Abstracts

Symposium

„Development and Regulatory Aspects of Topical Dermatics Today“

BfArM

Session 1 and 2

1: Dermatics – a world of its own

2: Identification and production of active pharmaceutical inredients

Symposium of GD in cooperation with BfArM

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Session 1: Dermatics – a world of its own

Which dermatics do we have?

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The most important milestone in the development of modern dermatics is the introduction of topical glucocorticoids which divides the history of derm-pharmacology in before C and after C. The study of the pharmacology of these compounds provided important observations which about the general pharmacology of dermatics. Dermatics exist for most dermatological indications and their application is limited by side effects and by the influence of the skin barrier. Advantages of dermatics can be that their side effects are less severe after topical application compared to systemic application. This includes even carcinogenic effects. New insights in the function of the skin barrier on the molecular level do not only include their relationship to some diseases such as the atopic dermatitis or the atopic march by e.g. filaggrin but also transporter molecules which influence different activities of drugs whether they are applied topically or systemically.

Session 2: Identification and production of active pharmaceutical ingredients

The regulatory framework for preclinical research

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In modern drug regulatory the common target in mind is a high safety potential of market drugs. This is the target of the regulatory authorities and the pharmaceutical entrepreneurs.

The progress in scientific disciplines needs an increasing number of requirements/criteria in the assessment of drug safety and drug quality to ensure a good risk/benefit ratio for patients. Nevertheless, one target of each drug development by a pharmaceutical entrepreneur is to be successful with an approval for the marketing of the developed drug within the planed time scheme.

Guidelines for the assessment criteria of the regulatory authorities can be a good guidance on the pathway from the idea to a marketing authorization for minimizing economic risk by a rational approach to development activities.

Session 2: Identification and production of active pharmaceutical ingredients

Target identification: general aspects and sphingosine-1-phosphate as an example

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Despite the increasing emphasis on proteomics in target identification, DNA microarray technology is still a powerful technique for identifying genes involved in susceptibility to diseases. Thus, differentially expressed genes may lead to identify genes that play key roles in disease pathways. As an example, up-regulation of sphingosine 1-phosphate phosphatase (SPP) was detected in samples of lesional skin of patients with psoriasis, whereas an up-regulation of sphingosine 1-phosphate lyase (SPL) was detected in patient with atopic dermatitis. It is of interest that both enzymes may lead to a degradation of the lipid mediator sphingosine 1-phosphate (S1P) suggesting that a decreased level of S1P is related in the biology of these diseases. Nevertheless, mRNA does not always correlate with production of protein, and even if more protein is produced it may not be active because it requires post-translational modification or relocalization. Therefore it is of interest to measure also levels of S1P in such patients. Indeed, in correlation with an enhancement of mRNA levels of S1P-degradadation enzymes, S1P levels are significantly decreased in patients with atopic dermatitis. One of the biggest challenges in drug target identification is to understand the underlying physiology of drug targets. The relevance of S1P in cellular processes has been emphasized by identifying its function as a ligand on a family of G-protein coupled receptors (GPCRs) termed as S1P1-5.. S1P exerts diverse cellular effects depending on the expression of the specific S1P receptor subtypes and their coupling to separate G proteins. But the role of S1P in skin cells has not been examined very well. Therefore, a characterization of the expression profile of S1P receptors in skin cells has been performed. Indeed, all investigated cell types including keratinocytes, fibroblasts and dendritic cells show a significant mRNA expression of all five S1P receptor subtypes. Moreover, the complex biology of S1P in skin cells has been explored in an efficient manner indicating that S1P is a crucial molecule in the homeostasis of both keratinocytes and dendritic cells. Thus, S1P has been identified as a bioactive molecule in keratinocytes as it inhibits their growth and initiates differentiation. Further experiments by downregulation of S1P receptors and the use of agonists/antagonists clearly indicate that the S1P2 receptor is dominantly involved in the S1P-induced keratinocyte growth arrest. Dendritic cells play a pivotal role in inflammation as they carry haptens from the skin through afferent lymphatic vessels to draining lymph nodes, where these haptens are presented to T cells. Our results indicated that S1P inhibits the function of dendritic cells leading to a decreased immune response. It is of interest that the S1P1 receptor subtype is essential to mediate this action. Thus,

it seems likely that S1P is an appropriate candidate for the treatment of hyperproliferative and inflammatory skin diseases. To prove this hypothesis, animal models of psoriasis and contact dermatitis were performed. Indeed both models indicate that S1P may beneficial in the treatment of inflammatory skin diseases.

Session 2: Identification and production of active pharmaceutical ingredients

Lead optimization: general aspects and MAP kinase inhibitors for psoriasis as an example

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Psoriasis is an immune-mediated inflammatory skin disorder characterized by skin-infiltrating lymphocytes causing hyperproliferation and abnormal differentiation of the keratinocytes. Dependent on the severity of the disease, in addition to phototherapy, several treatments including topically and orally administered small molecules or parenterally administered biological drugs are available. Cytokines, in particular TNF, play a pivotal role during onset and maintenance of the disease, which explains the clinical success of anti-TNF-therapies. The source of TNF in psoriasis is unclear but T-cells, keratinocytes and dendritic cells are involved. Analysis of psoriatic skin has shown, that the activity levels of ERK1/2 and p38 MAP kinase, as well as MAPKAP-2, a protein kinase which is activated by p38 MAP kinase, are increased. Results of immunohistochemical analysis of psoriatic skin and in vitro experiments with keratinocytes indicate an important role of the p38 MAP kinase/MAPKAP-2-signaling pathway in the generation of TNF. Therefore, modulation of this pathway by small molecules represents an attractive approach for development of new anti-psoriatic therapies. However, in other autoimmune diseases, such as rheumatoid arthritis and Crohn's disease, p38 MAP kinase inhibitors failed to demonstrate clinical efficacy. The reasons for the disappointing outcome are not clear. Recent comments on published clinical studies suggest the induction of alternative, p38 MAP kinase circumventing signalling pathways as a potential reason for the disappointing clinical results obtained with p38 MAP kinase inhibitors. Another explanation points to potential drug-induced systemic side effects, particularly in the liver, which may induce pro-inflammatory signalling pathways thereby neutralizing the beneficial anti-inflammatory effects of p38 MAP kinase inhibition. However, data which support these hypotheses are not available.

In this presentation, the discovery of p38 MAP kinase will be reviewed with a focus on the potential use of drug candidates for treatment of inflammatory skin diseases. The different requirements regarding selectivity profile, metabolic stability and physico-chemical properties of clinical candidates which are to be developed either for systemic or topical treatment of psoriasis will be discussed.

Session 2: Identification and production of active pharmaceutical ingredients

Target identification and lead optimization: fungal secreted aspartic proteinase inhibitors as a paradigm

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Candida albicans (*C. albicans*), the most virulent representative of the *Candida spp.*, can cause severe mucosal and life-threatening systemic infections in immuno-compromised hosts. In particular, the secreted aspartic proteinases (Saps), encoded by the SAP gene family with ten members, appear to play a major role in *Candida albicans* virulence. It has been shown that SAP1-3 contribute significantly to tissue damage and invasion of oral epithelium and cutaneous epidermis, while SAP4-6 are important for systemic infections. In contrast to the usual mode of action of antifungal drugs, to interfere with fungal cell wall production, our group identified a major virulence factor, namely the Saps as a prospective target for intelligent drug design.

By x-ray crystallography we solved the crystal structures of Sap1, Sap3 and as first member of the second subgroup Sap5. On this basis we performed molecular modelling studies of possible Sap-inhibitors with special regard to size and structure also adressing electric charges of the active centre of the different Sap's. We took special care to identify regions of similarity to the human aspartic proteinases to prevent cross-reactivity. 9 Inhibitors belonging to 3 inhibitor libraries were obtained by production of statine and usage of the protecting group strategy. These 9 inhibitors were tested in a fluorescence assay to evaluate their inhibitory potential regarding the different Saps. Keeping the results in mind additional new inhibitors were synthesized and tested.

The newly-synthesized inhibitors inhibit the tested Saps, as pepstatin A, in nanomolar range.

Session 2: Identification and production of active pharmaceutical ingredients

Production of active pharmaceutical ingredients: general aspects and sphingosine-1-phosphate as an example

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In this talk I like to give an introduction to a production process of sphingosine-1-phosphate with a view on the regulatory aspects related to it. Chemical processes are usually originated in a small scale in the laboratory. In many cases the first synthesis was developed by academic groups to test the product for pharmaceutical or biological mode of action. These groups care about the finding of new drugs, discovery of new biological interaction or just new ways of synthesising complex chemical structures.

Starting at the moment of the finding a new potential drug, the need of a sufficient and economic process for synthesis is raised.

The development is normally carried out by conducting the reaction steps successively larger before transferring to full size production for commercialization.

While the importance of drug impurity profiles has been recognized since the beginning of pharmaceutical drug therapy, regulatory considerations of stereochemistry has really only come to the force in the last decades. Increasing regulatory attention to stereoisomeric drugs is due to both technological advances in stereoselective analytical methods and the increased commercial practicality of stereoselective synthesis.