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LABORATORY INVESTIGATION

Essam Saber
Jan McDonnell
Kerry M. Zimmermann
Javier E. Yugar
Steven E. Feldon

Extraocular muscle changes in experimental orbital venous stasis: some similarities to graves' orbitopathy

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E. Saber · J. McDonnell
K.M. Zimmermann · J.E. Yugar
S. E. Feldon
Department of Ophthalmology,
University of Southern California School of Medicine, Los Angeles, California, USA

J. McDonnell · K.M. Zimmermann
J.E. Yugar · S. E. Feldon (✉)
Doheny Eye Institute, 1450 San Pablo St,
Los Angeles, CA 90033, USA

S.E. Feldon
Department of Neurological Surgery,
University of Southern California School of Medicine, Los Angeles, California, USA

Present address:
[1] Zagazig University, Banha, Egypt

Abstract ● Background: Graves' orbitopathy (GO) is generally considered to have an autoimmune etiology. Recently, however, it has been hypothesized that orbital venous obstruction may contribute significantly to the clinical manifestations. To determine whether such obstruction could induce histologic and clinical findings consistent with GO, we developed an animal model of orbital venous obstruction by ligating the draining ophthalmic veins of the right eyes of four cats. ● Methods: The branches of the ophthalmic veins were isolated and ligated following a lateral orbitotomy. Weekly photographs and echographs were taken of the cats; one cat was killed at each of four time points, namely 1, 2, 3, and 4 weeks after surgery. Histologic stains were applied to isolated orbital tissues to characterize pathologic changes. ● Results: Clinically, there was onset of marked proptosis, chemosis, and exotropia. Histological findings within the extraocular muscles included activation and the presence of acid mucopolysaccharides 1 week after ligation, increased collagen and the presence of lymphoid cells at 2 weeks after ligation, and persistent interstitial lymphocytic infiltrates the 3rd and 4th weeks after ligation. ● Conclusion: Without evoking a primary orbital inflammation or inducing a systemic autoimmune disease, an animal model has been developed that closely mimics many of the advanced clinical and histologic changes that occur in GO.

Introduction

Extraocular muscles are commonly affected in Graves' orbitopathy. The histopathologic changes include interstitial infiltration of the extraocular muscles with mononuclear cells and fibroblasts; in addition, there is edema and acid mucopolysaccharide deposition [12, 15, 19, 20]. Although both light and electron microscopy have failed to reveal destroyed or degenerated muscle fibers, [9, 12, 15, 20] the findings have been considered to be consistent with an autoimmune process. Serum antibodies to extraocular muscle [1, 2, 7, 11] and cell-mediated reactivity to extraocular muscle antigen have been identified [5, 21], but there is limited understanding of how an autoimmune attack results in the pathologic changes that occur in the extraocular muscles of patients with Graves' orbitopathy.

Recently, Hudson et al. [8] reported nine cases (13 orbits) of Graves' orbitopathy that were without significant extraocular muscle enlargement. Computed tomographic (CT) scans of these orbits revealed that mild enlargement of the superior rectus muscle alone was correlated with markedly increased volume of intraconal fat, increased vascular markings within the intraconal space, and enlargement of the superior ophthalmic vein – signs of congestion that have previously been described in Graves' orbitopathy [13, 22]. Hudson et al. [8] hypothesized that venous obstruction, rather than inflammation, contributed significantly to many of the clinical manifestations of this disease. Herein, we describe an animal

model of orbital venous obstruction and describe the clinical, echographic and histologic findings. These findings are compared with clinical and histologic descriptions of Graves' orbitopathy.

Material and methods

The studies described herein conformed to the ARVO Resolution on the Use of Animals in Research. Animals were maintained in facilities fully accredited by the American Association of Laboratory Animal Science. Domestic cats (*Felis domesticus*) were used because of their well-defined orbital venous drainage system [14], not dissimilar to that found in humans, which is lacking in rats and rabbits.

Surgical procedure

Experimental animals (n=4) were injected subcutaneously with atropine (0.5 mg/0.45 kg) and acepromazine (1 mg/0.45 kg) for sedation followed by an intramuscular injection of ketamine hydrochloride (10 mg/0.45 kg). An endotracheal tube was then passed to maintain normal breathing. Sodium thiamylal 2% (Biotal; 1 mg/4.5 kg), administered intravenously, was used as a general anesthetic.

With the anesthetized cat in lateral recumbency, a right lateral orbitotomy was performed. After shaving the hair and cleansing the skin with alcohol, a skin incision was made directly over the zygomatic arch. The incision was begun just posterior to the lateral palpebral ligament on the frontal process of the zygomatic bone and was extended caudally to the posterior one-third of the temporal process of zygomatic bone. The superficial and deep bellies of the temporalis muscle were dissected free to isolate the zygomatic arch. The frontal process of the zygomatic bone was nibbled away with a rongeur, and the short orbital ligament, located between the frontal process of the zygomatic bone and the zygomatic process of the frontal bone, was cut. The periorbita was incised, following which the branches of the inferior orbital vein and the superior ophthalmic vein were located and ligated with an 8-0 Vicryl suture. The temporalis muscle fibers were reattached to the zygomatic arch with 4-0 catgut suture, and the skin flaps were sutured with 4-0 silk.

Postsurgical analgesia included oxymorphone hydrochloride 0.25–0.5 mg and acepromazine 0.1 mg/4.5 kg, given subcutaneously every 8 h for 2 days. Amoxicillin antibiotic ointment was applied twice a day for 1 week.

Clinical measures

Photographs of the cats' heads were obtained on a weekly basis. B-scan orbital echography (Ultrascan 404) was performed weekly to assess changes in volume of the extraocular muscles and of the draining veins.

Histologic preparation

Cats were killed by barbiturate overdose at 1 week, 2 weeks, 3 weeks, and 4 weeks following venous ligation and were immediately infused with 5% formaldehyde via the intracardiac route. The orbits were then exenterated and placed in 5% formaldehyde solution. Coronal sections were obtained from the level of the equator of the globe, from the level of the exit of the optic nerve from the globe, and from a level approximately 1–2 mm posterior to the globe. Sections 5 μm thick were cut, and adjacent sections were stained with hematoxylin and eosin to evaluate morphology, with Gomori's one-step trichrome stain to evaluate collagen deposition, and with Alcian blue to evaluate presence of acid mucopolysaccharide.

Controls

In two cats (killed at the conclusion of weeks 3 and 4), no surgery was performed on the left eye. In the other two cats (killed at weeks 1 and 2), orbitotomy was performed in a manner identical to that of the eye undergoing orbital venous ligation, but no ligatures were placed (sham surgery).

Results

Clinical appearance

All of the animals demonstrated similar clinical findings. These consisted of marked, progressive conjunctival edema, lid swelling, limitation of ocular motility with exodeviation of the involved eye, and proptosis, all of which persisted to the time of sacrifice (Fig. 1). Minimal swelling of the soft tissue over the surgical site and mild conjunctival edema were present on the sham-operated side of the two animals that underwent sham surgery; this resolved completely within 48 h after operation.

Echographic appearance

B-scan echography of the experimental animals demonstrated thickening of the extraocular muscles, primarily in the midportion. The insertions appeared relatively spared (Fig. 2).

Histology

Orbital tissue adjacent to the operative site demonstrated fibrosis, consistent with healing, and did not undergo further examination. One week after vein ligation the extraocular muscles demonstrated areas of myocyte activation, characterization by rounding of the cytoplasm and central migration of the nuclei. With Alcian blue stain, acid mucopolysaccharide deposition between muscle bundles was apparent (Fig. 3). At 2 weeks after vein ligation, activated myocytes were again noted adjacent to foci of round cell infiltration within the interstitial space. Trichrome stain demonstrated the presence of diffuse inflammatory round cell infiltrate (Fig. 4) as well as interstitial collagen, consistent with fibrosis (Fig. 5). At 3 weeks (Fig. 6) and at 4 weeks (Fig. 7), focal areas of lymphocytic infiltration persisted within the interstitial spaces of the extraocular muscles. There was no histologic evidence of arterial occlusion or of tissue necrosis.

Fig. 1 Two weeks after venous ligation of the right eye, proptosis, exodiviation, and chemosis are apparent. All experimental animals demonstrated similar characteristics

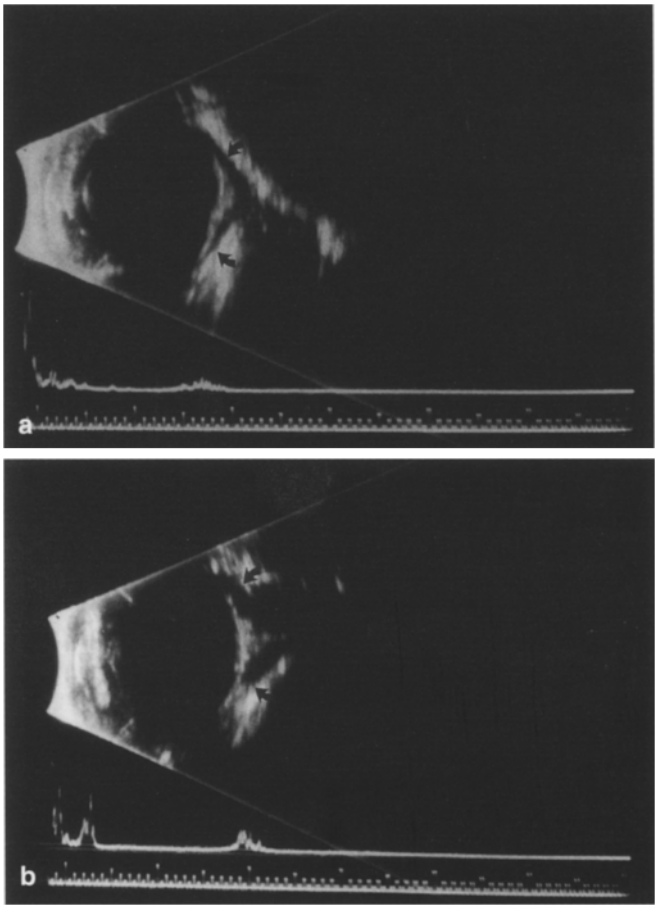

Fig. 2a, b Orbital echographs of control (**a**) and experimental (**b**) eyes, taken at 2 weeks after ligation, demonstrate enlarged superior and inferior rectus muscles (*arrows*) in the experimental eye compared with those of the control eye

Discussion

In a 1992 report of an experimental study of Volkmann's ischemic paralysis, Brooks [4] stated that "the pathologic process following the marked obstruction to venous return of blood from tissues in which the arterial supply is not obstructed is of the nature of an inflammatory rather than a degenerative process." He described a leukocyte infiltration with edema, followed by progressive fibrosis and contracture; degeneration of muscle tissue was minimal. Except for the type of leukocyte observed (polymorphonuclear as opposed to mononuclear), this description is consistent with both the clinical and the histologic muscle changes observed in our animal model.

There is ample evidence of venous obstruction in Graves' orbitopathy. Inoue et al. [10] described changes, demonstrable by CT, that included dilation of orbital veins. Peyster and colleagues [13] and Hudson et al. [8] have also demonstrated prominence of the superior ophthalmic vein by CT. Recently, a case of slow flow retinopathy in a patient with Graves' disease has been described (S.C. Benes, presented at 23rd Annual Meeting of the Frank B. Walsh Society, Salt Lake City, 1991). Increased episcleral venous pressures may raise intraocular pressure (IOP) in some patients with orbital venous obstruction. Similarly, IOP has been noted to increase in proportion to the degree of Graves' exophthalmos [17].

Based upon the lack of direct evidence of an autoimmune process directed against the extraocular muscles, and on the reported compromise of venous outflow from the orbit, the model described herein may accurately reflect many of the findings of Graves' orbitopathy. However, the pathophysiology that leads to venous obstruction in Graves' disease remains unexplained. Hudson et al. [8] proposed that the inflammatory process might extend along septa or the perimysium and produce periphlebitis or localized compression that affects the superior ophthalmic vein in the region of the superior rectus muscle. If this is the case, the pattern of venous compromise, especially of the superior ophthalmic vein, might be critical in determining the extent of the orbital changes. As the extraocular muscles increase in size, crowding at the orbital apex may produce additional obstruction to orbital venous outflow. Alternatively, there may be localized changes in the permeability of capillaries due to immune- or hormone-mediated alteration of vascular endothelial cells.

Venous obstruction explains some but not all of the findings in Graves' orbitopathy. Most of the evidence for an immune response in patients with Graves' orbitopathy is derived from the isolation of serum antibodies that bind to extraocular muscle, and usually to other skeletal striate muscle as well [1, 2, 7, 11]. In addition, B and T cell reactivity to striate muscle has been demonstrated

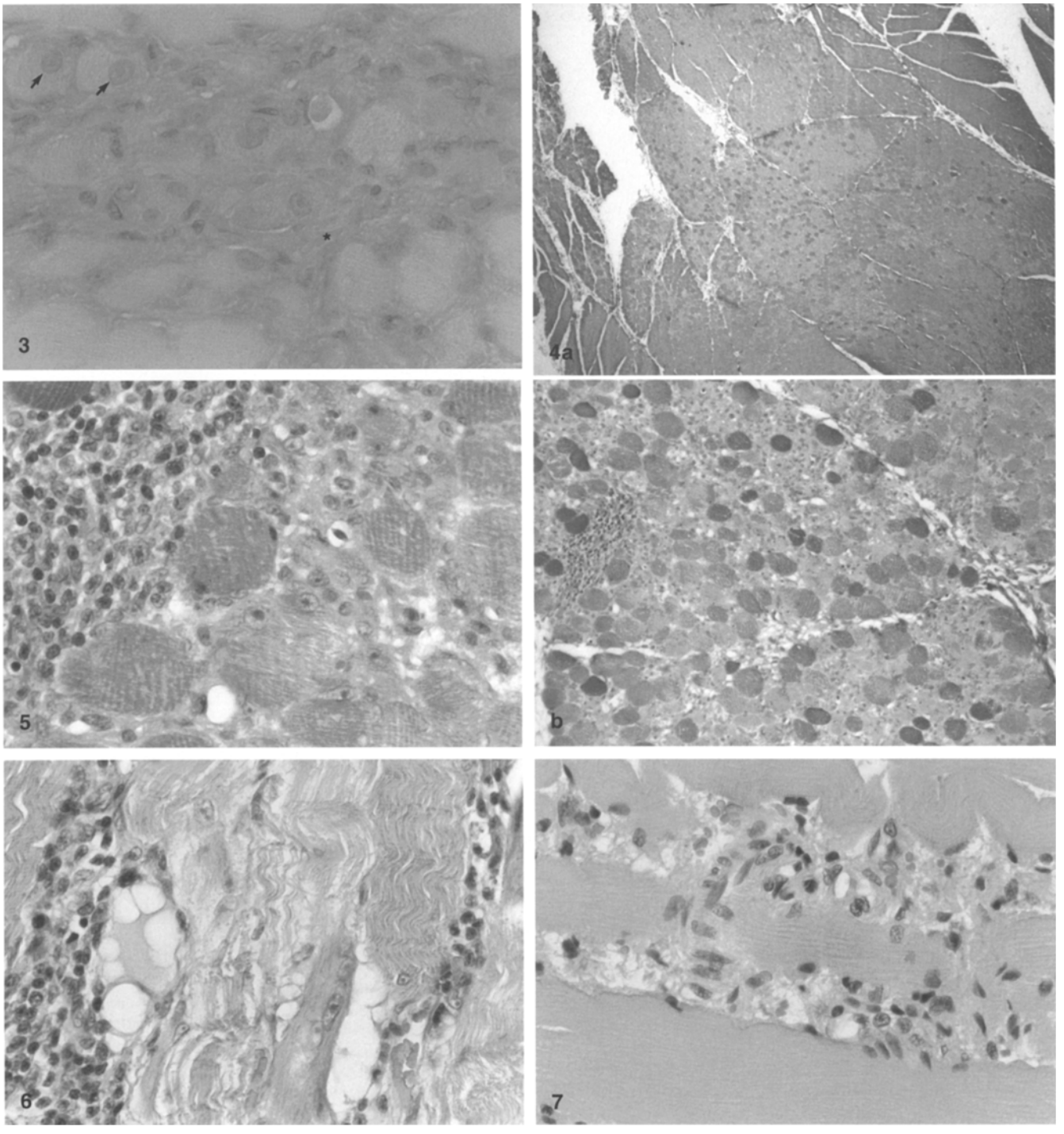

Fig. 3 Extraocular muscle from an experimental cat one week post-ligation shows reactive myocytes with internalized nuclei (*arrows*). The interstitium is expanded and contains acid mucopolysaccharide deposition (*asterisk*) (Alcian blue, pH 2.5×200)

Fig. 4a, b Widespread, diffuse, infiltration of inflammatory cells, insinuating between muscle bundels, is demonstrated at 2 weeks post-ligation at ×50 (**a**) and ×100 (**b**) magnification (Gomori's one-step trichrome)

Fig. 5 In this cat at 2 weeks post-ligation, the expanded interstitium contains increased collagen, as demonstrated with the trichrome stain. A collection of lymphoid cells is present at the *upper left*. The nuclei of the myocytes are enlarged and have prominent nucleoli (Gomori's one-step trichrome, ×200)

Fig. 6 Focal lymphocytic infiltrate persists at 3 weeks post-ligation (hematoxylin and eosin, ×200)

Fig. 7 This cat, 4 weeks post-ligation, also has infiltrates of lymphoid cells between muscle fibers (hematoxylin and eosin, ×200)

[21]. However, no primary degeneration of muscle fibers has been observed at either the light or electron microscope level [5, 21]. Furthermore, a relative paucity of inflammatory cells and a lack of class II antigen expression by extraocular muscle led Tallstedt and Norberg [18] to conclude that "biopsy findings may argue against a clinically significant immunologic reaction to the extraocular muscle antigen."

The question remains as to why circulating antibodies and cellular immune reactivity to extraocular muscles are features of patients with Graves' disease. The activation of myocytes noted in this experimental model of venous stasis may provide an important clue. This activation may be accompanied by expression of surface antigens and by cytokine elaboration, possibilities that require further investigation. However, it is reasonable to postulate that the immune reactivity to extraocular muscle is a secondary effect, rather than a primary cause of Graves' orbitopathy.

Attention has been directed also to the increased interstitial tissue that is present in extraocular muscle of many Graves' disease patients [9, 16, 19]. In addition, several investigators have reported evidence of autoimmunity related to orbital fibroblasts [3, 6]. Interstitial fibrosis is a feature of Volkmann's paralysis as well.

When considered with the constitutional symptoms and signs, there must be other immune and/or hormonal factors present to initiate the constellation known as Graves' disease. It is important to determine exactly which aspects of the orbitopathy can be explained solely by venous obstruction. Using this animal model, we are planning experiments to study length-tension curves of the affected extraocular muscles as well as other aspects of the orbital process, such as episcleral venous pressure and intraocular pressure. This model also affords the opportunity to determine whether muscle-specific antibody may circulate solely as a result of venous obstruction.

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