COMPARATIVE BIOSCIENCES, INC.
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH
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Delayed Type Hypersensitivity: Ear Thickness Model in Mice

J. Christensen • Robert Sukhu • Qin Zhang • Isaac Hakim • Carol Meschter
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Overview

Hypersensitivity reactions are classified into four types (Gell and Coombs, 1963). Type IV or delayed type hypersensitivity (DTH) is T-cell-mediated and its normal function is to mediate the immune response to intracellular pathogens. Pathologically, DTH is manifested clinically as irritant dermatitis, allergic contact dermatitis, or atopic dermatitis, among others. As a T-cell-driven model, it may also be considered a screening model for such diseases as multiple sclerosis. DTH models utilize "haptens" to induce the cell-mediated response. Haptens are low molecular weight chemicals that are non-antigenic themselves, but may cause sensitization when linked to a protein or other large molecule. In the case of DTH models, the haptens are typically alkylating agents that modify cell-surface proteins to mimic the presentation of intracellular antigens to CD4+ T-cells by MHC Type 1 or 2 MHC proteins at the cell surface. The pathophysiology has two distinct phases: sensitization and elicitation. The sensitization phase, (or the afferent or inductive phase) occurs as contact of the hapten with epidermal-specific proteins leads to the generation of antigen-specific T-cells.

In the Mouse Ear Thickness Model, a variety of haptenizing reagents are commonly used. These include oxazolone, FTNB, CTNB, FDNB, CDNB and other structurally-related haptenizing reagents. The different haptens are associated with some differences in T-cell and cytokine expression, but the methodology for use and assessment of activity is essentially the same.

Basically, in the mouse ear thickness test, BALB/c mice are exposed to topical hapten (generally presented in olive oil and ethanol) on two occasions at intervals of about two weeks. The inducer is applied to shaved abdominal skin or the foot pads. About a week later, there is elicitation by application to the ears. The edema due to the T-cell response can be readily measured as an increase in ear thickness. Drug treatment is administered at this time, and the ear thickness is measured using calipers hours to days later. Steroids such as dexamethasone are standard controls.

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Historical Aspects

CBI has performed this methodology for over 10 years both using mouse ears and guinea pig back skin. CBI has used a variety of sponsor-requested haptens including. oxazolone, FTNB, CTNB, FDNB, CDNB. CBI has standardized the concentration of the hapten used for induction and elicitation phase as well as the dose of dexamethasone used for the positive control. CBI has worked with sponsor test articles that were administered either systemically or topically. The technical staff is trained in all phases of this methodology including induction, elicitation, dosing, measurement, and scoring.

CBI Study Directors work closely with the sponsor to take into consideration the various scientific aspects needed to assure a successful Ear Thickness Study, including the mechanism of action of the test article, selecting the proper positive and negative controls, assessment of the vehicle, and appropriate hapten.

Study Design

The following is a basic protocol in the ear thickness model. The protocol may be customized to the sponsor's requirements.

Group	n	Sensitization with FDNB	Challenge DNB	Treatment
1	8	+	+	None
2	8	+	+	Vehicle
3	8	+	+	Test article
4	8	+	+	Test article
5	8	+	+	Test article
7	8	+	+	Positive control*

*Positive control as relevant for the individual study. Dexamethasone, SC is commonly used.

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Objective

The purpose of the study is to assess the activity of Sponsor Test Articles in a Delayed Type Hypersensitivity model in mice by measuring ear thickness.

Animals

BALB/c mice, adult female 6-8 wk old, 8/group (designed to identify test articles with ¼ the potency of dexamethasone with 90% power)

Reagents

Sensitizing reagent: 0.5 % FDNB, designated hapten in vehicle (acetone: olive oil 4:1)

Challenge reagent: 0.5 % DNB or designated hapten in vehicle (acetone: olive oil 4:1)

- Test article: Sponsor's choice.
May be topical or systemic

- Positive Control: Dexamethasone, 2 mg/kg SC twice daily for up to 4 days, or sponsor's choice

Sensitization and Challenge Procedure

Day - 21: Sensitization:
Groups of mice are sensitized by a two paintings with 25 µl FDNB on the pre-shaved abdomen and 5 µl on each footpad. The application site is carefully shaved the day before application to remove hair, but not traumatize the skin surface. Sensitization occurs on Day-21 and Day-4.

Day 0: Challenge (5 days following the final sensitization): After ear thickness is measured, 10 µl Challenge FDNB is applied to both side of each ear for each animal.

Test Article Administration

Test article is administered as per the sponsor's requirements. CBI is experienced in all routes of administration.

Assessment

Ear thickness is measured pre-challenge and at various timepoints thereafter. The response to the nitrobenzene haptens is somewhat stronger and more prolonged; measurements are usually performed daily for 4 to 6 days. Ears are measured using a constant-pressure micrometer (example: Mitutoyo). Measurements are conducted by the same trained biologist throughout the study. Results may be expressed as raw data or change in ear thickness from baseline. Typical results are shown in Figures 1 and 2.

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Histopathology

Histopathology and immunohistochemistry may be added upon request.

Other Parameters

Blood and lymph node collection may be added upon request. Other parameters may be added upon request.

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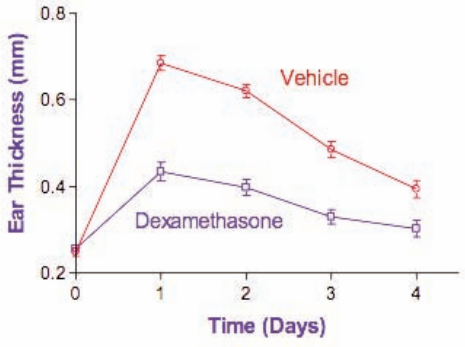
Report

A complete report with data tables, graphs, and statistics is prepared.

Results



Fib 1. Results of an FDNB experiment.

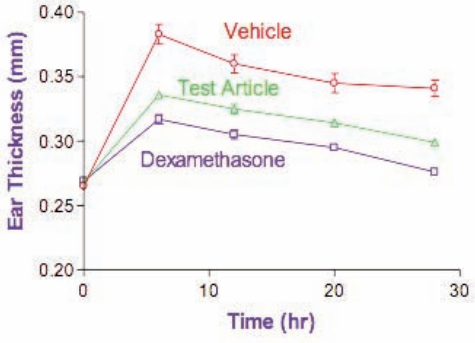


Fig 2. Results of an Oxazolone experiment

References

Gell PGH, Coombs RRA, eds. Clinical Aspects of Immunology. 1st ed. Oxford, England: Blackwell; 1963