

C₁₆-methyl corticosteroids are far less allergenic than the non-methylated molecules

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Summary

Background. It has been confirmed that binding to amino acids in skin proteins takes place at C₂₁ after oxidation of the corticosteroid molecule, which gives to the constituents of the D-ring an essential role in cross-reactivity patterns. In 2000, Matura et al. subdivided the corticosteroid esters of the D-group into two subgroups: D1, the 'stable' esters; and D2, the 'labile' esters. Recent data have indicated that non-methylated corticosteroids selectively react with arginine to form stable cyclic adducts, which are probably implicated in sensitization to corticosteroids.

Objectives. To compare the patch test results and reactivity of C₁₆-methylated and non-methylated corticosteroids.

Methods. Three hundred and fifteen subjects with a proven corticosteroid contact allergy underwent patch testing with an extended corticosteroid series. Statistical analysis was performed with the Wilcoxon signed rank test to compare the number of reactions to molecules with and without C₁₆-methyl substitution.

Results. Positive patch test reactions to corticosteroid molecules without C₁₆-methyl substitution groups A and D2, were, with statistical significance, much more frequently observed than to those with a C₁₆-methyl group, groups D1 and C.

Conclusions. C₁₆-methyl substitution, interfering with protein binding, and halogenation, seem to reduce the allergenicity of corticosteroid molecules. Hence, when indicated, C₁₆-methylated corticosteroids should be preferentially prescribed.

Key words: C₁₆-methyl substitution; corticosteroids; halogenation; hydrolysis; patch testing; protein binding.

In addition to the increased skin penetration capability of the more lipophilic ester-type corticosteroid molecules, skin metabolism plays an important role in their allergenic potential and also influences cross-reaction profiles (1). The reactions involved in the biotransformation of

corticosteroids have not yet been fully determined, although hydrolysis is one of the most important steps in this process (2). Indeed, for some corticosteroids, chemical or enzymatic hydrolysis of the ester function is often necessary to obtain sufficient biological activity. The extent of hydrolysis depends on the stability and size of the ester, the formulation of a topical corticosteroid-containing medicament, and the individual's enzymatic patterns. C₂₁-corticosteroid esters are less stable (labile) and thus easily hydrolysed, whereas the C₁₇ esters are much more resistant. However, molecules with both a C₁₇ mono-ester function and a C₂₁ hydroxyl group, for example hydrocortisone-17-butyrate, are also unstable

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in an acidic or alkaline environment; after hydrolysis of the C₂₁ ester, the C₁₇ ester can rapidly undergo non-enzymatic conversion into the C₂₁ mono-ester. Enzymatic hydrolysis then quickly follows, converting it into a free alcohol, which is liable to be oxidized. Assuming that all corticosteroids interact with proteins in the same manner, steroid glyoxals or 21-dehydrocorticosteroids (aldehydes), which are the principal metabolites, have been put forward as the haptens that bind to nucleophilic protein residues (3).

After binding to guanine (a nucleic base of arginine), the molecules generated by corticosteroid degradation create stable immune complexes, which are responsible for delayed hypersensitivity (1, 4). It has been shown that steroid glyoxals can bind to every amino acid except proline and hydroxyproline. The arginine reaction, however, is clearly predominant—and also irreversible (5, 6). Wilkinson et al. (5) have shown that corticosteroids with a greater capacity to bind to arginine do have stronger allergenic properties, and Berl et al. (6) recently confirmed that they selectively react with arginine to form important adducts. Moreover, data from Claudel, Lepoittevin and co-workers (7) confirmed that binding to arginine in skin proteins takes place at C₂₁ after oxidation of the corticosteroid molecule.

More recently, Debeuckelaere et al. (8) observed that, in contrast to C₁₆-methylated compounds, non-methylated C₁₆-corticosteroids selectively reacted with arginine to form important stable cyclic adducts that are very probably involved in sensitization to corticosteroids.

With regard to allergic contact reactions, Coopman et al. (9) have classified corticosteroids into four reacting groups, namely, groups A, B, C, and D. In 1995, Lepoittevin et al. (10) carried out conformational analysis of the observed cross-reactivities between corticosteroids, supporting this classification. Later, Matura et al. (1), according to the particular behaviour of certain constituents of group D corticosteroid esters, proposed a further subdivision into two subgroups, the stable D1 and labile D2 esters.

We analysed and compared the patch test results obtained with different esterified corticosteroids in order to confirm the greater reactivity/allergenicity of non-C₁₆-methylated compounds in relation to their chemical structure and reactivity with proteins, as well as with regard to skin metabolism.

Patients, Materials and Methods

We reviewed the results of patch testing with an extended corticosteroid series of 315 corticosteroid-allergic patients in the Dermatology Department at the K. U. Leuven

University Hospital during an 18-year study period (January 1990 to June 2008) (11).

Sixty-six corticosteroids, including those from the baseline series (tixocortol pivalate, budesonide, and hydrocortisone-17-butyrate, as added in our department), were tested. All corticosteroids (except for the two screening agents present in the European baseline series (12)) were prepared in the Contact Allergy Unit by a laboratory technician; the substances used for these preparations had previously been obtained from the pharmaceutical companies marketing them. Dexamethasone sodium phosphate was introduced from November 1990, and difluprednate (Sicor, Milan, Italy), a corticosteroid only recently (2008) marketed for the treatment of ocular pain (13), was included from October 1994 onwards. The test solutions were freshly prepared every 6–12 months.

Ethanol was the vehicle used to test all corticosteroid molecules, except for tixocortol pivalate and budesonide, for which petrolatum worked well (14, 15). Because of their weak trans-epidermal penetration, hydrocortisone and cortisone acetate were diluted in an equal mixture of ethanol and dimethyl sulphoxide (DMSO) (14). Both vehicles were included as controls in the extended series.

The concentrations of the test solutions were defined according to the methods of Isaksson and co-workers (16, 17) (Table 1). For budesonide, lower concentrations were also tested, that is, 0.01% pet. and 0.002% ethanol.

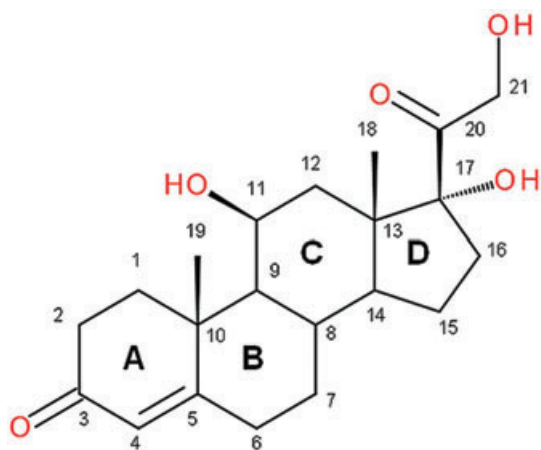
The patch test materials used were van der Bend® Chambers (van der Bend, Briele, The Netherlands) on Mepore® (3M, St. Paul, MN, USA), covered on the upper back with Mefix® (Mölnlycke, Göteborg, Sweden). The patches were removed after 2 days, and the patch test reactions were evaluated at day 2, day 4, and, in many cases, also after 7 days, in accordance with the International Contact Dermatitis Research Group criteria. All clearly positive test results were taken into account in this study. In general, dubious reactions were excluded from our analysis, although weak irritant reactions to the DMSO/ethanol (even in the presence of hydrocortisone) could not always be ruled out with certainty.

The positive reactions obtained with the different molecules were collected, and the results were analysed in relation to the structural characteristics of the molecule, that is, methyl or halogen substitution, and the location of the ester (at C₁₇ and/or at C₂₁) (Fig. 1, molecules of group A without substitution on the D-ring except for a very short chain ester or a thioester on C₂₁, which are immediately hydrolysed after skin penetration; Fig. 2, halogenated molecules of group C with a C₁₆ methyl substitution on the D-ring, without a side chain on C₁₇, but possibly with a side chain ester on C₂₁; Fig. 3, halogenated molecules of group D1 with methyl substitution on C₁₆,

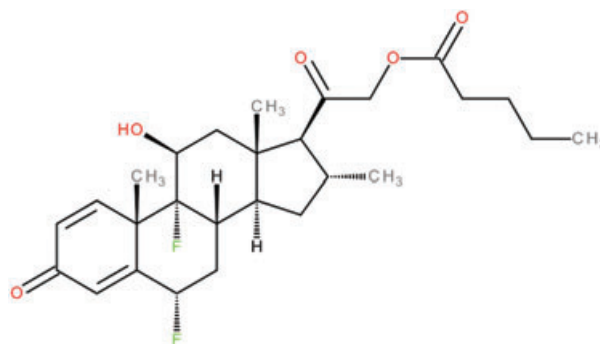
Table 1. Positive patch test reactions to the C₁₆-methylated corticosteroids*

	Corticosteroid molecules	Ester localization	Halogenation type and localization	Group according to the classification of Coopman and Matura	Concentration/vehicle	Number of positive test reactions (n = 315)
1	Beclomethasone dipropionate	C ₁₇ /C ₂₁	C ₉ chlorine	D1	0.1%/ethanol	22
2	Betamethasone	C ₂₁	C ₉ fluorine	C	0.1%/ethanol	36
3	Betamethasone dipropionate	C ₁₇ /C ₂₁	C ₉ fluorine	D1	0.1%/ethanol	19
4	Betamethasone sodium phosphate	C ₂₁	C ₉ fluorine	C	0.1%/saline	9
5	Betamethasone-17-valerate	C ₁₇ /C ₂₁	C ₉ fluorine	D1	0.1%/ethanol	19
6	Clobetasol propionate	C ₁₇ /C ₂₁	C ₉ fluorine	D1	0.1%/ethanol	16
7	Clobetasone butyrate	C ₁₇ /C ₂₁	C ₉ fluorine	D1	0.1%/ethanol	25
8	Desoxymethasone	None	C ₉ fluorine	C	0.1%/ethanol	18
9	Dexamethasone	None	C ₉ fluorine	C	0.1%/ethanol	27
10	Dexamethasone acetate	C ₁₇ /C ₂₁	C ₉ fluorine	C	0.1%/ethanol	18
11	Dexamethasone sodium phosphate	C ₂₁	C ₉ fluorine	C	0.1%/ethanol	9
12	Difflorasone diacetate	C ₁₇ /C ₂₁	C ₆ and C ₉ fluorine	D1	0.1%/ethanol	10
13	Difflocortolone valerate	C ₂₁	C ₆ and C ₉ fluorine	C	0.1%/ethanol	6
14	Flumethasone pivalate	C ₂₁	C ₆ and C ₉ fluorine	C	0.1%/ethanol	6
15	Flucortolone	None	C ₆ fluorine	C	0.1%/ethanol	7
16	Flucortolone caproate	C ₂₁	C ₆ fluorine	C	0.1%/ethanol	13
17	Flucortolone pivalate	C ₂₁	C ₆ fluorine	C	0.1%/ethanol	9
18	Fluprednidene acetate	C ₁₇	C ₉ fluorine	C	0.1%/ethanol	10
19	Fluticasone propionate	C ₁₇ /C ₂₁	C ₆ and C ₉ fluorine	D1	0.1%/ethanol	14
20	Halometasone	None	C ₆ and C ₉ fluorine	C	0.1%/ethanol	6
21	Mometasone furoate	C ₁₇ /C ₂₁	C ₉ chlorine	D1	0.1%/ethanol	15

*Including previously suggested 'stable' ester corticosteroids.

**Fig. 1.** Corticosteroid molecule of group A (for example, hydrocortisone with the conventional numbering of carbon atoms).

halogen substitution, a side chain ester on C₁₇, and possibly a side chain on C₂₁, i.e. the 'stable' esters; Fig. 4, molecules of group D2 with no methyl substitution on C₁₆, no halogen substitution, a side chain ester on C₁₇, and possibly a side chain on C₂₁, i.e. the 'labile' esters.) It is difficult to position difluprednate, as it contains two fluorine atoms in the B-ring of the corticosteroid molecule,

**Fig. 2.** Corticosteroid molecule of group C (for example, diflucortolone valerate).

but not a methyl at position C₁₆ of the D-ring. Moreover, alclomethasone dipropionate, flucortin butylester and meprednisone seem to have some structural particularities (Fig. 5).

For each patient, the number of positive reactions to both the C₁₆-methylated and non-methylated corticosteroids were computed. Statistical analysis was performed with the Wilcoxon signed rank test in order to compare the mean number of reactions to the corticosteroids with

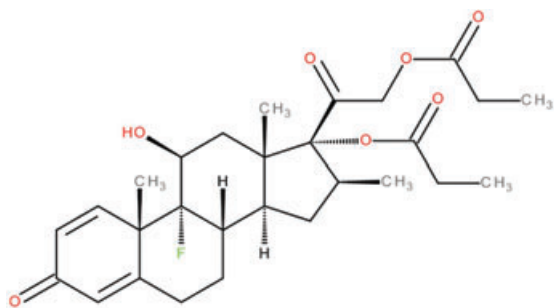


Fig. 3. Corticosteroid molecule of group D1 (for example, betamethasone dipropionate).

and without C₁₆-methyl substitution. The software used was SAS 9.2.

Results

The positive patch test reactions to each of the C₁₆-methyl-substituted corticosteroids from groups C and D1,

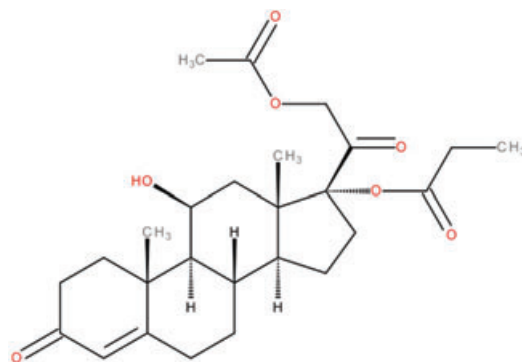


Fig. 4. Corticosteroid molecule of group D2 (for example, hydrocortisone aceponate).

as well as the non-C₁₆-methyl-substituted molecules from groups A and D2, are listed in Tables 1 and 2, respectively. The results presented in terms of mean and standard deviation are summarized in Table 3.

The C₁₆-methyl-substituted corticosteroids produced significantly fewer positive reactions than the ones

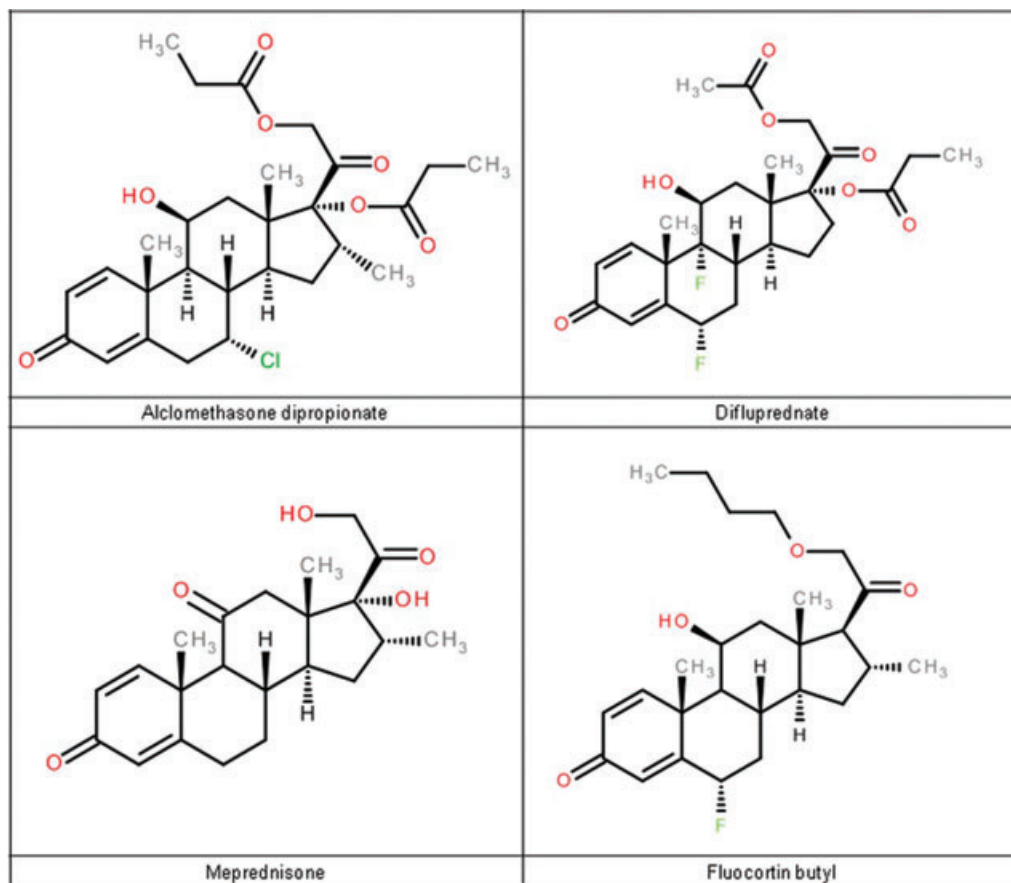


Fig. 5. Particular corticosteroid molecules.

Table 2. Positive patch test reactions to the C₁₆-non-methylated corticosteroids*

	Corticosteroid molecules	Ester localization	Halogen type and localization	Group according to the classification of Coopman and Matura	Concentration/vehicle	Number of positive test reactions (n = 315)
1	Cortisone acetate	C ₂₁	None	A	0.5%/ethanol/DMSO	67
2	Cloprednol	C ₂₁	C ₆ chlorine	A	0.1%/ethanol	61
3	Dichlorisone acetate	C ₂₁	C ₉ chlorine	A	0.1%/ethanol	30
4	Fludrocortisone acetate	C ₂₁	C ₉ fluorine	A	0.1%/ethanol	25
5	Fluprednisolone acetate	C ₂₁	C ₆ fluorine	A	0.1%/ethanol	15
6	Hydrocortisone aceponate	C ₁₇ /C ₂₁	None	D2	1%/ethanol	86
7	Hydrocortisone	No	None	A	0.5%/ethanol/DMSO	99
8	Hydrocortisone acetate	C ₂₁	None	A	0.1%/ethanol	48
9	Hydrocortisone-17-butyrate	C ₁₇ /C ₂₁	None	D2	0.1%/ethanol	96
10	Hydrocortisone-21-butyrate	C ₂₁	None	A	0.1%/ethanol	66
11	Hydrocortisone hemisuccinate	C ₂₁	None	A	0.1%/ethanol	15
12	Isofluprednone acetate	C ₂₁	C ₉ fluorine	A	0.1%/ethanol	14
13	Mazipredone	C ₂₁	None	A	0.1%/ethanol	67
14	Medrysone	C ₂₁	None	A	0.1%/ethanol	23
15	Methylprednisolone aceponate	C ₂₁	None	D2	0.1%/ethanol	105
16	Methylprednisolone acetate	C ₁₇ /C ₂₁	None	A	0.1%/ethanol	58
17	Methylprednisolone hemisuccinate	C ₂₁	None	A	0.1%/ethanol	17
18	Pivalate tixocortol	C ₂₁	None	A	0.1%/petrolatum	135
19	Prednicarbate	C ₁₇ /C ₂₁	None	D2	1%/ethanol	111
20	Prednisolone	No	None	A	0.1%/ethanol	55
21	Prednisone	No	None	A	0.1%/ethanol	43
22	Prednisolone caproate	C ₂₁	None	A	0.1%/ethanol	70
23	Prednisolone sodium metasulphobenzoate	C ₂₁	None	A	0.1%/ethanol	48
24	Prednisolone pivalate	C ₂₁	None	A	0.1%/ethanol	46
25	Prednisolone hemisuccinate	C ₂₁	None	A	1%/ethanol	13

DMSO, dimethyl sulphoxide.

*Including previously suggested 'labile' ester corticosteroids.

Table 3. Mean ± standard deviation (SD) of positive patch test reactions to corticosteroids belonging to the C₁₆-methylated and C₁₆-non-methylated groups

Group	Mean ± SD
C ₁₆ -methylated corticosteroid (C-D1)	13.8 ± 5.7
C ₁₆ -non-methylated corticosteroid (A-D2)	55.0 ± 36.2
C ₁₆ -non-methylated group A corticosteroid	44.5 ± 31.6
C ₁₆ -non-methylated group D2 corticosteroid	99.5 ± 10.9

without this particular substitution ($p < 0.0001$), except for certain molecules, for example fluocortin butyl, for which irritant reactions could not always be entirely ruled out (16 of 42 patients presented with isolated positive reactions to this molecule within group C), and alclomethasone dipropionate, which, according to the present results, co-reacts more often with the so-called labile esters than with the stable esters, as previously suggested (1) (Fig. 6). Moreover, the non-halogenated

meprednisone also produced slightly more positive reactions (Table 4).

Among the corticosteroids without C₁₆-methyl substitution, frequent reactions were observed to molecules from the 'labile' D2 esters, particularly to methylprednisolone aceponate ($n = 105$) and hydrocortisone-17-butyrate ($n = 96$), but also to hydrocortisone aceponate ($n = 86$) and prednicarbate ($n = 111$), the latter two not being commercialized in Belgium (Fig. 6). Most patients reacted to more than one molecule from this group, and 37 patients reacted to all four.

Difluprednate produced relatively few positive reactions ($n = 15$) (Table 4).

Within this group of corticosteroids without C₁₆-methylation, positive reactions were significantly less frequently observed to single C₂₁ esters belonging to group A than to the mono-ester hydrocortisone-17-butyrate and also to the double C₁₇ and C₂₁ esters belonging to group D2 ($p < 0.0001$).



Fig. 6. Positive patch test reactions to non-methylated group D2 molecules and to alclomethasone dipropionate in the same patient.

Table 4. Particular corticosteroid molecules

	Corticosteroid	Particular characteristics	Group according to the classification of Coopman and Matura	Concentration/vehicle	Number of positive tests (n = 315)
1	Alclomethasone dipropionate	C ₁₇ /C ₂₁ esters C ₁₆ -methyl Exocyclic chlorine	D1	0.5%/ethanol	57
2	Difluprednate	C ₁₇ /C ₂₁ esters No C ₁₆ -methyl halogenation	D2	0.1%/ethanol	15
3	Fluocortin butyl	C ₂₁ ester C ₁₆ -methyl halogenation Frequent isolated reactions	C	0.1%/ethanol	42
4	Meprednisone	No C ₁₇ /C ₂₁ esters C ₁₆ -methyl No halogenation	C	0.1%/ethanol	24

Discussion

C₁₆-methylated corticosteroids

Both C₁₆-methyl substitution and halogenation seem to stabilize corticosteroid molecules and thus influence their sensitization potential. C₁₆-methyl substitution, because of steric hindrance, has been considered to protect them against C₂₁ hydrolysis, probably resulting in less rapid conversion into aldehydes. Recent studies (8) have indicated that C₁₆-methyl substitution does not prevent hydrolysis, but avoids the formation of stable cyclic adducts with arginine that leads to sensitization.

Non-methylated corticosteroids

Molecules with single (C₂₁) or double (C₁₇ and/or C₂₁) esterification, but without halogenation or C₁₆-methyl substitution, undergo more rapid metabolic degradation, and form stable cyclic adducts with arginine, rendering them much more apt to sensitization. The double esters, that is, methylprednisolone aceponate, hydrocortisone aceponate, and prednicarbate, reacted positively more often than the mono-esters (except for hydrocortisone-17-butyrate, which is easily converted into hydrocortisone-21-butyrate); this can be explained by enhanced skin penetration resulting from increased lipophilicity. Indeed, metabolization occurs both with mono-esterified and double esterified molecules, and, hence, depends only on

the patient's enzymatic equipment; therefore, the presence of a double ester that needs to be hydrolysed twice does not protect against sensitization.

The low frequency of reactions to difluprednate, which does not contain a C₁₆-methyl substitution, was comparable to that for the D1 ester group. This could be explained by halogenation of the corticosteroid structure, the importance of which has been elucidated by Wilkinson et al. (18). Indeed, they showed that corticosteroids with halogen substitutions undergo less rapid metabolic degradation, and therefore bind less easily to arginine than their non-fluorinated derivatives, and are hence less likely to induce allergic reactions. Although the fluorine atom is similar in shape and size to the hydrogen atom, the difference in reactivity between halogenated and non-halogenated molecules may be caused by charge differences. It is also possible for a halogen atom in position C₉ to exert an electrical attraction for the carbon atom at C₁₁, conferring higher stability to the corticosteroid and therefore limiting the creation of allergenic by-products (12). This could explain the rather frequent reactions observed with alclomethasone dipropionate, which theoretically belongs to group D1; it contains only an exocyclic chlorine (and not an endocyclic one) that is located further away and probably does not interfere with the C₁₁ carbon atom.

However, whereas positive allergic responses are less frequent with fluorinated derivatives in groups A, C and D1, the halogen substitution in group B corticosteroid molecules does not influence the more pronounced allergenic properties of this group (11). It is possible that the C16, C17 *cis* ketal or diol structure on the D-ring, characteristic of group B molecules, plays an important role in protein binding and immune recognition.

Conclusion

Positive patch test reactions were, with statistical significance, much more frequently observed to corticosteroid molecules without C₁₆-methyl substitution in the D-ring, that is, groups A (esterified or not) and D2, than to those that are halogenated and have a methyl group at C₁₆, that is, groups D1 and C. C₁₆-methyl substitution, which interferes with protein binding, and also halogenation of corticosteroid molecules, which plays an important role in their stabilization, seem to protect them from being sensitizing. Also, the presence of a long ester chain at C₁₇, as present in the so-called 'stable' esters, seems to protect the molecule from rapid degradation (4). This confirms recent experimental data showing that the different reactivities of methylated and non-methylated corticosteroids depend on the stability of the cyclic adducts formed with arginine during protein binding, the non-methylated ones forming more stable adducts. Therefore, it seems to be more appropriate to refer to 'C₁₆-methylated' and 'C₁₆-non-methylated' corticosteroids than to 'stable' and 'labile' esters, as the non-esterified compounds are also concerned.

C₁₆-methylated corticosteroids should be prescribed preferentially if a topical corticosteroid treatment is needed, while, of course, taking into account their required potency.

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