Experiment of vitreous liquefaction induced by C_3F_8

Jing Wang and Qiuming Li
Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Abstract: To investigate the effect and safety of vitreous liquefaction induced by C_3F_8 (an inert gas) injected into vitreous cavit of rabbit eyes. 24 rabbits (48 eyes) were randomLy divided into four groups, named group A, group B, group C and group D, with 6 rabbits in each group. The right eye in each rabbit was taken as the experimental eye while the left as the control eye. The experimental eyes in group A were injected with 0.1mL disinfectant air; the experimental eyes in group B, group C and group D were all injected with C_3F_8 0.1mL, 0.2mL and 0.3mL respectively after receiving anterior chamber penetration; and the controlled eyes in all group were injected with 0.1mL balanced salt solution (BSS). During the first 7 d after injection, all the rabitts' eyes were examined by slit lamp, ophthalmoscope, intraocular pressure (IOP) and dark-adapted retina Electroretinography (ERG) each day. After that, the examination of IOG and ERP were reviewed weekly. Besides, B ultrasound should be examined to observe the situation of posterior vitreous detachment (PVD) in the 4^{th} and 8^{th} weeks. The rabbits were killed in the end of the 8^{th} week, with their specimens examined by the light microscope, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Except group A, all the experimental eyes were produced with vitreous liquefaction. In group C and group D, in addition to the produced vitreous liquefaction, posterior vitreous detachment (PVD), even complete PVD, were induced in different extent. But in group B, the vitreous body was returned to the gel state at 2 weeks after gas absorption. In group C and group D, the vitreous body was not found to recover its original state at 8 weeks. In group D, there was a little increase of intraocular pressure, a mild delay of wave a and wave b after ERG in the 4^{th} day after the gas injection. While there was no such situation in other groups. After the examination of B ultrasound in the 8^{th} week, the complete PVD occurred in group C (with 2 experimental eyes occurred) and group D (with 4 experimental eyes occurred). Light microscope and transmission electron microscope examination showed no obvious abnormality. Smooth inner limiting membrane could be seen in the eye with PVD occurred when scanning electron microscope used. the injection of C_3F_8 into rabbit eyes can improve the vitreous liquefaction of the vitreous body and a certain volume of C_3F_8 can successfully and safely induce the PVD, and a larger volume of C_3F_8 was also effective but with a transient high IOP in rabbit eyes.

Keywords: C_3F_8, vitreous liquefaction, posterior vitreous detachment

INTRODUCTION

Posterior vitreous detachment (PVD) is a kind of pathological changes related to the age. Some clinical experiment showed that the occurrence of PVD can indeed make some fundus diseases under control, and even self healing (Ebato K *et al*, 2000; Akiba J *et al*, 1990). In some literature, PVD can improve the prognosis of the retinal related diseases, such as the proliferative and non-proliferative diabetic retinopathy, macular hole, and retinal vein occlusion (Ferris JD, 1997). In addition, the vitrectomy after the artificial induction of PVD can significantly shorten the operation time and reduce the incidence of iatrogenic retinal breaks and bleeding caused by mechanical stretch during the operation. (Han DP *et al*, 1988; Vander JF *et al*, 1992). In particular, young patients can greatly reduce the possibility of iatrogenic retinal breaks, and even can replace surgery. Proliferative vitreous retinopathy (PVR) is a common complication of ocular injury-induced vitrectomy, which can be reduced greatly if the artificial induction of PVD can be used before operation. For these many reasons, the drug-PVD has been attached important to the experimental study. Nowadays, a lot of drugs like Dispase, hyaluronidase and tissue-type plasminogen activator can induce PVD. However, study of Lincoff showed that inert and expansive gas can destroy the structure of the vitreous body and cause liquefaction, and even can induce the formation of PVD (Lincoff H *et al*, 1984). In the present study, we adopted the method of injecting C_3F_8 with different volume into the vitreous body to induce fluid vitreous and even the formation of PVD, and then to evaluate the safety of drug.

MATERIAL AND METHODS

Materials

Experimental animals

24 adult healthy rabbits from New Zealand were provided by Labora Laboratory Animal Center of Henan Province, weighing 2.5~3.0Kg, both male and female

Reagent

C3F8 was from in Tianjin Jingming New Technological Development Co. Ltd., with the concentration of 99.5%.

**Corresponding author:* e-mail: zmby519@163.com

Main instruments

B ultrasonic: ODM-2100, was made in Tianjin Maida Medical Technology Co. Ltd.; Electrophysiological: GT-2000NV, was produced in Chongqing Guote Medical Equipment Co., LTD; Scanning electron microscopy (SEM): Hitachi HS-3500N type; Transmission electron microscopy (TEM): Hitachi H-7500.

Methods

24 New Zealand rabbits were randomLy divided into four groups, namely group A, B, C and D with 6 rabbits in each group. The right eye of each rabbit was regarded as the observation eye and the left as the controlled eye. Before operation, their conjunctival sac was washed by normal saline, and dropped with Tobra Dex eye ointment.

This experiment used the methods of anterior chamber penetration followed by injecting drugs in ventreous bodies. Ketamine hydrochloride was injected into the muscles for anesthesia with the concentration of 40 mg/Kg. After that, a 1mL injector was used to extract the aqueous fluid whose volume was same with injected gas through limbus paracentesis. And then after being filtered by the 0.22 micron filter, another new 1mL injector was used to extract the C3F8 through the pars plana corporis ciliaris with needle inserted vertically so as to prevent from damaging the crystal. Then C_3F_8 in the injector was quickly injected into the vitreous body to form a large bubble after the syringe needle was indeed in the vitreous body. When the bubble floated onto the upper part of the vitreous body, the injector was pulled out immediately, and then sterilized cotton was used to pressg on the pinhole for 2 min to prevent from air leakage. The experimental eyes in group A were injected with 0.1mL sterilized air, and group B,C and D were injected with 0.1 mL, 0.2mL and 0.3mL C_3F_8 respectively after conducting anterior chamber penetration. All the controlled eyes were injected with 0.1mL BSS.

Postoperative effect and safety

After operation, the experimental eyes were dropped with Pranoprofen Eye Drops to keep from infection. In the 1^{st}, 2^{nd}, 4^{th} and 8^{th} hour in each day of the first week and in the 2^{nd} 3^{rd} 4^{th} and 8^{th} week, compound tropicamide was used to enlarge pupils of the rabbits. And the direct ophthalmoscope and slit lamp were used to observe the gas expansion condition, vitreous liquefaction, whether the turbidity existed in posterior lens capsule and fundus situation. The each day IOP measurement in the postoperative 1h, 2h, 4h and 8h during the first two weeks was replaced by once a week in the last several weeks. They were fixed by rabbit cage after being dilated for 30 min dark adaption. Then they were anesthetized by Oxybuprocaine Hydrochloride with the corneal electrode put in the eyes. Also the silver needle reference electrode was put on the right ear and under the forehead skin to have a standard flashing ERG examination. B ultrasound was carried out to examined whether there were formation of PVD in the vitreous retina in postoperative 4^{th} and 8^{th} week.

Ultra-structure observation

The rabbits were killed by arterial air embolism, with the eyeballs immediately extirpated. Light microscopy specimen: they were fixed for 24 h with the stationary liquid, and then dehydrated by 95% alcohol and dyed with HE. And then light microscopy specimen was made by paraffin embedding slice technique. Scanning electron microscopy (sem) specimen: they were fixed with 2.5% glutaraldehyde solution. $1mm^2$ total tissue mass of eyeball were extracted from the upper and lower equator parts as the SEM specimens. Transmission electron microscopy (sem) specimen: 1 mm^2 total tissue mass of eyeball was fetched to make sem specimen after being fixed by 4% glutaraldehyde solution. Then the Ultra structure was observed and recorded.

Medical ethics review

This project had been censored by medical research ethics committee of the First Affiliated Hospital of Zhengzhou University. The content and process of this research followed the ethical requirements of related biomedical research issued by international and some nations. This project was agreed to carry out experimental study.

STATISTICAL ANALYSIS

All statistical analyses were analyzed by SPSS10.0 software, and the results were expressed by x±s. The data must conform to the normal distribution and equal variance, and to check by *t* test and variance analysis; Otherwise the nonparametric test would be used.

RESULTS

Expansion and gas absorption

The gas in the vitreous body of the experimental eyes of group B, C and D were expanded to the maximum at third days after operation. The bubble in group B was floated on the upper part of the vitreous body, occupying less than 50% glass body cavity; the bubble in group C took up more than 70% glass body cavity of the vitreous body; and the bubble in group D was full of the vitreous body. The gas in group A and group B was absorbed in the (6±1.6) days and (20±2.1) days respectively; while the absorption time of gas in group C and D were (25±1.7) days and (27±1.4) days respectively.

The general observation of the crystalline lens, vitreous, and retina

Preoperation

There was no lens opacity, no vitreous opacity, liquefaction or posterior detachment, no retinal hemorrhage, leakage or abnormal pigment.

Table 1: The amplitude of wave a and wave b of the experimental groups

Group n		Amplitude of wave a Amplitude of Wave b					
		Pre-op	Post-op		Pre-op	Post-op	
			3 d	7 d		3 d	7 d
Normal	6	-42.45±10.45	-43.41±17.23	-44.62±26.71	205.11±21.73	200.65±7.78	215.23±24.81
A	6	-36.60±17.10	-39.47±10.45	-41.36±16.80	213.64±28.10	221.90±25.24	204.20±19.21
B	6	-41.94±17.31	-41.66±15.33	-43.08±12.07	210.37±21.45	219.90±25.24	215.14±46.01
C	6	-42.79±16.47	-43.71±10.45	-44.68±16.31	198.74±31.62	201.00±32.97	204.20±25.81
D	6	-43.38±8.36	-21.13±18.67	-39.23±16.12	220.10±42.41	128.79±16.83	215.37±30.29

Table 2: The incubation period of wave a and wave b of the experimental groups

Group n		incubation period of wave a			incubation period of wave b		
		Pre-op	Post-op		Pre-op	Post-op	
			3d	7d		3d	7d
Normal	6	13.09±0.55	13.20±0.84	13.60±0.89	36.80±2.28	36.40±2.09	36.30±1.84
A	6	13.40±0.77	13.93±0.21	14.00±1.02	35.80±1.85	35.80±1.84	36.00±1.22
B	6	13.80±0.93	13.60±1.07	13.63±1.07	35.80±0.91	36.30±1.30	36.68±1.83
C	6	13.90±1.00	14.20±1.34	14.40±1.12	36.20±1.93	36.42±1.34	37.40±1.34
D	6	13.60±1.14	20.90±1.70	14.60±1.34	35.68±1.28	47.30±1.22	37.80±0.84

Note: P<0.05 vs pre-op D group with 3d.

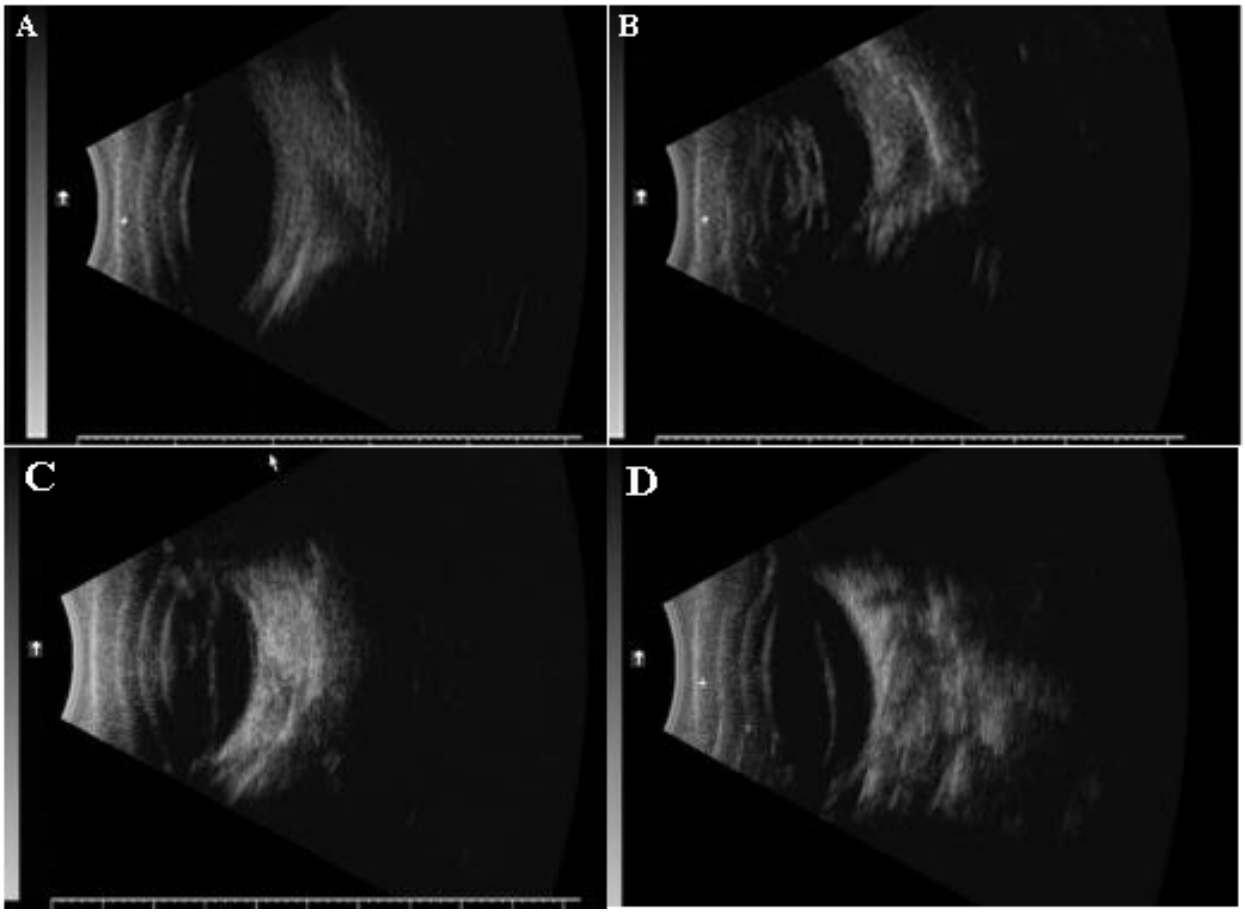

Fig. 1A: Normal vitreoretina in rabbit eyes;
Fig. 1B: Vitreous opacity
Fig. 1C: Vitreous opacity combined with incomplete PVD
Fig. 1D: Complete PVD

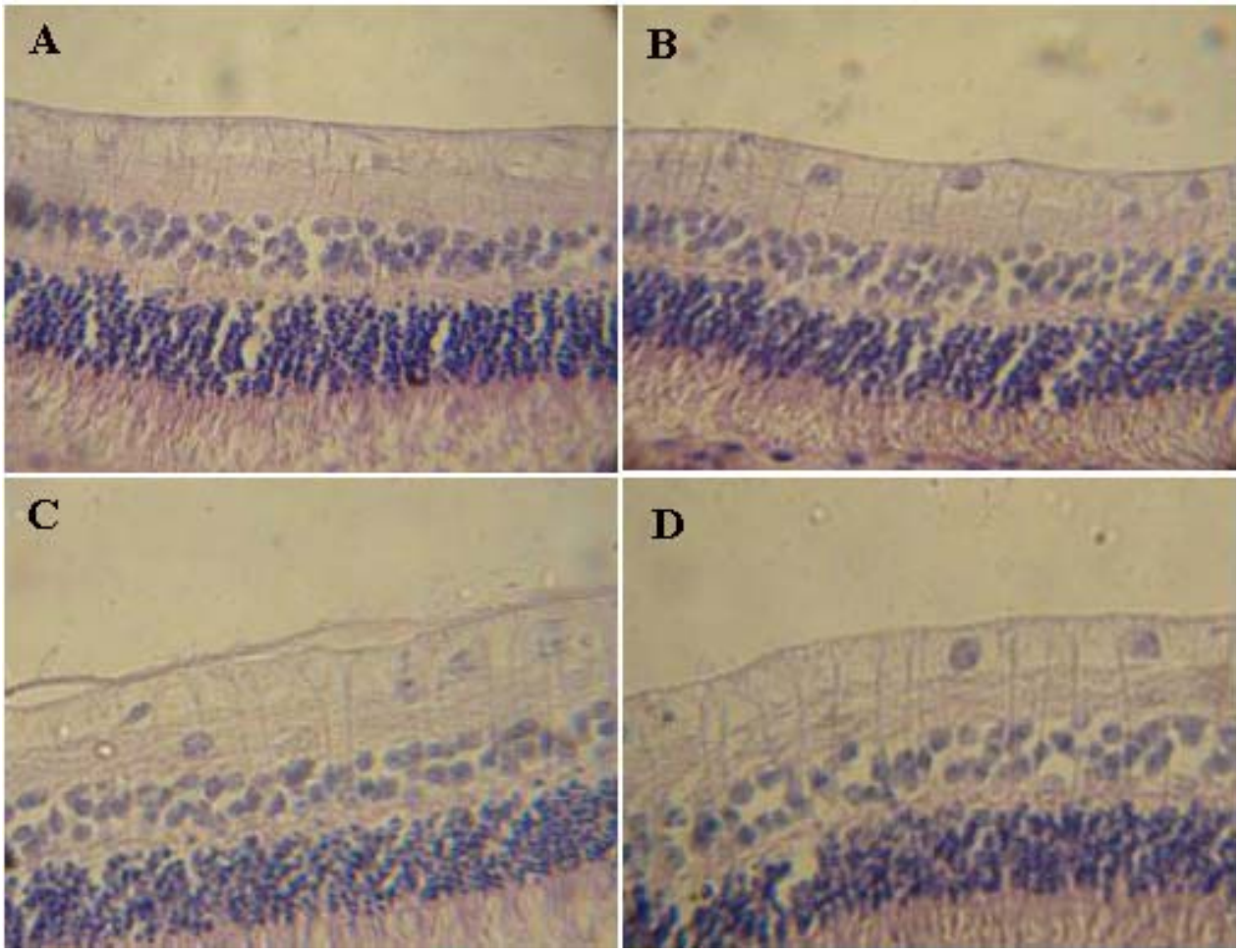

Fig. 2A: The control group, with order retinal tissue
Fig. 2B: Group A and group B under the light scope with clear layers without PVD
Fig. 2C: Partial PVD, with orderly retinal structure
Fig. 2D: Complete PVD and the retinal structure without notable difference compared with the control group

Postoperation

There were no corneal disease, anterior chamber fibrin exudation, phacoscotasmus, vitreous opacity, liquefaction and posterior vitreodetachmant during the continuous observation in group A and the control group. There was no direct change which can be observed in the fundus, compared with before surgery. Except group A, different degrees of vitreous liquefaction were all observed in the experimental eyes in group B, C, and D. Uneven vitreous opacity could be observed during the process of gas absorption. The vitreous liquefaction gradually disappeared after gas absorption in group B. In group D, there could be seen Wiess ring in some experimental eyes in the 4th week. Dotted lens opacity with different degrees appeared in the posterior capsule after 3 days in the experimental eyes of group B, C and D and disappeared after gas absorption finished, suggesting that it mignt be related to the mechanical friction caused by the direct contact of gas with the posterior capsule. Moreover, there were no abnormal performance in the fundus in the experimental groups.

Intraocular pressure

In addition to the group D that part of the experimental eye (2/6) appeared a slight increase in intraocular pressure in the 3rd day after the gas injection, the rest of the group did not appear abnormal intraocular pressure. The IOP of group D returned to normal level in 6 days after operation

Electrophysiological examination

The preoperative electroretinogram in control group showed that the mean amplitude of wave a was -62.92±38.34 and wave b of 207.04±56.43. And there were no notable changes after operation examination.

The amplitude of wave a and wave b in the experimental eyes were shown in table 1 and the incubation period were shown in table 2. There was a transient amplitude decrease of wave a and wave b shown in ERG and a mild delay of the incubation period only in experimental group D, and the difference was statistically significant (t test, $P<0.05$). There was no statistically significance difference between the rest groups.

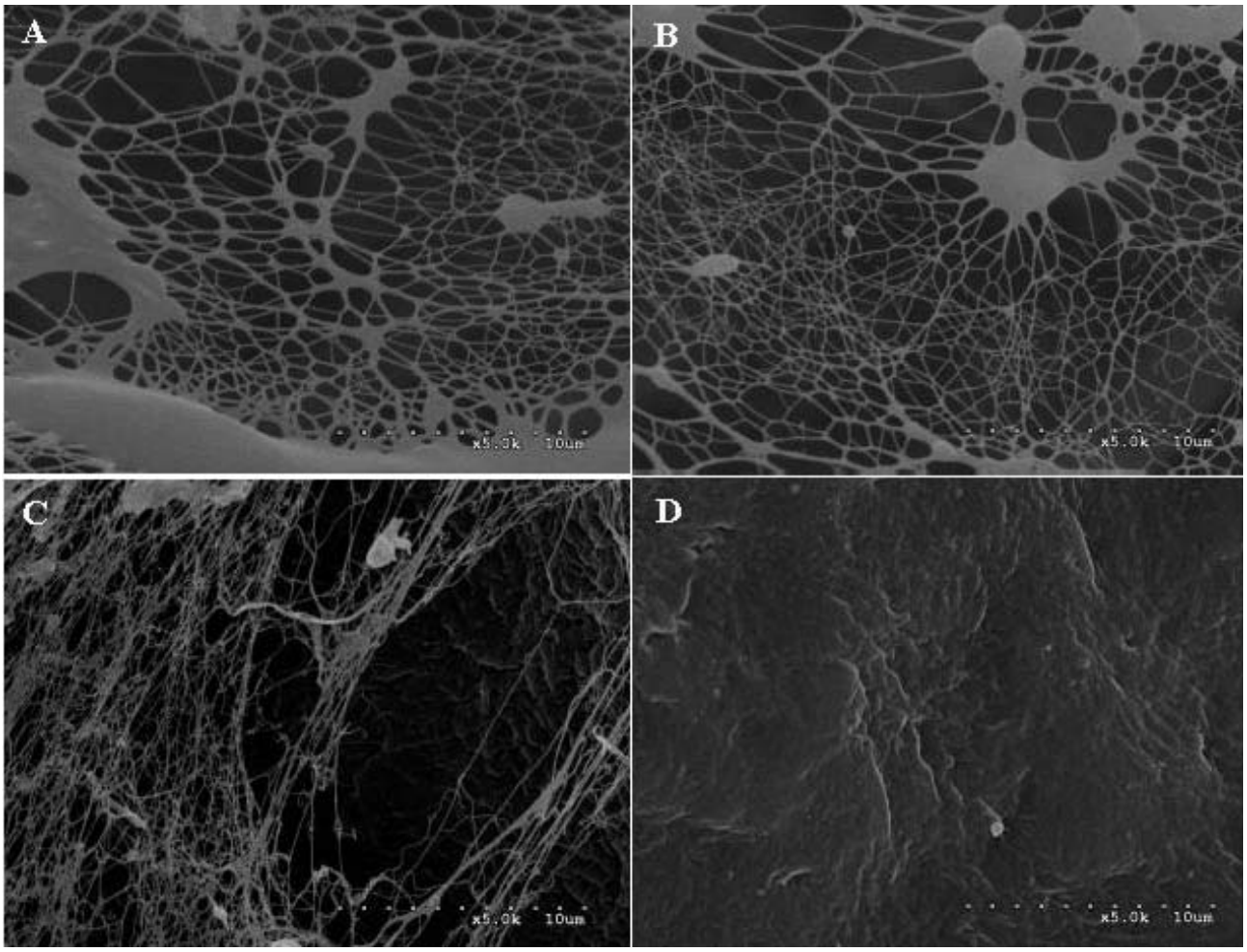

Fig. 3A: Numerous vitreous fiber woven mesh in the normal vitreous cavity
Fig. 3B: Group A and group B under the light scope with clear layers without PVD
Fig. 3C: Abnormal sparse vitreous fiber combined with local smooth internal limiting membrane and partial PVD
Fig. 3D: Complete PVD and smooth internal limiting membrane

B Ultrasound observation

There were no notable changes in group A compared with preoperative state and control eyes. It showed there was vitreous turbidity in group B in the 4th week and disappeared in the 8th week, and the changes was not notable compared with the operative state and the control group. There were 6 cases showed partial PVD combined with vitreous turbidity in group C in the 4th week and 2 cases showed complete PVD in the 8th week. In group D, there were 4 cases showed complete PVD and 2 cases showed partial PVD in the 4th week and no obvious change showed in B ultrasound in the 8th week. The experimental eyes with PVD could seen the midum echo membranous substance in vitreous body, with an active motivation and with nothing to do with the optic disk. As shown in fig. 1. Fig. 1A was normal vitreoretina in rabbit eyes; fig. 1B was vitreous opacity in group B; fig. 1C was vitreous opacity combined with incomplete PVD in group C; fig. 1D was complete PVD occurred in part rabbit eyes in group D.

Light microscopic observation

Under light microscope, the structure of the retina of the control group was complete, and arranged neatly. No abnormal changes were found. The cone and nod cells were complete, with clear structure and normal retinal ganglion cells. And PVD could be seen. As shown in fig. 2. Fig. 2A was the control group, with regular retinal tissue; fig. 2B was group A and group B under the light scope with clear layers without PVD; fig. 2C was partial PVD, with orderly retinal structure; fig. 2D was complete PVD and the retinal structure without notable difference compared with the control group.

Scanning electron microscopy

The observation range of scanning electron microscopy was the upper and lower equator and postretina of the eye. The difference in group A compared with the control group was not notable, with a large number of vitreous fibers covering on the surface of the retina. In group B the

difference was not notable compared with the control group, only with the local vitreous fiber breakage in the upper equator and postretina. In group C, partial smooth internal limiting membrane and partial vitreous fiber in the equator region could be seen, and smooth internal limiting membrane could be seen in the posterior pole. In group D, smooth internal limiting membrane in the equator region and posterior pole could be seen, indicating the formation of complete PVD. Shown in fig. 3.

Transmission electron microscopy

There was no significant difference between the experimental groups and the control group. Normal retinal ultrastructure could be seen, with smooth internal limiting membrane and complete lower foot-plate. As shown in fig. 4.

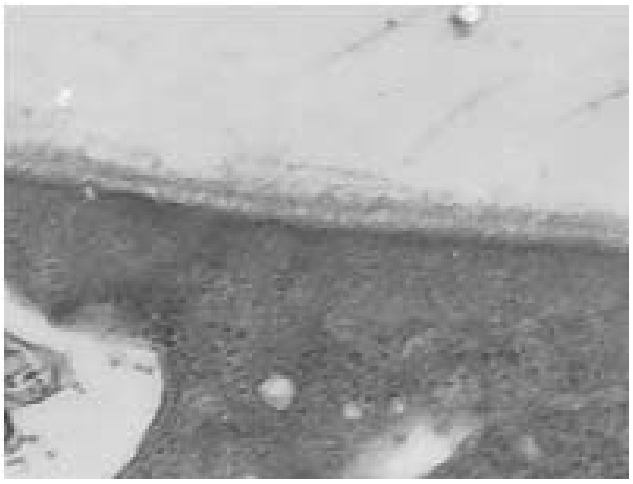

Fig. 4: smooth internal limiting membrane and normal cell structure of the lower cells

DISCUSSION

The meaning of complete PVD

PVD is a kind of common clinical pathological process, usually in the elder and myopia patients, and most of them are local PVD. Some researches show that local PVD sometimes leave the vitreous cortex connected to the inner surface of the retina, which are harmful to many vitreoretinal disease and even caused macular hole (Kakehashi A *et al*, 1997). During the natural process of the vitreoretinal disease, the adhesive effect of vitreous-body-retina is very clear. If there are complete PVD in retinal diseases in the patients with diabetes, their prognosis is better than those with local PVD or without PVD. The incidence of proliferative vitreoretinopathy (PVR) is directly related to prognosis of the patietns with retinal detachment. The preoperative formation of complete PVD will shorten the time and effect of vitrectomy. Therefore, it is necessary to artificially create a complete PVD. In some related retinal diseases, complete PVD is not only conducive to the treatment of vitreous surgery, but also conducive to the treatment and recovery of retinal diseases. (Liu HF *et al*, 2004).

The organizational structure for PVD formation induced by C_3F_8

PVD is the process of liquefaction and concentration of the vitreous body, which promotes the separation process of the posterior vitreous cortex (PVC) from the internal limiting membrane (ILM). Vitreous body is actually a kind of extremely thin corneal stroma-like substance (Scott J, 1992), with macromolecules of hyaluronic acid, collagen protein, polysaccharide, and non-collagen proteins. Under the electron microscope, the fine collagen fibers are randomLy arranged in the reticular tissue, with hyaluronic acid filled between the spaces. being connected with the unclear non-bonding. This structure is benefit to the hyaluronic acid liberation, collagen collapse and vitreous liquefaction, keeping the fiber in the separated with no agglutinate state (Verstraeten TC *et al*, 1993). In human vitreous body, collagen is mainly fine fiber, which is composed of many types, in which collagen type II is the main collagen component in the vitreous body, accounting for about 70%-80% (Bishop P, 1996). In addition, there is a molecular interaction between the vitreous cortex and the retina, that is, the interactive effect of the fibronectin on the retinal surface, fiber fibronectin and chondroitin sulfate (Sebag J, 1996; Sebag J, 1991). The normal vitreous cortex and retina mainly depend on the adhesion of collagen fibrils, which is hard to occur detachment. The true meaning of PVD is the seperation of II-type collagen of the vitreous body from the IV-type collagen whether on the aspect of the adhesion of collagen fibrils or the large molecular action. Then the so-called PVD is the damage of the vitreous gel state and then the dissolution of the II-type collagen and IV-type collagen on the interface of PVC-ILM (Zhou ZY, 2002). C_3F_8 is a common expansive gas. With its expansion character, on one hand, we break the stablility of hyaluronic acid and collagen, to release the hyaluronic acid and aggregate fine fibre; on the other hand, The mechanical tension and shear stress of the fine fibers of vitreous body cause the concentration and liquefaction of the vitreous body, and then segregation (Lincoff H *et al*, 1984). At present, the combination of expansive gas with enzyme produce complete PVD is common. In Thresher Experiment, C_3F_8 was used in the monkey eyes to make PVD (Thresher RJ *et al*, 1984).

The comparison of the observation methods in this experiment

Due to the close connection of posterior vitreous cortex with inner limiting membrane, as well as the posterior vitreous cortex as a thin layer of yarn, the judgment of posterior detachment of vitreous body is always a problem for the researchers. This study finds out that the clinical routine inspection such as slit lamp and ophthalmoscope can directly find the general changes of

the vitreous body, such as the vitreous opacity and post detachment, which can be taken as the preliminary judgment of PVD. B-type ultrasound can observe the general changes of the vitreous body after absorbing gas, which is a reliable method to judge the existence of PVD. However, the observation will be affected if the gas is not absorbed in the vitreous body. Moreover, the operation requires much attention, like multidimensional scanning, especially in the judgment of the early stage of PVD. Light microscopic examination is a kind of objective examination method which can directly observe the changes of the retinal tissue cells in each layer (Shen LP *et al*, 2004). Since there are so much water in the vitreous body, it is possible to cause man-made artifacts in the process of fixation and dehydration. Therefore, it is difficult to make an accurate and objective judgment on the results of the light microscopic examination for posterior vitreous detachment, which can only be used as an analytical method of safety. TEM can clearly reflect the two-dimensional image of neurons cell structure in each layer in the retina and the fine two-dimensional image of the posterior vitreous cortex and internal limiting membrane, which is also an objective method to evaluate the posterior detachment of vitreous body. However, from the view of specimen sampling and its own limitation, it is also hard to make an accurate and objective judge. SEM can display the retinal surface structure, and the vitreous fiber and internal limiting membrane clearly. Someone also think that it could be taken as the only method of examination of the posterior vitreous detachment (Hikichi T *et al*, 2000). And this study also indicates that this method is of great significance in judging the posterior detachment of vitreous body.

CONCLUSION

This study shows that except group A, all the experimental eyes were produced with vitreous liquefaction. In group C and group D, in addition to the produced vitreous liquefaction, posterior vitreous detachment (PVD), even complete PVD, were induced in different extent. But in group B, the vitreous body was returned to the gel state at 2 weeks after gas absorption. In group C and group D, the vitreous body was not found to recover its original state at 8 weeks. In group D, there was a little increase of intraocular pressure, a mild delay of wave a and wave b after ERG in the 4th d after the gas injection. All in all, the injection of C_3F_8 into rabbit eyes can improve the vitreous liquefaction of the vitreous body and a certain volume of C_3F_8 can successfully and safely induce the PVD and a larger volume of C_3F_8 was also effective but with a transient high IOP in rabbit eyes.

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