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Evaluation of the Vasoconstrictive Effects of Topical Steroids by Laser-Doppler-Perfusion-Imaging

ANJA SOMMER[1], JOEP VERAART[1], MARTINO NEUMANN[1] and ALFONS KESSELS[2]

Departments of [1]Dermatology and [2]Epidemiology, University of Maastricht, Maastricht, The Netherlands

Corticosteroids are one of the most frequently prescribed local therapeutic treatments. Their potency and bioavailability are tested with different methods. One of the most accepted methods is the skin-blanching test designed by McKenzie. In this study we investigated whether the skin-blanching test designed by McKenzie for screening topically active corticosteroids, producing vasoconstriction, is sufficiently detectable by a laser-Doppler-perfusion-imager (LDPI).

Eight sites in two rows on the right forearm of 10 healthy volunteers were treated with a topical glucocorticosteroid (clobetasol-17-propionate 0.05% (Dermovate®), and the blood-flow at each site was measured by the LDPI at different time-steps. Four sites per row were chosen to evaluate the dependency of bioavailability according to anatomical differences due to skin changes within the forearm. Furthermore, half of the sites were occluded to demonstrate the difference between occluded and non-occluded sites in bioavailability.

The results show that the LDPI can easily detect changes in bloodflow due to the vasoconstriction caused by topical corticosteroid. The results showed significant changes during the different measurements, with a maximum reaction 30 h after the application of the corticosteroid. The sites under occlusion showed a slower decrease of laser values than those without occlusion, so that it can be pointed out that occlusion prolongs the bioavailability of corticosteroids but does not influence the speed of onset.

So far we conclude that this technique is a simple and non-traumatic method for assessing steroid potency. Blanching, as a result of vasoconstriction, can be quantified by LDPI measurement. However, LDPI measurements have to be compared with other techniques, such as the non-traumatic ^{133}Xe washout technique, to find out if the two technologies respond in a similar way. *Key words: corticosteroid potency; blanching; corticosteroids; bloodflow; McKenzie study.*

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Anja Sommer, Department of Dermatology, Academisch Ziekenhuis Maastricht, P. Debyelaan 25, NL-6202 AZ Maastricht, The Netherlands.

It is now generally accepted that all anti-inflammatory steroids act through a common mechanism, involving binding to specific glucocorticoid receptors (1). Specific binding of glucocorticoids has been shown to take place in a wide variety of cells, including human fibroblasts and keratinocytes (2). Next to the anti-inflammatory effect, transmitted through releasing factors, glucocorticosteroids work antimitotically (3), such as in psoriasis.

Skin blanching, assessed by the vasoconstrictor assay technique of McKenzie & Stoughton (4), has been used for evaluation of percutaneous absorption of corticosteroids in humans. Different approaches to quantify the skin blanching have been more or less reliable and reproducible. In an attempt to record the degree of skin blanching at each site more quantitatively, the vasoconstrictor activity has been measured with several techniques, e.g. using instrumental colour-readings (tri-stimulus colorimeter) (5, 6), the reflectance spectrophotometric quantification (7–12), thermography (13), the ^{133}Xe washout technique (14, 15), laser-Doppler-flow (9, 16) and the paper-patch-test (17). These methods have often been supported by visual scoring of the blanching effect (18–20).

A new possibility was given by the LDPI, which measures the perfusion of cutaneous microcirculation. In this study we wanted to investigate whether this rather new technique was sensitive enough to measure changes in microcirculation of the skin after applying corticosteroids.

The LDPI is appropiate for measuring the microcirculatory changes of the skin of individual subjects after local corticosteroid application and is a technique that produces reliable and accurate data and determines the degree of vasoconstriction. Compared to the above-mentioned tests for assessing vasoconstriction after corticosteroid application, the major advantage of the LDPI is that it is easy to handle. Furthermore, the measurements take place without any physical skin contact. The LDPI has been used quite often during the last years for determining advances in wound healing (21) and for detecting abnormal bloodflow in pathologically changed skin (22).

The purpose of the study was to assess the changes in flow of the skin microcirculation after application of topical corticosteroid.

PATIENTS AND METHODS

Ten healthy Caucasian subjects were studied, and consent was obtained in each case. The average age was 28 years (range 22 to 36 years; 5 females, 5 males). The subjects had no skin disease, nor had they used topical or systemic corticosteroid preparations in the previous 2 months. All patients were nonsmokers. The skin blanching method used was a modification of that described by McKenzie & Stoughton (4) and Haigh & Kaufer (23). A nonoccluded and an occluded assay were used on two rows containing each four sites of the flexor aspect of the right forearm, avoiding the 4-cm strips of skin adjacent to the wrist and elbow, which Burdock (24) reported to be poor test areas in the vasoconstrictor assay. The corticosteroid formulation (clobetasol-17-propionate, Dermovate® ointment, Glaxo, The Netherlands) was drawn into a plastic syringe and 0.02 ml steroid was applied to each of the test areas (with a diameter of 18 mm per test site) of forearm skin and spread evenly with a glassrod. The application was repeated after 1, 2 and 3 h to maximize the percutaneous absorption, as described earlier (14, 15). Then one row, consisting of four test sites, was occluded with Tegaderm® (3M, Canada), which was removed at least half an hour before the measurements.

Laser-Doppler measurement was performed before the first and 8, 24, 30, 48 and 72 h after the last steroid application. Measurements were made under standard conditions with respect to posture and pre-measurement equilibration time (30 min) to room temperature (25°C). All measurements were performed with the PIM.Lisca version 2.4 (Linköping, Sweden). A full description of the LDPI together with its

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evaluation of the laser technique is given elsewhere (25). Briefly, the instrument generates a colour-coded image of the spatial distribution of tissue perfusion. The LDPI comprises a 2-mW helium-neon laser whose beam is directed at the tissue via an optical scanner. This consists of two mirrors controlled by two stepping motors which sequentially measure at maximum 4,096 points (pixels). The back-scattered light is detected by a photo diode at a distance of about 15 to 20 cm from the tissue surface. A maximum area of 150 cm^2 can be scanned by this technique with a spatial resolution of 2 mm corresponding to 4 mm^2 (26). The scanner is controlled by a personal computer, which is also used for image display and storage. The images are displayed with colours representing a scale of average blood velocity.

In our study the distance between the scanner head and the test site was 18 cm. A low resolution mode was used, with an image format of 36×36 pixels. The threshold background was 6.20.

To determine the influence of location, the measurement time and occlusion status of the site on the laser measurement, a four-way Anova was performed with location, the patient, the moment of measurement and occlusion as independent factors. For the occluded and non-occluded sites a three-way Anova with the three factors location, moment of measurement and patient was performed separately.

In four subjects the proximal occluded test site showed irritation due to Tegaderm® after 24 h, lasting for 3 days, so that LDPI measurements of these test sites were not included in the study.

RESULTS

In total, 480 measurements were performed. All values were compared with measurements before the application of the corticosteroid, revealing a mean flow of 0.38 mV (arbitrary units, range: 0.25–0.48 mV). An increase of the laser values (arbitrary units) could be found until the third measurement (8 h after last application). After this (the next measurement took place at 24 h after the last steroid application), the laser values decreased significantly to rise again with a second peak 30 h after last application (0.534 mV, sd 0.017; without occlusion and 0.608 mV, sd 0.018 under occlusion). From their maximum they decreased steadily within the following measurements until they reached the same values as in the beginning of the study 72 h after the last application of the corticosteroid (0.37, sd 0.018 without occlusion and 0.47 mV, sd 0.02 under occlusion). These data are shown in Fig. 1.

The most obvious differences between occluded and non-occluded sites were found after 30 h (see Fig. 2).

Measurements on the distal sites showed higher laser values (mean: 0.49 mV) than on the proximal ones (0.32 mV), which is also shown in Fig. 2.

DISCUSSION

It was our intention to evaluate in this study whether LDPI can be used in the evaluation of skin blanching after local steroid application. When one interprets the results of LDPI on the human skin several factors have to be borne in mind. The magnitude of blanching may be influenced by a number of variables, such as ambient temperature and relative humidity. Standard conditions before measuring are therefore important. Similarly, the differences in the vascularization of the dominant and nondominant arms may affect the degree of blanching induced by topical corticosteroids (18). Also the vehicle of the tested ointment could influence the microcirculation and should therefore always be tested separately.

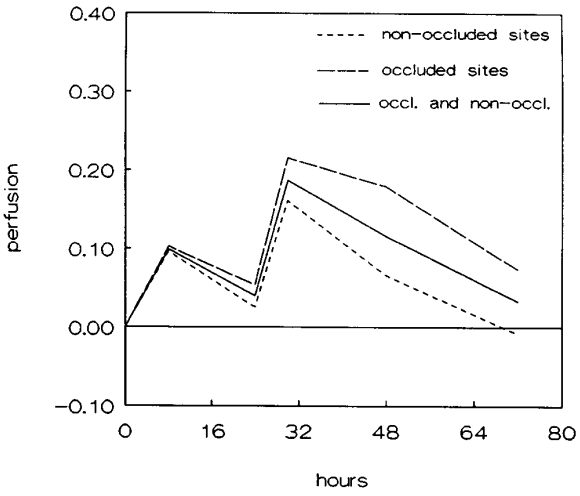

Fig. 1. Perfusion: Change in perfusion compared to the baseline perfusion.

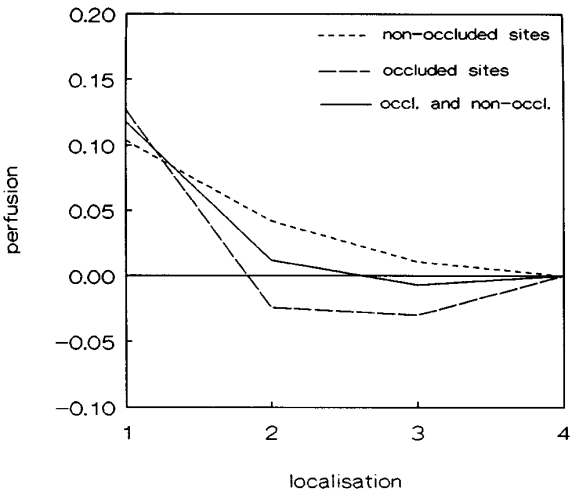

Fig. 2. Higher measurements are shown on the distal sites than on the proximal ones, which is seen at any time of measurement, but most obviously 30 h after application.

The vehicle used in our study, clobetasol-17-propionate (Dermovate® ointment excipient), was investigated in a former study and was reported to have no significant influence on the microcirculation (15).

It is still a problem to visually classify changes in skin colour with the eye. The results of other studies obtained by visual scoring were largely comparable with our results. Also a biphasic vasoconstriction was found after topically applied steroids (8). The authors explained the second peak as a reactive vasodilative phase. To exclude the possibility of an influence of the circadian steroid release we made control measurements with the time table shifted 12 h. The decrease of the LDPI signal remained the same. Other studies, however, did find an influence of blood cortisol levels on the skin colour changes after steroid application (12, 27). In our opinion, so far no good explanation exists for this obvious biphasic curve, also found in earlier reports.

At first the results of our measurements were surprising to us, since a decrease of LDPI flow was expected after local steroid application. However, the measured flow represents a ratio of volume per time-unit (i.e. the product of the total amount of red blood cells multiplied with the amount of moving red blood cells). Vasoconstriction leads to a decrease in capillary diameter. Subsequently velocity increases and the blood volume decreases. The last two changes are in accordance with an increase in the product of blood cell volume and speed (and therefore the LDPI signal), provided that the velocity increases more than the blood volume is reduced.

A second physical explanation could be that erythrocytes in a small blood vessel (10–20 μm) pass one after the other and not in groups of adhesive erythrocytes. This leads to the phenomenon that the laser is counting relatively more erythrocytes in constricted blood vessels. This has been supported by in vitro studies on laser Doppler values within different hematocrits, showing an increase in signal with an increase of the hematocrit (28).

Furthermore a change in flow pattern in the capillaries can explain part of our results. The velocity of erythrocytes in the middle of a blood vessel is higher than at the margin. Vasoconstriction therefore induces an increased LDPI signal.

Recently different techniques have been compared in one study (29) in order to evaluate their sensitivity to assess skin blanching. Beside visual scoring and laser Doppler velocimetry two measurements with LDPI were performed (before application and 1 h after removal of the glucocorticoid). Glucocorticoids (betamethasone dipropionate) were also applied to the volar side of the forearm. The measured site included the test site and the surrounding skin. Afterwards the mean flux was calculated of the central 15×15 mm square. The results of this investigation showed that LDPI was of no use assessing skin blanching. Measurements before and after application of the steroid did not show a significant difference. In our opinion it is necessary to measure only the test sites to minimize the influence of physiological changes in blood flow. As we know that corticosteroids influence the blood flow of the skin for at least 72 h, it is not sufficient to measure only before and after application of the corticosteroid.

In our study we also evaluated the effect of occlusion and the location on vasoconstriction due to local steroid application. Under occlusion the corticosteroid remains longer active on the skin. This is due to an increased percutaneous absorption as a result of epidermal maceration and increased skin temperature (30). Occlusion does not seem to accelerate the bioavailability of the applied corticosteroid (Fig. 1). Nearly the same values for occluded and nonoccluded sites in the first 30 h were found. After this, the LDPI signal of the occluded sites was significantly higher than that of the nonoccluded sites. We could therefore establish that occlusion prolongs the bioavailability of corticosteroids without influencing the time of onset.

Concerning the site of steroid application we found a clear-cut gradient of decreasing blanching response from wrist to elbow. These differences in local reactions have been observed by others (19, 31). The exact nature of this site-dependent response to topically applied steroids is not known. It is likely that factors relating to the vasculature are responsible. Another possible explanation could be local differences of subcutaneous fatty tissue. At this moment it should therefore be recommended to test each preparation at several sites along the forearm of each volunteer, in order to obtain an accurate comparative assessment of corticosteroid release from topical delivery vehicles.

In conclusion we can state that LDPI is a simple and non-traumatic method for studying local skin blanching after steroid application. In contrast to visual scoring it is an objective method for assessing skin blanching, and especially when it becomes almost impossible for the observer to distinguish any blanching at all the LDPI is of great advantage. It can become a valuable instrument for the assessment of steroid potency in dermatology.

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