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IN FOCUS
ITR Newsletter

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Immunologic Sensitization of Guinea Pigs via Inhalation

The potential of a new drug to adversely affect the immune system is usually determined during routine nonclinical toxicity evaluations. However, immunotoxicologic findings from such routine evaluations may warrant the need for more detailed assessments of immune function effects such as immunosupression, immunogenicity, hypersensitivity, autoimmunity or adverse immunostimulation. Many methods have been developed to determine the potential of a drug administered via the inhalation route to induce a hypersensitivity reaction. Such a model was recently validated at ITR Canada for the evaluation of respiratory hypersensitivity using guinea pigs to comply with the FDA Guidance for Industry entitled "Immunotoxicology Evaluation for Investigational New Drugs", published in October 2002.

Study Design:

Groups of 10 male guinea pigs were exposed by nose-only inhalation to control (saline) and positive control (ovalbumin) for 5 consecutive days, followed by a 17 day latency period and a single challenge inhalation exposure. The positive control substance (ovalbumin) is known to elicit an allergic response following inhalation exposure in guinea pigs.

The study design, including how a test article would be incorporated, is presented in the table below:

Group No.	Phase					
	Sensitization			Challenge		
	Saline	Ovalbumin	Test Article	Saline	Ovalbumin	Test Article
1	+			+		
2	+				+	
3	+					+
4		+		+		
5		+			+	
6			+	+		
7			+			+

During all phases of the study clinical observations and body weights were performed on all animals. Respiratory parameters (respiratory rate, tidal volume and derived minute volume) were obtained from all animals during inhalation exposures on the first and last day of the sensitization phase and during the exposure of the challenge phase. In order to confirm the positive control animals were adequately sensitized prior to the inhalation challenge, these animals were intradermally administered ovalbumin to elicit a local hypersensitive reaction determined by Draize scoring. Twenty-four hours after the challenge exposure, a bronchoalveolar lavage (BAL) of all the lung lobes was performed on all animals at

necropsy and respiratory tract tissues were retained and examined histologically for changes indicative of immunological responses.

Results:

All animals from all groups tolerated the 5 day sensitization inhalation phase, and the presence of erythema and edema in all positive control animals following intradermal injection confirmed the sensitization phase was successful. Following initiation of the challenge exposure, specific changes related to hypersensitivity were observed in positive control animals, as follows:

Parameter	Finding
Respiratory Function	A statistically significant increase in respiratory rate and decrease in tidal volume was observed during the challenge dosing when compared to the other groups and when compared to pre-exposure values.
Broncho-alveolar lavage	Increases in white blood cell counts, mainly eosinophils and neutrophils, as well as the presence of macrophages.
Histopathology of Respiratory Tissues	Mild to moderate alveolar/peribronchiolar inflammatory cell infiltrate of the lungs associated with minimal to moderate focal alveolar hemorrhage. Mucosal eosinophilic infiltrate in the trachea, carina and larynx.

None of the above changes were noted in the saline control groups.

Conclusion:

The immunologic sensitization of male guinea pigs by inhalation exposure to a known sensitization agent (ovalbumin) for 5 consecutive days, followed by a 17 day latency period and a single challenge inhalation exposure, was successfully achieved. Specific changes related to hypersensitivity were observed in respiratory function measurements, broncho-alveolar lavage constituents and inflammatory cell infiltrates determined histologically in respiratory tissues. Saline control groups were unaffected during all phases of the study. The above validated model is suitable for the evaluation of respiratory hypersensitivity to comply with the FDA Guidance for Industry entitled “Immunotoxicology Evaluation for Investigational New Drugs”.

OTHER NEWS:

ITR Canada will be present at:

- **ANNUAL BIOTECH SYMPOSIUM:** September 14-16 in La Jolla, California
- **SAFETY PHAMACOLOGY SOCIETY 9th ANNUAL MEETING:** September 15–18 in Strasbourg, France.
- **ASSOCIATION OF INHALATION TOXICOLOGISTS 2009 ANNUAL MEETING:** October 14-16 in Chester, United Kingdom