Pharmacology Discovery Services

A Eurofins Discovery Partner Lab

White Paper

Atopic Dermatitis - Selecting an Appropriate *In Vivo* Model to Evaluate Drug Candidates

Atopic Dermatitis

Atopic dermatitis (AD), also called atopic eczema, is a chronic inflammatory skin disorder characterized by cutaneous hyper-reactivity to environmental triggers and dry itchy skin and it is estimated to afflict up to 5% of adults in industrialized nations and nearly 15% of children in the United States.[1,2] While the pathogenesis of AD is not entirely understood, it is believed to involve genetics, environmental factors (such as mite antigens), skin barrier defects and immune system dysregulation.[3,4] Two forms of AD, extrinsic and intrinsic, are defined. The majority of clinical cases (70-80%) are extrinsic AD with a heavily Th2/IgE mediated immune response.[5] In mild-to-moderate cases of AD topical corticosteroid or calcineurin inhibitors like tacrolimus or phototherapy are often an effective means of disease management. For severe cases of AD where topical and phototherapy have failed the use of immunomodulatory therapies are the next line of treatment. U.S. FDA approval in 2017 for Dupilumab that targets IL-4Rα and thus inhibits both IL-4 and IL-13 signaling pathways demonstrates the clinical utility of immunomodulatory therapeutics for AD. In addition, there are hundreds of active AD clinical trials in the United States with a variety of immunomodulatory candidates such as mAb against IL-31R and JAK inhibitors (ClinicalTrials.gov).[6]

> Acute atopic dermatitis is mainly a Th2-dominant inflammatory response. The 2017 approval of Dupilumab demonstrates the clinical utility of immunomodulatory therapeutics.

Epicutaneous exposure to and sensitization with allergens are viewed as the first steps in initiating AD. Skin dendritic cells loaded with antigens migrate to the draining lymph nodes where they prime and activate T cells to promote a cascade of inflammatory responses. Immunologically, acute AD is mainly Th2-dominant inflammation characterized by dermal infiltration of $CD4^+$ T cells and eosinophils with increased skin expression of Th2 cytokines and increased serum IgE.[4,7]

In Vivo Models of Atopic Dermatitis

During drug discovery the *in vivo* efficacy of AD drug candidates may be assessed in a variety of animal models. AD models can be categorized into three groups: sensitizer (e.g. haptens or allergens)-induced, transgenic or knockout animals and spontaneously developed AD.[8] With respect to an initial *in vivo* evaluation of drug candidates, AD that is induced with sensitizers provides the best, and most reliable approach. Transgenic/knockout mice are an excellent tool to elucidate immune mechanisms but are not representative of the disease in the clinic. Spontaneous murine models (e.g. NC/Nga strain[9]) do mimic human AD reasonably well but as a spontaneously developed disease model it may be more appropriate for mechanistic and pathophysiological studies rather than pharmacodynamic ones. Its relatively low incidence and different onset among test animals do make the model challenging to use.

Pharmacology Discovery Services (PDS) has two validated models of AD that use sensitizers to induce the disease state, one being the hapten 2,4-dinitrochlorobenzane (DNCB) and the other being allergan from the house dust mite (HDM) *Dermatophagoides farinae*. Both models use the NC/Nga mouse, an inbred mouse strain that exhibits a high Th2 and IgE immune response (NC/Nga was the first mouse strain used to develop a model for AD).[9] Thus, we have taken advantage of a mouse strain that is genetically prone to the development of AD as well as using either a hapten or an allergen to induce synchronized disease.

Data for the DNCB (Item #555700) and HDM (Item #555710) models from PDS are provided in this white paper. With each model multiple readouts are available to assess the *in vivo* efficacy of AD drug candidates and they include: scratching, macroscopic lesions, ear thickness and a total clinical skin severity score. Other immunological assays also available from PDS include Th1/Th2/Th17 cytokine analysis in blood and/or tissue, flow cytometry, complete blood count (CBC) and histopathology etc. Both prophylactic and therapeutic dosing regimen can be implemented in these models to evaluate pharmacological effects of either small molecule or biological drug candidates.

2,4-Dinitrochlorobenzene (DNCB)-Induced AD Model

Haptens such as DNCB, trinitrochlorobenzene (TNCB) or oxazolone, when given twice at 5-7 days apart, typically induces allergic contact dermatitis (note: oxazolone can induced colitis as well, please refer to our white paper on IBD) which primarily involves Th1 dominated responses. However, repeated challenges with these haptens over a period of time induce skin inflammation with a shift from a typical Th1 dominated delayed type hypersensitivity response to a Th2 dominated inflammatory response that is similar to human AD.[10,11] NC/Nga mice, after repeated exposure to DNCB in the dorsal skin, develop scratching behavior, followed by the rapid development of AD-like skin lesions such as erythema, lichenification with edema, hemorrhage, erosion (excoriation), and scaling (dryness) over a period of several weeks. Histological examinations have revealed hyperplasia with hyperkeratosis, intercellular edema and accumulation of eosinophils and mast cells in skin lesions. In addition to these skin changes, NC/Nga mice challenged with DNCB exhibit elevated levels of serum IL-4, TNFα and total serum IgE as well as skin levels of IL-4 and IL-13.[12] Thus DNCB induced skin inflammation model is a good model for AD.

Procedure Summary

Female 8 to 10-week-old NC/Nga mice are randomly divided into groups of eight each and the dorsal skin and ears are sensitized with 200 µl of a 1% DNCB on Day 4 followed by challenges with 150 µl of 0.2 % DNCB every three days (Days 7, 10, 13). Test compounds and vehicle are administered daily by gavage starting on Day 1 through Day 14. Scratching assay is performed every 3 days (Days 2, 5, 8, 11 and 14). The experiment is terminated on Day 15. Macroscopic lesions are scored and ear thickness is measured. The severity of skin lesions on the face, ears, neck, and dorsal skin is evaluated. The total clinical skin severity score is defined as the sum of the individual scores: 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe.

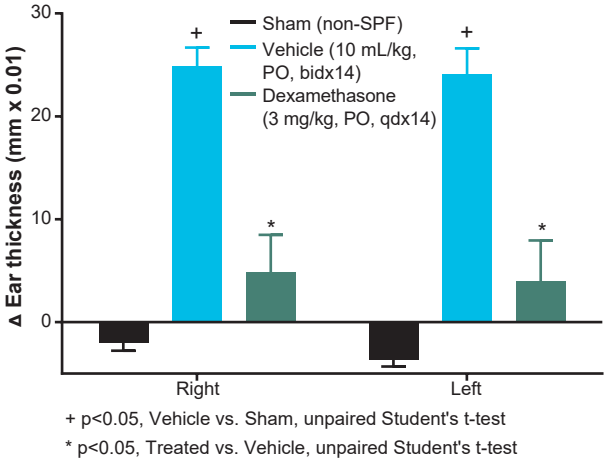

Figure 2. Ear Thickness Measurement in the DNCB model.

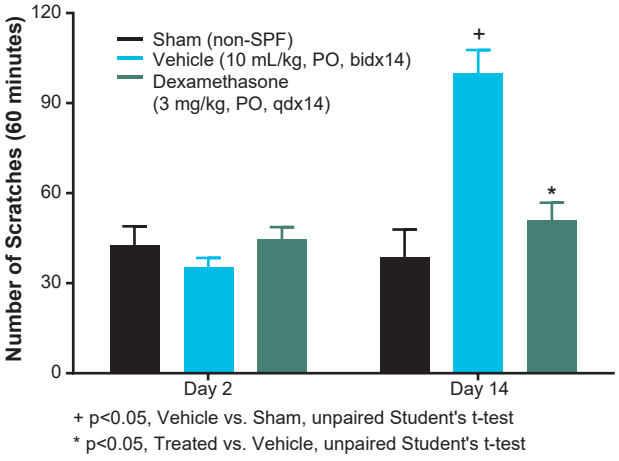

Figure 1. Scratching Endpoint in the DNCB model.

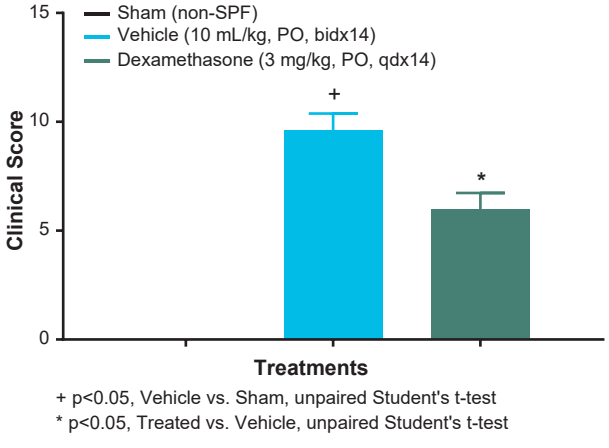

Figure 3. Clinical Score in the DNCB model.

House Dust Mite (HDM)-Induced AD Model

Mite allergen, extracted from *Dermatophagoides farinae*, has been shown to induce stable clinical AD-like skin diseases in NC/Nga mice under SPF conditions as well as in other strains of mice. Skin changes in NC/Nga mice are evidenced by scratching behavior, followed by the rapid development of erythema, lichenification with edema, excoriation and scaling. Histological/immunohistochemical examinations have revealed epidermal hyperplasia with hyperkeratosis and accumulation of $CD4^+$ T cells, eosinophils and degranulated mast cells in skin lesions. In addition, NC/Nga mice challenged with mite antigens also exhibit elevated levels of total plasma IgE. Immunologically, lymph node cells from mice immunized with mite Ag secret Th2 cytokines such as IL-4, IL-5 but not IFNγ.[13,14] Thus mite antigen challenged NC/Nga mice show clinical, immunological and histopathological aspects similar to patients with AD. This suggests that this model is excellent for studying the pathogenesis of human AD and pharmacological effects of drug candidates.

Procedure Summary

Female 8 to 10-week-old NC/Nga mice are randomly divided into groups of eight each. The Biostir®-AD (100 mg/mouse; mite dust, from *Dermatophagoides farinae*) is applied twice a week for three weeks (Days 1, 4, 8, 11, 15, and 18), a total of 600 mg Biostir® per mouse. Test compounds and vehicle are administered daily by gavage starting on Day 1 through Day 21. The dosing regimen may change per client's requests. Scratching assay, the severity of skin lesions, and ear thickness are performed weekly on Days 0 (baseline), 7, 14, and 21. Severity of skin lesions is characterized by the presence of erythema/hemorrhage, edema, excoriation/erosion, and dryness/scaling. The total clinical skin severity score is defined as the sum of the individual scores: 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe.

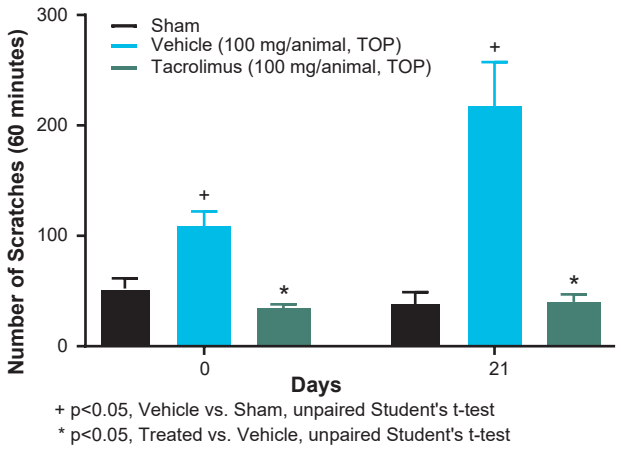

Figure 4. Scratching Endpoint in the HDM model.

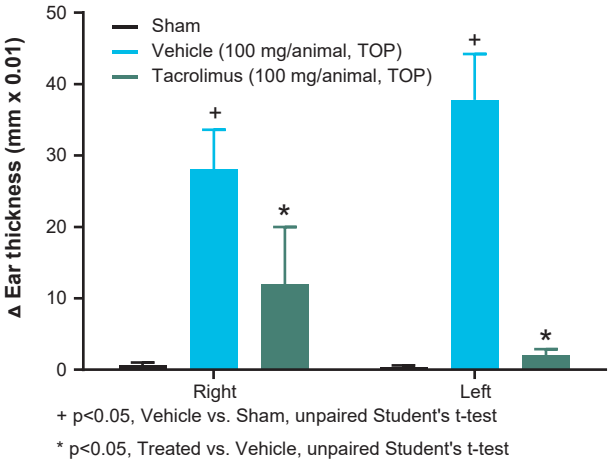

Figure 5. Ear Thickness Measurement in the HDM model.

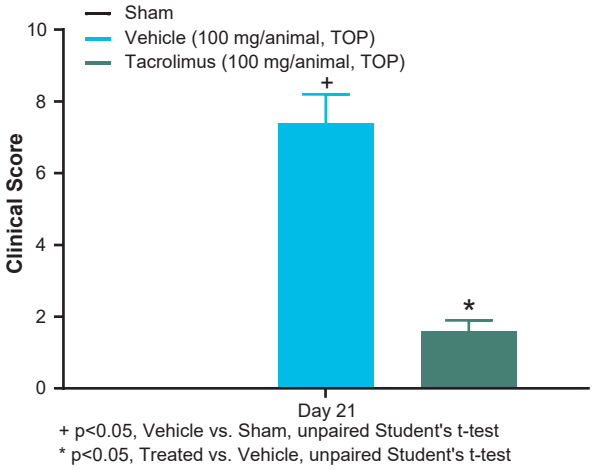

Figure 6. Clinical Score in the HDM model.

Summary

Both DNCB and HDM induce synchronized AD-like skin lesions and scratching in NC/Nga mice that are shown to spontaneously develop AD if housed under conventional conditions. The models have similar clinical, immunological and histological changes (e.g. both are more Th2 mediated responses at least in the acute stage) and have high serum IgE levels. The DNCB model, because of its reproducibility, relatively short duration and relatively low cost, is widely used for evaluating mechanisms of pathogenesis and drug candidates for AD. HDM is a major source of indoor aeroallergen, which induces allergic diseases including asthma and AD. There are reports that AD patients are hyperallergic to allergens, particularly HDM, a majority of AD patients are positive for IgE against mite Ag and clinical symptoms of AD improve after mites are removed or controlled.[15,16,17,18] Thus in our opinion, HDM model is more clinically relevant in this regard. When choosing the right AD model, one has to consider other factors such as reproducibility, test article formulation and route of administration (topical vs. systemic), and a test article's mechanism of action. In the study design phase Technical Directors from PDS are able to consult with clients to determine if the DNCB or HDM model is most appropriate to meet their study goals and objectives.

There are *in vitro* systems available (e.g. HaCat cells a human immortalized keratinocyte cell line) to model AD for studying its cellular and molecular mechanisms in response to allergens such as dust mites and DNCB.[19] Eurofins Discovery, a partner lab of PDS, provides an *in vitro* HaCat assay (Item# 307650-0) that may be used to evaluate test articles against AD.

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