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Letters to the Editor

Concerns About a Dog Model of Dry Eye Disease

We are writing to express our deep concerns about the article "Establishment of a Beagle Dog Model of Dry Eye Disease" by Li et al.[1] Our concerns relate to the paper's (1) scientific content; (2) ethical integrity; and (3) ethics approval.

1) Scientific content.

The study aimed to establish the Beagle dog as a model for dry eye disease with the objective of using this model for minor salivary gland transplantation. Although there is a need to improve the management of dry eye disease in humans and dogs, we do not believe that the model selected is based on robust scientific evidence.

Keratoconjunctivitis sicca (KCS) is a very common clinical condition in the dog and, as such, naturally occurring dry eye disease in dogs has long served as a model for the human disease. Calonge et al. (2010) state that "we have learned from KCS dogs that most of the time we face an immune-based disease. Also, abnormalities in the biochemistry of ocular surface mucin were first reported in spontaneous canine KCS. The effectiveness of cyclosporine treatment has been amply studied in spontaneous diseased dogs by analyzing the improvement in clinical appearance, tear production, goblet cell mucin production, and inflammatory cell infiltration."[2] Sebbag and Mochel (2020) aptly title their review paper "An eye on the dog as the scientist's best friend for translational research in ophthalmology: Focus on the ocular surface."[3] In addition, Kol et al. state in a *Science Translational Medicine* paper that "naturally occurring disease in companion animals that display the defining attributes of similar, if not identical, diseases in humans hold promise for providing predictive proof of concept in the evaluation of new therapeutics and devices."[4] Simply put, we can learn so much from the naturally occurring disease in dogs that there is no reason for an induced canine model. In fact, we would argue that the induced model is inappropriate for studying the human disease, as it will likely be too severe to represent human patients with Sjögren's syndrome. Most human patients with aqueous deficient dry eye (ADDE) have a Schirmer Tear Test (STT) of 9.6 ± 1.7 mm in the most recent dry eye assessment and management (DREAM) study published.[5] Therefore, the dramatic sicca achieved after removal of all aqueous secreting tissue, as seen in the Li et al. study (STT $= 2.2 \pm 0.5$ mm), resulted in a severe chronic injury to the ocular surface, a gross exaggeration of the human condition.

The main reason given by Li et al. for not considering canine cases of naturally occurring KCS in this study was their belief that KCS affects the salivary glands in the dog. However, our combined clinical experience in veterinary ophthalmology (totaling more than 200 years of cumulative clinical practice) challenges this assumption. The reference cited for xerostomia is a 44-year-old report on 2 Miniature Poodles from a small family of experimental dogs suffering from multiple conditions (systemic Lupus erythematosus, Sjögren's syndrome, autoimmune thyroiditis, rheumatoid arthritis, diabetes, and celiac disease).[6] The development of dry eye in these two dogs is likely to have been secondary to these diseases. Sjögren's syndrome was not confirmed and is rarely, if ever, associated with dry eye in the dog, although Sjögren's-like syndromes have been described. As there are only three reports in the literature of Sjögren's syndrome in dogs, all being individual case reports, the likelihood of these cases being representative of the vast number of spontaneous ADDE cases is extremely low. Indeed, Leonard et al. state that "the most common cause of ADDE in dogs is an insidious immune-mediated compromise of lacrimal gland function, analogous to human patients with Sjögren syndrome, although a salivary component is only rarely observed in dogs and no systemic manifestation of autoimmune disease has been reported in dogs to date."[7]

There is an increasing awareness that animal models are often not predictive for outcomes in humans, as frequently the results do not translate successfully to human medicine in most studies. This will certainly be the case if an inappropriate model is chosen. Multiple animal models, other than dogs, do already exist for the study of KCS and it is not clear (as suggested

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by Li et al.) why one of the existing models would not be suitable for minor salivary gland (MSG) transplantation, if such a study was deemed appropriate. There is insufficient justification given for developing yet another model. Furthermore, the reference given for this ablation model goes back 49 years,[8] so the model presented is not "new" as claimed by the authors in the last paragraph of the discussion.

MSG transplantation is already performed in humans, but it has been shown to increase STT by 2 to 4 mm in human patients, and those receiving submandibular gland transplantation had a greater effect.[9] It is unclear why one would need to induce a complete loss of aqueous tear production in dogs to demonstrate an effect of such transplantation in humans. In fact, now that the transplantation is being performed in humans with severe ADDE, they would represent the most ideal "model" to test any new techniques. We suggest that a human-relevant approach is more robust, more cost-effective, and more likely to lead to successful outcomes in managing this complex disease.

2) Ethical integrity

Surgical removal of the lacrimal gland and the entire third eyelid with associated connective tissues causes considerable postoperative pain and distress. This is indicated by the authors' statement that "because of the swelling and pain caused by the surgery, tear break-up time and corneal fluorescein staining scores were unable to be measured during the first three weeks after operation."[1] Ophthalmic surgery is commonly performed on dogs in veterinary clinics worldwide. This includes biopsy of tissue, conjunctival pedicle flaps, and intraocular surgery, among others. In our collective experience, if dogs receive proper peri-surgical analgesics and anti-inflammatory medications, it is rarely too painful to perform fluorescein staining and measuring STT in dogs. In fact, most animals undergoing ophthalmic surgery have one or both of these diagnostics performed within hours of surgery or the following day. The lack of cooperation due to excessive swelling and pain reported by Yi et al. suggests that adequate systemic analgesics and anti-inflammatory medication were not administered.

Serious ethical concerns are also raised regarding the way these 6 dogs were managed over a 6-month period. There is no mention of how they were cared for over this period and there does appear to be a sad lack of consideration for their general care and welfare. The authors indicate that the dogs were in severe pain and distress as evidenced by their lack of cooperation. In addition to postoperative swelling, corneal injury was reported to have persisted for up to 6 months. Severe ocular dryness and inflammation were also noted in the postoperative period.[1]

The ocular surface disease resulting from a near complete absence of aqueous tear production is a painful pathological process. Patients with ADDE most certainly describe ocular surface pain. A recent Dry Eye Workshop published a committee report on ADDE pain and sensation report stating that "in dry eye disease, reduced tear secretion leads to inflammation and peripheral nerve damage. Inflammation causes sensitization of polymodal and mechanonociceptor nerve endings and an abnormal increase in cold thermoreceptor activity, altogether evoking dryness sensations and pain."[10] Yet no consideration appears to have been given to recording levels of discomfort, pain, and distress in the dogs, or, more importantly, to their alleviation, which should have been mandatory. To ethically conduct this research, the investigators should have monitored for pain using a validated ocular pain scale and treated the dogs with appropriate analgesia.

Furthermore, from the data presented in the publication, it is unclear where the spontaneous remission of corneal disease occurred. In fact, the data suggest that by 2 months post-surgery, the primary end points of STT, tear film breakup time, fluorescein staining, and inflammatory cytokines had already hit their maximum pathological values in nearly all tests. It is unclear why this study was prolonged for 6 months to demonstrate that effect.

In summary, analgesia does not appear to have been considered at any stage in the study, which is a very serious omission. No treatment was given for the ocular problems or consequential adverse effects, other than antibiotics. The study by Li et al. demonstrates a lack of understanding and awareness in basic care for the welfare of experimental animals and a failure to relieve their suffering. In some countries, this would be an offence under experimental animal and animal welfare legislation.

3) Ethics approval

Surprisingly, this study purports to adhere to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and was approved by the ethics committee at Peking University School and Hospital of Stomatology. This approval does not absolve the responsibilities of those involved with this study. The ARVO guidelines are permissive for animal research. However, the guidelines do state an adherence to some basic principles in the care and use of animals, which

do not appear to have been complied with in this study. Other more detailed guidelines are available, not least the ARRIVE and PREPARE guidelines that have been published in numerous languages.[11,12] In conclusion, we are concerned that this study represents unethical and inappropriate research with little regard for the welfare of the experimental animals, and does not appear to meet the aspirations of *Translational Vision Science & Technology* or the legal and ethical responsibilities of the researchers.

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