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Comparison of the effect of Healon, Healon GV, Healon 5, Viscoat, and OcuCoat on platelet aggregation under *in vitro* conditions

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PURPOSE. *To study the effect of Healon, Healon GV, Healon 5, Viscoat, and OcuCoat on platelet aggregation under* in vitro *conditions.*
METHODS. *Ocular viscoelastic devices including Healon, Healon GV, Healon 5, Viscoat, and OcuCoat 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 one-way analysis of variance (ANOVA) test.*
RESULTS. *The tests with ADP as aggregating agent revealed that the percentages of maximal aggregation were a mean of 75 ± 4.35% for ADP only, 67 ± 4.35% for Healon, 59.33 ± 3.51% for Healon GV, 70 ± 3% for Healon 5, 58 ± 3.46% for Viscoat, and 64 ± 2% for OcuCoat. Kruskal-Wallis one-way ANOVA 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 80.66 ± 2.08%. The mean percentage of maximal aggregation was 67 ± 3% for Healon, 77.66 ± 4.04% for Healon GV, 77 ± 4% for Healon 5, 80.6 ± 4.04% for Viscoat, and 65 ± 5% for OcuCoat. Statistical analysis showed no significant difference. With collagen, maximum aggregation was 74 ± 5.29% for controls, 65 ± 4.35% for Healon, 54 ± 2% for Healon GV, 51 ± 2.64% for Healon 5, 59 ± 2% for Viscoat, and 72.66 ± 1.52% for OcuCoat. Kruskal-Wallis one-way ANOVA test revealed no significant change in the percentage of maximal aggregation for all tested substances in the experiments.*
CONCLUSIONS. *Ophthalmic viscosurgical devices like Healon, Healon GV, Healon 5, and Viscoat that contain glycosaminoglycans and OcuCoat that contains hydroxypropylmethylcellulose have inhibitory effects on platelet aggregation but the effect is not statistically significant and there is no difference among the ocular viscoelastic devices in regard to inhibitory effect on platelet aggregation. (Eur J Ophthalmol 2006; 15: 306-10)*

KEY WORDS. *Healon, Healon GV, Healon 5, OcuCoat, Platelet aggregation, Viscoat*

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INTRODUCTION

The introduction of ophthalmic viscosurgical devices (OVDs) has revolutionized anterior segment surgery. OVDs are useful in protecting the corneal endothelium, separating the tissue, stabilizing a space, and occupying a virtual space within the eye (1).

On the other hand, achievement and maintenance of good hemostasis is an integral part of the success of surgical interventions. Concerning hemostasis, platelets are important components of blood (2). Platelets are the first actors to play in the coagulation process. When exposed to a procoagulant material, i.e., subendothelial tissue, glass, or a foreign surface, they adhere and become activated, forming the primary hemostatic plug, and also provide some other factors and phospholipid membrane, which augments the process.

Hyaluronic acid is a ubiquitous, non-sulfated glycosaminoglycan component of the extracellular matrix. It has been shown that hyaluronic acid inhibits platelet aggregation and platelet adhesion as well as prolongs bleeding time measurements when administered systemically (3, 4).

To our knowledge, there are no data comparing the effect of OVDs on platelet aggregation. We studied the effect of different commercially available viscoelastic products that are most commonly used in ophthalmic surgery on platelet aggregation under *in vitro* conditions.

METHODS

In vitro studies were performed using sodium hyaluronate 1% (Healon, Pharmacia & Upjohn), high viscosity sodium hyaluronate 1.4% (Healon GV, Pharmacia & Upjohn), sodium hyaluronate 10% (Healon 5, Pharmacia & Upjohn), 1:3 mixture of 4% chondroitin sulfate and 3% sodium hyaluronate (Viscoat, Alcon Labs), and 2% hydroxypropylmethylcellulose (HPMC) (OcuCoat, Bausch and Lomb) in syringes devised for immediate surgical application.

Subjects and aggregation study

Three healthy male volunteers (mean age 32.3 ± 4.8 years), who denied having taken any drugs during the preceding 2 weeks and whose personal and family histories were negative for hemorrhagic diathesis and whose platelet count, prothrombin time, and activated partial thromboplastin time values were within normal limits, 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 109/L before measurement of platelet aggregation was performed.

The aggregometer was model 560 CA lumiaggregometer (Chronolog Corp., Havertown, PA). Aggregation was induced using three agonists including adenosine 5'-diphosphate (ADP), epinephrine (EPI), and collagen, with final concentrations of 10 µmol/mL for ADP, 4.4 µg/mL for collagen, and 100 µM/mL for EPI. In order to evaluate the effects of Healon, Healon GV, Healon 5, Viscoat, and OcuCoat on platelet aggregation, equal volumes of these substances were added to PRP samples at 37.5 ˚C 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 (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. The tests were repeated three times for each viscoelastic material.

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 (ANOVA) by ranks test (UNISTAT Statistical Package version 5.1.03). Multiple comparisons between different viscoelastics were done with Dunn tests.

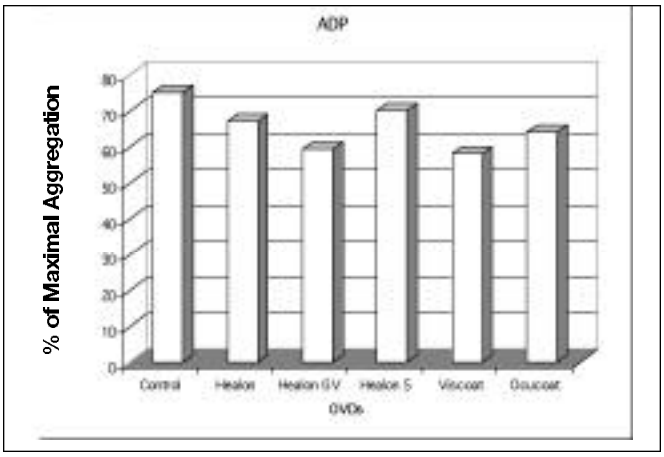

Fig. 1 - *Effect of Healon, Healon GV, Healon 5, Viscoat, and OcuCoat on adenosine diphosphate (ADP)-induced platelet aggregation. OVD = ophthalmic viscoelastic device.*

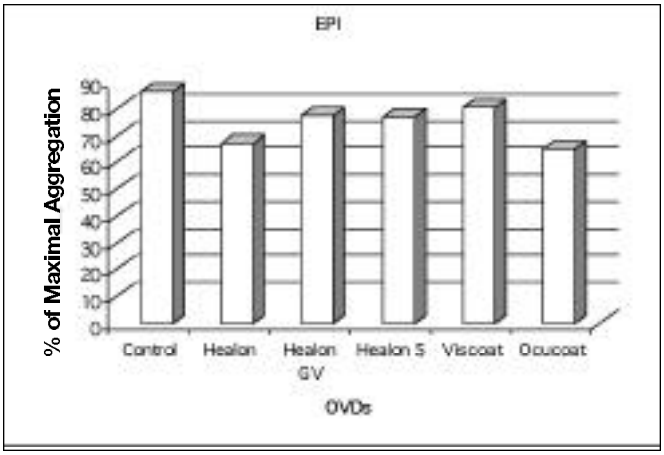

Fig. 2 - *Effect of different ophthalmic viscoelastic devices (OVDs) on epinephrine (EPI)-induced platelet aggregation.*

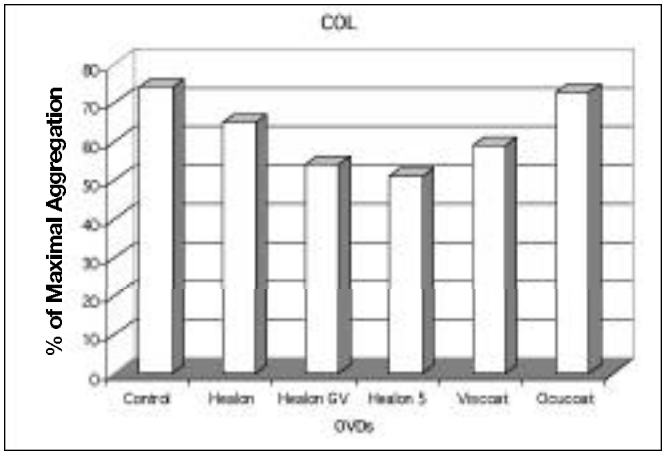

Fig. 3 - *Effect of Healon, Healon GV, Healon 5, Viscoat, and OcuCoat on collagen (COL)-induced platelet aggregation.*

RESULTS

Changes of maximal aggregation of Healon, Healon GV, Healon 5, Viscoat, and OcuCoat treated in response to ADP, epinephrine, and collagen are shown in Figures 1 through 3.

When control samples were stimulated by ADP, aggregation was induced in all with a mean of 75 ± 4.35%. The mean percentage of maximal aggregation was 67 ± 4.35% for Healon, 59.33 ± 3.51% for Healon GV, 70 ± 3% for Healon 5, 58 ± 3.46% for Viscoat, and 64 ± 2% for OcuCoat. The percentage of maximal aggregation decreased with all the substances tested compared to control specimens containing only ADP. Kruskal-Wallis one-way ANOVA test revealed no significant difference in the percentage of maximal aggregation for all tested substances.

With EPI, aggregation was induced in all control samples with a mean of 80.66 ± 2.08%. The mean percentage of maximal aggregation was 67 ± 3 for Healon, 77.66 ± 4.04% for Healon GV, 77 ± 4% for Healon 5, 80.66 ± 4.04% for Viscoat, and 65 ± 5% for OcuCoat. Kruskal-Wallis one-way ANOVA test revealed no significant decrease in the percentage of maximal aggregation for all the substances tested.

When control samples were induced by collagen, aggregation was detected in all with a mean of 74 ± 5.29%. The mean percentage of maximal aggregation was 65 ± 4.35% for Healon, 64 ± 2% for Healon GV, 51 ± 2.64% for Healon 5, 59 ± 2% for Viscoat, and 72.66 ± 1.52% for OcuCoat. Kruskal-Wallis one-way ANOVA test revealed no significant change in the percentage of maximal aggregation for all tested substances.

DISCUSSION

There is a general agreement that the introduction of OVDs such as Healon, Healon GV, Healon 5, and Viscoat has implied a significant step forward in ocular surgery. Sodium hyaluronate (NaHa) is a biopolymer occurring in many connective tissues throughout the body including both the aqueous and vitreous humour. Chondroitin sulfate (CDS) is another viscoelastic biopolymer that is found as one of the three major mucopolysaccharides in the cornea. Its structure is similar to hyaluronic acid, consisting of the

same repeating disaccharide unit. Healon, Healon GV, Healon 5, and Viscoat contain these glycosaminoglycans whose repeating disaccharide units bear resemblance to those of the anticoagulant heparin. Previous laboratory investigation demonstrated an anticoagulant effect of sodium hyaluronate and chondroitin sulfate (5, 6). Pandolfi et al (6) showed that CDS had a clotting inhibitory effect that was probably the consequence of structural similarity to heparin. However, Healon turned out to have almost no anti-thrombin activity *in vitro* in concentrations of up to 0.6 mg/mL. The study concluded that Healon does not inhibit coagulation, contrary to CDS (6). Additionally, it has been shown that hyaluronic acid inhibits platelet aggregation and platelet adhesion as well as prolongs bleeding time measurements when administered systemically (3, 4). On the other hand, the clinical study by Packer et al showed that Healon had procoagulant effects after phakic diabetic vitrectomy (7).

We studied the effect of Healon, Healon GV, Healon 5, and Viscoat, which all contain glycosaminoglycan, on platelet aggregation. We used ADP, EPI, and collagen for inducing platelet aggregation. We tested each OVD with each agonist. Our results showed that Healon, Healon GV, Healon 5, and Viscoat have little inhibitory effect on ADP-, EPI-, and collagen-induced platelet aggregation, but the inhibitory effect is not statistically significant. We tried to test three kinds of NaHa preparations (Healon, Healon GV, Healon 5) that have different concentrations of sodium hyaluronate to investigate the effect of increased concentration of material. Only the experiments performed with collagen as an aggregating agent revealed inhibitory action that is proportional with the increasing concentration of NaHa. Other experiments performed with ADP and epinephrine as aggregation inducing agents did not show this concentration induced enhancement of platelet aggregation inhibitory action.

HPMC is another viscoelastic material used for intraocular procedures. Unlike the previous three viscoelastic compounds, it does not occur naturally in animals but is distributed widely as a structural substance in plant fibers such as cotton and wood (8). OcuCoat is highly purified, synthetic, nonprotein, nontoxic preparation of 2% HPMC. OcuCoat has been marketed as a viscoadherent rather than a viscoelastic because of its significant coating ability and its relative lack of elastic properties. We also investigated the effect of OcuCoat on platelet aggregation. Our study showed that OcuCoat has little inhibitory effect on ADP-, EPI-, and collagen induced platelet aggregation. Its inhibitory effect was not statistically different from Healon, Healon GV, Healon 5, and Viscoat and controls.

OVDs like Healon, Healon GV, Healon 5, and Viscoat, which contain glycosaminoglycans, and OcuCoat have little inhibitory effect on platelet aggregation that apparently would not compromise surgical maneuvers and there is no difference among the OVDs in regard to inhibitory effect on platelet aggregation.

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REFERENCES

1. Arshinoff SA. Dispersive and cohesive viscoelastic materials in phacoemulsification. Ophthalmic Pract 1998; 16: 24-32.
2. Born GVR. Adenosine diphosphate as a mediator of platelet aggregation *in vivo*: an editorial point of view. Circulation 1985; 72: 741-2.
3. Barbucci R, Ito Y, Favia P. New materials containing HyalSx. Soc Biomaterials Trans 1998; 21: 171.

4. Mitchell JD, Lee R, Hodakowski GT, et al. Prevention of postoperative pericardial adhesions with a hyaluronic acid coating solution: experimental safety and efficacy studies. J Thorac Cardiovasc Surg 1994; 107: 1481-8.
5. Teien AN, Abildgaard U, Hook M. The anticoagulant effect of heparan sulfate and dermatan sulfate. Thromb Res 1976; 8: 859-67.
6. Pandolfi M, Hedner U. The effect of sodium hyaluronate and sodium chondroitin sulfate on the coagulation system *in vitro*. Ophthalmology 1984; 91: 864-6.
7. Packer AJ, Brooks VM, Hutton WL, Ramsay RC. Procoagulant effects of intraocular sodium hyaluronate (Healon) after phakic vitrectomy. Ophthalmology 1989; 96: 1492-4.
8. Rosen ES, Gregory RPF, Barnett F. Is 2% hydroxy propylmethylcellulose a safe solution for intraoperative clinical applications? J Cataract Refract Surg 1986; 12: 679-83.