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Resistance to enzymic hydrolysis as a parameter in drug potency

Y.W. Cheung [1], A. Li Wan Po [2] and W.J. Irwin [3]

[1] *Department of Health and Human Sciences, National Health Institute, Bethesda, MD 20205 (U.S.A.);*
[2] *Department of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, Belfast, Northern Ireland; and* [3] *Department of Pharmacy, University of Aston, Birmingham (U.K.)*

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Summary

The enzymic hydrolysis of hydrocortisone 17- and 21-butyrates and hydrocortisone-21-acetate are studied. The results show that the 17-ester is essentially resistant to hog liver and mouse skin esterases while the 21-esters are highly susceptible thus confirming earlier results obtained with betamethasone valerates. The usefulness of these observations in the design of topically active drugs and prodrugs is discussed.

Introduction

In an earlier paper (Cheung et al., 1985), it was shown that betamethasone-17-valerate and betamethasone-21-valerate possessed marked differences in their susceptibility to liver and skin esterases. This was postulated as a possible reason for their marked differences in potency reported by McKenzie and Atkinson (1964). Their spontaneous non-enzymic hydrolyses are also known to be markedly different (Yip et al., 1983) and because of this, interpretation of experimental data on their metabolism can be difficult. Experiments in which spontaneous decomposition of the esters is not accounted for are not justifiable (Rawlins et al., 1979) since the

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Correspondence: A. Li Wan Po, Department of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, Northern Ireland, U.K.

steroid-17-ester also undergoes isomerisation to the 21-ester (Gardi et al., 1963; Yip and Li Wan Po, 1979; Bundgaard and Hansen, 1981).

The results on the differential rates of enzymic hydrolysis of the isomers (Cheung et al., 1985) have important implications for the design of such molecules because of first-pass metabolism by cutaneous or hepatic esterases following their application to the skin or other tissues of the body. The concept of using active esters which are metabolized to inactive molecules may lead to minimization of systemic toxicity and is therefore attractive particularly for delivering highly toxic molecules topically (Reckers, 1977). To explore these aspects further, the enzymic hydrolysis of hydrocortisone-17- and 21-valerates were studied. This is particularly pertinent since work by Wieriks et al. (1976) indicates that plasma esterases from both rats and man can hydrolyze hydrocortisone-17-butyrate.

Experimental

The buffer systems and materials used were, unless otherwise stated below, the same as those reported earlier (Cheung et al., 1985).

Hydrolysis studies

The hydrolyses were carried out in 20% propylene glycol in Tris buffer solution pH 8.10 ± 0.05 at 37°C. At this relatively high pH, the enzymic activity is optimum but non-enzymic hydrolysis also takes place. To overcome this problem during the preparation of the steroidal solutions, the steroids were predissolved in propylene glycol before adjusting the final solutions which contained 20% propylene glycol, 6.0575 g of tris(hydroxymethyl) aminomethane and 279 ml 0.1 N hydrochloric acid per litre.

Hog liver esterase studies

The concentrations of steroid chosen for the hydrolytic studies were, 0.03 mg/ml for hydrocortisone-21-acetate and 0.1 mg/ml for hydrocortisone, hydrocortisone-17-butyrate and hydrocortisone-21-butyrate. Test solutions were prepared and sampled just prior to addition of 0.1 ml of hog liver esterase solution to 50 ml of the remaining solution. The strength of the standard hog esterase solution was 960 units/ml, each unit hydrolyzing 1 μmol of ethyl butyrate to butyric acid per minute at pH 8 and 25°C. No adjustment was made for the 0.1 ml volume addition to the test solution as the error introduced was negligible. One ml samples of the test solution were taken at intervals and the reaction quenched by the addition of acidified acetonitrile and assayed by HPLC.

Mouse skin homogenates were prepared as previously described (Cheung et al., 1985). Skin homogenate solution, buffer and steroid in buffer were mixed so as to produce final solutions containing 0.02 mg/ml steroid in 20% propylene glycol–aqueous Tris buffer. One-ml samples were withdrawn at intervals, quenched as described above and assayed by HPLC.

High-performance liquid chromatography

The hardware used was as previously described. The mobile phase, however, consisted of 50% acetonitrile delivered at 1 ml · min^{-1}. The internal standard used in the assays for hydrocortisone-21-acetate was 0.01 mg/ml hydrocortisone-21-butyrate while in the assays for the hydrocortisone butyrates, 0.06 mg/ml hydrocortisone-21-acetate was used instead. Both internal standard solutions were made in 50% aqueous acetonitrile acidified so as to be 0.024 M in hydrochloric acid. Control experiments showed that addition of the acidified internal standard solutions quenched the hydrolyses of the systems under study sufficiently to enable test solutions to be stored for at least 1 week at 4°C without detectable decomposition.

Results and Discussion

Fig. 1 shows the hog liver enzymic and non-enzymic hydrolyses of hydrocortisone-17-butyrate in 20% propylene glycol–Tris buffer. It can be seen that as previously reported for betamethasone-17-valerate, the non-enzymic hydrolysis of hydrocortisone-17-butyrate proceeds via an intermediate, hydrocortisone-21-butyrate. The composite reaction can be adequately modelled by the scheme shown below:

hydrocortisone-17-butyrate ⇌ hydrocortisone-21-butyrate
↓
hydrocortisone

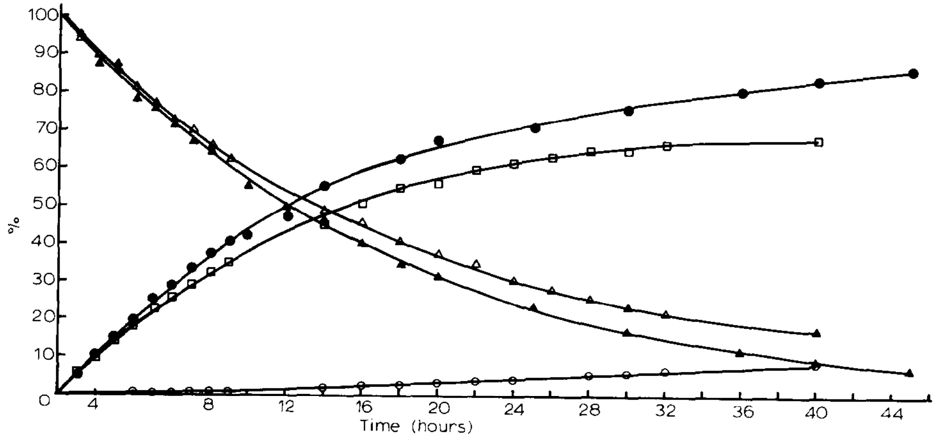

Fig. 1. Enzymic and non-enzymic decomposition of hydrocortisone-17-butyrate, 0.1 mg/ml, in 20% propylene glycol–Tris buffer, pH 8.14, 37°C.

Key: ▲, hydrocortisone-17-butyrate remaining } in the presence of 0.2% esterase
●, hydrocortisone formed
△, hydrocortisone-17-butyrate remaining } in the control
□, hydrocortisone-21-butyrate formed
○, hydrocortisone formed

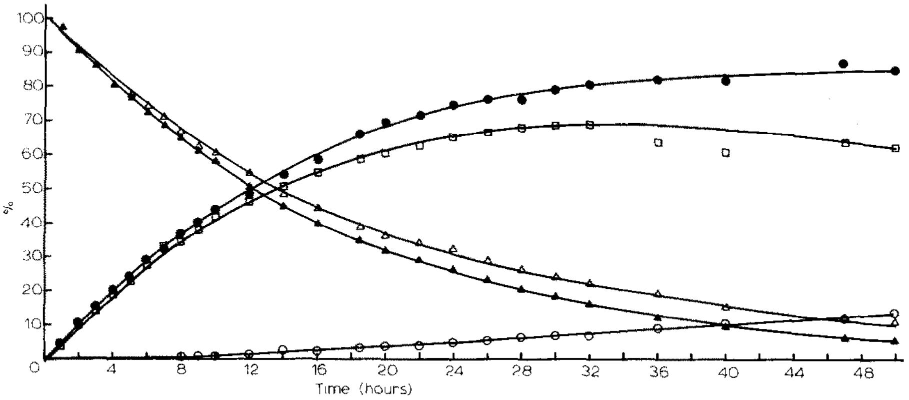

Fig. 2. Enzymic and non-enzymic decomposition of hydrocortisone-17-butyrate, 0.1 mg/ml, in 20% propylene glycol–Tris buffer, pH 8.14, 37°C.

Key: ▲, hydrocortisone-17-butyrate remaining
■, hydrocortisone-21-butyrate formed } in the presence of 0.01% esterase
●, hydrocortisone formed
△, hydrocortisone-17-butyrate remaining
□, hydrocortisone-21-butyrate formed } in the control
○, hydrocortisone formed

The integral equations used for modelling this scheme have previously been reported (Li Wan Po et al., 1979). Examination of the data obtained from the enzymic study indicates that no hydrocortisone-21-butyrate could be detected throughout the run. Although it is tempting to conclude that hydrocortisone-17-butyrate was rapidly hydrolyzed by the enzyme to the steroid alcohol, previous data with betamethasone valerates (Cheung et al., 1985) would suggest that it is more likely that following spontaneous isomerization to hydrocortisone-21-butyrate the latter was rapidly hydrolyzed so that none of it was detected during the reaction. To test for this possibility, the enzymic hydrolysis was repeated with a lower concentration of enzyme, 0.01% v/v instead of 0.2% v/v. Some hydrocortisone-21-butyrate could indeed be detected under the new conditions although only in trace amounts (Fig. 2).

The observed profiles for hydrocortisone-17-valerate in the enzymic and non-enzymic systems indicate that in the latter the concentrations of the parent compound were consistently above the levels observed in the control systems (Figs. 1 and 2). A logical conclusion would be that the enzyme also hydrolyzes the 17-ester although the 21-ester is much more susceptible. An alternative explanation is provided by the reversibility of the isomerization step. If the 21-ester is rapidly hydrolyzed by the enzyme then the isomerization back to the 17-ester will proceed at a slower rate than in the control system and hence higher concentrations of the 17-ester will be observed in the latter system. To determine the relative rates of hydrolysis of the 17- and 21-hydrocortisone esters by the enzyme, the hydrolysis of the 21-ester was monitored and the first-order rate constant calculated (Table 1). The rate constants for all the reactions discussed so far are also listed for comparison.

In calculating the individual rate constants, non-linear regression analysis was used in order to dissociate the reverse rate constants from the forward rate constants

TABLE 1

ENZYMIC AND NON-ENZYMIC TRANSFORMATION RATE CONSTANTS OF STEROIDS IN 20% PROPYLENE GLYCOL–TRIS BUFFER, pH 8.14, AT 37°C

Steroid	Initial concentration (mg/ml)	Enzymic hydrolysis		Non-enzymic degradation (control)	
		% v/v esterase	Observed rate constant [a] (h^{-1})	Observed rate constant [b] (h^{-1})	Corrected rate constant [c] (h^{-1})
Hydrocortisone-21-acetate	0.03	0.2	9.87 (0.165 min^{-1})	No change after 30 min	
Hydrocortisone-17-butyrate	0.1	0.2	0.059	0.0567	
	0.1	0.01	0.057	0.0514	0.0573
	0.1			0.0473	0.0524
Hydrocortisone-21-butyrate	0.1	0.2	instantaneously	No change after 30 min	
	0.1	0.01	9.59	0.0110	0.00602

[a] Rate constants calculated according to the disappearance of the starting compound, have not been corrected for the non-enzymic decomposition.

[b] Rate constants calculated according to the disappearance of the starting compound.

[c] True non-enzymic rate constant, calculated by non-linear regression analysis. With correction for the isomerization of the 21-esters back to the 17-esters.

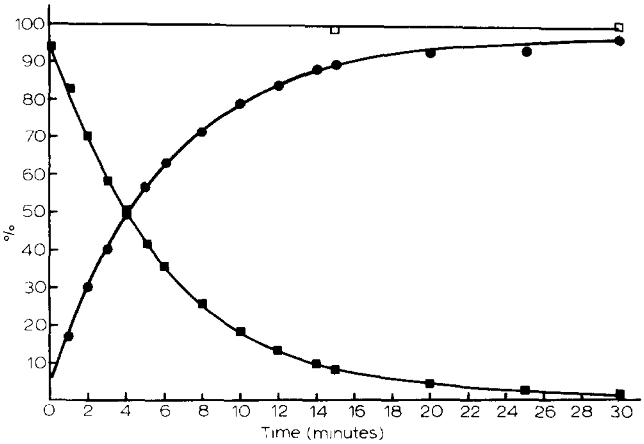

Fig. 3. Hydrolysis of hydrocortisone-21-acetate, 0.03 mg/ml, by 0.2% esterase, in 20% propylene glycol–Tris buffer, pH 8.14, 37°C.

Key: ■, hydrocortisone-21-acetate remaining } in the presence of 0.2% esterase
●, hydrocortisone formed
□, hydrocortisone-21-acetate remaining in the control

and both the corrected and the uncorrected first-order rate constants are listed in Table 1. Close examination of the data (Table 1) for the hydrolysis of hydrocortisone-17-butyrate in the presence and absence of the enzyme is interesting. In the presence of enzyme, the rate constant for disappearance of the 17-ester was higher than in the control system. However, if the rate constant for the non-enzymic hydrolysis of hydrocortisone-17-butyrate was corrected for reversibility by non-linear regression, a rate constant identical to that observed in the enzymic system was obtained. The enzymic rate constant does not need to be corrected for reversibility since only trace amounts of the 21-ester could be detected throughout the run (Fig. 2). One can therefore conclude that, on the basis of these observations, hydrocortisone-17-butyrate is essentially resistant to the hog esterase and isomerization to the 21-ester is required prior to hydrolysis to hydrocortisone. The 21-ester is highly susceptible with a rate constant of 9.59 h^{-1} in the system studied. These observations are in accordance with data reported on the enzymic and non-enzymic hydrolysis of betamethasone esters (Cheung et al., 1985). Under identical conditions, the enzymic hydrolysis rate constant for betamethasone-21-valerate was found to be 26.6 h^{-1}. Hydrocortisone-21-acetate was also highly susceptible to the hog liver esterase (Fig. 3).

To test for extraneous enzymic activity, in the enzyme preparations used, hydrocortisone was incubated under identical conditions. No additional peaks were observed in the chromatograms of the solutions assayed and there was no decrease in hydrocortisone content.

To investigate the behaviour of the hydrocortisone butyrates towards cutaneous esterases, the hydrolysis of the two esters were followed in the presence of mouse skin homogenates. Figs. 4 and 5 illustrate the results. Once again these show that the

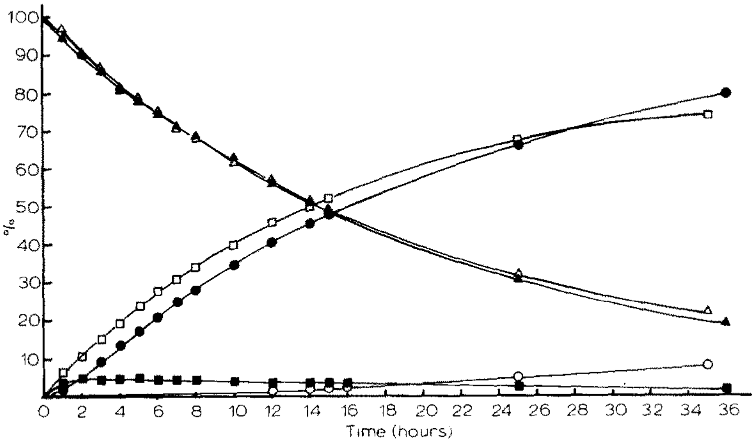

Fig. 4. Biotransformation of hydrocortisone-17-butyrate, 0.02 mg/ml, by mouse skin homogenates, in 20% propylene glycol–Tris buffer, pH 7.93, 37°C.

Key: ▲, hydrocortisone-17-butyrate remaining
■, hydrocortisone-21-butyrate formed
●, hydrocortisone formed
} in the presence of mouse skin homogenates
△, hydrocortisone-17-butyrate remaining
□, hydrocortisone-21-butyrate formed
○, hydrocortisone formed
} in the control

17-ester was essentially resistant to the esterase while the 21-ester was highly susceptible. The respective rate constants, 0.0472 and 77.76 h^{-1}, illustrate how dramatic the differences were. In fact, the disappearance of the 17-ester was

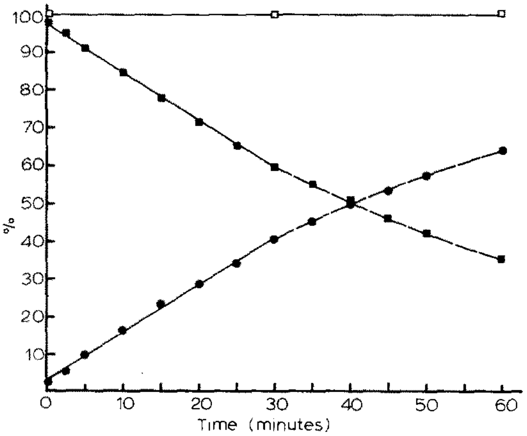

Fig. 5. Biotransformation of hydrocortisone-21-butyrate, 0.02 mg/ml, by mouse skin homogenates, in 20% propylene glycol–Tris buffer, pH 7.93, 37°C.

Key: ■, hydrocortisone-21-butyrate remaining
●, hydrocortisone formed
} with mouse skin homogenates
□, hydrocortisone-21-butyrate remaining in the control

probably again solely due to non-enzymic hydrolysis since in a control system a rate of 0.0479 h^{-1} was obtained.

The studies on hog-liver esterase and cutaneous esterase therefore strongly suggest that hydrocortisone-17-butyrate not only acts as a prodrug of hydrocortisone but is itself also an active entity. In fact, its role as a prodrug is probably limited. Modification of the transport properties of the steroid follows from increased lipophilicity as well as from increased resistance to enzymic hydrolysis. In assessing enzymic lability, the source of esterase is also critical since work by Wieriks et al. (1976) has shown that contrary to our results with hog liver and mouse skin esterases, hydrocortisone-17-butyrate was metabolized by both rat and human plasma esterases. Interspecies differences can, however, not be ruled out.

Conclusion

The data reported here confirm the results of an earlier study which showed that the corticosteroid-17-esters are essentially resistant to hog liver and mouse skin esterases while the 21-esters are highly susceptible. This difference probably largely accounts for the reported differences in the topical activity of the isomers. This study therefore provides useful information for those active in the design of topically active drugs. However, care must be exercised in using this information since the change in structure may also alter substantivity to the skin and may hence also enhance the likelihood of adverse reactions (Somerma et al., 1984).

In evaluating the potential of body tissues to act as metabolic barriers it is important not to confine the study to de-esterification. The skin for example is known to possess reductase (Takayasu et al., 1980), oxidase (Greaves, 1971), and decarboxylase (Lesiewicz and Goldsmith, 1980; Murray et al., 1980) activities. The enzymes may indeed be endogenous as well as exogenous, being produced by skin microflora.

The increased emphasis being placed on metabolic activation and deactivation of steroids (O'Neill and Carless, 1980) and other drugs (Duggan, 1981; Mizushima, 1982) in the context of drug design and delivery is likely to lead to increasingly selective and safer drugs. The data generated should also help in explaining why the percutaneous absorption of drugs such as the steroids increases on long-term administration (Wester et al., 1980).

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