The Effect of an Air Bubble in the Anterior Chamber on the Change in Intraocular Pressure (IOP)

Amir Samsudin¹,²,³*, Ian Eames², Steve Brocchini³,⁴ and Peng Tee Khaw³

¹Department of Ophthalmology, University of Malaya, Kuala Lumpur, Malaysia.
²UCL Department of Mechanical Engineering, London, United Kingdom.
³NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom.
⁴UCL School of Pharmacy, London, United Kingdom.

Authors’ contributions

This work was carried out in collaboration between all authors. Author AS designed the study, analysed the data, and wrote the first draft of the manuscript. Authors IE, SB and PTK all assisted in the study design and corrected the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Purpose: To compare rate of change in IOP with and without air bubbles in the anterior chamber (AC).

Methods: Enucleated porcine eyes were infused with Balanced Salt Solution (BSS) at 10µl/min. In one experiment, 5 eyes each were injected with 0.15 or 0.30ml bubbles without prior removal of aqueous humour while 5 controls were not injected. In another experiment, 9 eyes were injected with 0.30ml bubbles with prior removal of 0.30ml aqueous humour while 8 controls were not injected. The rate of change in IOP from 5mmHg to 20mmHg was compared. Statistical analysis involved the unpaired t-test and one–way ANOVA. P<.05 was considered statistically significant.

Results: In the first experiment, after initial spikes IOP settled to +3.5+/-3.4mmHg and +4.7+/-5.1mmHg from the original baseline with 0.15ml and 0.30ml respectively (P=.13). The rate of change was 0.25+/-0.09mmHg/min for controls, 0.31+/-0.12mmHg/min for 0.15ml and 0.46+/-0.10mmHg/min for 0.30ml. The difference between the control and 0.30ml groups was significant (P=.02). In the second experiment, IOP after injection was

*Corresponding author: E-mail: amir@ummc.edu.my;
5.3+/−1.6mmHg compared to 5.9+/−0.7mmHg in the control group (P=0.30). The rate of change in IOP was 0.28+/−0.09mmHg/min with bubbles and 0.30+/−0.08mmHg/min without (P=0.68).

Conclusion: An air bubble in the AC does not affect the rate of increase in IOP.

Keywords: Aqueous humour; anterior chamber; intraocular pressure; IOP; trabeculectomy; glaucoma.

1. INTRODUCTION

One of the common complications of trabeculectomy is immediate postoperative hypotony or low intraocular pressure (IOP) [1,2]. This occurs due to various reasons including ciliary body shutdown, overfiltration and wound leaks. Prolonged hypotony may in turn lead to serious sequelae such as choroidal effusion, macular hypotony, corneal oedema, synechiae formation, cataracts and loss of the filtering bleb [3,4]. Not infrequently, an air bubble is injected into the anterior chamber (AC) at the end of surgery as a tamponade to prevent the chamber from shallowing for the first 1-2 days. Surface tension and buoyancy of the bubble would help to maintain the space between the cornea, iris and lens [5]. Air bubbles are also injected into the AC for treatment of Descemet’s membrane tears after cataract surgery [6,7], prevention of Staphylococcus epidermidis endophthalmitis [8], treatment of corneal hydrops in keratoconus [9], and during Deep Lamellar Endothelial Keratoplasty (DLEK) and Descemet’s Stripping Automated Endothelial Keratoplasty (DSEK) to attach donor endothelium [10].

Air bubbles in the AC can affect IOP. Landry et al. reported that air bubbles of 0.7ml in feline eyes caused IOP to increase transiently by around 10mmHg, but this returns to its baseline level within 24 hours [11]. In DSEK, when a full AC bubble is injected, IOP can increase by 40-60mmHg on digital palpation. This is maintained only for a few minutes however, until the air is replaced by BSS from an infusion [12]. Miyata et al. reported that air bubble injections did not cause ocular hypertension, but they removed 0.1ml of aqueous humour from the AC prior to injecting their 0.1ml air bubble [9]. Other complications of air bubbles in the AC include endothelial damage [13-15], pupillary block [12,16,17], anterior subcapsular cataract formation [18] and fixed dilated pupils secondary to iris ischemia (Urrets-Zavalia syndrome) [19]. Additionally, they have also been reported to cause iridocorneal adhesion and secondary angle closure glaucoma in cases where they migrate behind the iris [20].

With air in the AC, Landry et al. found a >50% decrease in bubble size after 24 hours, although after that the remaining air lasted up to 9 days before completely disappearing [11]. Thompson reported that the half-life of air in the eye was 1.6 days in phakic and 0.9 days in aphakic eyes [21]. The absorption of an intraocular gas bubble depends on the rate at which the molecules of gas leave the bubble and diffuse into the surrounding tissue fluid [22]. IOP creates a pressure gradient which facilitates this diffusion. The higher the IOP, the higher the pressure gradient becomes. This increases the rate of gas diffusion from the bubble into the surrounding fluid. As this gas accumulates in the surrounding fluid, the pressure gradient becomes lower. Fluid flow around the bubble removes the accumulated gas and restores the pressure gradient. As such, higher IOP is associated with a shorter half-life of an intraocular gas.

In this experiment, changes in IOP with air bubbles in the AC are described. We aimed to see if there was modulation of IOP by these bubbles and whether there was a difference in
the rate of change in IOP with and without them. This information would be useful for surgeons while performing glaucoma surgery and also anterior segment surgery in general, in deciding whether to leave an air bubble in the AC after surgery.

2. MATERIALS AND METHODS

The intracameral pressure sensing equipment comprised of a 27-gauge winged cannula (0.42mm outer diameter, 0.21mm inner diameter; Becton Dickinson & Co, NJ, USA) connected via a 3-way tap to an infusion pump (Cole-Parmer, IL, USA) and a pressure transducer (model 162PC01D; Honeywell International, NJ, USA) connected to an interface board/ voltmeter (model VM110; Velleman NV, Gavere, Belgium) and personal computer (PC) (Fig. 1a). Balanced Salt Solution (BSS; Alcon Laboratories, Inc., Texas, USA) and non-expansile, low compliance silicone tubing (outer diameter 6mm, internal diameter 3mm; Alcon Laboratories, Inc., Texas, USA) was used throughout the system.

After calibration of the pressure transducer, we measured the resistance of the 27-gauge cannula and silicone tubing. This was done by infusing BSS at 1, 2, 4, 8 and 16µl/min through the system with the cannula tip opening into a beaker. Again, the cannula tip and the pressure transducer inlet port were positioned at the same level. The resistance was determined from the slope of the pressure vs. flow rate line. Compliance of the silicone tubing was also measured. We infused 10, 20, 30 and 40µl of BSS into the system with the cannula blocked and measured the resulting pressure changes. The compliance was derived from the slope of the volume infused vs. pressure line.

Eyes from 6-12 month old mixed breed pigs were used (Fig. 1b). Whole pig heads were obtained from an abattoir and the eyes enucleated and used within 36 hours of slaughter [23]. The eyes were discarded if they had any discernible damage on them. They were mounted facing forward on an acrylic block in a covered and moistened container at a room temperature of between 20-21ºC. The 27-gauge winged cannula was inserted through the inferior part of the cornea, near and parallel to the inferior limbus and positioned in the posterior chamber to prevent deepening of the AC and artefactual increase in outflow facility [24,25]. Prior to the main study, we measured ocular outflow facility (the reciprocal of outflow resistance) in 4 eyes. We used a constant flow perfusion method where BSS was infused into the eye at 2.0, 2.5 and 3.0µl/min and the equilibrium IOP noted. Outflow facility was determined from the slope of the flow rate vs. equilibrium IOP line.

This experiment was designed to look at two conditions. In the first one, there was no removal of aqueous humour prior to the air bubble injection so that there was a higher volume of the AC. In the second, a similar volume of aqueous humour to the air bubble to be injected was removed so that the volumes of the AC were similar between the no air bubble and air bubble groups.

2.1 Higher AC Volume

5 eyes were used for each of these groups–no air bubble (control), 0.15ml air bubble and 0.30ml air bubble. There was a large variation in initial IOP (Table 1) so we decided to prime these eyes prior to their usage. To do this, we infused BSS at a rate of 10µl/min (0.6ml/h) until their IOP reached 15mmHg. At that point, the infusion was stopped to let the IOP decrease to 5mmHg. For the eyes without air bubble injections, the infusion was restarted at 10µl/min and the time needed for the IOP to increase to 20mmHg was noted. For the eyes
with air bubble injections, the pre-determined amount of air was injected over 1 second into the superior part of the AC using a separate 30-gauge needle (0.32mm outer diameter, 0.16mm inner diameter; BD, NJ, USA) before the infusion was restarted at 10µl/min. The immediate post-injection IOP change was noted and also the time taken for IOP to increase to 20mmHg.

Fig. 1. a) Photograph of actual setup (without moisture container). b) Close-up view of porcine eye with air bubble in the AC. The cannula tip was in the posterior chamber.
Table 1. Data for all eyes (higher AC volume experiment)

| Eye   | Initial IOP (mmHg) | Transient maximum (mmHg) | Change in Baseline (mmHg) | Rate of increase in IOP (mmHg/min) |
|-------|--------------------|--------------------------|--------------------------|-----------------------------------|
| No bubble |
| 1     | 5.4                | NA                       | NA                       | 0.159                             |
| 2     | 6.4                | NA                       | NA                       | 0.298                             |
| 3     | 1.2                | NA                       | NA                       | 0.365                             |
| 4     | 3.4                | NA                       | NA                       | 0.176                             |
| 5     | 5.4                | NA                       | NA                       | 0.243                             |
| 0.15ml bubble |
| 6     | 0.9                | 6.2                      | -1                       | 0.16                              |
| 7     | 3.6                | 3                        | 2.3                      | 0.293                             |
| 8     | 2.1                | 10.4                     | 7.5                      | 0.291                             |
| 9     | 3.9                | 6.7                      | 6.2                      | 0.294                             |
| 10    | 2.6                | 14.1                     | 2.7                      | 0.493                             |
| 0.30ml bubble |
| 11    | 3.4                | 5.7                      | 4.7                      | 0.395                             |
| 12    | 6.4                | 17.4                     | 5.7                      | 0.442                             |
| 13    | 2.6                | 10.9                     | 9.9                      | 0.541                             |
| 14    | 2.1                | 1.2                      | 3.7                      | 0.355                             |
| 15    | 1.7                | 16.4                     | 7                        | 0.588                             |

2.2 Similar AC Volume

In this part of the experiment, we tried to ensure similar AC volumes in both groups before the start of the infusion. This time, we looked at only 2 groups, 0.3ml air bubble and no air bubble (control). For the air bubble group, after priming, 0.3ml of fluid was removed from the AC prior to injection of a 0.3ml air bubble. This was done using two separate 30-gauge needles and superior puncture sites. The infusion was then restarted and the time taken for IOP to increase to 20mmHg noted.

Statistical analysis was performed using Prism 4 software (Graph Pad Software, Inc., CA, USA). The unpaired t-test was used when comparing 2 groups while one–way ANOVA with Bonferroni’s multiple comparison post-test was used when comparing 3 groups. \( P < .05 \) was considered statistically significant.

3. RESULTS

The resistance of the 27-gauge cannula and silicone tubing was 0.06mmHg/µl/min and this was taken into consideration when calculating the actual IOP. The compliance of the silicone tubing was 1.60µl/mmHg and in the 4 eyes which we measured outflow facility, the value was 0.11+/-0.07 µl/min/mmHg.

3.1 Higher AC Volume

Actual data is shown in (Table 1). The typical IOP response curves with no air bubble and with a 0.30 ml air bubble are shown in (Fig. 2). Baseline IOP before priming was 3.4+/−1.8 mmHg (n=15). In practice, during the air bubble injection, a small amount of fluid would leak out of the AC through the paracentesis tract as there was a measure of AC volume.
equilibration. After injections, 0.15 ml of air would fill 30-40% of the AC while 0.3 ml of air would fill 70-80% of the AC. This percentage was determined by the ratio of the air bubble diameter to the corneal diameter. We also noted that a small amount of air could enter the posterior chamber but it was limited by the anterior vitreous face.

The initial spike in IOP was +8.1 +/- 4.3 mmHg with 0.15 ml bubbles and +10.3 +/- 6.9 mmHg with 0.30 ml bubbles (significant difference between each group and no bubble group, P=.01). Spikes did not last >90 seconds in any eye. This was followed by an increase of +3.5 +/- 3.4 mmHg and +4.7 +/- 5.1 mmHg from the original baseline with 0.15 ml bubbles and 0.30 ml bubbles respectively. However, there were no significant differences between any groups (P=.13). The rate of change in IOP was 0.25 +/- 0.09 mmHg/min for no air bubbles, 0.31 +/- 0.12 mmHg/min for 0.15 ml air bubbles and 0.46 +/- 0.10 mmHg/min for 0.30 ml air bubbles (Fig. 3a). There was a significant difference between the no air bubble and 0.30 ml air bubble groups (P=.02). 0.15 ml air bubbles did not produce any significant difference in the rate of change in IOP compared to both other groups (P=.22).

3.2 Similar AC Volume

Actual data is shown in (Table 2). The baseline IOP in the no air bubble group was 5.9 +/- 0.7 mmHg (n=9) while the IOP in the 0.3 ml air bubble group after injection was 5.3 +/- 1.6 mmHg (n=8) (Fig. 3b). This difference was not significant (P=.30). The rate of change in IOP was 0.30 +/- 0.08 mmHg/min with no air bubbles and 0.28 +/- 0.09 mmHg/min with 0.30 ml air bubbles. This difference was also not significant (P=.68) (Fig. 3c).
Fig. 3. a) Rate of change in IOP in higher AC volume experiment. There was a significant difference between the no air bubble (0.00ml) and 0.30ml groups (*). b) Baseline IOP in similar AC volume experiment. c) Rate of change in IOP in similar AC volume experiment. For b) and c), the differences between groups were not significant. Note that error bars indicate 1 SD.

We also looked at the time it took for IOP to increase by 5, 10 and 15mmHg in both groups. For 5mmHg, it took 19.5+/-8.0 minutes for the no bubble group and 27.7+/-11.1 minutes for the air bubble group. For 10mmHg, it took 38.7+/-11.4 minutes for the no bubble group and 46.8+/-15.7 minutes for the air bubble group. For 15mmHg, it took 53.6+/-15.2 minutes for the no bubble group and 57.9+/-17.9 minutes for the air bubble group. However, these differences were all not significant (P=.23).
### Table 2. Data for all eyes (similar AC volume experiment)

| Eye | Initial IOP (mmHg) | Rate of increase in IOP (mmHg/min) | Time to increase by 5mmHg(min) | Time to increase by 10mmHg(min) | Time to increase by 15mmHg(min) |
|-----|---------------------|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|
| **No bubble** | | | | | |
| 1   | 5.6                 | 0.185                             | 18.3                          | 53.3                          | 81.3                          |
| 2   | 5.6                 | 0.377                             | 10.3                          | 22.3                          | 39.8                          |
| 3   | 6.9                 | 0.411                             | 10.5                          | 22.5                          | 36.5                          |
| 4   | 6.1                 | 0.253                             | 28.8                          | 49.0                          | 59.3                          |
| 5   | 4.9                 | 0.287                             | 22.5                          | 42.0                          | 52.3                          |
| 6   | 6.0                 | 0.273                             | 14.3                          | 37.3                          | 54.9                          |
| 7   | 5.0                 | 0.229                             | 33.9                          | 46.7                          | 65.6                          |
| 8   | 6.5                 | 0.380                             | 21.1                          | 45.2                          | 39.5                          |
| 9   | 6.5                 | 0.299                             | 15.9                          | 30.3                          | 53.6                          |
| **0.30ml bubble** | | | | | |
| 1   | 4.7                 | 0.343                             | 24.6                          | 38.4                          | 43.8                          |
| 2   | 3.2                 | 0.212                             | 36.8                          | 58.9                          | 70.9                          |
| 3   | 5.9                 | 0.258                             | 27.1                          | 45.8                          | 58.2                          |
| 4   | 3.7                 | 0.179                             | 46.4                          | 71.4                          | 83.8                          |
| 5   | 3.8                 | 0.191                             | 35.0                          | 62.3                          | 78.6                          |
| 6   | 7.6                 | 0.341                             | 21.8                          | 35.5                          | 44.0                          |
| 7   | 6.5                 | 0.308                             | 17.2                          | 36.0                          | 48.6                          |
| 8   | 6.8                 | 0.421                             | 12.7                          | 26.3                          | 35.7                          |

### 4. DISCUSSION

This experiment was designed to simulate leaving air bubbles in the AC after surgery and record the pressure changes which occur. In a trabeculectomy, after a partial thickness scleral flap and sclerostomy are created, aqueous humour release is expected, along with lowering of the IOP. Soon after, the scleral wound is sutured close and the continuing aqueous humour production allows the IOP to increase. This is another basis of priming our porcine eyes by infusing them to 15mmHg and then stopping the infusion to let the IOP decrease to 5mmHg before re-starting the infusion and recording the time for the IOP to increase by 15mmHg. We decided to look at the time it took for the IOP to reach around 20 mmHg as it was a reasonable post-operative outcome to expect. Additionally, the outflow resistance and outflow facility are relatively constant in this range. Previous studies have shown that outflow resistance increases (while outflow facility decreases) with higher IOP and this was due to the collapse of the outflow passages [24,25]. We measured outflow facility in our porcine eyes to be 0.106+/-0.067µl/min/mmHg. This was similar to the values reported for enucleated porcine eyes, 0.10-0.42µl/min/mmHg measured by Vaudaux et al. [26] and 0.164µl/min/mmHg measured by Shaarawy et al. [27].

From our results, injecting an air bubble into the closed AC caused a spike in IOP but this effect was transient and lasted only 1-2 minutes. This was due to the filling and increased volume in the AC immediately after injection, followed by a measure of equilibration from aqueous humour leakage through the paracentesis tract and outflow through the remaining physiological outflow pathways. The IOP then reduced, but settled higher than the initial baseline. Although the difference was not significant, this higher baseline occurred in 80% of eyes and it is likely that they were fuller than at the beginning. In this state, subsequent
filling of the AC translated to a faster increase in IOP compared to an AC without an air bubble in it.

With the similar AC volume protocol, we tried to ensure similar AC volumes in the two groups and this was reflected in the similarity in baseline IOP. We found no significant differences in the rate of increase in IOP to 20mmHg or the times it took for IOP to increase by 5, 10 and 15mmHg between both groups. This mirrors the findings of Miyata et al. [9] where AC volumes were also kept the same. The higher IOP reported in other studies [12,28] are then likely to be due to the increased content of the AC when no aqueous humour is removed.

For a typical IOP of 20mmHg, the absolute value is atmospheric pressure+IOP i.e. (760+20)mmHg. With aqueous humour flow rates being 2-3μl/min, changes in IOP will be relatively small in terms of absolute pressure. As such, the bubble does not undergo significant volume changes due to pressure and there is no dampening effect on the increase in IOP. Our experiment shows that even with a flow rate at least 3 times higher than in the living eye, the effect of compressibility was not demonstrated. One possible confounding factor here is that the young porcine eyes used may not have increased outflow resistance, such as that found in open angle glaucoma or ocular hypertension, which could mask any possible demonstration of compressibility. However, the difference in effect may not be large, as to have a completely blocked aqueous humour outflow pathway is uncommon; in most cases of open angle glaucoma and ocular hypertension the outflow facility is between 0.1 and 0.2μl/min/mmHg, which implies that a certain amount of aqueous humour drainage still occurs [29-32].

In our experiment, we used porcine eyes in view of their low cost, widespread availability and the fact that their size and volume, particularly of the AC (0.3ml), are closer to the human than any other species reported [33]. Their use as trabeculectomy surgery models have been reported by Jacobi et al. [34] and Lee et al. [35], while they have also been utilised in other glaucoma-related experiments such as by Wagner et al. [36] to measure uveoscleral outflow, Shaarawy et al. [27] and Xu et al. [37] to model non-penetrating glaucoma surgery and Hernandez-Verdejo et al. [38] to investigate the change in IOP during LASIK surgery.

We used the relatively high and non-physiological infusion rate of 10μl/min. In our preliminary tests, when using physiological aqueous humour flow rates of 2-3μl/min, the IOP change was very slow and each run could take up to 12 hours to complete. The lower flow rate would have been time consuming and associated with tissue dehydration and deterioration. We were also keen to minimise the effect of the washout phenomenon in our porcine eyes. This phenomenon occurs in all animal eyes except humans [39-41]. Prolonged, continuous infusion of fluid into the eye results in a decrease in resistance and increase in outflow facility which can affect IOP readings. It is thought to be due to the separation of the inner wall of the aqueous plexus from the juxtacanalicular connective tissue. Additionally, the infusion rate we used was also similar to that reported for previous experiments on enucleated eyes [42-45].

We acknowledge some limitations of our experiment. We did not specifically measure the volumes of the AC in our test eyes. This would be helpful to confirm that our results are completely due to volume differences. However, we believe that in the case of a trabeculectomy, there is no significant increase in AC volume as aqueous humour is displaced through the trabeculectomy or paracentesis wound while the air bubble is injected.
In our study too, partial thickness scleral flaps and sclerostomies were not performed on the porcine eyes. Although these steps would make the trabeculectomy simulation more accurate by allowing more realistic volume equilibration when the air bubble enters the AC and displaces BSS from it, we did not want the potential variability in outflow with the creation of these flaps to affect our measurements. Finally, we also did not look at the influence of increased aqueous humour viscosity in our experimental model. These situations may occur after surgery due to the increased levels of circulating protein and fibrin, especially in cases such as uveitic glaucoma [46,47].

5. CONCLUSION

In this enucleated porcine eye model, an air bubble left in the AC at the end of surgery does not affect the subsequent rate of increase in IOP when AC volumes are kept similar. There is no significant compressibility of the air bubble to dampen the rate of increase in IOP.

CONSENT/ ETHICAL APPROVAL

This was deemed not applicable due to the nature of the specimens/ materials used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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