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Effect of Hydrocortisone-17-butyrate, Hydrocortisone, and Clobetasol-17-propionate on Prolyl Hydroxylase Activity in Human Skin

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Anti-inflammatory steroids, perhaps the local pharmaceuticals used most abundantly in dermatology, have many beneficial properties, but the long-term usage of potent steroids can lead to skin atrophy, teleangiectasia, acne rosacea like eruptions, and other side effects [6]. One reason for corticosteroid-induced atrophy is reduced collagen synthesis [1, 3–5, 10].

It has been noted in animal experiments that corticosteroids reduce the activity of prolyl hydroxylase [1, 4, 5], which is necessary in collagen biosynthesis [3]. It is not known, however, whether corticosteroids have the same decrease effect on this enzyme activity in human skin.

The effect of hydrocortisone-17-butyrate, hydrocortisone acetate, and clobetasol-17-propionate on the activity of prolyl hydroxylase are studied here in human skin. Commercial topical preparations of these steroids (Locoid creme 0.1 %, hydrocortisone 1 % in the base of Locoid creme, Dermovat ungt 0.05 %) and the base of Locoid creme as a control were applied to the back of voluntary hospital patients under occlusion twice daily for 14 days. Before treatment the skin was stripped ten times with adhesive tape and original Finn Chambers developed for epicutaneous tests served as occlusive dressings. After the treatment period 6 mm punch biopsies were taken from each test site for analysis.

Some samples were divided, one half being used to study collagen synthesis while the other was taken for the enzyme assays. The samples for enzyme assay were stored in −24° C until analyzed. Samples for collagen synthesis were analyzed immediately by incubating skin slices in Hepes-Krebs medium containing 100 UI penicillin and 50 µg streptomycine per milliliter for 10 h with [^{14}C]-proline [11]. After incubation, the samples were homogenized, dialyzed, and hydrolyzed; and total radioactivity and hydroxy-[^{14}C]-proline activity were assayed by a specific procedure [2].

The samples for the assay of prolyl hydroxylase activity were minced with scissors for 5 min in a cold solution consisting of 0.2 M NaCl, 0.1 M glycine, 50 µM dithiotreitol, 0.1 % (W/V) Triton-X 100 and 20 mM Tris-HCl buffer,

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Table 1. Effect of hydrocortisone-17-buterate, hydrocortisone, and clobetasol-17-propionate on prolyl hydroxylase activity in human skin

No. of patient	C	HB	Change (%)	HC	Change (%)	CP	Change (%)
1	5.70	2.45	−57	6.05	+ 6	3.05	−46
2	7.75	3.30	−57	3.45	−55	3.70	−52
3	3.30	3.55	+ 8	3.35	+ 1.5	2.20	−33
4	1.75	1.85	+ 6	1.90	+ 9	1.20	−31
5	3.33	2.60	−20	3.45	+ 3.6	2.50	−25
6	4.20	3.85	− 8	3.20	−24	3.15	−25

Commercial topical steroids of hydrocortisone-17-butyrate (Locoid creme 0.1%), hydrocortisone (hydrocortisone 1% in the base of Locoid creme), clobetasol-17-propionate (Dermovate ungt 0.05%), and the base of Locoid creme were applied on the back skin under occlusion for 14 days. Prolyl hydroxylase activities were assayed from punch biopsy specimens. The values are expressed as $10^{-3} \times$ d.p.m. per 100 mg wet weight. C (the base of Locoid creme), HB (hydrocortisone-17-butyrate), HC (hydrocortisone), CP (clobetasol-17-propionate)

Table 2. Effect of hydrocortisone-17-butyrate, hydrocortisone and clobetasol-17-propionate on the synthesis of hydroxy-[^{14}C]-proline in skin samples

Patient No. 2	C	HB	Change (%)	HC	Change (%)	CP	Change (%)
Total-[^{14}C]10^{-5} radioactivity d.p.m.	6.5	5.2	−20	3.9	−40	5.2	−20
Hydroxy [^{14}C] $\times 10^{-2}$ proline d.p.m.	15.0	7.1	−59	7.5	−50	11.0	−27

Ointments were applied as indicated on the back skin under occlusion for 14 days. One half of each punch sample was used to study collagen synthesis by incubating thin skin slices with [^{14}C]-proline for 10 h. Total radioactivity and hydroxy-[^{14}C]-proline were assayed as described. The values are expressed as d.p.m. per 100 mg wet weight. Abbreviations as in Table 1

pH 7.5, at 4° C and homogenized with an Ultra-Turrax homogenizer three times for 5 s at 0° C [8]. The samples were maintained at 4° C for 1 h and then centrifuged at 15,000 × *g* for 30 min. Prolyl hydroxylase activities were assayed from the supernatant using [^{14}C]-proline-labeled protocollagen as the substrate [7]. The enzyme assays were carried out in triplicate.

A standard curve was determined to ensure that assays were carried out under conditions in which the enzyme activity/product formation relationship was linear.

Clobetasol-17-propionate reduced prolyl hydroxylase activity in all six patients, markedly so in two of them (Table 1), and hydrocortisone-17-butyrate similarly to a marked extent in two and to a slight degree in two others (Table 1). It should be noted that hydrocortisone also reduced the activity of prolyl hydroxylase in two patients, markedly in one and slightly in the other (Table 1). This agent is also known to reduce prolyl hydroxylase activity in chick-embryo tendon cells [4].

The alterations in collagen synthesis were studied in one patient. All the steroids tested reduced collagen synthesis in the skin when measured in terms of synthesis of hydroxy-[^{14}C]-proline (Table 2). This decrease was even more pronounced than that in the synthesis of other proteins (Table 2).

There was a considerable variation in the enzyme activities even in the control samples when expressed per wet weight (Table 1). One reason for this may be that enzyme activities were not analyzed at once although all samples from one patient were assayed at the same time, so that the activities are comparable for each patient. Another reason may be that the patients were not all of the same age, since it has been reported earlier that prolyl hydroxylase activity may vary with age [8].

The decrease in collagen synthesis correlated rather well with the decreases in prolyl hydroxylase activity (Tables 1, 2), indicating that steroids may affect collagen synthesis in human skin by two mechanisms; by reducing collagen synthesis directly and by reducing prolyl hydroxylase activity. This is the first report to suggest that steroids can lead to a decrease in prolyl hydroxylase in human skin.

Since the material was small, no statistical conclusions can be reached, but it does seem that the potent steroid clobetasol-17-propionate reduces prolyl hydroxylase activity more often than does hydrocortisone-17-butyrate or hydrocortisone. This may be one reason for the high risk of atrophy with potent steroids. It should be noted, however, that even hydrocortisone reduced prolyl hydroxylase activity and collagen synthesis in two patients. The reasons for such individual variations may lie in the differences in the penetration, metabolism, and affinity of the steroids to the tissues.

It has been demonstrated earlier that prolyl hydroxylase is present in the skin as an active tetramer or inactive monomer [7]. This ratio was not studied here. Steroids do not alter this ratio in rats [1] and chick embryo cells [4], even though enzyme activity is changed indicating that steroids reduce enzyme protein synthesis but do not affect the activation of this enzyme.

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