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Review Article

Assessment of topical corticosteroid preparations: the human skin blanching assay

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Introduction

Since the introduction of topical corticosteroid formulations, their use has become widespread, being prescribed for a large variety of dermatological conditions. This widespread use has created a need for a reliable method of assessing the various dosage forms of these compounds. Clinical trials are laborious, costly and difficult to mount as well as being impractical for the screening of large numbers of drugs. Patients suffering from dermatological complaints are not ideal subjects for the testing of topical corticosteroid preparations as it is difficult to obtain standardized lesions which are necessary for the comparison of results between patients (Baker and Sattar, 1968). For these reasons a number of methods have been developed for the screening of novel corticosteroids and testing of topical corticosteroid formulations.

Of all the in vivo methods currently in use, the human skin blanching assay is one of the most reliable and convenient. The production of blanching in human skin is a side-effect of topical corticosteroid application and it was first observed by Hollander et al. (1950). They noted that intra-articular administration of corticosteroids produced blanching of the engorged synovial membrane. It was later observed (Ashton and Cook, 1952) that apparent vasoconstriction occurred in the superficial corneal vasculature when treated with subconjunctival corticosteroids. It was not until 1962 (McKenzie and Stoughton) that it was realized, after observation that topical corticosteroids under occlusion produced blanching of psoriasis lesions and the surrounding skin, that this blanching might be used as a measure of the percutaneous absorption of corticosteroids from topical formulations. Since that time numerous workers have modified and extended the human skin blanching assay which today provides a reliable and precise means of testing topical corticosteroids and their formulations (Barry, 1976). Although this method has been cited in numerous publications as the vasoconstrictor test or assay, since the exact mechanism of the observed response has not yet been fully elucidated and since the

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measurements are based upon the degree of blanching produced and not vasoconstriction per se, we prefer to call this technique the human skin blanching assay.

Blanching assay methodology

The human skin blanching assay has undergone many modifications since it was first suggested and the methodology currently in use in our laboratories is similar to that used in other parts of the world. Healthy male and female Caucasian volunteers are used who have not received topical or systemic corticosteroids for at least 6 weeks prior to the investigations; normally 10–15 volunteers are used in each experiment. In order to minimize the possible effects of environmental variables such as ambient temperature and humidity, all volunteers partaking in any one trial are processed concurrently at intervals of 5 min. Adhesive labels from which two independent 7 × 7 mm squares had been punched are applied to the flexor aspect of both forearms of each volunteer, producing 12 discrete application sites per arm. Occlusion is achieved by using a non-porous plastic tape; a single piece (70 × 25 mm) being applied over each pair of application sites. In unoccluded experiments the sites are protected with a cardboard frame held in place with surgical tape to prevent accidental removal of the test preparations. In both modes of application the adhesive labels are left in place for the duration of the application time. The preparations to be tested are coded by a person not directly involved with the trial, as are the application sites. Normally 4 different application charts are used in any one trial to prevent the appearance of a recognizable pattern on the arms of the volunteers. Four 7 mm stripes of each preparation are applied to each application site. The standard mass is extruded from 1 ml disposable tuberculin syringes, the needles of which had been cut to 5 mm in order to facilitate the extrusion of the formulated product. The mode of application allows a precise amount of preparation to be applied (Magnus et al., 1980). The syringes are filled immediately prior to use to minimize any possible interaction between the preparation and the plastic matrix of the barrel of the syringe. The syringes are discarded after use. The extruded formulations are spread over the application site using a different glass rod for each preparation. During lotion and solution assessments, fixed volumes are applied from a disposable tip using an autopipette. The formulations are allowed to remain in contact with the application sites for 6 h after which time the labels, occlusion tapes and protective covers are removed. All adhesive materials are removed slowly to reduce erythema and to prevent possible stripping of the epidermis. The sites are then gently washed with soap and warm water and patted dry with a towel. The forearms often show puckering and slight erythema around the application sites but this normally disappears within half an hour. Three independent observers are used to visually assess the degree of skin blanching. Assessments of blanching are made 7, 8, 9, 10, 12, 14, 16, 18, 28 and 32 h after application of the formulations which allows for the establishment of a blanching profile. Standard lighting by overhead fluorescent lamps is used throughout the investigation. The arms of the volunteers are placed horizontally on a desk directly in front of the observer and the readings of all 3 observers are used to analyze the data.

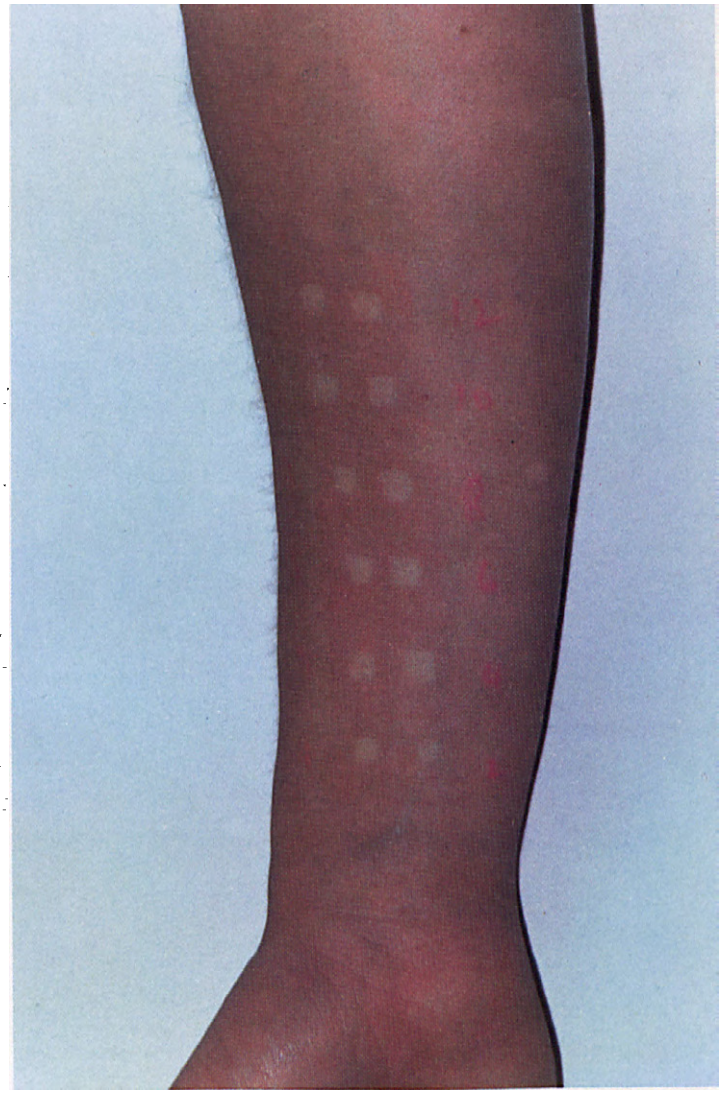

Fig. 1. A typical blanching response after the application of some topical corticosteroid formulations.

Three methods have been used in our laboratories to evaluate the results of skin blanching. The first involves a simple yes/no evaluation of whether or not blanching is present at each application site. These data are normally reported in terms of the total number of sites responding to a given formulation. Of the three methods used this is the least sensitive as it gives no indication of the degree of observed blanching. The second method involves the determination of the degree of observed blanching at each application site for each time interval. An arbitrary 0–4 intensity scale has been defined where 0 represents no blanching and 4 represents intense blanching over the whole application site with the values of 1, 2 and 3 representing the respective grades of blanching between the two extremes (Meyer et al., 1981). These data are usually presented in terms of the percentage of the total possible score

calculated using the following method:

The maximum score per site	= 4
The number of independent observers	= 3
The number of sites per preparation per arm	= S
The number of volunteers	= V
Total possible score (TPS)	= 4 × 3 × S × V

$$\text{Percent total possible score (\% TPS)} = \frac{Actual\ score}{TPS} \times 100$$

The profiles generated by use of the second method are also amenable to area under the curve (AUC) analysis. The trapezoidal rule is used to calculate AUC values. The third method of analysis makes use of a paired comparison of adjacent application sites (Poulsen et al., 1974). This method is extremely useful for the direct comparison of different formulations applied as pairs. Comparisons are made along the x and/or y axes on the forearm in a two-dimensional plane. For each pair of application sites one of the following decisions is required: (1) one site exhibited a greater degree of blanching than the other; (2) both sites exhibited an equal degree of blanching; (3) blanching was not observed at either site. The results obtained from all three of the above methods of evaluation may be analyzed statistically if required by χ^2, ANOVA or other appropriate techniques.

Provided the protocol of this method is strictly adhered to by experienced workers, the assay has been shown to be sensitive, accurate and reproducible (Barry and Woodford, 1978). Obvious advantages of using the human skin blanching assay are that healthy normal skin is used, it is not painful to the volunteers and several preparations can be evaluated simultaneously.

Mechanism of blanching

The exact mechanism of blanching as produced by topical corticosteroids is not yet fully understood. A number of researchers have implied that a relationship exists between the release of endogenous noradrenaline in response to corticosteroid application but others have demonstrated that noradrenaline is not the only factor involved. It was found (Fritz and Levine, 1951) that vasoconstriction by noradrenaline in the mesoappendix of adrenalectomised rats does not occur unless the cortical extract is applied topically, suggesting that corticosteroids may support vascular tone by potentiating the pressor action of noradrenaline. Subsequent studies (Reis, 1960) demonstrated that adrenocortical steroids have a local and direct effect on normal bulbar conjunctival vessels as well as potentiating noradrenaline, but a later report (Juhlin, 1964) showed no evidence of such a potentiation in the vascular reaction of normal skin treated with fluocinolone acetonide. It was therefore assumed that blanching was due to a vasoconstrictor effect of the corticosteroid itself. Further research showed (Frank et al., 1964; Altura, 1966) that corticosteroids do not act directly by constricting any of the muscular components of the capillary bed, but enhance vascular reactivity to various constrictors whilst suppressing histamine, bradykinin and tetrahydrofurfuryl alcohol induced vasodilation as well as potentiat-

ing noradrenaline. It has been demonstrated (Solomon et al., 1965) that guanethidine inhibits vasodilation in normotensive subjects, suggesting that corticosteroid vasoconstriction may be mediated by noradrenaline released from previously demonstrated (Moller, 1962) cutaneous stores of unknown location. Induced tachyphylaxis to topically applied corticosteroids has been demonstrated (Du Vivier and Stoughton, 1975). As a result of these studies, it has been suggested that the cutaneous stores of noradrenaline might be situated in nerve vesicles and that topically applied corticosteroids may act indirectly by releasing this endogenous noradrenaline or influencing its metabolism or re-uptake. Alternatively, it was suggested that the steroid may attach itself to a receptor site causing the release of an intracellular mediator such as adenosine monophosphate or guanosine monophosphate.

The effects of site occlusion on skin blanching

It is well recognized that the degree of hydration of the horny layer of the epidermis affects its permeability for corticosteroids and their transport through the skin. Hydration results from water diffusing from underlying epidermal layers or from the accumulation of perspiration after occlusion. The stratum corneum is changed from a tissue that normally contains little water (5–15%) to one that may contain as much as 50% water, and permeability increases in the order of 4–5 times (Idson, 1975). Hydration opens up the compact substance of the stratum corneum and not only increases the rate of percutaneous absorption, but also increases the possibility of the formation of a reservoir of corticosteroid (Vickers, 1963). In a study involving the use of both creams and ointments it was found (Coldman et al., 1971b) that ointments produced a greater degree of blanching of the skin than creams containing the same corticosteroid in the same concentration. It has been postulated that the higher degree of blanching observed for ointments may be due to the high placebo response of white soft vaseline (Coldman and Lockerbie, 1971; Barry and Woodford, 1972), although it is more likely due to the occlusive nature of the ointment preparations, resulting in an increased degree of skin hydration. In an early study (McKenzie and Stoughton, 1962) it was found that the use of occlusion resulted in a 100-fold increase in the absorption of triamcinolone acetonide, fluocinolone acetonide and dexamethasone when compared to an unoccluded topical application. Occlusion with plastic film provides the single most effective mechanism for increasing the penetration of topical corticosteroids. It has been shown (Maibach, 1976) that hydrocortisone is absorbed in 10-fold greater amounts with plastic occlusion compared to unoccluded. This difference could be clinically significant; many refractory dermatoses could become responsive due to this factor alone. Although occlusion with plastic film also causes an increase in skin temperature, the practical importance of temperature effects in topical therapy is likely to be of minor importance. Occlusion increases the temperature by preventing evaporation and reducing the loss of heat by radiation. The permeability change induced by this small increase in temperature is probably slight relative to that produced by the increased hydration of the stratum corneum.

The skin reservoir

The existence in the skin of a depot or reservoir for topical corticosteroids was first suggested by Malkinson and Ferguson (1955). Vickers (1963) demonstrated the existence of this reservoir in the stratum corneum and found it to be of considerable capacity. After the initial application of corticosteroid to the skin, the test areas were occluded for 16 h and on removal of the occlusion material blanching was visible, but faded after 10–16 h. The test sites were subsequently re-occluded every 2–3 days and on removal of the occlusion blanching was again observed. In some volunteers it was possible to demonstrate blanching up to 15 days after the initial application. The existence of this reservoir has been confirmed by a number of workers (Stoughton, 1965; Carr and Wieland, 1966; Brode, 1968; Woodford and Barry, 1974). Feldmann and Maibach (1965) have suggested that the deeper layers are implicated as well as the stratum corneum and that subcutaneous fat may also be involved. The retention of a corticosteroid in the skin should also be considered as a factor affecting its relative potency as the existence of a reservoir allows the steroid to be released slowly over a period of time. It has been shown (Barry and Woodford, 1974, 1975, 1976; Woodford and Barry, 1974) that the degree of blanching produced following re-occlusion of proprietary formulations was proportional to the clinical efficacy of the preparation. This was only in the case of the more potent corticosteroids as it was not possible to demonstrate the existence of a reservoir for hydrocortisone preparations. The same authors, in their assessment of blanching using a multiple dosage regimen (Barry and Woodford, 1977), found that the reservoir for the more potent corticosteroids was erratic and it was again not possible to demonstrate a reservoir produced by hydrocortisone preparations. In order to correlate blanching response with corticosteroid skin concentration, hydrocortisone concentrations were determined in skin samples from subjects in whom blanching was also assessed (Wallace et al., 1979). The epidermal skin concentration of hydrocortisone was significantly greater in those subjects who exhibited blanching. The data suggested that a minimal epidermal reservoir of 200 μg hydrocortisone/g lyophilized tissue is necessary to elicit a definite blanching response.

Correlation of blanching with clinical efficacy

The strong degree of correlation between blanching activity and clinical efficacy has been the subject of a number of papers. This correlation has been observed irrespective of the type of clinical study employed (Williams et al., 1964; Barrett et al., 1965; Portnoy, 1965; Munro et al., 1967; Reid and Brookes, 1968; Moore-Robinson, 1971; Burdick, 1972a, 1972b; Stoughton, 1972, 1976; Smith et al., 1973; Kaidby and Kligman, 1974; Fredriksson et al., 1975; Whitefield and McKenzie, 1975; Bluefarb et al., 1976; Haleblian et al., 1977; Miller and Munro, 1980). Other topical corticosteroid screening tests have been shown to be inconsistent with clinical efficacy (Sparkes and Wilson, 1974). The human skin blanching assay has been described as an excellent model in evaluating the efficacy of topical corticosteroids prior to their final testing during clinical trials (Haleblian, 1976).

Correlation of blanching with in vitro release

Whilst good correlations have been reported between the degree of blanching caused by topical corticosteroid formulations and their clinical efficacy as described above, attempts have also been made to establish a relationship between the degree of blanching and in vitro rate of release. Busse et al. (1969) utilized two in vitro systems. One consisted of a vessel containing two phases, alcohol/water and chloroform, onto which betamethasone 17-valerate preparations were layered. It was shown that a relationship existed between the blanching observed in vivo and the degree of release measured using this in vitro system. The second in vitro system using isopropyl myristate impregnated filter paper as the receiving phase also showed a direct correlation between the degree of blanching and in vitro release. In order to determine the effect of vehicle composition on the release of fluclorolone acetonide from several cream and ointment formulations, Malone et al. (1974) used a teflon cell with water as the receiving phase for the ointments and isopropyl myristate for the creams. A positive correlation was found between in vitro release and the in vivo blanching response. The release of the active components from a number of different fluocinolone acetonide and fluocinonide topical preparations was assessed in vitro using a diffusion cell and excised whole human abdominal skin (Poulsen, 1970, 1973; Katz and Poulsen, 1971, 1972; Ostrenga et al., 1971b). The relationships between the physical properties of the drug and vehicle were shown to correlate well with the nature of the in vitro release profiles and skin blanching. In the development of a fluocinonide gel formulation a high degree of correlation between the in vitro release into isopropyl myristate and skin blanching has been shown (Haleblian et al., 1979). Further work (Barry and Woodford, 1978) using an in vitro system consisting of a bag formed from soaked cellulose dialysis tubing containing one of several betamethasone 17-benzoate gel formulations was employed to measure the release of the corticosteroid into a chloroform receiving phase. Results from these studies showed that the amount of corticosteroid released in vitro was proportional to the amount of formulation applied to the skin and the degree of blanching observed in vivo. Correlations between in vivo blanching response and amount of corticosteroid released in vitro from various formulations containing betamethasone 17-valerate (Amundsen et al., 1981) or fluclorolone acetonide (Rosvold et al., 1982) have been demonstrated. The in vitro system was similar to that used by Poulsen et al. (1968) using isopropyl myristate as the receiving phase, the study involving an assessment of the effect of propylene glycol concentration on both the blanching response and the in vitro release of corticosteroid.

The assessment of variables affecting the blanching response

The biological model

There is considerable variability between individuals with regard to their ability to blanch. Some people do not show the phenomenon at all, even on application of large amounts of the most potent types of corticosteroid formulations under occlusion. For this reason volunteers should be screened before their inclusion into

experiments of this kind. Amongst those people who do exhibit this phenomenon there are those who blanch excellently, averagely and poorly. People who blanch excellently tend to be poor discriminators; many preparations of different types and strengths of corticosteroids elicit maximal blanching in people of this type. Poor blanchers are similarly difficult to use; it is often difficult to determine the difference between poor blanching and normal skin mottling. Dark-skinned races do not show blanching at all, presumably because, although blanching may occur, it is situated below the melanin layer and is not observable.

The skin site

It is a well recorded fact that a topical formulation of a particular corticosteroid will produce different degrees of blanching according to the part of the body to which it is applied (Cronin and Stoughton, 1962; Rushmer et al., 1966; Ishihara, 1976; Kidd, 1975; Stüttgen, 1976; Osamura, 1982; Idson, 1983). Work of this kind has standardized on the flexor aspect of the forearm for two main reasons, firstly this area shows reasonably good permeability and secondly the convenience factor. Volunteers are ambulatory and only slightly inconvenienced by the cardboard guards, and at observation times undressing is unnecessary. A disadvantage with regard to the use of the forearm is that inconsistencies of blanching have been observed in sites close to the elbow and wrist (McKenzie and Atkinson, 1964; Burdick, 1974; Barry and Woodford, 1978). It has recently been demonstrated (Kirsch et al., 1982) that there is a blanching gradient along the forearm increasing in intensity from elbow to wrist. This disadvantage can be overcome by ensuring that application sites for each preparation include sites over the whole forearm.

The amount of formulation applied

Early work in this field on alcoholic dilutions of a number of topical corticosteroids indicated that different concentrations, when applied to the skin, produced differing degrees of blanching (Falconi and Rossi, 1972; Engel et al., 1974; Barry and Brace, 1975; Clanachan et al., 1980). In the case of formulated products, the application of reproducible amounts to the test site is extremely difficult, and it has been reported (Barry and Woodford, 1974) that for betamethasone 17-valerate cream and ointment, a variation in mass of 3-8 mg spread over a 7×7 mm application site did not significantly effect the degree of observed blanching. A later report (Magnus et al., 1980) on the assessment of some variables affecting the blanching caused by betamethasone 17-valerate cream showed that considerable differences were observed when different masses were applied to a 7×7 mm application site. The observed blanching increased when from 1.6 to 4.8 mg of cream were applied, but over 4.8 mg did not increase it further. Since the corticosteroid is released from the surface of the cream in contact with the skin, the thickness of the layer of cream applied will be significant; over a certain thickness no increased release of corticosteroid would be expected. This critical thickness of the layer of cream may well have been achieved with 4.8 mg of betamethasone 17-valerate cream. It may also be that, at the concentration level of 4.8 mg of cream per 7×7 mm site, the maximum blanching activity of the corticosteroid has been reached.

Occlusion time

As discussed earlier, it is well known that occlusion of topical corticosteroid formulations with a non-porous plastic film increases the penetration of the corticosteroid through the skin. Different occlusion times have been used by a number of workers in the field, but only one report has been published (Magnus et al., 1980) concerning the effect of several different occlusion times on the same topical corticosteroid preparation. The formulation used was a commercially available betamethasone 17-valerate cream and the occlusion times studied were 2, 4, 6, 8, 10 and 12 h. The results of the study showed that the observed blanching increased with an increased occlusion time, a plateau stage being reached at 10 h. The 10-h occlusion maximum may represent the maximum blanching ability of the corticosteroid or it may be that the 10-h occlusion time causes maximum hydration of the stratum corneum. Workers in this field have standardized on a 6-h occlusion time as this permits maximum differentiation between products.

Blanching measurement

The most commonly used method for the determination of the degree of blanching is visually, using an arbitrarily defined scale. Because of the possibility of differences between individuals, we use 3 independent experienced observers in our laboratories, the results of all 3 observers being pooled in the analysis of the data. Workers in Germany (Zaun and Altmeyer, 1973; Altmeyer and Zaun, 1974a, 1974b, 1976a, 1976b; Altmeyer and Krumney, 1978) and England (Dawson et al., 1980; Feather et al., 1982; Ryatt et al., 1982) have developed instrumental techniques to determine differences in skin colour and consequently the degree of blanching produced by topical corticosteroids. These techniques are cumbersome and expensive and no real advantages over the visual method have been demonstrated by their use.

Applications of the human skin blanching assay

Screening of compounds for topical activity

Since the degree of blanching produced by topical corticosteroids has been correlated with their clinical efficacy, it follows that the human blanching assay can be used as a suitable method to screen large numbers of compounds for possible therapeutic use. The assay has been used by a large number of workers to this end. Experiments are normally carried out by dissolving the pure substance in an organic solvent (usually ethanol) and comparing the degree of blanching observed to that produced by a standard topical corticosteroid, usually betamethasone 17-valerate or fluocinolone acetonide. Compounds which have been studied in this fashion include beclomethasone dipropionate (Moore-Robinson and Christie, 1970; Moore-Robinson, 1971), betamethasone (Child et al., 1968; Stoughton, 1969; Place et al., 1970), betamethasone dipropionate (Place et al., 1970; Gruvstad and Bengtsson, 1980), betamethasone 17-valerate (Child et al., 1968; Stoughton, 1969; Moore-Robinson and Christie, 1970; Moore-Robinson, 1971; Bickhardt, 1972; Weirich and Lutz,

1973; Lutz and Weirich, 1974; Gruvstad and Bengtsson, 1980; Somera, 1980), betamethasone esters (McKenzie and Atkinson, 1964), budesonide (Gruvstad and Bengtsson, 1980), clobetasol 17-propionate (Somera, 1980), desonide (Gruvstad and Bengtsson, 1980), fluandrenolone (Child et al., 1968; Moore-Robinson and Christie, 1970; Moore-Robinson, 1971), fluclorolone acetonide (Moore-Robinson and Christie, 1970; Moore-Robinson, 1971), flucortolone caproate (Moore-Robinson and Christie, 1970; Moore-Robinson, 1971), flumethasone pivalate (Child et al., 1968; Moore-Robinson and Christie, 1970; Moore-Robinson, 1971; Weirich and Lutz, 1973), fluocinolide (Place et al., 1970), fluocinolone (Stoughton, 1969; Burdick, 1974), fluocinolone acetonide (Heseltine et al., 1964; Child et al., 1968; Stoughton, 1969; Place et al., 1970; Bickhardt, 1972; Weirich and Lutz, 1973; Burdick, 1974; Gruvstad and Bengtsson, 1980), fluocinolone acetonide acetate (Bickhardt, 1972), fluocinonide (Weirich and Lutz, 1973; Burdick, 1974), fluocortolone analogues (Baker and Sattar, 1968), fluperolone acetate (Child et al., 1968), fluprednylidine acetate (Bickhardt, 1972; Weirich and Lutz, 1973), hydrocortisone (Child et al., 1968; Place et al., 1970; Weirich and Lutz, 1973; Somera, 1980), hydrocortisone acetate (Heseltine et al., 1964), hydrocortisone 17-butyrate (Gruvstad and Bengtsson, 1980; Somera, 1980), paramethasone (Place et al., 1970), paramethasone acetate (Place et al., 1970), triamcinolone (Place et al., 1970), triamcinolone acetonide (Heseltine et al., 1964; Child et al., 1968; Place et al., 1970; Bickhardt, 1972; Weirich and Lutz, 1973), triamcinolone acetonide acetate (Place et al., 1970) and a large range of experimental and novel steroids and their derivatives (McKenzie, 1962; Bagatell and Augustine, 1974; Barry and Brace, 1975; Cimarusti et al., 1976).

Biopharmaceutic assessments and bioavailability

If different manufacturing concerns produce formulations containing the same topical corticosteroid in the same concentration it might be expected that those preparations would have the same clinical efficacy. In recent years it has been shown that this is not necessarily the case; the term bio-inequivalence has become commonplace in modern pharmaceutical research. The bioavailability of a drug is defined as the rate and extent to which the drug in its formulation is delivered to the general circulation. Clearly this definition cannot be applied to a substance intended for topical use and perhaps should be redefined in this case as the rate and extent to which the drug is delivered to the site of action. It is difficult to measure the amount of a topical corticosteroid which reaches the site of action, but since the degree of observed blanching is proportional to this amount, it can be used as a measure of the bioavailability of a particular corticosteroid. More specifically, Woodford and Barry (1982) have defined bioavailability in this context as the relative absorption efficiency for a corticosteroid, as determined by its release from the formulation followed by its penetration through the epidermis into the dermis, to produce the characteristic blanching response. A number of studies have appeared in the literature comparing the bioavailabilities of topical corticosteroid formulations containing the same corticosteroid in the same concentration. Topical corticosteroids assessed using the skin blanching assay include bethamethasone 17-valerate (Meyer et al., 1981 Meyer et al., 1983), diflucortolone valerate (Wendt and Reckers, 1976,

Coleman et al., 1978; Reckers and Wendt, 1980), fluclorolone acetonide (Garnier, 1971), fluocinolone acetonide (Coleman et al., 1979), fluocinonide (Reinstein et al., 1972). fluoromethalone (Tissot and Osmundsen, 1966) and hydrocortisone (Barry and Woodford, 1976; Woodford and Barry, 1979). Extemporaneous formulations of triamcinolone acetonide have been compared with commercially available formulations (Burdick et al., 1970). A novel drug delivery system, the quick break foam, has been assessed for betamethasone benzoate (Woodford and Barry, 1977b) and experimental formulations for amcinonide (Woodford and Barry, 1977a; Woodford and Haigh, 1979). The blanching assay has also been used to study interactions between active ingredients in topical corticosteroid preparations (Raab, 1973; Raab and Windisch, 1973), mixtures of different corticosteroids in the same formulation (Poulsen et al., 1978) and the placebo effect of different bases (Barry and Woodford, 1972; Durocher and Bielmann, 1975).

Potency ranking

A great deal of emphasis has been placed on the screening of different commercially available topical corticosteroid preparations and many papers have been published describing the comparative blanching ability of these formulations (Christie and Moore-Robinson, 1970; Coldman et al., 1971a; Ishihara, 1972, 1975; Burdick et al., 1973; Stewart et al., 1973; Woodford and Barry, 1973; Barry and Woodford, 1974, 1975; Zaynoun and Kurban, 1974; Szadurski et al., 1976; Haleblian et al., 1977; Agrup et al., 1978; Poulsen and Rorsman, 1980). The results of these studies have led to the establishment of the now common potency tables for topical corticosteroid formulations (Anonymous, 1977, 1983).

Influence of formulation factors

Extemporaneous dilution. Extemporaneous dilutions of topical corticosteroid formulations are commonly prescribed by dermatologists and general practitioners (Smith, 1982). That the vehicle composition has a direct effect on the release of corticosteroids from topical formulations is well documented (Ostrenga et al., 1971a, 1971b, 1971c; Hadgraft, 1972). For this reason, manufacturers contend that interference with well researched products by dilution may result in a reduction in clinical efficacy (Mooney and Pierce, 1974; Busse, 1978). One published study has been concerned with the effect of dilution on the blanching ability of topical corticosteroid preparations (Magnus et al., 1981). The conclusions drawn were that for a commercially available betamethasone 17-valerate cream, dilution with a number of different vehicles did not adversely effect the delivery of corticosteriod from the dilution for up to 3 months after preparation. However, generalizations may be premature; because a base has been found to be compatible with a particular corticosteroid in a particular formulation, it does not imply that the same base will be equally compatible with all corticosteroid containing formulations. Although the release characteristics may remain relatively unaffected as a result of the dilution, a decrease in clinical efficacy may occur through the injudicious choice of a diluent due to increased risk of degradation as a consequence of a pH or other change. Other studies in this field (Barry and Woodford 1978; Tanner and Woodford, 1981;

Woodford, 1981; Gibson et al., 1982) have also shown that the blanching assay is a useful method for studying the effect of dilution on corticosteroid release.

Accelerants. Probably the most important feature of a topical corticosteroid formulation in terms of its clinical efficacy is its ability to release the active ingredient into the skin. It has been observed that several adjuvants, when incorporated into topical formulations increase the release of the corticosteroid from the preparation, consequently much research has been carried out on this aspect of formulation. Accelerant compounds which have been studied using the human blanching assay include dimethylacetamide (Sarkany et al., 1965), dimethylsulphoxide (Sarkany et al., 1965), 1-dodecylazacycloheptan-2-one (Stoughton, 1982), isopropyl myristate (Pepler et al., 1971), propylene glycol (Ostrenga et al., 1971b; Pepler et al., 1971; Amundsen et al., 1981; Rosvold et al., 1982), salicylic acid (Wienert and Blazek, 1981), sodium polyhydroxycarboxylate (Gloor and Lindemann, 1980), sodium pyrrolidone carboxylate (Gloor and Lindemann, 1980), sulphur (Gloor and Lindemann, 1980), tetrahydrofurfuryl alcohol (Sarkany et al., 1965) and urea (Gloor and Lindemann, 1980).

Stability

The human blanching assay has also been used to monitor corticosteroid degradation in a commercially available betamethasone 17-valerate cream which had been diluted extemporaneously (Magnus et al., 1981). The transformation of betamethasone 17-valerate to betamethasone 21-valerate over a period of time was determined utilizing a high-performance liquid chromatographic analytical method and the decrease in blanching observed correlated with the reduction in concentration of the active ingredient.

Establishment of dosage regimens

It has recently been shown (Du Vivier and Stoughton, 1975, 1976; Du Vivier, 1976) that repeated application of topical corticosteroid formulations to the same skin site caused tachyphylaxis of the blanching response. The first application caused the usual degree of blanching, but on subsequent application the blanching response showed a dramatic decrease. After a resting period of 4 days the sites apparently recovered since new application again produced blanching but on repeated application, tachyphylaxis was again observed. The original work on this subject was extended (Altmeyer and Cremer, 1977) to other corticosteroids under occlusion; tachyphylaxis was again demonstrated. The basic concept of tachyphylaxis to repeated application of topical corticosteroid formulations to the same skin site has been utilized to establish ideal dosage regimens for a number of different formulations (Barry and Woodford, 1977; Woodford et al., 1983).

Future areas of investigation

It is quite clear that the human skin blanching assay is now well-established as a valuable method for assessing topical corticosteroids. There are, nevertheless, several

aspects which deserve further attention. Considering the assay procedure itself, optimization of the method of application of the test materials to the application sites would enhance the efficiency of the system. The development of an automated quantitative instrumental method of measuring the degree of blanching would reduce the tedium of the many visual observations necessary; in addition an increase in accuracy, precision and reproducibility would result.

Since the mechanism of blanching is not fully understood, more investigations into this area are required. Many questions need to be answered in this respect, i.e. what is the exact mechanism of blanching? why do some individuals blanch and others not? why do those individuals who do not blanch still respond to topical corticosteroid therapy? what effect does skin metabolism of the corticosteroid have on the blanching response? what part does the reservoir play, if any, in the production of the blanching response?

Whilst many of the biopharmaceutical factors affecting the release of corticosteroids from topical preparations have been elucidated, it remains necessary to continue with these investigations and assess the influence of the ever-increasing range of novel adjuvants and excipients being introduced for use in semi-solid dosage forms. In this respect, new formulations containing well-established corticosteroids should not be considered bioequivalent with previously assessed preparations. Comparative bioavailability data obtained using the human skin blanching assay could provide valuable information to the regulatory authorities concerned with the registration of new products.

The introduction of nighly potent topical corticosteroid preparations has given rise to concern relating to systemic absorption and consequent undesirable side effects. Research designed to determine whether a correlation exists between the blanching ability and serum/plasma concentrations of applied corticosteroids could be of use in toxicity studies.

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