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COMMENTARY

Need for Animal Models of Meibomian Gland Dysfunction

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ABSTRACT

Evaporative dry eye has gained increasing interest in recent years in academia, pharmaceutical, and medical device industries. The main cause of this type of dry eye is attributed to meibomian gland dysfunction (MGD). MGD is a diffuse abnormality of the meibomian glands characterised by terminal duct obstruction and eventually leading to signs and symptoms of dry eye. There have been only a few reported animal models of MGD, but recent advances are likely to lead to new models and better ways to assess the pathology in these animals. Recent models reported include one based on cautery of the meibomian glands in mice and another based on aggravated allergy in mice. These developments will enable better pre-clinical assessment of novel therapies in the future.

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COMMENTARY

Dry eye is a common problem worldwide, and it has been reported that 70% of dry eye has an evaporative component [1]. The major cause of evaporative dry eye is meibomian gland dysfunction (MGD). In MGD, the occlusion of the terminal ducts leads to a cascade of changes including retention of meibomian lipids, abnormal tear lipid layer, signs of evaporative dry eye, as well as atrophy and

loss of meibomian glands. A recent international workshop by international experts mentioned that there is an unmet medical need in MGD, research in MGD is a priority, and one essential tool for translational research is an appropriate animal model. With increased clinical interest in MGD as it relates to tear stability and tear lipid chemistry, development of an animal model for MGD is paramount. New therapeutic medical devices for MGD are now available. There is increased interest in the development of lipid-containing eyedrops. It is advantageous to evaluate efficacy and safety of these new treatments in pre-clinical studies [2]. Currently available animal models resemble only one or more of these pathophysiological aspects, but are dissimilar to human MGD in other ways, either in the chronicity of the disease or the requirement for glandular occlusion to be the initiating event in the disease. It is important to avoid excessive pain when inducing the MGD features in animals.

An ideal animal model for MGD should include anatomical features of human disease, such as obstruction of the glands, keratinisation of the ducts, and dilatation of the ductules. As in human disease, the observable characteristics should diffusely affect the entire lids in a chronic or progressive manner, with fibrosis or edema of the eyelid margin. Even though the lipids of murine meibum are quite different from humans [3], there is likely biochemical abnormality in the animal tear or meibum that can be measured after induction of disease, such as changes in saturation of wax esters, decreases in O-acyl ω-hydroxyl fatty acids (OAHFAs), or increases in lysophospholipids. Ideally, there should be a high success rate for induction of the MGD, and animals involved should have normal lifespan and have little to no involvement of other systemic organs.

Here we review the features of the reported animal models (mouse and rat) that exhibit abnormalities in meibomian glands (Table 1). Animal models that report primarily blepharits [4] or reflect an ocular surface dessication model with secondary MGD [5] are not included in the discussion. There are some pros and cons for each of the models reported. Several animal models are suitable for evaluation of developmental abnormalities of meibomian glands, for example, a mutation in the EDARadd gene resulted in rats with abnormal meibomian, sweat, mammary, and other exocrine glands [6]. Such models may or may not reflect acquired disease that obstruct the meibomian glands. As MGD is more common in older people, a model that used superoxide dismutase deletion in mice reflects human disease in that these mice also show age-related MGD features. Furthermore, this model also presents with evaporative dry eye features that improved after treatment [7]. Superoxide dismutase is an important enzyme that sequesters free radicals, and in the absence of this enzyme, free radicals tend to increase, which is a phenomenon observed in aging cells and tissues. A mouse model that used cautery was successful in the induction of MGD after 4 and 8 weeks due to induction of post-cautery fibrosis, suggesting that glandular changes were secondary to obstruction [8]. Another model in rabbits using cautery of meibomian glands has also been reported [9]. Two other interesting novel models of MGD include one that is HR-1 mice fed with a lipid limiting diet [10], the other an aggravated allergic model that involved infiltration of eyelid neutrophils and IL17-mediated inflammation [11].

There is much unknown in this exciting research area. Some of the more recent studies

Table 1 Animal models showing abnormal meibomian glands

Model	Description	Animal	MGD features	Other defects[a]
Pikus et al. [13]	Modulation of bone morphogenic protein (BMP)	K14-Noggin transgenic mouse	Abnormal meibomian glands	Abnormal sweat glands, ectopic cilia, distal limb agenesis, hyperpigmentation of claws, interdigital webbing, reduced foot pads
Cascallana et al. [14]	Overexpression of glucocorticoid receptor	K5-GR mice	Lack meibomian glands	Underdeveloped sweat glands, preputial glands; abnormal hair follicles, teeth, and palate
Chang et al. [15]	Elevation of EDAR signalling	Transgenic mice	Enlarged meibomian glands	Excessively branched mammary and salivary glands
Kuramoto et al. [6]	EDARadd gene missense mutation	Sparse-and-wavy (swh) rat	Defective meibomian glands	Defects in the sweat, mammary, preputial, and tongue glands
Cui et al. [16] and Wang et al. [17]	Mutation of X-linked anhidrotic ectodermal dysplasia (EDA) gene	"Tabby" mice	Lack meibomian glands, reduced tear break up times, blepharitis	Corneal neovascularization, ulceration, keratinization, reduced corneal epithelial microvilli, conjunctivitis[b]
Lin et al. [18]	Mice lacking fatty acid transport protein (FATP)4	Tg (IVL-Fatp4) transgenicmice	Under developed meibomian glands with thickened ducts	Abnormal sebaceous glands, thick skin with defective barrier
McMahon et al. [19]	Mutation in ELOVL4 (enzyme for synthesis of extremely long chain fatty acid)	heterozygous Stgd3 mice, mixed 129SvEv and C57BL6	Protruding meibomian gland orifice, intragland anatomical changes, tooth paste like meibum, intense staining for ELOVL4 in glands	Inability to open eyes fully, increased blink rates
Ikeda et al. [7]	Superoxide dismutase (SOD)-1 knock out	Mice with F1 background	Age related meibomian gland abnormalities (reduced glandular oil-red O staining)	Corneal fluorescein and lissamine staining, reduced tear secretion (phenol red thread readings)
Gilbard 1989 [9]	Cautery of meibomian gland orifice	Rabbits	Increased tear film osmolarity, decrease in conjunctival goblet cells and corneal epithelial glycogen levels	Nil

Table 1 continued

Model	Description	Animal	MGD features	Other defects[a]
Nichols et al. [8]	Cautery of meibomian gland orifice	Mice	Meibomian gland ductal engorgement and cyst formation, gland dropout, cheesy glandular inclusions	Nil
Miyake et al. [10]	Lipid limiting diet	HR-1/HR-AD hairless mice	Plugged meibomian gland orifice Hyperkeratinisation of meibomian duct epithelium; loss of acini and gland atrophy	Atopic dermatitis [20]
Reyes et al. [11]	Immunisation with ovalbumin and pertussis, then topical challenge	C57B6 mice	Plugged meibomian gland orifice, presence of neutrophils around meibomian glands	Nil reported

[a] Genetic models refer to homozygous mice
[b] Not in mice expressing the EDA-A1 isoform transgene

evaluated here have only been reported in conference proceedings, and there is no doubt that the search is ongoing for a better and more convenient animal model of MGD. Newer imaging modalities and robust biochemical techniques will be employed in the evaluation of these future animal models. Novel image analysis algorithms would be useful for in vivo confocal microscopy, which is a non-destructive process. In tissue sections non-linear optical imaging and volumetric analysis of meibomian glands is a promising technique [12]. In cases where inflammation is to be evaluated, observation of leukocytes in two-photon live microscopy may be useful. These novel techniques will likely further our understanding of newer therapies of MGD such as probing and intense pulse light, as to date there are no suitable models to assess the effects of these therapies at the microscopic level.

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