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Research Paper

Intravitreal clearance and volume of distribution of compounds in rabbits: *In silico* prediction and pharmacokinetic simulations for drug development

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ABSTRACT

The aims of this research were to (1) create a curated universal database of intravitreal volumes of distribution ($V_{ss,\ ivt}$) and clearances (CL_{ivt}) of small molecular weight compounds and macromolecules and (2) to develop quantitative structure property relationship (QSPR) and pharmacokinetic models for the estimation of vitreal drug concentrations based on the compound structure.

$V_{ss,\ ivt}$ and CL_{ivt} values were determined from the available literature on intravitreal drug administration using compartmental models and curve fitting. A simple QSPR model for CL_{ivt} of small molecular weight compounds was obtained with two descriptors: $\log D_{7.4}$ and hydrogen bond donor capacity. The model predicted the internal and external test sets reliably with a mean fold error of 1.50 and 1.33, respectively ($Q^2Y = 0.62$). For 80% of the compounds the $V_{ss,\ ivt}$ was 1.18–2.28 ml; too narrow range for QSPR model building. Integration of the estimated $V_{ss,\ ivt}$ and predicted CL_{ivt} parameters into pharmacokinetic simulation models allows prediction of vitreous drug concentrations after intravitreal administration.

The present work presents for the first time a database of CL_{ivt} and $V_{ss,\ ivt}$ values and the dependence of the CL_{ivt} values on the molecular structure. The study provides also useful *in silico* tools to investigate *a priori* the intravitreal pharmacokinetic profiles for intravitreally injected candidate compounds and drug delivery systems.

1. Introduction

Diseases affecting the posterior segment of the eye are becoming more and more prevalent in the ageing populations. These disorders include age-related macular degeneration, diabetic retinopathies, glaucoma, and rare retinal degenerations [1]. Currently, intravitreal drug administration is the best option to ensure therapeutic concentrations of drug in the vitreous humour, retina

Abbreviations: BAB, blood-aqueous barrier; BRB, blood-retinal barrier; CL, clearance; CL_{ivt}, intravitreal clearance; $C_{ss,\ ivt}$, steady state drug concentration in the vitreous; CV%, coefficient of variation; E, extraction ratio; $E_{aqueous\ humour}$, extraction ratio from vitreous to the anterior chamber; $E_{ciliary\ body}$, ciliary body extraction ratio; $E_{choroid}$, choroid extraction ratio; E_{iris}, iris extraction ratio; E_{ocular}, ocular extraction ratio; E_{retina}, retina extraction ratio; DDS, drug delivery system; FRB, freely rotatable bonds; HA, hydrogen bond acceptors; HD, hydrogen bond donors; Htot, total number of putative hydrogen bonds i.e. HD + HA; K_p, distribution coefficient between the tissue and vitreous; MW, molecular weight; PCA, principal component analysis; PLS, linear partial least square; PSA, polar surface area; Q, blood flow; $Q_{aqueous\ humour}$, aqueous humour flow; $Q_{ciliary\ body}$, blood flow in the ciliary body; $Q_{choroid}$, blood flow in the choroid; Q_{iris}, blood flow in the iris; Q_{ocular}, ocular blood flow; Q_{retina}, blood flow in the retina; QSPR, quantitative structure property relationship; RPE, retinal pigment epithelium; $t_{1/2}$, half-life; $t_{1/2,\ ivt}$, intravitreal half-life; V_{ss}, volume of distribution; $V_{ss,\ ivt}$, intravitreal volume of distribution.

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and choroid [2]. The drug concentrations in the vitreous are governed by $V_{ss,\ ivt}$ and CL_{ivt} of the drug. After intravitreal injection the dissolved drug will diffuse throughout the vitreous, distribute into the ocular tissues and eliminate into the systemic circulation. There are two main ocular barriers in the eye that affect the intravitreal pharmacokinetics: the blood-aqueous barrier (BAB) located in the anterior part of the eye and the blood-retinal barrier (BRB) located in the posterior part. Both barriers have epithelial and endothelial components with intercellular tight junctions. The BAB is formed by the inner non-pigmented ciliary epithelium and posterior iris epithelium and the endothelium of iris capillaries. The BRB consists of the endothelium of retinal capillaries and retinal pigment epithelium (RPE).

The volume of distribution (V_{ss}) is an apparent volume that describes the extent of drug distribution and binding to the tissues. As we have described elsewhere [3], the systemic V_{ss} values after intravenous administration can oscillate between 4 l (V_{ss} of erythropoietin contained in plasma) and 49,000 l (V_{ss} of hydroxychloroquine that accumulates in tissues). After intravitreal administration, the $V_{ss,\ ivt}$ should reflect the extent of drug distribution and binding to the ocular tissues, but these values are still mostly unknown. The CL_{ivt} represents the ocular volume that is being cleared of drug per unit of time. Thus, CL_{ivt} quantitatively describes irreversible drug elimination from the vitreous. In general, clearance (CL) can be defined by the Eq. (1):

$$CL = Q \times E \tag{1}$$

where Q is the blood flow of the organ and E the extraction ratio that varies between zero and one. Thus, CL_{ivt} is dependent on the aqueous humour flow ($Q_{aqueous\ humour}$), the blood flow in the ocular tissues (Q_{ocular}), the ability of the drug to reach the anterior chamber and get across the barriers (BRB, BAB) to the blood circulation (ocular extraction ratio or E_{ocular}). The CL_{ivt} values are rarely determined or related to the chemical structure of the drug or to the fluid flow (blood, aqueous humour) values in the eye. The intravitreal half-life ($t_{1/2,\ ivt}$) is more widely used, but this is a secondary parameter that is dependent on the primary pharmacokinetic parameters ($V_{ss,\ ivt}$ and CL_{ivt}) according the general equation of half-life ($t_{1/2}$):

$$t_{1/2} = \frac{ln2 \times V_{ss}}{CL} \tag{2}$$

QSPR approach is used to establish relationship between the chemical structure and pharmacokinetics. Intravitreal $t_{1/2}$ of compounds can be predicted with QSPR models [4,5], but the values and prediction tools of the primary intravitreal pharmacokinetic parameters, $V_{ss,\ ivt}$ or CL_{ivt}, are not available. Universal $V_{ss,\ ivt}$ and CL_{ivt} values and derived QSPR models would be very useful allowing structure based calculation of these parameters early in drug discovery. Unlike $t_{1/2,\ ivt}$, the estimated $V_{ss,\ ivt}$ and CL_{ivt} values can be used to predict vitreal drug concentrations after their inclusion in the pharmacokinetic simulation models.

In this study, we determined the $V_{ss,\ ivt}$ and CL_{ivt} values using all published literature reports with adequate quality. These values for small molecular weight (MW) compounds were used for QSPR and pharmacokinetic model building to provide tools for vitreal drug concentration predictions in drug discovery and development.

2. Materials and methods

2.1. Intravitreal primary pharmacokinetic parameters

The primary pharmacokinetic parameters for intravitreally injected compounds (CL_{ivt}, $V_{ss,\ ivt}$) were calculated from all published studies that met the quality requirements using the following procedure.

A search in PubMed database was conducted using different combinations of the key words: "intravitreal" "rabbit" and "pharmacokinetic" or "clearance". The intravitreal rabbit studies were used, since the available human data are too limited. The search yielded 367 references (1947–2013), but the number was reduced to 158 studies. For example, the studies in diseased or manipulated rabbit eyes or with suspensions and drug delivery systems (DDS) were removed, because suspensions and DDS retain in the vitreous much longer than free drug and they release drug gradually. Therefore, total and free intravitreal drug concentrations after suspension and DDS administration do not represent purely pharmacokinetics (CL_{ivt}, $V_{ss,\ ivt}$) of the drug. Further selection of data from injected intravitreal solutions was carried out (Fig. 1). When drug quantities instead of concentrations were used in the publications, the amounts were divided by the reported volume of the vitreous or by the reference value of 1.15 ml (for reference volume, see Supplementary data, Table A.1a). Average concentration values were used in the pharmacokinetic analyses.

Curve fitting with *WinNonlin® software (version 5.3, Pharsight Inc., St. Louis, USA)* was used for the analysis of $V_{ss,\ ivt}$ and CL_{ivt}. The numerical values were collected from the tables or they were extracted from the pharmacokinetic graphs using *GetData Graph Digitizer® (version 2.24. Digital River, Inc., Cologne, Germany).* For salts the equivalent dose of the free drug was used. Different compartmental models and weighting schemes were used to achieve the optimal data fitting. The level of correlation between the primary pharmacokinetic parameters had to be below 0.95 and their coefficient of variation (CV%) below 35%. The model with the lowest Akaike's information criterion (AIC) was considered to be the best one.

2.2. Generation of molecular descriptors

From small molecules, the ∗.sdf format of the structure was obtained and used as input in *ACDlabs® software (version 12, Advanced Chemistry Development, Inc., Toronto, Canada)* to generate 30 molecular descriptors: pK_a for the most acidic molecular form, pK_a for the most basic form, Log*D* at pH 5.5 and 7.4, Log*P*, MW, PSA (polar surface area), FRB (freely rotatable bonds), HD (hydrogen bond donors), HA (hydrogen bond acceptors), Htot (HD + HA), rule of 5, molar refractivity, molar volume, parachor, index of refraction, surface tension, density, polarizability, C ratio, N ratio, NO ratio, hetero ratio, halogen ratio, number of rings and number of aromatic, 3-, 4-, 5- and 6-membered rings. No computational descriptors were generated for macromolecules, because their 3D structures were not accessible.

2.3. Multivariate QSPR model generation

Multivariate QSPR models were generated for the 40 small MW molecules with $V_{ss,\ ivt}$ and CL_{ivt} values (Fig. 2). Principal component analysis (PCA) and linear partial least square (PLS) (*Simca plus®, version 10.5, Umetrics AB, Umeå, Sweden*) were used to analyse the chemical space and the relationship between the primary kinetic parameters ($V_{ss,\ ivt}$ and CL_{ivt}) and the molecular descriptors of the compound set. The descriptors were transformed with unit variance scaling and mean centring. The parameters requiring normal distribution were logarithmically transformed. Before data analysis, an external set was randomly chosen. These compounds were not used for model building, but for validating the model.

In the PCA analysis the chemical space of the compounds was defined, and outlier compounds and descriptors with too narrow range were excluded. Based on the scatter plot of the final PCA