Pharmacokinetics after intravitreal injection of a new anti-angiogenic therapeutic compound in rabbit eyes

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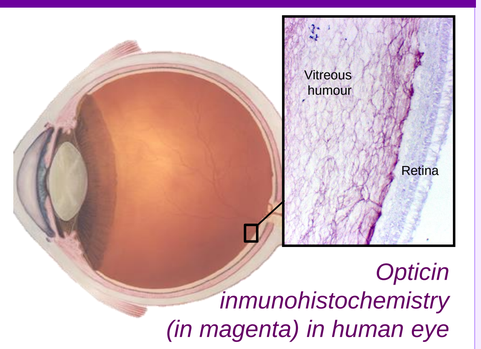
Purpose

Opticin is an endogenous, anti-angiogenic, extracellular glycoprotein found in the vitreous either in free form or bound to the vitreous collagen fibrils. We have previously demonstrated that it is synthesised by the non-pigmented ciliary epithelium[1,2]. Opticin has potential therapeutic value as an anti-angiogenic agent[3,4] in conditions such as proliferative diabetic retinopathy. The purpose of this study was to investigate the pharmacokinetic (PK) profile of intravitreally delivered human opticin in rabbit eyes. Moreover, the levels of endogenous rabbit opticin, both free and collagen-bound, were also investigated.

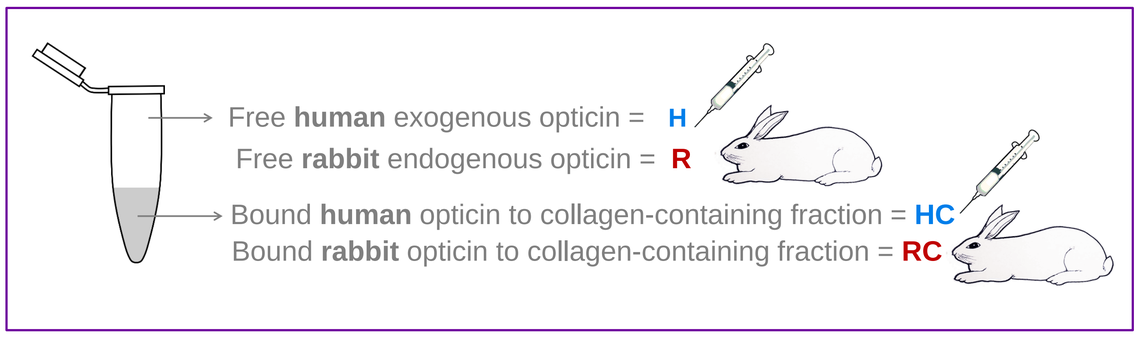


Opticin inmunohistochemistry (in magenta) in human eye

Methods

In vivo studies

Eighteen New Zealand rabbits were intravitreally injected in both eyes with 40 µg of human opticin (MW= 35 kDa) or vehicle (50 µl PBS). Eyes were enucleated at 5 h, 24 h, 72 h, 7 days, 14 days, 28 days post-injection (n=5-6). The vitreous was extracted and the concentration of ***human opticin*** was measured in the supernatant (**H**) and in the collagen-containing pellet (**HC**) by mass spectrometry using selected reaction monitoring. Additionally, the concentration of free (**R**) and bound to collagen-containing fraction (**RC**) of ***rabbit opticin*** were also measured using the same method.



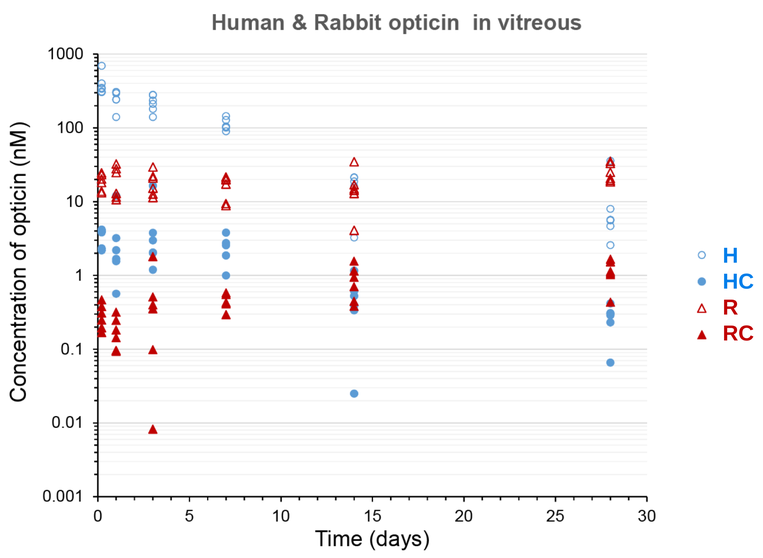
PK analysis

The resulting PK data was analysed by Nonlinear Mixed Effects Method by maximum likelihood using the stochastic approximation expectation maximization (SAEM) algorithm (MONOLIX software®) and employing a one compartmental model and combined residual error model. The interplay between the exogenous and endogenous opticin in the vitreous was also investigated.

Results

Time-concentrations profiles

Fig.1. Time-concentration profiles of the free and bound forms of human (**H** & **HC**) and rabbit opticin (**R** & **RC**) in rabbit vitreous after intravitreal injection of 40 µg of human opticin (1143 pmoles).



Human opticin PK results

The overall fit of **H** & **HC** and the resulting PK parameters estimates for **H** & **HC** are shown in Fig. 2 and Table 1 respectively:

Fig. 2. Visual Predictive Check plot: the solid lines represent the empirical percentiles (5%, 50%, 95%) of the observed data (blue dots) which are inside the prediction confidence intervals of 90 % (shaded areas).

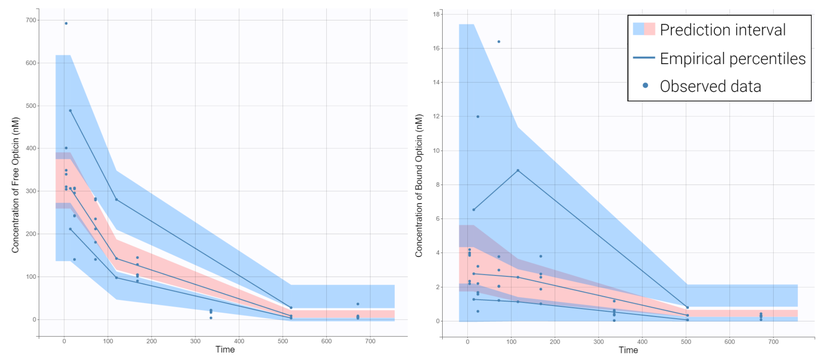
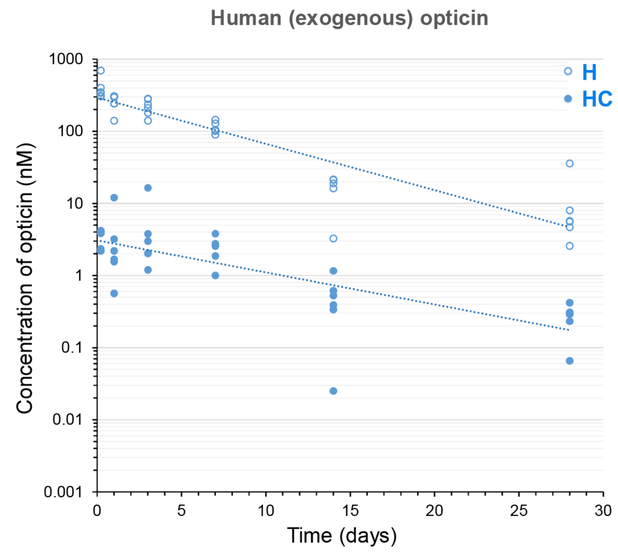


Fig.3. The estimated PK parameter values of volume of distribution (V), clearance (CL) and half-life ($t_{½}$) for **H** and $t_{½}$ for **HC** together with the concentrations profiles of free (**H**) and bound (**HC**) human opticin in rabbit vitreous together with a table of the RSE: Residual standard error.

H ○	**Values**	**Standard Error**	**RSE(%)**
V(ml)	3.18	0.313	9.84
CL (ml/h)	0.022	0.002	9.09
Half-life (h)	95.9	10.8	11.3
Half-life (days)	**4**		
HC ●	**Values**	**Standard Error**	**RSE(%)**
Half-life (h)	180	22.3	12.4
Half-life (days)	**7.5**		



Rabbit opticin PK results

Regarding the endogenous opticin in control eyes, most of it was in free form **R** (94% at least). In the injected eyes, **R** concentration was relatively constant (~19.2 nM, n=5 per time point) and similar to the basal levels in the control rabbit eyes (17.1 nM, n=9). Assuming that k_{el} is the same for **H** and **R**, and the anatomical vitreous rabbit volume of 1.5 ml, the synthesis rate (R_{syn}) can be calculated:

$$\frac{d\boldsymbol{R}}{\mathrm{d}t} = R_{syn} - k_{el} \cdot \boldsymbol{R} \text{ ; at steady state } R_{syn} = k_{el} \cdot \boldsymbol{R} \text{ ; } \boldsymbol{R_{syn} = 0.15\ \mu g/day}$$

The injected **H** reached concentration 20-fold greater than **R**, with an apparent partial replacement of **RC** from the collagen fibrils. This effect was reversed as the human opticin was cleared from the eye.

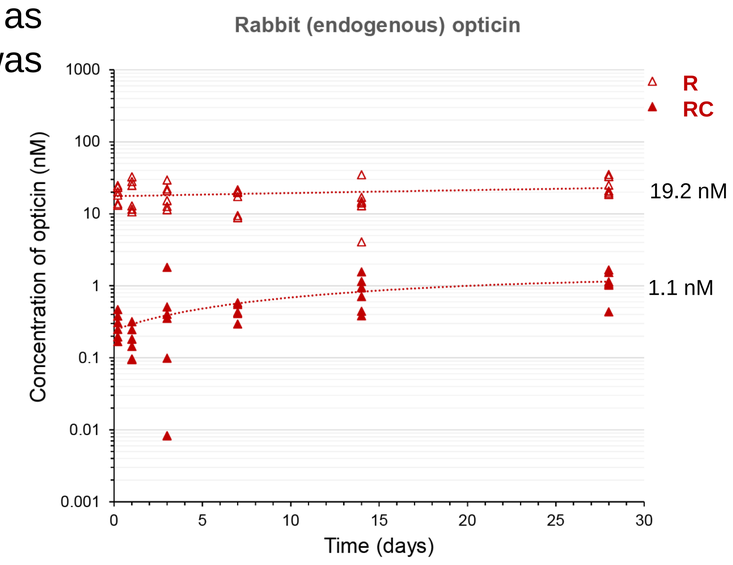


Fig.4. Concentrations profiles of free (**R**) and bound (**RC**) rabbit opticin in rabbit vitreous.

Conclusion

Most of the endogenous opticin in the vitreous is in its free form and the rate of synthesis is estimated to be 0.15 µg opticin/day. The exogenous free human opticin presented a comparable half-life to that of Eylea® and induces a reversible displacement of the natural opticin bound to the collagen fibrils.

References: *(1)Takanosu et al. IOVS Sep; 42:2202-10, 2001, (2) Bishop PN et al. Eye (Lond); 16:454-60, 2002, (3) Le Goff et al. IOVS; 53:228-34, 2012 and (4) Le Goff et al.J Biol Chem; 287:28027-36, 2012.*

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