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Effect of cyclodextrins on the solubility and stability of a novel soft corticosteroid, loteprednol etabonate

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To increase the aqueous solubility and stability of the soft corticosteroid loteprednol etabonate (LE), drug complexation using various cyclodextrins (CDs), such as γ-cyclodextrin (γ-CD), 2-hydroxypropyl-β-cyclodextrin (HPBCD), maltosyl-β-cyclodextrin (MBCD), mixture of glucosyl/maltosyl-α-, β-, and γ-cyclodextrin (GMCD), and heptakis (2,6-di-O-methyl)-β-cyclodextrin (DMCD), were attempted. The solubilizing and stabilizing effects of CD by itself or combined with various co-solvents were also investigated. Micronized (5 micron) LE was mixed in various aqueous CD or CD with co-solvent solutions. After equilibration and filtration at 23 °C, the solubility of LE was determined by HPLC. Subsequently, the stability of LE in the solutions was also determined by following the LE concentration change in the solution for an appropriate period. CD complexation significantly increased the aqueous solubility and stability of LE. The increase in solubility displayed a concentration dependency on CDs (0–50%). Among the five CDs used, DMCD showed the highest effects on the solubility (4.2–18.3 mg/ml in 10–50% DMCD) and stability (t_{90} > 4 years at 4 °C, when LE 0.5 mg/ml was dissolved in 10% DMCD solution) of LE. By adding co-solvents, such as glycerol, propylene glycol (PG), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP-10), the solubility of LE in DMCD solutions was further increased. Degradation of LE to the corresponding metabolites, Δ^1-cortienic acid etabonate (AE) and Δ^1-cortienic acid (A), in aqueous CD solutions appeared to be a predicted, two-step kinetics. Differential Scanning Calorimetry (DSC) was used to assist explaining the solubilizing and stabilizing activity differences between CDs. LE/CD mixture or lyophilized LE/CD complex was scanned at a rate of 20 °C/min. The exothermic peak found in the DSC diagram with LE/DMCD sample, but not with LE/HPBCD samples, suggests a stronger complex formed between LE and DMCD, resulting in higher solubility and stability of LE in DMCD than in HPBCD.

1. Introduction

The soft corticosteroid loteprednol etabonate (17α-ethoxycarbonyloxy-Δ^1-cortienic acid chloromethyl ester), LE, was developed by structural modification based on the "inactive metabolite" of soft drug concept [1–3]. It was developed mainly for topical use in various clinical fields, and the anti-inflammatory activities of LE have been proved [4–6]. However, the solubility of LE is relatively poor, and this can sometimes limit the clinical use of this drug. Also, while the hydrolytic conversion is a facile process *in vivo*, a slow hydrolysis can occur in the drug formulations. To overcome these formulation disadvantages, the complexation of LE with CDs has been attempted in this study.

CDs are cyclic oligosaccharides with hydroxyl groups on the outer surface and a cavity in the center. Their outer surface is hydrophilic, and the cavity shows hydrophobic character. By formation of an inclusion complex, the hydrophobic drug interacts with the hydrophobic cavity of the cyclic starch, while the exterior of the cyclic starch is hydrophilic and provides for the necessary aqueous solubility of the complex. In addition to the improved solubility, by formation of an inclusion complex, the rate of degradation of a drug within the complex frequently could be slowed. This results in an increased stability of a drug in the aqueous solution [7].

In this study, various CDs were used for LE/CD complex formation to increase the aqueous solubility and stability of LE. The main objectives were to compare the effects of various CDs on the solubility and stability of LE, to investigate the change of solubility and stability of LE in CD solutions under various conditions, to clarify the degradation pathway of LE in aqueous CD solutions, and to rationalize the significant solubility and stability differences of LE between different LE/CD complexes using a DSC method.

2. Investigations, results and discussion

2.1. Solubility of LE in aqueous CD solutions

Various CDs were used in this study. In Table 1, the results indicate that the solubility of LE alone in de-ionized water was very low (<0.001 mg/ml); however, by forming a drug/CD complex, a remarkable increase in the aqueous solubility of LE was obtained.

Among the five CDs used, DMCD showed the largest solubility enhancement (>18,000 times when 50% of DMCD was used), followed by MBCD, γ-CD, HPBCD, and GMCD. The solubilizing effect of CDs displayed a concentration dependency; thus, a larger effect was obtained when higher concentrations of CD were used. In the case of γ-CD, a lower concentration range (1–15%) was used due to the low water solubility of γ-CD as compared to other CDs (23.3% and >50%, respectively). For solubility investigations, de-ionized water and not buffer was used to avoid the effect of ion strength on the solubility of LE in CD solution. The pH of LE in each CD solution was determined to be about $6 \sim 7$ throughout the study.

To investigate the effect of various co-solvents on the solubility enhancing effect of CD, DMCD was chosen for its highest solubilizing activity. In Table 2, the results indicate that by adding PVA or PG as co-solvent, the solubility of LE in DMCD solution changed, although not significantly, at low DMCD concentrations (<10%) and was largely increased at higher DMCD concentrations (>20%). In general, the increased solubility of a drug resulting from complexation is directly proportional to the drug's saturated solubility. Therefore, the increased LE solubility with these co-solvents should easily account for the increase in solubility of the complex. The results also indicate that at higher concentrations of DMCD, the effect of glycerol and PVP-10 was about the same as that of PVA

Table 1: Solubility of LE in various cyclodextrin solutions at 23 °C[a,b]

CD[c]	% of CD	Solubility (mg/ml)
None	0	<0.001
γ-CD	1	0.024
	3	0.086
	5	0.14
	10	0.26
	15	0.35
GMCD	10	0.12
	20	0.27
	30	0.44
	40	0.74
	50	1.20
HPBCD	10	0.25
	20	0.40
	30	0.48
	40	0.89
	50	1.46
MBCD	10	0.26
	20	0.77
	30	1.69
	40	2.99
	50	4.10
DMCD	10	4.21
	20	5.33
	30	8.44
	40	12.3
	50	18.3

[a] Values are mean of two trials [b] Cyclodextrins were dissolved in de-ionized water [c] γ-CD : γ-cyclodextrin; HPBCD : 2-hydroxypropyl-β-cyclodextrin; MBCD : maltosyl-β-cyclodextrin; GMCD, mixtures of glucosyl/maltosyl-α-, β- and γ-cyclodextrin; DMCD : heptakis (2,6-di-O-methyl)-β-cyclodextrin

and PG. In the case of adding 10% ethanol, the solubility of LE in DMCD solution was, however, slightly decreased. To explain this result, the solution and CD complexation theory was used. Ethanol can reduce the complexation between drug and CD in aqueous solutions by acting as competing guest molecule at low concentration and alter solvent dielectric constant at higher concentrations. Similar results were reported in the cases of testosterone and ibuprofen solubility studies [8–9].

2.2. Stability of LE in aqueous CD solutions

CD complexation can have no effect or it can alter the stability of the compound by increasing or inhibiting the degradation of the labile compound through various ways, such as mimicking enzymatic catalysis or inhibition, saturation kinetics, substrate binding prior to reaction, competitive inhibition, and stereo-specific interactions [7]. In a preliminary study, the stability of saturated LE in various CD solutions at room temperature (23 °C) was investigated. The resulting t_{90}s of LE in 20% DMCD, MBCD, GMCD, and HPBCD solutions were 130, 7, 4, and 4 days, respectively, indicating that DMCD was the most effective stabilizer in preventing LE degradation. Therefore, DMCD was chosen for further stability studies. Table 3 shows the effect of DMCD concentration on the stability of LE at 37 °C. In the DMCD concentration range of 0–20%, the increase in stability of LE versus DMCD concentration displayed a linear relationship ($r = 0.999$); however, the effect leveled off when the DMCD concentration reached 20%. As a result, a more than 60 times increase in the LE stability can be obtained by 20% or more of DMCD at 37 °C.

Table 2: Effects of co-solvents on the solubility of LE in aqueous DMCD solutions, at 23 °C[a]

Co-solvents	% of DMCD	Solubility (mg/ml)
None	0	<0.001
	10	4.21
	20	5.33
	30	8.44
	40	12.3
	50	18.3
Ethanol 10%	0	<0.01
	10	1.48
	20	4.00
	30	7.64
	50	11.8
PVA[b] 1.4%	0	<0.01
	10	4.62
	20	11.3
	30	18.4
	50	21.5
PG[c] 1%	0	<0.01
	10	4.64
	20	10.2
	30	16.6
	50	23.5
Glycerol 1%	0	<0.01
	20	12.2
PVP-10[d] 1%	0	<0.01
	20	9.60
	30	13.8

[a] Values are mean of two trials Cyclodextrins were dissolved in de-ionized water [b] Polyvinyl alcohol [c] Propylene glycol [d] Polyvinyl pyrrolidone (MW 10,000)

The effect of temperature on the stability of LE was studied and the results are displayed in Table 4. LE was dissolved in 10% of DMCD at a concentration of 0.5 mg/ml. The rate of degradation (k, day^{-1}) of LE was investigated at 21, 37, and 56 °C. Using an Arrhenius plot ($r = 0.999$), the molar activation energy (E_a) was calculated as 25.5 kcal/mol. By extrapolation, the shelf life (t_{90}, day) of LE in 10% of DMCD (0.5 mg/ml) at 4 °C was estimated as more than four years.

Table 3: Effect of DMCD concentration on the stability of LE at 37 °C[a,b]

DMCD (%)	k (d^{-1})[c]	t_{90} (d)[d]	r[e]
0	2.62×10^{-1}	0.40	0.999
5	1.51×10^{-2}	7.0	0.995
10	8.69×10^{-3}	12.1	0.996
20	4.34×10^{-3}	24.3	0.999
30	3.61×10^{-3}	29.3	0.992
40	3.31×10^{-3}	31.9	0.996

[a] Values are mean of two trials [b] The original LE concentration in 0% DMCD solution was 0.00045 mg/ml, in 5%–40% DMCD solutions was 0.5 mg/ml. [c] Pseudo-first-order rate constant [d] Shelf-life [e] Correlation coefficient

Table 4: Effect of temperature on the stability of LE in 10% DMCD aqueous solutions[a,b]

Temperature	k (d^{-1})[c]	t_{90} (d)[d]	r[e]
56	1.14×10^{-1}	0.9	0.999
37	8.69×10^{-3}	12.1	0.999
21	1.11×10^{-3}	95.0	0.995
4	7.08×10^{-5} [f]	1490.2 [f]	–

[a] Values are mean of two trails. [b] LE concentration in DMCD solution was 0.5 mg/ml. [c] Pseudo-first-order rate constant [d] Shelf life [e] Correlation coefficient [f] Estimated values

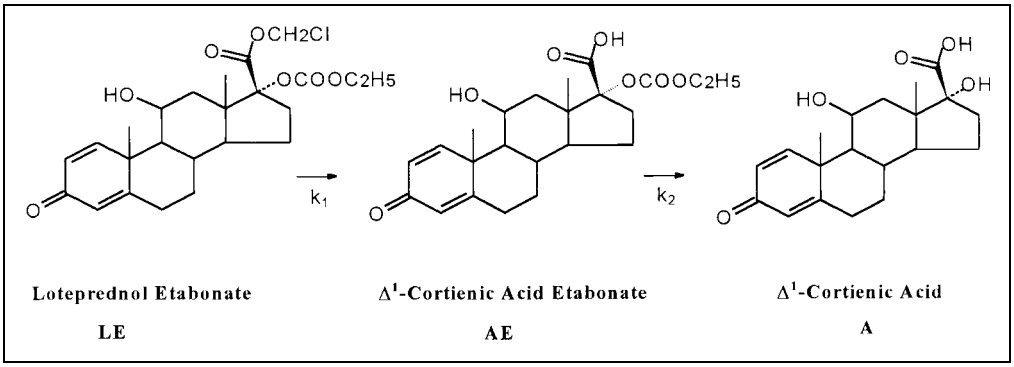

Fig. 1:
Degradation pathway of loteprednol etabonate (LE) in aqueous cyclodextrin solution

The degradation pathway of LE (0.5 mg/ml) in 10% DMCD solution (Fig. 1) was confirmed by HPLC using standard compounds of LE and its two predicted metabolites, AE and A. In the stability study at 56 °C, the concentration of each compound at various time points was quantitatively determined for two months. The results were well fitted to a two-step kinetics model with $k_1 = 4.77 \times h^{-1}$ and $k_2 = 4.60 \times h^{-1}$ corresponding to the LE → AE → A sequence [10].

The effect of co-solvent on the stability of LE was also investigated in 10% and 30% DMCD aqueous solutions at 37 °C (Table 5). The results indicate that no significant change in the stability of LE was induced by adding co-solvents, 1% PG or 1% PVA.

Table 5: Effect of co-solvent on the stability of LE in aqueous DMCD solutions at 37 °C [a, b]

DMCD/Co-solvent	k (d^{-1}) [c]	t_{90} (d) [d]	r [e]
10% DMCD	8.69×10^{-3}	12.1	0.999
30% DMCD	3.60×10^{-3}	29.3	0.999
10% DMCD + 1% PG [f]	8.09×10^{-3}	13.0	0.999
30% DMCD + 1% PG	4.03×10^{-3}	26.2	0.999
10% DMCD + 1% PVA [g]	7.30×10^{-3}	14.4	0.998
30% DMCD + 1% PVA	3.55×10^{-3}	29.7	0.998

[a] Values are mean of two trials [b] The original LE concentration in DMCD solutions was 0.5 mg/ml [c] Pseudo-first-order rate constant [d] Shelf life [e] Correlation coefficient [f] Propylene glycol [g] Polyvinyl alcohol

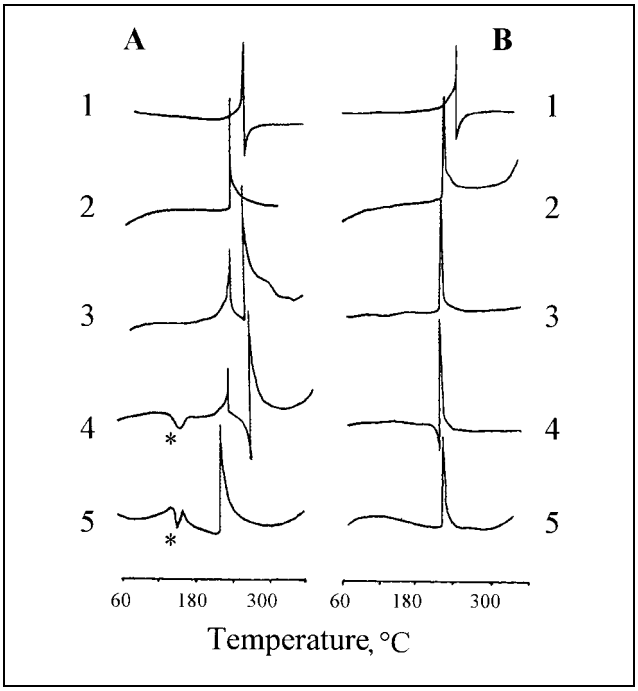

Fig. 2: Differential scanning calorimetry (DSC). A. 1: LE; 2: DMCD; 3: Physical mixture of LE & DMCD (ratio = 1:1); 4: Dried sample of ethanol treated LE & DMCD mixture; 5: Lyophilized sample of saturated LE in 20% DMCD aqueous solution. B. 1: LE; 2: HPBCD; 3: Physical mixture of LE & HPBCD (ratio 1:1); 4: Dried sample of ethanol treated LE & HPBCD mixture; 5: Lyophilized sample of saturated LE in 50% HPBCD aqueous solution. * Exothermic peak

2.3. Differential Scanning Calorimetry (DSC)

DSC was used to explain the solubilizing and stabilizing activity differences between CDs. The DSC system was operated at a scan rate of 20 °C/min. Samples of LE, DMCD, and HPBCD only, or samples of various LE/DMCD complexes and LE/HPBCD complexes were analyzed. The results displayed in Fig. 2-A indicate that LE or DMCD only, or physical mixture of LE and DMCD did not show any peak before the main peaks (temperature higher than 180 °C). However, both of the dried and lyophilized LE/DMCD samples (method 2 and method 3) showed an exothermic peak in the temperature range of 140–150 °C. These results indicate that an energy producing process took place during the heating of the sample, which can be considered as a rearrangement of the complex formed by LE and DMCD. On the other hand, in Fig. 2-B, all samples of LE, HPBCD, and LE/HPBCD mixtures did not show any sign of significant release of energy during the DSC heating process. Therefore, it can be considered as proof of a weaker binding between the LE and HPBCD molecule compared to the case of LE and DMCD complex. These findings may explain the fact that LE in DMCD aqueous solution is more soluble and stable than in HPBCD solutions.

3. Experimental

3.1. Materials

Loteprednol etabonate, LE, was supplied by courtesy of Otsuka Pharmaceutical Co. (Japan). Δ^1-Cortienic acid etabonate (AE), and Δ^1-cortienic acid (A) were supplied by Xenon Vision Inc. (FL, USA). γ-Cyclodextrin (γ-CD) and 2-hydroxypropyl-β-cyclodextrin (HPBCD) were obtained from Pharmatec Inc. (FL, USA). Maltosyl-β-cyclodextrin (MBCD) and mixtures of glucosyl/matosyl-α-, β-and γ-cyclodextrin (GMCD) were obtained from Ensuiko (Japan). Heptakis (2,6-di-O-methyl)-β-cyclodextrin (DMCD) was obtained from Sigma Chemical Company (MO, USA). All other chemicals, such as alcohol, glycerol, propylene glycol (PG), polyvinyl alcohol (PVA, molecular wt. 10,000), polyvinylpyrrolidone (PVP), and HPLC solvent, were commercially available products of special reagent grade.

3.2. Analytical method

HPLC was used for the quantitative analysis of LE, AE, and A. The system consisted of a Spectra-Physics 8810 isocratic pump, a Rheodyne 7125 injector (injection volume 10 µl), a Spectra-Physics 8450 UV/VIS detector (operated at 254 nm), a Spectra-Physics 4290 integrator, and a Waters NOVA-PAK phenyl column (4 µm, 3.9 mm × 7.5 cm, operated at room temperature). The mobile phase consisted of acetonitrile, water, and glacial

acetic acid at a ratio of 50 : 50 : 1. At a flow rate of 1.5 ml/min, the retention time of LE, AE, and A was 2.22, 0.88, and 0.52 min, respectively. Concentrations of the compounds were determined by comparing the sample peak area with calibration standards.

3.3. Solubility study

The solubility of LE was determined as follows. First, an excess amount of LE was added into the aqueous solutions containing various excipients (CD and/or co-solvent), then the mixture was sonicated in an ultrasonic bath for 45 min and agitated for three days at room temperature until the equilibrium state was reached (determined daily). Subsequently, the mixture was filtered through a 0.45 μm Millipore HV membrane filter, then the filtrate was diluted with mobile phase, and analyzed by HPLC.

3.4. Stability and kinetic studies

LE in water or LE in various concentrations of CD solutions were kept at different temperatures (21, 37, and 56 °C). At appropriate time intervals, an aliquot was taken, diluted, and analyzed by HPLC. Pseudo-first-order rate constant, k, for the total loss of LE from the aqueous solutions were determined by linear regression of a natural logarithm of the concentration of LE versus time plot. Shelf life (t_{90}) was used to express the stability of LE. For the kinetic study, concentrations of LE, AE, and A in each sample were determined, and k_1 and k_2 were calculated using the following equations [10]:

$$X_t = X_0\, e^{-k1\, t} \tag{1}$$

$$Y_t = X_0\, [k_1 /(k_1 - k_2)] \cdot (e^{-k2\, t} - e^{-k1\, t}) \tag{2}$$

$$Z_t = X_0\, [1 - (k_2\, e^{-k1\, t} - k_1\, e^{-k2\, t}) / (k_2 - k_1)] \tag{3}$$

where X_0 represents the concentration of LE at time zero; X_t , Y_t, and Z_t represent the concentration of LE, AE, and A at time t; k_1 and k_2 represent rate constants for the first order degradation of LE to AE, and AE to A, respectively.

3.5. Differential scanning calorimetry (DSC)

The DSC apparatus consisted of a Perkin Elmer TAC7/PC instrument controller, a Perkin Elmer DSC7 Differential Scanning Calorimeter, and an IBM computer workstation. A Cahn electromicrobalance (0.001 mg accuracy) was used for weighing the samples. The amount of LE, CD, or LE/CD complex used for each analysis was between 1 and 2 mg. A nitrogen purge was performed during each analysis. Baseline runs were made each day prior to analysis of samples, and the same condition was kept for each sample analysis. Experiments were carried out at a scan rate of 20 °C/min. The output record was a result of any emerging heat difference between the sample pan and an empty pan being heated at the same time. Samples of LE and CD (DMCD or HPBCD) were used as is. The other three types of LE/CD complex samples were made by various methods as follows: 1) Physical mixtures of a 1 : 1 ratio of LE and CD. – The sample was made by carefully mixing without causing significant heat of friction to the mixture. 2) Dried sample of ethanol treated LE and CD mixture – Appropriate 1 : 1 molar ratio of LE and CD were weighed in a vial. To the mixture, 5 ml of ethanol were added. The mixture was then shaken for at least three days at 21 °C and dried. 3) Lyophilized sample of saturated LE in 20% DMCD or 50% HPBCD aqueous solution – Excess amount of LE was added in 20% DMCD or 50% HPBCD water solution. The mixture was sonicated for two hours and filtered through a 0.45 μm micro-filter. The filtrate was then lyophilized. To dry or lyophilize the LE/CD mixture in method 2) and 3), a Virtis 10–324 lyophilizer was used and set at −45 °C, 100 millitorr.

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References

1 Bodor, N.; in Christophers et al. (Eds.): Topical Corticosteroid Therapy: A Novel Approach to Safer Drugs, p. 13, Raven Press Ltd., New York, 1988
2 Bodor, N.: Adv. Drug Res. **13**, 225 (1984)
3 Bodor, N.: Med. Res. Rev. **3**, 449 (1984)
4 Druzgala, P; Hochhause, G; Bodor, N.: J. Steroid Biochem. **38(2)**, 149 (1991)
5 Bodor, N.; in van der Goot, H. et al. (Eds.): Designing Safer Ophthalmic Drugs, p. 145, Elsevier, Amsterdam, 1989
6 Bodor, N.; Varga, M.: Exp. Eye. Res. **50**, 183 (1990)
7 Loftsson, T.; Brewster, M.: J. Pharm. Sci. **85**, 1017 (1996)
8 Loftsson, T.; Olafsdottir, B. J.; Fridriksdottir, H.; Jonsdottir, S.: Eur. J. Pharm. Sci. **1**, 95 (1993)
9 Loftsson, T.; Fridriksdottir, H.; Sigurdardottir, A. M.; Ueda, H.: Int. J. Pharm. **110**, 169 (1994)
10 Martin, A.; Swarbrick, J.; Cammarata, A. (Eds.): Kinetics, p. 362, Lee & Febiger, Philadelphia, 1983

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