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Natural Evolution of Experimental Vitreous Hemorrhage and Effects of Foreign Body on Its Course

Dragan Vukovic University of Belgrade,Faculty of Medicine, Clinic of Eye Disease,Clinical Centre of Serbia, Pasterova 2

Antoaneta Adzic Faculty of Medicine, Clinic of Eye Disease,Clinical Centre of Montenegro,Podgorica,

ABSTRACT

Introduction :The course and prognosis in vitreous hemorrhage are hard to predict, while its pathogenesis is yet not clear enough, in spite of so far published experimental and clinical studies.

Material and methods: The experiment was performed on 19 Chinchilla rabbits (Oryctolagus cuniculus), both male and female (38 eyes), weighting 2500-3000 g, which have been treated with autologous blood (B) from marginal ear vein – the first group, the second group being treated with both , blood and foreign body (B+FB), and the third group treated with blood, corticosteroid and foreign body (B+CS+FB). The control groups were determined by the following approach: for the examined group treated with B+FB+CS, the control group was the one treated with B+FB.

We have examined the course of vitreous hemorrhage by making drawings of fundus using indirect ophthalmoscopy, following number and type of cells within the vitreous body (by assessing central and peripheral coagulum, next to ciliary body and retina), fibrinolysis, and hemolysis in consecutive time periods of 5 minutes, 6 days, 2, 3, 4, 6, 9, 10 and 12 weeks after intravitreous injection. For this occasion we want just to present the course of fibrinolysis.

Results: Our results have shown that the fibrinolysis of vitreous hemorrhage is slow, compared to hemorrhage in other types of tissue, which is due to composition of the vitreous hyaluronan-collagen matrix which allow limited passage of blood cells that leads to absence of early polymorphonuclear cellular reaction. That leads to persisting of fibrin deposits that are normally being degraded by polymorphonuclear cells.

KEYWORDS : vitreous haemorrhage,natural course,foreign body, fibrinolysis

METHODS:

Hemorrhage within the vitreous body in all cases originate from the periphery, or from the vessels penetrating into the vitreous in certain pathological conditions, thus leading to the condition known as vitreous hemorrhage, or hemophthalmus.

The experiment was conducted on 19 adult Chinchilla rabbits (Oryctolagus cuniculus) of both sexes, weighting 2500-3000 g. The experimental procedure was the following:

Animals were anesthetized by intramuscular injection of ketamine hydrochloride, using dose of 14-15 mg/kg.

Both eyes were softened by withdrawal of 0.15 ml of liquid vitreous using 27-gauge needle (with outer diameter of 0.40 mm and inner diameter of 0.20 mm) and tuberculin syringe. Using plastic silicone coated syringe we collected 0.2 ml of full autologous blood from the marginal ear vein, without using anticoagulants. The blood was immediately injected into the central part of the vitreous body, using standardized procedure of via pars plana approach to the upper temporal quadrant, 3 mm away from limbus. The procedure was conducted under ophthalmoscopic control and using 27-gauge needle.

Eyeballs were perforated by sclerotomy, in all cases using 19 gauge needle (outer diameter 1.05 mm and inner diameter 0.65 mm) and pars plana approach, 3 mm away from limbus in the upper temporal quadrant, assuring to cause minimal physical trauma to the eye, equivalent to the one caused during usual surgical procedures during vitrectomy.

Animals (eyes) were categorized into 2 basic groups:

1) Firs group (right eyes) – full autologous blood only was injected into the central vitreous body;
2) Second group (right eyes) – full blood was administered together with non-sterile copper foreign body of 1 mm3 in volume

The first group of eyes in which blood was injected, and in which the evolution of coagulum was analyzed in detail, was in the same time the control group for the other group of animals (eyes), which suffered the same traumatic procedure of intravitreous injection of blood and blood and foreign body

In the group 2, copper foreign body, approximately 1 mm3 in volume (copper wire measuring 1 mm in diameter, cut in parts of 1 mm in length) was introduced using tweezers, through the same scleral incision into the mid-vitreous (1-1.5 mm in depth from the sclerotomy incision); later, it was allowed to spontaneously take its position within the vitreous, coagulum or retinal surface.

Experimental group intended to be used for assessment of possible adverse effects of ocular trauma.

In periods of time, eyeballs were frozen in acetone after enucleation, and than analyzed anatomically and physiologically.

Interventions were completed by administration of 1% atropine solution and 1% chloramphenicol oil suspension into the cojunctival sac.

Based on literature (1,2), time intervals after which it was found that the material was useful for investigation were: 5 minutes, 6 days, 2, 3, 4, 6, 9, 10, and 12 weeks after intravitreous injection of blood.

Animals were euthanized using ketamine overdose or by causing gas embolism: one 5 minutes after vitreous injection of blood, and two each time after 6 days (one week), 2, 3, 4, 6, 9, 10, and 12 weeks.

Blood clearance from vitreous body was assessed weekly, using indirect ophthalmoscopy and making drawings of the observed image, until 12th week after intravitreous injection of blood. In addition to that, drawing of ophthalmoscopic findings was made 5 minutes after intravitreous injection of blood, too.

Eyeballs were enucleated using standard technique.

Eyeballs fixed in formaldehyde were cut through frontal plane, behind the lens, while eyeballs frozen in acetone were cut vertically, through sagittal plane, so the topographic characteristics of changes in vitreous body would remain preserved.

For the purpose of histological analysis, we sampled vitreous coagulums together with the surrounding tissue, wedge-like slices of eyeball tissue with parts of ciliary body and adjacent tissue, parts of retina, as well as granulomas or vitreoretinal proliferative bands, if any.

All slices were stained using haematoxylin & eosin (HE), periodic ac-

id-Schiff (PAS) and van Gieson (VG) staining methods. Furthermore, we used special staining method for platelets (Mallory trichrome), for iron (Prussian blue), for monocytes (Masson), and for elastic collagen (VG).

Histological slices of vitreous and other surrounding structures mentioned above were carefully analyzed and photographed.

Gross and histological findings were classified for every eyeball together with clinical findings based on indirect ophthalmoscopy and, in few cases, on fundus photography.

Based on the findings obtained using indirect ophthalmoscopy and fundus camera, we made drawings or photographs of the appearance of vitreous body and coagulum, as well as retina during the first couple of weeks of experiment, in order to assess changes that took place during coagulum evolution, compared to the changes in eyeballs which were treated with foreign body and blood.

RESULTS

The appearance of vitreous body assessed using indirect ophthalmoscopy was recorded on drawings in all two groups treated by different procedures. The extent of blood elimination from vitreous was arbitrary defined through 5 stages.

Firstly, we would like to present the results of blood elimination from vitreous space obtained by indirect ophthlamoscopy in time periods of 5 minutes, 1 week, 2, 3, 4, 6, 9, 10, and 12 weeks.

We examined two groups of eyes (rabbits): those treated with blood only (B), and those treated with blood and foreign body (B+FB). During the experiment, both eyes of each rabbit were used; one was treated with blood (B), and the other with B+CS(blood and corticosteroid), or one was treated with B+FB, and the other with B+FB+CS. We want to present two groups of eyes for this occasion (without groups with B+CS and B +FB+CS).

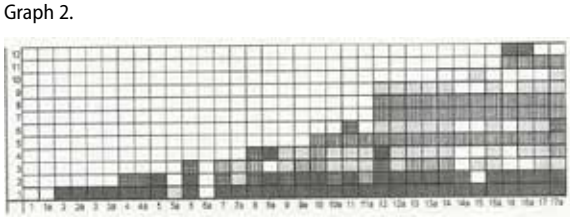
Rabbits, eye, type of experimental injections, number of samples, and examination duration (i.e. time interval before rabbits were euthanized) are presented in Figure 1.

Ophthalmoscopic appearance of vitreous body was recorded using drawings in all four groups of eyes. The extent of vitreous blood elimination, which was assessed ophthalmoscopically, was arbitrarily segregated in 5 stages:

– Stage 1 – total vitreous opacity
– Stage 2 – increased red reflex
– Stage 3 – fundus is visible through patchy vitreous opacities
– Stage 4 – central vitreous is clean with presence of small residual opacities
– Stage 5 – totally clean vitreous

Characteristics of elimination processes in the two groups treated with B,and B+FB, is shown on Graphs 2 and 3.

In rabbits treated with blood only, changes in vitreous opacities begin during fourth week after injection. Almost all rabbits, which survived the experiment, reached stages 4 or 5 after sixth week, as shown on Graph 2.

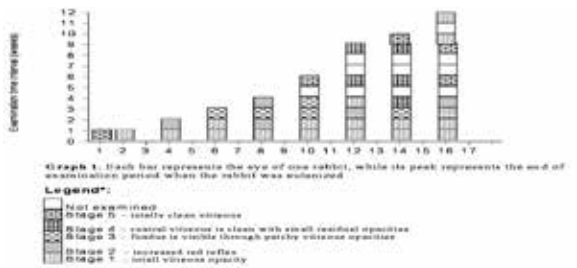


Horizontal: Each bar represents the eye of one rabbit while their peaks represent the end of examination period when the rabbit was eutanized.

Vertical:Examination time interval (weeks).

Figure 1 .Collective data of number of rabbits, eyes, type of experimental injections and examination duration (i.e. time interval before rabbits were euthanized) and the extent of vitreous blood elimination arbitrarily segregated in 5 stages.

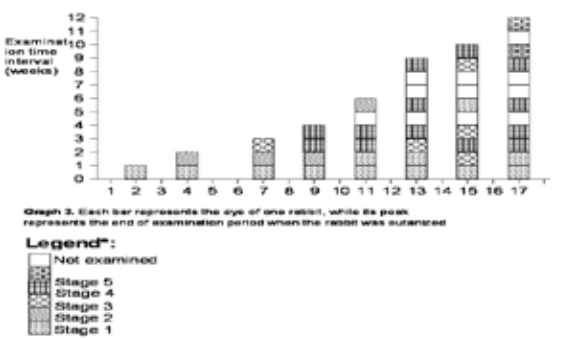
Figure 2. BLOOD AS INJECTANT



Each bar represents the eye of one rabbit, while its peak represents the end of examination period when the rabbit was eutanized.

The changes in vitreous opacities when eyes were treated with blood and foreign body are shown on Graph 3. Clearance begins during third week after injection, while almost all rabbits (except one) reached stages 4 or 5 after sixth week.

Figure 3. BLOOD + FOREIGN BODY AS INJECTANT



Each bar represent the eye of one rabbit, while its peak represents the end of examination period when the rabbit was eutanized.

Based on ophthalmoscopic and gross anatomical findings on samples frozen in acetone, all examined eyes from all 2 groups showed diffuse vitreous opacification during first week, which we characterized as Stage 1.

Hemolysis was assessed based on its cardinal sign – vitreous opacifi-

cation by hemolyzed blood.

On Graphs 2 and 4**,** where eyes were treated with blood, it is clearly shown that all of the specimens were in stage 1 (100%) during the first week. During the second week, from 7 examined eyes, 5 (71.43%) remained in stage 1, while two (28.57%) reached stage 2.

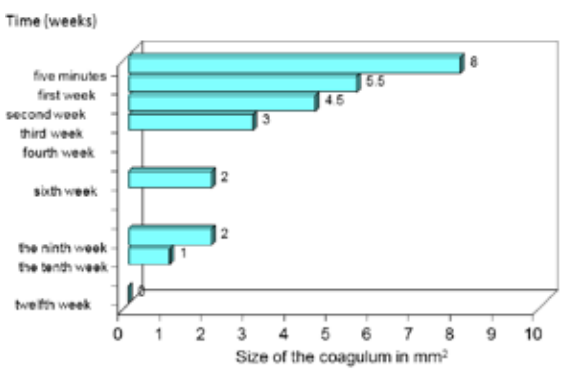
During third week, one out of 6 examined eyes remained in stage 1 (16.6%), one reaches stage 2 (16.6%), while the remaining 4 reach stage 3 (66.4%).

During fourth week, out of five examined eyes, one (20%) paradoxically reaches stage 1 (after previously being in stage 2), one eye (20%) reaches stage 2, two eyes (40%) reach stage 3, while one eye (20%) reaches stage 4.

During sixth week of experiment, out of four examined eyes, two (50%) were in stage 4, while two (50%) were in stage 5.

If we consider stages 4 and 5 to be indicative of vitreous clearance (i.e. elimination of hemolyzed blood), we can conclude that, in the group in which eyes were treated with blood, clearance was completed during sixth week.

Figure 4. Fibrinolysis in function of coagulum reduction – blood as the injectant.



On Graphs 3 and 5 we can see that out of 8 examined eyes from the group treated with blood and foreign body, all of them (100%) were in stage 1.

During the second week of examination, out of 7 examined eyes, 3 (42.84%) were in stage 1, while 4 (57.12%) were in stage 2.

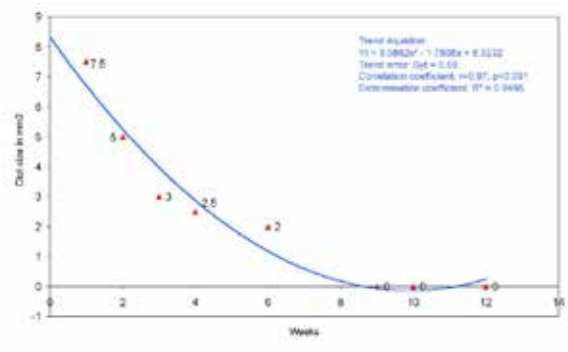
During the third week of examination, out of 6 examined eyes, 2 eyes (33.2%) were in stage 3, while 4 (66.4%) were in stage 4.

During the fourth week of examination, out of 5 examined eyes, one eye (20.0%) was in stage 3, while 4 eyes (80.0%) were in stage 4.

During the sixth week of examination, out of 4 examined eyes, one (25%) was in stage 2, one (25%) in stage 3, while 2 eyes (50.0%) were in stage 4.

In this group of rabbits, eye 19 showed continuous opacification, which was in stage 3 during ninth week, to advance to stage 4 during the tenth week.

Figure 5. Fibrinolysis as function of coagulum reduction - blood and a foreign body as the injectant



Changes in vitreous opacities in cases where full blood only was injected, start during fourth week after injection. All rabbits that survived reached stages 4 and 5 after sixth week, as shown on Graph 2.

Changes in vitreous opacities in cases where eyes were treated with blood and foreign body are shown on Graph 3. Changes resulting in clearance start during third week after application of blood and foreign body, resulting in almost all rabbits (except one) reaching stage 4 or 5 after sixth week.

DISCUSSION

Vitreous hemorrhage is a major cause of vision loss (3).

Hyaluronan is a major macromolecule of the vitreous.(4,15). Studies have shown that the vitreous contains collagen type II, a hybrid of types V/XI, and type IX collagen in a molar ratio of 75:10:15, respectively.(5,6).

Such structure of the vitreous body makes clearance of the vitreous hemorrhage very different than in other tissues.

Fibrinolysis was indirectly assessed by analyzing the size of the coagulum that was primarily composed of fibrin network, with erythrocytes trapped it the interfibrillar spaces. We were also able to assess fibrinolysis gross-anatomically and ophthalmoscopically, based on the change of the color of coagulum to white.

If the injected substance, as mentioned above, was always the same amount of autologous blood (0.2 ml), we had an opportunity to determine the area of the coagulum by measuring its length and width using gross-anatomical specimens (this can also be seen on anatomical photographs), and therefore gaining the comparable parameter for the analysis in our experiment. On Graph 4. we can see that, when blood was injected, the size of coagulum started to decrease from 8mm^2, five minutes after the blood was injected, to negligible size of 1 mm^2, after the tenth week.

On the Graph 5. we can see that, when blood and foreign body were injected, the coagulum decreased from the initial 7.5mm^2 through the process of gradual disintegration until the sixth week when it measured 2 mm^2, and becoming immeasurable in the ninth week.

Fibrinolysis as a function of coagulum reduction, in cases when blood was injected, has a form of a second order polynomial:

Yt=0.0535X2-1.1591X+6.9746, with a trend error SYt=0.77

which could be used for interpolation and extrapolation of data in the cases that have the characteristics of the analyzed specimen.

The correlation of the coagulum size and time in weeks, in cases when the blood was injected is statically highly significant (r=0.95; p<0.001). Explanatory factor of time impact (in weeks) on the reduction of the size of the coagulum is 91.2%, while the remaining 8.8% of variability is attributed to other factors.

Fibrinolysis as a function of coagulum reduction, in cases when blood

and foreign body were used, has a form of a second order polynomial:

Yt=0.0862x2-1.7086x +8.3232, with a trend error Syt= 0.60

which could be used for interpolation and extrapolation of data for the cases that have the characteristics of the analyzed specimen.

The correlation of size of the coagulum and time in weeks, in cases when the blood with a foreign body was used, is statically highly significant (r=0.97; p<0.001). Explanatory factor of time impact (in weeks) on the reduction of size of the coagulum is 95%, while the remaining 5% of variability is attributed to other factors.

We analyzed cellular response to vitreous blood and blood and foreign body, by counting and establishing average cell number seen within 3 visual fields at characteristic spots, using large magnification (400x). For this occasion we want to present just course of fibrinolisis through coagulum disintegration.

There were no significant modifications in fibrinolysis rate within the three examined groups of rabbits.

We may conclude that the vitreous fibrin clearance represents slow process, which in cases of blood only, terminate during 9th or 10th week. Cellular penetration into the vitreous is very poor.

Vitreous response to presence of coagulum was characterized by persistence of coagulum until 4th week. Our slices show that original fibrin network still persists after one month, when the majority of erythrocytes becomes disintegrated and removed from coagulum.

The loss of coagulum fibrin network is not observed before 4th-6th week after injection of blood. This suggests that disintegration of vitreous coagulum, i.e. fibrinolysis, is very slow.

On the other hand, rapid vitreous coagulum formation is confusing, knowing that thromboplastin activity within vitreous tissue is low. During our experiment, we clearly observed that blood coagulum was formed and clearly demarcated from surrounding vitreous structures, after no more than 5 minutes.

Concerning the fact that coagulum in rabbit skin is removed within week or two (7) in abundant presence of PMN cells, and that the same process in vitreous takes 6-9 weeks in presence of extremely small number of PMN cells, we may conclude that the reason for slow fibrin elimination is nothing else but complete absence or modest amount of PMN cells, which is compatible with results of other autors (8,9,10).

Furthermore, this leads to fibrin degradation products, which are known to stimulate PMN cellular migration (11,12,13).

Graph 5. shows coagulum, i.e. vitreous fibrin disintegration rate, in case where blood and foreign body were injected. The coagulum decreased from the initial 7.5 mm2 on the first week, to 2 mm2 on the 6th week, to become immeasurable on the 9th week.

The conclusion is that the process of vitreous fibrin removal is timely process, which terminates during 9th-10th week in cases where blood only is injected. Cellular penetration into the vitreous coagulum is very modest.

There were no significant differences in fibrinolysis rate in the remaining group of rabbits where blood and foreign body were injected.

CONCLUSION

Analyzing the obtained results from our experimental study, whose purpose was to demonstrate normal intravitreous hemorrhage evolution over the successive periods of time, as well as to assess any possible modifications caused by foreign body, we can conclude that normal intravitreous hemorrhage evolution had the following course:

Five minutes after the blood was injected, very rapidly, the coagulum was formed with distinctive margins and clearly demarcated from the rest of the vitreous.

On the second week, the coagulum decreases in size. Comparison of the fibrinolysis curves suggests that the progressive fibrinolysis allows the release of erythrocytes.

Close examination of coagula areas shows that they linearly decreased in size during the following weeks and that on third week the fibrin network was still clearly evident. By the time the fourth week is reached, the size of the coagulant was still reducing. The coagulant was rather small in size on the sixth week (around $2mm^2$). On the ninth week, the size of the coagulant was practically negligible (around $1mm^2$) along with a low number of new cellular elements. During this time interval, the specimens displayed the fusion of old and new macrophages.

On the tenth and twelfth week, the coagulant was immeasurable. Therefore, the vitreous hemorrhage differs from other types of hemorrhage that affect tissues other than eye, by following characteristics: rapid formation of blood coagulum and slow fibrinolysis.

In our experimental study we have been tested is FB can not significantly modify the rate of vitreous clearance.

Various pharmacologic vitreolysis agents (hyaluronidase, urea,tPA, plasmin,dispase) have been tested to date, and so far none have met with sufficient success to result in widespread use (13).

Various enzymes have been investigated to dissolve the vitreous hemorrhage or modify the structural characteristics of the vitreous to allow diffusion of blood out of the visual axis Enzymatic vitreolysis has several advantages over conventional surgery, including ability to treat the hemorrhage earlier, in an office setting,and lower cost. With pharmacological vitreolysis, complications of surgery such as cataract, endophthalmitis, retinal hemorrhage, tear or detachment, and anesthesia-related complications can be avoided.(14)

Our results have shown that the fibrinolysis of vitreous hemorrhage is slow, compared to hemorrhage in other types of tissue, which is due to composition of the vitreous hyaluronan-collagen matrix which allow limited passage of blood cells which are necessary for the iniciation of fibrinolysis process.

Then, our experiment has shown that is reasonable to wait up to 9^{th} weeks after vitreous hemorrhage to be cleaned spontaneously,unlike in other types of tissues (for example skin) it is reasonable to wait up to 3 weeks. After that period of time vitrectomy has to be performed for those eyes with traumatic vitreous hemorrhage (of course if severe inflammation is not associated) following analogy between human and animals eyes.

We hope that our experiment could be very useful in general medical practice as some explanation why we have to wait much more time for vitreous hemorrhage (up to 9 weeks) instead of 2-3 weeks for other types of tissues..

REFERENCES

1.Forrester JV, Grierson I. The cellular response to blood in the vitreous: An ultrastructural study.J Pathol 1979;129: 43-52. 2. Forrester JV, Grierson I, Lee WR. The pathology of Vitreous Hemorrhage – Gross and Histological Appearance.Arch Ophthalmol 1978;96: 703-710. 3. Spraul CW,Grossniklaus HE. Vitreous hemorrhage. Surv Ophthalmol 1997;42(1): 3-39. 4. Sebag J. molecular biology of pharmacologic vitreolysis. Trans Am Ophthalmol Soc 2005;103:473-494. 5. Bishop PN. Structural macromolecules and supramolecular organization of the vitreous gel. Prog Ret Eye Res 2000;19:323-344. 6.Sebag J. The Vitreous- structure, function and pathobiolog. Springer-Verlag, New York, 1989. 7. Riddle JM,Barnhardt MI. Ultrastructural study of fibrin dissolution via emigrated polymorphonuclear neutrophil.Am J Pathol 1964;45: 805-823. 8. Gloor BP .On the question of the origin of macrophages in the retina and the vitreous following photocoagulation.Graefes Arch Ophthalmol 1974;190: 183-194. 9. Forrester JV, Edgar W, Prentice CR. The effect of fibrinolytic inhibition in the resolution of experimental vitreous hemorrhage.Am J Ophthalmol 1977;84: 810-814. 10.Klemen UM,Freyler H, Kulnig W. Electron microscopical studies on the resorption of vitreous hemorrhage.Graefes Arch Ophthalmol 1980;201: 214-251. 11. Guerra A, Pastor J.C. Retinal cryoapplication and fibrinolysis in experimental vitreous hemorrhage.Curr Eye Res : 1991, Vol. 10, 8: p. 697-701 . 12. Paulo A.A. Mello-Filho, Cardillo JA, Farah ME . Intravitreal gas injection for the treatment of experimental vitreous hemorrhage in rabbits.Curr Eye Res: 2002, Vol. 25, 5: p. 261-265. 13 .Regnault FR. Vitreous hemorrhage: An experimental study. A Macroscopic and isotopic study of the evolution of whole blood and hemoglobin. II Hemoglobin degradation.Arch Ophthalmol 1970;83: 458-474. 14.Hugo Quiroz-Mercado,John B Kerrson,D. Virgil Alfaro,William F. Mieler,Peter E Ligget. Macular surgery,2th edition, Wolters Kluver/Lippincott Williams&Wilkins, Philadelphia,2011, pp 115-118. 15. J. Sebag. Vitreous in Health and Disease, Springer New York Heidelberg Dordrecht London,2014,pp 437-477.