed with LiChrosorb RP-18 10 μm (Merck Co., Ltd.). Other conditions were as follows; eluent, mixture of water–methanol (4 : 6); flow rate, 1.0 ml/min; detector, ultraviolet (UV) spectra at 254 nm; sensitivity, 0.02—0.16 absorbance unit full scale (AUFS); injection volume, 10 μl; column temperature, 40 °C. The standard DA solution for HPLC was prepared by using the same solvent for each sample.

Vasoconstrictor Activity——It is difficult to evaluate the percutaneous absorption of corticosteroids by using animals, because the amount of absorbed drug is very small. Therefore, the method used was an adaptation of the human vasoconstrictor assay, which has been reported to show high sensitivity.[9] About 50 mg of each cream was applied at random on the flexor surface of the left forearm of ten healthy male volunteers using a Finn chamber for patch testing (Taisho Pharm. Co., Ltd., Tokyo). The total number of test sites was 10 per cream.

At 4 h after application of the creams, the Finn chamber was removed and the sites were wiped with gauze soaked with 70% ethanol. Then, at 8 h after application, the blanching of the test site was graded as showing no blanching, slight blanching, distinct blanching, or very distinct blanching (scored as 0, 1, 2, and 3, respectively). The average score for each cream was obtained by dividing the total score by the number of test sites.

Results and Discussion

Solubility of DA in the Creams

Since creams are semisolid, it is difficult to determine the solubility of DA in creams by ordinary methods. In order to do this, therefore, the solubility of DA in each component of the creams was determined, and the solubility of DA in the creams was obtained by calculation. The solubility of DA in each component is shown in Table II. The DA solubility in polysorbate 80 was measured for an aqueous solution containing various concentrations of polysorbate 80, because polysorbate 80 is considered to be present in the aqueous phase as micelles and at the oil: water interface. The solubility of DA in these solutions was linearly related to the concentration of polysorbate 80, as shown in Fig. 1. The solubility of DA in the polysorbate 80 micelles was calculated from the slope of the plot. The results showed DA to be very soluble in polysorbate 80, but insoluble in stearyl alcohol.

Table I. Formulation of Model Creams

Component	% (v/v)
DA[a]	0.0125—0.1
Polysorbate 80[a]	1.0—5.0
Isopropyl myristate	20.0
Light mineral oil	15.0
Stearyl alcohol	10.0
Purified water	Add to 100.0

a) % (w/v).

Table II. Solubility of DA in Components of Cream at 30 °C

Component	Solubility of DA (μg/ml)
Isopropyl myristate (20) + Light mineral oil (15)	79
Stearyl alcohol	≃0
Polysorbate 80	14800
Purified water	15

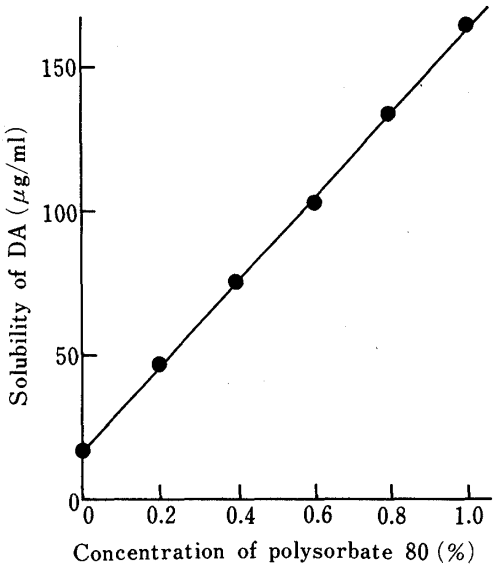

Fig. 1. Solubility of DA in Polysorbate 80 Aqueous Solution at 30 °C

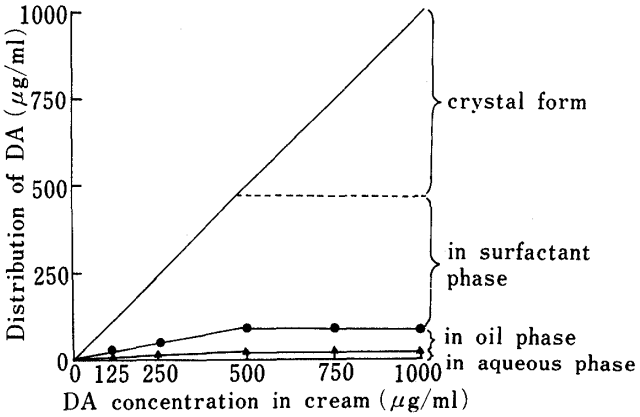

Fig. 2. Distribution of DA in Creams Containing 3% Polysorbate 80

Table III. Solubility of DA in Model Creams at 30 °C

Formula No.	DA (μg/ml)	Polysorbate 80 (%)	Calculated DA solubility (μg/ml)	Percentage of dissolved DA (%)	DA crystals observed microscopically
1-1	125	1.0	184	100	None
1-2	250			74	Present
1-3	500			37	Present
1-4	750			25	Present
1-5	1000			18	Present
2-1	125	3.0	480	100	None
2-2	250			100	None
2-3	500			96	Present
2.4	750			64	Present
2-5	1000			48	Present
3-1	125	5.0	775	100	None
3-2	250			100	None
3-3	500			100	None
3-4	750			100	None
3-5	1000			78	Present

The solubility of DA in the cream was calculated by summation of the products of the solubility in each component and its volume fraction. The solubility of DA in creams which contained 1, 3, or 5% polysorbate 80 was thus calculated; the results are shown in Table III, which also shows the solubilization ratios of DA in creams which contained 125—1000 μg/ml DA and the presence or absence of DA crystals in these creams after storage for 2 weeks at 30 °C. DA crystals were not detected in creams in which the DA solubilization ratio obtained by calculation was 100%, but they were detected in the other creams.

Therefore, it is considered that this calculation method is convenient and useful for determining the DA solubility in creams.

Distribution of DA in Creams

DA is considered to be distributed in the oil phase, the surfactant phase at the interface of the emulsion, and the aqueous phase, which includes the micelles of polysorbate 80. If the DA concentration in the cream exceeds its solubility, DA is partially present in the solid state in the cream. The measurement of DA distribution in the cream was performed by the same method as described in a previous paper.[8] However, the amount of DA crystals was estimated from the calculation of DA solubility in the cream, because it could not be measured directly.

The distribution of DA in creams which contained various concentrations of DA and 3% polysorbate 80 is shown in Fig. 2. In creams with DA concentrations under about 500 μg/ml, in which DA was completely solubilized, the amounts of DA in the aqueous and oil phases increased as the DA concentration increased, but in creams in which the DA concentration was over 500 μg/ml, the amount of DA in each phase showed a constant value.

In cases where DA is solubilized, the distribution is considered to depend simply on a distribution law, as in the case of fluocinolone acetonide cream.[8] However, when DA is partially present in a crystalline state, the DA concentration in each phase is saturated.

Release of DA from Cream

DA release was examined from creams which contained various concentrations of DA and polysorbate 80. It is known that the amount of drug released from suspension-type creams is approximately proportional to the square root of time.[10] When the amounts of DA released from creams were plotted against the square root of time, straight lines were obtained, as shown in Fig. 3. The amount of DA released from cream containing 3%

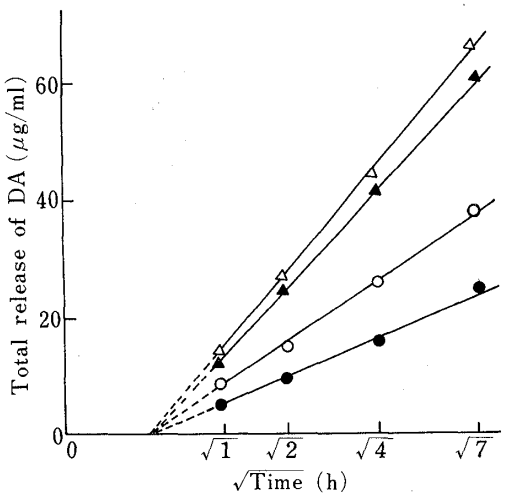

Fig. 3. Release of DA from Creams Containing 3% Polysorbate 80 at 30 °C

DA concentration in cream: ●, 125 μg/ml; ○, 250 μg/ml; ▲, 500 μg/ml; △, 1000 μg/ml.

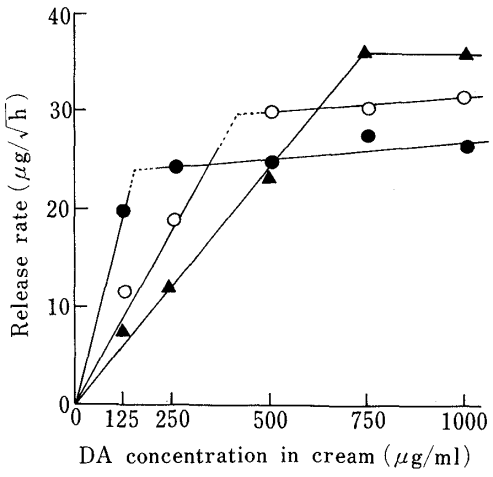

Fig. 4. Release Rate from Creams Containing Various Concentrations of DA and Polysorbate 80

Concentration of polysorbate 80 in cream: ●, 1.0%; ○, 3.0%; ▲, 5.0%.

polysorbate 80 increased as the DA concentration increased, but in creams with DA concentrations over 500 μg/ml, in which DA crystals were observed, the release was almost the same (Fig. 3).

The relationship between the release rate and the DA concentration in creams which contained various concentrations of polysorbate 80 is shown in Fig. 4. When DA was solubilized in the cream, the release rate from creams containing each concentration of polysorbate 80 increased as the concentration of DA increased, but showed an almost constant value when DA was partially present in the solid state.

In creams of low DA concentration, the release rate decreased as the concentration of polysorbate 80 increased. On the other hand, in creams of high DA concentration, the release rate increased as the concentration of polysorbate 80 increased.

The results can be interpreted as showing that in creams of low DA concentration, DA release decreased because DA was trapped in the polysorbate 80, but in creams of high DA concentration the solubilization ratio of DA increased as the concentration of polysorbate 80 increased, thus increasing the DA release rate.

Vasoconstrictor Activity

The vasoconstrictor activity was assayed for creams which contained various concentrations of DA and polysorbate 80. The results are shown in Fig. 5 in terms of the average score of vasoconstrictor activity at 8 h after the application of the creams. In creams of low DA concentration, the DA was completely dissolved, and the vasoconstrictor activity decreased as the concentration of polysorbate 80 increased. This result agreed with that of the release test. On the other hand, in creams of high DA concentration, containing DA crystals, the activity was almost constant, regardless of the solubilization ratio.

The relationship between vasoconstrictor activity and the concentration of free DA in the aqueous phase is shown in Fig. 6. The vasoconstrictor activity increased with the concentration of free DA in the aqueous phase and showed a maximum at the saturated concentration of DA (15 μg/ml). This result is considered to indicate that the activity is primarily dependent on the concentration of free DA in the aqueous phase, because the amount of steroid absorbed by the skin is very small.[11]

Accordingly, in order to enhance the efficacy of the drug, it is necessary that the free DA concentration in the aqueous phase should be increased by selecting suitable amounts of

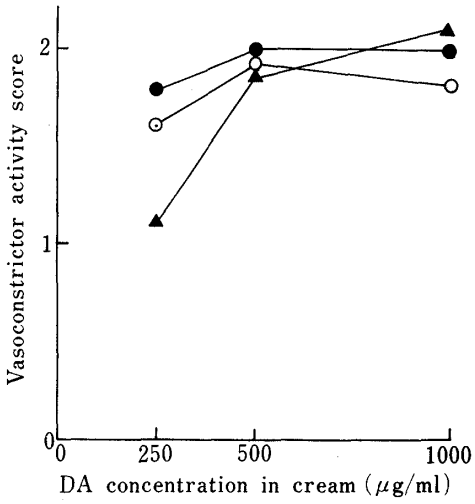

Fig. 5. Vasconstrictor Activity of Creams at 8 h after Application

Concentration of polysorbate 80 in cream: ●, 1.0%; ○, 3.0%; ▲, 5.0%.

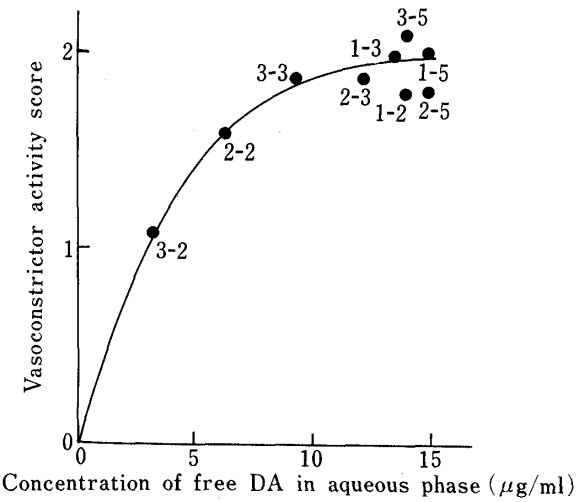

Fig. 6. Relationship between Free DA Concentration in the Aqueous Phase and Vasoconstrictor Activity at 8 h after Application

The number shows the formula No. (see Table III).

surfactant and other components. However, in the case of creams containing DA crystals, which showed the highest free DA concentration in the aqueous phase, care is necessary that crystal growth does not occur during storage, since large crystals are considered to be undesirable for practical use.

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