## Mouse Meibomian Gland Dysfunction Model

Meibomian gland dysfunction (MGD) is the leading cause of dry eye disease (DED), which afflicts countless people throughout the world (e.g. >40 million in the United States), and is one of the most frequent causes of patient visits to eye care practitioners.[1] There is no global cure for MGD.

Our goal is to develop novel therapeutics to treat MGD and its associated evaporative DED. To help achieve this objective, we sought to use a mouse model of obstructive MGD to determine the capacity of specific treatments to ameliorate this condition. We chose the mouse model reported by Miyake et al.,[2] that appears ideal and is induced in Hos:HR-1 (HR-1) hairless mice by feeding them a special diet with limited lipid content (HR-AD). Following this diet for 4 weeks, mice develop hyperkeratinization of the Meibomian gland (MG) ductal epithelium, a toothpaste-like meibum, obstructed MG orifices, loss of MG acini, and MG atrophy.[2] Hyperkeratinization of the ductal epithelium and reduced meibum quality, in turn, contribute significantly to the luminal plugging found in human obstructive MGD.[3] In addition, topical azithromycin (AZM) treatment in this HR-1 mouse model significantly alleviates the diet-induced MG sequelae.[2] Hence, this model mimics many of the characteristics of human MGD[3,4] and is responsive to treatment with AZM, which is a member of a class of pharmaceuticals (i.e. cationic amphiphilic drugs [CADs]) that we wish to test for therapeutic efficacy.

The albino hairless mouse strain used by Miyake et al.[2] was developed by Hoshino Laboratory Animals, Inc., from a spontaneous mutation originally discovered in 1968. The HR-AD feed seems to have been created by Nosan, Corp.,[5] but is also sold by Hoshino. Both the mice and the feed are distributed by Japan SLC, Inc. An SLC representative informed us in February 2018 that we could purchase the HR-AD feed with or without sterilization and that the smallest unit equaled 10 kg. We were also told that only male HR-1 mice could be ordered and exported from Japan, in order to prevent breeding of these animals in other countries.

Six months later, we sought to order the HR-AD feed and 48 of the 10-week-old HR-1 male mice. We also asked about the survival rate of mice being shipped from Japan to Boston. The SLC representative informed us that the company had changed its policy, and would no longer export the HR-AD feed. In addition, we were told that HR-1 mice older than 7 weeks of age fight and that it was recommended to ship younger animals (i.e. 6 weeks). There was uncertainty about how many of these mice would survive transport, and it was unclear whether there would be an issue with fighting once the mice arrived at our animal facility.

A question then became how do we replicate this mouse model of obstructive MGD in Boston? More specifically, what diet and what mouse strain should we use? We learned that the Japanese creator of the HR-AD diet would not disclose its ingredients. We learned that most of the general ingredients in the diet were published,[5] but because the nature of these ingredients was not specified, the diet could not be reproduced by a US company. We also learned that another diet (Research Diets, Product #D03052309)[6] was apparently similar to that of HR-AD, and we chose to use that diet. Amid concerns about the mouse strain, we found that both the Hos:HR-1 and an SKH1 strain available from Charles River Laboratories were developed directly from the Skh:HR strain,[7] so we opted to purchase the SKH1 mice from Charles River.

We then conducted a pilot study to examine whether a 4-week exposure of SKH1 mice ($n = 10$) to this modified, irradiated diet would result in the development of MGD. We discovered that administration of this low lipid diet to SKH1 mice did not lead to plugged MG orifices, or the loss of MGs or MGD. In effect, we could not duplicate the Miyake et al. model in Boston.[2]

To paraphrase the poet Robert Burns,[8] the best-laid plans of mice and men often go awry. The Miyake et al. model[2] seems ideal, but turned out to be irreproducible in our hands in Boston. Consequently, in order to test our CADs for therapeutic efficacy, we need, again, to identify an animal model of human MGD that will demonstrate the human MGD signs, as well as the tear film and ocular surface sequelae associated with MGD and evaporative DED.

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