ELSEVIER

Journal of the European Academy of Dermatology and Venereology
3 (1994) 468–474

JEADV

B-Scanning assessment of the anti-inflammatory activity of methylprednisolone aceponate on experimentally induced allergic reactions

Stefania Seidenari *, Anna Di Nardo, Alberto Giannetti

Department of Dermatology, via del Pozzo 71, 41100 Modena, Italy

Abstract

Background The inhibitory activity of methylprednisolone aceponate (MPA) on experimentally induced allergic reactions was assessed by an echographic method employing a B-scanner and a dedicated software, and compared to the effects of corticosteroids of known potency.

Material and methods Experimental lesions, which were induced by patch testing 12 sensitized subjects with 5% nickel sulfate (pet.), were treated with two medications of different steroids (clobetasol propionate, fluocinolone acetonide, clobetasone butyrate, and methylprednisolone aceponate), performed 16 and 40 h after the application of the nickel patch tests. Clinical and echographic evaluations were carried out at the beginning of the experiment, and 64 hours after the induction of the reactions. Values of skin thickness and of extension of hypo-reflecting dermal areas were determined by image processing on echographic recordings.

Results Rank order of the potency of the tested corticosteroids, as evaluated by echography, was the same as the one obtained by visual scoring. MPA proved to be less effective than clobetasol propionate, more effective than clobetasone butyrate, and equally as effective as fluocinolone acetonide.

Conclusion This experiment indicated MPA can be considered a potent steroid.

Keywords: Topical corticosteroids; Methylprednisolone aceponate; Allergic contact dermatitis; Nickel sulfate; B-scanning echography; Image analysis

1. Introduction

Determination of relative potency of corticosteroids is accomplished by several procedures, which employ spontaneous diseases or experimentally induced reactions, as evaluated by clinical and, more recently, also by instrumental assessments [1–5]. Inhibition of experimentally induced contact dermatitis by topically applied corticosteroids, as a model for evaluating the strength of the anti-inflammatory effect of these molecules,

* Corresponding author. Tel. +59 360321; Fax +59 367151.

0926-9959/94/$07.00
SSDI 0926-9959(94)00048-5

was first employed by Haxthausen, who induced eczematous reactions in sensitized volunteers [6]. Subsequently, the same test was used for assessing patients who were contact sensitized to various allergens [7–11]. Crijns et al. demonstrated a correlation between the blanching scores on normal skin and the anti-inflammatory action on induced contact dermatitis [11]. In general, the rank order, as evaluated by this method, corresponded to the internationally used classification of clinical potency of corticosteroids [12,13]. Recently, we described an echographic method for evaluating the intensity of patch test responses based on image processing of 20 MHz B scan recordings, enabling the determination of the amount of low reflecting amplitude echoes at positive patch test sites [14]. This method has been employed for objective assessment of the inhibition of patch test reactions after pharmacological treatment [15]. Our study aimed at determining the anti-inflammatory activity of methylprednisolone aceponate (MPA), a non-halogenated esterified molecule, using the echographic evaluation of the inhibition of experimentally induced allergic reactions in nickel sensitized subjects, and comparing its efficacy to that of other topical corticosteroids of known potency.

2. Materials and methods

2.1. Patients

12 nickel sensitized women, aged 18 to 45, underwent patch tests with 20 mg of 5% nickel sulfate in petrolatum (Trolab, Hermal Chemie, Germany) on 5 sites of the right volar forearm skin after informed consent. The upper test area was fixed at 5 cm below the antecubital fossa, the lower site at 5 cm above the wrist crease, and the other 3 test sites were lined up at equal distances along a line running from the middle of the elbow to the middle of the wrist crease. Patch test sites were covered with large aluminium Finn Chambers with an internal diameter of 11 mm (Epitest Ltd., Finland) fixed to the skin by Scanpor tape (Norgesplaster, Norway).

2.2. Topical treatment

After 16 and 40 h, 100 μl of different corticosteroids were applied on 4 areas, which had been nickel patch tested, in a double blind randomized manner. The second application of the steroids was performed in the same order as the first. The test sites were once again covered by large Finn Chambers until evaluation at 64 h. One test area, which was covered by an empty chamber, served as control.

The commercial preparations used for the pharmacological treatment contained clobetasol propionate 0.05% (Clobesol cream, Glaxo) (CP), classified as a very potent steroid, fluocinolone acetonide 0.025% (Localyn cream, Recordati) (FA), considered a potent steroid, clobetasone butyrate 0.05% (Eumovate cream, Glaxo) (CB), which is moderately potent [16,17], or methylprednisolone aceponate 0.1% cream, whose inhibitory activity on experimentally induced allergic reactions was still to be determined.

2.3. Clinical evaluation

The clinical evaluation was performed blindly at 64 h, 30 min after patch test removal, by the same examiner. Scoring was attributed using a numerical scale, as follows: normal skin = 0; erythema with slight infiltration and papules = 1–2; erythema, infiltration, papules and vesiculation = 3–4; intense erythema, infiltration, intense vesiculation and bullae = 5.

2.4. Echographic evaluations

Echographic evaluations were carried out at the beginning of the test and at 64 h, 30 min after patch test removal.

2.5. Ultrasound equipment

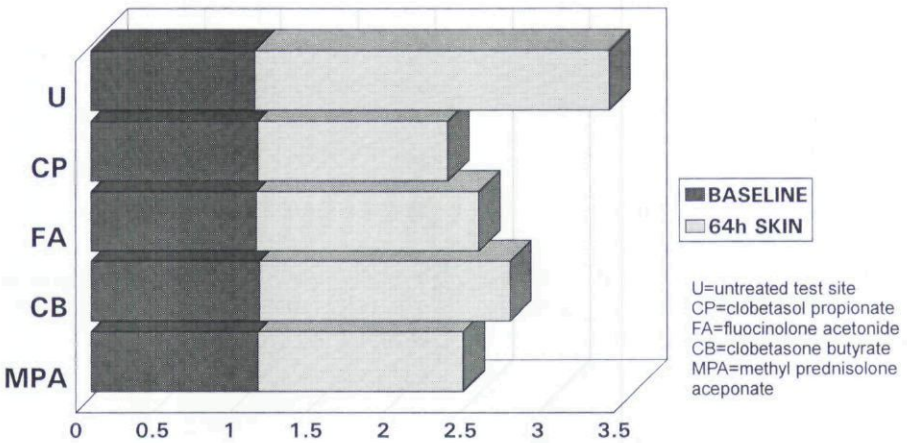
A B-scanner, provided with a 20 MHz transducer (Dermascan C, Cortex Technology, Hadsund, Denmark), which produces images representing a cross section of the skin, was used. The system nominally has an axial resolution of 50 μm and a lateral resolution of 350 μm. Sound is

coupled from the transducer to the tissue by water in the scanning head. A water based gel (Cogel, Comedical, Italy) was used to provide contact between the scanning head diaphragm and the skin surface. Equipment and calibration methods have already been described in detail elsewhere [18,19]. Evaluations were performed by employing the zoom function in the axial direction at the first magnification (at factor 2), which enables exploration of the tissue to a depth of 6.71 mm. During recordings the distance between probe membrane and the skin was kept at 1.7 ± 0.2 mm. Assessment of skin thickness was performed in B mode, determining the extension of the whole skin block appearing on the screen by the ROI function and dividing its value by 22.4 (corresponding to the length of the image) [18].

After the acquisition, echographic images were processed by a dedicated software package (Dermavision 2D, Cortex Technology, Hadsund, Denmark), set up on an IBM compatible system, which ascribes an arbitrary numerical scale, ranging from 0 to 255, to the amplitude values of the echoes. This programme provides a numerical description of the picture data, enabling the selection of homogeneous amplitude bands, and the calculation, in number of pixels, of the extension of areas reflecting within the same amplitude intervals [19]. When an inflammatory reaction takes place in the dermis, ultrasound is reflected with low amplitude levels. If patch test images are recorded by a constant gain of 22 dB, an amplitude band, ranging from 0 to 30, highlights most of the tissue, site of inflammation. As already shown, with an increase in the allergic response, the extension of the area reflecting within this amplitude band increases. Conversely, at the same gain level, the amount of ultrasound reflection in this range for normal volar forearm skin of subjects under 45 years of age is low.

3. Statistics

Significance of variations of thickness and pixel numbers between baseline and 64 h measurements at the same patch test sites were evaluated by Student's t test. Since rotating patterns of application were used, baseline values of skin thickness and pixels were homogeneous. Thus, the efficacy of the inhibition of the eczematous reaction by different corticosteroidal molecules



	UNTREATED	CP	FA	CB	MPA
BASELINE					
mean	1.07	1.09	1.08	1.1	1.09
sd	0.25	0.24	0.21	0.25	0.23
64h					
mean	2.3	1.23	1.44	1.62	1.33
sd	0.72	0.62	0.55	0.65	0.64

Fig. 1. Skin thickness values at untreated test sites and at skin areas treated with different corticosteroids.

was calculated by comparing the 64 hour values of skin thickness and number of pixels reflecting within 0 to 30 at test sites treated with different corticosteroids with those calculated from the image of a non-medicated reaction, using the ANOVA for repeated values. Further differentiation between the groups was evaluated by the Student Neuman Keuls test (SNK).

4. Results

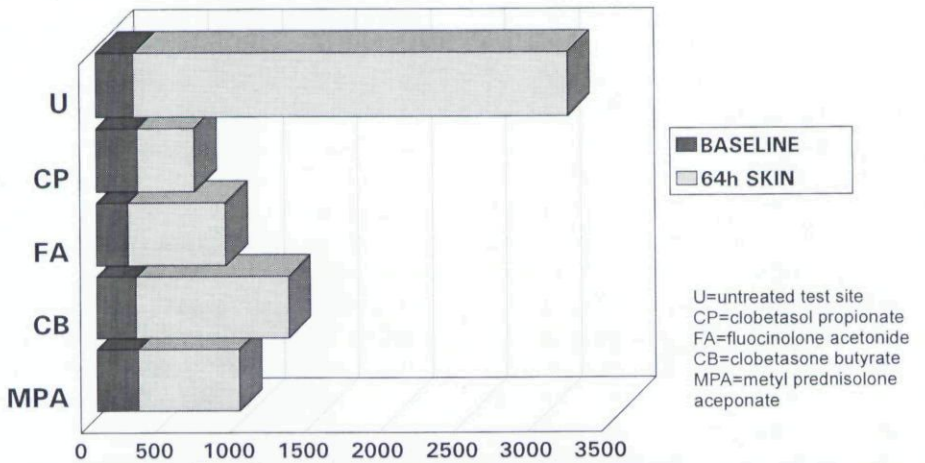
4.1. Clinical evaluation

At 64 h, 30 min after removing patch tests, the allergic reactions had fully developed at the control test site, which had had no medication 16 and 40 h after patch test application. Test areas, which had been treated with steroid creams, showed milder or no reactions. Total score per area was: 75 for untreated test sites, 26 for clobetasol propionate, 36 for fluocinolone acetonide, 43 for clobetasone butyrate, and 34 for methylprednisolone aceponate treated areas.

4.2. Echographic evaluation

Baseline thickness and 0–30 pixel values at different test sites were homogeneous, showing no statistical differences. Baseline and 64 h skin thickness mean values are illustrated in Fig. 1. 64 h fully developed reactions at untreated test areas showed a thickening of the skin up to two times the initial value. Skin thickness increased on average by 0.14 mm at the test site treated with clobetasol propionate (moving from 1.09 to 1.23 mm), by 0.36 for fluocinolone acetonide (1.08 to 1.44), and by 0.52 for clobetasone butyrate (1.11 to 1.62). The mean increase in skin thickness at sites medicated with methylprednisolone aceponate was 0.24 mm. Statistical differences were present between baseline and 64 h thickness values, and between 64 h thickness values of treated and untreated patches. However, no differences could be demonstrated between skin sites treated with different corticosteroids.

At positive 64 h patch test responses, we observed in the dermis a hypo-echogenic area whose extension, as evaluated by image processing, var-



	UNTREATED	CP	FA	CB	MPA
BASELINE					
mean	252.9	278.62	212.72	264.5	279.62
sd	198.24	204.3	118.21	182.76	112.45
64h					
mean	2922.13	384.55	655.88	1024.81	676.75
sd	859.22	142.12	285.67	293.84	387.36

Fig. 2. Extension of the hypo-echogenic dermis area (in number of pixels) at untreated test sites and at skin areas treated with different corticosteroids.

ied according to the intensity of the inflammatory reaction. Mean 0–30 pixel values referring to baseline skin and to 64 h test areas are illustrated in Fig. 2. Untreated patch test areas showed a mean increase in the extension of the hypo-echogenic area of about 1155%. At medicated test areas 0–30 pixel values were approximately 1/7, 1/3 and 1/5, respectively, for CP, CB, and FA and MPA, compared to the mean value recorded at sites where the skin response had fully developed. Statistical differences were present between baseline and 64 h pixel values at treated and untreated sites. Differences between 64 h pixel values recorded from patch test reactions inhibited by different corticosteroids and 64 h values at untreated skin sites were statistically significant. Moreover, mutual comparison of the data corresponding to different corticosteroids, showed statistical differences between all the groups, except FA and MPA.

5. Discussion

After basic activity of topical corticosteroids is determined by means of animal experiments, human pharmacological test models must be used to verify their efficacy, and to ascertain the degree of induction of local and systemic side effects. Since the level of blanching caused by corticosteroids generally correlates well with the extent of clinical efficacy, the vasoconstriction test is frequently used because it is simple [3,12,13,20, 21]. However, this test is quite unspecific, since the phenomenon of vasoconstriction is not coupled to the receptor-mediated activity of the steroids [16]. The main purpose of topical glucocorticoids is the reduction of inflammatory processes in the skin. For this reason, in order to evaluate the anti-inflammatory activity of these molecules, many models have been employed for reproducing inflammation on human skin, such as the croton oil-kerosene test, the UV erythema test, the pyrexal erythema test, etc. Moreover, disease models (psoriasis, eczema) were utilized for testing the activity of these drugs in a way which has a highly predictive value regarding the therapeutical action in clinical practice. The first attempts to imitate spontaneous allergic conditions through a human experimental model were carried out by Haxthausen, who induced eczematous reactions in sensitized subjects by iontophoresis with various allergens [6]. Test sites were treated under occlusion with the corticoid formulations to be assayed and readings were performed 24 and 48 h later. This experimental procedure demonstrated that 9-α-fluor-hydrocortisone acetate has a greater inhibitory effect on eczematous reactions than hydrocortisone acetate. Kaidbey and Kligman performed patch testing with Rhus oleoresin on volunteers with a common poison-ivy dermatitis [7]. After 48 h patch testing, repeated applications of corticosteroids were performed for 4 consecutive days. The Rhus-oleoresin induced dermatitis regressed in accordance with the therapeutic efficacy of the topical corticosteroid applied.

The anti-inflammatory action of 7 topical glucocorticoids was studied by Crijns et al. on experimentally induced eczematous reactions of subjects with allergies to nickel sulfate, *p*-phenylenediamine, colophony and potassium dichromate [11]. In these cases skin areas were treated with the corticosteroid formulations twice, 48 and 72 h after patch testing.

Böttger et al. analysed the inhibitory effect of various steroid formulations on the development of eczematous reactions by pre-treating skin areas, where patch testing was subsequently performed in nickel sulfate and potassium dichromate sensitized subjects [9].

Finally, Queille-Roussel et al. studied the effects of two steroids and two anti-inflammatory drugs on experimental lesions induced by patch testing nickel-sensitized subjects. Drugs were applied after removing patch tests, 48 h after application, and twice a day during the following days. Evaluations were performed clinically and instrumentally, by colorimetry, laser-Doppler velocimetry, and evaporimetry. Only Dermaval cream proved effective, whereas hydrocortisone cream, Parfenac and indomethacin 2.5% were unable to stop the reaction [10].

Even if directly testing the therapeutical capacities of corticosteroids, these procedures were rarely used, mainly owing to the difficulty in

objectively quantifying the inhibition of the inflammatory reaction. These problems have been partially solved by carrying out both clinical evaluation and instrumental assessment [10,15].

The echographic investigation associated with image analysis, as well as allowing the detection of subclinical responses and the distinction between visually equivalent reactions, enables the quantification of the eczematous reaction, by using more parameters at the same time: namely the determination of skin thickness, and the evaluation of the extension of the hypo-echogenic area, whose values vary according to the intensity of the inflammatory reaction [14].

Pharmacological investigations in animals were first performed to evaluate the anti-inflammatory activity of MPA compared to clobetasol propionate and hydrocortisone butyrate by means of the rat ear test and the oxazolone test [22]. The therapeutic action of MPA clearly exceeded that of hydrocortisone butyrate since it proved three times more effective. Investigations in humans, using the UV erythema inhibition test, demonstrated an anti-inflammatory activity comparable to that of mometasone furoate [22].

In our experiment we compared the inhibitory activity of MPA on experimentally induced eczematous reactions to that of three other corticosteroids belonging to different categories of potency. The action of corticosteroids appeared both with a reduction of skin thickness, and with a decrease of the hypo-echogenic area of the dermis, corresponding to edema and inflammatory infiltration. However, although thickening of the skin at test sites treated with corticosteroids was less remarkable in respect to that recorded at sites where the eczematous reaction had fully developed, no statistical differences were evident between values concerning sites treated with different corticosteroids. On the contrary, pixel numbers defining the extension of the hypo-echogenic dermis area were able to discriminate between corticosteroids of different potency. MPA proved to be less effective than clobetasol propionate, more effective than clobetasone butyrate, and as effective as fluocinolone acetonide. Rank order of the potency of the tested corticosteroids, evaluated by echography, was the same as the one obtained by visual scoring. Thus, on the basis of this experimental procedure, MPA can be considered a potent steroid.

References

[1] Bisgaard H, Kristensen JK, Sondergaard J. A new technique for ranking vascular corticosteroid effects in humans using laser-Doppler velocimetry. J Invest Dermatol 1986;86:275–278.

[2] Serup J, Holm P, Stender IM, Pichard J. Skin atrophy and telangiectasia after topical corticosteroids as measured non-invasively with high frequency ultrasound, evaporimetry and laser Doppler flowmetry. Methodological aspects including evaluations of regional differences. Bioeng Skin 1987;3:43–58.

[3] Queille-Roussel C. Le test de vasoconstriction en peau saine: techniques et applications. Ann Dermatol Venereol 1988;115:491–503.

[4] van der Valk PGM, Maibach HI. Do topical corticosteroids modulate skin irritation in human beings? Assessment by transepidermal water loss and visual scoring. J Am Acad Dermatol 1989;21:519–521.

[5] Broby Johansen U, Karlsmark T, Petersen LJ, Serup J. Ranking the antipsoriatic effect of various topical corticosteroids applied under a hydrocolloid dressing: skin thickness and blood flow and colour measurements compared to clinical assessments. Clin Exp Dermatol 1990;15:343–348.

[6] Haxthausen H. Preliminary and short report. A comparison of the inhibitory effect of 9 alpha fluor-hydrocortisone acetate and hydrocortisone acetate ointments on eczematous reactions produced by electrophoresis. J Invest Dermatol 1956;26:111.

[7] Kaidbey KH, Kligman AM. Assay of topical corticosteroids. Efficacy of suppression of experimental Rhus dermatitis in humans. Arch Dermatol 1976;112:808–810.

[8] Burrows WM, Stoughton RB. Inhibition of induction of human contact sensitization by topical glucocorticosteroids. Arch Dermatol 1976;112:175–178.

[9] Böttger E, Koch E, Tronnier H. Klinisch experimentelle Prüfung auf antientzündliche Wirksamkeit von Korticosteroid-externa. Berufsdermatosen 1977;25:53–62.

[10] Queille Roussel C, Duteil L, Padilla JM, Poncet M, Czernielewski J. Objective assessment of topical anti-inflammatory drug activity on experimentally induced nickel contact dermatitis: comparison between visual scoring, colorimetry, laser Doppler velocimetry and transepidermal water loss. Skin Pharmacol 1990;3:248–255.

[11] Crijns MB, Nater JP, van Oostreen F, van der Valk PGM. Vasoconstrictor test and the anti-inflammatory effects of 7 corticosteroids. Contact Dermatitis 1984;11: 108–111.

[12] Cornell RC, Stoughton RB. Correlation of vasoconstrictor assay and clinical activity in psoriasis. Arch Dermatol 1985;121:63–67.

[13] Gipson JR, Kirsch JM, Darley ER. Assessment of relationship between vasoconstrictor assay findings, clinical efficacy and skin thinning effects of a variety of undiluted and diluted corticosteroid preparations: Br J Dermatol 1984;111(suppl 27),204–207.

[14] Seidenari S, Di Nardo A. B-scanning evaluation of allergic reactions with binary transformation and image analysis. Acta Derm Venereol (Stockh) 1992;suppl 175:3–7.

[15] Seidenari S, Di Nardo A, Giannetti A. Assessment of topical corticosteroid activity on experimentally induced contact dermatitis: echographic evaluation with binary transformation and image analysis. Skin Pharmacol 1993;6:85–91.

[16] Bateman D.N. Clinical pharmacology of topical steroids. In: Handbook of Experimental Pharmacology. 1989 Vol. 87/II. Springer-Verlag.

[17] Niedner P. Klassifikation topischer Kortikosteroide. Therapiewoche 1990;Sonderheft Juli:40.

[18] Seidenari S, Di Nardo A, Pepe P, Giannetti A. Ultrasound B scanning with image analysis for assessment of allergic patch test reactions. Contact Dermatitis 1991;24:216–222.

[19] Seidenari S., Di Nardo A. Cutaneous reactivity to allergens at 24 h increases from the antecubital fossa to the wrist: an echographic evaluation by means of a new image analysis system. Contact Dermatitis 1992;26:171–176.

[20] Stoughton RB, Wullich K. The same glucocorticoid in brand name products. Does increasing concentration result in greater topical bioactivity? Arch Dermatol 1989;125:1509–1511.

[21] Smith EW, Meyer E, Haigh JM, Maibach HI. The human skin blanching assay as an indicator of topical corticosteroid bioavailability and potency; in Bronaugh R, Maibach HI (eds): Percutaneous Absorption, 2nd edn., New York, Dekker 1989, pp. 443–460.

[22] Zaumseil RP, Kecskés A: Methylprednisolone aceponate – a new therapeutic for eczema: a pharmacological overview. J Dermatol Treat 1992;3,suppl 2:3–7.