

An Improved Immunogenic Uveitis Test in Rabbits for Evaluation of Anti-inflammatory Compounds (34682)

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Immunologic techniques for producing experimental uveitis in rabbits have been reported for a number of years. Numerous antigens, immunizing techniques, methods of challenge, and grading systems have been presented (1-5). Immunogenic uveitis in rabbits has been used successfully as a biological assay for the evaluation of a number of therapeutic agents (6-9). However, these methods were found too lengthy when many potentially active anti-inflammatory compounds are submitted for pharmacologic screening. For our purposes, a test of no more than 2-weeks duration was desired.

In this study, uveitis dose-response data is presented for two serum antigens described in the literature and one serum antigen (normal canine serum), which, to our knowledge has not been previously reported. These sera were chosen because of availability. In addition, the response of several compounds with known anti-inflammatory activity will be reported in this test model.

Materials and Methods. Experimental Animals. Albino rabbits of either sex, 1.25 to 2.25 kg, free from ocular lesions and disease were used throughout this study.

Immunizing antigens. Normal canine serum, bovine anticorynebacterium pasteurella serum (Haver-Lockhart Laboratories, Kansas City, Missouri) and normal equine serum (Professional Biological Company, Denver, Colorado) were used as supplied.

Dose-response techniques. One hundred forty-four test animals were separated into 24 groups of 6 animals each. Twelve groups received 1.0, 2.5, 5.0, or 10.0 ml/kg of an antigen injected subcutaneously into the suprascapular area of the back on day 1. The

immunization procedure was repeated on days 3 and 5.

The remaining groups of animals received injections of antigen intravenously via the marginal ear vein on day 1. Each group received 0.2, 0.3, 0.5, or 1.0 ml/kg of an antigen. As with the subcutaneous dose response groups, intravenous immunization procedure was repeated on days 3 and 5.

On day 8 the right eyes of all test animals were anesthetized by topical application of two drops (0.1 ml) of tetracaine hydrochloride 0.5% (Alcon Laboratories, Inc., Fort Worth, Texas). The anesthetized eyes were then carefully proptosed and 0.05 ml of the same immunizing antigen was injected into the vitreous chamber using a 25-gauge needle. Puncture of external ocular musculature and visible blood vessels was avoided. Left eyes of all groups remained unchallenged and served as negative controls.

Uveitis grading. All eyes were observed biomicroscopically (Carl Zeiss slit lamp, Model 100/16) before challenge and at 24-hr intervals after challenge until day 12. The following arbitrary system was used for grading the uveitis: Grade 0 = no response; Grade 1 = slight congestion of iris vessels (usually only part of iris affected); Grade 2 = mild congestion of iris vessels; Grade 3 = moderate congestion of iris vessels, mild iris edema; and Grade 4 = marked congestion and dilation of iris vessels, moderate iris edema.

Anti-inflammatory compound testing. Results of the preliminary dose-response study were utilized to determine the antigen to be used, the dose of antigen and the method of administration of antigen which together produced optimal uveitis. Animals immunized by this method were utilized for testing steroid

and nonsteroid anti-inflammatory compounds. On day 8, animals were separated into groups of six, and one drop (0.05 ml) of the test solution or suspension was instilled topically into the conjunctival sac of right eyes only. One group was not treated and served as a uveitis control. Treatment was continued at hourly intervals throughout the day for a total of 10 treatments/day. After the fifth treatment on day 8, right eyes of all animals were challenged intravitreally with 0.05 ml of the immunizing antigen. Treatment was continued on a 10 times/day schedule on experimental days 9 through 11, and until biomicroscopic examination on day 12. All eyes were examined biomicroscopically, as in the dose-response study, with treatments and observations made under blind conditions.

Compounds tested by this method were as follows: dexamethasone alcohol and dexamethasone 21-phosphate at concentrations of 0.1, 0.01, and 0.001% dissolved or suspended in 0.5% hydroxypropyl methylcellulose; indomethacin (Merck Sharp & Dohme, West Point, Pennsylvania) at a concentration of 0.5% in 0.5% hydroxypropyl methylcellulose; prednisolone acetate at a concentration of 0.25% in hydroxypropyl methylcellulose; and phenylephrine hydrochloride at a concentra-

tion of 5.0% in 0.85% saline.

Results. Mean uveitis grades for the groups immunized subcutaneously and intravenously are presented in Tables I and II. An inflammatory response was observed at all levels of immunization for each serum antigen. The unchallenged eyes (negative controls) remained "normal." The most severe uveitis responses were observed in the groups immunized subcutaneously with canine serum, 2.5 and 5.0 ml/kg (Fig. 1).

These data were used to select an antigen, antigen level, and regimen of administration of antigen for use in testing of anti-inflammatory compounds. Canine serum at 2.5 and 5.0 ml/kg, administered subcutaneously, produced the highest scores for uveitis. A dose level of 2.5 ml/kg was chosen instead of 5.0 ml/kg, even though the uveitis responses were similar, to insure that the response was submaximal and consequently would be more readily affected by administration of drugs.

The pharmacologic activities of selected compounds tested are shown in Fig. 2 compared with the untreated, uveitis control group. Concentration response data for dexamethasone alcohol and dexamethasone 21-phosphate are depicted in Fig. 3, representing replicate test of six eyes each and incor-

TABLE I. Biomicroscopic Scores for Antigen Dose-Response in Rabbits Following Subcutaneous Immunization and Intravitreal Challenge.

Serum antigen	(ml/kg) S.Q.	(hr):	Mean biomicroscopic scores ^a			
			24	48	72	96
Canine	1.0		1.5 ± 0.25 ^b	0.5 ± 0.25	0.3 ± 0.22	0 ± 0
	2.5		0.5 ± 0.31	2.2 ± 0.28	2.8 ± 0.44	2.3 ± 0.19
	5.0		2.0 ± 0.33	3.3 ± 0.30	2.3 ± 0.30	2.0 ± 0.24
	10.0		0.6 ± 0.36	1.0 ± 0.35	0.8 ± 0.65	0.3 ± 0.22
Bovine	1.0		1.8 ± 0.54	1.5 ± 0.56	0.8 ± 0.41	0.3 ± 0.22
	2.5		1.0 ± 0.33	1.5 ± 0.31	1.4 ± 0.46	0.6 ± 0.22
	5.0		1.4 ± 0.36	2.0 ± 0.63	1.9 ± 0.72	1.0 ± 0.40
	10.0		1.0 ± 0.50	1.7 ± 0.54	1.5 ± 0.56	0.7 ± 0.27
Equine	1.0		0.5 ± 0.31	1.0 ± 0.24	1.0 ± 0.33	0.8 ± 0.15
	2.5		1.2 ± 0.37	1.5 ± 0.39	1.7 ± 0.30	1.0 ± 0
	5.0		0.5 ± 0.22	0.8 ± 0.33	1.2 ± 0.44	1.4 ± 0.22
	10.0		0.7 ± 0.45	1.2 ± 0.44	1.3 ± 0.56	0.8 ± 0.37

^a *n* = 6.

^b Mean ± standard error.

TABLE II. Biomicroscopic Scores for Antigen Dose-Response in Rabbits Following Intravenous Immunization and Intravitreal Challenge.

Serum antigen	(ml/kg, iv)	Mean biomicroscopic scores ^a			
		(hr): 24	48	72	96
Canine	0.2	0 ± 0 ^b	0.7 ± 0.30	1.0 ± 0.33	0.7 ± 0.30
	0.3	0.8 ± 0.22	1.3 ± 0.54	0.5 ± 0.43	0.3 ± 0.22
	0.5	0.2 ± 0.15	0.2 ± 0.15	0.3 ± 0.30	0.7 ± 0.30
	1.0	0.2 ± 0.15	0.2 ± 0.15	0 ± 0	0.3 ± 0.30
Bovine	0.2	0.3 ± 0.19	0.8 ± 0.37	0.8 ± 0.44	0.5 ± 0.28
	0.3	0.6 ± 0.36	0.8 ± 0.52	1.0 ± 0.40	1.2 ± 0.33
	0.5	0.2 ± 0.18	0.4 ± 0.36	1.3 ± 0.18	1.8 ± 0.36
	1.0	0.8 ± 0.33	1.4 ± 0.46	1.0 ± 0.28	0.6 ± 0.22
Equine	0.2	0.6 ± 0.22	1.4 ± 0.46	1.2 ± 0.72	0.8 ± 0.52
	0.3	0.5 ± 0.20	0.5 ± 0.20	0.3 ± 0.19	0.3 ± 0.19
	0.5	0.5 ± 0.20	0.3 ± 0.19	0.5 ± 0.31	0.7 ± 0.30
	1.0	1.3 ± 0.18	1.4 ± 0.46	1.0 ± 0.49	0.8 ± 0.33

^a n = 6.^b Mean ± standard error.

porating data shown in Fig. 2 for the 0.1% levels.

Figure 2 shows the mean scores for the animals tested plotted vs. time (days). The responses to all compounds tested, with the exception of 5.0% phenylephrine, were significantly more active than control ($p < .05$).

To obtain the values depicted in Fig. 3, the mean responses for each treatment group

were added together for days 9 through 12. This cumulative value was expressed as a percentage of the value obtained for the respective control group calculated in the same manner. The relative activities of dexamethasone alcohol and dexamethasone 21-phosphate at lower concentrations are shown. No significance existed between treatment groups at the levels tested.

Comment. When evaluated as a test model in which duration of the experiment was a major factor, equine and bovine sera were able to produce only a mild uveitis. In previous tests in which more time was allowed for immunization, severe uveitis developed following use of either sera. Canine serum produced a moderately severe uveitis in this test system when administered subcutaneously at 2.5 and 5.0 ml/kg to the rabbits. This suggests a superiority of canine serum on a volume/volume basis for the production of uveitis with a rapid onset.

Also noted was the marked decrease in uveitis reaction in the 10.0 ml/kg subcutaneous canine serum group. Antibody suppression by larger doses of antigen has been previously established (10, 11). Additional experiments with canine serum, in which immunization on a daily basis with large doses of serum also produced low uveitis reactions,

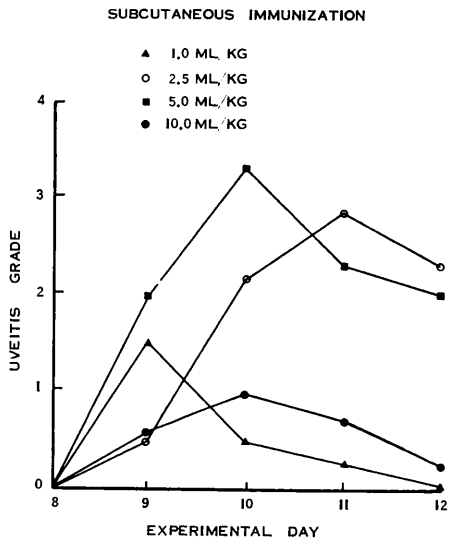


FIG. 1. Grade of uveitis obtained following subcutaneous immunization and intravitreal challenge with canine serum ($n = 6$ eyes).

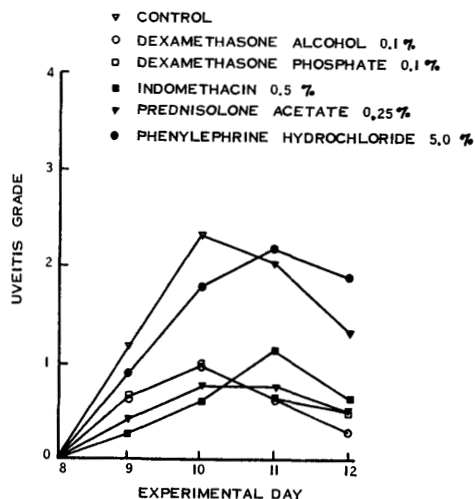


FIG. 2. Activity of selected compounds in the test model for induced uveitis (n = 6 eyes).

further suggested immunoparalysis. Since this effect was not as marked in either the bovine or equine serum groups at this level, it may be speculated that, in this test model, canine serum administered to rabbits subcutaneously is a stronger antigen. In preliminary experiments the biomicroscopic grading system included several observable parameters of the inflammatory process (iris hyperemia and edema, aqueous flare, hypopyon, keratitic

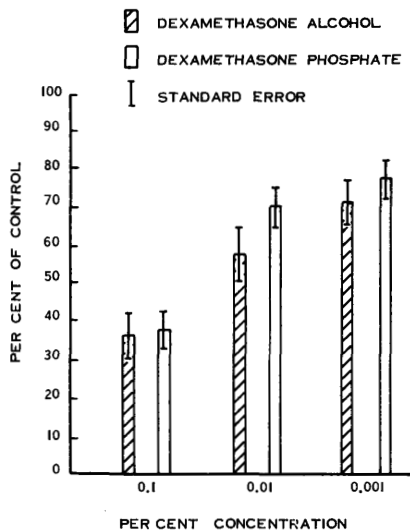


FIG. 3. Concentration response data for dexamethasone alcohol and dexamethasone 21-phosphate in the test model for induced uveitis (n = 12 eyes).

plaques, posterior synechia and corneal opacity). However, with steroid treatment, all but the vasoconstrictor activity on the iris were found extremely variable and better reproducibility was obtained by using the iris grade only.

Vasoconstrictor activity of topically applied steroids has been studied (12, 13) and found to be an adequate measure of activity for topically (skin) applied steroids. In the present test model, vasoconstrictor activity did not appear to be involved in the mechanism of the ocular anti-inflammatory activity which was measured. When 5.0% phenylephrine hydrochloride was used as a treatment for canine serum uveitis, no vasoconstrictor activity on the iris was noted. Some mydriasis was observed with phenylephrine hydrochloride but, due to the edema and posterior synechia formation, was not as pronounced as would be expected and did not significantly interfere with the grading system.

Dexamethasone alcohol, dexamethasone 21-phosphate and prednisolone acetate all significantly reduced the inflammatory reaction produced by this test model. Indomethacin, a nonsteroid anti-inflammatory agent, was also active in this test model, thus confirming Hanna's observations (8).

This study demonstrates a testing procedure of short duration for evaluating, in rabbits, ocular anti-inflammatory activity of potentially useful compounds. By using the techniques described, adequate assessment of compound activity can be accomplished in 12 days.

Summary. Dose-response data are reported for three serum antigens evaluated in a rapid screening test suitable for evaluation of anti-inflammatory compounds. Canine serum, when administered subcutaneously, was found to be a more useful antigen than bovine or equine sera for producing an immune response with rapid onset and relatively short duration. The test model described may be completed in 12 days. Dexamethasone, dexamethasone 21-phosphate, prednisolone, and indomethacin were successful in reducing the canine serum uveitis.

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