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Tools and data towards supporting quantitative systems pharmacology of the eye – an overview

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Abstract

Good eyesight belongs to the most-valued attributes of health, and diseases of the eye are a significant health care burden. Case numbers are expected to further increase in the next decades due to an aging society. The development of drugs in ophthalmology, however, is difficult due to limited accessibility of the eye, in terms of drug administration and in terms of sampling of tissues for drug pharmacokinetics (PK) and pharmacodynamics (PD). Ocular quantitative systems pharmacology (QSP) models provide the opportunity to describe the distribution of drugs in the eye as well as the resulting drug-response in specific segments of the eye. In particular, ocular physiologically-based pharmacokinetic (PBPK) models are necessary to describe drug concentration levels in different regions of the eye. Further, ocular effect models employing molecular data from specific cellular systems are needed to develop dose-response correlations. We here describe the current status of PK/PBPK as well as PD models for the eye and describe cellular systems, data repositories as well as animal models in ophthalmology. The application of the various concepts is highlighted for the development of new treatments for post-operative fibrosis after glaucoma surgery.

Keywords

ophthalmology, glaucoma, fibrosis, quantitative systems pharmacology, bioinformatics

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Clinical needs and current challenges in the development of ophthalmic drugs

Diseases of the eye

The eye is the most important sensory organ in the human body and visual impairment places a huge burden on affected patients. Leading causes for irreversible visual impairment are cataracts, age-related macular degeneration, glaucoma, diabetic retinopathy and retinitis pigmentosa (1). For a good visual acuity of the eye, the refractive cornea is of crucial importance. Due to its transparency and its high refractive power it is responsible for the optimal focusing of the incident light on the retina. Various diseases of the cornea, such as keratoconus, can lead to a severe deterioration of vision (2). Additionally, trauma, surgical intervention, or wound healing processes of the cornea, induced by infection such as trachoma, can trigger fibrotic processes and neovascularization (3), which can subsequently lead to a loss of corneal transparency and progress to complete stromal opacification and blindness (4). One of the most common irreversible causes of blindness in industrialised countries is glaucoma. The term glaucoma covers various eye diseases in which the optic nerve is damaged by a progressive course of the disease, which can initially lead to a decrease of the visual field, and in later stages, to blindness. In 2010, 60.5 million people worldwide were affected (5) and this number is expected to increase to 111.8 million people in 2040 due to an increasing life expectancy (6, 7). Ten percent of glaucoma patients are bilaterally blind (8).

Challenges in the development of ophthalmic drugs

The complex anatomy of the eye hampers the quantification of drug exposure at pharmacological sites of action. Likewise, the permeation of therapeutic agents within the eye across different layers and barriers is difficult to track. Ocular drug delivery is governed by the highly specialised anatomy of the eye (6, 9), which consists of an anterior and a posterior segment. The anterior segment includes the cornea, conjunctiva, iris, ciliary body and the lens while the posterior one encompasses the vitreous, retina, choroid, and the optic nerve (Figure 1). Ocular drug administration includes non-invasive routes of administration such as topical or oral applications, as well as posterior, periocular, and intravitreal injections (6). Topical administration is the main route of drug delivery in ophthalmic pharmacotherapy, since it is easily applied. For this reason, topical solutions, ointments and suspensions comprise 90% of ocular drug administrations (10). However, a considerable drawback of topical as well as periocular drug administration is limited bioavailability due to tear film turnover, which limits topical residence time (6), often rendering drug levels in the vitreous and retina insufficient (11, 12). Posterior segments of the eye such as the vitreous, retina, and the retinal pigment epithelium are usually accessible by intravitreal application (13). However, most macromolecules have a relatively short half-life, such that repeated intravitreal administration is often necessary (14). Other periocular routes of drug administration such as subconjunctival, suprachoroidal, subretinal, and trans-scleral injections may provide alternatives (14).

Oral application is another possibility, yet it leads to systemic drug distribution with potential off-target adverse drug effects. Also, many ocular tissues such as cornea and lens are avascular so that any convective transport of drugs in blood plasma is hardly possible. uTranscellular permeation through the cornea is generally favored by drug lipophilicity while higher paracellular permeation correlates with smaller molecule sizes (15). Hence, small hydrophilic drugs are frequently administered intraocularly, e.g., by injection under the conjunctiva (15).

Maintaining sufficiently high drug levels is generally difficult in the eye. In particular, several physiological barriers such as the endothelial monolayer between corneal stroma and aqueous humor hamper drug distribution within the eye (15). A potential alternative is the local administration of small molecules with slow release formulations, based on hydrogels, microparticles or nanoparticles (14). A quantitative understanding of drug half-life is of utmost importance since clearance determines the minimum required release rates in order to maintain the required intraocular therapeutic drug level.

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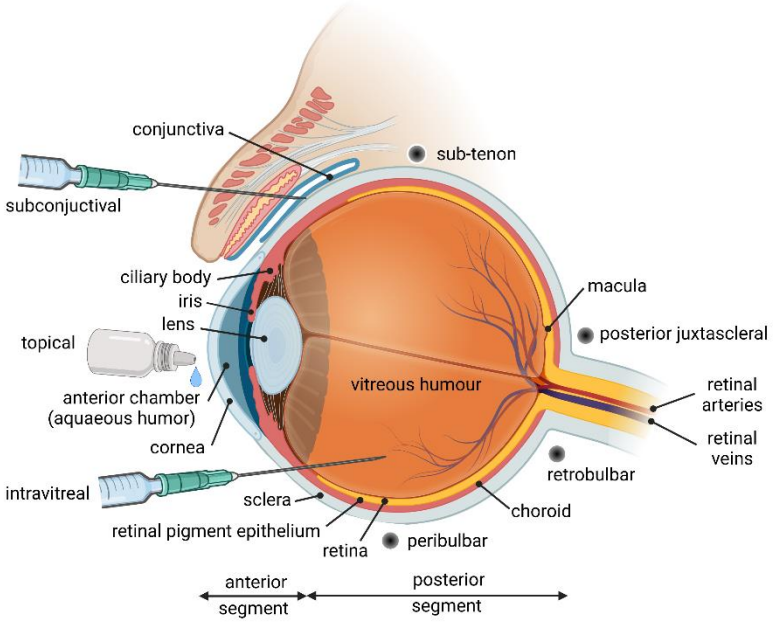

Figure 1: A cross-section of the eye. Different segments of the eye as well as standard routes of administration are illustrated (adapted from (16)).

Ocular quantitative systems pharmacology (QSP)

Animal models

The distribution of drugs in the eye can rarely be measured directly in humans. Our understanding of the various physiological processes governing ocular pharmacokinetics in humans is hence incomplete, and animal models are still widely used in ophthalmology. Rabbits, pigs, dogs, cats, mice, rats, and monkeys are standard model species for pharmacokinetic studies of the eye despite physiological differences with human morphology and physiology. For example, the consistency of the vitreous is different in rabbits, mice and humans (9) as is the melanin content (17). There are also several specific differences in the physiology of the eye between humans and rabbits, the most widely used animal model in ophthalmology. For example, the serum compartment, the retina vascular density, the vitreous cavity as well as the conjunctival surface area are comparatively larger in humans than in rabbits and vice versa for the cornea and lens (9). Moreover, rabbits have a lower blinking rate. As a consequence, drug pharmacokinetics may differ significantly between rabbits and humans. For example, the half-life of ranibizumab was 2.18-2.88 days in rabbit but 7.19 days in human eyes (14). Similarly, the half-life of bevacizumab was 4.32-7.56 days in rabbits and 9.82 days in humans (14). Nevertheless, animal models are often the only way to investigate pathological mechanisms. For this reason, animal models have been established for many diseases of the eye, see (18-20) and below. Again, the consistency of the tissues is a major issue.

The 3R principles urge researchers to minimise animal suffering. Here, physiologically-based pharmacokinetic (PBPK) modeling has the potential to significantly limit the need for animal experiments through cross-species extrapolation (Thiel et al., 2015). Whole-body PBPK models for rabbits, mice, rats and humans are available (Thiel et al, 2015; Mavroudis et al., 2018; Kuepfer et al., 2016). This, together with a clear understanding of inter-species differences in ocular physiology as outlined above and the use of cell systems derived for

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humans and animals significantly supports model-based cross-species extrapolation. Omics data (such as gene expression data) can be a further aid in the understanding of molecular mechanisms.

Cell systems

Cell lines as well as primary cells of animal and human origin are used in ophthalmology while organoid models of the eye are still in their infancy (21). Cell systems may include primary human cells of one type or co-cultures in which different cell types of one tissue are cultivated together to account for intracellular, e.g., cytokine-mediated communication (22, 23). Using co-culture systems, it is possible to replicate complex ocular structures such as the retina for experimental studies *in vitro* (24, 25). The use of polymer-based scaffolds in co-culture systems allows colonisation by the different cells and leads to the formation of cellular interactions within a spatial 3D structure *in vitro*, allowing the interaction patterns to partially reproduce *in vivo* situations (26), (22) (27), (28). The cornea for example is a complex tissue containing five well-defined layers: the epithelium, Bowman's layer, Descemet's membrane, and the endothelium (29). The cornea is the main barrier that topically administered drugs must overcome. Corneal models based on human primary cells are now commercially available and can be used to assess drug permeability (27).

Cytokine-mediated intercellular communication of the various corneal cell types mediates corneal homeostasis, but it also initiates a targeted response to challenges, such as injury or drug application (30), (31). Concerning the conjunctiva, a simplified fibrotic cell culture model was developed based on the cultivation of human primary fibroblasts of Tenon's space, allowing to describe fibrotic processes by gene expression. Comparing this description to changes in gene expression triggered in cancer cell lines allowed us to identify an antibiotic counteracting the fibrotic processes (32). The mechanisms of action of this antibiotic is currently investigated in cell systems of several species. The long-term goal of cell systems in ophthalmology is to understand the complex molecular processes in all cells in an affected tissue, or ideally in the entire organism, in order to be able to predict its response to a specific intervention.Bioinformatics and omics data support

Omics data may reflect drug PK e.g. by highlighting the abundance of genes and proteins related to absorption, distribution, metabolism and excretion (ADME) in specific cells or tissues. Likewise, omics data may provide insight in drug PD e.g. by describing the regulation of drug targets such as receptor proteins. The gene expression (transcriptome and proteome) of cells and tissues can thus be decisive about specific drug effects, and modern *single-cell* molecular data can provide detailed descriptions of tissues, down to the individual cell types and their molecular capabilities, sometimes at spatial resolution (33). High-throughput omics data are thus becoming an invaluable tool in drug development despite their inherent noisiness due to biological variability and measurement challenges (34). In particular, omics data hold great promise to enable the identification of molecular biomarkers, which may predict treatment response, or toxicity. Aggregating genes into gene sets or pathways and checking their enrichment among the over- or underexpressed genes frequently matches existing knowledge or provides the basis for further analyses. For instance, in toxicology, "adverse outcome pathways" are frequently checked for their activation based on gene expression data, and "points of departure" can be estimated on this basis (35).

In ophthalmology, omics data are still scarce. Tissues or cells from the eye are not usually covered by large databases such as GTEx, ENCODE or LINCS (36), irrespective of species. Most of the work in dedicated studies deals with the cornea (37) and the retina (38). Human gene expression data are often derived from post-mortem tissue, since for many eye tissues it is difficult to justify biopsies. The aim of describing, as closely as possible, the *in vivo* situation

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in humans is thus hard to accomplish, and artifacts caused by cell culture, tissue degradation or the use of a model organism are often substantial.

A long-established dataset is the OTDB database (genome.uiowa.edu/otdb/, GSE41102) which comprises microarray data of ten human eye tissues, that is, retina, optic nerve head, optic nerve, ciliary body, trabecular meshwork, sclera, lens, cornea, choroid/retinal pigment epithelium and iris. More recently, the *Eye in a Disk: eyeIntegration* (39) provides data from cornea, eyelid, lens, retina, retinal epithelium and RPE (choroid). The *Mega Single Cell Transcriptome Ocular Meta-Atlas* focuses exclusively on retinal tissues (40 BioRxiv). The *eye-transcriptome.com dataset* (41 BioRxiv), was derived from 11 healthy (conjunctiva, cornea, eyelid, lacrimal gland, optic nerve, retina periphery, retina centre, choroid/RPE, retinal microglia, and hyalocytes) and 9 diseased tissues.

The low prominence of omics data use in ophthalmology may be explained in part by the scarcity of such data, and by the difficulty of finding and handling them (32, 38). The increasing adoption of FAIR data principles and the increasing precision, sophistication and utility of the data types (cf. single-cell) and methods of analysis and integration (e.g., deep learning, transfer learning) for an increasing number of ocular tissues for more and more species give hope that ophthalmology will profit more and more from omics data efforts, similarly to research in fields such as cellular senescence (42).

Ocular PK/PBPK modelling

Ocular pharmacokinetic (PK) models describe drug distribution in different regions of the eye (43). A basic example for small molecule PK is a two-compartmental model of the precorneal area as well as the aqueous humor levels following topical application in rabbit eyes (44). Similarly, a four-compartment model was used to describe retinal pharmacokinetics, considering periocular space, choroid-containing transfer compartment, retina, as well as an additional distribution compartment (45). For proteins, a two-compartment model of the vitreous and the aqueous chamber was used to simulate vascular endothelial growth factor (VEGF) suppression by the antibody ranibizumab in ocular fluids (46). This model was extended by geometrical and biophysical considerations to investigate intravitreal pharmacokinetics. It was found that the ocular half-life of large molecules is proportional to the vitreous diffusion time which in turn can be estimated from the Stokes-Einstein-relation for the diffusion coefficient. The proportionality factor in turn follows from the fractional area of the vitreous/aqueous chamber interface (46). These results were confirmed in an extended model which additionally included the retina (47) to describe ocular pharmacokinetics following IVT injections. The model predicts the same half-lives for all three compartments, calculated from the hydrodynamic radius of each molecule. The model also estimated the permeabilities in the retinal pigment epithelium (RPE) and the internal limiting membrane (ILM) as well as the efficacy of clearance pathways between the retina and the choroid. Diffusion from the vitreous into the aqueous humor was found to be the main elimination pathway, while a minor part of the drug is transported between vitreous and retina, and between the retina and choroid. This three-compartment model was then further extended by additionally including permeability coefficients between retina and vitreous or choroid which were identified from rabbit PK data. Ranibizumab−VEGF binding kinetics were next included in the model to simulate ranibizumab treatment of human patients with wet-AMD (age-related *macular degeneration*) (48), (49). Notably, the extended model allows to couple intravitreal pharmacokinetics with VEGF suppression profiles in retina and aqueous humor as a pharmacodynamics readout.

Similar models for small molecules pose additional challenges. In particular, molecular structure becomes increasingly important and in consequence permeability-dependent elimination, e.g., vitreal clearance (13), may govern PK. Vitreal clearance of small molecules can be based on quantitative structure−property relationships (QSPR) (50) or on correlations

with physicochemical drug properties such as lipophilicity, polar surface area or molecular weight (16, 51). The contribution of ADME genes, in particular cytochrome P450 or phase II metabolism to drug metabolism in the vitreous appears to be limited to a few active enzymes (52). Caco-2 cell permeability was used as a surrogate for permeability through the posterior segment tissues (13). Further ocular PK models were developed specifically addressing drug transport across the cornea and the conjunctiva including clearance by tear drainage (53) or the simulation of diffusion kinetics of the transient solute transport through the cornea for periocular drug administration (54). Distribution across blood-ocular barriers was simulated to describe the dependency of systemic circulation on vitreous drug levels (55). In this model, vitreal clearance was estimated from QSPR data (56) and used analogously for the distribution clearance between plasma and vitreous. Simulations of vitreal drug concentrations correlated well with experimental measurements in rabbit eyes (55).

A notable extension of compartmental PK models are PDE-based (PDE: partial differential equations) models pioneered by the work of Missel (57) who developed an anatomically accurate geometric model to simulate intravitreal injections in rabbits, monkeys, and humans. The model considers outer surfaces as well as interior structures in specific coordinate systems to account for mass flow, pressure, and concentration. It was validated for rabbits using drugs with a molecular weight up to 157 kDa. Extensions of this anatomically accurate geometric model for rabbit eyes describe diffusion of IgG and Fab in the retina and the RPE/choroid (58), (59). The model was also used as a spatiotemporal model for drugs against macular degeneration as well as to describe drug delivery from an episcleral implant (60).

First examples for physiologically-based pharmacokinetic (PBPK) modelling in ophthalmology include representations of cornea, iris, lens and aqueous humor to describe the intraocular distribution of pilocarpine in rabbits (61) or timolol in a physiological ocular model in rats (62). An extension of these earlier models is the OCAT model (Ocular Compartmental Absorption and Transit) which encompasses aqueous and vitreous humor, retina, ciliary body, iris, choroid, cornea, lens, and sclera (63). The model also includes pathways to and from the systemic circulation. The model was in particular used to investigate the bioavailability of topical ophthalmic suspension formulations. A PBPK model has also been developed for therapeutic antibodies, considering segmentation into the aqueous humor, retina, and vitreous humor (64).

Ocular effect models

The biochemical and physiological effect of a drug to the body can be described through pharmacodynamic (PD) modelling. Classically, such PD models describe the dose-response relation at a single molecular target or pathway. Alternatively, associated clinical endpoints such as tumor volume can be considered. With regard to ophthalmology, there are several mechanistic studies on eye biomechanics (65) as well as on eye development (66). In contrast, there are only few descriptions of PD models of the eye; among these are descriptive effect correlations used for PK/PD modelling (46) (67) (68). Detailed network models from cellular systems biology, however, are even more rare. Such network models may generally involve stoichiometric models of cellular metabolism (69), ODE-based intracellular signalling cascades (70) or interaction maps (71). One of the few studies using such extended models for the metabolism of the eye concern the identification of marker metabolites in the aqueous humor from cataract patients (72) or the role of sphingolipids in retinal pathophysiology (73). The reason for the limited availability of systems biology network models may largely lie in the lack of adequate large-scale molecular data from different eye tissues which would otherwise provide a knowledge-base for subsequent network analyses. An exception in this regard is the work of the EYE-RISK Consortium (37), focused on cornea, which enables the cross-omics investigation of metabolomics, genomics and disease pathways. Existing signaling models of

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the eye mainly address the functioning of photoreceptors to describe a phototransduction model in rod cells (74). A single-cell atlas was compiled of cornea, iris, ciliary body, NR, RPE and choroid in humans and pigs which was also used develop a disease map of genes involved in different eye disorders (75).

Ocular QSP

Quantitative systems physiology (QSP) aims for the integration of cellular models from computational systems biology into PBPK models to overcome the focus on isolated drug targets (76). Considering the whole body, such multiscale models allow to simultaneously describe drug exposure in plasma as well as the resulting drug-induced response within the cellular networks. QSP models have amongst others been used to simulate cellular signalling models within the context of whole-body PBPK models (77, 78). Likewise, metabolic network models have been integrated in PBPK models (79, 80). Finally, gene expression data have been correlated with on-and-off target drug exposure (81, 82). Not unexpectedly, many QSP studies deal with central internal organs such as the liver, kidney or the heart. Applications for the eye, however, are lacking. This is not surprising given the limited amount of cellular effect models in ophthalmology, as discussed above. However, QSP concepts bear great promise in ophthalmology, since they allow pharmacodynamic analyses in segments of the eye which are experimentally not accessible, at least not in humans. Here, cellular systems play a crucial role because ideally they allow a systematic and dense sampling of data, in terms of both time and drug exposure, without the need of animal sacrifices (83, 84). This allows to experimentally track the longitudinal emergence of drug responses at different medium concentrations in cell systems. Such data can in turn be contextualised in QSP models to correlate downstream drug responses at the cellular scale with upstream dose administration at patient level. This includes both markers for drug efficacy as well as toxicity. The latter can be described in detail by adverse outcome pathways (AOP). Ocular QSP models can thus be used to screen the therapeutic window of a drug for various treatment regimes.

Ocular QSP models should be based on physiologically-based descriptions of the eye or at least of its relevant segments to quantify *in vivo* drug exposure (Figure 1). Ocular PBPK for both small molecules as well as therapeutic proteins are an important prerequisite so that relevant regions of the eye are mechanistically represented. Drug effects can then be described with mechanistic effect (PD) models to support the establishment of mechanistic dose-effect models. Given the rather limited number of cellular systems biology models in ophthalmology, molecular-mechanistic gene/protein interaction and regulation networks appear to be a promising approach. With the help of a dense sampling scheme including different doses and time points it is then possible to establish dose-effect correlations for marker genes or pathways to perform differential network analyses. The integration of effect models in PBPK models supports the contextualization of omics data in a systemic context, allowing detailed dose-response correlations through reverse dosimetry. In the future, ocular QSP models can enable important insights in ophthalmology including the identification of optimal dosing schedules or the comparison of different routes of administration as well as formulations. Ocular QSP models may also be used to analyse adverse side-effects in the eye by quantifying the effects of off-target drug exposure, for example after oral drug administration.

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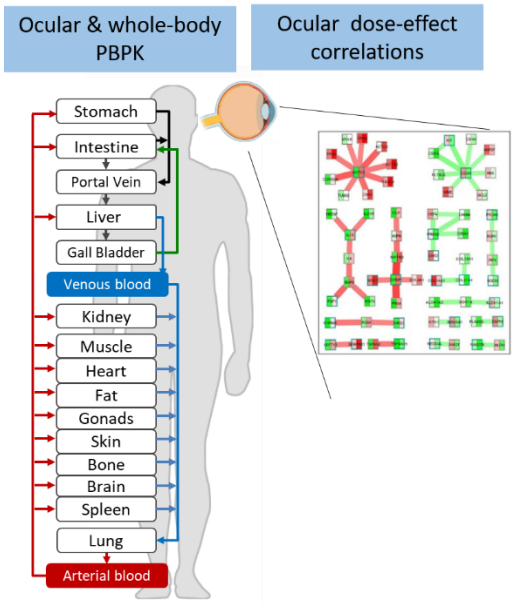

Figure 2: Ocular QSP. PBPK models can be used to describe systemic and ocular drug distribution, while dose-response correlations can be used to characterise the resulting drug response over time (16).

Use case: post-operative glaucoma

In the following, the application of ocular QSP for the development of new drug therapies will be exemplarily discussed for the case of glaucoma, one of the most severe eye diseases worldwide. The main risk factor for the development of glaucoma is the increase in intraocular pressure (IOP). The reduction of IOP is currently the only therapy that has been proven to slow the progression of the disease (6). In the majority of glaucoma patients, IOP can be adjusted to physiological values by daily application of hypotensive eye drops. The active substances and the mechanisms of action vary and are selected according to the cause of the glaucoma disease, the patient's age and the level of IOP. However, side effects of these therapies, such as dry eye disease caused by preservatives (85), allergic reactions (86) (87), but also insufficient therapy efficiency or adherence (88) may make alternative, permanent forms of therapy necessary for the treatment of glaucoma. Besides coagulation procedures to reduce aqueous humor production (89) (90) or laser trabeculoplasty, which increase the outflow of aqueous humor and thus lower the IOP (91) (92) (93), surgical interventions are frequently used for long-term glaucoma therapy. Fistulating interventions such as trabeculectomy (94) and deep sclerectomy (95) (96) predominantly drain aqueous humour into the subconjunctival space via surgically created drainage channels (97). Conventional surgical glaucoma therapies using trabeculectomy and deep sclerectomy as well as the implantation of alloplastic glaucoma drainage implants are often associated with problems in terms of long-term efficiency. The long-term success rates of trabeculectomies are estimated to lie around 40-50 % (98) (99). Frequently, a renewed increase in IOP requiring additional interventions is caused by excessive fibrotic scar formation during healing, resulting in drainage resistance or closure, triggering increase of IOP and therapy failure (100) (101) (102).

In order to prevent fibrosis in fistulating glaucoma therapy, cytostatic agents are currently used which inhibit the proliferation of fibroblasts and their transformation into myofibroblasts, thereby slowing down or preventing scar formation and maintaining the functionality of the surgery-created drainage pathways in the longer term. The main drugs used are mitomycin C (MMC) and 5-fluoruracil (5-FU), which both inhibit fibroblast cell division (103). Due to the non-

specificity of the cytostatic effect, however, the use of these drugs is associated with side effects (104) (105) (106) (107) and often requires a renewed surgical intervention.

As outlined above, establishment of an adequate cellular system is of key importance to analyse drug effects *in vitro*. In this use case, the anterior chamber, the trabecular meshwork (through which a major fraction of the aqueous humor exits the eye), the tenon (Tenon's space, which dominates the fibrotic scarring after glaucoma surgery), and the conjunctiva are of particular interest. Tenon fibroblasts are considered to occupy the conjunctival main drainage area, and their myofibroblast transformation triggers post-surgery fibrosis. RNA-seq gene expression data (for rabbit) in a time series after trabeculectomy were reported (108) as well as a mouse dataset (109)describing a glaucoma filtration surgery model. Primary human tenon fibroblasts (hTF) were also used to investigate fibrotic processes after trabeculectomy in glaucoma therapy (32).

An important question here is the validity of this specific cell system since cultivation of the primary tenon fibroblasts causes molecular changes which may already turn them fibrotic, at least in part. The molecular changes are characterised by an increased proliferation rate compared to the *in vivo* situation. Nevertheless, protein analyses of the primary tenon fibroblast cell culture system showed that the typical fibrosis marker alpha smooth muscle actin (α-SMA), which also marks the transformation of fibroblasts into fibrotically active myofibroblasts, is only expressed to a very low extent. Only the stimulation with cytokines such as transforming growth factor beta (TGF-β1) induces notable α-SMA expression and thus indicates myofibroblast transformation, which are also characterised by an increased expression rate of other fibrotic markers of the extracellular matrix (collagens, fibronectin) (110) whereby the cellular behaviour resembles the *in vivo* situation. It can hence be concluded that primary human tenon fibroblasts are not already "profibrotic", so that valid conclusions for the investigation of fibrotic scarring after glaucoma surgery can be expected.

Having established a cellular system, drug-induced responses need to be identified and characterised next. For the cellular system of primary human tenon fibroblasts, specific molecular mechanisms behind fibrosis are the upregulation of actins, the downregulation of CD34, and the upregulation of inflammatory cytokines such as IL6, IL11 and in inflammation involved BMP6. The macrolide antibiotic Josamycin (JM) reverses these molecular mechanisms according to human cancer cell line data from the CMap (111), indicating that JM could be an inhibitor of fibrosis. Follow-up experiments validated the predictive value of the cellular system, JM indeed showed an inhibitory effect on hTF proliferation in a concentration-dependent manner, and suppressed the synthesis of extracellular matrix (ECM) components. In hTFs stimulated with TGF-β1, JM specifically inhibited α-SMA expression, suggesting that it inhibits the transformation of fibroblasts into fibrotic myofibroblasts. In addition, a decrease of components of the ECM such as fibronectin, which is involved in *in vivo* scarring, was observed. Thus, JM may be a promising candidate for the treatment of fibrosis after glaucoma filtration surgery or drainage device implantation *in vivo*.

Generally, *in vitro* studies require that the *in vitro* medium concentration of any bioactive agent is chosen at physiologically relevant conditions. To this end, *in vivo* drug concentrations in specific eye segments need to be identified through simulation with ocular PBPK models. Similar PBPK-based concepts for assay design have been previously applied for the liver (84, 112). A two-dimensional assay design which covers both different drug concentrations in the medium at different times would be an ideal experimental setup (83). An accurate assay design significantly supports the identification of exposure-effect correlations for molecular markers. In the case of post-operative glaucoma this could be α-SMA levels as a function of both

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concentration and time, such that drug pharmacodynamics at the cellular levels can be established.

Exposure-effect correlations are an important prerequisite for PK/PD correlations. Thus, reverse dosimetry can be used to identify doses that have to be administered *in vivo* at the whole-body or whole-organ level such that an observed drug effect can be achieved (81, 113). PBPK models are of particular interest here since they allow simulation of PK profiles in tissues from which specific cell systems originate. With regard to glaucoma research, a physiologically-based model representation of the anterior segment of the eye is of particular relevance. Having developed such a model, drug exposure in different eye segments could be specifically quantified and, combined with *in vitro* cell system data, be used to optimise a required treatment design. Ocular PBPK models could thus be used for the development of dedicated QSP models which simultaneously describe drug PK and the resulting drug response at the molecular level. These QSP models could then be applied for forward dosimetry to convert drug doses at the whole-body or whole-organ level to the expected cellular biomarker levels. Of note, this includes markers for both efficacy and toxicity, enabling a systematic screening of the therapeutic window.

QSP models can be validated further by animal model data. In particular, the accuracy of the computational models can be assessed by comparing the simulation results at the cellular scale with specific physiological endpoints in animals. In the case of postoperative fibrosis, that may involve the comparison of α-SMA concentrations in Tenon's subspace with IOP. Some of the endpoints may only be accessible in animal models where invasive studies are possible. However, the development of a dose-response correlation in a rabbit or rodent QSP already significantly reduces the need for animal experiments, if the *in vitro/in silico* approach reflects the in-vivo situation sufficiently well. Animal QSP models can be further embedded in a parallelogram approach (114), for inter-species comparisons between animals and humans. In such a parallelogram approach, PBPK models are established for both species, together with species-specific cell systems and effect models with common molecular markers. Clearly, even a validated animal QSP model does not validate its human equivalent due to the numerous inter-species differences. However, careful translation of the animal model to humans, e.g. through PBPK-based extrapolation of drug PK and functional, structural or evolutionary analyses of the similarity of the PD marker genes (115), (Figure 2) may help to develop accurate human QSP models.

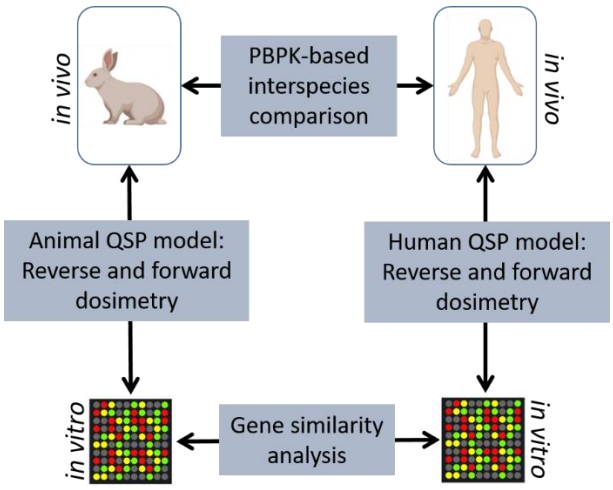

Figure 3: Parallelogram approach.

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4. Conclusion

Many eye diseases significantly reduce quality of life of affected patients. Development of new therapies, however, is difficult due to an incomplete physiological understanding of the factors governing drug distribution in the eye. Likewise, drug effects are almost impossible to characterise *in vivo* in the human eye. Ocular QSP models, building upon ocular PBPK and advanced effect (PD) models, bear the promise of providing new concepts for ocular drug development. We described and discussed the current status of the specific building blocks, i.e. PK/PBPK models, dedicated cell systems and animal models, and systems biological effect PD models. We highlighted their application for the case of fibrosis after glaucoma surgery. Ocular QSP models shall eventually allow the prediction of drug disposition and action in the human *in vivo* situation. We are convinced that the concepts here discussed will increasingly be used in ocular pharmacology in the future.

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