Role of Blood Components in Ocular Silicone Oil Emulsification

Studies on an In Vitro Model

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**Purpose.** To develop an in vitro model for silicone oil emulsification and to explore the blood components involved in this process.

**Methods.** The capacity of various blood components to support silicone oil (1000 CS) emulsification was studied by applying 0.5 ml oil on top of 0.5 ml saline containing various blood components. Each tube was sonicated for 150 seconds and centrifuged at 5000g for 20 minutes. Three phases were noted in the tube: At the top was clear silicone oil, in the middle was emulsified silicone oil, and at the bottom was aqueous solution. The tubes were photographed, and the percentage of the phase length containing emulsified silicone oil (middle) of the total length of the three phases was calculated from the projected image of each tube.

**Results.** Emulsified silicone oil in plasma or serum was initiated after 100 seconds of sonication and quickly reached maximum (approximately 80%) at 120 seconds. The size of these oil droplets prepared in vitro was 0.0467 ± 0.028 mm, closely resembling that observed in oil samples removed from a patient's anterior chamber (0.038 ± 0.018 mm). Under these conditions, silicone oil emulsified in the presence of whole blood cells occurred only at a concentration of 120 μg protein/ml; in the presence of red blood cell membranes, it occurred at a concentration of 60 μg protein/ml. Lipoprotein-deficient serum failed to support emulsification; however, samples of high-density lipoprotein and low-density lipoprotein supported this process. Purified high-density lipoprotein-apolipoproteins supported oil emulsification. The addition of phosphatidylcholine further enhanced this process, but phosphatidylcholine alone failed to support emulsification.

**Conclusions.** A simple and fast in vitro model to study factors affecting silicone oil emulsification was developed. Using this model, red blood cell membranes, plasma lipoproteins, and purified HDL-apolipoproteins supported silicone oil emulsification. Lipids did not, but they had the capacity to enhance the apolipoprotein-supported emulsification. Invest Ophthalmol Vis Sci. 1996; 37:2694-2699.

Silicone oil is used extensively in complicated vitreoretinal operations.¹² Its use, though, may be associated with side effects, one of which is oil emulsification, with a reported incident rate up to 56%.³ In phakia, the oil drops are small enough to travel between the zonules or, as in aphakia, directly through the pupil into the anterior chamber, where trabeculum dysfunction and secondary glaucoma can occur. The droplets, rising to the superior part of the anterior chamber, may accumulate to such an extent that all or part of the pupil is covered, which interferes with vision and may damage the corneal endothelium and its metabolism because of the subsequent development of corneal edema and band keratopathy.⁴

Many factors may enhance emulsification. An oil bubble is spherical and is stabilized by the surface tension of the interface between the aqueous medium and the oil. For example, when the surface tension is reduced by detergents or surfactants, oil may break...
up into smaller drops if an external mechanical force is applied. This process is called emulsification. The lower the oil surface tension, the easier it is to emulsify.

Proteins, lipids, and, in particular, phospholipids in the eye have been proposed as substances whose presence may reduce surface tension of the silicone oil, accelerating its emulsification. Additionally, the presence of impurities in the form of low molecular weight components may also enhance emulsification. The use of high-viscosity oil has been suggested as a means to reduce the incidence of emulsification; breaking the silicone oil into small bubbles requires a mechanical force whose magnitude is related directly to the viscosity of the oil. In a given patient, the amount of mechanical force generated by eye movement is more or less constant; hence, it can be expected that for any given amount of eye movement, more viscous oil will emulsify less.

In vitro models attempting to imitate this process have used an orbital shaker, which may take days or weeks to cause emulsification, or a vortex instrument, which requires only a few minutes. The lipid droplets created by both were larger than the droplets found in the patient eye.

Other investigators have focused on the properties of silicone oil, such as its viscosity and the presence of impurities. We were intrigued by the outstanding fact that silicone oil from the same batch may emulsify early and massively in some patients and late and little or not at all in others. This suggests that factors in the intraocular environment are the cause for emulsification; it does not mean that factors in the oil itself may make one type of oil less emulsifiable than another.

To determine the reason for these individual variations in silicone oil emulsification, it was established that major cellular and liquid components of the blood are emulsifiers. In this investigation, a simple and fast in vitro model was developed to study silicone oil emulsification and to specify the biochemical components of the blood elements that have the highest potential as emulsifiers.

**METHODS**

**Preparation of Blood Components**

**Plasma and Serum.** A sample of human whole blood was placed in two glass test tubes. Heparin (20 U/ml) was added to one of the tubes to obtain plasma. After 24 hours of incubation at 4°C, tubes were centrifuged (1000g), and the supernatant was collected representing serum and plasma, respectively. Blood samples for the preparation of plasma, serum, white blood cells, and red blood cells were taken in compliance with the Declaration of Helsinki, and proper consent and approval were.

**Preparation of Lipoproteins and Lipoprotein-Deficient Serum.** Lipoproteins were prepared according to Havel et al. Briefly, desired fractions were separated by differential ultracentrifugal flotation from blood bank human plasma. Lipoproteins were separated according to their specific density: low-density lipoproteins (LDL), 1.019 < d < 1.063 g/ml; high-density lipoproteins (HDL), 1.07 < d < 1.21 g/ml; and lipoprotein-deficient serum (LPDS), d > 1.25 g/ml. The protein concentration of each fraction was determined by the modification of Markwell et al of the method of Lowry et al.

**Preparation of White Blood Cells.** Fifty milliliters of blood were incubated with 20 U/ml of heparin for 1 hour. After centrifugation at 4°C for 5 minutes at 100g and the removal of platelets, red blood cells (RBC) in the precipitation were lysed in hypotonic solution, and the remaining white blood cells (WBC) were washed twice with phosphate-buffered saline by centrifugation at 100g for 10 minutes. White blood cells were suspended in 10 ml of phosphate-buffered saline. The WBC count was 10,400 cells/μl. The protein content of this solution was 12.4 mg/ml as determined by the method of Lowry et al.

**Red Blood Cell Membranes (Ghosts).** Red blood cells were precipitated from whole blood by centrifugation at 100g at 4°C for 10 minutes, washed with saline solution for 20 minutes, and centrifuged. No platelets were found. From the precipitate, 2 ml were added to 28 ml hypotonic phosphate buffer (20 mM, pH 7.4) and centrifuged at 20,000g at 4°C for 40 minutes. The pellet was rewashed twice with the same hypotonic buffer. After the third wash, the buffer solution was transparent. The protein content was determined as described above (10.9 mg/ml).

**Preparation of Apolipoproteins.** Apolipoproteins of HDL (HDL APO) were prepared by extracting the lipids from HDL by ethanol:ether solution (3:2 vol/vol) at −20°C for 24 hours, and this was followed by centrifugation at 2000g at 4°C for 20 minutes. The protein sediment was washed twice with ether and dried. Purification of apolipoprotein A-I (APO A-1):HDL APO powder was dissolved in 8 M urea solution containing 1 mM EDTA and 10 mM Tris buffer (pH 8.6), and subjected to a Sephacryl (Pharmacia, Uppsala, Sweden) S-200 column (2.6 × 90 cm) equilibrated with the same buffer. Separation of the purified APO A-I was performed as previously described.

**Preparation of Phosphatidylcholine-Coated Tubes.** Chloroform solution (0.5 ml) containing phosphatidylcholine (625, 1250, or 2500 μg/ml) was placed in glass test tubes. After evaporating the chloroform, the lipids coated the internal surface of the test tube.

**Silicone Oil Emulsification Procedure.** Phosphate-
buffered saline (0.5 ml) with or without APO A-I or various blood components was added to glass test tubes, precoated with phosphatidylcholine or uncoated. Tubes were shaken by a vortex instrument for 20 seconds. Silicone oil (1000 CS; Dow Corning, Corning, NY) (0.5 ml) was added to each test tube. This action was performed carefully to preserve the separate aqueous and silicone oil phases. Each tube was placed in a stand in the center of a sonication instrument (Ultrasonic Cleaner; Astrason, Farmingdale, NY) and activated for 150 seconds or as indicated. The test tube was then centrifuged at 2800 rpm for 20 minutes in a Sorvall GLC-2B centrifuge (DuPont Instruments, Newtown, CT). After centrifugation, three phases were noted in the test tube: The top contained clear oil, the middle contained emulsified silicone oil, and the bottom contained aqueous solution. The test was performed in duplicate for each concentration of potential emulsifier in the aqueous solution. All tests were conducted at room temperature; the water temperature in the sonication instrument was maintained at 20°C to 24°C. Test tubes were photographed on a slide film. Measurement of the phase length of emulsified silicone oil (middle) in the test tubes was done on the projected slide image. The percentage of the length of this emulsified phase of the total length of the three phases was calculated.

Assessment of Emulsified Silicone Oil Drops. Samples of emulsified silicone oil were produced by sonication or by a vortex emulsification for 60 seconds as previously described. The size of the sample droplets, as well as those taken from a patient’s anterior chamber during surgery, was measured. Preparations were observed and photographed using a phase-contrast microscope (Olympus, Tokyo, Japan), and the diameters of 200 drops were measured for each solution. The minimal drop diameter detected by this technique was 0.001 mm.

RESULTS

Emulsification of Silicone Oil as a Function of Sonication Time

To determine the optimal time required to obtain maximal stable emulsification, sonication of silicone oil was performed with plasma for different time periods (Fig. 1). At sonication times shorter than 100 seconds, little emulsification (approximately 20%) was achieved. After 100 to 120 seconds, a steep, fast increase in the degree of emulsification (approximately 80%) was observed; it only slightly increased after that. Based on these results, sonication for 150 seconds was chosen for all subsequent assays.

Drop Size of Emulsified Silicone Oil

The size of an emulsified silicone oil drop removed from a patient’s anterior chamber was 0.038 ± 0.018 mm (mean ± SD; n = 200). The oil drops prepared by in vitro sonication with plasma were 0.0467 ± 0.028 mm, whereas drops prepared by shaking the oil–plasma mixture on a vortex instrument for 60 seconds were 0.189 ± 0.168 mm.

Role of White Blood Cells and Ghosts in Silicone Oil Emulsification

The involvement of WBC and RBC ghosts in the emulsified silicone oil was studied (Fig. 2). Increasing concentrations of RBC membranes (ghosts) were sonicated in the presence of silicone oil. The percentage of emulsified silicone oil formed in the presence of blood cells was measured. Only partial emulsification occurred at a WBC concentration as high as 120 μg protein/ml, whereas in the presence of ghosts, it oc-
Ocular Silicone Oil Emulsification

Role of Plasma, Serum, and Lipoprotein-Deficient Serum Fraction in Silicone Oil Emulsification

Silicone oil was sonicated with plasma, serum, and LPDS at increasing concentrations, and the percentage of oil emulsification is presented in Figure 3. Both plasma and serum supported emulsification, but plasma was slightly more effective. The degree of emulsification was proportional to the increase in concentration of plasma and serum, reaching a level of 80% to 90% emulsification at a concentration of 100% plasma or serum. It should be noted that no emulsification was observed without plasma or serum, and LPDS failed to induce significant emulsification even at a concentration of 100%. A slight emulsification (approximately 30%) was observed in the presence of 25% LPDS.

Role of Lipoproteins in Silicone Oil Emulsification

Both LDL and HDL were sonicated with silicone oil at increasing concentrations; the percentage of oil emulsification is presented in Figure 4. Although LDL and HDL supported emulsified silicone oil, HDL was more efficient than LDL. To explore the active component in HDL, the ability of phosphatidylcholine coated onto the test tube to support silicone oil emulsification was examined. These experiments indicated that pure phosphatidylcholine could not support the emulsification of silicone oil (data not shown). Protein constituents of HDL (HDL APO) were prepared and tested (Fig. 5). The mixture of all HDL APO supported emulsified silicone oil at a concentration of 200 to 300 μg/ml at a level of 40% to 50%. Although phosphatidylcholine did not support emulsification, its effect, in combination with the major HDL APO—APO A-I, was tested. There was an increase in emulsification when the APO A-I-phosphatidylcholine mixture was used, and it was dependent on the phosphatidylcholine concentration (Fig. 6).

DISCUSSION

In this study, a new in vitro method using a sonication technique to evaluate silicone oil emulsification was developed. It is a quick and easy assay that provides the opportunity to study the contribution of blood components or various silicone oils to the process of silicone oil emulsification. The droplets created in vitro were similar in size to the emulsified silicone oil obtained from patients, indicating that this method may be more relevant than others to the emulsification process occurring in vivo.
Among serum components, the role of the lipoprotein effect on silicone emulsification. This observation protein concentration range of 100 to 300 μg/ml. Fur- therserved. Proteins serving as lipid carriers in the blood fraction in the emulsification of silicone oil was ob- served. Furthermore, phosphatidylcholine enhanced the APO A-I effect on silicone emulsification. This observation may be explained by the ability of apolipoproteins to attach to both the aqueous and the lipid phases, a property essential to their natural role as lipid carriers. It is not surprising that materials that do not dissolve in water (cholesterol, phosphatidylcholine) cannot reach the interface between the aqueous and the silicone oil phase. Consequently, there was no emulsification, only the enhanced effect of apolipoproteins.

Why did membranes have substantial emulsifying activity? The structure of membranes, including both protein and lipid structures (especially phospholipids), allows emulsification even at low protein levels, as was shown in the combination of phosphatidylcholine and APO A-I. In vivo, the relevance of membranes in emulsification may be even smaller because there are not many dead cells that release membranes, whereas the natural levels of lipoproteins such as HDL are relatively high. Much like membranes, HDL contains phospholipids. It is probably important as a cause of emulsification.

The basic question is, what is the main cause for emulsification in the natural environment—the vitreous cavity—in which it occurs? Two factors determine this property in vivo. The first is the affinity of the serum component to the silicone oil, and the second is the concentration of this substance in the ocular environment in which the silicone oil exists. The main lipid carriers in plasma are LDL and HDL. A large complex (approximately 1,000,000 Da), LDL does not enter the vitreous cavity. Conversely, HDL is smaller and can enter the vitreous cavity, reaching significant levels when the blood−retinal barrier is broken.

It is more difficult to evaluate the significance of pathologic conditions of the eye (e.g., hemorrhage, inflammation, infection) during and immediately after surgery and in the late postoperative stage, when proliferative vitreous retinopathy or recurrent or longstanding hemorrhage may occur. All these conditions raise the concentrations of blood protein and other blood components, which may enhance emulsification on contact, especially prolonged, with the silicone oil.

In this study, it was shown that intraocular proteins present in relatively high levels and under certain clinical conditions have the potential to facilitate the emulsification of silicone oil. It is reasonable to implicate variations in the penetration of these substances into the eye under various clinical conditions as the underlying cause for the observed, sometimes extreme, variability in emulsification of the same batch of silicone oil in different patients.

**Key Words**

apolipoproteins, emulsification, lipoproteins, silicone oil, vitreoretinal operation

**References**

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