Author Response: Surface Area of the Exposed Eye

The author thanks Millar[1] for his insightful and critical comments. His comments refer to a discussion paragraph that speculates how much phospholipid theoretically would be available to the surface of tears. The exposed ocular surface area given in the paragraph is taken directly from an article by Campbell et al.[2]: "The area of this polar interface layer can be calculated if we use 1,2 dipalmitoyl-sn-glycero 3-phosphatidyl choline (DPPC) as a model lipid for this layer. DPPC has a minimum area per molecule of DPPC of 85 square angstroms ($Å^2$)[48] and the area of the ocular aperture has been estimated at between 1.5 3 10^7 and 3.5 3 10^7 ($Å^2$).[49]" Details of the calculation are not immediately apparent in Campbell et al.[2] but seem much different from the reference cited.[3] Perhaps one mathematical error was omission of the sum of the exponents in converting from cm^2 to $Å^2$.

Correction of the calculated surface area enhances the findings of the article. The polarization modulated fourier transform infrared reflective absorption spectroscopy (PM-IRRAS) and ellipsometry data show evidence of phospholipid on the surface of tears, but measured thicknesses of the surface layer are inconsistent with a monolayer of phospholipid. Implementing the corrected ocular surface area of $1 E + 16$ $Å^2$ results in a close match between calculated phospholipid molecules and that observed by PM-IRRAS. If each phospholipid molecule is 85 $Å^2$, then the required number of phospholipid molecules to form a monolayer is $1 E + 16$ $Å^2$/85 $Å^2$/molecule = $1.18 E + 14$. The concentration of potentially free phospholipid calculated theoretically from a conservative dissociation constant for a fluorescent lipid with tear lipocalin was 0.1 µM. The number of molecules available for spreading on the surface is then $1 E - 7$ mol/L * $6.023 E + 23$ molecules/mol * $6.5 E - 6$ L or roughly $3.9 E + 11$ molecules. This is roughly 2 orders of magnitude less than needed to form a monolayer. However, application of the less conservative dissociation constant for the complex of tear lipocalin and native phospholipid, $Kd = 1.5$ instead of 0.15, results in 1 order of magnitude higher amount of available phospholipid. Therefore, about 1/10 the amount of phospholipid required to form a monolayer could be available from dissociation from tear lipocalin.

As Millar[1] suggested, the data from my article should be reinterpreted in light of the corrected surface area. Figure 5 shows the relative intensity of absorption for the phospholipid signal in tears versus a known 1,2 dipalmitoyl-sn-glycero 3-phosphatidyl choline (DPPC) monolayer. From the scale and peaks, the integrated signal intensity in tears is roughly 1/10 to 1/30 of that of the DPPC monolayer. This presumes that the absorption signal is proportional to the number of molecules. The data match closely the revised theoretical calculations of the number of phospholipid molecules available purely from dissociation from tear lipocalin in the subphase.

Several caveats apply. The numbers are highly speculative as the absorption signal depends not only on the number of phosphate groups in tear phospholipid but also on molecular orientation, two unknowns. Biologic variation in tear film thickness has been well documented,[4] and unknown factors such as phospholipid transfer protein have been posited to directly transport lipids to the surface.[5] In addition, interactions between phospholipids and other surface lipids may alter the composition of the surface film. However, the implication of the lesser phospholipid is the absence of a complete monolayer. Absence of a phospholipid monolayer may accrue some advantages, as shown by the experiment in Figure 8. When a DPPC monolayer is added to the surface of tears, the surface layer becomes thinner. The resident surface lipids (probably mainly wax and cholesterol esters) appear displaced by the monolayer of phospholipid. Assuming that a thicker layer of lipid may be preferable, an amount of phospholipid less than needed for a surface layer may avert displacement of other lipids and permit favorable interactions with other components. Perhaps a more stable tear film would be promoted.

In summary, I concur with Millar[1] regarding mathematical errors in Campbell et al.[2] about the exposed ocular surface area. After recalculation, the theoretical amount of phospholipid available to the surface layer of tears now matches the data in the article quite well. However, many assumptions have been made that need more experimentation.

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