Robot-assisted retinal vein cannulation in an in vivo porcine retinal vein occlusion model

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ABSTRACT.
Purpose: To evaluate the feasibility of robot-assisted retinal vein cannulation for retinal vein occlusion.
Methods: Prospective experimental study performed in in vivo porcine eyes. A standard three port pars plana vitrectomy was followed by laser-induced branch retinal vein occlusion. Consequently, a retinal vein cannulation with the help of a surgical robot and a microneedle was performed. Complete success was defined as a stable intravenous position of the needle tip confirmed by blood washout for at least 3 min. Secondary outcomes were the occurrence of intra-operative complications and technical failures.
Results: Cannulation was successful in 15 of 18 eyes with a complete success rate (duration of infusion of more than 3 min) of 73% after exclusion of two eyes from analysis due to failure in establishing a blood clot. There were no technical failures regarding the robotic device. The intravessel injections of ocriplasmin in two of two eyes led to a clot dissolution. In a subset of five eyes, a second cannulation attempt at the border of the optic disc resulted in a stable intravessel position and infusion during 362 (±138) seconds.
Conclusion: Robot-assisted retinal vein cannulation with prolonged infusion time is technically feasible. Human experiments are required to analyse the clinical benefit of this new therapy.

Key words: ocriplasmin – retinal endovascular surgery – retinal vein cannulation – retinal vein occlusion – robot-assisted intra-ocular surgery

Introduction
Retinal vein occlusion (RVO) is the second most common retinal vascular disease and profoundly affects visual acuity (Klein et al. 2000; Rehak et al. 2008). Clot formation can occur in the central retinal vein or in branch retinal veins, leading to central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO), respectively. Particularly, clots will form at places where two arteries and veins cross (BRVO) or where the central vein enters the lamina cribrosa (CRVO) to exit the eye (Jefferies et al. 1993). It is thought that retinal arterial disease can affect vein perfusion, especially when both vessels lie in close contact (Zhao et al. 1993). Risk factors include all those known to be associated with systemic cardiovascular disease (Kolar 2014). When RVO occurs in one eye, the contralateral eye has a 7% chance of developing RVO within 3 years (Hayreh et al. 1994). Branch retinal vein occlusion is considered the most common form and has a prevalence between 0.5 and 1%, whereas CRVO is thought to be 3–6 times less prevalent (Rogers et al. 2010). The Beijing Eye Study states, on the basis of a sample of 4439 patients, that the 10-year incidence of BRVO is 1.6 per 100 subjects and the incidence of CRVO is 0.3 per 100 persons (Kolar 2014). As advancing age also is a major risk factor, the prevalence of BRVO among those aged 60 or older increases to be over 1% for every decade.

Currently, treatment is focused on the complications induced by the sudden stop in the retinal circulation. Intravitreal injections with anti-vascular endothelial growth factor (VEGF) or corticosteroids and retinal laser photocoagulation are widely accepted as effective treatments against secondary neovascularization and retinal oedema (Berger et al. 2015). Some did investigate causative treatments aimed at removing or dissolving the clot. Radial optic neurotomy, arteriovenous sheathotomy and chorioretinal venous
anastomosis are all non-thrombolytic mechanical approaches either to decrease compressive forces of the adjacent artery on the affected vein or to provide a bypass for the interrupted circulation (McAllister & Constable 1995; Yamamoto et al. 2004; Opremcak et al. 2006; Arevalo et al. 2008). Others have tried thrombolysis to restore blood flow by intravitreal injection of recombinant tissue plasminogen activator (rtPA) and systemic or local intravenous rtPA administration (Glacet-Bernard et al. 2000; Weiss & Bynoe 2001; Hattenbach et al. 2009). The latter is possible when performing a retinal vein cannulation and is referred to as retinal endovascular surgery (REVS). When using this technique, some reported good results while others could not confirm a gain in visual acuity and experienced significant complications (Feltgen et al. 2007). Cannulation of a vessel with a diameter of approximately 150 μm followed by holding the needle tip in place for several minutes is considered a difficult surgical task. Furthermore, the fibrinolytic action of rtPA at the clot depends on the amount of plasminogen that can be converted to active plasmin in order for clot lysis to start (Gurman et al. 2015). Moreover, plasmin has a short half-life because it is inactivated by an abundant amount of alpha-2-antiplasmin present in blood (Nogueira et al. 2009). Ocriplasmin (Jetrea®, Thrombo- genesics, Leuven, Belgium) is a small molecule containing the active part of the larger plasmin. It is an EMA- and FDA-approved drug for intra-ocular use to treat vitreomacular traction (Stalmans et al. 2012). Furthermore, its efficacy in clot lysis was already shown in a study investigating acute peripheral artery occlusion treatment (Verhamme et al. 2012) and in a coronary artery occlusion model (Dommke et al. 2010). Systemic administration of a high dose did not seem to influence the outcome in stroke patients probably because of rapid inactivation by alpha-2-antiplasmin (Thijs et al. 2009). As a powerful fibrinolytic, ocriplasmin might be effective in the treatment of RVO (obviating the need of plasminogen to plasmin conversion).

Using rtPA, most researchers report to succeed in a local intravenous retinal bolus injection (Weiss & Bynoe 2001; Bynoe et al. 2005; Yamamoto et al. 2009; Kadonosono et al. 2013). Others managed to maintain infusion for about 1.5 min (van Overdam et al. 2015) but did not find a significant increase in visual function.

To overcome the technical difficulties associated with such delicate procedure, a surgical robot was designed to increase the surgeon’s precision and stability (Gijbels et al. 2013). This study aims at investigating the possibility of a prolonged retinal vein cannulation in an in vivo porcine RVO model with the use of a robotic device.

Materials and Methods

The surgical robot is designed to work around a remote centre of motion (RCM) which coincides with the sclerotomy. This allows movement of the instrument in four dimensions (X-Y-Z and rotation), while the eye remains stabilized. The robot is operated in comanipulation modus with the surgeon’s hand in direct contact with the instrument. The needle is attached to the robotic device, but only moves when the surgeon exerts a force on the shaft as illustrated in Video Clip S1. The robotic device is thus a surgical assistive stabilizer and does not operate on its own. The surgeon can select the amount of resistance by using a foot pedal. Based on the pedal’s inclination, the electromagnetic motors of the robot are controlled to render higher or lower levels of viscous damping, hence filtering out more or less physiologic hand tremor.

Before initiating the animal experiments, the robot was extensively tested in vitro. These tests revealed the necessity of constructing a mobile platform for the robotic device, attached to the surgical table. A 3-sled orthogonal positioning stage was developed to position the robot over a range of 70 mm in all three dimensions and optimally align the robot’s working range with respect to the scleral entry point as illustrated in Video Clip S1.

A glass microneedle was developed following the specifications described by Pournaras et al. (2012). The 500-μm-long tip has an outer diameter of 30 μm and is angled 30° with respect to the shaft (Fig. 1). A custom built 18-G trocar with silicon valve was designed to be inserted in the sclerotomy and guide the needle shaft during the surgery.

Pilot experiments ex vivo and in vivo illustrated the necessity to redefine the RCM point during surgery in order to tilt the eye for optimal visualization and needle alienation. Therefore, a separate footswitch to reposition the entire robot in the horizontal plane was developed and used during this trial.

A total of 18 eyes from nine domestic pigs (Medanex facility, Diest, Belgium) weighing approximately 20–30 kg were included for this study. All animals received humane care in compliance with the FELASA guidelines and recommendations. The study was approved by the Ethical Committee for Animal Research at Medanex Clinic (EC MXCI-2013-017), and all experiments were done in accordance with the ARVO statement on the use of animals in ophthalmic and vision research.

At first, animals were premedicated with an intramuscular injection of tile-tamine–zolazepam 4.4 mg/kg (Zoletil 100®; Virbac, Leuven, Belgium) and xylazine 2.2 mg/kg (Xyl-M® 2%; V.M.D.nv/sa, Arendonk, Belgium). An IV catheter with fixation wings was placed in an auricular vein and fixed with a single suture (Vicryl 3.0, Ethicon Inc., Somerville, NJ, USA).

Animals were intubated with a cuffed endotracheal tube (internal diameter of 7–7.5 mm) (Kruase, Langeskov, Denmark). The animal was placed in lateral recumbency on the operating table and connected to the mechanical ventilator. Anaesthesia was maintained with 1.5–2% isoflurane (IsoFlO®; Euphar, Oostkamp, Belgium). Mechanical ventilation was provided with a volume-controlled ventilator (Cicero; Dräger, Lübeck, Germany) at a tidal volume of 8–10 ml/kg body weight with an inspiratory oxygen fraction (FiO2) of 0.5. A saturation probe was attached for measurement of SaO2.

The vital parameters (heart rate, respiratory rate, saturation, end tidal CO2) were continuously monitored throughout the surgery.

The pig was installed on a surgical table with adapted head rest and platform for mounting of the robot. An eyelid speculum was placed, and a retrobulbar injection with balanced salt solution (BSS, Alcon, Puurs, Belgium) was made to induce proptosis, improving access to the eye. A standard 3-port 23-gauge pars plana vitrectomy with posterior vitreous detachment (PVD) induction was performed using the EVA vitrectomy machine (DORC,
Zuidland, the Netherlands). Intravenous rose Bengal was administered systemically, and a large superiorly running retinal vein was selected for focal photocoagulation with a 532-nm intra-ocular laser (Oculight tx; Iridex, Mountain view, CA, USA). Laser burns were applied directly on the vessel until a white clot became apparent and the distal part of the vein started to swell. Occurrence of dot and blot haemorrhages surrounding the affected vein served as confirmation of definite venous closure (Fig. 2).

A sclerotomy was made with a 20G MVR blade (Laseredge 20G MVR blade; Bausch & Lomb Incorporated, Rochester NY, USA) and enlarged to allow subsequent placement of the 18-G trocar. The needle was installed on the robot, and the cannula was aligned with the RCM point. Next, the needle was inserted into the eye and the protecting shaft retracted. Infusion was done in an on/off pattern with an average amplitude of approximately 85 μm following the cardiac and respiratory cycle and the limited flexibility of the glass micropipette tip of 60 μm (de Kinkelder et al. 2011). An estimated standard deviation of 30 μm around the breaking point of the glass pipette resulted in a sample size of at least 14 eyes for a power of 0.80 with a type I error of 0.05 to be able to test the feasibility of cannulation without bias from needle breakage. This number was increased to 18 to account for other possible technical failures. Both eyes of each animal were used to decrease the total number of animals.

Results

In two eyes, clot induction failed resulting in marked vessel wall damage and extensive intra-ocular bleeding. These two eyes were excluded for further analysis.

In one eye, with the needle intra-venously, a torsional force transferred from the infusion line to the needle holder resulted in breakage of the glass capillary. The protective metal sleeve was readvanced and the remaining intraretinal part of the tip could be removed with an endoforceps. To prevent such problem from reoccurring, a mechanical brake was placed at the needle shaft.

Cannulation was successful in the remaining 15 eyes. The average time of intravenous needle tip position was 177 ± 73 seconds. The complete success rate (intravenous tip position during more than 3 min) was 73%. A double puncture occurred in two eyes resulting in a limited subretinal injection. The needle was retracted, and a second attempt resulted in a successful cannulation (Video Clip S2).

The infusion pattern translated into a clearly visible periodic washout of the blood column inside the vein. In five eyes, the clot was dislocated as a whole and flushed towards the central retinal vein removing the obstruction. In two eyes, a haemorrhage occurred at the site of occlusion during the cannulation. When the needle was retracted, a self-limiting haemorrhage was seen in all eyes, but more apparent in the ones with persistent occlusion. In two eyes, ocirplasmin (Jetrea®; Thrombogenics) was infused. Thrombolysis was effective in both after a minimum of 4 min, but after needle retraction, a longer time with increased intra-ocular pressure was needed to reduce bleeding from the puncture site.

After removing the vitreous haemorrhage, a second cannulation was performed in a hemivein at the border
of the optic disc in five eyes as illustrated in Video Clip S3. If necessary, the eye was tilted using the XYZ platform of the robot to gain access to the targeted vessel in an optimal angle and plane. A stable intravenous needle tip position could be achieved in all five eyes with an average of 362 (±138) seconds with two eyes well over 10 min. Infusion was done with a 10% Indian ink solution to clearly monitor effective infusion because the washout of blood could be confused with normal venous pulsations when the cannulation is done at the optic disc. The dark colourant rendered enough contrast to monitor effective infusion. Cannulations at the border of the optic disc were considered to be more straightforward because of less rolling of the vessels, with respect to distal branch retinal veins. After needle retraction, there was no haemorrhage.

**Discussion**

Retinal vein cannulation is a surgical challenge because of the small size of the veins and the physiologic tremor which is estimated to be as large as the width of the targeted vessel (Singh & Riviere 2002). To overcome technical difficulties, a comanipulation robotic system has been developed. The robot is meant to assist the surgeon by offering increased stability and precision. It reduces tremor and can maintain a certain position for long period of time. Furthermore, always working around a RCM, it not only stabilizes the instrument, but as the globe is prevented from rotating, it also stabilizes the eye. Next to the viscous damping supplied by the electromagnetic motors, there is also a downscaling effect of motions because the surgeon holds the instrument at a larger distance from the RCM than the diameter of the globe. This study showed that robot-assisted retinal vein cannulation in a porcine model is possible and that prolonged cannulation times are feasible. Comanipulation proved to work intuitively and safe because the instrument is unable to move unless when the surgeon applies a force. Furthermore, in contrast to robotic solutions that follow a teleoperation approach, the surgeon can stay close by his/her patient and move the instrument around the RCM similar as he/she would be doing during purely manual procedures, that is without connecting the robotic end-effector to the instrument. The possibility to move the entire robot (and thus the RCM) in all three dimensions through a stable intravenous position and a flow direction towards the optic disc. The robot-assisted cannulation attempt. Defining the RCM in the beginning of the surgery and place it at the sclerotomy, makes sure that the eye remains stable and in that exact position without being able to tilt the eye anymore. To improve visibility or to change, taking into account the limited work space of the robotic device, the useful range of the needle, the surgeon can change the position of the RCM in the horizontal plane during the surgery. This implies a tilt of the eye and a different approach to the targeted vessel. Although the surgeon needs to build up experience with such device, and plan the approach carefully, the learning curve for a comanipulated approach is relatively short. It also needs to be emphasized that the surgeon always remains in charge of the entire surgery; there is no remote controlling of the robot. There were no technical failures related to the robotic device during the conducted experiments. One needle broke because of torsional forces transferred from the stiff micro volume infusion line.

Thrombus induction using intravenous rose Bengal and direct argon green laser burns resulted in most of the eyes in a typical BRVO image. The consequent venous dilatation allowed for a more realistic cannulation model with respect to human RVO where venous dilation is one of the key characteristics in early RVO. The largest difference, however, is that in porcine eyes the vessels lie on top, whereas in humans they are embedded within the internal layers of the retina. During the experiments, extensive rolling of the veins complicated the surgical procedure when cannulating these vessels. This is expected to be much less a problem in humans. The latter was illustrated in cannulations at the border of the optic disc where rolling of the vessels was eliminated and prolonged cannulation (more than 6 min on average) was successful in all five cases.

The needle tip has a 30° angle and a bevel up configuration allowing an optimal almost horizontal approach for a cannulation. The tip itself, measuring 500 µm in length, can be advanced in the vessel lumen assuring a stable intravenous position and a flow directed towards the optic disc. The glass has a certain flexibility. This was found beneficial as it allows compensating for physiologic cardiovascular- and respiratory-induced movements of the retinal structures without breaking nor occluding the puncturing method described by van Overdam et al. (2015) with an approach reaching over the optic disc to the contralateral hemivein was not tested in this trial because of the large variation of retinal venous anatomy in the porcine eyes. Furthermore, the cannulations performed at the optic disc border were all successful and very well controlled with a clear direct view on the needle tip, targeted vessel and intraluminal tip advancement. The importance of visual feedback is undescribed by Hattenbach et al. (2012) who performed ex vivo cannulations with a glass microneedle connected to an endoscope. They reported to be successful in cannulating retinal veins on different locations next to and away from the optic disc.
A postneedle-retraction haemorrhage was seen in all eyes after branch retinal vein cannulation regardless of clot removal. This is in contrast to the findings of Pournaras et al. (2012) who reported haemorrhages but only with larger needle tip diameters. Noteworthy is that there were no haemorrhages after a cannulation at the optic disc border.

Following the histologic findings reported by Pournaras et al. (2012) on vessel wall healing without clot formation at the puncture site, two eyes received local intravenous ocriplasmin (125 μg/ml) to assess clot lysis and subsequent possible haemorrhages. In both eyes, the clot could be removed after a prolonged infusion of ocriplasmin. Up until now, rtPA is the most commonly used thrombolytic agent. Its ultimate thrombolytic effect depends on the amount of conversion of natural plasminogen to the active enzyme plasmin. Consequently, both the amount of plasminogen and the amount of rtPA present at the clot determine the efficacy and duration of the effect. The use of ocriplasmin as an active thrombolytic agent to treat RVO locally was initially suggested by M. De Smet (anecdotally). From a pharmacological point of view, the local administration of small doses of ocriplasmin is an appealing option and could become a causative treatment for RVO. The experiments in this trial do suggest that a prolonged infusion period is needed to have an exposure time long enough for the enzymatic effect to take place and restore blood flow.

It is reassuring that in vivo porcine tests showed that robot-assisted retinal vein cannulation at the optic disc is feasible for periods extending to 10 min and beyond, possibly offering targeted thrombolysis for CRVO patients in the near future.

Limitations of this study consist mainly of the use of young, healthy pigs with a normal and reactive retinal vasculature. Inducing a clot resulted in marked swelling of the veins probably reaching high intravenous blood pressures. In humans, one would expect the intravenous pressures to be lower because of the, usually, associated arterial disease. Furthermore, following their anatomic placement on top of the retina, retinal vein cannulation in pigs probably is more difficult than in humans. Another limitation is the aggressive, laser-assisted clot induction. In two eyes, direct laser burns caused an uncontrollable vitreous haemorrhage, and in two other eyes, a haemorrhage occurred during cannulation at the occluded site due to laser-induced vessel wall damage. To overcome these limitations, human trials in RVO patients are required to assess the efficacy effectivity and long-term clinical effect of this treatment. Although the golden standard for assessing the retinal circulation and perfusion times is fluorescein angiography, the advent of OCT angiography and retinal oximetry offers reproducible, fast and non-invasive alternatives in the assessment of blood flow restoration (Hardarson et al. 2006; Rechtman et al. 2009; Hardarson & Stefansson 2010; Spaid et al. 2015). These imaging methods will become important in the future studies investigating the clinical applicability of retinal endovascular surgery.

Conclusion

Prolonged retinal vein cannulation is possible and safe with the help of a camouflaged robotic device. The possibility of exposing the thrombus for a longer time to an active thrombolytic agent might increase the chance of successfully removing the clot in patients suffering from recent RVO.

References


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video Clip S1. Demonstration of functionalities of the robotic device and XYZ platform. First, the principle of working around a remote centre of motion (RCM) rendering a completely stable eye despite movements of the instrument is illustrated. Second, alignment of the RCM with the sclerotomy by using the orthogonal stage to position the entire robot (and thus RCM) with respect to the eye is shown.

Video Clip S2. Double puncture with subretinal injection. After repositioning of the needle, a successful retinal vein cannulation was performed.

Video Clip S3. Successful cannulation at the optic disc border with infusion of 10% Indian ink. Notice the stable position of the needle tip inside the vessel and on/off pattern of infusion.