

Corticosteroid cross-reactivity: clinical and molecular modelling tools

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Abstract

Background: Corticosteroids have been classified into following four cross-reacting groups in function of their contact-allergenic properties: A, B, C and D, the last subdivided into D1 and D2. Recent data indicate that C₁₆-methylated and non-methylated molecules need to be distinguished, the latter selectively binding with arginine to form stable cyclic adducts and producing considerably more positive reactions than the former. This study compares molecular modelling and patch-test results to determine cross-reactivity patterns.

Methods: The patch-test results obtained with 66 corticosteroid molecules in 315 previously sensitized subjects were analysed and correlated with modelling and clustering in function of the electrostatic and steric fields of these molecules.

Results: The classification obtained after *in silico* hydrolysis of C₂₁ and C₁₇ esters was selected with an optimal cut into three clusters: the patients who reacted positively to cluster 2 (halogenated molecules from group B, with C₁₆/C₁₇ cis ketal or diol structure) and cluster 3 (halogenated molecules from groups C and D1, C₁₆-methylated) also reacted to cluster 1 (molecules mostly from groups A and D2, without C₁₆-methyl substitution or halogenation and budesonide). The reverse, however, was not the case.

Conclusion: Two patient profiles with probably different areas of immune recognition are identified as follows: the profile 1 patients were allergic to the frequently positively reacting cluster 1 only, for whom electrostatic fields (molecular charge) seem important; the profile 2 patients reacted to clusters 1 and 2 and/or 3, for whom steric fields (structure) are determinant and who probably presented a global recognition of the corticosteroid skeleton. A modified classification is thus proposed.

In 1989, Coopman et al. (1) classified corticosteroids (CSs) into four reacting groups A, B, C and D in function of contact-allergic reactions and structural characteristics. In 1995, Lepoittevin et al. (2) supported this classification and the central role of constituents of the D ring by means of conformational analysis of the observed cross-reactions (Data S1 in supporting information).

Later on, Matura et al. (3), observing the particular behaviour of certain constituents of group D CS esters, proposed a further subdivision into two subgroups, i.e. the stable D1 and labile D2 esters (Table 1).

In addition to the C₁₆/C₁₇ substitutions, Wilkinson et al. (4) considered that the A-ring was a second reactive centre and that halogenation of the CS structure (C₆ and/or C₉) was of critical importance in their cross-sensitivity pattern.

We observed (5) that positive patch-test reactions occurred much more frequently to CS molecules with no C₁₆-methyl substitution or halogenation, i.e. groups A and D2, than to those that were halogenated and had a C₁₆-methyl group, i.e. groups C and D1. Indeed, C₁₆-methyl substitution, which interferes with protein binding, and halogenation, which plays an important role in the stabilization of CS molecules

Table 1 Corticosteroid classification according to Coopman et al. (1)

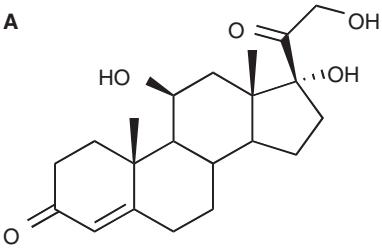
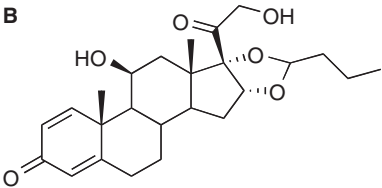
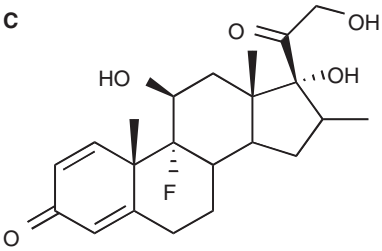
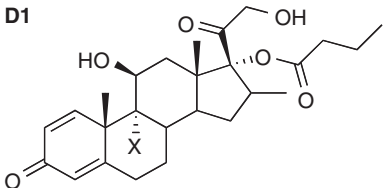
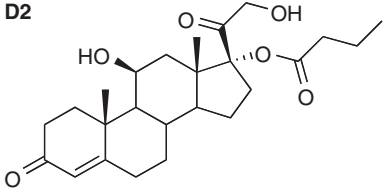
Group	Characteristics of the group	Typical members	Possible cross-reactions
A 	No substitution on the D ring except a short chain ester or a thioester on C ₂₁	Cloprednol Cortisone acetate Dichlorisone acetate Fludrocortisone acetate Fluorometholone Fluprednisolone acetate Hydrocortisone Hydrocortisone acetate Hydrocortisone 21-butyrate Hydrocortisone hemisuccinate Isofluprednone acetate Mazipredone Medrysone Methylprednisolone acetate Methylprednisolone hemisuccinate Prednisolone Prednisolone caproate Prednisolone pivalate Prednisolone sodium metasulpho benzoate Prednisolone succinate Prednisone Tixocortol pivalate	D2
B 	C ₁₆ , C ₁₇ cis Ketal or diol structure Possibly a side chain on C ₂₁	Amcinonide Budesonide Desonide Fluchloronide Flumoxonide Flunisolide Fluocinolone acetonide Fluocinonide Halcinonide Triamcinolone Triamcinolone acetonide Triamcinolone benetonide Triamcinolone diacetate Triamcinolone hexacetonide	
C 	C ₁₆ methyl substitution on the D ring Halogen substitution No side chain on C ₁₇ Possibly a side chain on C ₂₁	Betamethasone Betamethasone sodium phosphate Desoxymethasone Dexamethasone Dexamethasone acetate Dexamethasone sodium phosphate Diflucortolone valerate Flumethasone pivalate Fluocortin butyl Fluocortolone Fluocortolone caprylate Fluocortolone pivalate Fluprednidene acetate Halomethasone Meprednisone	

Table 1 (Continued)

Group	Characteristics of the group	Typical members	Possible cross-reactions
D1 	Methyl substitution on C ₁₆ Halogen substitution Side chain ester on C ₁₇ Possibly a side chain on C ₂₁	Alclomethasone dipropionate Beclomethasone dipropionate Betamethasone dipropionate Betamethasone 17-valerate Clobetasol propionate Clobetasone butyrate Diflorasone diacetate Fluticasone propionate Mometasone furoate	
D2 	No methyl substitution on C ₁₆ No halogen substitution Side chain ester on C ₁₇ Possibly a side chain on C ₂₁	Difluprednate Hydrocortisone aceponate Hydrocortisone 17-butyrate Methylprednisolone aceponate Prednicarbate	A Budesonide (S isomer)

(4), seem to protect them from sensitization properties. It has been found (6) that CSs selectively react with arginine to create immune complexes (adducts), which are responsible for delayed hypersensitivity and that binding to arginine in skin proteins takes place at C₂₁ after oxidation of the CS molecule (7, 8). Hence, hydrolysis of the C₂₁ ester is one of the most important steps in the biotransformation of CSs (9). Recent experimental data (10) show that the different reactivity of methylated and nonmethylated CSs depends on the stability of these adducts, the nonmethylated being more stable than the methylated. Therefore, it seems to be more appropriate to refer to 'C₁₆-methylated' and 'C₁₆-nonmethylated' CSs, instead of 'stable' and 'labile' esters, because the nonesterified compounds are also involved.

Materials and methods

Clinical data

We reviewed and analysed the results of patch testing with an extended CS series of 315 CS-sensitized patients in the Dermatology Department at the *Katholieke Universiteit Leuven* compiled in the course of 18 years (January 1990 to June 2008) (11).

Sixty-six CSs, including those from the baseline series (tixocortol pivalate, budesonide and hydrocortisone 17-butyrate as added in this department), were tested. All CSs (except for the two screening agents present in the European baseline series) (12) had been prepared in the Contact Allergy Unit by a laboratory technician. The substances used for these preparations had been obtained from the pharmaceutical companies that marketed them. Dexamethasone sodium phosphate was introduced in November 1990 and difluprednate in October 1994. The test solutions were freshly prepared every 6–12 months.

Ethanol (eth.) was the vehicle used to test all of the CS molecules except for tixocortol pivalate and budesonide, for

which petrolatum (pet.) worked well (13, 14). Because of their weak trans-epidermal penetration, hydrocortisone and cortisone acetate were diluted in an equal mixture of ethanol and dimethyl sulfoxide (DMSO) (13). Both vehicles were included as controls in the extended series.

Owing to the anti-inflammatory properties of CSs, 0.1% was the test concentration used instead of 1%, as classically described in the literature (15, 16). For budesonide, lower concentrations were also tested: 0.01% pet. and 0.002% eth (16). The corticosteroid series, along with test concentrations and vehicles, are listed in the Data S2.

The patch-test materials used were van der Bend[®] chambers (van der Bend, Briele, The Netherlands) on Mepore[®] (3M, Saint 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 ICDRG criteria. All clearly positive tests were taken into account in this study. In general, dubious reactions were excluded from our analysis although weak irritant reactions to the DMSO/eth. could not always be ruled out with certainty (even in the presence of hydrocortisone).

The positive reactions obtained with the different molecules were collected, and the results were analysed in function of the structural characteristics of the molecule, i.e. methyl- and/or halogen substitution, and location of the ester (at C₁₇ and/or C₂₁).

Molecular modelling

The C₂₁ and C₁₇ esters were removed *in silico* to mimic as much as possible the natural hydrolysis of these esters, and the resulting structures were modelled. Out of the 66 molecules, 47 unique molecules could be obtained. For each model, geometric optimization was performed using the Sybyl

X-1.2 molecular modelling package (Tripos Inc. St. Louis, MO, USA) with the Merck molecular force field (MMFF94). Each conformation was checked and then added to a chemical structural database, which was rigidly aligned using atoms of the steroid rings, which gives excellent superposition of all molecules despite a certain degree of molecular diversity. The lateral chains of the molecules were also aligned by rotation of the free torsion angle to the common conformation. For each molecule of which the conformation had been modified, a second energy minimization was performed and the internal energy was checked; a difference less than 20 kJ/mol was necessary to confirm the reliability of the conformation (see the image in the Data S3).

Molecular field calculations for 3D-QSAR (3-dimensional quantitative structure–activity relationship) and clustering

Starting from the aligned structural database, the molecules were arranged in a Cartesian grid extended by 4 Å° in all space directions from the larger molecules (see the image in the Data S4). A C₃-type carbon atom with a partial charge of +2 was used as a probe to describe the molecular field in the QSAR and the clustering study. The electrostatic and the steric interaction energy descriptors were used to describe molecular fields with a cut-off set to 50 kcal/mol.

Clustering & QSAR

Using the above alignment and molecular fields, a hierarchical clustering was computed using the complete method with an autoscale set to comparative molecular field analysis (CoMFA) standard. The CoMFA method is particularly well suited for treating a set of molecules with a common core (17–19).

Probes were filtered with a cut-off set to 1 kcal/mol. The input data were selected to normal.

For QSAR studies, the partial least square method was chosen with an optimal number of components set to 7. The different values of steric and electrostatic descriptors were filtered with a value of 1 kcal/mol to avoid statistical artefacts. To improve the relationship, the 'leave one out' (LOO) validation strategy was chosen, and the activities of all the molecules of the set were predicted.

Results

Patch-test results

Eighty-five per cent of the 315 CS-sensitized patients had reacted positively to more than one molecule. Positive patch tests to molecules to which patients had never been exposed (these CSs had not been available in Belgium at the time) confirmed cross-reactions.

However, the C₁₆-methyl-substituted CSs produced significantly fewer positive reactions than the ones without this particular substitution ($P < 0.0001$) (5).

While positive allergic responses were less frequent with fluorinated derivatives in groups A, C and D1, the halogen

substitution in group B CS molecules did not influence the more pronounced allergenic properties of this group.

One hundred and forty-three patients reacted exclusively to nonmethylated and in most cases nonhalogenated CSs (molecules from groups A and D2 and also budesonide), with no cross/concomitant reactions to the C₁₆-methylated and halogenated molecules from groups C and D1 or to molecules with a C₁₆, C₁₇ cis ketal or diol structure being halogenated in most cases (the molecules from group B). Except in six cases, all other patients reacting to methylated and halogenated molecules and/or to those with a C₁₆, C₁₇ cis ketal or diol structure always also tested positively to nonmethylated molecules and/or budesonide. Budesonide cross-reacted in 15 cases exclusively with the acetone B Group.

Molecular classification (dendrogram)

A hierarchical dendrogram was computed using the 47 molecules obtained 'after *in silico* hydrolysis' of labile esters in C₂₁ and C₁₇ (Table 2). Two molecules were completely isolated and removed from the dendrogram and are shown in black in Table 2. An optimal cut into three clusters was selected, i.e. cluster 1 with 25 molecules (mostly from groups A and D2, with no C₁₆-methyl substitution and also budesonide), cluster 2 with 10 molecules (from group B, with a C₁₆, C₁₇ cis ketal or diol structure) and cluster 3 also with 10 molecules (mostly from groups C and D1, C₁₆-methylated and all of which halogenated).

Clinical and molecular classification correlation

The correlation with the clinical data indicates that 310 patients had shown positive tests to molecules including those from cluster 1, 110 to those including cluster 2 and 85 to those including cluster 3. One hundred and eighty-two (58%) patients tested positively to molecules from one unique cluster with no cross/concomitant reactions to the two other ones, i.e. 176 (56%) patients to molecules from cluster 1 and one and four patients (2%) to clusters 2 and 3, respectively. Except in the last five cases for which there were several possible explanations (e.g. false-negative or false-positive results, irritant reactions), all of the patients testing positively to cluster 2 and cluster 3 also reacted positively to cluster 1 but not on the reverse.

Two patient profiles were thus identified: profile 1 for those who reacted to molecules from cluster 1 only ($N = 176$) and profile 2 for those who reacted to molecules from at least two different clusters ($N = 134$) (Fig. 1). Age, sex and reaction type were not statistically different between these two patient groups.

QSAR

Two 3D-QSAR studies were performed. The first one was performed with the 25 molecules of cluster 1 (profile 1 patients) and the second with all 45 molecules (profile 2 patients). The percentage of positive responses for the 176 profile 1 patients was reported per molecule and used as

Table 2 Hierarchical dendrogram of the 47 molecules, all being hydrolyzed *in silico* in Positions 17 and 21

CAS number	Coopman classification	Molecule name	Group
124-94-7	B	Triamcinolone	1
	C	Fluprednidene acetate*	
61951-99-3	A	Tixocortol pivalate	
806-29-1	A	Difluprednisolone	
152-97-6	C	Fluocortolone	
382-67-2	C	Desoxymethasone	
50-02-2	C	Dexamethasone	
	A	Flumethasone pivalate*	
	D1	Aldomethasone dipropionate*	
50-23-7	A	Hydrocortisone	
50-24-8	A	Prednisolone	
53-34-9	A	Fluprednisolone acetate	
5251-34-3	A	Cloprednol	
83-43-2	A	Methylprednisolone	
127-31-1	A	Fludrocortisone	
7008-26-6	A	Dichlorisone	
53-03-2	A	Prednisone	
53-06-5	A	Cortisone	
50629-82-8	C	Halomethasone	
1247-42-3	C	Meprednisone	
	D1	Clobetasone butyrate*	
2668-66-8	A	Medrysone	
426-13-1	A	Fluorometholone	
51333-22-3	B/D2	Budesonide	
13085-08-0	A	Mazipredone	
58497-00-0	B	Procinonide	2
60135-22-0	B	Flumoxonide	
	B	Amcinonide*	
76-25-5	B	Triamcinolone acetonide	
638-94-8	B	Desonide	
3693-39-8	B	Fluchloronide	
	B	Fluocinonide*	
67-73-2	B	Fluocinolone acetonide	
3385 03 3	B	Flunisolide	
3093-35-4	B	Halcinonide	
	D1	Mometasone fuorate*	3
	C	Diflucortolone valerate*	
378-44-9	C	Betamethasone	
338-95-4	A	Isoflupredone	
	D1	Beclonmethasone Dipropionate*	
	D1	Diflorasone diacetate*	
	D1	Fluticasone propionate*	
	D1	Clobetasol propionate*	
	B	Triamcinolone diacetate*	
2392-39-4	C	Dexamethasone Na-phosphate	
41767-29-7	C	Fluocortin butyl	
151-73-5	C	Betamethasone Na-phosphate	

The name of molecules with (*) was not changed in the QSAR studies, but hydrolyzed models were used. Comparative molecular field analysis dendrogram was computed with the complete method, and three clusters were determined.

activity data for the QSAR study. When ‘*in silico* hydrolysis’ of several CS resulted in the same molecule, the mean of the positive responses obtained was assigned to it. Using the CoMFA method, steric and electrostatic grid structure–activ-

ity relationships were devised for the two profiles. For profile 1, the coefficient of correlation in the calibration phase was $r^2 = 0.984$ and $q^2 = 0.685$ for LOO cross-predicted values and, for profile 2, $r^2 = 0.951$ and $q^2 = 0.718$. For each 3D-

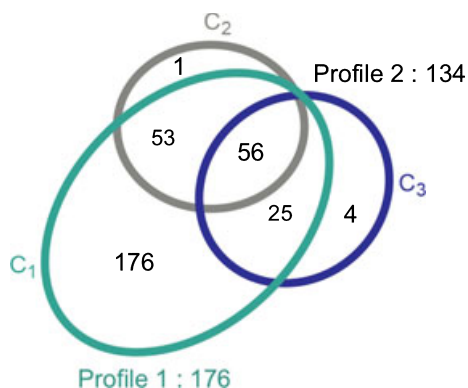


Figure 1 Two patient profiles: profile 1 patients react to molecules from cluster 1 only ($N = 176$) and profile 2 patients react to molecules from at least two different clusters ($N = 134$).

QSAR, a graph of fields was plotted as shown in Fig. 2 for profile 1 and Fig. 3 for profile 2, respectively. The isosurfaces were plotted with the classic CoMFA colour types (blue and red representing the electrostatic fields and green and yellow the steric ones) and the same values.

Electrostatic fields mainly represented the isovolumes revealed for the profile 1 patients (Fig. 2), which means that the charge of the CS molecule is very important. Indeed, CS molecules are very different from each other from the electrostatic point of view. A positive contribution to allergenicity is located near position C_6 (blue), i.e. a positive charge enhances allergenicity and a negative charge such as halogen substitution reduces the risk of sensitization. The halogenated halomethasone, flumethasone pivalate, flucortolone and difluprednate, and the halogenated molecules from clusters 2 and 3, which produced positive reactions less frequently, confirmed this.

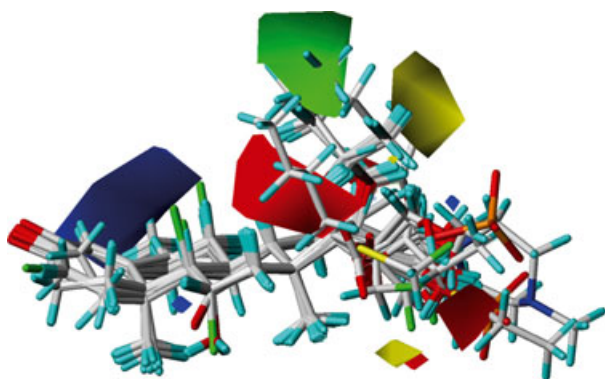


Figure 2 3D-QSAR study for the profile 1 patients: superposition of 25 molecules, with electrostatic fields (blue and red) being mainly represented; in particular, the blue volume near position C_6 exerts a positive contribution to allergenicity, i.e. a positive charge enhances allergenicity, and, hence, a negative charge such as halogen substitution reduces the risk of sensitization.

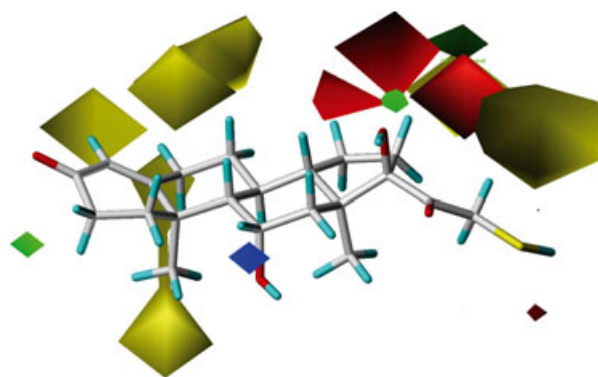


Figure 3 3D-QSAR study for the profile 2 patients: one of the 45 molecules with steric fields (yellow and green) being predominant.

For the profile 2 patients, the analysis of the volumes revealed that, in these cases, steric fields are the most important (Fig. 3), which indicates that the sensitization risk is mainly influenced by the molecular structure. Indeed, CSs are similar to each other in terms of shape, particularly from C_5 to C_{19} .

Discussion

Good concordance between the patch-test data and molecular results was observed despite some discrepancies. In the dendrogram, the halogenated and/or C_{16} -methylated molecules, i.e. meprednisone, halomethasone, dexamethasone, desoxymethasone, flucortolone and fluprednidene acetate, which belonged to group C, clobetasone butyrate and alclomethasone dipropionate, which belonged to group D1, and triamcinolone, a group B molecule, all clustered within cluster 1. However, as previously noted (5), alclomethasone dipropionate, which theoretically belongs to group D1, coreacts more often with the so-called labile esters than with the stable esters, and the nonhalogenated meprednisone also produced slightly more positive reactions than expected. Moreover, triamcinolone, despite previously classified in the acetonide group, does not present with a C_{16}/C_{17} cis ketal/diol structure. Finally, the group C molecules, such as dexamethasone, desoxymethasone or flucortolone, despite their structural similarity to group A, have no unifying characteristics in terms of structure or volume.

The cross-reactivity of budesonide exclusively with acetonides in some patients relate to its R-isomer, its S-isomer resembling Group 1 molecules (2, 20).

Of course, we must take into account that, although patch testing remains essential in diagnosing contact allergy, it is a biological test with the possibility of false-negative reactions, particularly because of the anti-inflammatory properties of CSs, and it may even give false-positive results (particularly with DMSO as the vehicle).

This study emphasizes the existence of two subgroups of patients: those who react to molecules from one unique group and those who may react to the entire spectrum of CS

Table 3 Reappraised corticosteroid classification

Groups	Characteristics	Typical members
1 (A, D2), budesonide	No C ₁₆ -methyl substitution No halogen substitution in most cases	Budesonide
		Cloprednol
		Cortisone acetate
		Dichlorisone acetate
		Difluprednate
		Fludrocortisone acetate
		Fluorometholone
		Fluprednisolone acetate
		Hydrocortisone
		Hydrocortisone aceponate
		Hydrocortisone acetate
		Hydrocortisone 17-butyrate
		Hydrocortisone 21-butyrate
		Hydrocortisone hemisuccinate
		Isofluprednone acetate
		Mazipredone
		Medrysone
		Methylprednisolone aceponate
		Methylprednisolone acetate
		Methylprednisolone hemisuccinate
		Prednicarbate
		Prednisolone
		Prednisolone caproate
		Prednisolone pivalate
		Prednisolone sodium metasulpho benzoate
		Prednisolone succinate
		Prednisone
		Tixocortol pivalate
		Triamcinolone
2 (B)	C ₁₆ /C ₁₇ cis ketal or diol structure Halogen substitution except*	Amcinonide
		Desonide*
		Fluchloronide
		Flumoxonide
		Flunisolide
		Fluocinolone acetonide
		Fluocinonide
		Halcinonide*
		Triamcinolone acetonide
		Triamcinolone benetonide
3 (C, D1)	C ₁₆ -methyl substitution Halogen substitution	Alclomethasone dipropionate
		Beclomethasone dipropionate
		Betamethasone
		Betamethasone 17-valerate
		Betamethasone dipropionate
		Betamethasone sodium phosphate
		Clobetasol propionate
		Clobetasone butyrate
		Desoxymethasone
		Dexamethasone
		Dexamethasone acetate
		Dexamethasone sodium phosphate
		Diflucortolone valerate
		Diflorasone diacetate
		Flumethasone pivalate

Table 3 (Continued)

Groups	Characteristics	Typical members
		Fluocortin butyl
		Fluocortolone
		Fluocortolone caprylate
		Fluocortolone pivalate
		Fluprednidene acetate
		Halomethasone
		Meprednisone
		Fluticasone propionate
		Mometasone furoate

molecules. The latter population probably presents with a powerful enzymatic hydrolysis system or recognizes the global skeleton of the steroid molecules rather than particular substitutions, as has been suggested (21).

Moreover, QSAR analyses indicate that the two patient profiles differ mainly with regard to the respective contribution of electrostatic and steric fields:

- For some patients, the sensitization risk depends mainly on the molecule charge, the presence of a halogen substitution on C₆ being protective. As the electrostatic characteristics of the CS molecules differ from each other, this accounts for why such patients reacted only to selected molecules and to the nonhalogenated ones, in particular.
- However, for other patients, it is the CS molecule structure that mainly influences sensitization. As all CSs, despite a degree of diversity, present similar steric characteristics and common structural cycles, such patients would react to any CS molecule. Compared with the first group, classifications, despite being valuable for orienting the search for a replacement molecule, cannot replace systematic, individualized evaluation of each patient's sensitization/tolerance profile.

Absence of age differences between the patients of the two groups could exclude the first group from becoming, with time, patients of the second group, they being simply younger.

This study also indicates that the ABCD (D1, D2) classification has to be partly reappraised. Hence, a simplification is proposed with three different groups, i.e. group 1 (cluster 1): nonmethylated molecules and nonhalogenated in most cases, including budesonide; group 2 (cluster 2): halogenated molecules in most cases with a C₁₆/C₁₇ cis ketal/diol structure; and group 3 (cluster 3): halogenated and C₁₆-methylated molecules (Table 3).

Conclusion

This study demonstrates the existence of two subgroups of patients with probably different areas of immune recognition: patients who react to molecules from one unique group and patients who may react to the entire spectrum of corticoster-

oids. Hence, simplification of the ABCD (D1, D2) classification is proposed:

Group 1: the nonmethylated, most often nonhalogenated molecules (groups A and D2, and budesonide);

Group 2: the halogenated molecules with a C₁₆/C₁₇ cis ketal/diol structure (group B or the acetonide group);

Group 3: the halogenated and C₁₆-methylated molecules (groups C and D1).

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Conflict of interest

None.

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Supporting Information

Additional Supporting Information may be found in the online version of this article found at: <http://www.wileyonlinelibrary.com>

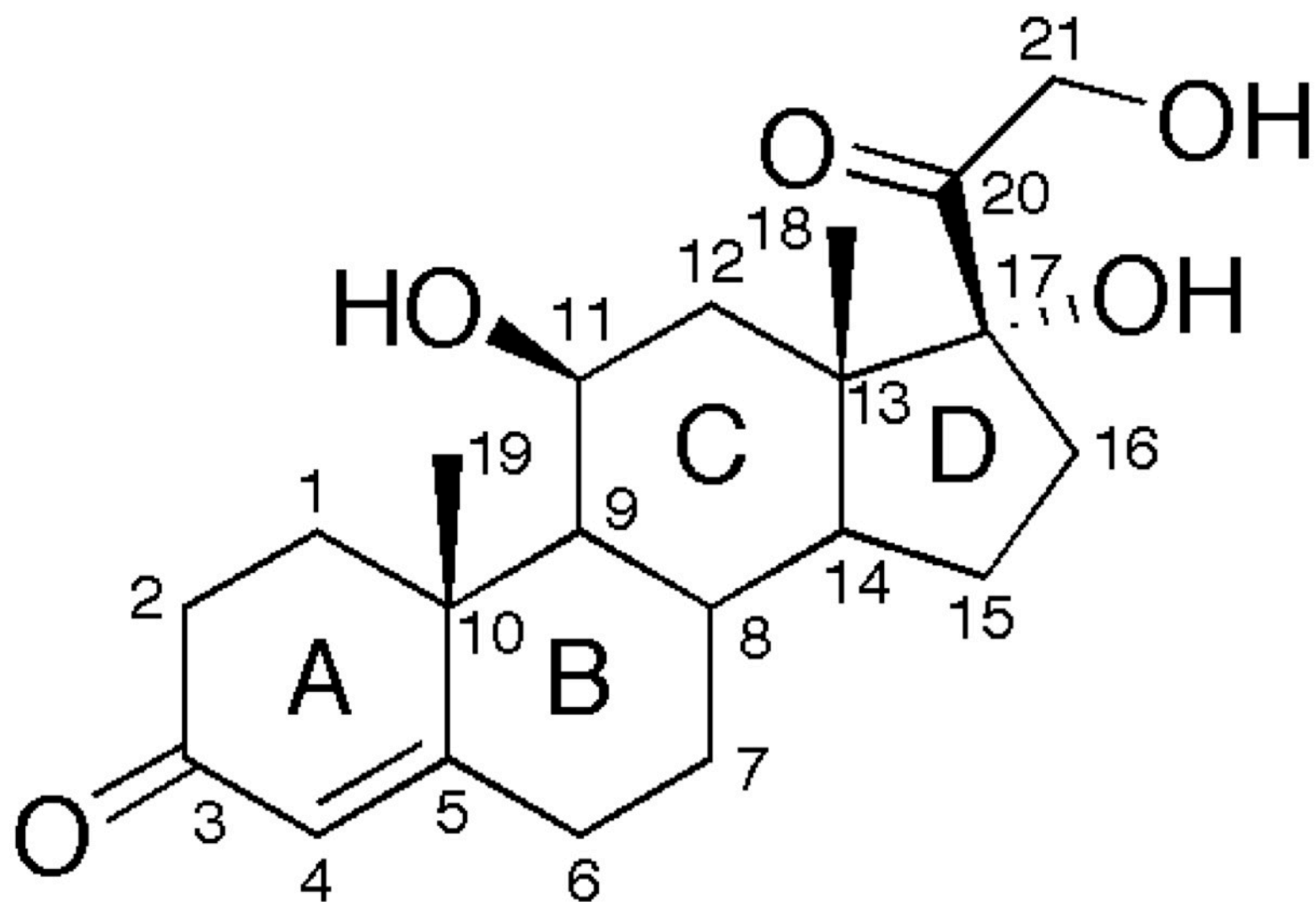
Data S1. Chemical structure of hydrocortisone with the conventional numbering of carbon atoms.

Data S2. Corticosteroid series, along with test concentrations and vehicles.

Data S3. Modelling of corticosteroid molecules with the Sybyl Molecular Modelling Suite using MMFF94 forcefield and alignment on their common steroid skeleton.

Data S4. Steric/electrostatic fields calculation with the aligned structure inserted in a Cartesian grid.

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Repository data 2: Corticosteroid series, along with test concentrations and vehicles

	Molecules	Vehicle /Concentration	CAS
1	Alclometasone dipropionate	0.5% ethanol	66734-13-2
2	Amcinonide	0.1% ethanol	51022-69-6
3	Beclomethasone dipropionate	0.1% ethanol	5534-09-8
4	Betamethasone	0.1% ethanol	378-44-9
5	Betamethasone dipropionate	0.1% ethanol	5593-20-4
6	Betamethasone sodium phosphate	0.1% saline	151-73-5
7	Betamethasone 17-valerate	0.1% ethanol	2152-44-5
8	Budesonide	0.01% petrolatum 0.002% ethanol	51333-22-3
9	Clobetasol propionate	0.1% ethanol	25122-46-7
10	Clobetasone butyrate	0.1% ethanol	25122-57-0
11	Cloprednol	0.1% ethanol	5251-34-3
12	Cortisone acetate	0.5% ethanol/DMSO	50-04-4
13	Desonide	0.1% ethanol	638-94-8
14	Desoxymethasone	0.1% ethanol	382-67-2
15	Dexamethasone	0.1% ethanol	50-02-2
16	Dexamethasone acetate	0.1% ethanol	1177-87-3
17	Dexamethasone sodium phosphate	0.1% ethanol	2392-39-4
18	Dichlorisone acetate	0.1% ethanol	79-61-8
19	Diflorasone diacetate	0.1% ethanol	33564-31-7
20	Diflucortolone valerate	0.1% ethanol	59198-70-8
21	Difluprednate	0.1% ethanol	23674-86-4
22	Flucloronide	0.1% ethanol	3693-39-8
23	Fludrocortisone acetate	0.1% ethanol	514-36-3
24	Flumethasone pivalate	0.1% ethanol	2002-29-1
25	Flumoxonide	0.1% ethanol	60135-22-0
26	Flunisolide	0.1% ethanol	3385-03-3
27	Fluocinolone acetonide	0.1% ethanol	67-73-2
28	Fluocinonide	1% ethanol	356-12-7
29	Fluocortin butyl	0.1% ethanol	41767-29-7
30	Fluocortolone	0.1% ethanol	152-97-6
31	Fluocortolone caproate	0.1% ethanol	303-40-2
32	Fluocortolone pivalate	0.1% ethanol	29205-06-9
33	Fluorometholone	0.1% ethanol	426-13-1
34	Fluprednidene acetate	0.1% ethanol	1255-35-2
35	Fluprednisolone	0.1% ethanol	53-34-9
36	Fluticasone propionate	0.1% ethanol	80474-14-2
37	Halcinonide	0.1% ethanol	3093-35-4
38	Halometasone	0.1% ethanol	50629-82-8
39	Hydrocortisone	0.5% ethanol/DMSO	50-23-7
40	Hydrocortisone aceponate	1% ethanol	74050-20-7
41	Hydrocortisone acetate	0.1% ethanol	50-03-3
42	Hydrocortisone-17-butyrate	0.1% ethanol	13609-67-1
43	Hydrocortisone-21-butyrate	0.1% ethanol	6677-99-2
44	Hydrocortisone hemisuccinate	0.1% ethanol	2203-97-6
45	Isofluprednone acetate	0.1% ethanol	338-98-7
46	Mazipredone	0.1% ethanol	13085-08-0
47	Medrysone	0.1% ethanol	2668-66-8

48	Meprednisone	0.1% ethanol	1247-42-3
49	Methylprednisolone aceponate	0.1% ethanol	86401-95-8
50	Methylprednisolone acetate	0.1% ethanol	53-36-1
51	Methylprednisolone hemisuccinate	0.1% ethanol	2921-57-5
52	Mometasone furoate	0.1% ethanol	83919-23-7
53	Pivalate tixocortol	0.1% petrolatum	55560-96-8
54	Prednicarbate	1% ethanol	73771-04-7
55	Prednisolone	0.1% ethanol	50-24-8
56	Prednisolone caproate	0.1% ethanol	69164-69-8
57	Prednisolone sodium metasulphobenzoate	0.1% ethanol	630-67-1
58	Prednisolone pivalate	0.1% ethanol	1107-99-9
59	Prednisolone hemisuccinate	1% ethanol	2920-86-7
60	Prednisone	0.1% ethanol	53-03-2
61	Procinonide	0.1% ethanol	58497-00-0
62	Triamcinolone	0.1% ethanol	124-94-7
63	Triamcinolone acetonide	0.1% ethanol	76-25-5
64	Triamcinolone benetonide	0.1% ethanol	31002-79-6
65	Triamcinolone diacetate	0.1% ethanol	67-78-7
66	Triamcinolone hexacetonide	1% ethanol	5611-51-8
	Ethanol	70% water	64-17-5
	Ethanol / DMSO	50/50% water	64-17-5/67-68-5
	Glutaraldehyde	1% water	111-30-8
	Glyoxal	2% water	107-22-2

