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In vitro effect of silicone oil and liquid perfluorocarbons on platelet aggregation

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PURPOSE. *To study the* in vitro *effect of silicone oil of different viscosities and liquid perfluorocarbons on platelet aggregation.*
METHODS. *Silicone oil with a viscosity of 5700 cs and 1000 cs and liquid perfluorocarbons of perfluoroperhydrophenantren and perfluorodecaline were studied to investigate the effect of these agents on platelet aggregation under* in vitro *conditions. The experiments were performed by using platelet rich plasma with an aggregometer. Aggregation was induced with three different agonists, including 5"-adenosine diphosphate (ADP), epinephrine (EPI), and collagen (Col). The results were obtained as a percentage of maximal aggregation and compared with controls using Kruskal-Wallis one way analysis of variance test.*
RESULTS. *The tests with ADP as aggregating agent revealed that the percentage of maximal aggregation was a mean of 72.66±3.51% for ADP only, 58.66±3.05% for silicone oil of 1000 cs, 62.66±2.08% for silicone oil of 5700 cs, 56.00±7.00% for perfluoroperhydrophenantren, and 52.3±3.1% for perfluorodecaline. With EPI, aggregation was induced in all control samples with a mean of 76±9.54%. The mean percentage of maximal aggregation was 66.7±3.06 for silicone oil of 1000 cs, 72.33±5.5% for silicone oil of 5700 cs, 71.67±3.79% for perfluoroperhydrophenantren, and 70.33±2.52% for perfluorodecaline. With collagen, it was 86.67±1.53% for controls, 83.67±3.51% for silicone oil of 1000 cs, 85.33±4.51% for silicone oil of 5700 cs, 83.33±4.93% for perfluoroperhydrophenantren, and 81.33±4.16% for perfluorodecaline. Statistical analysis revealed no significant change in the percentage of maximal aggregation for all tested substances in the experiments.*
CONCLUSIONS. *Silicone oil of different viscosities and perfluoroperhydrophenantren and perfluorodecaline have minimal antiaggregating effect on platelets. The level of effect is not statistically significant. (Eur J Ophthalmol 2004; 14: 550-4)*

KEY WORDS. *Liquid perfluorocarbons, Platelet aggregation, Silicone oil*

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INTRODUCTION

During the past decade, silicone oil and perfluorocarbon liquids (PFCL) have become commonly used agents in vitreoretinal surgical procedures and have facilitated surgical management of complex retinal detachment (1, 2). Both substances are clinically well known and there are many clinical and experimental studies on the physicochemical and clinical properties of these substances (3, 4). On the other hand,

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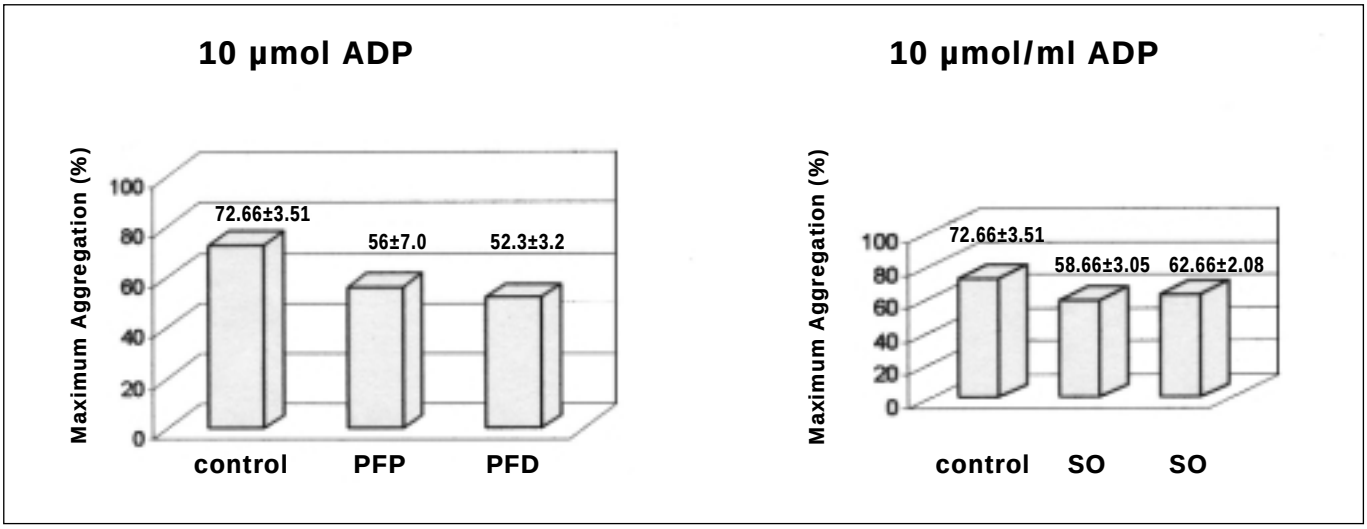

Fig. 1 - *Effect of silicone oil of different viscosities and liquid perfluorocarbons on adenosine 5'-diphosphate (ADP) induced platelet aggregation (PFP = Perfluoroperhydrophenantren; PFD = Perfluorodecaline).*

achievement and maintenance of good hemostasis is an integral part of the success of these surgical interventions. Concerning hemostasis, platelets are important components of blood. The actions of biomaterials on platelet aggregation (5) are well known, but there is no study on the role of silicone oil and PFCLs on platelet aggregation.

In the present study, we investigated the *in vitro* effect of silicone oil and PFCLs on platelet aggregation.

METHODS

In vitro studies were performed using silicone oil with a viscosity of 5700 cs and 1000 cs (Oxane, Chauvin Opsia SA, France) and two different PFCLs: perfluoroperhydrophenantren (Vitreon, Vitreophage, Lyons, IL) and perfluorodecaline (D-K Line, Chauvin Opsia SA, France).

Subjects and aggregation study

Three healthy male volunteers (mean age 32.3±3.2 years) who denied having taken any drugs during the preceding 2 weeks were included into the study.

Following overnight fasting, venous blood samples were obtained from each subject at 8 AM and were anticoagulated with 3.8% trisodium citrate (blood/anticoagulant: 9/1). Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by centrifugation at 1000 rpm for 10 minutes and at 3500 rpm for 10 minutes, respectively. When necessary, autologous PPP was used to adjust the platelet count of PRP to 200 x 10^9/L before measurement of platelet aggregation was performed.

The aggregometer was model 560 CA lumiaggregometer of Chronolog Corporation (Chronolog Corp., Havertown, PA). Aggregation was induced using three agonists, including adenosine 5'-diphosphate (ADP), epinephrine (ADR), and collagen, with final concentrations of 10 µmol/mL for ADP, 4.4 µg/mL for collagen, and 100 µM/mL for ADR. In order to evaluate the effects of silicone oil of 5700 cs, silicone oil of 1000 cs, perfluoroperhydrophenantren, and perfluorodecaline on platelet aggregation, equal volumes of these substances were added to PRP samples at 37.5 ^oC 5 minutes before the addition of agonists.

Aggregation was recorded as light transmission of the PRP at 37 °C with continuous stirring by a magnetic stirrer and stirring speed was set at 1000 rpm. The initial light transmission of PRP was set at 0% while that of PPP was set at 100%. Aggregation traces were recorded for a minimum of 6 minutes up to a maximum of 15 minutes until maximal aggregation

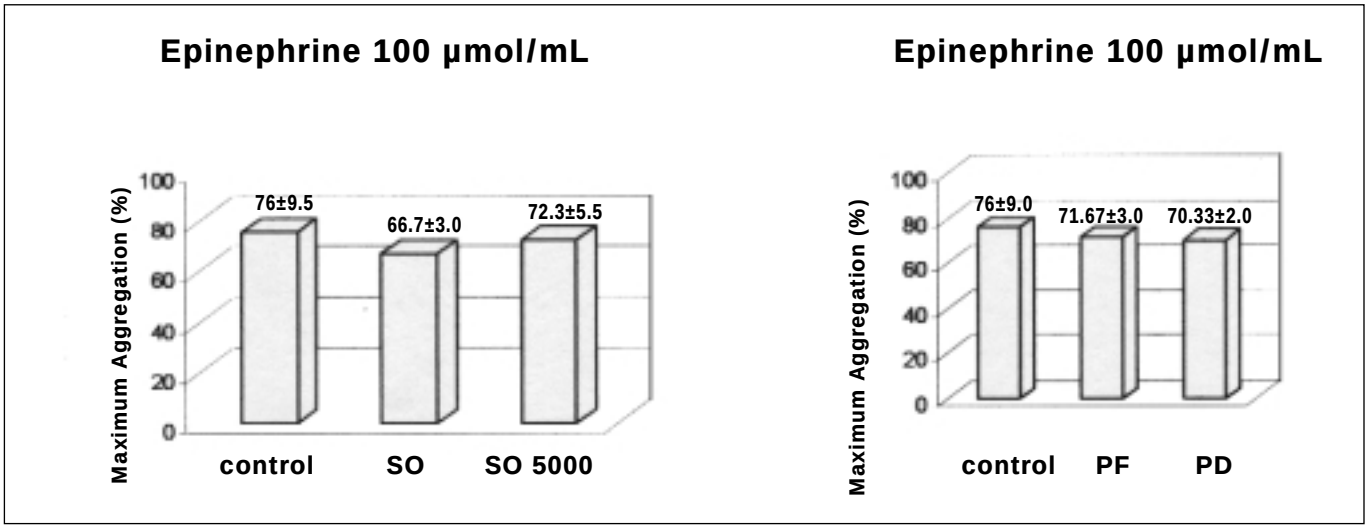

Fig. 2 - *Effect of silicone oil of different viscosities and liquid perfluorocarbons on epinephrine induced platelet aggregation (SO = Silicone oil; PFP = Perfluoroperhydrophenantren; PD = Perfluorodecaline).*

(percent of maximal light transmittance after addition of the aggregating agent) was determined.

For the quantification of platelet responses to agonists, the reading of percentage aggregation was used as an endpoint measurement indicating the extent of aggregation, whereas the slope of the trace, automatically calculated by the instrument, was taken to represent the rate of the aggregation reaction. Tests were repeated three times for each substance.

The results obtained for each tested substance were compared with the results obtained from the samples containing aggregating agent only, using Kruskal-Wallis one way analysis of variance test (UNISTAT Statistical Package version 5.1.03).

RESULTS

Changes of maximal aggregation of silicone oil of different viscosities and different PFCLs treated in response to ADP, epinephrine, and collagen are shown in Figures 1, 2, and 3.

When control samples were stimulated by ADP, aggregation was induced in all with a mean of 72.66±3.51%. The mean percentage of maximal aggregation was 58.66±3.05% for silicone oil of 1000 cs, 62.66±2.08% for silicone oil of 5700 cs, 56.00±7.00% for perfluoroperhydrophenantren, and 52.3±3.1% for perfluorodecaline. The percentage of maximal aggregation was decreased with all the substances tested when compared to control specimens including only ADP (Fig. 1). Kruskal-Wallis one way analysis of variance test revealed no significant decrease in the percentage of maximal aggregation for all tested substances.

With EPI, aggregation was induced in all control samples with a mean of 76±9.54% (Fig. 2). The mean percentage of maximal aggregation was 66.7±3.06 for silicone oil of 1000 cs, 72.33±5.5% for silicone oil of 5700 cs, 71.67±3.79% for perfluoroperhydrophenantren, and 70.33±2.52% for perfluorodecaline. Kruskal-Wallis one way analysis of variance test revealed no significant decrease in the percentage of maximal aggregation for all tested substances.

When control samples were induced by collagen, aggregation was detected in all with a mean of 86.67±1.53% (Fig. 3). The mean percentage of maximal aggregation was 83.67±3.51% for silicone oil of 1000 cs, 85.33±4.51% for silicone oil of 5700 cs, 83.33±4.93% for perfluoroperhydrophenantren, and 81.33±4.16% for perfluorodecaline. Kruskal-Wallis one way analysis of variance test revealed no significant

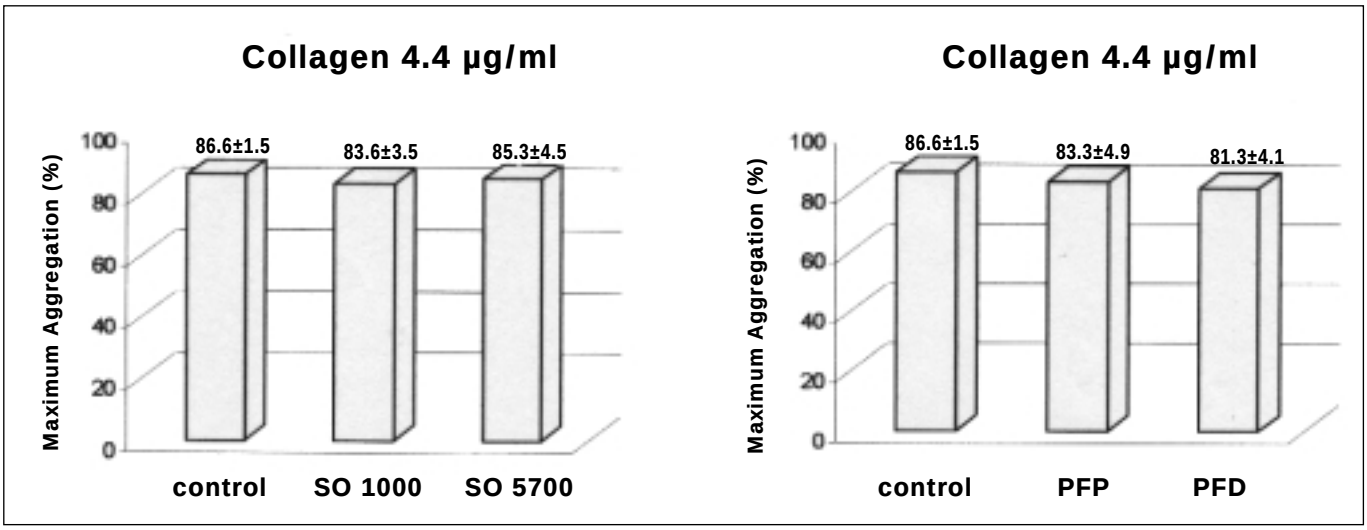

Fig. 3 - *Effect of silicone oil of different viscosities and liquid perfluorocarbons on collagen induced platelet aggregation (SO = Silicone oil; PFP = Perfluoroperhydrophenantren; PD = Perfluorodecaline).*

change in the percentage of maximal aggregation for all tested substances.

DISCUSSION

Progress in the development of biomaterials has been hindered by the lack of basic information on the nature of the tissues, organs, and systems that are replaced, repaired, or supported. The aspects of surface chemistry, surface degradation, and physical structure are critical concerning the reaction of blood with artificial surfaces. Under normal conditions platelets do not adhere to endothelial cells in the circulating blood. Exposure of blood to abnormal surfaces activates the coagulation system, often accompanied by activation of the immune system, and leads to thrombus formation and deposition of a layer of proteins and cells.

In this study, we aimed to evaluate the *in vitro* effect of silicone oil of different viscosities and two different PFCLs, substances which are commonly used intraoperatively and postoperatively in vitreoretinal surgical procedures, on agonist-induced human platelet aggregation.

We used ADP, epinephrine, and collagen as aggregating agents separately and measured the percentage of maximal aggregation of thrombocytes. Then, we repeated these experiments by adding silicone oil of different viscosities and two kinds of PFCLs. Although the percentage of maximal aggregation measured using all substances was lower than the measurements using aggregating agent only, the differences were not statistically significant.

PFCLs are immiscible and have a surface tension of approximately 14 to 16 dynes/cm against air. PFCLs generally have a high oxygen-carrying capacity. Mice immersed in an oxygenated bath of low viscosity PFCLs can survive by breathing this liquid. PFCLs have potential application as an oxygen reservoir in the vitreous cavity (6). It was shown that acute and chronic hyperoxygenation decreases platelet aggregation and adenosine triphosphate release from thrombocytes (7). The high oxygen carrying capacity of PFCL might have caused minimally decreased platelet aggregation that was demonstrated in our study.

Silicone oil is immiscible with water and has surface tension of approximately 54 dynes/cm measured against air. The study by Gemmell et al demonstrated that silicone shows low platelet activation in *in vitro* experiments (8). From the findings of the electron microscopic study on silicone coated polypropylene hollow-fiber oxygenator, it appeared that silicone coating reduces platelet adhesion on the sur-

face of hollow fibers (9). Our study showed that silicone oil did exert an *in vitro* antagonistic effect on platelet aggregation although it did not reach statistical significance. Our *in vitro* results also showed that viscosity of the silicone oil does not influence the effect of silicone oil on platelet aggregation.

Silicone oil of different viscosities and perfluoroperhydrophenantren and perfluorodecaline have little antiaggregating effect on platelets. This effect is probably not substantial enough to be able to compromise surgical maneuvers.

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