Appendix I

Estimation of fluorescein bioavailability from coated microneedles

To experimentally estimate the bioavailability of fluorescein delivered from a coated microneedle, we inserted fluorescein-coated microneedles into intact cadaver rabbit cornea (Pel-Freez, Rogers, AR) for 30sec. The corneal tissue at the site of insertion was immediately excised from the rabbit eye using a surgical blade and incubated in a 2-ml bath of phosphate-buffered saline (PBS) at room temperature for 1h to extract the fluorescein delivered into the tissue. The microneedle was similarly incubated in a separate 2-ml PBS bath to dissolve off any residual fluorescein coating. The fluorescein content of each solution was measured by spectrofluorimetry (Photon Technology International) and compared to determine the fraction of coated fluorescein delivered into the corneal tissue (i.e., bioavailability).

The above in vitro estimate was supplemented with independent pharmacokinetic modeling of the in vivo data from the rabbit study. Using the anterior segment as the compartment in a one-compartment model, a mass balance on fluorescein in the aqueous humor of the rabbit eye was performed

\[ M_{in} = M_{out} + M_{acc} \]  

where \( M_{in} \) is the fluorescein mass inflow rate from the cornea, \( M_{out} \) is the fluorescein outflow rate via aqueous humor drainage, and \( M_{acc} \) is the fluorescein accumulation rate in the aqueous humor. This approach neglects fluorescein outflow by routes other than aqueous humor drainage, such as uptake into the lens.

The fluorescein outflow rate can be calculated as:

\[ M_{out} = C_f \cdot v_{aq} \]  

where \( C_f \) is the fluorescein concentration in the aqueous humor, which is assumed to be spatially uniform (which is an imperfect assumption, as shown by the spatial variation in concentration seen in Fig. 4) and therefore equal to the average experimental measurement, and \( v_{aq} \) is the aqueous humor volumetric flow rate, which is reported as 4.2–4.5µl/min in the rabbit eye.\(^1\)

The fluorescein accumulation rate can be estimated as

\[ M_{acc} = \frac{d(V_{aq} \cdot C_f)}{dt} \approx \frac{V \Delta C_f}{\Delta t} \]  

\( \text{(3)} \)
where $\Delta C_{fl}$ is the change of fluorescein concentration in the aqueous humor between two measurements; $V_{aq}$ is the aqueous humor volume, which is approximately 0.3ml in the rabbit eye; and $\Delta t$ is the time between measurements.

By substituting Eqs. (2) and (3) into Eq. (1), the fluorescein mass inflow rate is estimated as

$$M_{in} = C_{fl} \times V_{aq} \times \frac{V\Delta C_{fl}}{\Delta t}$$  

(4)

and the total amount of fluorescein entering the aqueous humor ($M_{in, total}$) is equal to

$$M_{in, total} = \sum_{i} M_{in_i} \times \Delta t_n$$  

(5)

where $i$ is the number of measurement points and $n$ is the corresponding measurement point number. In this endpoint calculation, the total amount of fluorescein that entered the aqueous humor equals the total amount that left the aqueous humor and the accumulation terms goes to zero. Bioavailability was determined as the ratio of the total amount of fluorescein entering the aqueous humor and the amount of fluorescein coated onto the microneedle.

References