Arch Pharm Res Vol 25, No 4, 556-559, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Effect of Crystal Form on *in Vivo* Topical Anti-Inflammatory Activity of Corticosteroids

Young-Taek Sohn and Sun-Young Kim

College of Pharmacy, Duksung Womenís University, 419, Ssangmun-Dong, Tobong-Gu, 132-714, Seoul, Korea

(Received February 1, 2002)

The aim of this study was to gain information on the effects of the crystal form of corticosteroids on the topical anti-inflammatory activity. Two different crystal forms, Form A and Form B, of the drugs of prednicarbate, hydrocortisone, betamethasone 17-valerate, prednisolone, and methyl prednisolone were prepared and their topical anti-inflammatory activities were measured using arachidonic acid induced ear edema assay in mice. Two crystal forms of the drugs showed differences in anti-inflammatory activity. Among the drugs examined, Form B of prednicarbate and betamethasone 17-valerate showed significantly more potent anti-inflammatory activities as compared to their Form A.

Key words: Corticosteroids, Crystal Form, Topical anti-inflammation

INTRODUCTION

Drug delivery system has to be developed to achieve suitable topical treatment in response to pathological or cosmetic requirements. However, pharmacological treatment of various skin diseases present some difficulties, particularly when the superficial layer of stratum corneum is not the target site and drug penetration into deeper skin strata is required. In order to achieve better drug penetration into deeper skin strata upon topical application, various strategies have been utilized including vehicle manipulation (liposome, etc.),[1,2] chemical penetration enhancer,[3] iontophoresis,[4] electrophoresis,[5] and ultrasound-aided penetration.[6] However, among the known chemical penetration enhancers, only a few are being used in clinical practice due to their irritability, irreversibility of action. Also, other physical methods such as ultrasound-aided penetration are time- and cost consuming in development and application.

The polymorphism of organic compounds can be regarded as the simplest form of stereoisomerism in which various isoforms are realized only in crystalline states. This difference in molecular arrangements in polymorphs leads to different degrees of thermodynamic stability that, in turn, leads to different physicochemical characteristics including solubility and dissolution kinetics. Even though many studies have been carried out to explore effects of different solubility of polymorphs on the drug efficacy of oral preparations and implants, few studies have been conducted on the relationship of polymorphs and topical activities of drugs. In this study, crystal forms of several corticosteroids, which are widely used in the treatment of skin diseases, are prepared and their topical anti-inflammatory activities are studied.

Correspondence to: Young-Taek Sohn, College of Pharmacy, Duksung Womenís University, 419, Ssangmun-Dong, Tobong-Gu, 132-714, Seoul, Korea
E-mail : ytsohn@center.duksung.ac.kr

MATERIALS AND METHODS

Materials

The corticosteroids examined in this study were obtained from the following commercial suppliers: Prednicarbate and hydrocortisone, Handok Pharm. Co., Korea; betamethasone 17-valerate, Goryo Pharm. Co., Korea; prednisolone, Glaxo Korea; methyl prednisolone, Chong Kun Dang Pharm. Co., Korea.

Preparation of crystal forms

The compounds examined and the various forms obtained are listed below.

-Prednicarbate ((11β)-17-[(Ethoxycarbonyl)oxy]-11-hydroxy-21-(1-oxopropoxy)pregna-1,4-diene-3,20-dione): Form A was obtained by a melting process at a rate of 5°C/min to

Table 1. X-ray powder diffraction data

Prednicarbate						Betamethasone 17-valerate						Hydrocortisone						Prednisolone						Methyl prednisolone					
Form A			Form B			Form A			Form B			Form A			Form B			Form A			Form B			Form A			Form B		
2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0	2θ	*d*	I/I_0
8.7	10.1	49.3	9.8	9.1	71.2	10.0	8.8	55.2	8.4	10.5	16.2	12.1	7.3	83.0	12.0	7.3	6.0	10.1	8.7	13.6	7.8	11.4	7.1	9.3	9.4	49.4	14.6	6.1	100
15.6	5.7	100	11.8	7.5	22.6	14.3	6.2	100	12.2	7.3	18.8	14.4	6.1	100	14.5	6.1	100	12.4	7.1	91.9	10.0	8.8	41.5	15.0	5.9	100	16.6	5.4	89.3
			14.3	6.2	100	17.1	5.2	64.4	14.4	6.1	100	18.6	4.8	62.5	17.3	5.1	47.4	16.3	5.4	100	12.0	7.4	13.5						
			17.0	5.2	93.1	24.1	3.7	44.0	17.2	5.1	54.3	21.1	4.2	33.0	19.5	4.5	15.2	22.2	4.0	64.8	15.0	5.9	32.1						
			20.3	4.4	34.3	26.7	3.3	38.8	19.3	4.6	17.9	24.6	3.6	12.8	23.4	3.8	7.3	25.2	3.5	14.2	15.9	5.6	100						
			22.5	3.9	20.4	30.5	2.9	34.1	21.7	4.1	4.3	28.6	3.1	13.2	27.3	3.3	7.3	28.1	3.2	20.8	17.7	5.0	87.9						
			24.8	3.6	20.3				24.1	3.7	27.3	30.9	2.9	15.3	30.0	3.0	7.8				20.7	4.3	16.7						
			26.8	3.3	12.3				27.0	3.3	12.0										22.6	3.9	21.2						
			28.1	3.1	15.8				30.2	3.0	10.1										25.5	3.5	18.1						
			30.4	2.9	13.3																26.7	3.3	13.1						
																					28.8	3.1	19.7						

2q=angle *d*=distance, I/I_0 (%)=(intensity of peak/intensity of the highest peak) × 100

180°C and slow cooling in oven to room temperature. Form B was the commercial product.

-Betamethasone 17-valerate (9α-Fluoro-16β-methyl-11β, 17a,21-trihydroxypregna-1, 4-diene-3,20-dione 17-valerate): Form A was obtained by saturating betamethasone 17-valerate in ethanol and drying at 18°C for 2-3 days. Form B was the commercial product.

-Hydrocortisone (11β,17α,21-Trihydroxypregn-4-ene-3, 20-dione): Form A was obtained by evaporation of chloroform solution (1.6 mg/mL) at 18°C. Form B was prepared by evaporation of ethanol solution (1.5 mg/mL) at room temperature.

-Prednisolone (11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione): Form A was the commercial product. Form B was obtained by evaporation of acetone solution at 37°C in water bath.

-Methyl prednisolone (6α-Methyl-11β,17α,21-trihydroxy-pregna-1,4-diene-3,20-dione): Form A was prepared evaporation of ethanol solution at 18°C. Form B was the commercial product.

Differential scanning calorimetry (DSC)

A Mettler differential scanning calorimeter (Switzerland) equipped with a data station (Thermal analyst 90, Mettler, Switzerland) was used to determine the DSC curves representing the rates of heat uptake with respect to temperature. The solid sample (2mg) was weighed into aluminum pan and heated with a rate 10Åé/min to 300°C.

Powder X-ray Diffraction (PXRD)

The PXRD patterns of polymorphic forms of the corticosteroids were determined using a diffractometer (Rigaku, Japan). Each sample was scanned with the diffraction angle, 2θ, increasing from 5° < 2q < 35°.

Anti-inflammatory activity

-Animals

Male mice (ICR, 20-22 g, Yeougbu Animal Farms, Kyungki-do, Korea) were maintained on standard diet with water *ad libitum* under controlled lighting conditions (lights on 0700-1900 h) for 1 week prior to use.

-Preparation of suspension

The particle size of each form (Form A and B) was selected in the range of 150~250 mm by a sieving process and the drug was finely suspended (100 mg/mL) in propyleneglycol 400 by sonication for 5 min.[7]

-Inhibition of arachidonic acid induced ear edema

The relative topical potency was measured using the arachidonic acid induced ear edema assay.[8,9] The initial ear thickness was measured with a dial thickness gauge (Ozaki Manufacturing Co., Japan) after which 20 μL of vehicle or drug suspension was applied to each surface of both ears at a dose of 2 mg/ear. Thirty minutes later, 20 μL/ear of 2.5% arachidonic acid in acetone was topically applied in the same manner. After 1 hour, at the peak of the inflammatory activity, the ear thickness was remeasured.

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC) and X-ray powder diffraction analysis are used to characterize polymorphism. For each drug, two forms, Form A and Form B, were selected for the study. Based on the results obtained from powder X-ray diffraction analysis (λ=1.5418Å) (Table 1) and DSC (Fig. 1), each form was

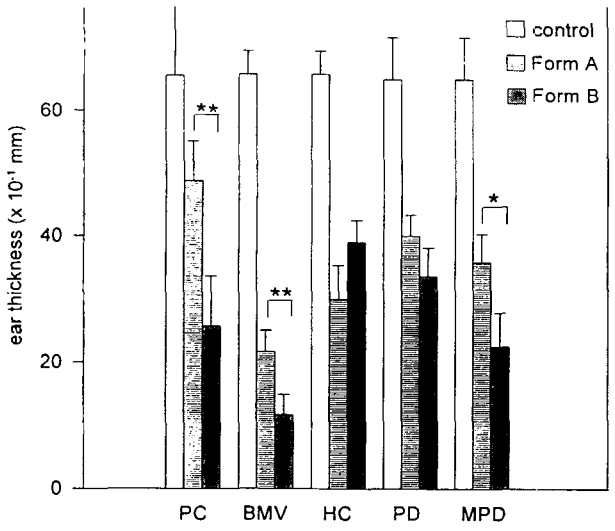

Fig. 2. Topical anti-inflammatory activities of the two crystal forms of the drugs.
Topical anti-inflammatory activities were measured using arachidonic acid induced mouse ear edema assay.
Experimental details are described in the text. PC, prednicarbate; BMV, betamethasone 17-valerate;
HC, hydrocortisone; PD, prednisolone; MP, methyl prednisolone. *$p<0.1$, **$p<0.05$.

characterized as follows.

-Prednicarbate

Form A: Colorless and glassy melt (Fig. 1).

Form B: Crystal form with mp. 188.6°C

-Betamethasone 17-valerate

Form A: The base line in X-ray diffraction showed a broad peak (identical with Form B in ref. 10).

Form B: Crystal form with mp. 199.2°C.

-Hydrocortisone

Form A: On desolvation of the chloroform complex, hydrocortisone-chloroform (2:1) solvate was formed (identical with $CHCl_3$ solvate form in ref. 11) with mp. 213.1°C. The chloroform vaporization peak was also found at 85.3°C.

Form B: It was always produced during recrystallization step with other solvents (identical with Form A in ref. 11) with mp. 242.6°C.

-Prednisolone

Form A: Melting range, T[10%-95%]=246.0-254.5°C (identical with modification I in ref. 12, 13. mp. 250.4°C).

Form B: Melting range, T[10%-95%]=231.0-243.5°C (identical with modification II in ref. 12, 13. mp. 242.6°C).

-Methyl prednisolone

Form A: Crystal form was identical with the reported form in ref. 14.

Form B: Haloform with mp. 238.2°C

Fig. 2 illustrates the anti-inflammatory activities of two forms of the drugs. In general, two different crystal forms of the drugs showed difference in anti-inflammatory activity. In particular, Form B of prednicarbate and betamethasone 17-valerate showed significantly more potent anti-inflammatory activities as compared to their Form A. These two drugs were the ones that had larger differences in crystallinity. The results obtained from this study clearly demonstrated the influence of crystal form on topical anti-inflammatory activity. The differences observed for different crystal forms might be related to different solubility in the vehicle. These results, therefore, suggested that crystal forms be considered in formulation studies, especially for topical application where other methods are being mainly employed to enhance penetration through the skin barrier.

REFERENCES

Idson, B., Vehicle effects in percutaneous absorption. *Drug Metab. Rev.*, 14, 207-222 (1983).

Massimo, F. and Giovanni, P., Corticosteroid dermal delivery with skin-lipid liposomes. *J. Controlled Rel.*, 44, 141-151 (1997).

Buyuktimkin, N., Buyuktimkin, S. and Rytting, J. H., Chemical means of transdermal drug permeation enhancement. In Ghosh, T. K., Pfister, W. R. and Yum, S. I. (Eds.). *Transdermal and topical drug delivery systems*, Interpharm Press Inc., Buffalo Grove, Illinois, pp.357, (1997).

Green, P. G., Flanagan, M., Shroot, B. and Guy, R. H., Iontophoretic Drug Delivery. In Walters, K. A. and Hadgraft, J. (Eds.). *Pharmaceutical skin penetration enhancement,* Marcel Decker, New York, pp.331, (1993).

Prausnitz, M. R., Pliquett, U., Langer, R. and Weaver, J. C., Rapid temporal control of transdermal drug delivery by electroporation. *Pharm. Res.*, 11, 1834-1837 (1994).

Murphy, T. M. and Hardgraft, J. A., Physicochemical interpretation of phonoporesis in skin penetration enhancement. In Scott, R. C., Guy, R. H. and Hardgraft, J. (Eds.). *Prediction percutaneous penetration : methods, measurements, modeling*, IBC Technical Service Ltd., London, pp 333, (1990).

Watson, W. S. and Finlay, A. Y., The effect of the vehicle formulation on the stratum corneum penetration characteristics of clobetasol 17-propionate. *Br. J. Dermatol.*, 118, 523-530 (1988).

Bird, J., Kim, H. P. and Lee, H. J., Anti-inflammatory activity of esters of steroid 21-oic acids. *Steroids*, 47, 35-40 (1986).

Kim, H. K., Namgoong, S. Y. and Kim, H. P., Anti-inflammatory

activity of flavonoids:Mouse ear edema inhibition. *Arch. Pharm. Res.*, 16, 18-24 (1993).

Mesley, R. J., The infra-red spectra of certain steroids and dissolution rate of their tablets. *Spectrochim. Acta,* 22, 889-917 (1966).

Gao, C. K. and Zahng, R. H., Polymorphic forms of certain steroids and dissolution rate of their tablets. *Pharm. Ind.*, 18, 301-306 (1987).

De Maury; G., Chauvet; A., Terol, A. and Masse, J., Etude thermoanalytique de quelques steroids IV. Prednisolone et derives. *Thermochimica Acta*, 97, 127- 142 (1986).

Veiga, M. D., Cadorniga, R., Fonseca, I. and Garcia-Blanco, S., Polymorphism characterization of prednisolone: spectrometric and diffractometric study. *Il Farmaco Ed. Pr.*, 42, 93-102 (1987).

Guillory, J. K., Heat of transition of methylprednisolone and sulfathiazole by a differential thermal analysis method. *J. Pharm. Sci.*, 56, 72-76 (1967).