

## Patch-testing with Serial Dilutions of Tixocortol Pivalate and Potential Cross-reactive Substances

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**Of patch-tested patients with dermatitis, 4–5% are allergic to corticosteroids. Four groups of corticosteroids are recognized (A–D), where substances from the same group may cross-react. We investigated the potential cross-reactivity pattern and dose-response relationship for several corticosteroids from group A. We also included the corresponding aldehyde to hydrocortisone, as this degradation product has been proposed to be immunogenic. Eleven patients shown to be allergic to tixocortol pivalate were patch-tested with several corticosteroids from group A, as well as with the aldehyde, all in serial dilutions. All 11 reacted to both tixocortol pivalate and hydrocortisone. The dose-response relationship for the corticosteroids tended to be similar to sensitizers lacking anti-inflammatory potential. Patients with simultaneous reactions to many substances had high patch-test reactivity to tixocortol pivalate and hydrocortisone, while patients with few such reactions showed low reactivity ( $p=0.001$  and  $0.003$ , respectively). Several patients reacted to the aldehyde, supporting the theory that it is an intermediate in sensitization. *Key words:* contact dermatitis; corticosteroids; contact allergy; 21-dehydrohydrocortisone; dose-response relationship.**

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Corticosteroid contact allergy is becoming increasingly common. Of patch-tested patients with dermatitis, 4–5% are allergic to 1 or more corticosteroids (1–3). Based on statistical calculations of patch-test results, corticosteroids can be divided into 4 groups, A–D (4). Conformational analysis of the electronic shape of these molecules has confirmed that groups A, B and D are highly homogeneous within each group in terms of molecular structure, but that there are significant differences between the groups (5). Substances from the same group thus have the ability to cross-react with each other, but between-group cross-reactions are seldom seen (4). Knowledge about cross-reaction patterns is important because patients reacting to corticosteroids need adequate information on which corticosteroids to use and, above all, which to avoid. This applies not only to topical formulations, but also to corticosteroids administered by other routes, e.g. systemically, intrarticularly, etc.

Tixocortol pivalate and hydrocortisone both belong to group A (5). According to Coopman et al. (4) other group A

corticosteroids should therefore be able to cross-react with both these substances.

In order to investigate the potential cross-reactivity pattern and dose-response relationship, a study was undertaken, in which patients allergic to tixocortol pivalate were patch-tested with other substances from group A.

Recent patch-test results (6) have shown, that positive patch-test results to the more recently developed methylprednisolone aceponate (MPA) (belonging to group D) correlate significantly ( $p<0.01$ ) with reactions obtained with group A corticosteroids. We therefore also included this non-halogenated corticoid diester in the patch-test material.

It has been hypothesized that differences in corticosteroid metabolism or degradation could explain why some corticosteroids seem to be more immunogenic than others. It has been shown that the number of allergic reactions to corticosteroids is dependant on the intrinsic ability of the corticosteroid to degrade and bind to protein (7). Degradation is seen at the C-17-ketohydroxyl side chain and both oxidative and non-oxidative degradation occur, leading to the formation of steroid “glyoxals” containing an aldehyde function at the C 21 position (8). Bundgaard proposed (9), that some degradation products, 21-dehydrocorticosteroids, may be potentially immunogenic. Patch-testing to the corresponding aldehyde to hydrocortisone, budesonide and hydrocortisone-17-butyrate was performed in corticosteroid-allergic patients with concordant reactions to the mother molecule in several patients, supporting this hypothesis (10). We therefore included the corresponding oxidation product of hydrocortisone, 21-dehydrohydrocortisone, in this study.

## MATERIALS AND METHODS

### Patients

Eleven patients previously shown to be hypersensitive to tixocortol pivalate gave written consent to join the study, which was approved by the Medical Faculty Ethics Committee, Lund University.

### Substances

All patients were patch-tested with 10-fold serial dilutions of tixocortol pivalate, starting at 1.0%, which is the traditional patch-test concentration for most corticosteroids, and equimolar concentrations of potentially cross-reacting substances, the aldehyde and MPA (Fig. 1). If possible, 1 maximal concentration was also used (depending on the physical solubility for the substance) in potentially cross-reacting substances in order to increase the possibility of contact allergic reactions. The vehicle used was ethanol (EtOH) 99.5% v/v,

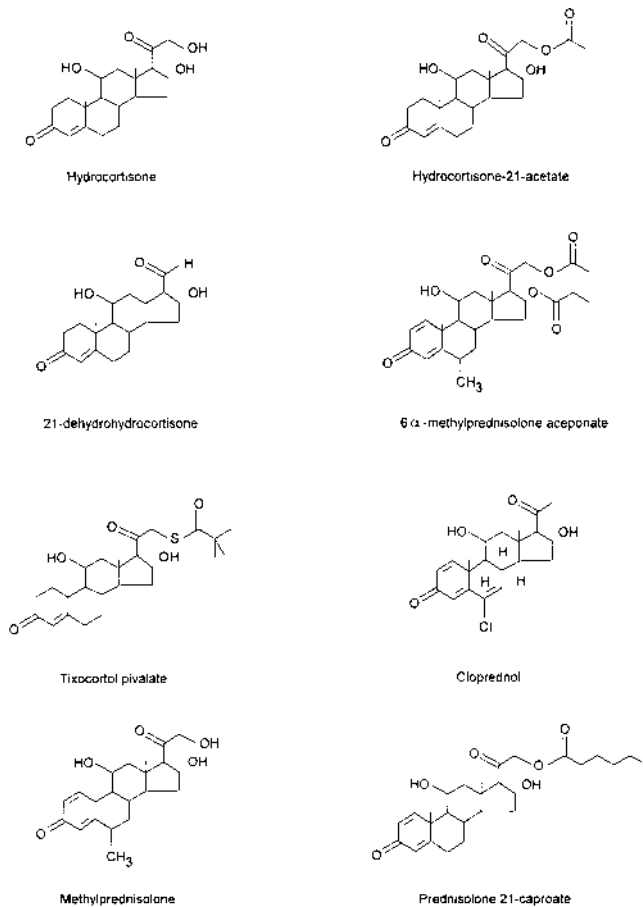


Fig. 1. The molecular structures of hydrocortisone, hydrocortisone-21-acetate, 21-dehydrohydrocortisone, 6 alpha methylprednisolone aceponate, tixocortol pivalate, cloprednol, methylprednisolone and prednisolone 21-caproate.

except for 1 dilution series with hydrocortisone, where dimethyl sulfoxide/ethanol (DMSO/EtOH) 50/50 v/v was used. The following substances were used: Tixocortol pivalate 99.9%, purchased from Chemotechnique Diagnostics, Sweden; hydrocortisone, obtained from Yamanouchi, The Netherlands; hydrocortisone acetate, obtained from Roussel Uclaf, France; methylprednisolone, obtained from Upjohn, Belgium; cloprednol, obtained from Syntex, Germany and prednisolone caproate and MPA, both obtained from Schering, Germany. 21-dehydrohydrocortisone 99.7% was prepared in Strasbourg (Laboratory of Dermatochemistry) from hydrocortisone by the method of Laenza et al. (11). Patch-testing consecutive patients in Leuven (10) with this aldehyde did not elicit any irritant reactions. Two blanks (EtOH and DMSO/EtOH 50/50 v/v, respectively) were also tested.

#### Patch-testing

With a micropipette 15  $\mu$ l of each dilution was drawn and tested in Finn Chambers on Scanpor and the patches were placed on the upper part of the back and left for 48 h. Readings took place on D3 and D7 according to ICDRG criteria.

All simultaneous positive patch-test reactions that were noted for tixocortol pivalate and substances from group A in one and the same patient are termed "cross-reactions" in this paper.

#### Statistics

For hydrocortisone in DMSO/EtOH and tixocortol pivalate, the association between the number of cross-reactions on the one hand and patch-test reactivity on the other was measured using Spearman's rank correlation coefficient ( $r_s$ ). The lowest patch-test reactivity for hydrocortisone and tixocortol pivalate was defined as the lowest concentration eliciting a 1 plus reaction (erythema and infiltration). The degree of reactivity in each patient between hydrocortisone in EtOH and the aldehyde was compared using the Sign test. A  $p$ -value less than 0.05 is referred to as statistically significant.

#### RESULTS

In Tables I and II the patch-test results for all the substances are shown.

All 11 patients reacted to both tixocortol pivalate and hydrocortisone. Patients 6 and 8 reacted to these 2 only, whereas the rest of the patients showed simultaneous reactions to various corticosteroids. Patients 3, 7 and 10 had positive patch-test reactions to all tested substances. The dose-response relationship for each sensitizer showed, that tixocortol pivalate evoked most positive patch-test reactions at all but 1 equimolar concentration. The exception was the lowest concentration (0.001 ppm ( $2.2 \times 10^{-6}$  mmol  $\times$  l $^{-1}$ )), where hydrocortisone acetate had 1 patient reacting and tixocortol pivalate none (Table I). There were positive patch-test reactions to tixocortol pivalate down to 0.01 ppm ( $2.2 \times 10^{-5}$  mmol  $\times$  l $^{-1}$ ). Independent of reading day, all 11 patients tested positively to tixocortol pivalate 1.0%, but when 1 single day was considered for 0.78% hydrocortisone (equimolar concentration) in DMSO/EtOH, 10 and 9 patients tested positively on D3 and D7, respectively, as patient 6 was negative to hydrocortisone on D3 and patient 9 and 11 had questionable reactions on D7.

The dose-response relationship for the aldehyde showed a higher or equal number of positive reactors compared with hydrocortisone in EtOH, but a lower number of positive reactors for any concentration tested compared with hydrocortisone tested in DMSO/EtOH and tixocortol pivalate. The time course of the positive patch-test reactions to the aldehyde was also similar to tixocortol pivalate and hydrocortisone, with most positive reactions to high concentrations and early readings (D3) and a lower number of positive reactions to lower concentrations and late readings (D7). When comparing the degree of reactivity between hydrocortisone in EtOH and the aldehyde in each patient, small differences were seen with a higher degree of reactivity for the aldehyde in patients 2, 3, 7, 9 and 11 and a lower reactivity in patient 4 compared with hydrocortisone. In patients 1, 5, 6, 8 and 10 there were no differences in reactivity. The Sign test gave a  $p$ -value of 0.32. Using Spearman's rank correlation coefficient ( $r_s$ ), a positive correlation for high patch-test reactivity and a high number of cross-reactions were found for both hydrocortisone ( $r_s=0.805$ ) ( $p=0.003$ ) and tixocortol pivalate ( $r_s=0.866$ ) ( $p=0.001$ ) (Fig. 2). Two of the 11 patients reacted to EtOH alone. In 1, a few papules were recorded on D3 followed by erythema and infiltration on D7. In the other erythema and infiltration was seen on D3, but at re-

Table I. Results of patch-testing with serial dilutions of tixocortol pivalate in EtOH (Tp EtOH), hydrocortisone in EtOH (Hc EtOH), hydrocortisone in DMSO/EtOH (Hc DMSO/EtOH), hydrocortisone acetate in EtOH (Hcac EtOH), 21 dehydrohydrocortisone in EtOH (21 dehydroHc EtOH), cloprednol in EtOH (Cloprednol EtOH) in 11 patients shown to be allergic to tixocortol pivalate. In all the blank boxes the tests were negative

Substance, vehicle	Conc. mmol l <sup>-1</sup>	1		2		3		4		5		6		7		8		9		10		11	
		D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7
Tp EtOH	22 (1.0 % w/v)	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2.2	+++	+	++	+++	+++	+++	++	++	+++	++	++	+++	+++	++	++	++	+	+++	++	+++	++	
	2.2x10 <sup>-1</sup>	+++	+	++	++	+++	+++	++	++	++	++	++	+++	+++	++	++	+++	+	+++	+	+++	+	
	2.2x10 <sup>-2</sup>	++	(+)	+	+	+++		++	+	++	++	+	+	+++	++			+++	+	++	+	+	
	2.2x10 <sup>-3</sup>	+				+		+		+	+			++	++			+	+++		+		
	2.2x10 <sup>-4</sup>	(+)				(+)								++	+				+++				
	2.2x10 <sup>-5</sup>													++	+				+				
Hc EtOH	55 (2.0 % w/v)							+		++			+++	++					++	++	++		
	22 (0.78 % w/v)					+++	++	++		+	+		+++	+					++	+	++		
	2.2					+		+					+++	+					++	+			
	2.2x10 <sup>-1</sup>												+++						+	+			
	2.2x10 <sup>-2</sup>												++										
	2.2x10 <sup>-3</sup>												++										
	2.2x10 <sup>-4</sup>												++							+			
	2.2x10 <sup>-5</sup>																						
Hc DMSO/EtOH	220 (7.8 % w/v)	++	+	++	+	+++	+++	++	++	++	++		+++	++	++	+	+	+	+++	++	++		
	22 (0.78 % w/v)	++	+	++	+	+++	+++	++	++	++	++	+	+++	++	++	+	+	+	+++	++	++		
	2.2	++	+	+		+++	+++	++	+	++	++		+++	++				+	+++	++	+		
	2.2x10 <sup>-1</sup>	+		+		++	+			+	+		+++	+					++	+			
	2.2x10 <sup>-2</sup>					+	(+)						++	+					++	+			
	2.2x10 <sup>-3</sup>												++	+							+		
	2.2x10 <sup>-4</sup>													+							(+)		
	2.2x10 <sup>-5</sup>												+								(+)		
Hcac EtOH	22 (0.87 % w/v)		+					+		++			++	++					++	++	++		
	2.2		+	+				+	+	++	++		++	+					++	++	+		
	2.2x10 <sup>-1</sup>									(+)	++		++	+					++	++			
	2.2x10 <sup>-2</sup>												++							+	++		
	2.2x10 <sup>-3</sup>												+								+		
	2.2x10 <sup>-4</sup>																						
	2.2x10 <sup>-5</sup>																				+		
21dehydroHc EtOH	220 (7.8 % w/v)					+++	++			+	+		+++	++			+	(+)	+++	++	+++		
	22 (0.78 % w/v)					+	+++	++		+	+		+++	++			+	(+)	+++	++			
	2.2					+	+						+++	++			+	(+)	+++	++	++		
	2.2x10 <sup>-1</sup>					+							+++	+			+		++	+			
	2.2x10 <sup>-2</sup>												++	+					(+)	+			
	2.2x10 <sup>-3</sup>												+										
	2.2x10 <sup>-4</sup>												++	+									
	2.2x10 <sup>-5</sup>												+										
Cloprednol EtOH	220 (8.5 % w/v)						++						++	++					++	++	++	++	
	22 (0.85 % w/v)					++	++			+			++	++					++	++	++	++	
	2.2					++	++						++	++					++	++	++	++	
	2.2x10 <sup>-1</sup>					+	+						+	+					++	+	(+)		
	2.2x10 <sup>-2</sup>												+							+			
	2.2x10 <sup>-3</sup>												+										
	2.2x10 <sup>-4</sup>												+								+		
	2.2x10 <sup>-5</sup>																				+		

testing the blank was negative. A test with EtOH 70.0% v/v in this second patient rendered a few papules on D3, and re-testing with hydrocortisone acetate  $2.2 \times 10^{-6}$  mmol l<sup>-1</sup> in the same patient, which had given a positive reaction on the actual reading occasion D3 in this study, led to a few

papules 3 days later. An additional 2 patients of the 11 exhibited a few papules to DMSO/EtOH, 1 on D3 and 1 on D7. The reactions to 70.0% and 99.5% EtOH were regarded as irritant, as were the reactions to DMSO/EtOH. In 7 patients there were no reactions to the 2 blanks.

Table II. Results of patch-testing with serial dilutions of prednisolone caproate in EtOH (Pred capr EtOH), methylprednisolone in EtOH (Methylpred EtOH) and methylprednisolone aceponate in EtOH (MPA EtOH) in 11 patients shown to be allergic to tixocortol pivalate. In all the blank boxes the tests were negative

Substance, vehicle	Conc. mmolx <sup>-1</sup>	1		2		3		4		5		6		7		8		9		10		11	
		D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7	D3	D7
Pred capr EtOH	220 (9.9 % w/v)					++	++			++		++	++										
	22 (0.99 % w/v)					++(+)	++			+		++	++							++	++	++	
	2.2					++	++			+		++	++							++	++		
	2.2x10 <sup>-1</sup>					++	++(+)			(+)		++	+							++	++		
	2.2x10 <sup>-2</sup>					+	+					++	+							++	++		
	2.2x10 <sup>-3</sup>													(+)									
2.2x10 <sup>-4</sup>																							
Methylpred EtOH	22 (0.81 % w/v)					++	++			+		+	++							++	+		
	2.2							+			+	++								++	++		
	2.2x10 <sup>-1</sup>																			++	++		
	2.2x10 <sup>-2</sup>																			++	+		
	2.2x10 <sup>-3</sup>																			++			
	2.2x10 <sup>-4</sup>																			++			
MPA EtOH	220 (10.0 % w/v)														++	+++				+	+++	++	
	22 (1.02 % w/v)														++	+++				++	+++	++	
	2.2														++	+++				+	+++	++	
	2.2x10 <sup>-1</sup>								+						++	+					++	+	
	2.2x10 <sup>-2</sup>														++	+					++	+	
	2.2x10 <sup>-3</sup>														++	+							+
	2.2x10 <sup>-4</sup>														++	+							
	2.2x10 <sup>-5</sup>														+								
2.2x10 <sup>-6</sup>																							

DISCUSSION

In this study, former patch-test results were reproducible, as all 11 patients reacted to tixocortol pivalate tested at 1.0% in EtOH on both D3 and D7. The dose-response relationship for tixocortol pivalate showed that 1.0%, 0.1% and 0.01% equally well picked up contact allergic patch-test reactions, as all 11 patients reacted to these concentrations on both D3 and D7. Compared with a potent corticosteroid such as budesonide, which, tested in serial dilutions, rendered positive patch-test reactions only to low concentrations in some patients (12), tixocortol pivalate showed a slope and time course of the dose-response curve for elicitation that corresponded more to a sensitizer lacking anti-inflammatory properties. This study also shows that tixocortol pivalate is a strong sensitizer with regard to its elicitation capacity, as positive patch-test reactions even were noted to extremely low concentrations (0.01 ppm (2.2 x 10<sup>-5</sup> mmol x l<sup>-1</sup>)). A guinea pig maximization test showed tixocortol pivalate to be a potent sensitizer (13). When comparing the degree of reactivity for tixocortol pivalate in the 11 patients with that of hydrocortisone in DMSO/EtOH on D3 (Table I), differences were found. Penetration and metabolism could explain this difference in reactivity, tixocortol pivalate penetrating more readily being a lipophilic ester and reacting more strongly with proteins than hydrocortisone (7).

When analysing the results from the patients with reactions to the same corticosteroids (Tables I and II) we saw that patients with low patch-test reactivity to tixocortol pivalate and hydrocortisone in DMSO/EtOH had positive patch-test reactions to few substances, whereas patients with a high degree of reactivity had reactions to more corticosteroids. Patients 6 and 8 only reacted to tixocortol pivalate

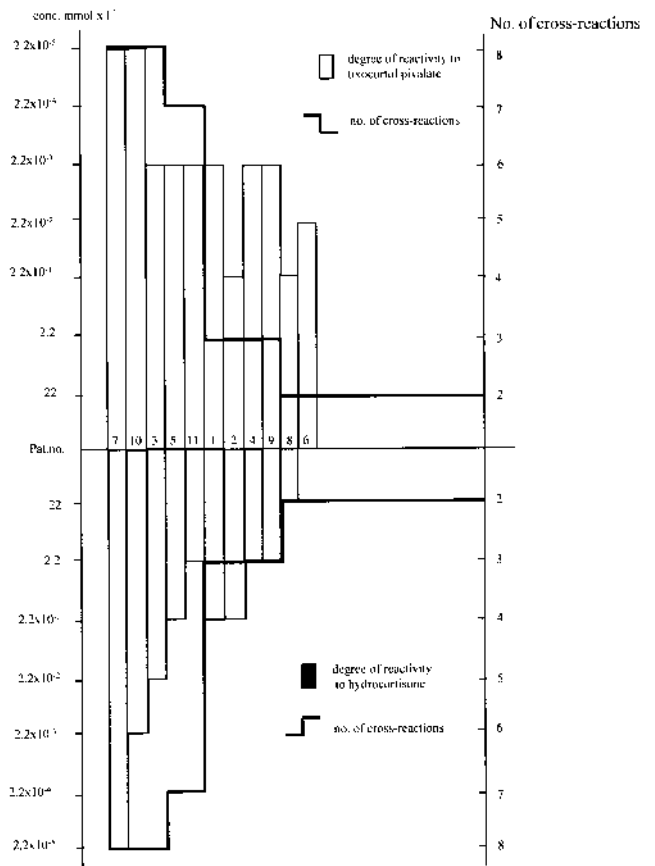


Fig. 2. The lowest eliciting patch-test concentration for tixocortol pivalate in ethanol and hydrocortisone in DMSO/ethanol for the respective patient on D3 correlated to the number of cross-reactions.

and hydrocortisone and they expressed low patch-test reactivity, in contrast to patients 3, 7 and 10, who had reactions to all tested substances in this study (Fig. 2). We also found a statistically significant positive correlation between the number of cross-reactions and the degree of reactivity for both tixocortol pivalate ( $p=0.001$ ) and hydrocortisone ( $p=0.003$ ). There may be several explanations for this phenomenon, i.e. the metabolic status of the individual, different subsets of lymphocytes reacting to the different sensitizers in group A but with overlapping action, a higher number of specific T-cells binding to tixocortol pivalate than to the other corticosteroids, a higher binding capacity on antigen-presenting cells for tixocortol pivalate, a high affinity of the T-cell receptor to the modified self structures of tixocortol pivalate (Rik Scheper, Department of Pathology, Free University Hospital, Amsterdam, The Netherlands, personal communication), the presence of cross-reactions, a common metabolite generated in the skin or a common contaminant. The purity of tixocortol pivalate and all putatively cross-reacting corticosteroids tested is not thoroughly investigated, and with patients reacting positively to tixocortol pivalate down to a concentration of 0.01 ppm ( $2.2 \times 10^{-5}$  mmol/l), the need for such purity investigations seems more than necessary.

Several observations in this study do not rule out the possibility of the aldehyde being the sensitizing molecule. When comparing the outcome for the aldehyde with hydrocortisone in EtOH, the number of positive reactors was similar and in fact a higher number reacted to the aldehyde. The patch-test reactivity for the aldehyde exceeded that of hydrocortisone in EtOH in some patients, though it was not statistically significant ( $p=0.32$ ; Sign test). Differences in penetration between the aldehyde and hydrocortisone tested in DMSO/EtOH can explain the variation in the patch-test results. Aldehydes can react with amines in the upper skin layers and consequently have a greater risk to be trapped in the skin proteins. Metabolism may be different from one person to another.

Three patients with the highest reactivity to tixocortol pivalate and hydrocortisone in DMSO/EtOH (Fig. 2) reacted to all tested compounds and strongly support the theory that substances in group A have the ability to cross-react with each other.

Furthermore, when patch-testing patients allergic to hydrocortisone with extended corticosteroid series, one often sees reactions also to MPA, a labile group D substance with ester functions both at C17 and C21. MPA is hydrolysed in the skin, forming a major metabolite, 6-methylprednisolone-17-propionate, which binds to the corticosteroid receptor, and this molecule is partly biotransformed non-enzymatically to methylprednisolone via the ester function moving from C17 to C21, where it is more accessible to hydrolysis (14). Patients allergic to group A substances could then react to this transformation product. 4/11 patients reacted to methylprednisolone (patients 3, 5, 7 and 10), 3 of these also to MPA (patients 3, 7 and 10) together with patient 11. As MPA penetrates more readily into the stratum corneum than does methylprednisolone due to the lipophilic diester grouping, positive patch-test reactions could be expected first to MPA. Reactions only to methylprednisolone could be due to a low reactivity to this substance and consequently no reaction to MPA would be expected. High

reactivity to methylprednisolone implies positive reactions to MPA at early readings. Diverging results in this study seem to speak for a considerable individual variation in the metabolism of the skin.

This study confirms, that tixocortol pivalate is the best one of all the tested substances to pick up contact allergies to group A molecules. Hydrocortisone in DMSO/EtOH rather than hydrocortisone in EtOH alone is superior for patch-testing due to the higher number of positive reactions found. The dose-response relationship for tixocortol pivalate and hydrocortisone differ from potent corticosteroids such as budesonide (12). The time course and slope of the dose-response curve for elicitation of tixocortol pivalate and hydrocortisone refer more to sensitizers lacking anti-inflammatory properties. The results in this study fit with the theory that molecules within group A should cross-react. Many cross-reactions were seen in patients who showed a high degree of reactivity to tixocortol pivalate and hydrocortisone, whereas few cross-reactions were seen in patients with a low degree of reactivity. This study is also compatible with the hypothesis that the aldehyde of hydrocortisone, dehydrohydrocortisone, may be an intermediate in contact sensitization and elicitation.

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