

Tixocortol pivalate contact allergy in the GPMT: frequency and cross-reactivity

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In spite of their intrinsic anti-inflammatory properties, corticosteroids can induce contact allergy. When studying the allergenic properties of corticosteroids it has to be considered that both the allergenic and anti-inflammatory effect may influence the induction phase as well as the elicitation phase and that such effects may be dose-dependent. A multiple dose guinea pig maximization test (GPMT) was therefore used to study the dose-response relationship of tixocortol pivalate. The GPMT was conducted according to OECD guideline #406, using a multiple-dose design and test results were analysed with logistic regression analysis. There was a significant tixocortol pivalate sensitization of the test animals compared to the control group ($p < 0.05$), after both challenge and re-challenge. The challenge with 1% tixocortol pivalate gave more positive reactions than the challenge with 3%. The highest frequency of positive animals was observed when the animals were treated with low to intermediate induction concentrations and intermediate to high challenge concentrations with tixocortol pivalate in the TRUE TestTM. Cross-reactivity was found between tixocortol pivalate and hydrocortisone, which was expected from their close molecular resemblance, whereas no cross-reactivity was seen between tixocortol pivalate and the 3 other corticosteroids: amcinonide, budesonide, and hydrocortisone-17-butyrate.

Key words: GPMT; guinea pig maximization test; tixocortol pivalate; amcinonide; budesonide; hydrocortisone-17-butyrate; dose response; guinea pig; TRUE Test[®]; allergic contact dermatitis; contact allergy; corticosteroids; medicaments. © Munksgaard, 2001.

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In recent years, it has become evident that corticosteroids, in addition to having intrinsic anti-inflammatory properties, also can induce contact allergy (1). 3 multi-center studies from 1995, 1996, and 2000 found an overall corticosteroid sensitization frequency in consecutive eczema patients ranging from 2.0–2.6% (2–4). However, sensitization and elicitation with a corticosteroid is influenced by its anti-inflammatory effect (5). The immunological activation and/or suppression may depend on the corticosteroid concentration. It is therefore of interest to evaluate the influence of induction and challenge concentration on the sensitization frequency.

It has been recognized that most patients react to several different corticosteroids caused by either concomitant sensitization or cross-reactivity. Distinguishing between cross-reactivity and concomitant sensitization may be difficult in patients, because they are often exposed to many corticosteroids in different amounts and in different concentrations.

The guinea pig maximization test (GPMT) with a multiple dose-design was used to study the sensitizing potential of tixocortol pivalate. Challenge was performed with tixocortol pivalate in Finn Chambers[®]. Simultaneously, challenge was performed in multiple doses with the TRUE TestTM.

A 2nd study was performed where the optimal sensitizing concentration of tixocortol pivalate, from the 1st study, was used to study the cross-reactivity between tixocortol pivalate and 4 other corticosteroids: amcinonide, budesonide, hydrocortisone, and hydrocortisone-17-butyrate.

Material and Methods

Chemicals

Freund's complete adjuvant (FCA) (Statens Serums Institute, Copenhagen, Denmark) and propylene glycol (Merck, Germany) were used as vehicle for intradermal induction, and petrolatum was used as vehicle for topical induction and challenge. Tixocortol pivalate (Chemotechnique Dia-

gnostics, Sweden) and Tixocortol pivalate in the TRUE Test™ (Pharmacia and Upjohn, Hillerød, Denmark). Hydrocortisone, amcinonide, budesonide, and hydrocortisone-17-butyrate (Lab. de Dermatologie, Strasbourg) were used for induction and/or challenge.

Test design

2 series of experiments were performed, as shown in Table 1.

The aim of the 1st series was to study the allergic potential of tixocortol pivalate. The GPMT procedure was modified with a multiple induction design (6): 30 animals were divided into 1 control group and 5 test groups of 5 animals. Tixocortol pivalate was used for challenge either in Finn Chambers® or in the TRUE Test™. Rechallenge was performed on day (D) 35 with tixocortol pivalate in Finn Chambers®.

The aim of series 2 was to study the cross-reactivity between tixocortol pivalate-sensitized animals and 1 of the following corticosteroids: hydrocortisone, amcinonide, budesonide, or hydrocortisone-17-butyrate. 60 animals were assigned into 4 test groups of 10 animals and 4 control groups of 5 animals. The intradermal and topical induction concentration sensitizing all animals were chosen from the 1st series. Each test group and 1 control group was challenged with tixocortol pivalate and 1 of the corticosteroids: hydrocortisone, amcinonide, budesonide, and hydrocortisone-17-butyrate.

Guinea pig maximization test (GPMT)

The guinea pig maximisation test (GPMT) was performed according to the original description

(7), but sodium lauryl sulfate (SLS) pretreatment was omitted.

Animals

Outbred albino female guinea pigs (Dunkin-Hartley, Sahlins, Malmö, Sweden), weighing between 300 to 400 g on receipt, were housed in groups of 2 or 3 in plastic cages at the Biomedical Laboratory, Odense University, Denmark. The animals were kept on a 12-h photoperiod at a room temperature of $21^{\circ}\pm 3^{\circ}$ and a relative humidity of $55\%\pm 15$, with food and water available ad libitum (standard guinea pig pellets, Altromin®, 3123, Chr. Petersen A/S, 4100 Ringsted, Denmark). Beech wood chips were used as bedding (Glamsbjerg Træindustri A/S, Glamsbjerg, Denmark). The animals were randomly assigned to control and test groups, ear marked with baby simplex ear tags (Chevillot®, Paris, France) and allowed adaptation for 1 week before use. The skin areas used for treatment were clipped and shaved with an electric razor prior to each treatment. All animals were weighed before each test procedure.

Dose finding. The concentration range for induction and challenge was determined from 2 pilot studies, 1 for each series, utilizing FCA-treated naive guinea pigs.

For the 1st series, 12 animals were divided into 3 groups of 4 animals. 2 weeks after FCA injections, each group of animals were treated with a range of tixocortol pivalate concentrations intradermally or topically with a closed patch or TRUE Test™ to the flank of the animal. Readings were performed after 3 and 24 h. The highest concentrations tolerated systemically and not producing skin necrosis were selected for induction. The

Table 1. Test design for series 1 and 2

Series	No. animals	Tixocortol pivalate				Cross-challenge (%)
		induction		challenge		
		Id (%)	top	Finn (%)	TRUE ($\mu\text{g}/\text{cm}^2$)	
1	5*	control		1 and 3	0.023 to 50	
	5	0.01	0.1	1 and 3	0.023 to 50	
	5	0.1	1	1 and 3	0.023 to 50	
	5	0.3	3	1 and 3	0.023 to 50	
	5	1	10	1 and 3	0.023 to 50	
	5	10	30	1 and 3	0.023 to 50	
2	10+5*	0.1	1	1		Hy (0.96 and 0.096)
	10+5*	0.1	1	1		Am (1.33 and 0.133)
	10+5*	0.1	1	1		Bu (1.14 and 0.114)
	10+5*	0.1	1	1		Hy17 (1.14 and 0.114)

* control animals.

Hy (Hydrocortisone), Am (amcinonide), Bu (budesonide), and Hy17 (hydrocortisone-17-butyrate).

Finn: Finn Chambers®.

TRUE: TRUE Test™.

Table 2. Induction with tixocortol pivalate in a multiple dose GPMT. 72 h challenge and re-challenge results of tixocortol pivalate in Finn Chambers®

Id.	Induction conc. (%)	Challenge (D21)		Re-challenge (D35)
		top	1% 3%	1%
	Control		0/5 0/5	0/5
0.01	0.1		5/5 0/5	3/4
0.1	1		5/5 3/5	4/4
0.3	3		5/5 3/5	4/5
1	10		5/5 2/5	2/4
10	30		2/5 1/5	0/5

highest non-irritating concentration and 1 lower concentration were chosen for challenge with the Finn Chambers® and the non-irritating concentrations with the TRUE Test™.

For the 2nd series, tixocortol pivalate at 1% was chosen for challenge. The other corticosteroids were tested for skin irritancy: hydrocortisone, aminonide, budesonide, and hydrocortisone-17-butyrate in a concentration equimolar to 1% of tixocortol pivalate and in a concentration 1/10 of the equimolar concentration. 12 animals were divided into 4 groups of 3 animals and each group treated topically with 1 of the corticosteroids. Both concentrations were used for challenge if they were non-irritating.

Induction. For intradermal induction, 3 pairs of 0.1 ml injections were given in 2 rows in the nuchal area of each guinea pig. Glass syringes with luer lock G23 needles were used for intradermal injections.

For topical induction, a 2×4 cm filter paper was applied (Whatmann no. 3MM) covered with a layer of petrolatum preparation. Leucoflex® (Beiersdorf AG, Hamburg, FRG) was used to ensure occlusion and secured by Acrylastic® (Beiersdorf AG, Hamburg, FRG). Concentrations used for induction in series 1 are shown in Table 1 and for series 2 a tixocortol pivalate concentration of 0.1% intradermally and 1% topically were chosen.

Challenge. Patches were placed on the flank using the TRUE Test™ and small Finn Chambers® 8 mm (Epitest Ltd., Helsinki, Finland) on Scanpor® (Norgesplaster A/S, Oslo, Norway) filled with petrolatum preparations. The patches were secured with Acrylastic® and Leucoflex®. Concentrations used for challenge are shown in Table 2.

Patch test reading. The challenge and rechallenge reactions were read blindly at D2 and D3 using the grading scale (7). Reactions graded ++ and +++ were regarded as positive (8).

Statistical method

Logistic regression analysis was used for analysis of the dose-response data. If there are no positive

responses in the control group the standard logistic dose-response model is used, $\log(p(x)/(1-p(x))) = a + b \log(x)$, giving the relationship between the probability $p(x)$ and the dose x , where a is the intercept and b is the slope of the linear logistic relationship. The fit of the model is tested by means of a χ^2 test with $n-p$ degrees of freedom, where n is the number of dosed groups and p is the number of parameters in the model. The significance of the dose-response relationship is tested by means of a χ^2 test with $p-1$ degrees of freedom. A PC-programme designed for analysis of multiple dose-response data version 1.0, copyright STSC Inc. and the Danish Environmental Protection Agency developed by Aage Vølund was used (9). A generalization of the Fisher exact test of the same response rate in all groups was carried out by means of the program StatExact® (Cytel Soft Ware Corporation, Cambridge, MA, USA) (9).

Results

Table 2 shows the tixocortol pivalate challenge and rechallenge results after induction with a multiple dose design. All control animals were negative. There was a significant sensitization of the test animals compared to the control group ($p < 0.05$), after both challenge and re-challenge. After challenge with 1% tixocortol pivalate all test animals were positive, apart from the highest induction group where only 2/5 animals were sensitized. The challenge with 1% tixocortol pivalate gave more positive reactions than the challenge with 3%.

Fig. 1 and Table 3 show the challenge results

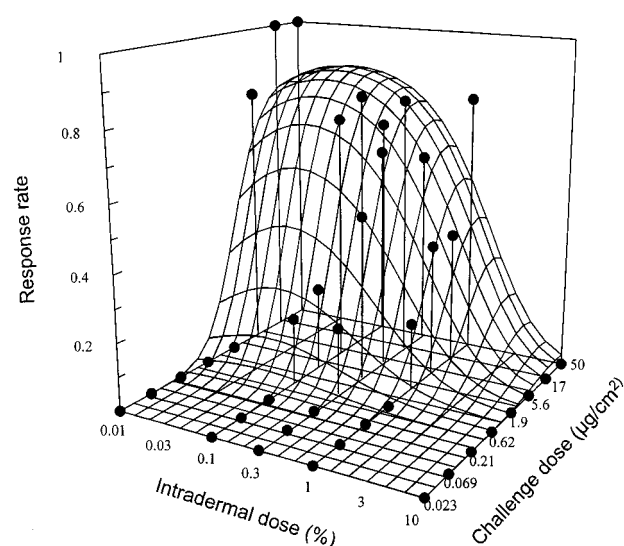


Fig. 1. The 3-dimensional dose response curve for tixocortol pivalate in the GPMT. TRUE Test™ was used for challenge and the responses were read after 72 h. ● is the observed response and — is the non-monotone logistic curve fit.

Table 3. Induction with tixocortol pivalate in a multiple dose GPMT. D3 challenge results with tixocortol pivalate in the TRUE Test™; the maximum sensitization frequency is observed in the highlighted area

Induction (%)		Challenge (TRUE Test™) $\mu\text{g}/\text{cm}^2$					
Id.	Top.	0.21	0.62	1.9	5.6	17	50
Controls		neg.	neg.	neg.	neg.	neg.	neg.
0.01	0.1	neg.	neg.	neg.	4/5	5/5	5/5
0.1	1	neg.	1/5	1/4	3/4	4/5	3/5
0.3	3	neg.	1/5	2/4	3/4	4/5	3/5
1	10	neg.	neg.	1/5	2/5	2/5	4/5
10	30	neg.	neg.	neg.	neg.	neg.	neg.

Table 4. Test reactions after challenge (D3) with tixocortol pivalate and hydrocortisone, budesonide, amcinonide, or hydrocortisone-17-butyrate in guinea pigs sensitized to tixocortol pivalate

Corticosteroid	Challenge concentration (%)	No. positive animals	Tixocortol pivalate	
			test	control
hydrocortisone	0.96	1/9	9/9	0/5
	0.096	5/9		
amcinonide	1.33	1/9	9/9	0/5
	0.133	2/9		
budesonide	1.14	0/8	8/8	0/5
	0.114	1/8		
hydrocortisone-17-butyrate	1.14	0/10	10/10	0/5
	0.114	0/10		

with tixocortol pivalate in the TRUE Test™ after D3. All control animals were negative. The challenge results with 0.023 and 0.069 $\mu\text{g}/\text{cm}^2$ were negative and not shown in the table. The combination of low to intermediate induction concentrations and intermediate challenge concentrations gave the highest frequency of positive animals, as shown by the highlighted area in Table 3.

Cross-reaction results between tixocortol pivalate-sensitized animals and hydrocortisone, amcinonide, budesonide, and hydrocortisone-17-butyrate are shown in Table 4. 4 animals were sacrificed prior to the challenge procedure due to irritation at the site of FCA injections. All test animals were sensitized to tixocortol pivalate and all control animals were negative. A significant number of animals (5/9) were positive to hydrocortisone and tixocortol pivalate ($p < 0.05$). However, the highest sensitization frequency was seen with the lowest hydrocortisone concentration. 2 animals also reacted to amcinonide and 1 to budesonide, which was not significant. No animals reacted to hydrocortisone-17-butyrate.

Discussion

The value of using a dose-response design in the GPMT has been demonstrated for a number of contact allergens. It provides the possibility of estimating the threshold concentration (the lowest concentration sensitizing) and the concentration giving the maximal sensitization frequency (6).

Furthermore, the reproducibility of the GPMT is increased. A dose-response design may be even more relevant for studying allergenicity of corticosteroids, because the allergenic and anti-inflammatory properties are dose dependent.

The sensitizing capacity of tixocortol pivalate in the GPMT was high, with a threshold below 0.01% intradermally. After Finn Chamber® challenge all test animals were positive, apart from the highest induction group given 10% intradermally and 30% topically, where 2/5 animals were positive. A slightly decreased sensitization frequency was found after rechallenge. This is in agreement with previous findings, where a high frequency of sensitization to tixocortol pivalate was demonstrated (10,11). Bruze and coworkers (10) obtained 20/24 positive animals in the standard GPMT with tixocortol pivalate, using an induction concentration of 1% intradermally and 5% topically. However, only 5/24 were positive after rechallenge. This may be explained by the anti-inflammatory effect of a booster injection with tixocortol pivalate given between challenge and rechallenge.

In this study, we did not establish the threshold concentration for sensitization because even the lowest induction concentration, 0.01% intradermally and 0.1% topically, sensitized all animals. The influence of challenge concentration on the sensitization rate was seen with the Finn Chambers® and to an even greater extent with the larger number of patches in the TRUE test™. Fig. 1 shows that, for tixocortol pivalate, the influence

of both the sensitizing and anti-inflammatory effect is dose dependent. The influence of concentration on elicitation has also been demonstrated for budesonide in humans, where lower concentrations detected sensitization better, especially at early readings(12).

In the 2nd study, all animals in the 4 test groups were sensitized to tixocortol pivalate. Cross-reactivity was seen between tixocortol pivalate and hydrocortisone but not amcinonide, budesonide, or hydrocortisone-17-butyrate. This is in agreement with the suggested cross-reaction pattern within molecular groups, where group-specific characteristics of volume and shape have been found. Tixocortol pivalate and hydrocortisone are in group A, while amcinonide and budesonide, with their acetal function, are placed in group B, and hydrocortisone-17-butyrate, with its ester function, in group D. Bruze and coworkers (10) did not find cross-reactivity between tixocortol-sensitized animals and hydrocortisone, mometasone furoate, budesonide, prednisolone, or triamcinolone acetonide (10). However, only the 5/24 animals positive to tixocortol pivalate after rechallenge could be evaluated for cross-reactivity. In conclusion, tixocortol pivalate is a strong sensitizer in the GPMT and the sensitization frequency and the ability to elicit the response is dose dependent.

Rechallenging results support tixocortol pivalate sensitization being truly allergic in nature. The results support testing with a dose-response protocol. The use of a multiple dose protocol may reduce some of the difficulties in testing chemicals with both antiinflammatory and sensitizing properties. Cross-reactivity was obtained between the 2 group-A corticosteroids tixocortol pivalate and hydrocortisone, as expected from epidemiological studies and group-specific electronic volumes of the molecules. The much lower response to amcinonide and budesonide is not statistically significant but does not exclude the possibility of cross-reactivity to these compounds. However, applying even lower induction concentrations would provide more information on the dose-response relationship for sensitization and the possibility of estimating the threshold concentration.

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