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Identification of esterase involved in the metabolism of two corticosteroid soft drugs

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ABSTRACT

The soft drug approach is successful in obtaining high local therapeutic efficacy without systemic adverse effects, because soft drugs are designed to be bioconverted to inactive form by hydrolytic enzymes in systemic circulation. However, there is little information about the exact nature of these metabolic enzymes. In this study, the human enzymes for biotransformation of soft drugs were investigated. Loteprednol etabonate (LE) and etiprednol dicloacetate (ED) were designed from Δ^1-cortienic acid (Δ^1-CA), the inactive metabolite of prednisolone, by introducing two labile ester bonds to restore the corticosteroidal activity. We found that LE and ED were mainly deactivated in human plasma rather than the liver. Inactive monoesters were produced, but the second hydrolysis to Δ^1-CA was much slower. ED was hydrolyzed 10 times faster than LE in plasma ($t_{1/2}$ = 1.35 ± 0.08, 12.07 ± 0.52 h respectively). Paraoxonase 1 that attached with high density lipoprotein (HDL) was found to be the major hydrolase for LE and ED in human plasma as demonstrated by enzyme inhibition and stimulation experiments and the hydrolysis in lipoproteins-rich plasma fractions. Human serum albumin (HSA) showed slight hydrolase activity against ED but not LE. LE was slowly hydrolyzed in liver (clearance: 0.21 ± 0.04 and 2.41 ± 0.13 ml/h/kg in liver and plasma, respectively) but ED wasn't hydrolyzed at all, so LE has superior metabolism in two sites. The difficult diffusion of HDL into tissues from blood suggests the stable presence of LE at the administration site, while ED might be deactivated by its relatively rapid chemical hydrolysis and hydrolase activity of HSA, in the interstitial fluid of the administration tissue. Moreover, deactivation in plasma and strong protein binding (around 98%) minimize the adverse effects of LE and ED in the systemic circulation.

1. Introduction

The 'Soft Drug' concept was developed in the late 1970s as a comprehensive, general methodology to design safer drugs based on drug metabolism. The general drug design principles, rules and classifications of different soft drugs (i.e. soft analogs, the inactive metabolite approach, the active metabolite approach, etc.) were outlined in 1980 together with examples of specific applications [1]. At the same time, a comprehensive new class of soft corticosteroids, based on the inactive metabolites derived from cortienic acid, substituted in the 17α-position with a unique carbonate and haloalkyl esters in the 17β-position were also developed. These molecules demonstrated remarkably high activities but low systemic side effects, thus providing a significantly improved therapeutic index. The progress of the soft drug field occurred rapidly and the concept became well established [2,3]. One of the main and distinctive principles of the soft drug design is to avoid oxidative metabolic processes and replace them with hydrolytic processes based on the various esterases, since oxidative metabolic processes are slow, easily saturable and usually implicate drug interactions. This can be achieved by starting the design process with an inactive metabolite, formed by oxidative

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processes, often a carboxylic acid compound. Synthetic activation of this acid to an isosteric/isoelectronic analog of the given drug molecule provides the desired activity, but strategic design renders soft derivatives which are substrates to esterases, hydrolytic deactivation forms the inactive metabolite with which the design process started.

The design of the soft corticosteroids successfully led to FDA approved, clinically important novel safe drugs with unique properties such as loteprednol etabonate (LE) and etiprednol dicloacetate (ED), both were designed based on Δ^1-CA (also called prednienic acid), an inactive metabolite of prednisolone. LE has ethyl carbonate ester and chloromethyl ester, while ED has dichloromethyl ester and ethyl ester at 17α- and 17β-positions, respectively [4], as shown in Fig. 1. The binding affinity of LE to glucocorticoid receptors is 4.3 times greater than that of dexamethasone [5], but its therapeutic index (ratio of toxic and effective doses) is 24 times greater than those of most currently-used corticosteroids. The carbonate substitution at 17α-position prevents the formation of the mixed internal anhydride which is presumably cataractogenic [6]. The structure of LE, which prevents the formation of the cataractogenic moiety, makes it ideal for the treatment of ocular inflammation, and it has already been approved by the FDA for this use [5,7]. In addition, a study was recently carried out to evaluate its potential in the treatment of rhinitis [8]. ED has a unique structure as it contains 17α-dichloroester, while no other known corticosteroid contains halogen substitution at the 17α-position. This structure improves the binding affinity of ED to glucocorticoid receptors to be even higher than that of LE [4] and provides fast hydrolysis of the ester bond [3]. ED was developed to be used mainly for the treatment of asthma [9].

It is expected that LE and ED are spontaneously hydrolyzed to their inactive metabolites by several esterases in systemic circulation. The previous *in vitro* hydrolysis studies of LE showed half-life of 4.9 min in rat plasma, and *in vivo* pharmacokinetics studies in rats showed that LE was metabolized to the inactive monoester, Δ^1-cortienic acid etabonate (the "carboxyl metabolite"), which can be excreted in bile and urine or be further metabolized to Δ^1-CA [10]. In dogs, the intact drug was undetectable in plasma after oral administration of LE, while only the carboxyl metabolite was detected but not Δ^1-CA, suggesting its first pass hepatic metabolism to the inactive monoester [11] and very fast elimination of Δ^1-CA. These data indicate the importance of plasma and hepatic hydrolases for the deactivation of LE. In spite of being already released to the market, no information was published about the actual metabolism and elimination of LE in humans. The metabolites of LE were not determined and only its half-life in plasma has been reported, half-life is 9.3 h of *in vitro* hydrolysis in plasma, and 2.2 h of *in vivo* elimination in plasma after intranasal administration [8,12]. In case of ED, less information is available about the pharmacokinetics in experimental animals and humans. Concerning the hydrolysis of ED in human tissues, the available information is only that the incubation of ED with human serum decreases its pharmacological activity by converting it to the inactive hydroxyl metabolite [13]. The results of the previous studies suggest probable metabolism of LE and ED in liver and plasma.

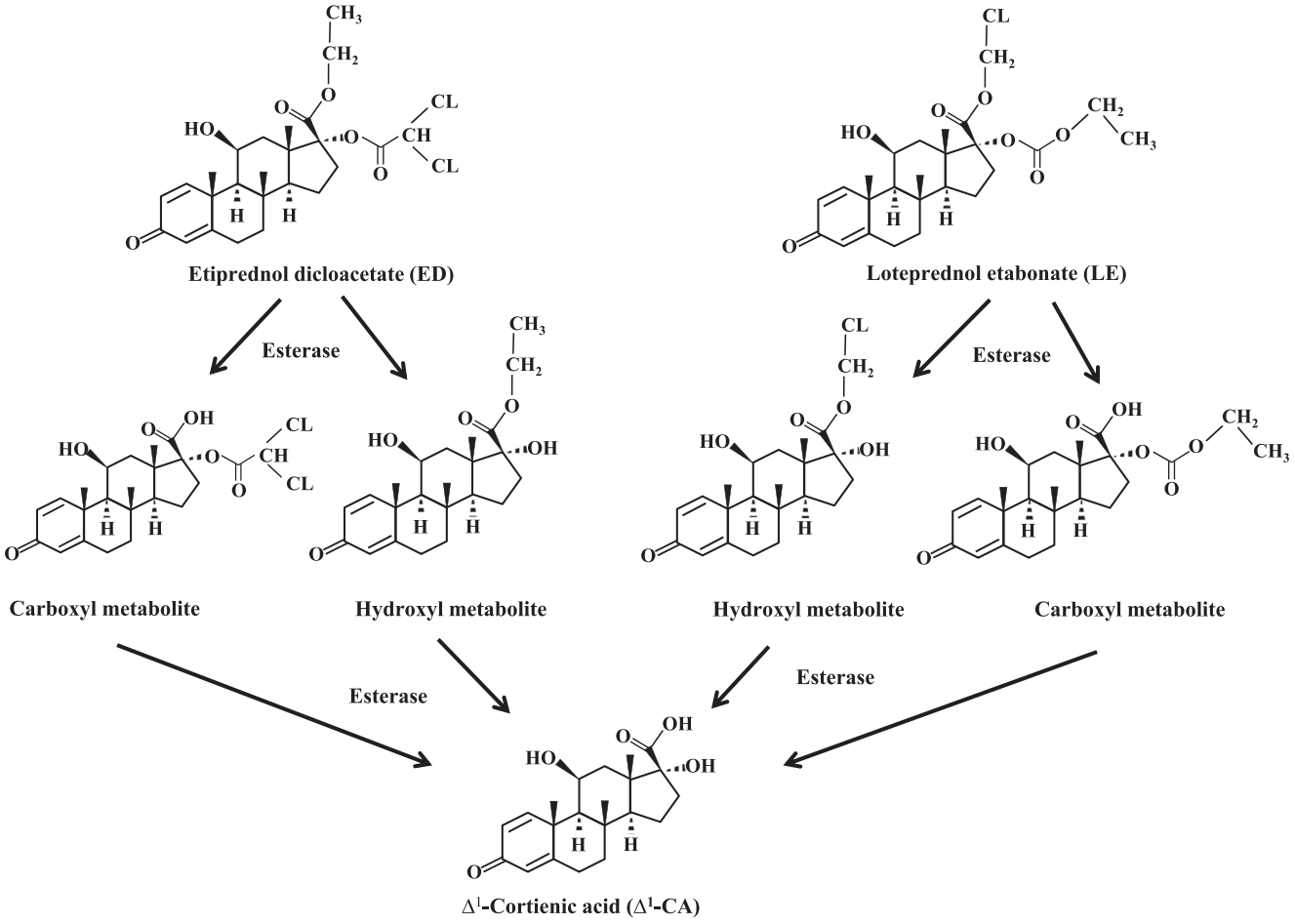

Fig. 1. Chemical formulas of loteprednol etabonate (LE), etiprednol dicloacetate (ED) and Δ^1-cortienic acid (Δ^1-CA), and the expected metabolism pathway of LE and ED in body.

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