Progress in Retinal and Eye Research 28 (2009) 483–498

Contents lists available at ScienceDirect

Progress in Retinal and Eye Research

journal homepage: www.elsevier.com/locate/prer

The Meibomian Puzzle: Combining pieces together

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Keywords:
Aqueous tears
Cholesteryl esters
Dry eye
HPLC
Human
Lipids
Mass spectrometry
Meibomian gland secretions
Meibum
Oleamide
Phospholipids
Tear film
Tear film lipid layer
Very long chain (O-acyl)-omega-hydroxy fatty acids
Wax esters

A B S T R A C T

The purpose of this review was to summarize the available information on lipidomic analysis of human meibum and tear film, and critically evaluate the pertinent past and present analytical procedures and results obtained in various laboratories. Human meibum was shown to be a very complex mixture of lipids of various classes. For decades, their exact structures have remained elusive. Because of the limitations of the then-current techniques, most of the complex lipids that constitute meibum could not be analyzed as whole molecules and required prior hydrolysis and/or transesterification of the entire lipid pool. These procedures effectively made it very difficult, and often impossible, to reconstruct the complete structures of the original intact compounds, which prompted us to call this *The Meibomian Puzzle*. Modern techniques such as high-performance liquid chromatography in combination with mass spectrometry help in solving this puzzle by allowing a researcher to detect and analyze intact molecules of complex lipid compounds, even if present in extremely low concentrations. This current de-facto standard procedure in lipidomic analysis of natural lipids and their mixtures is compared with other experimental techniques such as nuclear magnetic resonance spectroscopy, infrared spectroscopy, gas chromatography, and thin layer chromatography, among the others. The results obtained by older techniques, and their limitations and deficiencies are discussed. It appears that some of the earlier findings did not withstand a scrupulous re-evaluation and need to be modified and/or corrected. The most intriguing development is the virtual absence in meibum of typical phospholipids – an important group of amphiphilic compounds whose role in the human tear film was thought to be to stabilize the entire tear film structure. Instead, another group of previously unidentified compounds, very long chain (O-acyl)-omega-hydroxy fatty acids, appears to be a stabilizing factor which might be related to tear film stability and deterioration. Thus, these compounds may become an important target in biochemistry and (patho)physiology of ocular surface and dry eye research.

Contents

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1350-9462/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.preteyeres.2009.07.002

1. Introduction

Meibomian glands (or *glandulae tarsales*), described in 1666 by the German physician and anatomist Heinrich Meibom (1666), are a variety of sebaceous glands that are located at the margins of the upper and lower eyelids of humans and mammals. The glands produce a lipid-rich secretion (Pes, 1897), also called *meibum* (Nicolaides et al., 1981), which is slowly, but constantly, released from the orifices of the glands, but could also be forced out of the glands in a burst-like manner by squeezing the eyelids upon forced blinking (Tiffany et al., 1998; Bron et al., 2004). The moving eyelids spread meibum across the ocular surface and mix it with *aqueous tears* (AT), which are produced by lacrimal glands. Mixing and spreading of meibum and AT result in a more or less continuous structure called *tear film* (TF), which covers the entire ocular surface and serves multiple purposes, including protective, lubricatory, nutritional, and antimicrobial, among others. TF was also linked to visual acuity because it provides a smoother ocular surface which improves the optical properties of the eye (Bron et al., 2004; Goto et al., 2006). However, TF is not homogeneous. This is not surprising considering that lipids do not easily form aqueous solutions and tend to separate by forming a clearly hydrophobic lipid-enriched subphase. A classical view on the TF structure presumes a three-layer organization of TF (Wolff, 1954; Holly, 1973; McCulley and Shine, 1997). As lipids are, typically, less dense than water, they accumulate on the surface of the aqueous subphase thus forming a lipid-enriched outer-most layer of TF [also called *tear film lipid layer*, or TFLL (McCulley and Shine, 1997)]. Beneath the TFLL is a much more hydrophilic *aqueous* layer enriched with water-soluble proteins, carbohydrates, salts, and other more or less hydrophilic compounds. The closest to the corneal epithelium is believed to be a relatively hydrophilic mucin-enriched *glycocalyx* layer, which is formed primarily of membrane-bound mucins (Gipson et al., 1992; Gipson, 2004; Butovich et al., 2008; Ramamoorthy and Nichols, 2008). By using interferometry, the depth of TFLL was estimated to be ~40–90 nm, while the aqueous layer was found to be much thicker at about 4 μm (King-Smith et al., 2004, 2009). It is important to realize that all three layers are soft and dynamic structures, where changes occur as a result of numerous simultaneously manifesting factors, e.g. mechanical movements of the eyelids, continuous secretion of meibum, aqueous tears and mucins, and AT evaporation and drainage through nasal ducts. One can hardly expect that under these conditions TF can preserve its classical three-layer structure for any extended length of time. Indeed, if the eye is forced to stay open without blinking, the human TF quickly deteriorates, thins, and breaks – a phenomenon know as tear break-up. The tear break-up time (TBUT) for humans is measured in seconds (Holly, 1993). It has long been considered an important and objective diagnostic parameter in evaluating the health of the ocular surface (Holly and Lemp, 1977). TBUT is widely used in ophthalmic practice to diagnose *dry eye* – a multifactorial condition (or disease) whose onset and progress is linked to the deterioration of TF in general, and TFLL in particular (Argueso et al., 2007). When the break-up occurs, the cornea becomes exposed to air, causing a discomfort to the patient. The incomplete coverage of the ocular surface with TF also increases the chances of damage to the corneal epithelium cells because of excessive dehydration, abrasions, irritation, inflammation, infections, etc. Another cause of the TF instability are meibomian glands incapable of secreting enough meibum of the necessary quality, e.g. because of meibomian gland dysfunction associated with meibomian gland inflammation and/or obstruction.

This review will focus on human meibum and its lipid composition. Those interested in other aspects of TF biochemistry and physiology are advised to read earlier comprehensive reviews on the topic (Holly and Lemp, 1977; Holly, 1993; Bron and Tiffany, 1998; Mathers and Lane, 1998; McCulley and Shine, 2004; Ohashi et al., 2006; Argueso et al., 2007; Foulks, 2007; Tiffany, 2008, among the others).

Before discussing the lipid composition of human meibum, it is important to review lipid classification and nomenclature, and the basics of analytical techniques most frequently used in, or suitable for, meibomian lipid studies. This necessary excursion will help the reader to follow the evolution and compare side-by-side the reliability and informativeness of various experimental approaches used in TF and meibum studies over the period of several decades. The second goal of the manuscript was to help in moving toward finding a solution of what can be called *the Meibomian Puzzle* – one of the hot areas of ocular surface science dealing with the lipid composition of meibum, TFLL, and TF in general.

Why a puzzle? This question brings us to the major problem that plagued the lipid analyses of meibum for decades. In most of the earlier studies, the lipids (typically, complex molecules) were hydrolyzed to their simpler "building blocks" before the analyses. Deducing the structures of the original compounds from their fragments is similar to solving anagrams, only more difficult. Consider a phrase "Eleven plus two" scrambled to a meaningless set of letters "e, e, e, l, l, n, o, p, s, t, u, w" (from http://www.anagramgenius.com) by means of "linguistic hydrolysis". The anagram could be solved as "Twelve plus one", which looks logical, is mathematically and statistically a correct answer, but still is not *the* correct answer. The second, equally wrong, answer is "To eleven lumps". Finally, one can arrive to a third (this time correct) answer "Eleven plus two". But, who is going to tell us which answer is indeed the correct one? Earlier, a researcher who analyzed meibum by using the then-standard experimental techniques that almost always include sample hydrolysis and transesterification faced exactly the same problem with no one to tell in which order to combine the complex mosaic of fragments into the correct starting structures. Consequently, over the period of decades, very little information was published on actual chemical structures of the detected lipids, with most of the researchers resorting to presenting them as *lipid classes*: note the absence of actual *molecular structures* in all but a few papers on the topic. Though important as a start, the information on lipid classes only is somewhat limiting as there are countless examples where even a slightest variation in a chemical structure of a compound leads to profound changes in its properties and physiological activity. Recent advances in bioanalytical techniques discussed below are changing this situation for the better as they enable us to perform much more detailed and accurate analyses of biochemical composition of whole meibum and its individual components than were feasible only a decade ago. There is little doubt that we will see rapid developments in the area with emphasis on complete structural characterization of individual meibomian compounds, their quantitation in meibum and TF, and studying the biophysical properties of individual lipids and their mixtures, in order to understand how TFLL and TF are organized and function.

2. Lipid classification and nomenclature

For general information on lipids, the reader is advised to visit the following Web sites: LipidLibrary.co.uk, Cyberlipid.org, hplc-ms.byrdwell.com, Lipidbanks.jp, and LipidMaps.org, among the others.

Lipids are an extremely diverse class of biomolecules, whose complexity manifests itself on several levels. Thus, there are multiple lipid classification systems.