Abstract
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Comparison of Endotoxin-Induced Uveitis Model and Experimental Autoimmune Uveitis Model in Lewis Rats for Drug Screening

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ABSTRACT

Purpose: The study is to compare Endotoxin-Induced Uveitis (EIU) Model and Experimental Autoimmune Uveitis (EAU) Model in Lewis Rats in order to benefit how to choose appropriate models for anti-inflammatory drug screening.

Methods: The EIU model was induced by endotoxin named lipopolysaccharides (LPS) through footpad injection on Day 0 in 49 Lewis rats included five groups: negative control of saline (Saline); positive control of dexamethasone + low dose lipopolysaccharides (Dex + LLPS); positive control of dexamethasone + high dose lipopolysaccharides (Dex+ HLPS); low dose lipopolysaccharides (LLPS); and high dose lipopolysaccharides (HLPS). The EAU model induced by a subcutaneous injection of peptide R16 of bovine interphotoreceptor Retinoid Binding Protein (IRBP)/Mycobacterium tuberculosis H37Ra on Day 1 in 30 Lewis rats included three groups: negative control of saline (Saline); positive control of dexamethasone + IRBP (Dex + IRBP) and IRBP. Animals were sacrificed on Day 2 or 3 (EIU), and on 21 or 28 (EAU) for histopathological or cytokine analysis. During studies, animals were observed for clinical observation daily, ophthalmic examinations and body weight daily (EIU) or weekly (EAU).

Results: In EIU, ophthalmic examination showed that both aqueous flare and vitreous body flare in Dex. + LLPS, Dex + HLPS, LLPS and HLPS groups scored significantly higher than the Saline group in a time-dependent manner ($P < 0.05$). Both aqueous flare and vitreous body flare scores in LLPS and HLPS groups were significantly more severe than scores in Dex + LLPS, Dex + HLPS groups ($P < 0.05$). The results demonstrated that LPS induced inflammation in eyes which presented a peak exposure at 48 hours following administration, and Dex. inhibited to a certain extent inflammation. Pathological reports showed that the inflammatory cells in eye tissues in Dex + LLPS, Dex + HLPS, LLPS and HLPS groups were significantly increased when compared to the Saline group in a time-dependent manner ($P < 0.05$). Cytokine analysis reports showed that concentration levels of ICAM-1, IL-6, MCP-1 and TNF-α in retina increased in various extents in Dex + LLPS, Dex + HLPS, LLPS and HLPS groups. In EAU, ophthalmic examination showed signs of inflammation on Day 15, and the peak of inflammation was at Day 21 in IRBP group. The clinical grading of EAU in IRBP group was significant different when compared to that in the Saline or Dex + IRBP groups ($p<0.05$). Pathological reports showed that mainly inflammatory and degenerative changes were detected in the retina of IRBP group. Cytokine analysis reports that concentration levels of IL-17, TNF-α, MCP-1 and IL-6 were not significantly different between groups ($p>0.05$).

Conclusion: The EIU and EAU models were successfully induced and the peak of inflammation was at 48 hours (EIU) or Day 21 (EAU) following administrations. Dex effectively inhabited the inflammation in both models. Both of EIU and EAU models may provide a stable, effective and reliable method for anti-inflammatory drug screening.

METHODS & MATERIALS

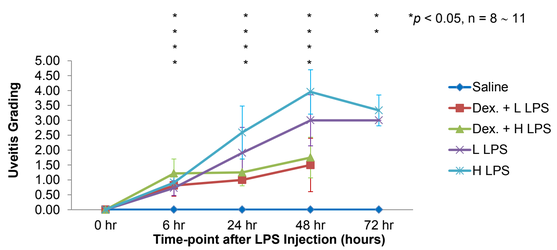
EIU: 49 female Lewis rats, 250 – 350 grams. **Groups:** (1) Saline: balanced salt solution; (2) Dex + LLPS: dexamethasone + low dose lipopolysaccharides; (3) Dex + HLPS: dexamethasone + high dose lipopolysaccharides; (4) LLPS: low dose lipopolysaccharides; (5) HLPS: high dose lipopolysaccharides. **Administration:** Day 0 injection of 200 µL/animal lipopolysaccharides (LPS) or saline (BSS) in footpads. **Treatment:** Day 0 – 2, 10 µL/eye BID Dex (TobraDex: 0.3% tobramycin and 0.1% dexamethason ophthalmic suspension).

EAU: 30 female Lewis rats, 250 – 350 grams. **Groups:** (1) Saline: balanced salt solution; (2) Dex + IRBP: dexamethasone + IRBP; (3) IRBP. **Administration:** Day 1, injection of 200 µL (30ug)/animal of peptide R16 of Bovine Interphotoreceptor Retinoid Binding Protein resides 1177 – 1191, sequence ADGSSWEGVGVVPDV or saline (BSS) subcutaneously at the base of the tail. **Treatment:** Day 12 – 16, 10 µL/eye BID Dex (TobraDex: 0.3% tobramycin and 0.1% dexamethasone ophthalmic suspension)

Scoring: Clinical Grading and Histopathological Grading following Methods in Molecular Medicine, 2010, Vol. 102: Autoimmunity: Methods and Protocols Edited by: A. Perl © Humana Press Inc., Totowa, NJ.

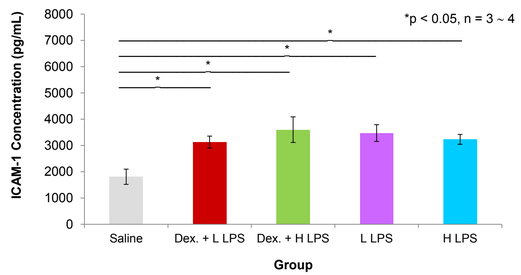
RESULTS

Ophthalmic Examination in the EIU Model

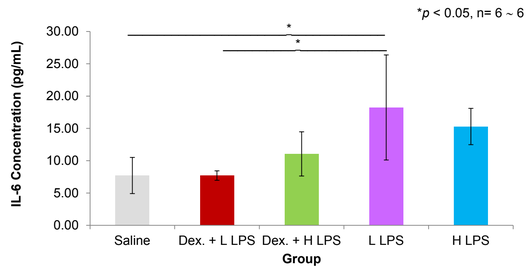


RESULTS (Cont.)

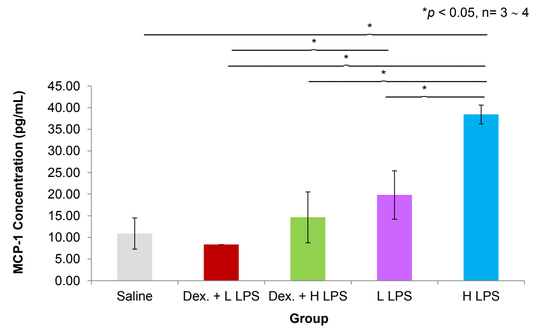
ICAM-1 in Retina-choroid complex in the EIU model



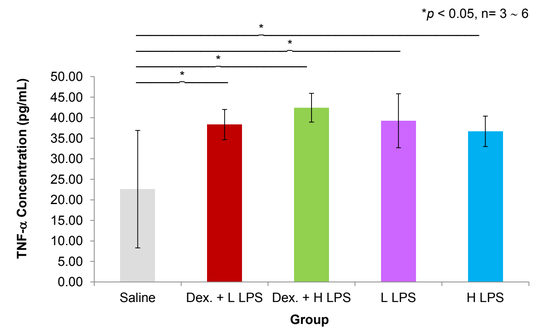
IL-6 in Retina-choroid complex in the EIU model



MCP-1 in Retina-choroid complex in the EIU model

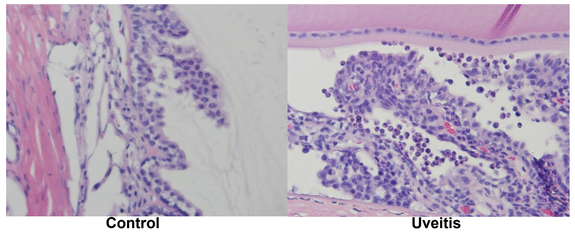


TNF- in Retina-choroid complex in the EIU model

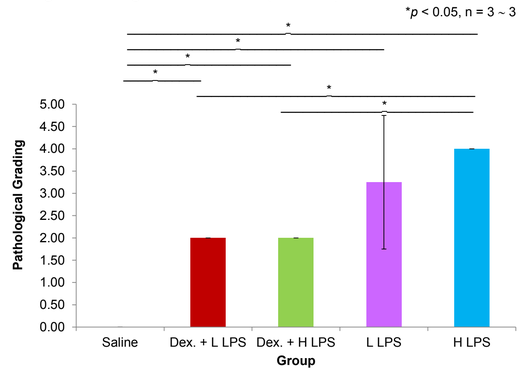


RESULTS (Cont.)

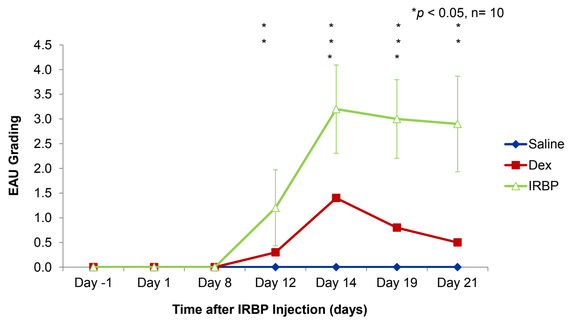
Histopathological Images of Iris-Ciliary Body in the EIU Model



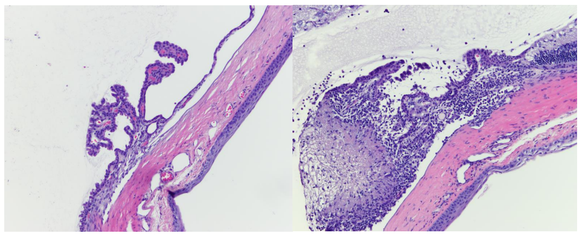
Pathological Grading in the EIU Model (48 hours)



Ophthalmic Examination in the EAU Model

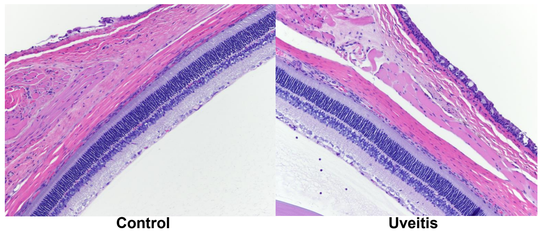


Histopathological Images of Iris-Ciliary Body in the EAU Model

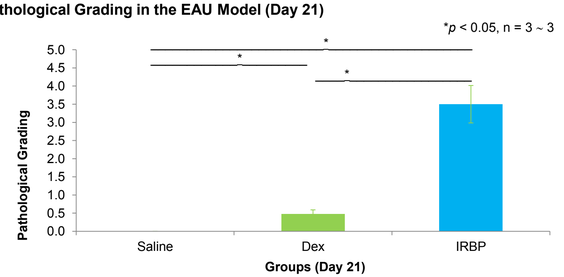


RESULTS (Cont.)

Histopathological Images of Retina in the EAU Model



Pathological Grading in the EAU Model (Day 21)



Comparison of EIU and EAU Models

Descriptions		EIU	EAU
Animal	Strain	Lewis rats	Lewis rats
Administration	Frequency	Once Day 1	Once Day 1
	Route	Footpad	subcutaneous
	Drugs	LPS	IRBP/H37Ra
Inflammation	Starting	6 hours	Day 12
	Peak	48 hours	Days 14-19
	Period	~ 3 days	~ 3 weeks
	location	Anterior Segment: Anterior Chamber, Iris-Ciliary body, Vitreous body	Anterior/Posterior Segments: Anterior Chamber, Iris-Ciliary body, Vitreous body, Retina/Choroid
	Compared to patients	As Secondary Uveitis	As Primary Uveitis
Treatment	Drugs	Dex	Dex
	Route	Topical	Topical Systemic (Optional)
Cytokines in Retina-Choroid Complex	Name	ICAM-1, IL-6, MCP-1, TNF-α, NF-κB.	ICAM-1, IL-6, MCP-1, TNF-α, NF-κB.
	Concentration	ICAM-1, IL-6, MCP-1, TNF-α, ↑	ICAM-1, IL-6, MCP-1, TNF-α, ↑
	Peak Time	24-48 hours	16-21 days
Study Period	Hour/Day	48 – 72 hours	21- 28 days
Selection for Drug Testing	Focus on	Iris-Ciliary body	Retina/Choroid
	Effective Time	Short	Long

SUMMARY

1. The EIU and EAU models were successfully induced and the peak of inflammation was at 48 hours (EIU) or Day 21 (EAU) following administrations.
2. Dexamethasone effectively reduced the inflammation in both models.
3. Both of EIU and EAU models may provide a stable, effective and reliable method for anti-inflammatory drug screening.