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Enhanced Activity of Topical Hydrocortisone by Competitive Binding of Corticosteroid-Binding Globulin

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ABSTRACT

Atopic dermatitis of sensitive areas such as the face, particularly in children, is a difficult disease to treat as the standard therapeutic, topical steroids, is contraindicated for this application in children. Hydrocortisone (HC) can be used in these instances because it has been shown to be safe, but is often ineffective as it is a relatively weak steroid, especially at over-the-counter concentrations. To enhance the local topical activity of HC, the terminal inactive metabolite of prednisolone, Δ^1-cortienic acid (Δ^1-CA), is added to HC, as Δ^1-CA preferentially binds transcortin, liberating more HC to elicit its therapeutic effect. Skin blanching studies, which are used to evaluate the potency of topical steroids, were employed to assess the ability of Δ^1-CA to enhance the activity of HC. The results demonstrate that Δ^1-CA, when applied in combination with HC, does indeed potentiate the vasoconstriction effect of topically applied HC, while having no effect alone. Thus, addition of the inert prednisolone metabolite Δ^1-CA can increase the therapeutic effect of over-the-counter concentrations of HC when applied topically.

Introduction

Glucocorticoids, such as cortisol, regulate a variety of physiological processes including cognition, reproduction, immune function, and many others, which can be exploited for therapeutic development.[1,2] For example, topical corticosteroids still represent the most effective treatment for inflammatory and allergic disorders of the skin. However, topical corticosteroids are not free of adverse effects. Some are topical, such as skin thinning, skin atrophy, and telangiectasia, while others relate to suppression of the hypothalamic-pituitary-adrenal axis and the immune system. Some specific side effects are ophthalmic, such as glaucoma and cataract. For this reason, most steroids cannot be used on the face, which represent a problem for treating children who have the highest incidence of atopic dermatitis and tend to have facial lesions.

The actions of glucocorticoids are mediated by the glucocorticoid receptor (GR), which is present in nearly all tissues, and it is their affinity for this receptor that dictates their relative strength of activity. This activity can be determined by using *in vitro* binding procedures, resulting in potencies being described as relative binding affinity (RBA). On a scale in which dexamethasone has by convention an RBA of 100, the natural hydrocortisone (HC) is considered weak (RBA = 10), while prednisolone (PR) is a stronger steroid, with an RBA of 40.

Another factor that characterizes corticosteroid activity is the binding to transcortin or corticosteroid-binding globulin (CBG). CBG is a 50-60 kDa glycoprotein with a range of glycosylation states[3] and is synthesized primarily in the liver. CBG belongs to the serine protease inhibitor (serpin) superfamily despite having no serpin activity. CBG varies in a diurnal manner opposite to that of total plasma cortisol,[4,5] ranging from 30 to 52 pg/mL. The primary role of CBG is to bind and transport anti-inflammatory steroids, including cortisol and progesterone.[6] The degree of corticosteroid binding to this protein determines the bioavailability of the molecule. Under normal circumstances, approximately 80%-90% of cortisol is bound to CBG, 10%-15% is bound with low affinity to albumin, and the remaining 5%-10% of cortisol is unbound or "free." The binding site is saturable (and molecules with a stronger binding affinity can displace those with weaker affinity); thus, as cortisol levels rise to over 400-500 nmol/L, CBG binding is saturated and free cortisol levels increase rapidly.[7]

CBG may also actively transport cortisol to inflammatory sites. Activated neutrophils are found in regions of inflammation, where they release high concentrations of elastase, a protease that cleaves

Abbreviations used: AUC, area under the curve; CA, cortienic acid; CBG, corticosteroid-binding globulin (a.k.a. transcortin); GR, glucocorticoid receptor; HC, hydrocortisone; LE, loteprednol etabonate; Me-Δ^1-CA, Δ^1-cortienic acid methyl ester; OTC, over-the-counter; Δ^1-CA, Δ^1-cortienic acid (a.k.a. prednienic acid).

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CBG between residues 344 and 345, disrupting the binding site for cortisol and reducing the affinity of CBG for cortisol by 10-fold, thus releasing cortisol.[4,8-10] The conformational transition that CBG undergoes after cleavage by elastase has been revealed by high resolution crystallographic studies.[8,9]

The relative affinities of steroids for the GR and CBG do not run in parallel. Moreover, many of the potent steroids used currently (dexamethasone, betamethasone valerate, ciclesonide, fluticasone propionate) do not bind CBG. However, the simple steroids HC and PR do bind to both sites. It was also found that the acidic inactive metabolite of HC, cortienic acid (CA), or of PR, Δ^1-cortienic acid (Δ^1-CA), while having no affinity for the GR (1/10,000 that of HC), bind relatively strongly to CBG (Table 1, unpublished data). These differential binding properties were initially exploited with loteprednol etabonate (LE), a non-fluorinated "soft" ophthalmic steroid, which also binds to CBG.[11] In these studies, the local activity of LE was found to be significantly enhanced when mixed with Δ^1-CA. The increase in human vasoconstrictor activity was dose dependent and the 24-h area under the curve (AUC) values indicated an up to 3-fold increase in local vasoconstriction, indicative of stronger anti-inflammatory activity.[11]

As shown in Table 1, while intrinsic GR activity of the CA derivatives 3, 4, and 5 is extremely low, their CBG binding is comparable in value to those of HC and PR. As was demonstrated with LE, the local activity of both HC and PR also could be increased by mixing with the inactive metabolites (compounds 3-5, Table 1). For the studies presented here, the Δ^1-derivatives (compounds 4 and 5) were selected, as they have higher binding properties and the Δ^1-double bond contributes to increased stability of the steroidal structure. HC was chosen as the active steroid as it is readily available as an over-the-counter (OTC) product at concentrations up to 1%.

The generally accepted human vasoconstrictor activity assay (Stoughton–McKenzie vasoconstrictor assay) was used to assess the relative local anti-inflammatory activity of various concentrations of HC and Δ^1-CA (or Δ^1-cortienic acid methyl ester [Me-Δ^1-CA]) combinations. This assay system was established as a means to assess *in vivo* bioequivalence and demonstrates relative potencies of topical dermatologic corticosteroids and is an accept method by the US Food and Drug Administration. The molecules were combined in an ethanol/propylene glycol (9:1) solvent mixture, loaded on filter paper disks, and then attached to a water impervious adhesive film. After evaporation of the ethanol, the filters loaded with samples were applied to the forearms of volunteers for 4 h. After removal, the vasoconstriction/blanching reaction was judged by the appearance of pallor at various time intervals, as described in Methods.

Following the studies conducted with ethanol/propylene glycol solutions, the encouraging results prompted the testing of transporter enhancement of the marketed HC creams. Δ^1-CA was added to the OTC HC creams (either 0.5% or 1.0% HC) and thoroughly mixing to final concentrations of either 0.5% or 1.0%. The blanching induced by the HC/Δ^1-CA mixtures was compared directly on the forearm of volunteers.

Table 1
IC50 Values of Selected Steroids for GR and CBG

Number	Compound	GR est. IC50 (μM)	CBG est. IC50 (μM)
1	HC	67	70
2	PR	30	7
3	CA	>1000	120
4	Δ^1-CA	>1000	50
5	Me-Δ^1-CA	>1000	30

I. Kuruc, N. Bodor (unpublished results).