Dermatological Models: A Sound Basis for Early Decision-Making

Whereas the early phase of drug development for systemically acting drugs is mostly driven by the presumed relationship between safety/efficacy and concentration of drug in the systemic circulation, this relationship does not exist for topically applied drugs intended for local or regional action. For these topical drugs, systemic absorption is not desired and systemic exposure can often be kept to very low or even negligible levels by controlling the size of the treatment area. In early clinical development this ability to limit systemic exposure often allows for initial assessment of efficacy in innovative models before investment in costly studies.

In dermatological models, discrete evaluation of treatment effects may be possible following simultaneous application of multiple formulations/actives to small treatment areas in parallel in one individual, without risk of crossover effects. By making intra-individual comparisons between treatments, the inter-subject variability is reduced, allowing meaningful interpretation of results with smaller panel sizes. Further, in these models non-invasive biophysical measurement and imaging methods to monitor skin function and structure provide alternative objective endpoints to support clinical evaluation, delivering additional information on structural and functional changes in the skin.

The following provides a brief overview of several established dermatological models and how they can be applied in clinical testing.

Psoriasis Plaque Test

In the psoriasis plaque test, also known as the psoriasis microplaque assay, anti-psoriatic efficacy of multiple formulations is assessed in parallel in small test areas located on stable psoriatic plaques in patients with psoriasis vulgaris. It may even be possible to test efficacy in this design as the first Phase I study, analog to an early Phase I study under an exploratory IND. In the original test design described by Dumas and Scholtz in 1972, the efficacy of corticosteroids for treatment of plaque-type psoriasis was evaluated clinically following standardised occlusive application over several days. Test fields were graded as follows: unchanged or less than full involution (0) or complete return of epidermis to normal (+). Whereas this design is well suited for testing of strong anti-psoriatics such as potent corticosteroids, the sensitivity may not be sufficient for assessment of weaker formulations or determination of slight differences between formulations.

Meanwhile biophysical measurement methods have been used in the PPT to objectively measure the inflammatory alterations accompanying psoriasis, greatly improving the sensitivity of the test. Since one of the most important clinical endpoints in psoriasis is the extent of the psoriatic infiltrate, objective measurement of the infiltrate depth with 20 MHz sonography is probably the most relevant outcome to use as a marker for treatment effects. Other methods that have been used in the plaque test include measurement of intensity of erythema by colorimetry, skin blood flow by laser-Doppler flowmetry, skin surface characteristics by profilometry, and skin temperature.

In a typical psoriasis plaque test design, patients (n=15-20) suffering from psoriasis vulgaris with stable plaques already existing for several months or years are suitable for inclusion. Plaques exhibiting spontaneous regression as well as exacerbation of psoriasis are excluded. Small test fields (e.g., 12 mm) are treated over a 12-28 day period. The proposed mechanism of action of the active pharmaceutical ingredient (API) should be taken into consideration to determine the length of treatment. Ideally all test fields are located on a single psoriatic plaque or on two plaques located in a similar body area and of comparable severity. Prior to baseline measurements and the first treatment, scales are removed from plaques. This is necessary since it is not possible to obtain sonographic images of good quality if images are taken through thick scales. A bandage is attached to the plaque in which the test fields have been punched out (figure 1). Treatments are preferably performed in an occluded manner once daily. More rarely treatments are performed in an open manner once or twice daily. In general, occlusion is preferred as this maximises absorption of drug, shortening the necessary treatment period. The primary variable is the depth of the psoriatic infiltrate measured in sonographic images taken at defined intervals during the treatment period. The echo-poor region directly underneath the entrance echo mainly represents the inflammatory infiltrate and is readily demarcated in good quality images (figure 2). Alternatively, full skin thickness (infiltrate plus dermis) can be measured to evaluate changes in severity of psoriasis, however this method is generally less sensitive than measurement of the psoriatic infiltrate alone. Clinical improvement is assessed as a secondary variable, but it is not possible to make the fine distinctions between treatments that are possible with
measurements of sonographic images. The intensity of erythema can also be measured in the test fields by colorimetry. Even though this measurement has proven particularly useful for the evaluation of corticosteroids where vasoconstriction plays a prominent role in the measurable effect, the overall relevance of additional assessment of redness is questionable for other drug classes. Redness is the last symptom to clear in psoriasis and some residual erythema may remain after all other skin alterations have cleared.

The psoriasis plaque test has been used to investigate efficacy of corticosteroids, vitamin D analogs, retinoids, oligonucleotides, and immunomodulators, among others. The test is very well suited for comparison of developmental candidates, alternative formulations or initial studies of dose response. Due to the maximised conditions it is to be expected that efficacy will be found if a formulation indeed possesses antipsoriatic activity.

**Irritant Dermatitis Model**
Acute or chronic eczematous skin reactions can be simulated by standardised repeated washing with the anionic surfactant sodium lauryl sulfate (SLS). The clinical appearance of the skin (erythema, scaling, fissuring) and biophysical parameters of skin condition (transepidermal water loss (TEWL), stratum corneum hydration, skin colour/erythema) show similarities between the irritation induced with SLS and irritant dermatitis.

In one such model used at bioskin comparable localised skin lesions are created on the forearms of healthy or atopic subjects using a highly standardised open washing procedure once daily for six days. Following this washing procedure, test fields are delineated by application of a bandage system with punched holes (figure 3). Treatments are then applied once or twice daily for up to four days. In a modification of this design the washing procedure may be continued during the treatment phase to better simulate chronic irritation.

This model is mainly suited for screening of barrier repair formulations or other anti-inflammatory formulations intended for treatment of conditions such as hand eczema. It may also be very useful for screening of suitable moisturisers and lubricating vehicles.

**UV-Induced Erythema Test**
The UV-induced erythema test is an experimental model to evaluate anti-inflammatory efficacy of steroidal and non-steroidal topical formulations. The model has the advantage that it is performed in healthy volunteers as opposed to patients with inflammatory skin disease. UV-induced inflammation is mediated by several possible mechanisms and involves the generation of a variety of inflammatory mediators such as prostaglandins, histamine, bradykinin, serotonin and leukotrienes.

In this model, a typical sample size is 20 to 40 subjects with Fitzpatrick skin type II to III. Since efficacy is correlated with the severity of the inflammatory response, success of the test is dependent on controlling the extent of inflammation, even in individuals with different inherent sensitivities. This is achieved by irradiating with a defined UVB dose which is a multiple of the Minimal Erythemal Dose (MED). The MED is the smallest amount of UVB light producing distinct erythema, and differs from individual to individual. The MED for each subject must be determined beforehand by parallel exposition of small fields to graduated UVB dosages.

After determination of the individual MED, test fields on the back are irradiated with the desired UVB dosages. The degree of inflammation can be varied by irradiating separate test fields with different UVB dosages, e.g. 1.25, 1.6 and 2 MED, in a single panel. Immediately after irradiation the test fields are treated for the first time with the test formulations. Since treatments are performed after irradiation, a sun protective effect can be excluded. Test products can be applied in an occlusive or open manner, however in the case of weak anti-inflammatory drugs, e.g. hydrocortisone, it may only be possible to measure an anti-inflammatory effect under occlusive conditions. The frequency of application (single or repeated applications) and the length of the treatment period (eight to 48 hours) can be varied.

The endpoint is the degree of erythema in the irradiated test fields. Diminished erythema compared to irradiated, untreated fields or vehicle-treated fields is indicative of anti-inflammatory efficacy. Objective measurements of erythema are done using colorimetry.

**Thermal Sensory Assessment**
Alterations in pain perception can be quantified using a thermal sensory analyser. Using this technique a thermal diode is placed on the subject’s skin to heat or cool the skin. The subject is then asked to respond to the temperature stimuli by pressing a button when pain is first perceived or becomes intolerable.

To take this methodology one step further, thermal hyperalgesia can first be induced in test fields by setting an inflammation with UVB light to increase pain sensitivity for testing antihyperalgesic drugs. To measure the heat pain threshold, the skin temperature is linearly increased using the thermode, and subjects are advised to stop the heat by pressing a button as soon as the heat becomes painful. The threshold temperature is recorded. The threshold is assessed prior to irradiation and at intervals beginning six to 12 hours following irradiation. Antihyperalgesic drugs lower the heat pain threshold.

**Wound Healing Models**
Aside from patients with wounds resulting from accidents or diseases, or wounds resulting from diagnostic or therapeutical measures (cryosurgical removal of skin lesions, punch biopsies...
and post-operative wound care), there are few ethical models to examine wound healing in humans. The production of wounds is inevitably an invasive procedure that may be associated with pain, bleeding, wound infection and scarring. There are however two good models for induction of superficial wounds in which the promotion of wound healing during the early phase of re-epithelisation can be measured.

The first is a recently developed model in which standardised abrasive wounds are induced which closely mimic the clinical picture of bagatelle abrasive wounds[12]. Multiple wounds are induced on the forearm by scrubbing the skin with a hard brush until punctate bleeding occurs. Healing and re-epithelisation is then followed for up to 14 days (figure 4). Panel sizes range from 10-30 subjects.

An alternative model involves making epidermal defects of defined size by raising suction blisters using negative pressure over a surgical pump. Following removal of the blister roof there is a large increase in water loss as a result of the missing epidermal barrier. It is well established that the transepidermal water loss (TEWL) is correlated with the degree of epidermal damage. The highest TEWL values are measured in fresh wounds with a continual decline until values from intact skin are reached at the end of healing. Therefore, the measurement of TEWL is a suitable parameter to determine the degree of re-epithelisation. The critical phase of epithelial regeneration already occurs during the first days following experimental wound induction in this model.

Expanded Flora Test

Bactericidal efficacy of topical preparations can be investigated in the expanded flora test[13]. In this model the bacteria on the surface of the skin are multiplied by occluding test fields located on the back with a plastic covering. In this way the resident cutaneous microflora can be expanded from its normal low density of about 10^2 colony forming units to 10^7-10^9 after 24-48 hours of occlusion[14,15]. Modifications of the original model allow for treatments before or after expansion of the skin’s bacteria to assess the antibacterial activity.

Conclusion

These as well as other “custom-made” models can help to make the all-important go/no go decision early in a topical product development programme. Further, test model results are valuable for planning of later therapeutic trials, for instance for sample size calculations, dose finding and ranking with comparators. These advantages should be exploited to the fullest to optimise clinical development programmes.

References: