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Research Article

The Study of Different Vitreous Contrasting Suspensions Dissolving (*In Vitro*)

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Abstract

Introduction: The search and development of an optimal endovitreal dye that provides clear visualization and identification of intravitreal structures of the vitreous body (VB) and retina is still an important problem today. One of the defining characteristics is the solubility of the contrasting agent.

Purpose: To conduct a comparative study of the solubility of suspensions of barium sulfate (Vitreocontrast) and triamcinalone acetonide (Kenalog-40) in an in vitro model.

Material and Methods: The experimental study was carried out on 12 weighed samples of 1 mg Barium sulfate and triamcinolone acetonide. Weighed samples were placed in test tubes containing 5 ml of 0.9% NaCl solution. After that tubes were installed in a horizontal shaker incubator Unimax 1010 with a temperature of 37°C and setting it to a rotation speed of 60 rpm. The pre-calculated volume of liquid was updated once a day. Drying of the sediment and determination of the mass of the residue in the dry state on the Ohaus Analytical plus AP210 analytical balance was performed on 10, 15, 20, 25, 30 days.

Results: During a comparative study of the solubility of BA sulfate and triamcinolone acetonide suspensions in an in vitro experiment, the complete dissolution time of the first sample was 27 days, and the complete dissolution of the second sample was 29 days.

Conclusion: Analysis of the results of a comparative study of the solubility of suspensions indicates a faster complete dissolution of the BA sulfate suspension, which is an advantage for its use in clinical practice.

Keywords: *Vital Dyes; Vitreous Body; Barium Sulfate ("Vitreocontrast"); Triamcinolone Acetonide ("Kenalog-40"); Solubility*

Introduction

Complete removal of difficult-to-see, nearly transparent vitreous layers in vitreoretinal surgery, is the key to enabling atraumatic and effective vitreoretinal surgery. The desire of surgeons to achieve maximum visualization of intravitreal structures and increase the efficiency of surgical interventions has led to the emergence and dynamic development of vitreocontrastography. Peter Kroll in 2002 proposed the name chromo-vitrectomy describing vitrectomy with the use of any dyeing agent injected into the VB to highlight its structures.

Today vitreoretinal specialists continue to search and develop a contrast agent that would combine such specific properties: high dispersity, selective affinity to tissues, ease of administration and the possibility of the removal through natural outflow pathways, absence of side effects [1].

Groups of researchers use the systemic approach to find a vital dye that would be characterized by good biocompatibility, absence of toxic effect on eye tissues and the selectivity of structures and the retina contrasting [2,3].

Currently, in ophthalmology most surgeons use Triamcinolone acetonide (TA) suspension. In 2000 Peyman G., *et al*. for the first time described the intravitreal use of TA as a contrast agent to visualize ocular structures during vitrectomy [4].

In the framework of the study, it was found that the suspension particles settle between vitreous cortical fibers staining them and are not washed out by the irrigation fluid, which provides clear visualization and makes it easier for surgeons to perform a vitrectomy.

TA ($C_{21}H_{27}FO_6$) is a non-water soluble synthetic glucocorticosteroid.

Injectable form of triamcinolone acetonide is also available on Russian pharmacological market - suspension containing 40 mg of TA and 9.9 mg of benzyl alcohol in isotonic sodium chloride solution Kenalog-40 (Bristol-Myers Squibb. USA). This drug has anti-inflammatory, anti-edema, anti-angiogenic and anti-proliferative effects. According to the literature, 4 mg of the active substance in 0.1 ml solution is most commonly used for intravitreal injection [5].

It should be mentioned that the application of Kenalog-40 suspension (Bristol-Myers Squibb. USA) is prohibited in the territory of the Russian Federation. The convenience of use, as well as the effectiveness of the chromo-vitrectomy method in the practice of the vitreoretinal surgeon, encourage researchers to continue the search for the ideal contrast agent.

In order to visualize the structures specialists from Research Experimental Production Eye Microsurgery LLC of the Fedorov Eye Microsurgery Federal State Institution of the Russian Ministry of Health developed contrast agent Vitreocontrast [6-8].

Vitreocontrast is based on the nonorganic ultradispersed suspension made on the basis of the inorganic salt of barium sulfate in isotonic solution with osmolarity of 300 - 350 mOsm. Barium sulfate is a crystalline substance of white color, insoluble in water and physiological liquids. It is a neutral and non toxic salt with the molecular weight of 233.43 g/mol.

The particle size of the Vitreocontrast suspension is less than 5 microns and the density is 4.4 gr/cm^3. Each 1.0 ml of sterile solution contains 140 mg of dry substance (barium sulfate). Barium sulfate, actively used in medicine, is not toxic to the body. In the course of preclinical toxicological studies (Test Report № 463 from 17.06.2009) of Vitreocontrast at the autonomous non-for-profit organization Biomir testing laboratory of preclinical studies of the Biomedical Research and Technology Institute (Moscow), it was found that that the contrast agent samples had no local irritating, sensitizing or toxic effect, was sterile and met the requirements for products that are in a long contact with the internal environment of the eye.

Experimental and clinical research showed a number of advantages of Videocontrast suspension for selective contrasting of vitreous structures and the inner limiting membrane, which makes vitreoretinal surgical manipulations much easier [9-12]. However, to date, a number of issues remain, one of which is the dissolution time of contrast agent residues after surgical intervention, which is important in assessing expected results and prognosis in the postoperative period.

To address this issue, an experimental study was carried out to compare the solubility of barium sulfate suspension (Vitreocontrast) and triamcinolone acetonide suspension (Kenalog-40) in an *in vitro* model.

Materials and Methods

The study of the barium sulphate suspension and triamcinolone acetonide (Kenalog-40) *in vitro* was carried out in accordance with the standard protocols of the study of medical products of the Russian Federation national standard GOST R ISO 10993-9-2009 [13]. The study of the proposed implant resorption was carried out on the basis of Research and Experimental Production Eye Microsurgery LLC in a fixed volume of 0.9% NaCl solution equal to 5.0 ml. Isotonic sodium chloride solution (NaCl) recommended in the international standard ISO 10993-5. The experiment was performed on 12 samples of Ba sulfate and 12 samples of Kenalog-40, each 1 mg, which were placed in sealed test tubes containing 5 ml of 0.9% NaCl solution with phosphate buffer pH = 7.4. All tubes were placed in a horizontal shaker incubator Unimax 1010 (Heidolph, Germany) with a shaking frequency of 60 rpm and a temperature of 37°C.

Daily a certain amount of pre-calculated volume of liquid was renewed. The volume of fluid intake from the test tube was 2 mm^3/min. It was calculated based on the value of the average rate of aqueous humor production. Consequently, about 3 ml of fluid flowed through the anterior chamber of the eye. Thus, 3.0 ml of liquid from the test tube was replaced with 3.0 ml of saline while maintaining the set temperature and its volume in the test tube. The condition of the sediment was visually assessed once a day. On the 10^{th}, 15^{th}, 20^{th}, 25^{th} and 30^{th} days in the ventilated isolated drying chamber Binder B10 (Germany) the sediment was completely dried up to the achievement of the constant weight. The temperature of drying was 80°C.

Using Ohaus Analytical plus AP210 scales (Ohaus, Switzerland) with a division value of 0.0001g, the sediment weight in a dry condition was determined. The weight of m0 to the 2^{nd} digit was measured in milligrams. Determination of the sediment weight loss was carried out from the beginning of the experiment until its complete visual disappearance in the test tube. Based on these results, a graph was plotted as a function of the sample weight loss versus time.

Results

The mean value of the original weight of barium sulphate and triamcinolone acetonide sediment is m m0 in a dry state was 0.1 mg. During the study the change of the color of the solution, suspended matter or sediment is not revealed.

On the 10^{th} day, the average weight of barium sulfate sediment was 0.63 + 0.33 mg. The average weight of Kenalog-40 sediment was 0.67 + 0.33 mg.

On the 15^{th} day the average weight of barium sulfate sediment was 0.44 + 0.02 mg. The average weight of Kenalog-40 sediment was 0.5 + 0.03 mg.

On the 20^{th} day the average weight of barium sulfate sediment was 0.26 + 0.01 mg. The average weight of Kenalog-40 sediment was 0,33 + 0,02 mg.

On the 25^{th} day the average weight of barium sulfate sediment was 0.07 mg. The average weight of Kenalog-40 sediment was 0.17 + 0.01 mg.

On the 27^{th} day during the visual control of the barium sulphate suspension sample no visible residue was detected.

On the 29^{th} day during the visual inspection of the Kenalog-40 suspension sample no visible sediment was detected. Thus, in the course of the experiment the time of the complete dissolution of barium sulfate was 27 days, the time of the complete dissolution of the Kenalog-40 suspension was 29 days.

Dynamics of changes in the weight of barium sulfate suspension dry residue and Kenalog-40 suspension residue as a function of time in model *in vitro* is presented in the graph (Chart 1).

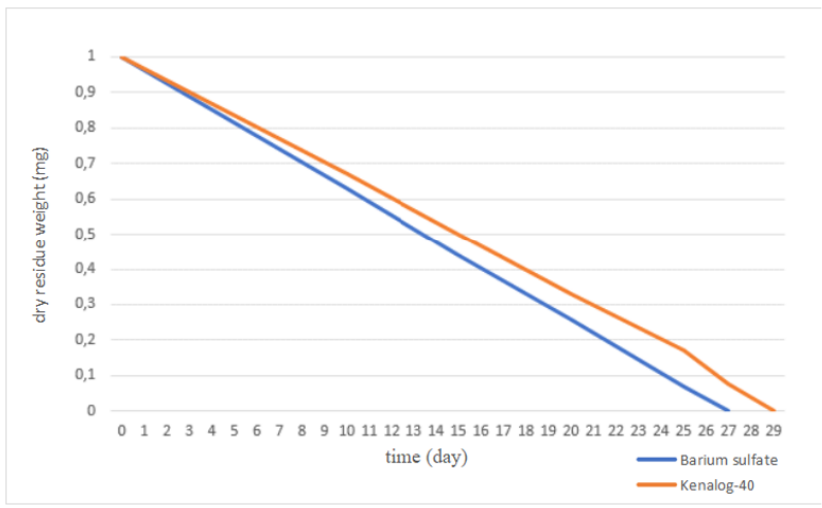

Chart 1: *Changes in the weight of barium sulfate suspension residue and Kenalog-40 suspension residue as a function of time in model in vitro.*

The results of the comparative study of the solubility of barium sulphate and triamcinolone acetonide suspensions *in vitro* experiments demonstrated that the time of barium sulphate suspension complete dissolution was 27 days, and the triamcinolone acetonide suspension complete dissolution was 29 days.

Discussions

One of the most difficult tasks for the surgeon and one of the main components of surgical treatment success is the safe removal of the transparent, seemingly structureless vitreous body and vitreoretinal interface structures: translucent ILM, epiretinal membranes during subtotal vitrectomy. To visualize the vitreous body intraoperatively, its native structures and abnormally changed areas are highlighted with biological and synthetic dyes.

The peak of intravitreal injection of dyes was in 2000, when a new surgical approach - chromovitrectomy - appeared, the essence of which is intraoperative injection of dyes to contrast the posterior segment structures of the eye. This technique provides for the better visualization of the VB and vitreoretinal interface intraoperatively, more thorough removal of vitreous cortical layers, facilitates "peeling" of the ILM and epiretinal membranes, reducing the risk of iatrogenic retinal damage. Alternative dyes for chromovitrectomy have been proposed: brilliant blue (BriB; Merck, Darmstadt, Germany), bromophenol blue (BroB; Sigma-Aldrich, Munich, Germany), congo red (CR; Merck), light green (LG; Merck), indigo carmine (IC; Merck), evans blue (EB; Merck), triamcinolone acetonide and others. And their number is constantly growing. However, according to the literature, the above drugs have a number of side effects, and as a result, there remains much controversy regarding the potential toxicity and safety of these substances.

Thus, in spite of the big experience gathered in this sphere, a number of questions related to the properties of intravitreal vital dyes, their interaction with surrounding anatomical structures, and the side effects of these substances remain unresolved until now. There is a need to develop a specialized contrast agent (contrast composition) for chromo-vitrectomy that would meet all the requirements for

intravitreal dyes, have no toxic properties, provide isolated contrasting of posterior segment structures, which would allow performing vitreoretinal surgical interventions as effectively and most safely as possible.

One of the determining factors when choosing a contrast agent in vitreoretinal surgery is the time of its complete dissolution in the vitreous cavity. In foreign practice, the main suspensions used by surgeons for VB visualization is triamcinolone acetonide suspension (Kenalog - 40.) At Fedorov Eye Microsurgery Institute together with LLC Research Experimental Production Microsurgery LLC Vitreocontrast staining suspension for the VB structures was developed. For this experiment, intraocular fluid exchange rate in the vitreous cavity and corresponding temperature parameters were simulated under the model *in vitro* conditions.

According to the data of comparative experimental study under *in vivo* conditions, it was found that Vitreocontrast barium sulfate suspension has an advantage over Kenalog 40 suspension in its dissolution rate (Figure 1-3).

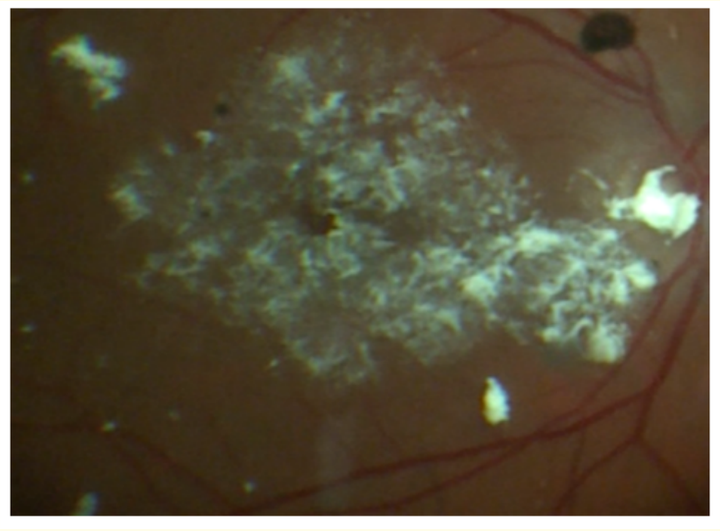

Figure 1: *Intraoperative photo of the fundus after the introduction of "Vitreocontrast" suspension.*

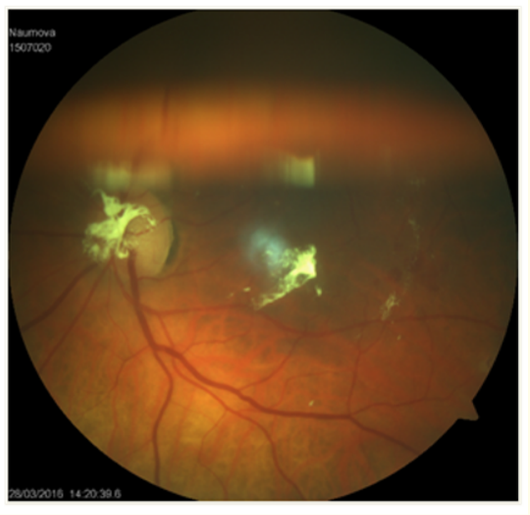

Figure 2: *Photo of the fundus 2 days after the introduction of the "Vitreocontrast" suspension.*

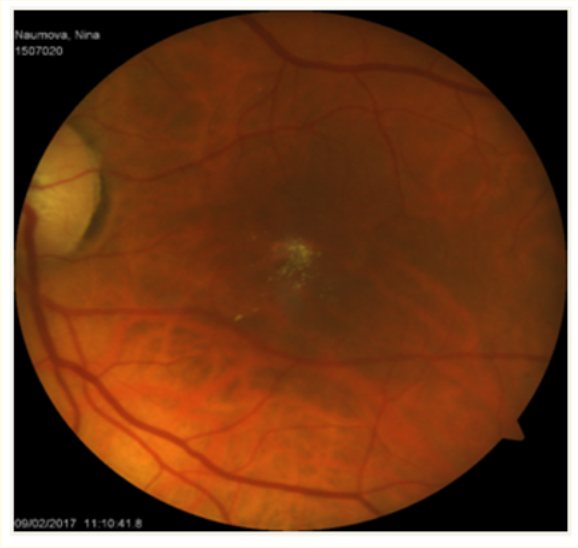

Figure 3: *Photo of the fundus 14 days after the introduction of the "Vitreocontrast" suspension.*

Conclusion

Analysis of the results of a comparative study of the solubility of barium sulfate and triamcinolone acetonide suspensions in the experiment *in vitro* indicates a more rapid complete dissolution of the barium sulfate suspension, which is an advantage for its use in clinical practice.

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