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Volume and Composition of Reflux Following Intravitreal Injection

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Abstract

Purpose—To quantify the amount of drug loss from cadaveric human eyes which are injected via the pars plana with a known volume of dye at variable intraocular pressures.

Methods—Eight cadaver eyes were divided into two intraocular pressure groups: normal (15 mmHg, 4 eyes) or high (30 mmHg, 4 eyes). Each eye was injected with 50 μl of hematoxylin dye and the subsequent reflux was immediately collected on a Schirmers test strip. The test strip was scanned and digitally analyzed to determine the area of saturation and total color intensity present. Using a previously established equation, total volume of reflux and amount of dye within that reflux were calculated.

Results—The average total volume of refluxed fluid was 1.68 μL (median: 0.62 μL), with a range of 0 μL to 8.05 μL. The average volume of refluxed dye was 0.37 μL (median: 0.08 μL), with a range of 0 μL to 2.15 μL. On average only 0.74% of the original 50 μL of injected dye was lost (median: 0.15%), with a range from 0% to 4.30%.

Conclusion—Although the presence of subconjunctival bleb formation after intravitreal injection may be concerning to the clinician, our data shows that only a very small amount of the injected therapeutic agent is lost in the reflux.

Keywords

reflux; intravitreal injection

INTRODUCTION

Intravitreal injections are commonly used to treat a variety of ophthalmologic diseases. A frequent occurrence after these injections is the reflux of fluid which presents as a

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subconjunctival bleb.[1] The reflux is a mixture of fluid which includes injected drug and vitreous.[2–5] If a significant amount of drug refluxes, physicians are concerned that the effect of the agent could be diminished.[2] Previous studies have attempted to estimate this volume of reflux indirectly by measuring the diameter of the resulting bleb[2,3] or by radiolabeling the injected drug.[4] A direct measurement of the volume of reflux and the composition of lost therapeutic agent versus vitreous fluid has not been previously examined.

We have previously described a method for determining the volume and composition of reflux after intravitreal injection (injected agent or vitreous) in porcine eyes.[5] That study suggested that reflux is primarily composed of liquid vitreous and very little of the injected drug or added solution is lost to reflux. The previous study, however, did not account for anatomic differences between porcine and human eyes. The effect of differences in vitreous quality and scleral thickness between porcine and human eyes on reflux following intraocular injection is unknown. The purpose of the present study was to examine the amount and composition of refluxed liquid after intravitreal injection in human eyes.

METHODS

Eye Preparation

Cadaveric human eyes were obtained from the Lion's Eye Banks of Delaware Valley, Tampa, and Miami. Approval by the Institutional Review Board is waived for cadaveric eye research at the University of Pennsylvania. Consents for tissue use in research were completed by the respective Lion's Eye Banks prior to harvesting. Eyes were stored in cooled moist media without any preservation agents immediately upon harvesting of tissue. Eyes were allowed to equilibrate to room temperature prior to use in this study. Time from death to injection in donor eyes is listed in Table 1.

Conjunctiva and Tenon's capsule were dissected from the globe in a quadrant marked for intravitreal injection. A 23-gauge Alcon vitreoretinal cannula (Fort Worth, Texas) connected to an infusion line of Balanced Salt Solution (BSS) was inserted 180 degrees from the dissected quadrant and 4mm posterior to the limbus.

Eyes were assigned to either a normal intraocular pressure (IOP) group (15mmHg, 4 eyes) or a high IOP group (30mmHg, 4 eyes). No two eyes from the same donor were placed in the same group. The bottle height was adjusted to obtain intraocular pressures of 15mmHg (20.5 cm above the eye) or 30mmHg (40.8 cm above the eye) and confirmed by Tonopen (Medtronic, Minnesota). The infusion line was then clamped.

Injection Technique

A 1ml tuberculin syringe was filled with 0.3 ml of a prepared dye (1 part Hematoxylin and 5 parts BSS). A 30-gauge needle was marked 5mm from the tip to regulate injection depth. The syringe was primed with a volume of 0.05 ml of prepared dye. Excess dye was removed from the external surface of the needle with clean gauze.

The dissected quadrant (180 degrees from the infusion cannula) was dried with Weck-cel sponges and a position four millimeters posterior to the limbus was marked with an

ophthalmic caliper. 0.05 ml of dye was injected using a straight technique (needle insertion at 90 degrees to the sclera) into the vitreous cavity at a depth of five millimeters. Immediately after the needle was withdrawn, a Schirmer test strip (test strip; Tianjin Jingming New Technological Development Company, China) was placed on the injection site and held for 30 seconds to capture any reflux. Care was taken to not exert pressure on the globe during this procedure.

The test strip was then immediately scanned at 1,200 dots per inch resolution into a digital image with a Canon LIDE 210 scanner (Canon USA, Melville, NY).

Image analysis was performed using *ImageJ* software, a free image analysis software package produced by the National Institute of Health (http://imagej.nih.gov/ij). Using the method described in Brodie *et al.*[5], the area of saturation was measured in pixels. The refluxed volume was calculated from the area of saturation using a previously created regression equation from known quantities of the same dye on the same test strips: Volume (μL)= 0.00004043 × Area (pixels).[5]

In order to determine the proportion of injected dye in the reflux, the pixel intensity of the dye on the test strip was measured. For this calculation, the image was first converted to gray scale and the pixel intensity scale was inverted so that dyed portion had greater pixel intensity values. The total cumulative pixel intensity was measured for the saturated portion of the test strip. Then, to adjust for any background pixel intensity contributed by the test strip itself, the average intensity of a non-saturated portion of the test strip was multiplied by the area of saturation and then subtracted from the initial cumulative intensity measurement. This provided a total background-adjusted value for dye intensity.[5–8]

The amount of dye refluxed was calculated from the background adjusted intensity value using the previously established regression equation on known values of dye: Amount of Dye (μL) = 0.00000072 × Background Adjusted Pixel Intensity.[5]

The Kruskal-Wallis rank test was used to compare the total volume of refluxed fluid and the proportion of dye in the refluxed fluid between different IOP groups. A P-value of less than 0.05 was considered significant. STATA® 12 (College Station, Texas) software was used for all statistical analyses.

RESULTS

Eight cadaveric eyes were injected with 50 microliters of dye to quantify the volume and composition of the resultant reflux (Table 1). The eyes came from donors ages 60 – 92 years (mean = 79 years). Eyes were injected 33 – 288 hours (mean = 87 hours) from donor death. The mean calculated total volume of refluxed fluid was 1.68 μL (SD: 2.65 μL) with a median of 0.62 μL (range: 0 μL – 8.05 μL). The mean calculated volume of refluxed dye was 0.37 μL (SD: 0.73 μL) with a median of 0.08 μL (range of 0 μL – 2.15 μL). The mean composition of the reflux was 14.07% dye (SD: 12.09%) with a median of 9.67% (range of 0% – 35.53%). From the 50 μL injection volume, the mean amount of dye lost to reflux was 0.74% (SD: 1.46%) with a median of 0.15% (range: 0% – 4.30%). There were no significant

differences in total volume of reflux and volume of refluxed dye between IOP group ($p>0.14$ for both comparisons).

DISCUSSION

The occasional clinical observation of the formation of a subconjunctival bleb after intravitreal injection raises concerns of loss of therapeutic dose. The delivery of a sub-therapeutic dose to the vitreous cavity could result in decreased efficacy of treatment or shorter duration of effect. In addition, there has been considerable recent discussion on the observation of tachyphalaxis during anti-VEGF therapy.[9–11] Loss of medication during treatment could be a confounding factor related to this issue. Despite these clinical observations, our data shows that very little of the injected volume is lost to reflux in human eyes. On average, only 0.74% of the original 50 μL injection was lost to reflux, with a maximum loss of 4.30%. Our results are similar to previous studies in porcine models that suggest that the amount of reflux following intravitreal injection is minimal.[5,12]

Measurement of reflux using digital image analysis was originally described by our group using a porcine model.[5] In that study of 20 pig eyes, we found that the reflux was predominantly composed of vitreous and that the average loss of injected dye was less than 1% of the injected volume. However, it was unclear whether differences in the composition vitreous and sclera between young porcine eyes and human eyes would be significant. The previous study was a pilot study to validate a method of quantitating reflux from intravitreal injection. The purpose of this study was to investigate the actual amount and composition of reflux in a human model. Data between the studies were fairly consistent: the average volume of total reflux was 1.68 μL in human eyes versus 1.19 μL in the pig eyes average refluxed dye was 0.37 μL in human eyes versus 0.47 μL in the pig eyes. Importantly, the average amount of injected dye lost in the human eye study was again less than 1%.

While the time from death to injection appears to be positively correlated with larger volumes of total and dye reflux as the 2 largest volumes for each were also the 2 eyes with the longest death to injection times, our numbers are not large enough to draw any conclusions from this observation. Previous work has shown that storage of post-mortem eyes decreases vitreous viscosity.[13] Cold storage of eyes, however, has shown to reduce degradation of vitreous viscosity and colder temperatures are known to increase vitreous viscosity.[13,14] Although eyes were allowed to equilibrate to room temperature in our study, eyes were not warmed to physiologic temperature and vitreous temperature was not measured. Thus, the effect of vitreous temperature on reflux after intraocular injection was not measured and could be future areas of investigation. Similarly, although anti-VEGF agents are routinely refrigerated prior to injection, the dye in our study was maintained at room temperature throughout the experiment. This could potentially bias the results towards a less viscous injection and larger volumes of reflux, however given that the injected dye was a minor component of the reflux, this is less concerning.

Initially, we intended to examine the effect of IOP on reflux, but again, our sample size was not large enough for meaningful comparisons ($p>0.14$ for both comparisons).

While our method provides a novel way of quantifying total reflux and specifically the loss of the injected agent, there are several considerations that may limit generalizability to clinical practice. The effect of vitreous syneresis on reflux from intravitreal injection is unknown. Younger eyes have more formed vitreous bodies, and the results of this study with an average age of 79 may not apply to a younger demographic who may require intravitreal injections for different clinical indications compared to older populations.

There are a few additional possible weaknesses that should be addressed. As discussed in the prior work using this method, cleaning the needle to remove excess dye could potentially absorb some of the dye out of the primed needle.[5] This would result in a smaller injected volume and potentially a smaller calculated proportion of reflux that is lost during injection. Conversely, the placement of the test strip directly onto the scleral after injection may wick out additional reflux that would not have left the eye during actual clinical practice. This would result in a larger calculated volume of reflux than might normally occur. However, given the already small volumes of reflux found, this is likely not a significant effect. Finally, any differences in molecular movement through a sclerotomy between hematoxylin dye and various therapeutic agents are unknown.

In summary, this study presented data from 8 cadaveric eyes using digital image analysis to measure the volume and composition of reflux following intravitreal injection. The data suggest that only a small amount, less than 5% of the original 50 μL injection, is lost to reflux. These data should be reassuring to clinicians when post-injection reflux or subconjunctival blebs are seen in clinical practice following intravitreal injection.

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Table 1

Reflux Data from 8 Human Cadaver Intravitreal Injections

Eye #	Donor Age (years)	Time from Death to Injection (hours)	IOP (mmHg)	Injection Depth (mm)	Injection Volume (μl)	Total Reflux Volume (μl)	Dye Refluxed (μl)	% Dye in Reflux	% of Injection Lost to Reflux
1	60	144	15	5	50	0.51	0.18	35.53%	0.36%
2	87	288	15	5	50	8.05	2.15	26.69%	4.30%
3	76	33	15	5	50	2.10	0.41	19.35%	0.81%
4	84	52	15	5	50	0.59	0.03	5.53%	0.07%
5	74	40	30	5	50	0.00	0.00	0.00%	0.00%
6	92	53	30	5	50	0.64	0.04	6.08%	0.08%
7	76	33	30	5	50	0.50	0.04	8.53%	0.09%
8	84	52	30	5	50	1.04	0.11	10.82%	0.22%

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