

Clinical relevance of tixocortol pivalate-positive patch tests and questionable bioequivalence of different hydrocortisone preparations

Daniel W. Shaw¹ and Howard I. Maibach²

¹Division of Dermatology, University of California, San Diego, CA 92122-1010, USA and ²Department of Dermatology, University of California, San Francisco, CA 94143-0989, USA

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Summary

Background. Tixocortol-21-pivalate (tixocortol pivalate) is used as a surrogate marker for contact allergy to hydrocortisone and other corticosteroids.

Objective. To review the results of repeated open application testing (ROAT) and patch testing with hydrocortisone creams and ointments in tixocortol pivalate patch test-positive patients.

Methods. ROAT was performed when the history of hydrocortisone contact dermatitis was negative or equivocal. Hydrocortisone cream and/or ointment patch tests were performed in patients with and without histories of hydrocortisone contact dermatitis. Results were excluded in patients allergic to vehicle ingredients.

Results. ROAT was positive in 15 of 20 patients (75%) with hydrocortisone cream versus 6 of 16 (38%) with hydrocortisone ointment ($p = 0.02$). Patch testing was positive with hydrocortisone cream in 23 of 27 patients (85%) versus 1 of 14 (7%) with hydrocortisone ointment ($p < 0.0001$). Only 1 of 26 tixocortol pivalate patch test-negative patients had a positive patch test with hydrocortisone cream caused by a vehicle ingredient.

Conclusions. Clinical relevance was shown in most patients with positive tixocortol pivalate patch tests. Hydrocortisone creams gave positive patch tests and positive repeated open application tests much more often than hydrocortisone ointments. There were also significant differences in patch test reactivity among different hydrocortisone creams. We hypothesize that these differences may be explained by differences in hydrocortisone transepidermal penetration in different vehicles.

Key words: allergic contact dermatitis; hydrocortisone cream; hydrocortisone ointment; patch testing; provocative use testing; repeated open application testing; tixocortol pivalate; transepidermal penetration.

Tixocortol pivalate is used in patch testing to screen for contact allergy to hydrocortisone and other structurally similar corticosteroids (1, 2). However, it has been noted (3) and has been our experience that many patients

with positive patch tests with tixocortol pivalate give a history of using topical hydrocortisone preparations without overt evidence of contact dermatitis.

Repeated open application testing (ROAT)/provocative use testing (PUT) is a technique used to assess the clinical relevance of positive patch test reactions (4). We report our experience with ROAT/PUT in patients with positive tixocortol pivalate patch tests but with negative or equivocal clinical histories of worsening of their dermatitis when using topical hydrocortisone preparations. We also report the results of patch testing with hydrocortisone creams and ointments in patients with and without a

Correspondence: Daniel W. Shaw, Division of Dermatology, University of California San Diego, 8899 University Center Lane Ste 350, San Diego, CA 92122-1010, USA. Tel: +858-657-8322; Fax: +619-741-6655. E-mail: dwshaw@ucsd.edu

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history suggestive of allergic contact dermatitis caused by hydrocortisone preparations.

Methods

Among patients referred for patch testing to the University of California, San Diego contact dermatitis clinic over a period of 16 years, 62 had positive patch tests with tixocortol pivalate 1% or 0.1% in petrolatum. In most of these patients, patch tests were performed with a series of 65–75 of the most common contact allergens, similar to the North American Contact Dermatitis Group baseline series (5), with IQ[®] or IQ Ultra[®] (Chemotechnique, Vellinge, Sweden) patch test chambers. This was usually supplemented with a series of 20–30 additional vehicle ingredients that varied over the years. The following ingredients that were present in one or more of the hydrocortisone creams¹ were individually tested in all patients: propylene glycol 30% aqua, benzyl alcohol 1–5% pet., and paraben mix 12–16% pet. Over half of the patients were also tested with sorbic acid 2% pet., cetearyl alcohol 20% pet., and either tocopherol 100%, tocopheryl acetate 10% pet., or both. Approximately 25% of patients were tested with stearyl alcohol 30% pet., cetyl alcohol 5% pet., glyceryl monostearate 15% pet., and sorbitan monostearate 2.5% pet. Patch tests were occluded for 48 hr. An initial reading was performed after patch test

removal on D2, a second reading was performed at D4 or D5, and, in many cases, a late reading was performed at D7. The presence of palpable induration, papules or vesicles was considered to be a positive reaction.

Twenty-two tixocortol pivalate patch test-positive patients who gave a negative or equivocal history of worsening dermatitis from the use of topical hydrocortisone underwent ROAT/PUT with various hydrocortisone creams and/or ointments (Table 1). Patients were instructed to apply small quantities one to two times per day to one antecubital fossa. In some patients, post-auricular or pre-auricular skin was used. When both cream and ointment were tested, they were applied right versus left. The duration of application was variable (Table 1). The products tested did not contain any of that patient's known vehicle allergens.

Twenty-nine of the 62 tixocortol pivalate patch test-positive patients also had patch tests performed with one or more hydrocortisone creams. Eleven of these were in the group that underwent ROAT/PUT, and the other 18 were not.

In order to further assess possible allergenicity or irritancy of hydrocortisone cream vehicles, patch test results with Cortizone 10[®] or Fougere[®] hydrocortisone cream were examined in 26 patients with suspected contact dermatitis who had negative tixocortol pivalate patch tests and who had used hydrocortisone cream.

Results

Vehicle ingredient patch test results

Multiple vehicle ingredients were patch test-positive in tixocortol pivalate patch test-positive patients. However, the only ones that were potentially confounding factors in the hydrocortisone cream patch test results were propylene glycol and benzyl alcohol. The hydrocortisone cream patch test results were adjusted to exclude these reactions (Table 2). There was no potentially confounding vehicle allergen detected that would have affected the ROAT/PUT results.

ROAT/PUT in tixocortol pivalate patch test-positive patients with a negative or equivocal history of hydrocortisone contact dermatitis

ROAT/PUT was positive with hydrocortisone cream and/or ointment in 16 of 22 patients (73%) (Table 1). Hydrocortisone creams were positive in 15 of 20 patients (75%), and hydrocortisone ointments were positive in 6 of 16 (38%) ($p = 0.024$, chi-square test). In each case in which cream versus ointment testing was performed,

¹ Vehicle ingredients of hydrocortisone 1% creams used in patch testing or ROAT/PUT were as follows. Cortizone 10[®] cream (Chattem, Inc., Chattanooga, TN, USA): *Aloe barbadensis* gel, aluminium sulfate, calcium acetate, cetearyl alcohol, glycerin, light mineral oil, maltodextrin, methylparaben, potato dextrin, propylparaben, purified water, sodium cetearyl sulfate, sodium lauryl sulfate, white petrolatum, and white wax. (Beeswax was added to the formula towards the end of the testing period.) Cortizone 10 Plus[®] cream (Chattem, Inc.): same as Cortizone 10[®] cream, plus cholecalciferol, isopropyl palmitate, propylene glycol, retinyl palmitate, tocopheryl acetate, and corn oil. Hytone[®] cream (no longer manufactured): isopropyl myristate, cholesterol and related sterols, polysorbate 60, sorbitan monostearate, polyoxyl 40 stearate, cetyl alcohol, glyceryl monostearate, propylene glycol, sorbic acid, and water. Hydrocortisone cream (E. Fougere & Co., a division of Altana Inc., Melville, NY, USA): glyceryl monostearate, polyoxyl 40 stearate, glycerin, paraffin, stearyl alcohol, isopropyl palmitate, sorbitan monostearate, benzyl alcohol, potassium sorbate, lactic acid, and water. Aveeno[®] hydrocortisone cream (Johnson & Johnson, Skillman, NJ, USA): *A. barbadensis* leaf juice, *Avena sativa* (oat) kernel flour, beeswax, cetyl alcohol, citric acid, glyceryl stearate, isopropyl myristate, methylparaben, PEG-40 stearate, polysorbate 60, propylene glycol, propylparaben, sodium citrate, sorbic acid, sorbitan stearate, stearyl alcohol, tocopheryl acetate, and water. Cortaid[®] Intensive Therapy Moisturizing Cream (Johnson & Johnson): benzalkonium chloride, butylparaben, carbomer, cetareth-6, cetyl alcohol, citric acid, dimethicone, ethylparaben, glycerin, isobutylparaben, methylparaben, mineral oil, petrolatum, propylparaben, sodium citrate, sodium hydroxide, stearyl alcohol, tetrasodium EDTA, tocopheryl acetate, and water.

Table 1. Repeated open application testing/provocative use testing (ROAT/PUT), one to two applications daily

Patient number	Hydrocortisone cream 1%	Hydrocortisone ointment 1%
Cream-positive, ointment-positive		
1	Positive 5 days, antecubital, brand unknown	Positive 18 days, antecubital, Cortizone 10 [®]
2	Positive 2 days, antecubital, brand unknown	Positive 4 days, antecubital, Cortizone 10 [®]
3	Positive 3 days, antecubital, Cortizone 10 [®]	Positive 12 days, antecubital, Cortizone 10 [®]
4	Positive 4 days, antecubital, brand unknown	Positive 8 days, antecubital, Cortizone 10 [®]
5	Positive 4 days, postauricular, Cortaid Intensive Therapy Moisturizing [®]	Positive 7 days, postauricular, Fougera [®]
Cream not tested, ointment-positive		
6	Not tested	Positive 10 days, antecubital, Fougera [®]
Cream-positive, ointment-negative		
7	Positive 4 days, antecubital, Cortizone 10 Plus [®]	Negative 30 days, antecubital, Fougera [®]
8	Positive 4 days antecubital, Cortizone 10 [®]	Negative 8 days, antecubital, Fougera [®]
9	Positive 2 days, antecubital, brand unknown	Negative 5 days, antecubital, Cortizone 10 [®]
10	Positive 3 days, antecubital, Cortizone 10 Plus [®]	Negative 5 days, antecubital, Taro [®]
11	Positive 3 days, antecubital, Fougera [®]	Negative 5 days, antecubital, Cortizone 10 [®]
12	Positive 28 days, antecubital, Fougera [®]	Negative 28 days, postauricular, Fougera [®]
13	Positive 5 days, antecubital, Cortaid Intensive Therapy Moisturizing [®]	Negative 30 days, antecubital, Taro [®] ; negative 16 days, postauricular, Taro [®]
Cream-positive, ointment not tested		
14	Positive 3 days, antecubital, brand unknown	Not tested
15	Positive 5 days, antecubital, Cortaid Sensitive Skin [®]	Not tested
16	Positive 5 days, antecubital, Cortizone 10 [®]	Not tested
Cream-negative, ointment not tested or negative		
17	Negative 21 days, antecubital, Cortizone 10 [®] and Fougera [®]	Not tested
18	Negative 21 days, antecubital, Cortizone 10 [®] ; negative 21 days, postauricular, Fougera [®]	Not tested
19	Negative 21 days, antecubital, Cortaid Intensive Therapy Moisturizing [®]	Not tested
20	Negative 30 days, postauricular, Cortizone 10 [®]	Negative 30 days, postauricular, Taro [®]
21	Negative 30 days, postauricular, Fougera [®]	Negative 30 days, postauricular, Fougera [®]
Cream not tested, ointment-negative		
22	Not tested	Negative 21 days, antecubital, Cortizone 10 [®] ; negative 18 days, preauricular, Cortizone 10 [®]
Totals: n positive/n tested (%)	15/20 (75)*	6/16 (38)*

Days to development of first sign of dermatitis in tixocortol pivalate patch test-positive patients.

*Totals: cream (75%) versus ointment (38%), $p = 0.024$, chi-square test.

if contact dermatitis developed, it occurred earlier with hydrocortisone cream than with hydrocortisone ointment (Fig. 1).

Hydrocortisone cream and ointment patch test results in tixocortol pivalate patch test-positive patients with or without a history of hydrocortisone contact dermatitis

On examination of the results for all tixocortol pivalate-positive patients and after adjustment for reactions to vehicle ingredients, 23 of 27 (85%) of those who had patch tests performed with one or more hydrocortisone creams had at least one positive patch test with a hydrocortisone cream (Table 2). Among the creams (Table 2), Cortizone 10[®] was more often positive than Aveeno[®] ($p = 0.015$,

chi-square test) and Fougera[®] ($p = 0.0002$). Likewise, Hytone[®] was more frequently positive than both Aveeno[®] ($p = 0.028$) and Fougera[®] ($p = 0.001$). In marked contrast to the creams, hydrocortisone ointment was positive in only 1 of 14 (7%) of patients ($p < 0.0001$) (Table 3).

In the group of 22 patients in whom ROAT/PUT was performed, and after adjustment for reactions to vehicle ingredients, 8 of 11 who had patch tests performed with one or more hydrocortisone creams had at least one positive patch test with a hydrocortisone cream (8 of 8 in the ROAT/PUT-positive group and 0 of 3 in the ROAT/PUT-negative group). In the group in which ROAT/PUT was not performed, 15 of 16 patients tested had a positive patch test result to one or more hydrocortisone creams.

Table 2. Number of positive patch tests with each hydrocortisone cream/number of tixocortol pivalate patch test-positive patients tested

Name of hydrocortisone cream	Total, unadjusted for vehicle allergenicity (%)	Total, adjusted for vehicle allergenicity (%)
Hytone [®] cream 1%*	9/10 (90)	8/9 (89)
Cortizone 10 [®] cream 1%	16/19 (84)	16/19 (84)
Aveeno [®] cream 1%*	5/11 (45)	4/10 (40)
Fougera [®] cream 1%†	4/16 (25)	3/15 (20)
Total number of patients with one or more positive hydrocortisone cream patch tests	25/29 (86%)	23/27 (85%)
<i>p</i> -values (chi-square test), adjusted for multiple comparisons		
Hytone [®] versus Aveeno [®] = 0.028		
Hytone [®] versus Fougera [®] = 0.001		
Cortizone 10 [®] versus Aveeno [®] = 0.015		
Cortizone 10 [®] versus Fougera [®] = 0.0002		

*Adjusted to exclude patients with positive patch tests with propylene glycol, present in this cream's vehicle.

†Adjusted to exclude 1 patient with a positive patch test with benzyl alcohol, present in this cream's vehicle.



Fig. 1. Repeated open application test result with hydrocortisone cream and ointment. Both were applied unoccluded twice daily. Hydrocortisone cream caused contact dermatitis more quickly than the ointment.

Hydrocortisone cream patch test results in tixocortol pivalate patch test-negative patients

We observed positive patch test reactions to hydrocortisone creams in this group only when the patient was patch test-positive to a vehicle ingredient. This occurred with 1 patient allergic to benzyl alcohol in Fougera[®] hydrocortisone cream. All 25 patch tests with Cortizone 10[®] cream were negative.

Discussion

Tixocortol-21-pivalate (CAS 55560-96-8) is a C-21 thioester of hydrocortisone (Fig. 2). Tixocortol pivalate is used in patch testing to detect allergy to hydrocortisone, because of the observed high false-negative rate when

Table 3. Number of positive patch tests with each hydrocortisone ointment/number of tixocortol pivalate patch test-positive patients tested

Name of hydrocortisone ointment	Totals
Cortizone 10 [®] ointment 1%*	0/7
Fougera [®] ointment 1%†	0/3
Taro [®] ointment 1%†	0/3
Fougera [®] ointment 2.5%†	1/1
Total number of patients with one or more positive hydrocortisone ointment patch tests	1/14 (7%)

*Vehicle of petrolatum only.

†Vehicle of petrolatum + mineral oil.

patch testing is performed with hydrocortisone. This is especially a problem when hydrocortisone is tested in a pet. vehicle, but also occurs with an ethanol vehicle (1, 6–8). For this reason, hydrocortisone patch testing in a vehicle of equal parts ethanol and dimethyl sulfoxide has been advocated in order to enhance hydrocortisone penetration (1, 9). Tixocortol pivalate patch test-positive patients usually have positive tests when injected intradermally with hydrocortisone-21-sodium phosphate, hydrocortisone-21-succinate, or hydrocortisone-21-acetate (10, 11). Given the higher sensitivity of intradermal testing with hydrocortisones than of patch testing with hydrocortisone, it has been proposed that the greater sensitivity of patch testing with tixocortol pivalate is attributable to greater transepidermal penetration of tixocortol pivalate than of hydrocortisone (11). However, to our knowledge, this hypothesis has not been tested by direct measurements of transepidermal penetration of these two corticosteroids.

Among our tixocortol pivalate patch test-positive patients, hydrocortisone creams gave positive patch tests far more often than hydrocortisone ointments (Tables 2

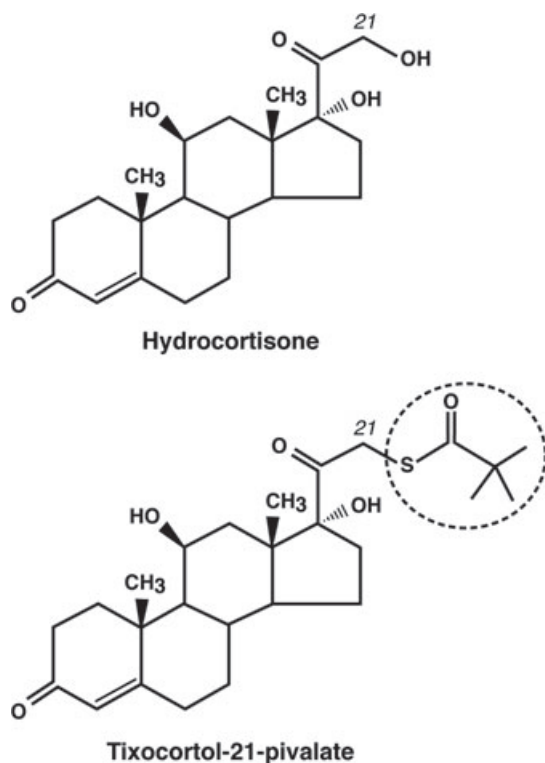


Fig. 2. Structures of hydrocortisone and tixocortol-21-pivalate. These molecules differ only by a C-21 thioester (circled) present in tixocortol pivalate.

and 3). A similar high frequency of false-negative patch tests with hydrocortisone in pet. has been observed previously (6–8, 12), even when patch testing was performed with hydrocortisone concentrations of 10% and 20% in pet. (6–8) (Table 4).

Consistent with the patch test findings, the hydrocortisone creams were also more likely than the ointments to elicit contact dermatitis in ROAT/PUT (Table 1). Furthermore, when both the cream and ointment reacted in ROAT/PUT, the cream always reacted more quickly (Table 1). It is possible, however, that more patients would have eventually reacted to ROAT/PUT with hydrocortisone ointment if the tests had been continued for longer periods, on different anatomical areas, or on areas of pre-existing dermatitis.

We also found statistically significant differences in patch test reactivity among different hydrocortisone cream formulations (Table 2), with Hytone[®] and Cortizone 10[®] creams giving the most positive patch test results. In a previous report of patch testing with hydrocortisone creams, all 10 tixocortol pivalate patch test-positive patients were patch test-positive with an unspecified hydrocortisone cream (12) (Table 4).

We propose several possible explanations for the greater reactivity of hydrocortisone creams than of ointments in patch testing and ROAT/PUT, and for the differences in patch test reactivity among different hydrocortisone cream formulations.

Coexistent hydrocortisone cream vehicle allergenicity or irritancy

Patients with positive tixocortol pivalate patch tests commonly had reactions to other contact allergens present in skin care products and topical medications. However, propylene glycol and benzyl alcohol were the only vehicle ingredients that gave potentially confounding reactions to the specific hydrocortisone creams used in patch testing of those patients. The patch test results were adjusted to exclude those cream reactions (Table 2). Likewise, the creams chosen for ROAT/PUT did not contain any of the patient's known vehicle allergens. Although not all ingredients of the creams were tested, those that were not tested are rare allergens, and so were unlikely to have accounted for many, if any, of the positive reactions in patch testing or ROAT/PUT. To more definitively assess the possibility of undetected vehicle allergenicity, we attempted to obtain placebo cream vehicles from each of the hydrocortisone cream manufacturers, but were unsuccessful.

Inherent irritancy under patch test conditions is also unlikely, given the negative patch test results with Cortizone 10[®] cream in 25 of 25 patients with negative tixocortol pivalate patch tests. Therefore, neither vehicle allergenicity nor irritancy is a likely explanation for our observations.

Inadequate hydrocortisone concentration in ointments

A recent evaluation found that the formulations of 1% hydrocortisone creams and ointments used in our testing were all close to the labelled 1%, so this is not a likely explanation (Vinod P. Shah, United States Pharmacopoeia, Rockville, MD, pers. comm. 2008).

Inadequate hydrocortisone release from ointments

In vitro release testing by use of a six-cell vertical diffusion cell apparatus with a polysulfone synthetic membrane was recently performed. Hydrocortisone release from Cortizone 10[®] ointment was similar to that observed from the hydrocortisone creams (13). Therefore, differences in hydrocortisone release do not appear to explain the differences in contact dermatitis potential.

Table 4. Number of patients with positive hydrocortisone patch tests/number of patients with positive tixocortol pivalate patch tests

	Hydrocortisone cream 1%	Hydrocortisone ointment 1%	Hydrocortisone 1% pet.	Hydrocortisone ointment 2.5%	Hydrocortisone 10% pet.	Hydrocortisone 20% pet.
Wilkinson et al. (8)	–	1/1	–	–	–	0/5
Lauerma (6)	–	0/20	0/20	–	0/20	–
Burden & Beck (7)	–	–	–	–	–	4/119
Freeman (12)	10/10*	0/10	–	–	–	–
Current study	23/27 [†]	0/13	–	1/1	–	–
Totals	33/37 (89%)	1/44 (2%)	0/20 (0%)	1/1 (100%)	0/20 (0%)	4/124 (3%)

*Concentration not specified.

[†]After adjustment for reactions to vehicle.

Transepidermal penetration differences

Topical corticosteroid ointments generally induce greater vasoconstriction than corresponding corticosteroid creams (14, 15). This usually correlates with greater clinical potency of the ointment (16). However, we could find only one published comparison of vasoconstrictor assay results of hydrocortisone cream and ointment (17). In this study, hydrocortisone cream gave greater vasoconstriction than the ointment, although the difference was not statistically significant.

However, *in vitro* assays with excised human skin (18) or cadaver trunk skin (19) have shown statistically significant greater hydrocortisone penetration from hydrocortisone creams than from ointments. These studies also found considerable differences in penetration among different hydrocortisone cream formulations (18, 19).

Consistent with these observations, we hypothesize that greater hydrocortisone transepidermal penetration explains the greater reactivity of hydrocortisone creams than of ointments in both patch testing and ROAT/PUT. In addition, it might also explain the differences in patch test reactivity among different hydrocortisone cream formulations.

The results of ROAT/PUT show that, even in patients without an apparent history of contact dermatitis caused by topical hydrocortisones, most (16 of 22, 73%) of the studied patients developed allergic contact dermatitis from hydrocortisone cream or ointment applied to normal skin. The possibility that the patch testing or ROAT/PUT might have increased the hydrocortisone sensitivity

in some of these patients cannot be excluded. In 6 patients, ROAT/PUT was negative; most of these applied hydrocortisone cream for 3–4 weeks (Table 1). This does not, however, prove clinical irrelevance of the tixocortol pivalate patch test reactions in these patients. It is possible that ROAT/PUT might have been positive in some or all of these 6 patients if testing had been performed for a longer duration and/or on areas of higher transepidermal penetration, such as the scrotum, eyelid, or lip, or an area of pre-existing dermatitis (20).

In summary, the results of ROAT/PUT showed that most tixocortol pivalate patch test reactions were clinically relevant, even in the absence of a positive overt history. Patch testing and ROAT/PUT were more often positive with hydrocortisone creams than with ointments. Furthermore, there were significant differences in patch test reactivity among different hydrocortisone cream formulations. Limited *in vitro* studies suggest that these observations may be attributable to differences in the transepidermal penetration of hydrocortisone in different vehicles. These findings suggest a lack of bioequivalence, not only between hydrocortisone creams and ointments, but also among different widely used hydrocortisone creams. Further *in vitro* and *in vivo* studies are needed to fully understand these possibly clinically significant issues.

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