MR imaging of model drug distribution in simulated vitreous

Abstract: The in vitro and in vivo characterization of intravitreal injections plays an important role in developing innovative therapy approaches. Using the established vitreous model (VM) and eye movement system (EyeMoS) the distribution of contrast agents with different molecular weight was studied in vitro. The impact of the simulated age-related vitreal liquefaction (VL) on drug distribution in VM was examined either with injection through the gel phase or through the liquid phase. For comparison the distribution was studied ex vivo in the porcine vitreous. The studies were performed in a magnetic resonance (MR) scanner. As expected, with increasing molecular weight the diffusion velocity and the visual distribution of the injected substances decreased. Similar drug distribution was observed in VM and in porcine eye. VL causes enhanced convective flow and faster distribution in VM. Confirming the importance of the injection technique in progress of VL, injection through gelatinous phase caused faster distribution into peripheral regions of the VM than following injection through liquefied phase. VM and MR scanner in combination present a new approach for the in vitro characterization of drug release and distribution of intravitreal dosage forms.

Keywords: magnetic resonance imaging; vitreous body; intravitreal injection; posterior vitreous detachment; vitreous model; eye movement system

1 Introduction

During the last years the imaging technologies, such as electron paramagnetic resonance spectroscopy, positron emission tomography or magnetic resonance imaging (MRI) have gained further importance for studying age-related changes of the volume, structure of eye tissues and drug distribution after periocular and intravitreal injection [1].

With advanced age the gelatinous fraction of the vitreous body decreases and liquid filled pockets are formed. This so-called posterior vitreous detachment (PVD) may cause the separation of the vitreous membrane from the retina [2]. With increasing amount of liquefaction, the convective flow in the vitreous cavity induces increased drug mobility [3]. As the vitreal liquefaction (VL) must be considered to investigate drug distribution in vivo, ex vivo and in vitro, the PVD was implemented in this study.

Beyond physicochemical features of the active agent, procedure of intravitreal injection [4], patient-age and progression of the PVD, the eye movement has a distinctive effect on the intravitreal distribution. Saccadic eye movements induce vitreous humor motion, determine intravitreal distribution processes and shear stresses along the surrounding membranes [3]. The distinctive effect of the eye movement, particularly under PVD conditions, was shown in vitro with the combination of the eye movement system (EyeMoS) and the vitreous model (VM [5]). With increasing amount of liquid in the VM, the influence of the movement gained importance. The usage of MRI as visualization technology implies many benefits such as a higher spatial resolution and a continuous monitoring without sampling [1].

2 Material and methods

Solutions of gadolinium-based contrast agents with different molecular masses, GadoSpin™ M (GSM, 938 Da), GadoSpin™ D (GSD, 17000 Da) and GadoSpin™ P (GSP, 20000 Da) obtained from Miltenyi Biotec GmbH (Bergisch-Gladbach, Germany) were used to investigate distribution effects depending on molecular weight over a period of
12 h. Sterile 0.9 % sodium chloride solution (NaCl) was purchased from B. Braun Melsungen AG (Melsungen, Germany). Rotiphorese®, ammoniumperoxodisulfate (APS) and tetramethylethylenediamine (TEMED) were supplied from AppliChem GmbH (Darmstadt, Germany). All chemicals were of analytical grade.

Fresh porcine eyes were obtained as animal byproducts from a registered slaughter facility. The eyes were excised immediately after the death of the animal and stored in Ringer buffer maximally for 1 h until use.

2.1 Vitreous model and eye movement system

The previously developed combination of the VM (Figure 1) and the EyeMoS allows simulation of the natural vitreous body in form, shape, selected physicochemical properties as well as several types of eye movement in vitro [5].

![Figure 1: Modified vitreous model (VM): schematic view (left) and image of VM with two vents to inject (right).](image)

To achieve closer conformity with the situation in vivo and to simulate the progress of PVD [6], additional experiments were performed by substituting 50 % of PAA-gel by Ringer buffer solution. After completely gelatinization the PAA-gel was overlaid with Ringer buffer solution. Through implementation of a second vent, the test solution was injected either through the gelatinous fraction or through the liquid phase to investigate the relevance of the injection procedure [4]. For distribution experiments the VM was mounted on the holder of the EyeMoS.

A periodic movement was accomplished for 6 h to investigate the initial drug distribution. The movement was interrupted for a period of measurement in the MRI device. To compare the distribution in VM with a commonly consulted animal model [6], contrast agents were injected into porcine vitreous (ex vivo).

2.2 MRI distribution studies

After completely gelatinization of PAA-gel 100 µL of the dissolved contrast agent were injected centrally into the PAAgel of the VM using a 22 gauge needle. Injection into the vitreous body of the porcine eye was performed lateral to the cornea through the sclera, following usual surgical technique. For injection into the VM the syringe was placed on a spacer on top of the vent to ensure the reproducibility of needle penetration depth.

Distribution in the model was investigated using a 7.1 Tesla magnetic resonance imaging device (ClinScan 70/30, Bruker Bio-Scan GmbH, Ettlingen, Germany) with a phased array surface coil (rat brain, Bruker Bio-Scan GmbH, Ettlingen, Germany) with 2 channels and 2 coil elements for each channel (T1weighted sequences, T1w). The applied MRI parameters are listed in Table 1. The acquisition time of a single measurement amounted to 8:02 minutes.

Table 1: Parameters for magnetic resonance imaging.

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
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<tbody>
<tr>
<td>Repetition time</td>
<td>500</td>
</tr>
<tr>
<td>Echo time</td>
<td>12</td>
</tr>
<tr>
<td>Field of view</td>
<td>40 mm x 40 mm</td>
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<tr>
<td>Matrix</td>
<td>130 pixels x 130 pixels</td>
</tr>
<tr>
<td>Spatial resolution</td>
<td>125 µm x 125 µm</td>
</tr>
<tr>
<td>Slice thickness</td>
<td>700 µm</td>
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<tr>
<td>Flip angle</td>
<td>90°</td>
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The analysis of the obtained images was accomplished with OsiriX™ imaging software [7]. By setting the range of signal intensities, the regions of interest and the resulting volumes of the injected contrast solution were tracked and calculated by the software. For the calculation of the volume, all determined slices of 700 µm thickness were included. The scale of the volume expansion in the VM or porcine vitreous body was determined by setting the volume short after injection as point of origin. The ratio between the start value and the volumes at the selected time points were calculated. All experiments were performed in triplicate.

3 Results

3.1 Distribution of contrast agents with different molecular weights

The molecular weight of the selected contrast agents corresponds broadly to those of therapeutically applied active agents. To estimate the distribution of clinical established
drugs with low molecular weight as dexamethasone [8] or triamcinolone acetonide [9], dissolved GSM was used. GSD and GSP were applied to simulate the distribution of macromolecules such as antibodies (e.g. bevacizumab, 150 kDa) and monoclonal antibody fragments (e.g. rituximab, 40 kDa) [10]. Slow pursuit eye movements were simulated [5].

As expected, with increasing molecular weight of injected contrast agent, the velocity of distribution decreased (Figure 2). Simultaneously the spatial volume of distribution in VM and in porcine vitreous humor decreased.

![Figure 2: Distribution of GadoSpin™ M (A), GadoSpin™ D (B), GadoSpin™ P (C) in the vitreous model (100 % polyacrylamide-gel) and GadoSpin™ P in porcine vitreous body (D). Depending on the molecular weight the initial wavy injection tracks diffuses up to peripheral area of the vitreous model (A, B). Injecting macromolecular solution (C, D) the initial spot stays in effect for 12 h.](image)

Following central injection into simulated native state of the VM (100% PAA-gel), hyperintense wavy injection tracks of the contrast agents were detected on T1w-images (Figure 2, t=0 h). Independent of the molecular weight, an initial backflow along the puncture duct was seen on MR images in VM as well as in enucleated porcine eyes. Similar effects have been observed in vivo [11].

In contrast to examined macromolecules the low molecular solution of GSM (Figure 2A) spread most rapidly in vitreous, justifiable with diffusion-controlled process at initial concentration gradient that provides motive force. Diffusion into more peripheral areas occurred more slowly lasting about 6 h. Visual equilibrium state was approximately achieved at point of 12 h.

Compared to GSM, GSD diffusion zone (Figure 2B) reached peripheral regions of VM at later time points. An equilibrium distribution was not achieved during the period of investigation.

Distribution of dissolved GSP in VM (Figure 2C) and in enucleated porcine eyes (Figure 2D) yielded similar results. The initial injection spots (t=0 h) became less distinct but were present still after 12 h. In either instance the peripheral distribution of the macromolecular GSP was slower than GSM and GSP but in porcine vitreous humor faster than in VM, justifiable with age of animal model and presentational liquefaction. The process of distribution was not completed.

### 3.2 Distribution studies under simulated posterior vitreous detachment

In clinical routine senior patients are treated with intravitreal injections because of posterior segment diseases, but vitreal degeneration is mostly not considered. In vitro advanced PVD with a liquefied vitreous of 50% [6] was simulated by filling the VM with a gelatinous fraction of 50% as well as Ringer buffer solution. As the injection technique has a distinctive effect on volume and velocity of the drug distribution in liquefied vitreous [4, 11] GSM was either injected through the gelatinous fraction or through the liquid phase. The results are shown in Figure 3.

![Figure 3: Distribution of GadoSpin™ M (A), GadoSpin™ D (B), GadoSpin™ P (C) in the vitreous model (100 % polyacrylamide-gel) and GadoSpin™ P in porcine vitreous body (D). Depending on the molecular weight the initial wavy injection tracks diffuses up to peripheral area of the vitreous model (A, B). Injecting macromolecular solution (C, D) the initial spot stays in effect for 12 h.](image)

Following drug injection through PAAgel, hyperintense wavy tracks near the puncture duct remained for at least 6 h and established the small visible volume of distribution in PAAgel (Figure 3A). Applying through liquefied buffer solution (Figure 3B) the mass transport within aqueous area largely overcomes diffusive transport in gelatinous area and induced initial higher distribution in VM. An initial injection track occurred as well (Figure 3, t=0 h). After 12 h a nearly uniform distribution of the macro-molecular contrast agent in VM was detected.
The distribution of GSP in gelatinous phase is much slower than in liquid phase, justifiable with the distinctive meshwork of PAA-gel. Structural components, also present in native vitreous body, impose mixing processes and drug distribution [6]. Additional intravitreal distribution processes, such as hydrostatic pressure, convective flow and aqueous and retinal clearance pathways are incompletely known [12]. At present the VM is limited by the closed circulation processes.

4 Conclusion

Biorelevant test systems may be helpful to gain a thorough knowledge about distribution processes and intravitreal pharmacokinetics of injections and implants. The investigated combination of VM and MR scanner presents a new approach for the in vitro characterization of drug release and distribution of intravitreal injected dosage forms. The achieved images with high spatial resolution indicated a drug distribution depending on the molecular mass and agerelated liquefaction of the simulated vitreous. Conceivable modified methods are the attachment of animal models to the EyeMoS or varied volume fractions of PAA-gel and buffer solution to approximate individual vitreal liquefaction and the situation in vivo. Investigating an MR-compatible EyeMoS provides an insight into the methodical potential of the presented setup.

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Author’s Statement

Conflict of interest: Authors state no conflict of interest. Material and Methods: Informed consent: Informed consent is not applicable. Ethical approval: The research conducted with animal byproducts was compiled in accordance with all the relevant national regulations and institutional policies regarding animal welfare.

References