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Enzymic and non-enzymic hydrolysis of a polymeric prodrug: Hydrocortisone esters of hyaluronic acid

L.G. Rajewski, A.A. Stinnett, V.J. Stella and E.M. Topp

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045-2504 (U.S.A.)

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Summary

The enzymic and non-enzymic hydrolysis of partial hydrocortisone esters of hyaluronic acid was investigated. Pseudo-first order hydrolysis was observed between pH 3.5 and 10.0 at 37°C and an ionic strength of 0.5 M. Between pH 5 and 10 hydroxide ion catalysis was observed. The apparent energy of activation at pH 7.4 was found to be 22.8 kcal/mol. Exposing the esters to porcine liver esterase for 500 h at pH 7.4 and 25°C did not increase the hydrolysis rate significantly, suggesting that the compound was not a good substrate for the enzyme. Exposure to bovine testicular hyaluronidase and to sequential hyaluronidase then esterase also produced no significant increase in hydrolysis rate. In human serum albumin and human plasma, the hydrolysis rate did not appear to be first-order, and was only slightly accelerated when compared to hydrolysis in an aqueous buffer solution. The implications of the results for use of these polymers in drug delivery are discussed.

Introduction

Hydrocortisone, has been quantitatively esterified to hyaluronic acid, an endogenous mucopolysaccharide (Sparer et al., 1983; Della Valle and Romeo, 1987). The repeating unit of hyaluronic acid consists of alternating units of β-(1–4)-D-glucuronic acid and β-(1–3)-N-acetylglucosamine. Esterification occurs at the C6 carbon of the glucuronic acid residue, as illustrated in Fig. 1.

This polymeric ester of hydrocortisone offers new alternatives for the controlled or sustained delivery of hydrocortisone. Low molecular weight esters of hydrocortisone and other steroids have been synthesized by various researchers in order to overcome solubility limitations which prevent suitable steroid formulation (Anderson et al., 1985; Johnson et al., 1985). Covalent linkage of hydrocortisone to a biocompatible and bioerodible polymer such as hyaluronic acid may provide a means to alter the solubility, stability and pharmacokinetic properties of the resulting drug conjugate. In addition, the polymeric properties of hyaluronic acid may permit the fabrication of novel drug delivery devices.

Drug delivery devices formed from steroid hyaluronate esters are being evaluated; these de-

Correspondence: E.M. Topp, Dept of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045-2504, U.S.A.

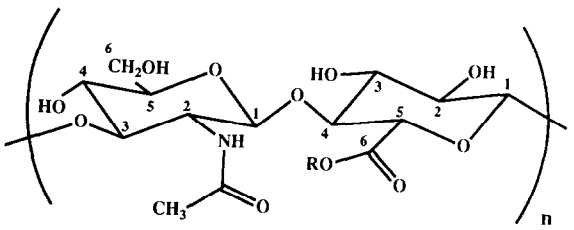

Fig. 1. Structure of hyaluronic acid repeating unit where 100% of R = H for hyaluronic acid; 25% of R = hydrocortisone and 75% of R = sodium salt for HYC13; 25% of R = hydrocortisone, 25% of R = ethanol, and 50% of R = sodium salt for HYC14.

vices include microspheres, films, and pellets (Goei et al., 1989; Joshi et al., 1989; Benedetti et al., 1990). In vitro, drug is released more slowly from devices of the steroid hyaluronate esters than from devices in which the drug is physically incorporated into the hyaluronate derivatives, regardless of the type of device studied. Under certain conditions, approximate zero-order release of drug has been observed and found to be independent of device size and geometry. Rapid hydration of the devices and rapid diffusion of small organic molecules through films of these esters have also been observed (Hunt et al., 1990), suggesting that hydrolysis of the steroid hyaluronate ester may control the release rate of drug from the device.

In vivo, the release of drug from these devices may be influenced by endogenous physicochemical conditions and by local enzyme activities. Although temperature and pH are expected to influence ester hydrolysis rates, fluctuations in these parameters are minimal in vivo. Esterases are known to cleave ester linkages, and hyaluronidases are known to cleave the β-1,4 bond of the hyaluronic acid backbone. In studying factors that will affect in vivo release rates, therefore, both non-enzymic and enzymic effects must be considered.

In this study, the aqueous stability of partial hydrocortisone esters of hyaluronic acid has been investigated. The effects of pH, buffer concentration and temperature on the hydrolysis of the ester bond have been measured. The possible acceleration of ester hydrolysis by porcine liver esterase as well as human serum albumin has also been evaluated. In addition, the polymer has been exposed to bovine testicular hyaluronidase, sequential hyaluronidase and esterase, and human plasma in order to more closely mimic potential enzymic conditions found in vivo.

Materials and Methods

Hydrocortisone hyaluronate esters were prepared by Fidia S.p.A. (Abano Terme, Italy). Two partial esters were used: HYC13, a hyaluronate derivative in which 25% of the carboxyl groups are esterified to hydrocortisone and the remaining 75% are present as the sodium salt, and HYC14, a derivative in which 25% of the carboxyl groups are esterified to hydrocortisone, 25% are esterified to ethanol and the remaining 50% are present as the sodium salt. By saponification in 0.1 M NaOH, the actual percent of hydrocortisone esterified is 24.8% $\pm$ 1.4 for HYC13 and 24.8% $\pm$ 0.9 for HYC14 (personal communication: Benedetti, Fidia S.p.A., 1992). Porcine liver esterase and bovine testicular hyaluronidase were purchased from Sigma Chemical Co. (St. Louis, MO). Buffer components and all other chemicals were obtained from commercial sources.

Non-enzymic hydrolysis

Hydrolysis of the HYC13 and HYC14 esters was studied in the pH range of 3.5–10.0. 5 mg/ml stock solutions of the polymers were prepared in deionized, distilled water and diluted with the appropriate buffer to 0.4 mg/ml at the beginning of each experiment. Buffer systems included: 0.025, 0.05 and 0.10 M formate (pH 3.5); 0.025, 0.05 and 0.10 M acetate (pH 4.5); 0.025, 0.05 and 0.10 M phosphate (pH 7.0 and 7.4); and 0.02 M borate (pH 9.0 and 10.0). Sodium chloride was added in appropriate amounts to each buffer system to maintain the ionic strength constant at 0.15 or 0.5 M. The reaction mixtures were placed in a 37°C water bath and samples were withdrawn at appropriate time intervals. At pH 10.0, 1-ml

samples were quenched with 0.10 ml acetate buffer (1.0 M) at pH 4.5 to prevent further hydrolysis prior to analysis.

Samples were analyzed for free hydrocortisone by a size exclusion chromatographic (SEC) assay. The chromatographic system consisted of a Shimadzu LC-6A pump leading to a Rheodyne six-port manual injector fitted with a 100 μl sample loop. Hydrocortisone was detected by a Shimadzu SPD-6A variable-wavelength UV detector at 248 nm; peaks were integrated by a Shimadzu C-R6A integrator. The SEC column was prepared in our laboratories. The 1 m $\times$ 4.6 mm i.d. column was packed at atmospheric pressure with glyceryl-CPG (Electro-nucleonics, Fairfield, NJ), which has a mean pore diameter of 77 Å and particle size of 200–400 mesh. Analyses were performed under isocratic conditions at ambient temperature with a flow rate of 1.5 ml/min. The completely aqueous mobile phase consisted of 0.1 M acetate buffer at pH 4.5. Under these conditions, the retention volume for free hydrocortisone was 15.3 ml.

At pH values between 7.0 and 10.0, pseudo-first order rate constants were determined from the slopes of linear regressions of $\ln(C_\infty - C_t)/C_\infty$ versus time, where C_∞ is the concentration of free hydrocortisone after complete hydrolysis and C_t denotes the concentration of free hydrocortisone at time, t. At pH 3.5 and 4.5, initial rate methods were employed due to the slow hydrolysis rate. The production of free hydrocortisone was followed for less than 20% of the reaction. The initial rate of hydrolysis was calculated for three initial polymer concentrations (0.4, 0.6, 0.8 mg/ml) and three buffer concentrations. The observed pseudo-first order rate constant was calculated by obtaining the slope from a linear regression of a plot of the initial hydrolysis rate (extrapolated to zero buffer concentration) vs the initial concentration of bound hydrocortisone. The initial amount of bound hydrocortisone was assumed to be equal to the amount of free hydrocortisone following complete hydrolysis and was determined by placing the polymer in pH 10 buffer and monitoring for free hydrocortisone until the hydrocortisone concentration remained constant.

Enzymic hydrolysis

The hydrolysis of HYC13 was studied in the presence of porcine liver esterase. A 0.001 M stock solution of HYC13 was prepared in deionized double-distilled water. The molarity of the solution was calculated as the moles of polymer repeat units per liter of solution, using the hyaluronic acid unit equivalent. The hyaluronic acid unit equivalent is calculated in the following manner: in HYC13, one repeat unit in four is esterified to hydrocortisone while the rest are present as the sodium salt of the carboxylic acid; the hyaluronic acid unit equivalent for HYC13 therefore is 485.9 g/mol.

At the beginning of each experiment, the stock solution was diluted with 0.02 M borate buffer at pH 8.0 and 1 ml of a 10 U/ml esterase solution was added. The solutions were then diluted to one of five final concentrations by the addition of buffer: 1, 50, 100, 150 or 200 μM. At each concentration, controls were prepared by repeating the dilutions above without the addition of the esterase solution. The esterase demonstrated full activity (330 U/mg) in 0.01 M borate buffer at pH 8.0 and 25°C which was determined by following ethyl butyrate hydrolysis as recommended by the supplier (Sigma, 1976).

The solutions were then placed in a 25°C water bath, and 1-ml samples were removed at appropriate time intervals. The production of hydrocortisone was followed by extracting the samples with 1 ml of ethyl acetate. The extraction procedure was repeated three times, after which the ethyl acetate was evaporated with nitrogen gas using a Meyer N-EVAP analytical evaporator. The dried samples were then dissolved in the mobile phase for separation and analysis by reverse-phase liquid chromatography with UV detection. The chromatographic system included an ODS Hypersil column, an Altex 110A solvent delivery pump, a Rheodyne manual injector fitted with a 20 μl loop, a Waters Model 440 UV detector set to 254 nm, and a Shimadzu C-R3A Chromatopac integrator. The mobile phase consisted of 70% water, 30% acetonitrile, and 1% glacial acetic acid. The flow rate was 1.0 ml/min and the retention volume of hydrocortisone was 6.1 ml. The extraction efficiency of hydrocorti-

sone from six aqueous solutions was determined to be 98 ± 1.9%.

The effect of sequential exposure of HYC13 solutions to bovine testicular hyaluronidase and porcine liver esterase was investigated. Two 0.56 mg/ml (1.15 mM) solutions of HYC13 in 0.025 M acetate buffer at pH 4.1 and 0.15 M NaCl were prepared; one solution contained 0.4 mg/ml bovine testicular hyaluronidase, the other contained no added hyaluronidase. The optimum pH for testicular hyaluronidase activity is reported to be near pH 4.0 (Meyer, 1971).

After 24 h at ambient temperature, each solution was diluted 2:1 with 0.05 M borate buffer at pH 10.0, resulting in a pH of 7.5. This dilution was necessary to elevate the solution pH to the optimal pH range reported for esterases (pH 7.5–9.0) (Kirsch, 1971). Sodium chloride was added to increase the ionic strength to 0.44 M. Immediately following this addition, a 3-ml sample was taken from each solution and monitored for changes in free hydrocortisone concentration. Another 3-ml sample was taken from each solution and 0.252 mg (70 U) of porcine liver esterase were added. These solutions were also monitored for free hydrocortisone. Each sample was analyzed for hydrocortisone using the size exclusion assay described previously.

The rate of hydrolysis of HYC13 was also studied in human plasma. A 4 mg/ml HYC13 stock solution was diluted to 0.4 mg/ml with human plasma previously equilibrated to 25°C. 200-μl samples were withdrawn at appropriate time intervals. 400 μl of acetonitrile was added to samples, vortexed, and centrifuged for 2 min at 12400 rpm using a Fisher Scientific microcentrifuge (Model 235C). The supernatant was injected onto an ODS Hypersil reverse phase column. Hydrocortisone was quantitated using the reversed-phase chromatographic assay described previously.

Investigation of HYC13 hydrolysis in human serum albumin was performed in the same manner as described for the plasma studies. Plasmin-5® (Cutter/Miles), a 5% human serum albumin solution, was used as the hydrolysis medium.

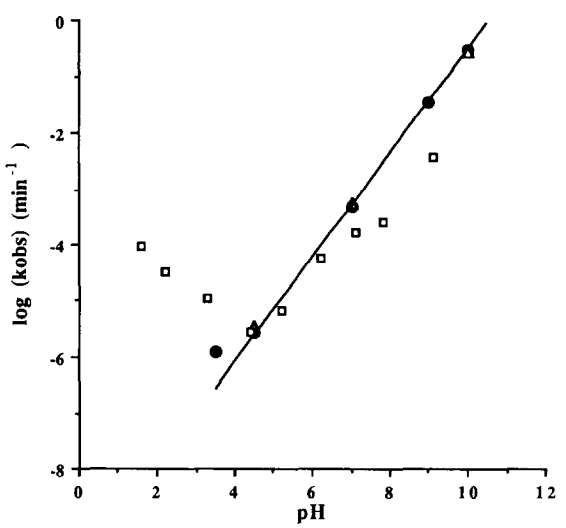

Fig. 2. pH-rate profile for HYC13 (●) hydrolysis and HYC14 hydrolysis (△) at 37°C and 0.5 M ionic strength, and pH-rate profile for hydrocortisone acetate (□) at 22°C; the line represents a linear regression of the HYC13 data.

Results and Discussion

Non enzymic hydrolysis

First-order hydrolysis of hydrocortisone hyaluronate was observed between pH 7 and 10 and was assumed between pH 3.5 and 4.5 where initial rates were measured for less than 20% of reaction. A small effect of buffer concentration on hydrolysis was observed for acetate and phosphate buffer systems. The relationship between the first-order rate constants and pH is illustrated in the pH-rate profile of Fig. 2. In cases where buffer catalysis was observed, the rate constant plotted is the extrapolated value at zero buffer concentration. The slope of a linear regression of the pH rate profile between pH 7 and 10 was 0.93, which indicates hydroxide ion catalysis in this region. This is typical of the hydrolysis of 21-esters of corticosteroids (Adams and Cripps, 1980; Anderson, 1985) and of esters in general (Conners et al., 1986; Lowry and Richardson, 1987; Sykes, 1987). Hydroxide ion catalysis usually arises from the attack of $^{-}$OH on the carbonyl function in the rate determining step of the reaction. Using the Eyring equation, the effect of temperature on the second-order rate constant,

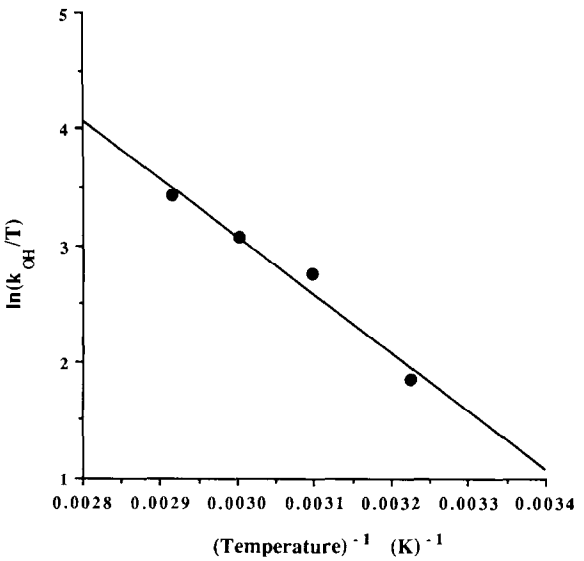

Fig. 3. Eyring plot for HYC13 hydrolysis at pH 7.41 and 0.5 M ionic strength. The line represents a linear regression of the data.

k_{OH} was determined at pH 7.41 (Fig. 3). At each temperature k_{OH} was calculated by dividing the observed rate constant, k_{obs}, by the hydroxide ion concentration previously corrected for temperature. From the Eyring plot illustrated in Fig. 3, $\Delta H^{\ddagger}$ was found to be 9.92 kcal/mol, and $\Delta S^{\ddagger}$ was calculated to be -19.48 cal/deg per mol, which are consistent with a bimolecular transition state in the rate-determining step. The Arrhenius equation was used to determine the effect of temperature on the observed rate constant for hydrocortisone hyaluronate at pH 7.41. The energy of activation was calculated to be 22.8 kcal/mol which is comparable to that of a steroid alkyl ester, hydrocortisone hemmisuccinate, reported to be 19.8 kcal/mol at pH 8.0 (Garrett, 1962). Between pH 3.5 and 4.5 in the pH-rate profile, the data deviate positively from the theoretical line, suggesting that spontaneous or water catalyzed hydrolysis may be important in this pH region. Below pH 3.5 the polymer was not sufficiently soluble to achieve detectable hydrocortisone concentrations with the current assay methods preventing the study of the hydrolysis rate in the 1–3 pH range.

As demonstrated in Fig. 2, the observed rate constants for HYC14 (25% HC, 25% EtOH, 50% Na^+ salt) and for HYC13 (25% HC, 75% Na^+ salt) are superimposable, suggesting that further esterification of the hyaluronate backbone did not affect the hydrocortisone ester hydrolysis rate. The figure also exhibits data of the hydrolysis of hydrocortisone-21-acetate previously reported by Adams and Cripps (1980). In the basic region the pH-rate profile for hydrocortisone acetate is similar to that of the hyaluronate esters, suggesting that hydrolysis is not hindered by the presence of the large polymer chain. The shoulder in the hydrocortisone acetate profile at pH 7.5 may be due to acyl migration between the C21 and C17 positions of hydrocortisone. The absence of this shoulder for hydrocortisone hyaluronate may suggest that acyl migration is sterically hindered by the large hyaluronate group.

Enzymic hydrolysis

Table 1 demonstrates the effect of porcine liver esterase on the first-order rate constants of HYC13 hydrolysis in solutions with polymer concentrations ranging from 1 to 200 μM. For the 1 μM concentration, no hydrocortisone was detected with the analysis procedure used, so the lack of effect of the enzyme on the hydrolysis rate cannot be verified at this concentration. An analysis of covariance was performed on the data of the other concentrations using the ANCOVA program of the BIOM package of Statistical Programs (Rohlf, 1985). The first order rate con-

TABLE 1

First-order rate constants for hydrocortisone hyaluronate hydrolysis in borate buffer solution, pH 8.0, in the presence and absence of porcine liver esterase at 25°C

HYC13 concentration (μM)	k_{obs} ($\times 10^3$) (h^{-1})	
	No esterase	Esterase [a]
50	8.1 ± 0.3 [b]	9.6 ± 0.5
100	7.1 ± 0.2	9.1 ± 0.8
150	8.4 ± 0.3	9.3 ± 0.3
200	8.7 ± 0.3	12.2 ± 0.6

[a] Esterase concentration was 10 U/ml.
[b] Standard error of the slope.

stants for HYC13 hydrolysis in the absence of enzymes at 50–200 μM are equal within the 99.9% acceptance region ($\alpha = 0.001$). In the presence of esterase, the first-order rate constants are equal within the 99.5% acceptance region ($\alpha = 0.005$). This suggests that there is no effect of concentration on hydrolysis in the presence and absence of esterases. At each HYC13 concentration, k_{obs} values in the presence and absence of esterase are equal within the 99% acceptance region ($\alpha = 0.01$), suggesting that the partial hydrocortisone ester of hyaluronic acid is either not a good substrate or at best a poor substrate for porcine liver esterase. In contrast, previous studies by Cheung et al. (1985) have demonstrated that 21-acetate and butyrate esters of hydrocortisone are rapidly cleaved by porcine liver esterase.

The size and polarity of the hyaluronate ester may contribute to its poor affinity for the esterolytic active site. The effect of polymer chain length on the susceptibility of HYC13 to esterases was studied by first digesting the partial ester with bovine testicular hyaluronidase and then adding porcine liver esterase. After the 24 h exposure to hyaluronidase, the HYC13 solution was analyzed by size exclusion chromatography. The elution of the hydrocortisone hyaluronate peak at a longer retention time suggested that the average molecular weight of the polymer had decreased. The absolute molecular weight was not determined, since suitable molecular weight standards for calibration of the size exclusion column have not yet been obtained. Bovine testicular hyaluronidases are known to cleave hyaluronic acid at the β-1,4 linkage ultimately producing disaccharides (10%) and tetrasaccharides (90%) (Meyer, 1971).

Following exposure to hyaluronidase, the pH of the solution was increased to 7.5 to achieve optimum pH for the subsequent esterase addition. The concentrations of hydrocortisone found at various times in each of the following four solutions are listed in Table 2. 'Control 1' was exposed to neither hyaluronidase nor esterase, 'Control 2' was exposed only to esterase, 'Control 3' was exposed only to hyaluronidase, and the 'Test solution' was exposed to both hyaluronidase and esterase. Within the time period studied, the concentrations of free hydrocortisone for control 2, control 3, and the test solution differed less than 4% from control 1, indicating that although the molecular weight of the hyaluronate polymer decreased on exposure to hyaluronidase, the resulting fragments are still poor substrates for porcine liver esterase.

These results are consistent with earlier studies on esterase substrate specificity. O'Neill et al. (1980) studied the effect of acyl carbon chain length on the hydrolysis of hydrocortisone esters by esterases in hamster and guinea-pig skin homogenates. For a series of straight chain esters, they observed a dramatic decrease in esterase

TABLE 2

Free hydrocortisone concentration at various time intervals under four different enzymic conditions at ambient temperature

Time (h)	Free hydrocortisone concentration ($\times 10^2$) (mg/ml)			
	Control 1 (no enzymes) [a]	Control 2 (esterase only) [b]	Control 3 (hyaluronidase only) [c]	Test (hyaluronidase and esterase) [d]
0.00	0.00	0.00	0.00	0.00
26.2	2.64	2.62	2.73	2.59
47.7	3.92	3.87	3.93	3.89

[a] pH 4.1 acetate buffer for the first 24 h, then diluted with borate buffer to pH 7.5.

[b] pH 4.1 acetate buffer for the first 24 h, then diluted with borate buffer to pH 7.5 upon which porcine liver esterase was added to a final concentration of 23 U/ml.

[c] pH 4.1 acetate buffer with 140 U/ml of bovine testicular hyaluronidase for the first 24 h, then diluted with borate buffer to pH 7.5.

[d] pH 4.1 acetate buffer with 140 U/ml of bovine testicular hyaluronidase for the first 24 h, then diluted with borate buffer to pH 7.5 upon which porcine liver esterase was added to a final concentration of 23 U/ml.

activity for acyl chains containing more than six carbon atoms. While the acyl groups in this study are not straight chains, they contain more than six carbon atoms; the smallest acyl groups produced by hyaluronidase digestion are disaccharides containing 14 carbon atoms. The size of the alkyl chain of esters also has been reported to have an effect on esterase catalyzed hydrolysis. Dixon and Webb (1964) studied the reactivity of C1–C8 alkyl chains of acetate and butyrate esters to horse liver carboxyesterase. They have found that a four carbon alkyl chain length demonstrated the highest reactivity. The polarity of the sugar residues of hyaluronic acid may also contribute to the lack of esterase activity observed here. The best substrates for carboxyl esterases are reported to be esters comprised of nonpolar acyl and alkyl components. These compounds exhibit the greatest binding affinity as well as the greatest hydrolytic activity (Dixon and Webb, 1964; Malhorta and Philip, 1966).

To study the potential for other enzymes to catalyze the hyaluronate hydrocortisone degradation, the hydrolysis of hydrocortisone hyaluronate was followed, in vitro, in the plasma of two healthy human volunteers for almost 60 h. The results indicate that hydrolysis does not follow zero-order or first-order kinetics as illustrated in Fig. 4A and B. While the observed non-linearity after 24 h may be indicative of enzymatic catalysis, possible acceleration of hydrolysis due to release of enzymes from bacterial growth at these later time points cannot be ruled out. The apparent pH was measured for both plasma solutions and found to be greater than the expected value of 7.4: pH 7.75 for volunteer 1 and pH 7.85 for volunteer 2. Using the data collected within the first 12 h, the first-order rate constant was calculated for each trial in plasma and compared to that measured in buffer at pH 7.4, as well as that extrapolated to the measured pH value of the plasma samples. These comparisons are listed in Table 3. Extrapolation was based on the k_{obs} measured in buffer solution at pH 7.4 and specific base catalysis in the 7.0–7.4 pH range. In each case, the hydrolysis in plasma was faster by a factor of 1.6–2.9. Lack of a first-order relationship as well as the more rapid hydrolysis rate suggests that catalytic factors affect hydrocortisone hyaluronate hydrolysis in plasma. However, while enzymes present in plasma may play a catalytic role, the very small acceleration of hydrolysis rate in plasma and in enzymic esterase solutions suggest that enzymic catalysis may not be occurring or may not be the

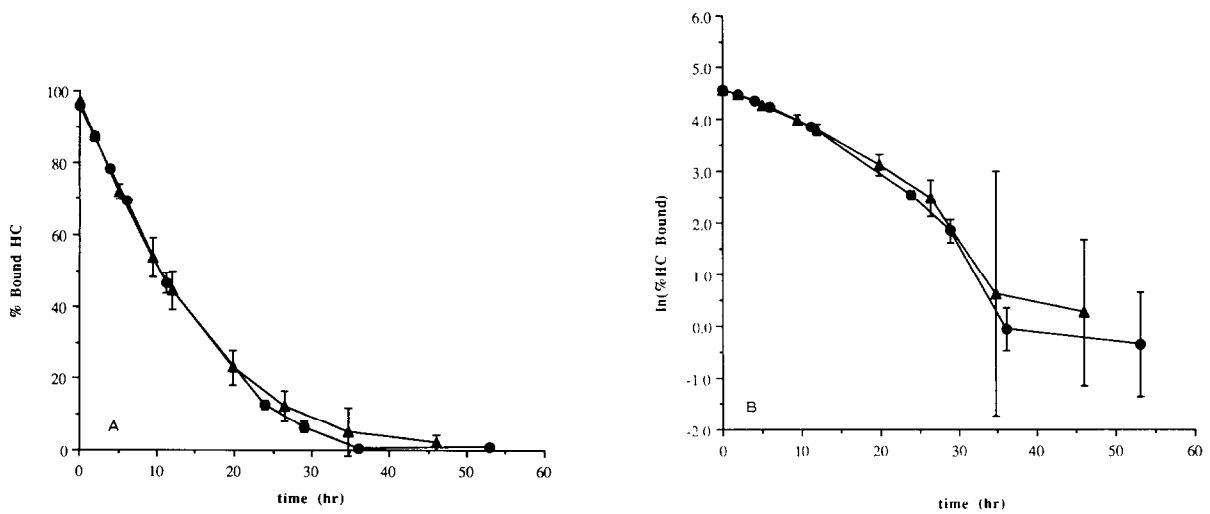

Fig. 4. (A) Zero-order plot and (B) first-order plot of HYC13 hydrolysis in human plasma for two healthy volunteers at 25°C. Two trials in the plasma of volunteer 1 (●) and volunteer 2 (▲) were performed.

only factor resulting in the increase in hydrolysis rate. Al-Habet and Lee (1990) have found the hydrolysis of alkyl esters of prednisolone to display similar kinetic behavior in rat, rabbit, and human plasma, but have observed a dramatic (460–3500-fold) increase in the hydrolysis rate. The authors have attributed this accelerated rate to esterases present in the plasma.

The results of the hydrolysis studies performed in human serum albumin (apparent pH 7.12) vs phosphate buffer (pH 7.12, $\mu = 0.15$ M) are demonstrated in Fig. 5. Again, hydrolysis in albumin does not appear first-order as it does in buffer solution. The first-order rate constant calculated for the first 10 h of the albumin study was approx. 1.6-times faster than the calculated value for buffer. This suggests that the acceleration of hydrolysis in plasma may be attributed in part to general catalysis by plasma proteins.

The results of these studies have implications for the use of hydrocortisone esters of hyaluronic acid as polymeric carriers for hydrocortisone. The small acceleration in enzymic preparations and biological fluids suggests that the release of free drug in vivo will be relatively insensitive to inter-individual variations in enzyme concentrations and activities, and will be determined largely by the pH of the surrounding biological fluid. Furthermore, the results suggest that solutions of hydrocortisone hyaluronate are not suitable formulations unless they can be chemically stabilized, since at the most stable pH studied (pH 3.5) the time for 10% of the hydrocortisone hyaluronate to be hydrolyzed at 37°C is only 58 days. While the rate of hydrolysis is too rapid to permit successful formulation of solutions, it may contribute significantly to the release rate of hydrocortisone from solid devices. In in vitro studies at pH 7.4 involving microspheres, pellets and films formed from slightly soluble hydrocortisone esters of hyaluronate, the time for half of the total amount of drug to be released is on the order of 100 h at neutral pH (Goei et al., 1989; Joshi et al., 1989; Benedetti et al., 1990). The 24 h half-life for hydrocortisone ester hydrolysis at this pH is a significant fraction of the time for half of the drug to be released, suggesting that the hydrolysis rate is a major determinant of release. Finally, the results suggest that the stability of new esters of hyaluronic acid might be predicted from published hydrolysis data on low molecular weight organic analogs. Studies are ongoing to relate release rates to hydrolysis rates and other kinetic phenomena, and to determine the relationship between hydrolysis rate and pH for other esters of hyaluronic acid.

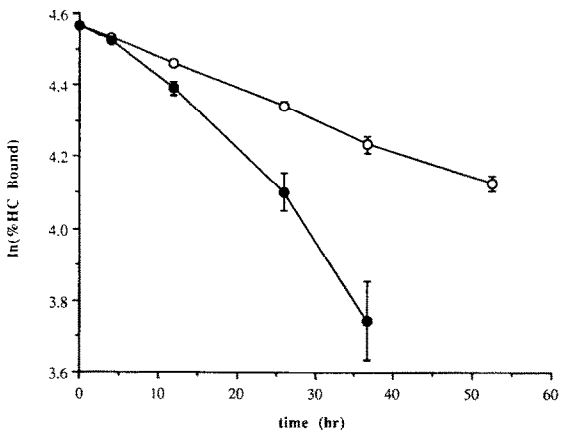

Fig. 5. First-order plot of HYC13 hydrolysis at 25°C in human serum albumin (●) and phosphate buffer (○).

TABLE 3

Experimental and theoretical first-order rate constants in plasma of volunteer 1 and volunteer 2

Condition	pH	k_{obs} [a] measured $(h)^{-1}$	k_{obs} [b] extrapolated $(h)^{-1}$	Fractional increase
Phosphate buffer	7.4	0.0134	–	–
Volunteer 1, trial 1	7.75	0.0694	0.0302	2.5
Volunteer 1, trial 2	7.75	0.0605	0.0302	2.9
Volunteer 2, trial 1	7.93	0.0733	0.0457	1.6
Volunteer 2, trial 2	7.93	0.0716	0.0457	1.6

[a] First-order rate constant measured in plasma solutions.
[b] First-order rate constant in phosphate buffer solution calculated at the measured pH of the plasma solutions.

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References

Adams, P.S. and Cripps, A.L., The stability of hydrocortisone-21-acetate in aqueous solution. *J. Pharm. Pharmacol.*, 32 (Suppl.) (1980) 47P.

Al-Habet, S.M.H. and Lee, H.J., In vitro hydrolysis of steroid acid ester derivatives of prednisolone in plasma of different species. *J. Pharm. Sci.*, 79 (1990) 916–918.

Anderson, B.A., Conrad, R.A. and Knuth, K.E., Strategies in the design of solution-stable, water-soluble prodrugs I: A physical-organic approach to pro-moiety selection for 21 esters of corticosteroids. *J. Pharm. Sci.*, 74 (1985) 365–374.

Benedetti, L.M., Topp, E.M. and Stella, V.J., Microspheres of hyaluronic acid esters-fabrication methods and in vitro hydrocortisone release. *J. Controlled Release*, 13 (1990) 33–41.

Cheung, Y.W., Li Wan Po, A. and Irwin, W.J., Resistance to enzymic hydrolysis as a parameter in drug potency. *Int. J. Pharm.*, 27 (1985) 325–333.

Connors, K.A., Amidon, G.L. and Stella, V.J., *Chemical Stability of Pharmaceuticals*, 2nd Edn, Wiley, New York, 1986, pp. 68–76.

Della Valle, F. and Romeo, A., Hyaluronic acid esters and their medical and cosmetic uses and formulations. *Eur. Pat. Appl. EP 216453*, April 1, 1987, 129 pp.

Dixon, M. and Webb, E.C., *Enzymes*, 2nd Edn, Academic Press, New York, 1964, p. 220.

Garrett, E.R., Alkaline hydrolysis of hydrocortisone hemisuccinate. *J. Pharm. Sci.*, 51, (1962) 445–450.

Goei, L., Benedetti, L.M., Topp, E.M. and Stella, V.J., Drug release from hydrocortisone esters of hyaluronic acid. *Pharm. Res.*, 6 (1989) S94.

Hunt, J.A., Joshi, H.N., Stella, V.J. and Topp, E.M., Diffusion and drug release in polymer films prepared from ester derivatives of hyaluronic acid. *J. Controlled Release*, 12 (1990) 159–169.

Johnson, K., Amidon, G.L. and Pogany, S., Solution kinetics of a water-soluble hydrocortisone prodrug: Hydrocortisone-21-lysinate. *J. Pharm. Sci.*, 74 (1985) 87–89.

Joshi, H.N., Stella, V.J., and Topp, E.M., Drug release from films of hyaluronic acid and its esters. *Pharm. Res.*, 6 (1989) S155.

Krisch, K., Carboxylic ester hydrolases. In Boyer, P.D. (Ed.), *The Enzymes*, 3rd Edn, Academic Press, New York, 1971, Vol. 5, pp. 43–69.

Lowry, T.H. and Richardson, K.S., *Mechanism and Theory in Organic Chemistry*, 3rd Edn, Harper and Row, New York, 1987, pp. 717–723.

Malhorta, O.P. and Phillip, G., Specificity of goat intestinal esterase. *Biochem. Z.*, 346 (1966) 386–402.

Meyer, K., Hyaluronidases. In Boyer, P.D. (Ed.), *The Enzymes*, 3rd Edn, Academic Press, New York, 1971, Vol. 5, pp. 307–320.

O'Neill, R.C. and Carless, J.E., Influence of side chain on the hydrolysis of some hydrocortisone esters. *J. Pharm. Pharmacol.*, 32 (Suppl.) (1980) 10P.

Rohlf, J.F., A Package of Statistical Programs to Accompany the Text: BIOMETRY, Department of Ecology and Evolution, State University of New York, Stony Brook, NY, 1985.

Sigma Chemical Co., Enzymatic assay of esterase (E.C. 3.1.1.1), February 1976.

Sparer, R.V., Ekwuribe, N. and Walton, A.G., Controlled release from gylcosaminoglycan drug complexes. In Roseman, T.J. and Mansdorf, S.Z. (Eds), *Controlled Release Delivery Systems*, Dekker, New York, 1983, pp. 107–119.

Sykes, P., *A Guidebook to Mechanism in Organic Chemistry*, 6th Edn, Wiley, New York, 1987, pp. 238–244.