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The effect of cyclodextrins on the solubility and stability of medroxyprogesterone acetate and megestrol acetate in aqueous solution

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Summary

The degradation of medroxyprogesterone acetate and megestrol acetate in aqueous buffered solutions was investigated over the pH range 1–10. The degradation followed first-order kinetics. The pH-rate profiles were of typical V-shape with a minimum at pH between 4 and 5. In aqueous solutions the shelf-life of the drugs at physiological pH and 25°C was estimated to be about 1 year. The effects of six cyclodextrin (CD) derivatives on the stability was investigated and 2-hydroxypropyl-β-cyclodextrin (HPβCD) of molar substitution (MS) 0.9 and methyl-β-cyclodextrin (MβCD) of degree of substitution (DS) 1.8 appeared to have the greatest stabilising effect. The drugs degraded 2.5–4-times slower within the HPβCD and MβCD cavities than outside the cavities. Of the two CD derivatives, MβCD DS 1.8 resulted in better solubilisation of the drugs.

Introduction

Medroxyprogesterone acetate and megestrol acetate are synthetic progesterone agonists, progestins, which are sometimes used in advanced progressing endometrial carcinoma previously treated by surgery and radiotherapy. The exact oral bioavailability of the two progestins is not known but when the area under the plasma level-time curve (AUC) after oral administration of medroxyprogesterone acetate was compared with those after intraperitoneal and intramuscular injections, the relative oral bioavailability was only 0.2–17.4% (Camaggi et al., 1985). Medroxyprogesterone acetate is given orally or intramuscularly. Megestrol acetate is given orally and is well absorbed after oral administration but it is possibly subject to first-pass metabolism (Lønning et al., 1992). Both medroxyprogesterone acetate and megestrol acetate are practically insoluble in water.

Cyclodextrins (CDs) are a group of structurally related cyclic oligosaccharides which form a whole new family of pharmaceutical excipients (Loftsson et al., 1991; Szejtli, 1991a,b). The important structural characteristics of the CD molecules are their cylindrical shape, somewhat hydrophobic central cavity and hydrophilic outer surface. CDs

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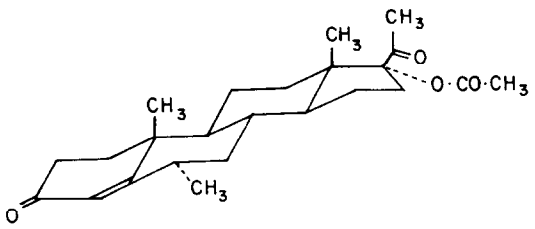
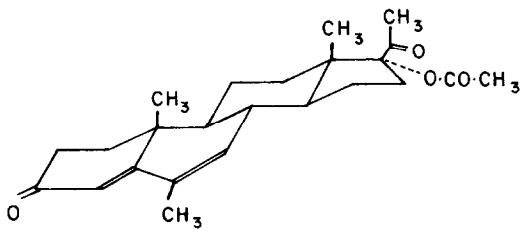


MEDROXYPROGESTERONE ACETATE



MEGESTROL ACETATE

Scheme 1.

are capable of forming inclusion complexes with many drugs by taking up a whole drug molecule, or some part of it, into the cavity. This type of molecular encapsulation will affect many of the physicochemical properties of the drugs. The drug-CD complexes can improve the clinical usage of the drugs by increasing their aqueous solubility, rate of dissolution and pharmaceutical availability. For example, due to their low aqueous solubility and a large first-pass effect, oral administration of gonadal steroids frequently results in a low and variable bioavailability. Pitha et al. (1986) have shown that when testosterone is complexed with 2-hydroxypropyl-β-cyclodextrin (HPβCD), it is readily absorbed from under the tongue, resulting in a rapid increase in plasma levels.

It is often difficult to introduce anticancer drugs into aqueous drug formulations due to their chemical instability. Frequently, the rate of degradation of a drug within the drug-CD inclusion complex is slower than out in the solution and addition of CDs to aqueous drug formulations can in many cases improve the drug's shelf-life (Loftsson et al., 1991). Thus, we have previously shown that CDs can be used to stabilise chlorambucil and melphalan (Loftsson et al., 1989), lomustine (Loftsson and Friðriksdóttir, 1990), doxorubicin (Brewster et al., 1992), estramustine (Loftsson et al., 1992), and tauromustine (Loftsson and Baldvinsdóttir, 1992). The purpose of this present work was to investigate the chemical stability of medroxyprogesterone acetate and megestrol acetate, and the effect of CDs on their stability and solubility in aqueous solutions.

Materials and Methods

Materials

Medroxyprogesterone acetate (6α-methyl-17α-hydroxyprogesterone acetate), medroxyprogesterone (6α-methyl-17α-hydroxyprogesterone) and megestrol acetate were obtained from Sigma Chemical Co. (U.S.A.). 2-Hydroxypropyl-α-cyclodextrin (HPαCD) of molar substitution (MS) 0.6, 2-hydroxypropyl-β-cyclodextrin (HPβCD) MS 0.6, HPβCD MS 0.9, 2-hydroxypropyl-γ-cyclodextrin (HPγCD) MS 0.6, methyl-β-cyclodextrin (MβCD) of degree of substitution (DS) 1.8 and hydroxyethyl-β-cyclodextrin (HEβCD) MS 0.6 were obtained from Wacker-Chemie (Germany). All other chemicals were commercially available products of special reagent grade.

Chromatographic conditions

The quantitative determination of medroxyprogesterone acetate, megestrol acetate and medroxyprogesterone was performed on a high-performance liquid chromatographic (HPLC) equipment consisting of a Milton Roy Consta-Metric 3000 solvent delivery system, a Merck-Hitachi Model AS-4000 autosampler with a temperature controlled sample rack, a Beckman Ultrasphere ODS 5 μm (150 $\times$ 4.6 mm) column and a Spectra-Physic SP8450 UV/Vis detector operated at 241 nm (medroxyprogesterone acetate and medroxyprogesterone) or 287 nm (megestrol acetate). The mobile phase used consisted of acetonitrile and water (6:4). The retention time was 4.6 min for medroxyprogesterone acetate, 2.6

min for medroxyprogesterone and 4.4 min for megestrol acetate at 1.50 ml/min flow rate.

Buffers

Acetate (pH 3.6–5.6), phosphate (pH 6.4 and 7.3), borate (pH 7.5), Tris (pH 8.0) and carbonate (pH 9.1–9.7) buffers were prepared by mixing aqueous solutions of the acid with aqueous solutions of its corresponding salt. The total concentration of the buffer salts ranged from 0.02 to 0.8 M. Hydrochloric acid solutions were used at pH 1.2–2.2, and sodium hydroxide solution at pH 10.3. The ionic strength of the buffer solutions was adjusted to 0.5 by addition of sodium chloride. In the kinetic studies the CD concentration in the buffer solutions ranged from 0 to 1.0% (w/v). The water used for the buffer preparation was distilled in all-glass apparatus.

Kinetic studies

The degradation studies were carried out by adding stock solution (3–5 μl) of the drug in methanol to aqueous buffer solution (1.5 ml), previously equilibrated at the desired temperature in the temperature controlled sample rack, and mixed thoroughly. Unless otherwise indicated, the initial medroxyprogesterone acetate concentration was 4.4×10^{-5} M, that of megestrol acetate 2.4×10^{-5} M and that of medroxyprogesterone 1.5×10^{-5} M. The pH of the final reaction mixture was determined with a pH-meter (PW 9420 (Philips), U.K.) standardised at appropriate temperature. All reactions were run under pseudo-first-order conditions. Aliquots (20 μl) were injected into the column at various time intervals, and the pseudo-first-order rate constants (k_{obs}) determined from the disappearance of the drug by linear regression of natural logarithm of the peak height vs time plots. The correlation coefficient was calculated for each run.

The enthalpy of activation ($\Delta H^{\ddagger}$) and the entropy of activation ($\Delta S^{\ddagger}$) were determined from linear plots of $\ln(k/T)$, where k is k_{obs}, vs $1/T$ based on the Eyring equation (Loftsson et al., 1992). The stability constant (K_c) for the CD complex and the pseudo-first-order rate constant (k_c) for the degradation of the drugs within the complex were calculated from Lineweaver-Burk plots assuming formation of a 1:1 complex (Loftsson et al., 1989).

Solubility studies

Excess amount of medroxyprogesterone acetate or megestrol acetate was added to aqueous solution containing 0–10% (w/v) HPβCD MS 0.9 or MβCD DS 1.8. The suspension formed was first sonicated in an ultrasonic bath (Kerry, U.K.) for 3 h and then heated in an autoclave (M7 Speed Clave from Midmark Corp., U.S.A.), in a sealed container, to 120°C for 20 min. After equilibration for 6 days at room temperature (approx. 23°C), the suspension was filtered through a 0.45 μm membrane filter (Millex-HV filter units from Millipore, U.S.A.), diluted with a mixture of methanol and water (7:3 v/v) and analysed by HPLC.

Results and Discussion

The degradation of medroxyprogesterone acetate and megestrol acetate followed first-order kinetics in aqueous buffer solutions at constant pH and temperature. At each pH the observed pseudo-first-order rate constant (k_{obs}) was determined at three different buffer concentrations and the value at zero buffer concentration (k'_{obs}) obtained by linear regression. With the exception of borate, all the buffer salts had a detectable catalytic effect on the degradation. The pH-rate profiles are of typical V-shape (Fig. 1) and the experimental results could be fitted to the following equation:

$$k'_{obs} = k_H[H^+] + k_O + k_{OH}[OH^-]$$

where k_H is the second-order rate constant for specific acid catalysis, k_O denotes the first-order rate constant for solvent catalysis (also called non-catalysis), and k_{OH} is the second-order rate constant for specific base catalysis. At 80.0 ± 0.1°C and ionic strength of 0.5 the values of k_H, k_O and k_{OH} were determined to be 3.5×10^{-2} M^{-1} min^{-1}, 2×10^{-5} min^{-1} and 20 M^{-1} min^{-1}, respectively, for degradation of medroxyproges-

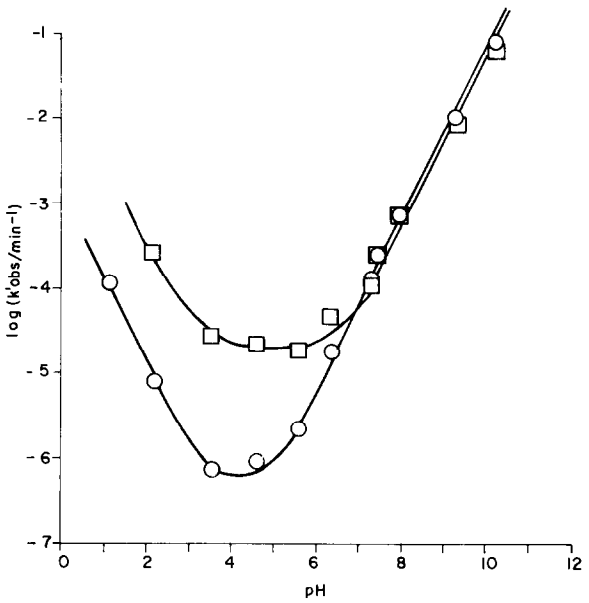


Fig. 1. The pH-rate profiles for the observed first-order degradation of medroxyprogesterone acetate (□) and megestrol acetate (○) at zero buffer concentration, ionic strength 0.5 and 80.0°C.

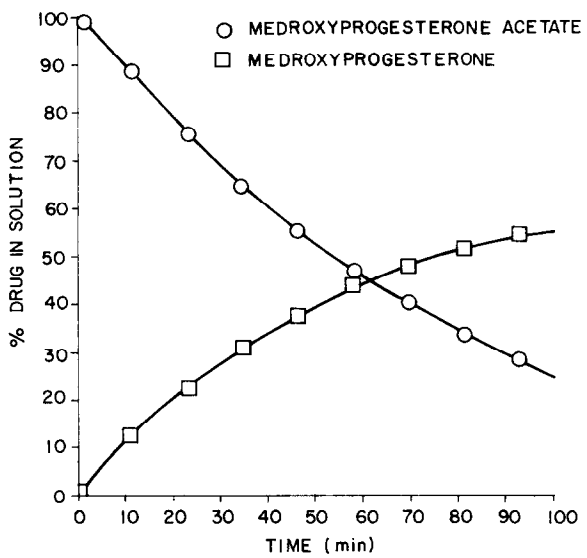


Fig. 2. Kinetics of degradation of medroxyprogesterone acetate in 0.05 M aqueous pH 9.11 carbonate buffer solution at 80°C.

terone acetate and 1.5×10^{-3} M^{-1} min^{-1}, 4×10^{-7} min^{-1} and 25 M^{-1} min^{-1}, respectively, for degradation of megestrol acetate.

In the specific base catalysis region (i.e., at pH greater than 6) of the pH-rate profile large amounts of medroxyprogesterone were formed during degradation of medroxyprogesterone acetate and, thus, the hydrolysis of the acetyl ester appears to be the main degradation pathway under basic conditions (Fig. 2). Formation of medroxyprogesterone could not be detected under acidic condition. Medroxyprogesterone was unstable in aqueous solutions with a half-life of 1.1 h at pH 1.2 and 8.9 h at pH 9.7 and 80°C. Under the same conditions, the half-life of medroxyprogesterone acetate was estimated to be 3–4 h at pH 1 and 1–2 h at pH 9.1. Since at pH 1 medroxyprogesterone was degraded 3–4-times

TABLE 1

Observed first-order rate constants (k_{obs}) and half-lives ($t_{1/2}$) for degradation in 0.05 M aqueous pH 9.4 carbonate buffer solution at various temperatures

Temperature		Medroxyprogesterone acetate		Megestrol acetate	
°C	K	k_{obs} ($\times 10^3$) (min^{-1})	$t_{1/2}$ (days)	k_{obs} ($\times 10^3$) (min^{-1})	$t_{1/2}$ (days)
80.0	353.1	7.53	0.06	10.4	0.05
67.5	340.6	2.38	0.20	3.14	0.15
60.0	333.1	1.09	0.44	1.42	0.34
25.0	298.1	–	23.6 [a]	–	20.7 [a]
5.0	278.1	–	259 [a]	–	341 [a]
$\Delta H^{\ddagger}$	(kJ/mol)	91.4		94.4	
$\Delta S^{\ddagger}$	(J/mol per K)	−62.0		−51.0	

[a] Calculated values.

TABLE 2

Pseudo-first-order rate constants (k_{obs}) for degradation of medroxyprogesterone acetate and megestrol acetate in 0.05 M aqueous pH 9.4 carbonate buffer solution containing no CD or 1.3×10^{-2} M CD at ionic strength 0.5 and 80°C

Cyclodextrin	k_{obs} ($\times 10^3$) (min^{-1})	
	Medroxy-progesterone acetate	Megestrol acetate
No CD	10.1	12.7
HPαCD MS 0.6	7.49	9.31
HPβCD MS 0.9	6.07	6.62
HPβCD MS 0.6	6.78	6.99
HPγCD MS 0.6	10.5	8.17
HEβCD MS 0.6	10.2	10.0
MβCD DS 1.8	2.65	4.40

The initial medroxyprogesterone acetate concentration was 8.8×10^{-6} M and that of megestrol acetate 6.8×10^{-6} M.

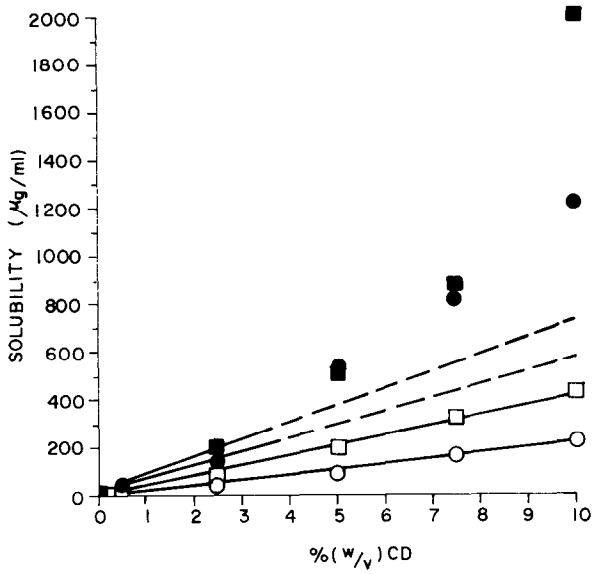


Fig. 3. Phase-solubility profiles of medroxyprogesterone in aqueous HPβCD MS 0.9 (○) and MβCD DS 1.8 (●) solutions, and that of megestrol acetate in aqueous HPβCD MS 0.9 (□) and MβCD DS 1.8 (■) solutions at room temperature (approx. 23°C).

faster than medroxyprogesterone acetate, the possibility that hydrolysis of the acetyl ester was one of the main degradation pathways under acidic conditions cannot be ruled out. At pH 9.1 medroxyprogesterone acetate was degraded much more rapidly than medroxyprogesterone which results in build-up of this hydrolysis product.

The enthalpy ($\Delta H^{\ddagger}$) and the entropy of activation ($\Delta S^{\ddagger}$) for the degradation were determined at pH 9.4 (Table 1). The half-lives of the drugs were estimated to be 20–23 days at pH 9.4 and 25°C. This corresponds to a half-life of 5–6 years and a shelf-life of about 1 year at physiological pH. As shown in Table 2 it was possible to increase their stability by CD complexation. HPβCD MS 0.9 and MβCD DS 1.8 appeared to have the strongest stabilising effect and were studied further (Table 3). The drugs degraded 2.5–4.1-times slower within the CD cavity than outside it (i.e., the k_O/k_c ratio is 2.5–4.1) and there was some difference, albeit not crucial, between the two CDs in this respect, however, MβCD DS 1.8 formed 6–8-times more stable complexes with the drugs than HPβCD MS 0.9 (i.e., the values of K_c in Table 3 are 6–8-times larger for MβCD DS 1.8 than for HPβCD MS 0.9). This can also be clearly seen from the phase-solubility profiles where MβCD DS 1.8

TABLE 3

First-order rate constants (k_c) for degradation of medroxyprogesterone acetate and megestrol acetate within the CD complex and the stability constant (K_c) for the complex in 0.14 M aqueous pH 7.4 phosphate buffer solution at ionic strength 0.5 and 80°C

CD	Medroxyprogesterone acetate		Megestrol acetate	
	k_c ($\times 10^5$) (min^{-1})	K_c (M^{-1})	k_c ($\times 10^5$) (min^{-1})	K_c (M^{-1})
HPβCD MS 0.9	7.56	263	6.59	333
MβCD DS 1.8	4.82	1610	7.19	2680

The value for the first-order rate constant (k_{obs}) for the degradation of medroxyprogesterone acetate when no CD was present in the solution was determined to be 1.97×10^{-4} min^{-1} and that for megestrol acetate to be 1.81×10^{-4} min^{-1}.

results in better solubilisation of the drugs (Fig. 3). This implies that of the two CD tested addition of MβCD DS 1.8 to aqueous formulations of the drugs will result in greater stabilisation and solubilisation than addition of HPβCD MS 0.9. However, since in cancer treatment the daily dosages of the drugs are relatively large (0.3–1.5 g) the solubilising effects of the two CDs are probably not sufficient to formulate the drugs as sublingual tablets or in aqueous solutions.

Acknowledgement

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