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RESEARCH PAPER

Study of the Complexation Behavior of Tenoxicam with Cyclodextrins in Solution: Improved Solubility and Percutaneous Permeability

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ABSTRACT

Complexation of tenoxicam (TEN) with γ-, HPγ-, β-, HPβ-, and Mβ-cyclodextrin (CD) in aqueous solution at pH 7.4 has been investigated using phase solubility diagrams. TEN formed soluble complexes with 1:1 stoichiometry with all the CDs studied, although the inclusion stability constants ($K_{1:1}$) obtained had low values. The presence of propylene glycol (PG) in the dissolution medium decreased the stability constants and led to a higher fraction of free drug by competitive displacement and by an increase in the lipophility of the media.

Among the CDs tested, MβCD was chosen for further studies since TEN–MβCD complexes yielded the best results: good solubility and the highest stability constant. The effect of MβCD and PG on the TEN partitioning coefficient was also studied in skin–buffer systems. Although each substance reduced the partitioning value, the combination of PG and MβCD increased this parameter.

The noticeable increase in solubility of the drug found in the presence of MβCD allowed the formulation of carbopol gels with higher doses of TEN and a reduced amount of cosolvent. The presence of MβCD improved the percutaneous penetration of TEN through abdominal rat skin by increasing the solubility of the drug in the vehicle and by affecting the partitioning behavior of TEN in the skin. In addition, TEN retention in the skin was found to be related to the flux values attained with the corresponding gels.

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Key Words: *Cyclodextrins; Partitioning coefficient; Percutaneous penetration; Skin retention; Stability constant; Tenoxicam*

INTRODUCTION

Tenoxicam (TEN) is a nonsteroidal anti-inflammatory (NSAID) agent of the oxicam family, with anti-inflammatory, analgesic, and antipyretic properties, often used in the treatment of rheumatic and arthritic diseases (1). However, like other NSAIDs, its use continues to be primarily limited by its untoward effects on the gastrointestinal tract, especially at the high-sustained dosages necessary for treatment of arthritis.

An interesting approach for reducing these side effects is via transdermal drug delivery. Nevertheless, percutaneous administration is greatly limited by the barrier function of the stratum corneum (SC) and many attempts have been made to increase its permeability. Besides, the physicochemical properties of TEN make difficult its percutaneous penetration.

Cyclodextrins (CDs), which are cyclic torus-shaped oligosaccharides, have been applied to optimize the transdermal delivery of drugs intended either for local or systemic use. Due to their potential to form noncovalently bonded reversible inclusion complexes with a wide variety of drugs, the CDs have recently been recognized as a new group of useful pharmaceutical excipients (2). The resulting complexes generally show some favorable changes of the characteristics of the guest molecule, such as increased solubility, improved stability, enhanced bioavailability, reduced side effects, etc. (3–5).

Attention should be directed towards the binding constant between the drug and the CD. A low inclusion stability constant means that the drug will be released easily from the complex, thus approaching saturation concentration and precipitating (6,7). A high stability constant means that the release rate may be slow or incomplete, thus possibly delaying bioavailability (8,9). Since only the free drug is absorbed through biological membranes, the absorption rate will depend on the magnitude of the drug–CD inclusion stability constant (10). Knowledge of this constant is very useful, because it governs the yield of inclusion formation as well as the behavior of the inclusion in liquid medium. In addition, the apparent stability constant of the complexes depends not only on the nature of the guest molecule and of the CD, but also on the nature of the surrounding medium.

The mechanism of action of the CDs by which they improve the penetration of drugs through the skin is not clearly understood. It has been described that the CDs can enhance the percutaneous absorption of drugs either by increasing the solubility of the drug in the vehicle (11–14), acting directly on the skin (15), or by influencing the distribution and partitioning of the drug in the skin (16,17).

The goal of the present study was to develop and optimize a topical gel formulation of TEN and to analyze the influence of CD complexation on the percutaneous penetration of the drug. Binding constants were calculated using the phase solubility technique. The partitioning coefficients of TEN between skin fragments and PG–buffer mixtures in the presence of methyl β-CD (MβCD) were also determined.

MATERIALS AND METHODS

Materials

Tenoxicam was kindly provided by Products Roche S.A. (Madrid, Spain). The CDs: γCD, hydroxypropyl γ-CD (HPγCD) (DS 0.6), βCD, and MβCD (DS 1.8) were generously supplied by Wacker S.A. (Barcelona, Spain); hydroxypropyl β-CD (HPβCD) (DS 0.9) by Cerestar S.A. (Indiana). Carbopol 940, triethanolamine 99% (TEA) and propylene glycol USP (PG) were purchased from Roig Pharma S.A. (Barcelona, Spain), absolute ethanol (EtOH) and potassium carbonate from Panreac Quimica (Barcelona, Spain), and tetrahydrofuran (THF) from BDH Laboratory Supplies (Poole, UK).

Methods

Preparation of Solid Inclusion Complexes of Tenoxicam with Cyclodextrins

Inclusion complexes of TEN and the different CDs were prepared in 1:1 molar ratio by coprecipitation. Drug–CD physical mixtures (PM), at the same molar ratio, were prepared by simple mixing

using an agate mortar and pestle. All the inclusion complexes in solid state were characterized elsewhere (18).

Determination of Physicochemical Properties of Complexes

Water Solubility of Free and Complexed Tenoxicam

The solubility of TEN and the different complexes with the CDs was determined at 25°C in aqueous buffer solution (pH 7.4) and in PG–buffer mixtures (20% w/w) for TEN–MβCD complex ($n = 8$–10). The buffer consisted of 1.787 g of KH_2PO_4 1/15 M and 9.531 g of Na_2HPO_4 1/15 M in 1 L with an ionic strength of 0.266 M. Free TEN or coprecipitated samples were suspended in buffer solution (pH 7.4) or in PG–buffer mixtures and maintained in a thermostated bath (Selecta Unitronic 320 OR) at 25°C with constant stirring (80 rpm) until equilibrium was reached. Then, an aliquot of each suspension was filtered (0.8 μm pore) and assayed spectrometrically at 368 nm (Diode Array HP 8452 A spectrophotometer) to determine drug concentration.

Determination of the Stability Constants of the Complexes ($K_{1:1}$)

Phase solubility diagrams were obtained as described by Higuchi and Connors (19) at two different temperatures (25 and 37°C) ($n = 5$–8). Excess amounts of TEN (80 or 100 mg, depending on the temperature) were added to aqueous solutions (20 mL) of each CD at different concentrations (0–2×10^{-2} M; 0–1×10^{-2} M for βCD that has a lower solubility). The suspension was shaken in a water bath at constant temperature until equilibrium was attained (3 or 7 days). Then, aliquots were filtered (0.8 μm pore) and the content of the solubilized TEN was determined spectrophotometrically at 368 nm after suitable dilution. The interaction between TEN and MβCD was studied not only in aqueous solution but also in water–PG mixtures (80:20 and 95:5 w/w).

Partition Coefficient Determination

The partition coefficient, P, of TEN alone or in the presence of 20% MβCD was determined between full-thickness rat skin and buffer solution (pH 7.4) or PG–buffer mixtures (0 and 20% w/v). One hundred milligrams of TEN were suspended in 10 mL of the aqueous phase and the initial concentration of drug was determined spectrophotometrically after 24 hr of shaking in a thermostated water bath at 25°C. One hundred and forty to one hundred and fifty milligrams of full-thickness skin were added and maintained in the water bath for 48 hr, time enough to reach equilibrium. Then, the aqueous phase was filtered and assayed spectrophotometrically.

P was determined by the following expression:

$$P = \frac{\left(C^{aq}_{initial} - C^{aq}_{final}\right)/W_{skin}}{C^{aq}_{final}/V^{aq}} \quad (1)$$

where $C^{aq}_{initial}$ is the initial concentration of drug in the aqueous phase; C^{aq}_{final} is its final concentration in the aqueous phase; W_{skin} is the skin's weight; V^{aq} is the volume of the aqueous phase.

Preparation of Tenoxicam Gels

Gels were prepared by dispersing 1% of carbopol 940 in mixtures of water, with PG having different concentrations of TEN, as free or complexed drug. The mixture was stirred for 12 hr. The dispersion was then neutralized (pH 7.4) and made viscous by adding TEA. The resulting gel was stored at room temperature for 24 hr prior to use. Gels with 1% TEN as free form and 20% PG will be referred to as control gel.

In Vitro Release and Permeation Studies

All the studies were carried out using a Franz-type diffusion cell with a diffusional area of 1.76 cm^2 (FDC-400, Crown Glass Company, Somerville, NJ). A certain amount of the gel (0.5 and 1 g for the release and permeation studies, respectively) was placed on the membrane surface in the donor compartment while the receptor one was filled with 11 mL of phosphate buffer solution (pH 7.4). During the experiments ($n = 5$–8), the receptor solution was stirred at 600 rpm and kept at 37±1°C. At designated time intervals, samples of receptor fluid were withdrawn and replenished with fresh buffer solution. Tenoxicam was always assayed spectrophotometrically at 368 nm.

Tenoxicam release rates (k) were measured through 0.2 μm cellulose nitrate membranes

(Sartorious AG, Goettingen, Germany) as they do not offer resistance to drug permeation and were calculated using the Higuchi equation (20).

Permeation studies were conducted in accordance with approved institutional protocols. The abdominal hair of male Wistar rats (230–240 g) was removed using electric razors. Animals were sacrificed, the abdominal skin was excised, and the adhering fat eliminated. The membranes were mounted on Franz-type diffusion cells with the dermis facing the receptor compartment. Tenoxicam steady-state fluxes (J) were estimated from the slope of the straight-line portion of the cumulative amount of drug absorbed against time profiles, and the lag time (t_L) from the x-intercept.

Extraction of Tenoxicam from Skin Samples

Following the permeation studies and the removal of the formulation, the area of diffusion was cut out and weighed. The skin samples were homogenized in an Ultraturrax (Euro Turrax T 20 basic) in phosphate buffer solution (pH 7.4) for 2 min. To 1 mL of skin homogenate were added 700 mg of potassium carbonate, 1 mL of THF, and 0.5 mL of EtOH. The tubes were vortex-mixed for 1 min, then centrifuged for 12 min at 2550**g** and 20°C. The supernatant was placed in a second test tube and evaporated to dryness at 60°C in an evaporator. The residue was then reconstituted in 1 mL of THF, vortex-mixed for 10 sec, and filtered with a 0.45 μm filter (Albet-JCR-045-15) prior to TEN determination by spectrophotometry at 382 nm.

RESULTS AND DISCUSSION

Solubility Studies

The aqueous solubilities of TEN and the different TEN–CD inclusion complexes at 25°C are presented in Table 1. The solubility of TEN was improved by the use of CD complexes, compared with that of the pure drug. The highest solubility was found for the TEN–HPβCD complex, followed by the MβCD complex.

The better solubility values obtained with the βCD complexes than with the γCDs may suggest a stronger interaction between the drug and the βCD cavity. The substitution of the hydroxyl groups of the parent CDs led to a higher solubility of the host and, therefore, of the complexes formed with these CDs. Thus, the complexes formed with the CD derivatives were more soluble than those with the parent CDs.

Table 1

Solubility of TEN and Its Inclusion Complexes at 25°C in Aqueous Buffer Solutions (pH 7.4). Values Are the Mean ± SEM of 8–10 Experiments

Compound	Medium	S (M)×10^4 (25°C)
TEN	Buffer	68.44 ± 0.15
TEN–γCD	Buffer	123.46 ± 0.32
TEN–HPγCD	Buffer	310.45 ± 3.27
TEN–βCD	Buffer	220.47 ± 2.18
TEN–HPβCD	Buffer	435.57 ± 0.78
TEN–MβCD	Buffer	343.53 ± 0.62
TEN	5% PG–buffer	89.84 ± 0.99
TEN	20% PG–buffer	163.21 ± 0.82
TEN–MβCD	20% PG–buffer	383.47 ± 0.92

The solubility of TEN in 20% w/w PG, $(163.21 \pm 0.82) \times 10^{-4}$ M, was higher than 100% pure aqueous buffer solution. In addition, TEN–MβCD complex in the presence of 20% w/w PG–buffer medium displayed a solubility value of $(383.47 \pm 0.92) \times 10^{-4}$ M, slightly higher than in pure buffer solution. This increase in the solubility was mainly due to the cosolvent effect of PG.

In all cases, over the concentration range examined a linear increase of TEN solubility was observed as a function of CD concentration at 25 and 37°C. The solubility curves obtained can be classified as A_L-type, which indicates the formation of water-soluble complexes with stoichiometry $n = 1$ in relation to the ligand. Our results are in good agreement with those described by other authors, who obtained the same type of solubility diagrams for TEN with βCD (21) and HPβCD (22).

The stability constants for TEN with the different CDs, assuming a 1:1 stoichiometry, are listed in Table 2. The binding constants decreased with increasing temperature, suggesting a typical drug–CD inclusion of an exothermic nature.

It seems that TEN tends to interact more strongly with βCDs than γCDs, suggesting the βCD cavity to be a more appropriate site for inclusion of TEN. Inclusion stability constants for the drug–CD complexes were slightly larger for the derivatives than for the parent CDs, with MβCD being the strongest.

Table 2

Tenoxicam Stability Constants ($K_{1:1}$) in Phosphate Buffer at pH 7.4 and in PG–Buffer Mixtures Determined by the Phase Solubility Method. "b" Represents the Slope and "a" the y-Intercept of Phase Solubility Diagrams. Values Are the Mean ± SEM of 5–8 Experiments

		25°C			37°C		
CD	Medium	$K_{1:1}$ (M^{-1})	b	$a \times 10^4$ (M)	$K_{1:1}$ (M^{-1})	b	$a \times 10^4$ (M)
γCD	Buffer	27 ± 1.0	0.16 ± 0.01	66.87 ± 0.61	16 ± 0.7	0.14 ± 0.01	99.29 ± 0.69
HPγCD	Buffer	34 ± 0.5	0.18 ± 0.01	66.18 ± 0.88	20 ± 1.3	0.16 ± 0.01	98.93 ± 2.17
βCD	Buffer	47 ± 0.7	0.24 ± 0.01	67.96 ± 0.86	35 ± 1.0	0.26 ± 0.01	103.41 ± 0.95
HPβCD	Buffer	53 ± 1.4	0.26 ± 0.01	65.07 ± 0.68	37 ± 0.1	0.28 ± 0.01	103.74 ± 0.58
MβCD	Buffer	57 ± 1.1	0.27 ± 0.01	65.05 ± 0.67	41 ± 0.8	0.30 ± 0.01	102.71 ± 0.64
MβCD	5% PG–buffer	38 ± 0.5	0.25 ± 0.01	89.84 ± 0.99	—	—	—
MβCD	20% PG–buffer	21 ± 1.3	0.26 ± 0.01	163.38 ± 0.88	12 ± 0.3	0.20 ± 0.01	202.75 ± 0.95

Because of the larger stability constant found with MβCD, further TEN experiments were carried out with MβCD. The presence of PG in the dissolution medium modified the TEN–CD interactions. The solubility diagrams obtained with MβCD in the presence of 5 or 20% PG were also A_L-type, although the stability constants decreased significantly as the amount of PG was increased. Propylene glycol markedly influenced TEN–MβCD complexation possibly due to competitive displacement, increasing the intrinsic solubility, or both.

Several authors have reported that PG and/or EtOH, at low concentrations, reduced the CD complexation of some drugs by acting as competing guest molecules (2,23,24). However, at high concentrations, they can reduce complexation through manipulation of the solvent dielectric constant (25–27).

Partitioning Coefficient

The partitioning coefficients of TEN between skin fragments and mixtures of PG (0 and 20% w/v) and phosphate buffer (pH 7.4), in the absence or presence of 20% MβCD, are represented in Table 3. The partition coefficient value determined in the absence of PG and CD was 1.27 mL mg^{-1}. If the amount of PG in the aqueous phase was increased, the partitioning value decreased as a result of the enhanced solubility of TEN in the media. Such a decrease has been reported previously (28).

Addition of 20% MβCD to the phosphate buffer medium reduced the coefficient due to a better solubilization of the drug. Similarly, the decrease in the partitioning coefficient obtained for dexamethasone in SC–buffer was ascribed to an increase in the drug solubility in the presence of β- and HPβCD (29).

Table 3

Partitioning Coefficients of TEN: Influence of PG and MβCD in Skin/Buffer Systems (n = 3, CV < 6%)

PG Content	CD Content	
	0%	20%
0%	1.27 ± 0.14	0.57 ± 0.12
20%	0.52 ± 0.15	1.20 ± 0.19

However, the combination of both PG and MβCD increased this parameter compared to the values obtained for each substance when studied separately. This could be due to the lower stability of TEN–CD complexes formed in the presence of PG and the possible inclusion of the cosolvent in the CD cavity. It can be concluded that the presence of CD in the formulations containing PG favored TEN partitioning into the skin since the CDs conferred on the drug more affinity for the membrane.

In Vitro Release and Permeation Studies

With regard to drug permeation through the skin from vehicles, a drug should first diffuse out from the vehicle to the skin and then penetrate into and

Table 4

Tenoxicam Release Rate Across Cellulose Membranes ($k \times 10^2$), Skin Permeation Parameters (J and t_L), and the ER. Values Are the Mean ± SEM of 5–8 Experiments

Gel	$k \times 10^2$ ($mg\,cm^{-2}\,min^{-1/2}$)	J ($\mu g\,cm^{-2}\,hr^{-1}$)	t_L (hr)	ER
Control (1% TEN:20% PG)	10.44 ± 0.48	1.74 ± 0.17	1.50 ± 0.16	1
1% TEN:5% PG		1.51 ± 0.27	1.20 ± 0.23	0.9
1% TEN–MβCD:20% PG	9.94 ± 0.30	2.62 ± 0.44	1.30 ± 0.21	1.5
1.5% TEN–MβCD:20% PG		3.39 ± 0.55	1.20 ± 0.32	1.9
2% TEN–MβCD:20% PG	14.94 ± 0.11	6.81 ± 0.88	1.19 ± 0.17	3.9
1% TEN–MβCD:5% PG	9.45 ± 0.11	2.43 ± 0.67	1.40 ± 0.19	1.4
2% TEN–MβCD:5% PG	17.88 ± 0.50	5.62 ± 0.55	1.28 ± 0.20	3.2
2% TEN–MβCD:10% PG		4.98 ± 0.55	1.06 ± 0.09	2.9
PM (1% TEN–MβCD:20% PG)		1.39 ± 0.14	1.82 ± 0.41	0.8
PM (2% TEN–MβCD:20% PG)		5.03 ± 0.37	1.24 ± 0.10	2.9

through the skin at the application site. In order to evaluate the influence of MβCD complexes on TEN diffusion through carbopol gels, the in vitro release of the drug from these vehicles containing different proportions of TEN, as free or complexed drug (1 and 2%), and PG (5 and 20%) was studied. The apparent release rates, k, were calculated and are listed in Table 4.

In general, the addition of TEN–CD complexes to the gels reduced the release of TEN compared to the pure drug since the fraction of free drug was lowered and only the free form can diffuse through the membranes. In addition, the CD may reduce the thermodynamic activity of the drug because of the higher solubility of TEN, and could also increase the viscosity of the vehicle (30).

The apparent release rate of TEN increased upon increasing the concentration of TEN, but not proportionally. These differences in the release values could be explained by the fact that any increase in TEN concentration would displace the equilibrium to complex formation, reducing the free fraction of the drug. Thus, gels with 2% TEN presented lower percentages of free drug than gels containing 1% TEN.

When increasing the PG content, the release rates from gels containing 1% TEN complexed with MβCD scarcely changed, while those from gels with 2% TEN decreased ($p < .01$). However, upon addition of PG a higher diffusion would be expected since PG tends to displace the drug–CD complex equilibrium to the free form. Besides, such obtained values could be ascribed to the fact that at 5% PG, TEN is completely solubilized and at a concentration very close to saturation, and thus its thermodynamic activity is high.

The percutaneous penetration of TEN from carbopol gels containing TEN–MβCD complexes was studied across full-thickness rat skin. The steady-state flux, J, and lag time, t_L, for each of these gels are also summarized in Table 4.

The presence of MβCD enhanced TEN percutaneous absorption in comparison with the control gel, providing enhancing ratios (ER) between 1.4 and 3.9. When the amount of TEN formulated as a complex was 1% there were no significant differences in the flux values, whereas with 1.5% an improvement in the flux was observed. As shown in Fig. 1, the TEN flux increased with drug concentration, practically in the same proportion and, thus, the greatest flux was achieved with 2% TEN and 20% PG.

Various amounts of PG displayed negligible changes in TEN flux at low and high drug concentrations. This may be explained by the fact that a decrease in PG content increased the thermodynamic activity in the vehicle, but at the same time it also decreased the free fraction of drug ready to be absorbed.

With the purpose of attributing the enhanced absorption observed to the CD complex or just to the presence of CD, gels containing PM of TEN and MβCD were also evaluated (Table 4; Fig. 1). The TEN–MβCD complexes favored the penetration of the drug slightly compared to physical mixtures, although the differences were not statistically

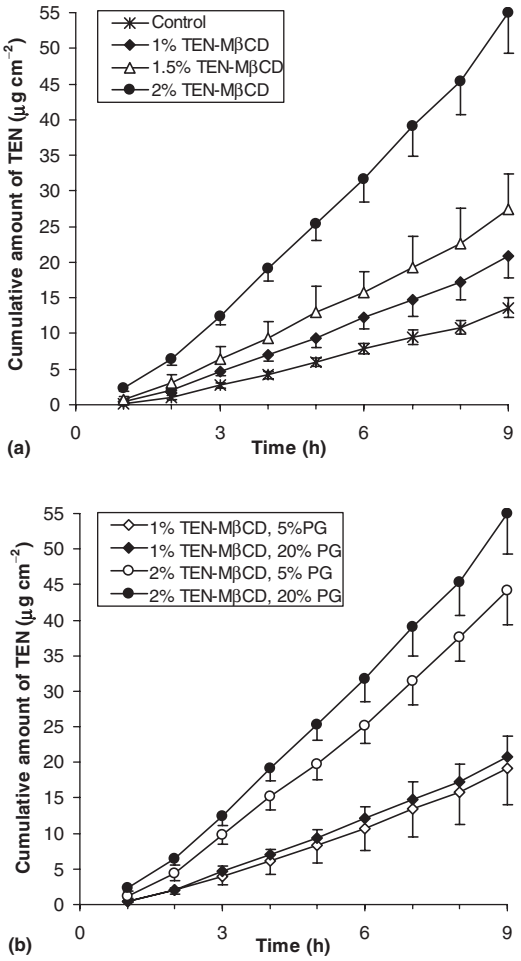

Figure 1. Permeation profiles of TEN across rat skin: (a) different drug concentration (20% PG); (b) different PG concentration. Values are the mean ± SEM of 5–8 experiments.

significant. However, it seems that the physical mixtures could not reduce so efficiently the lag times.

CDs may affect percutaneous absorption by means of two mechanisms: indirectly, influencing physicochemical properties of the drug (10–13) and/or directly, influencing the skin permeability (14). In the case of TEN, a solubilizing effect of the CDs on the drug was observed, making it possible to formulate gels with more TEN in solution and attain higher flux values. In addition, the time required to achieve the steady state was slightly shortened in the presence of MβCD, which may suggest that the CDs favored the partitioning of TEN into and through the skin, attaining the equilibrium faster. The increased penetration of the drug found with the CDs could justify the first mechanism. In fact, as previously verified, the partitioning coefficient of the drug was increased by the combination of MβCD and PG in the vehicle.

When the amount of TEN retained in the skin after the application of these gels was studied, it was found that the amount of drug present in the tissue increased proportionally with percutaneous absorption. Tenoxicam within tissue was calculated to be $(7.9\pm0.3)\times10^{-2}$, $(6.2\pm0.6)\times10^{-2}$, and $(12.5\pm0.8)\times10^{-2}\,\mu g\,mg^{-1}$ for the control gel, 1% TEN, and 2% TEN as MβCD complex, respectively. Therefore, the accumulation of the drug in the skin was related to the flux values obtained in each case.

In conclusion, gels containing TEN–MβCD complexes enhanced the percutaneous penetration of the drug by improving the solubility. The increase in solubility enabled us to double the amount of TEN formulated, subsequently increasing the release and absorption parameters. Addition of the CD complexes to the carbopol gels increased the partitioning behavior of the drug into the skin, resulting in an effective delivery of TEN.

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