



EXPERIMENTAL MODEL OF INTRAVITREAL INJECTION TECHNIQUES

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Purpose: To evaluate the amount of drug reflux and vitreous leakage from the needle tract after various intravitreal (IVT) injection techniques in porcine cadaver eyes.

Methods: The reflux after IVT injection was quantified by methylene blue injection through the pars plana of fresh pig eyes (0.05 mL per eye, $n = 150$) and the vitreous incarceration measured after balanced salt solution (BSS) IVT injection (0.05 mL per eye, $n = 150$) into eyes with vitreous previously stained with methylene blue. Blue spots observed on the ocular surface after injection quantified both reflux and vitreous incarceration. We tested different needle sizes (27, 30, and 32 gauge) and different techniques (depth and speed of injection). We used an ocular endoscope to observe the flow and diffusion of injected methylene blue and the vitreous incarceration at the puncture site after IVT injection using the different techniques.

Results: Thirty-gauge needles showed less drug reflux than the 32-gauge or 27-gauge needles ($P < 0.01$). Thirty-two-gauge needles demonstrated less incarceration of vitreous at the tract site ($P < 0.01$), but with the endoscope, all needle tracts showed vitreous incarceration at their internal aspect. Deep IVT injection showed less reflux than superficial IVT injection, but vitreous incarceration did not differ. The delay between the scleral puncture and the injection did not modify the reflux or the vitreous incarceration.

Conclusion: Thirty-gauge needles and deep placement of the needle tip into the vitreous before injection may reduce reflux and vitreous incarceration. This could maximize the therapeutic effect of IVT injection and may decrease the rates of severe complications such as retinal detachment and endophthalmitis.

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Intravitreal injection is a routinely performed ophthalmic procedure. Usually, the indications for IVT injection are treatment of age-related macular degeneration with medications designed to block the effects of vascular endothelial growth factor and vitreous inflammation, endophthalmitis, or macular edema. However, drugs are injected directly into the vitreous cavity through the pars plana via a needle. Intravitreal drug injections may considerably limit systemic side effects and bypass the blood–ocular barrier by delivering drugs adjacent to the intraocular tissues. However, some complications related to the injection have been described, including cataract formation, in-

crease in intraocular pressure (IOP), uncontrolled drug reflux responsible for subconjunctival bleb formation, retinal detachment, vitreous hemorrhage, and endophthalmitis.^{1–6}

The safety and efficacy of IVT injections of anti-vascular endothelial growth factor medications have been assessed for the treatment of exudative age-related macular degeneration.^{7–9} Nevertheless, these studies did not compare varying parameters of the IVT injection procedure itself. Thus, while some general guidelines exist, the exact details of needle depth, needle size, speed of injections, and other variables are typically at the discretion of the injecting ophthalmologist and could differ dramatically.¹⁰ Drug reflux through the injection site may limit the therapeutic efficacy of medications delivered by this route.^{11,12} Vitreous incarceration in the scleral wound after an IVT injection procedure may predispose the eye to retinal detachment or endophthalmitis. The purpose of this study was to evaluate various techniques for IVT

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Table 1. Characteristics of Each Subgroup Designed for Drug Reflux Evaluation (Group A) and Vitreous Incarceration Evaluation (Group B): Division of Study Eyes

Group Name	Injection Depth	Waiting Time (Seconds)	Withdrawal Pressure	Needle Gauge
A1, B1	Deep	3	No	27, 30, 32
A2, B2	Deep	3	Yes	27, 30, 32
A3, B3	Deep	None	No	27, 30, 32
A4, B4	Shallow	3	No	27, 30, 32
A5, A5	Shallow	None	No	27, 30, 32

injection using a pig eye model to explore the reflux and incarceration rates associated with the procedure.

Methods

Eye Preparation

We evaluated the different IVT injection techniques using 327 freshly harvested pig eyes (Sierra for Medical Science, Whittier, CA), according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. The porcine eyes were affixed to styrofoam platforms with pushpins placed through the Tenon capsule. We filled eyes with either BSS or methylene blue (to both fill and stain the vitreous) to stabilize the IOP between 15 mmHg and 23 mmHg. Volumes of BSS injected to regulate IOP ranged between 0.5 mL and 1.5 mL per eye. The methylene blue injection to stain the vitreous was injected at least 30 minutes before the experimental IVT injection of BSS. Intraocular pressure was verified to be between desired IOP range by using a tonopen (Medtronic, Minneapolis, MN) before injection. All intraocular injections were completed within 1 minute of the IOP check.

Design of the Study

Drug reflux was investigated in Group A, which consisted of 150 eyes (Table 1). After filling the eyes with BSS, we injected 10% methylene blue in the vitreous to study the reflux of injected volumes. Vitreous incarceration was assessed in Group B, which

consisted of 150 eyes (Table 1). We injected BSS to assess the extrusion of the previously methylene blue-stained vitreous. In Groups A and B, 5 subgroups ($n = 30$ eyes per subgroup) using 27-gauge, 30-gauge, and 32-gauge needles ($n = 10$ eyes per size) were defined to assess each injection parameter (needle depth, waiting time of ~ 3 seconds, injection pressure).

Fluid dynamics during IVT injection were evaluated in Group C, which consisted of 27 eyes (Table 2). We used a 20-gauge lighted ocular endoscope (model E2, probe ref OME 200 SMA-EA, Endo-optics, Little Silver, NJ) inserted through the pars plana at the 12-o'clock position to directly observe the fluid flow into the vitreous, vitreous incarceration, and drug reflux during IVT injection performed at the 3-o'clock position. We injected 10% methylene blue to study the reflux ($n = 18$ eyes) or IVT triamcinolone acetate to better visualize the vitreous incarceration ($n = 9$ eyes). In Group C, 3 subgroups ($n = 9$ eyes) using each needle size ($n = 3$ eyes per size) were defined to assess depth and waiting time. We endoscopically videotaped the IVT injection procedures to document the fluid dynamics inside the eye.

Technique of Injection

For the IVT injection procedure assessment, injections were performed at the 9-o'clock position 3.5 mm posterior to the limbus. Into each eye, we injected a planned volume of 0.05 mL of BSS (Group B) or 10% methylene blue (Groups A and C). We used 27-gauge (305106, Becton, Dickinson & Co., Franklin Lakes, NJ), 30-gauge (305109, BD), and 32-gauge needles

Table 2. Characteristics of Each Subgroup for Intravitreal Injections Observed by Ocular Endoscope

Group Name	Injection Depth	Waiting Time (Seconds)	Injected Substance	Needle Gauge
C1	Deep	3	Methylene blue	27, 30, 32
C1	Deep	3	Triamcinolone	27, 30, 32
C2	Deep	None	Methylene blue	27, 30, 32
C2	Deep	None	Triamcinolone	27, 30, 32
C3	Shallow	None	Methylene blue	27, 30, 32
C3	Shallow	None	Triamcinolone	27, 30, 32

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Table 3. Statistical Comparison of Various Needle Gauges and Injection Parameters Regarding Drug Reflux and Vitreous Incarceration

Variable Comparison	Outcome Measure	Chi Square	P
27-gauge versus 30-gauge needles	Reflux	19.253	<0.001
	Incarceration	0	1
27-gauge versus 32-gauge needles	Reflux	3.56	0.059
	Incarceration	0	1
30-gauge versus 32-gauge needles	Reflux	7.441	0.006
	Incarceration	0	1
Deep versus shallow injection	Reflux	4.94	0.026
	Incarceration	0.015	0.902
Waiting versus no waiting before withdrawal	Reflux	1.504	0.22
	Incarceration	0.334	0.563
Pressure on plunger versus no pressure	Reflux	0.089	0.765
	Incarceration	4.29	0.038

(Steriject 3213, TSK, Tochigi, Japan). All needles were 12 mm in length. After drawing the methylene blue or BSS into the syringe through the needle, we cleaned the tip of the needle gently to eliminate any droplet present. Deep injections were performed by inserting at least two thirds of the needle length (8 mm) through the pars plana into the vitreous cavity, and shallow injections were performed by inserting the needle less than $\frac{1}{3}$ of the needle length (4 mm) into the vitreous.

The waiting time between the fluid injection and the removal of the needle from the globe was instantaneous for “no wait” injections and ~3 seconds for delayed removal technique or “wait” injections.

We also evaluated the effects of pressure on the syringe plunger for the fluid injections. In the group labeled “pressure,” we applied pressure on the plunger from the end of the injection until the removal of the needle was complete.

As a preliminary sham control group, we used syringes filled with 10% methylene blue topped with 3 gauges of needles (27, 30, 32). We touched the needle tips to the sclera but did not penetrate it. The procedure was repeated 10 times with each needle.

Scoring of the Fluid Reflux and the Vitreous Incarceration

We scored the methylene blue reflux or vitreous incarceration by measuring the diameter of the blue spot size visible at the entry site. No blue spot observed at the needle entry site was given a score of zero. A trace punctate blue spot was scored as 1, whereas a clearly visible spot <2 mm in diameter was scored as 2. Spots >2 mm were graded as 3.

The authors used a calibrated micropipettor (Eppendorf, Haverhill, MA) to measure the volume of methylene blue needed to obtain the blue spots graded from

1 to 3 on a pig eye sclera. Grade 1 corresponded just to a “trace” of methylene blue, Grade 2 corresponded to a volume of 0.5 μ L of methylene blue, and Grade 3 corresponded to a volume >1.5 μ L of methylene blue.

We performed data analysis using the SAS software (SAS Institute, Cary, NC). Comparisons were made using chi-square and Fisher exact tests when appropriate. We intended to test each hypothesis individually, and we considered $P < 0.05$ as statistically significant.

Results

The results of the experiments regarding the effect of needle size and injection technique on fluid reflux and vitreous incarceration are summarized in Table 3. In the sham control group, we did not observe a blue spot in any of the three needle gauges tested.

Evaluation of the Drug Reflux

When comparing needle sizes based on the overall amount of reflux (none or Grade 1 versus Grade 2 or 3+), there were differences observed between needle gauges. Thirty-gauge needles showed less reflux than 32 gauge ($P < 0.01$). Thirty-gauge needles also showed less reflux than 27 gauge ($P < 0.01$). Thirty-two-gauge needles tend to have less reflux than 27 gauge as well, but not significantly ($P = 0.06$).

We observed no difference in reflux whether we waited 3 seconds before withdrawing the needle from the scleral puncture versus immediate withdrawal ($P = 0.22$). Similarly, pressure on the syringe plunger while withdrawing the needle did not seem to alter the reflux profile of any given technique or needle size ($P = 1.0$). However, inserting the needle more than $\frac{2}{3}$ of its length through the vitreous showed less reflux of injected substance than inserting the needle less than $\frac{1}{3}$ length ($P = 0.03$).

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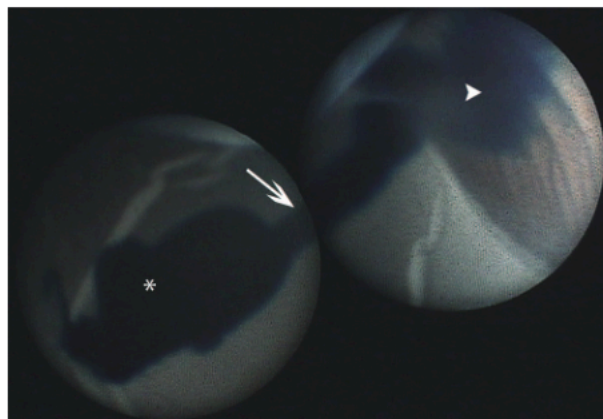


Fig. 1. Accumulation and mild diffusion of the drug (*) is visible at the end of the funnel (arrow) created by the needle bevel at the time of the injection. Note the reflux of the drug along the funnel (arrow) toward the entry site (arrowhead).

Evaluation of the Vitreous Incarceration

We found differences in vitreous incarceration through the needle tract between needle sizes. The amount of blue-stained vitreous present at the pars plana injection site or needle wound was significantly lower after injection with a 32-gauge needle than with a 30-gauge or 27-gauge needle injection when considering whether any blue-stained vitreous was present at all at the external entry site (Grade 0 versus others; $P < 0.01$).

There was no difference in the incarceration of blue-stained vitreous observed whether the needle tip was placed deep or superficial in the vitreous cavity ($P = 0.9$). Similarly, there was no difference in vitreous incarceration regardless of whether we allowed a 3-second delay to elapse after the injection and before removal of the needle ($P = 0.56$). Pressure on the syringe plunger while withdrawing the needle from the globe was associated with higher incarceration rates ($P = 0.01$).

Evaluation of Fluid Dynamics With the Ocular Endoscope

Examination of IVT injections with the ocular endoscope confirmed the observations from Groups A and B. The injected methylene blue was confined to the tract created by the needle and a small area of surrounding vitreous (Figure 1). Vitreous was noted to be adherent to the internal aspect of the entry site wound, if only a very mild amount, in 100% of injections performed under observation with the endoscope. Interestingly, some reflux from the vitreous cavity to the needle tract was also visualized after injection of triamcinolone acetate (Figure 2).



Fig. 2. Endoscopic injection of triamcinolone acetate staining vitreous incarcerated in a needle wound.

Discussion

The results of this study showed that varying methods of fluid (drug) injection into the vitreous cavity through the pars plana can alter the amount of injected fluid reflux and vitreous incarceration. Our study assesses multiple IVT injection factors such as needle gauge, depth of insertion, and time to injection, as well as reflux of fluid and vitreous from the needle tract.

This study used a porcine eye model for the IVT injections. Pig eyes are easy to obtain and are widely used for ophthalmic surgical training because of their anatomic similarities to human eyes. The use of methylene blue to stain the vitreous and as a proxy for IVT antivascular endothelial growth factor medication made reflux or vitreous incarceration easy to identify against the white background of the sclera.

When performing IVT injection, clinicians aim to deliver the proper amount of the drug close to the targeted tissue and to avoid complications. Reflux of drug may jeopardize the quantity of active therapeutic agent and the effectiveness of the injection. The ensuing vitreous incarceration may increase the risk of retinal detachment or cystoid macular edema.^{1–6} Moreover, inflammation or endophthalmitis could be promoted by a vitreous wick at the incision site.

In the analysis of the data, we chose to stratify the scoring of fluid reflux slightly differently than vitreous incarceration. When evaluating reflux, the needle filled with methylene blue inevitably has some methylene blue present on the tip or shaft of the needle

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from drawing up the drug. This may lead to slight staining that might just reflect the scleral entrance site even if no reflux is truly present. Therefore, Grade 0 (no blue spot visualized) and Grade 1 (trace blue spot seen) were actually considered as no reflux. Because of this, Grade 0 and Grade 1 versus Grades 2, 3, and 4 were the 2 categories used in the statistical tests for the associations of reflux with needle size and injection technique.

A volume of 0.05 mL was used because this is the standard amount of drug given during an IVT injection of ranibizumab or bevacizumab for age-related macular degeneration. These medications are the most commonly used in IVT injections.

The sham control group, in which the needle tip containing methylene blue was simply touched to the ocular surface, ensured that just touching the sclera with the tip of the needle would not interfere with our grading scale of the blue spot on the ocular surface.

Based on our observations of methylene blue reflux on the ocular surface and the diameter of the blue spot seen on the ocular surface, the amount of drug reflux (Grade 0–Grade 4) is ~0 mL to 0.003 mL (<10% of the amount injected). It has been observed that the complete amount of drug injected is highly variable even when performed with an incrementally marked syringe by a trained surgeon.^{11,12}

There were differences in the amount of liquid reflux among the different needle sizes. The diameter of a 27-gauge needle is 0.360 mm (surface area of 0.101 mm²), whereas the diameter is 0.255 mm (surface area of 0.05 mm²) for 30-gauge and 0.200 mm (surface area of 0.031 mm²) for 32-gauge needles. This makes the scleral wound created by a 27-gauge needle twice as large as a 30-gauge scleral puncture wound. Twenty-seven-gauge needles had greater reflux than 30-gauge needles, probably because the scleral wound created by the 27 gauge is a larger opening. Interestingly, the 32-gauge needles also had greater reflux than the 30-gauge needles. One plausible explanation is that the path of the needle creates a core into the vitreous gel that is larger for a 30-gauge needle than a 32 gauge. This creates more space for the drug to stay in the eye and probably less pressure at the wound site. There may be a balance between these two factors having opposite effects to explain the reflux observations. It is possible that the entry wound size of 27-gauge needles is large enough that it overcomes the benefit of the larger vitreous core space created by placement of the wider 27-gauge needle, thus leading to more reflux and incarceration. However, the self-sealing properties of the scleral wound created with the 32-gauge needle we used for this

experiment may not be as good as those obtained with the 30-gauge needle, leading to more reflux.

Insertion of the needle at least two thirds of the way to the hub was associated with less drug reflux. This finding may also be explained by the fact that deeper insertion created a larger core in the vitreous gel, inside which the drug was deposited. In contrast, a waiting time or delay after injection of the fluid did not affect reflux. Examination with the ocular endoscope helped to analyze the dynamics of the fluid injection. Endoscopic evaluation demonstrated that the injected fluid stayed localized within the needle tract and surrounding vitreous gel immediately after injection. This may explain the observation that waiting a few seconds before removing the needle did not alter the amount of reflux. Diffusion of the drug through the vitreous gel to the targeted tissue likely occurs over a much longer period of time.

Recently presented data suggest that fluid reflux is greater with 32-gauge needles compared with 30-gauge needles.¹³ However, the authors of this study graded reflux by the size of the conjunctival bleb over the scleral wound and not by observation of colored fluid reflux as with our study. It is possible that that study and our study are, in reality, measuring two different things.

Many factors may affect the intraocular pharmacokinetics and distribution of injected drug. These factors may include ocular volume (myopia), vitreous characteristics (syneresis or posterior vitreous detachment), previous ocular surgery (aphakia or vitrectomy) among others. Given the pharmacokinetic strategy used with monthly or periodic injections, namely, bolus followed by exponential decay, it is feasible to hypothesize that when even 10% of the drug is lost at the time of injection, the pharmacokinetic curves may be lowered enough to alter clinical outcomes in certain circumstances. Thus, drug loss to reflux at the time of injection may play a significant role affecting a given outcome or treatment failure.

One may argue that injecting BSS into the vitreous cavity to regulate IOP changed the consistency of the vitreous gel before the experimental injections, thus contaminating the data. However, the injected volumes of BSS were quite small relative to the overall volume of vitreous. In addition, the experimental injections were performed 180° away from the injection of BSS, thus measuring reflux and vitreous incarceration on relatively pristine vitreous. In the compromise between altering vitreous consistency and mild hypotony, we felt the avoidance of hypotony more important in making the animal model more physiologic.

Although it is true that IOP may affect the rate of incarceration, the IOP in our studies eyes was mea-

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sured to be within normal limits between 15 mmHg and 20 mmHg at the time of injection. Most eyes of otherwise normal human patients will also be at this level at the time of the injection.

A fair criticism of these experiments centers on the variability of human vitreous in the elderly as opposed to freshly harvested pig eyes where the vitreous is relatively more formed and a posterior vitreous detachment is uncommon. However, patients with diabetic macular edema or vein occlusions may be a bit younger and without a posterior vitreous detachment. Therefore, they may be more closely approximated by pig vitreous. Furthermore, posterior vitreous detachment appears less commonly among patients with exudative age-related macular degeneration than patients with dry macular degeneration¹⁴ and may modify the response of the disease to treatment.¹⁵

When observing the vitreous incarceration experiment results, we needed to assume that virtually the entire vitreous volume was stained with methylene blue before injecting BSS. For this reason, we waited at least 30 minutes after the initial methylene blue injection before studying the eyes for vitreous incarceration. Regarding vitreous incarceration, 32-gauge needles showed less incarceration (visible on the ocular surface/Grade 1 or greater) than the 30-gauge or 27-gauge needles. This finding is likely because the entrance wound from the thinner needle is much smaller than a wider needle, which does not allow as much formed vitreous to exit the globe. Therefore, a smaller scleral wound acts as more of a barrier to the semisolid vitreous gel than the liquid drug. The semisolid character of the vitreous gel may also help explain the observation that delaying needle removal and depth of injection did not seem to affect the amount of vitreous incarceration.

When we performed an examination using the ocular endoscope, it became clear that some amount of vitreous incarceration is always present at the needle tract, even if only a minute amount. This type of minute amount of vitreous coming to the internal portion of the sclerotomy correlated with Grade 0 as observed on the ocular surface. This observation is in keeping with seminal pars plana entry site studies performed by Kreiger, showing an essentially universal rate of vitreous incarceration at surgical sclerotomies.^{16–18} In the surgical entry site studies, the vitreous wick is not truly present at the ocular surface because the conjunctiva is closed, so risk of endophthalmitis may be minimized. Using the endoscope to better observe the dynamics of the drug in the vitreous cavity helped us to understand and validate the results of the study.

The second type of vitreous incarceration observed with the endoscope is obvious dragging or streaming of the vitreous gel into the scleral wound. This type of vitreous capture is consistent with the observation of Grade 1 or higher of methylene blue at the ocular surface. Such vitreous wicks could serve as an entry point for bacteria, which highlights the risk of endophthalmitis after IVT injection. It is intriguing that the rate of endophthalmitis is so low in published reports given the high prevalence of vitreous incarceration. Nonetheless, these data emphasize the need for meticulous preparation and aseptic technique and may also suggest a need for conjunctival displacement when performing an IVT injection. The conjunctiva is another layer covering the scleral wound at least partially that could considerably limit the risk of endophthalmitis. Furthermore, dragging of vitreous toward the wound might result in early or late retinal tears because of the forces that occur acutely and as healing progresses.¹⁷

We hypothesized that a small amount of suction could be created at the needle tip when keeping pressure on the syringe plunger before withdrawing the needle. This could theoretically cause more reflux or incarceration. Although pressure on the plunger when removing the needle did not affect the rate of reflux, pressure was associated with greater vitreous incarceration. Minimal reduction in manual pressure on the syringe plunger while withdrawing the needle may lead to incarceration of vitreous gel into the needle and consequently into the sclerotomy. When the results of pressure versus no pressure were stratified by needle gauge, there was no significant difference in incarceration between the different needle sizes.

One might intuitively expect more reflux and more incarceration with the same needle type. We hypothesize that the reason for this difference is that vitreous gel is different than fluid and that incarceration and reflux are different phenomena. During the removal of the needles, vitreous incarceration was seen with the endoscope in 100% of the eyes. Partial or complete plugging of the sclerotomy site with vitreous gel may consequently decrease drug reflux while increasing the observed vitreous incarceration at the sclerotomy site. This would explain the seemingly disparate results between reflux and incarceration.

Several recent studies have evaluated the reflux of drugs such as pegaptanib, bevacizumab, and triamcinolone after IVT injection using an oblique needle insertion.^{19,20} Other authors have advocated displacement of the conjunctiva with a cotton tip application or forceps before IVT injection.²¹ Although these authors showed a mild reduction in drug reflux or subconjunctival bleb formation, these methods are not

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routinely performed in a clinical setting. We elected not to evaluate the use of oblique insertion or conjunctival displacement because of sample limitations and the relative scarcity of this technique in actual clinical practice.

New types of IVT drug therapies and delivery systems are on the horizon, and the indications for IVT injections continue to evolve.²² Understanding the flow of the drug inside the globe after the different techniques of IVT injection has important implications for clinical care. Given the small amount of drug used in IVT injections, it is important to maximize delivery to the target tissues. In addition, minimizing vitreous incarceration may help to decrease the rate of serious complications. Evaluation with the ocular endoscope showed vitreous adherence to the entry site, which was present in a small amount with every injection. The use of 30-gauge needles and placement of the needle tip deep into the vitreous before injection may decrease reflux and maximize the therapeutic use of each injection.

Key words: intravitreal injection, vitreous leakage, anti-vascular endothelial growth factor, choroidal neovascularization, drug administration, intraocular drug administration.

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