The psoriasis plaque test on the stage

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SUMMARY

To detect drugs with potential antipsoriatic activity a number of different methods are available (in vitro investigations, animal models, investigations in humans). Scientific interest has been focused on the development of clinical models because of the lack of a corresponding animal model for psoriasis. The psoriasis plaque test (PPT) as a classical within-patient trial is an important tool to study topical antipsoriatic drug activity in vivo. Many modifications in performing the PPT permit conclusions aiming at additional information, e.g. about the antipsoriatic drug activity per se as well as information on the mechanism of action, the dose-response relationship, the choice of vehicle, the application frequency or the local tolerability. An important prerequisite for correct performance of the PPT is the knowledge of toxicological and pharmacological data of the investigational compounds. Otherwise, the PPT is relatively simple, suitable and useful. The PPT can be seen as a reference model for the evaluation of drugs with potential antipsoriatic activity. Nevertheless, the results of the PPT must be confirmed by controlled clinical trials.

KEY WORDS

psoriasis, psoriasis plaque test, antipsoriatic drugs, evaluation

1. Introduction

Psoriasis is a chronic skin disease of unknown etiology (1). Certain clinical forms indicate that psoriasis may even be a systemic disease. It affects about 2-3% of the population in western countries, but is less common among Eskimos, American Indians and Japanese. Additionally, an early as well as a late onset of psoriasis has been characterized (2). It is generally accepted that the disease is precipitated by a number of various non-genetic triggering factors (e.g. trauma (Koebner phenomenon), stress, drugs, infections, hormones) (3). Since psoriasis is oligogenic and multifactorial there is no cure for this disorder. Nowadays, every psoriasis therapy remains symptomatic.

Over the last few years progress has been made in molecular biology with regard to etiopathogenetic aspects, e.g. the function of endothelial cells, the importance of adhesion molecules or the discovery of chemokines. The observation of activated $CD4^+$-T-cells expressing surface HLA-DR molecules and interleukin-2 receptors in psoriatic lesions provides evidence for

an ongoing immune response (1). From the dermatological point of view, the lesions are characterized to a large degree by the combination of lesional redness, scaling and infiltration. The corresponding histological changes are well documented. They reflect disturbances of three major mechanisms: proliferation/differentiation, immunological regulation and inflammatory response. The morphology of the skin involvement may vary considerably and a dynamic picture may even be observed within a single lesion (4). Thus the evaluation of topical antipsoriatic drug has to be based on a reliable choice of psoriasis type and severity, respectively. This can be achieved by restricting the evaluation to chronic stable plaque psoriasis without any change of disease severity for several weeks or months.

2. The ranking of the psoriasis-plaque test (PPT) within the spectrum of methods for the determination of potential antipsoriatic activity

Three possible approaches to the assessment of the efficiency of a potentially antipsoriatic compound have been employed so far:

2.1. In vitro examinations of the test compounds ability to influence the mediators relevant for disease induction (e.g. inhibition of leukotriene B_4 synthesis by 5-lipoxygenase inhibitors, influence on the IL-2 receptors)

The disadvantage of these investigations is that, in contrast to the established complexity of the mediator network in the etiopathogenesis of psoriasis, only individual, selective and constricted mechanisms are recorded. In the case of 5-lipoxygenase inhibitors and leukotriene B_4 receptor antagonists it has been proved, for example, that the in vitro efficacy does not necessarily correlate with the efficacy in the clinically involved skin (5, 6).

2.2 Animal models

2.2.1. Artificially induced hyperproliferation of the epidermis in animals

Several different animal screening models have been described (7, 8). The assessment of a test substance depends on the extent of its inhibition of artificially induced hyperproliferation which is in most models temporary. The in vivo character of these investigations serves them as an advantage. The disadvantages are not only the possibility of a species specifity but also the assessment based on a monosymptomatic registration of primarily anti-proliferative effects, i.e. inflammatory and immunological phenomena are registered secondarily, if at all. Additionally, there are numerous drawbacks of the practical accessibility of these models, such as 'Lack of essential fatty acids model' used in rats and mice (7), due to cost restrictions. Experiments in animals, for example, therefore revert to the use of certain tumor promoters such as 12-0-tetra-decanoyl-phorbol-13-acetat (TPA) or the chemically-induced papilloma model. A striking example for the usefulness of the latter in pre-clinical trials is the successful development of retinoids by Bollag (8).

2.2.2. Mouse tail test and other models with abnormal keratinization

The mouse tail test is closely related to the two different types of keratinization – the appearance of parakeratotic and orthokeratotic horny layers – in the adult mouse tail. With antipsoriatic drugs it is possible to convert the parakeratotic to orthokeratotic differentiation. This was first described by Jarrett and Spearman in 1964 and later confirmed by other investigators (9, 10, 11). Until now many compounds with antipsoriatic activity have been tested using the mouse tail test. The only disadvantage inherent to this model is the physiologic character of the parakeratotic keratinization of mouse epidermis, which therefore raises questions about its relevance for psoriatic patients. Nonetheless, the mouse tail test offers a promise for simple drug screening. Additionally, other models, such as the nude mouse xenograft or psoriasiform dermatitis in a rhesus monkey have been reported (7).

2.3. In vivo investigations in humans

Investigations in humans can be attributed to three categories: 1. Pharmacological investigations in healthy volunteers; 2. Investigations by means of the PPT; 3. Classical clinical trials.

In the case of the investigations in healthy volunteers, individual, selective mechanisms are investigated which are pathogenetically linked to the clinical picture of psoriasis with regard to their pharmacological target. Van de Kerkhof and co-workers demonstrated in a series of experiments the effect of antipsoriatic substances on leukotriene B_4-induced chemotaxis of polymorphonuclear leukocytes (12). The buccal epithelium in normal humans was also proposed by Harrison and Skerrow (13). The advantages of these models are obvious. They involve human in vivo investigations and therapeutic concentrations can be determined. Further-

more topical therapy, a dermatological preference, can be employed. The major disadvantage of these models is the examination of isolated pathogenetic events.

3. The PPT as a screening model

The PPT employs standardized observation of the three skin symptoms of psoriasis *erythema, infiltration, scaling*, limited to indicator lesions. Dumas and Scholtz inaugurated this bio-assay in 1967 and introduced it on the occasion of the 13th International Congress of Dermatology. Both authors described it in detail in 1972 (14). Today the term psoriasis plaque test (PPT) has asserted itself. In 1981, Weinstein and co-workers were able to confirm the validity of the PPT for a variety of antiproliferative substances in an extensive multi-centre study (15). Numerous modifications have been made to the test procedure of the PPT in the last few years. However, the principle of the PPT has remained unchanged.

Performing the PPT, either a large area of psoriasis (e.g. on the back) or several small areas (e.g. on the forearms) are used. When choosing a large psoriatic plaque, numerous smaller, defined psoriatic test sites can be outlined at a sufficiently large distance from each other. Smaller psoriatic plaques may be used as one test site, if the plaque-size exceeds that of the Finn-Chamber employed. In order to include patients with uniform lesions only each patient's form of psoriasis must be defined exactly, before starting the investigation. The corresponding inclusion and exclusion criteria must also be defined, as is generally the case for clinical studies. After a wash-out phase, which as a rule is shorter for topical therapy than for systemic therapy (e.g. 14 days for topical and 1 to 2 months for systemic drugs), the individual test sites are numbered and the initial scoring for each test area is assessed by reading the respective parameters according to a given scale (e.g. 0 = absent, 1 = weak, 2 = medium, 3 = strong). For a fixed period of time (e.g. 5 - 15 days) the test substances are applied daily, with or without occlusion, but always including the corresponding vehicle controls without the active ingredient (placebo). The scoring for the individual symptoms of redness, scaling and infiltration can either be made daily, whereby information on the dynamics of the activity of the disease can be recorded related to time, or can be made once at the end of the investigation.

Possible modifications of the PPT procedure may become necessary due to the following criteria:

1. Number of the test sites
2. Size of the test sites
3. Application of the test substances (open, under occlusion, semi occlusion/occlusion by Finn-Chamber)
4. Frequency of application
5. Overall application time
6. The reading of the individual scores for the single symptoms of redness, scaling and infiltration
7. The registration of the severity of the disease by means of "objective" measuring techniques (e.g. laser-doppler, skin-reflectance spectrometry, ultrasound, photography) (16, 17, 18, 19).

4. The PPT - which statements are possible?

4.1. Assessment of antipsoriatic activity

There is a clear information of the antipsoriatic activity of the tested compound if, during the course of the investigation, any decrease (or increase) in the score is recorded. Experience with the PPT reveals that all antipsoriatics clinically employed exhibit an antipsoriatic activity in the PPT. This applies not only to the standard antipsoriatic anthralin but also to topical glucocorticosteroids, vitamin D3 analogues and immunosuppressive agents (20). Independent of the time of the year, the group of patients with a double-blind reading fluocinolone acetonide 0.025%, for example, achieved an average amelioration of psoriasis of approximately 95% under occlusive conditions on the 11th to 15th day of application (21, 22). When interpreting the results of different groups of investigators the conditions of application, especially occlusion, must be kept in mind. Clinical experience has shown that occlusion alone applied to psoriatic lesions (e.g. by hydrocolloid dressing) leads to a reduction in symptom scores. Under occlusion there is an increase in the penetration of the drugs, an inhibition of the mitotic index or cell division, an increase in hydration and a decrease in certain enzyme activities (23, 24, 25)). It is therefore necessary to carry out corresponding vehicle controls without the active substance in order to be able to differentiate beyond doubt between the effect of the occlusion and the pharmacodynamic effect of the test-compound. The psoriasis symptoms can decrease continually, particularly when the occlusive technique is applied over a longer period of time (e.g. 2-3 weeks). The long-term application of topical glucocorticosteroids of different strenghth under occlusive plastic film also leads to improvement. This means that the antipsoriatic effect of the steroids can no longer be distinguished from each other adequately. Therefore, it is good common practice not to perform the PPT for longer than a maximum of three weeks under occlusion. Generally, it should be possible to prove the antipsoriatic effect employing either the occlusive technique by plastic film or by Finn-Chamber or in an open application without dressing (26). In the latter case the trial compounds should be applied once or twice daily always at the same time of a

day by rubbing. If, for example, on the 14th day two or more test substances display identical efficacy with regard to the remaining psoriasis symptoms, a difference between a fast and a slow onset of action is still possible by recording the effect over time, i.e. by making daily or every other day readings.

4.2. Assessment of the mechanism of antipsoriatic activity

By recording the redness, the scaling and the infiltration it is possible to detect the tendencies with regard to the mechanism of antipsoriatic activity. In the PPT, the vitamin D3 analogue calcipotriol, for example, is very effective in suppressing the infiltration and the scaling. The redness, however, is only slightly suppressed (21). As the inflammatory activity is primarily expressed by the symptom of redness, it is justifiable to conclude that this vitamin D3 analogue has a strong antipsoriatic effect on the infiltration and the scaling but weak anti-inflammatory activity. Consequently, a combination of calcipotriol and glucocorticosteroids has been proposed. In principle, such distinctions are also possible with other antipsoriatic compounds.

4.3. Assessment of the dose-response relationship of topical antipsoriatics

When a substance is applied in different concentrations incorporated into the same vehicle including the corresponding active ingredient-free controls, it is possible to determine the respective minimum concentration of the test compound which exhibits antipsoriatic activity. If a sufficient number of different concentrations is chosen, it is possible to determine the optimum concentration as well as the concentration which induces further improvement. The PPT is therefore particularly suitable as a screening model for the dose-response relationship of potential antipsoriatics.

4.4. Assessment of the optimum test substance - vehicle

The PPT enables the investigators to establish the optimum base or galenic preparation from which a given active compound, e.g., a topical glucocorticosteroid, can best be liberated. However, it is important that, in this case, a constant concentration of the active substance is incorporated in the different vehicles, such as fatty ointment, ointment, lotion or solution.

4.5. Assessment of the efficiency of different forms of application

Beside its suitability to test different substances in different concentrations, the PPT also offers the possibility of testing user-orientated forms of application (e.g. once or twice daily applications of topical glucocorticosteroids (27) or combination treatment with different topical glucocorticosteroids (28).

4.6. Assessment of local tolerability of test compound and vehicle

The PPT as a classical within-patient trial with simultaneous administration of multiple formulations further allows the evaluation of local tolerability of drugs and vehicles. The occlusive application used in the PPT enables to detect any irritant or allergic potential of the investigational compounds or vehicles far better than an open application mode.

5. Prerequisites for carrying out the PPT

The most important prerequisite for using PPT is the knowledge of the toxicological data of the investigated compound (e.g., LD_{50}, mutagenic, carcinogenic potential and side effects). It is also desirable to have data on pharmacodynamic/kinetic parameters such as liberation, penetration and metabolism. As there is a known heterogeneity of psoriasis itself, only those patients should be included in a respective examination whose skin manifestations have been relatively stable for at least three weeks. Confirmation that the test sites are stable can be made by comparison between the pretreatment evaluation (e.g. day –21) and a repeated evaluation at the start of the treatment phase (e.g. day 0). When selecting test sites on the extremities, it is also necessary to switch the test sites from proximal to distal or vice versa from patient to patient. This takes into consideration an effect, which has been observed in the topical therapy of psoriasis, namely that proximal sites tend to improve earlier than sites on a distal location. If a test has the potential to percutaneous absorption, this must be controlled by appropriate investigations, as it may so affect other test sites.

6. Advantages and disadvantages of the PPT

If an almost standardized psoriatic lesion is used in PPT, all disadvantages of the in vitro and animal investigations can be ignored. The relatively small test site allows the simultaneous examination of several compounds in different concentrations and in different vehicles.

Apart from a semiquantitative scoring, the test can

also be evaluated by objective measuring techniques and even histological examination (29). The PPT is therefore especially suitable as a screening method. By recording the score daily or every other day, the kinetics of the anti-inflammatory, the antiproliferative or the antipsoriatic activity of a test substance can be determined. Additionally, it is possible to establish the local tolerability of the test-drugs and/or the formulations used (20). Compared to everyday psoriasis therapy, the only disadvantage of the PPT is that the occlusive technique or the use of an occluding chamber may have special pharmacodynamic effects of their own (24, 30). Therefore the data obtained by PPT may not necessarily be obtained by an open treatment protocol. The appropriate controls with vehicle only (placebo) are necessary. The results of the PPT, however, serve as a proof-of-concept which, in a second step, has to be proven by controlled clinical trials.

7. Summary: evaluation of the PPT

The PPT is an important in vivo model, which is disease-specific and allows the simultaneous screening of several investigational compounds over a short period of time. Under standardized conditions and with sufficient experience with the PPT, reproducible data on the antipsoriatic efficacy of a given compound can be obtained. Furthermore, other dermato-pharmacological parameters, e.g. the different effects on the psoriasis activity, the dose-response relationship and the galenical formulation can be evaluated. The most important prerequisite for performing the PPT is the knowledge of the toxicological profile of the respective compound. Today, the PPT is clinically established and therefore regarded not only as a reference model for the examination of topical glucocorticosteroids but also of other topical compounds with expected antipsoriatic activity.

REFERENCES

1. Christophers E. The immunopathology of psoriasis. Int Arch Allergy Immunol 1996; 110: 199-206.

2. Henseler T. Genetics of psoriasis. Arch Dermatol Res 1998; 290: 463-76.

3. Boehncke WH, Dressel D, Zollner TM, Kaufmann R. Pulling the trigger on psoriasis (letter). Nature 1996; 379: 777.

4. Van Scott EJ. Lesional heterogeneity in psoriasis and therapeutic relevance. In Farber EM, Nall LM, Morrhenn V, Jacobs PH, ed. Proceedings of the Forth International Symposium. Stanford University, July 6-11, 1986. Elsevier Science.

5. Newton JA, Boodle KM, Dowd PM, Greaves MW. Topical NDGA (nordihydroguaiaretic acid) in psoriasis. Br J Dermatol 1988; 119: 404-6.

6. Hunter I, Skerrow D. The effect of increased tissue turnover on the keratinization of human epidermis. Biochem Biophys Acta 1981; 674: 155-9.

7. McCullough JL, Weinstein GD, Ziboh VA. Cell kinetics in psoriasis. The use of animal and human skin models. In: Maibach HI, Lowe NJ, ed.: Models in Dermatology. Basel, Karger, 1985; 1: 51-8.

8. Bollag W. The development of retinoids in experimental and clinical oncology and dermatology. J Am Acad Dermatol 1983; 9: 797-805.

9. Wrench R. Assessing drugs for psoriasiform diseases and their antiparakeratotic mechanisms using the mouse tail test. In: Maibach HI, Lowe NJ, ed. Models in Dermatology. Basel, Karger, 1985; 2: 76-91.

10. Jarrett A, Spearman R. Vitamin A and the skin. Br J Dermatol 1970; 82: 197-9.

11. Bladon PT, Cooper NE, Wood EJ, Cunliffe WJ. Biochemical markers in the mouse tail model of psoriasis. In: Marks R, Plewig G, ed. Skin Models; Models to study function and disease of skin. Springer-Verlag Berlin Heidelberg New-York Tokyo 1986: 172-82.

12. Wozel G, Chang A, Zultak M, Czarnetzki BM, Happle R, Barth J, van de Kerkhof PCM. The effect of topical retinoids on the leukotriene B_4-induced migration of polymorphonuclear leukocytes into human skin. Arch Dermatol Res 1991; 283: 158-61.

13. Harrison PV, Skerrow D. A comparative study of psoriatic and non-psoriatic buccal mucosa. Br J Dermatol 1982; 106: 637-42.

14. Dumas KJ, Scholtz JR. The psoriasis bio-assay for topical corticosteroid activity. Acta Derm Venereol (Stockh) 1972; 52: 43-8.

15. Weinstein GD, McCullough JL, Eaglestein WH, Golub A, Cornell RC, Stoughton RB, Clendenning W, Zackheim H, Maibach H, Kulp KR, King L, Baden HP, Tayler JS, Deneau DD. A clinical screening program for topical chemotherapeutic drugs in psoriasis. Arch Dermatol 1981; 117: 388-93.

16. Wolff HH, Kreusch JF, Wilhelm KP, Klaus S: The psoriasis plaque test and topical corticosteroids: Evaluation by computerized laser profilometry. In: Korting HC, Maibach HI, ed. Topical glucocorticoids with increased benefit/risk ratio. Basel, Karger 1993; 21: 107-13.

17. Marks J, Rogers S, Chadrik B, Shuster S. Clearance of chronic plaque psoriasis by anthralin - subjective and objective assessment and comparison with photochemotherapy. Br J Dermatol 1981; 105: 96-9.

18. Marks R, Barton SP. Assessment of disease progress in psoriasis. Arch Dermatol 1989; 125: 235-40.

19. Savolainen L, Kontinen J, Alatalo E, Röning J, Oikarinen A. Comparison of actual psoriasis surface area and the psoriasis area and severity index by the human eye and machine vision methods in following the treatment of psoriasis. Acta Derm Venereol (Stockh) 1998; 78: 466-7.

20. Mrowietz U, Graeber M, Bräutigam M, Thurston M, Wagenaar A, Weidinger G, Christophers E. The novel ascomycin derivative SDZ ASM 981 is an effective anti-psoriatic compound when used topically under occlusion. Br J Dermatol 1998; 139: 992-6.

21. Wozel G. Effektivität der Kombinationsbehandlung mit Calcipotriol und Fluocinolonacetonid im Psoriasis-Plaque-Test. Akt Dermatol 1994; 20: 155-8.

22. Wozel G, Barth J. Vergleichende Untersuchungen über die Wirksamkeit topischer Steroide bei Psoriasis. Dermatol. Monatsschr 1986;172:550-3.

23. Nieboer C, Bruynzeel DP, Boorsma DM. The effect of occlusion of the skin with transdermal therapeutic system on Langerhans cells and the induction of skin irritation. Arch Dermatol 1987; 123: 1499-1502.

24. Berardesca E, Maibach HI. Skin occlusion: Treatment or drug-like device? Skin Pharmacol 1988; 1: 207-15.

25. Lindberg M, Johannesson A, Forslind B. The effect of occlusive treatment on human skin. An electron microscopic study on epidermal morphology as affected by occlusion and dansyl chloride. Acta Derm Venereol (Stockh) 1982; 62: 1-5.

26. Behrendt H, Korting HC. Klinische Prüfung von erwünschten und unerwünschten topisch applizierbarer Glucocorticoide am Menschen. Hautarzt 1990; 41: 2-8.

27. Wozel G, Barth J. Fluocinolonacetonid und Psoriasis - kontinuierliche oder alternierende Therapie?. Dermatol Monatsschr 1986; 172: 620-3.

28. Wozel G, Barth J. Untersuchungen zur Wirksamkeit von topischer Corticosteroidkombination bei Psoriasis. Z Klin Med 1989; 44: 2279-81.

29. Christophers E, Mrowietz U. The inflammatory infiltrate in psoriasis. Clin Dermatol 1995; 13: 131-5.

30. Wozel G. Einschätzung von Calcipotriol (MC 903) im Psoriasis-Plaque-Test. Dermatol Monatsschr 1993; 179: 209-11.

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