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Effect of High-Molecular-Weight Hyaluronic Acid on the Viscosity of Rabbit Pericardial Fluid Measured with a Cone and Plate Viscometer

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To determine whether or not the high-molecular-weight ($>2\times10^6$) hyaluronic acid in rabbit pericardial fluid contributes to the changes in the viscosity of the pericardial fluid, we compared the viscosity of rabbit pericardial fluid with that of various glycosaminoglycan solutions by using a cone and plate viscometer at 37 °C. Rabbit pericardial fluid showed non-Newtonian flow behavior and had an apparent viscosity of about 1.03—1.04 cP at a shear rate of $750\,s^{-1}$ at 37 °C. On the basis of the data on the viscosity of pericardial fluid and of high-molecular-weight hyaluronic acid solutions, the high-molecular-weight hyaluronic acid (about 0.01% (w/v)) could contribute to the increase in the viscosity and the rheological characteristics of the pericardial fluid. These results suggest that the high-molecular-weight hyaluronic acid in the pericardial fluid could provide the heart tissue with resiliency and compliance when the tissue is subjected to stretching or compressive forces.

Keywords——viscosity; rabbit pericardial fluid; hyaluronic acid; cone/plate viscometer; rheology

Introduction

The functional roles of the pericardium and pericardial fluid are confined to lubricating the moving surfaces of the heart and providing mechanical support for the contraction of the ventricle.[1] The possibility that the pericardium of the heart might secrete some substance which lubricates the moving surfaces of the heart has received little attention. Recently, we found for the first time the occurrence of high-molecular-weight ($>2\times10^6$) hyaluronic acid (about 80—100 μg/ml) in the rabbit pericardial fluid.[2] Furthermore, incubation experiments revealed that the high-molecular-weight hyaluronic acid was actively synthesized by the pericardium from [^{3}H]glucosamine and released into the pericardial fluid.[2]

To determine whether or not the high-molecular-weight hyaluronic acid in rabbit pericardial fluid contributes to the changes in the viscosity of the pericardial fluid, we compared the viscosity of rabbit pericardial fluid with that of various glycosaminoglycan solutions by using a cone and plate viscometer (biorheolizer) at 37 °C because only small amounts of samples were available.

We report here that the high-molecular-weight hyaluronic acid in the pericardial fluid does contribute to the increase in the viscosity and the rheological characteristics of the pericardial fluid.

Materials and Methods

Materials——The following commercial materials were used: hyaluronic acid (molecular weight, $4—6\times10^4$) from hog skin, and chondroitin 4-sulfate from whale cartilage from Seikagaku Kogyo, Tokyo, Japan; hyaluronic acid (molecular weight, $>2\times10^6$) from human umbilical cord from Sigma Chemical Co., St. Louis, MO., U.S.A.

Preparation of Rabbit Pericardial Fluid and Serum——A Japanese white rabbit (3.0—3.5 kg) was anesthetized with sodium pentobarbital (39 mg/kg i.v.) and the pericardial fluid (250—300 μl) was obtained from the cardiac cavity with a plastic syringe. About 0.5—1 ml of pericardial fluid was obtained from 2—4 rabbits.

Blood was obtained from the carotid of rabbits with a plastic tube. The blood was kept for 30 min at room temperature and then centrifuged at 1000 rpm for 10 min. Serum was separated and used for subsequent experiments.

Measurement of Viscosity——The values of viscosity of rabbit pericardial fluid, serum, and hyaluronic acid solutions prepared from human umbilical cord and from hog skin, and chondroitin 4-sulfate solution prepared from whale cartilage were measured with a cone and plate viscometer (biorheolizer, BRL-500, Toki Sangyo Co., Tokyo, Japan) at 37 °C.[3] The sample volume used was 0.5 ml.

Results and Discussion

Viscosity of a fluid is defined as the ratio of shear stress to shear rate.[3] Since a cone and plate viscometer can record absolute values of shear stress and shear rate and permit the rapid analysis of small samples (0.5—1 ml) of fluids,[3] we carried out a comparative study on the viscosity of rabbit pericardial fluid and various glycosaminoglycan solutions by using a cone and plate viscometer (biorheolizer) at 37 °C.

Rabbit pericardial fluid showed non-Newtonian flow behavior and had an apparent viscosity that changed with the shear rate (Fig. 1). The apparent viscosity was about 1.03—1.04 cP at a shear rate of 750 s^{-1} at 37 °C (Table I). Since the real concentration of the high-molecular-weight ($>2\times10^6$, as judged from the elution position (void volume) from a Sepharose CL-2B) hyaluronic acid in the pericardial fluid was about 80—100 μg/ml,[2] high-molecular-weight ($>2\times10^6$) hyaluronic acid solution (100 μg/ml saline) was prepared from human umbilical cord as a model system and its viscosity was measured. As shown in Figs. 1 and 2 and Table I, the viscosity of the rabbit pericardial fluid was similar to that of high-molecular-weight hyaluronic acid solution (100 μg/ml saline; hyaluronic acid from the human umbilical cord) as a model system. However, at the same concentration (100 μg/ml saline), the

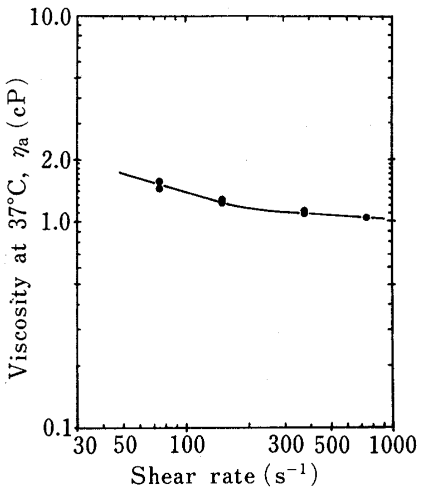

Fig. 1. Non-Newtonian Viscosity of Rabbit Pericardial Fluid

The viscosity of the rabbit pericardial fluid (0.5—1 ml) was measured with a cone and plate viscometer (biorheolizer) at 37 °C. The apparent viscosity (η_a, the ordinate) of two samples of rabbit pericardial fluid was plotted against the shear rate (the abscissa) on log-log graph paper.

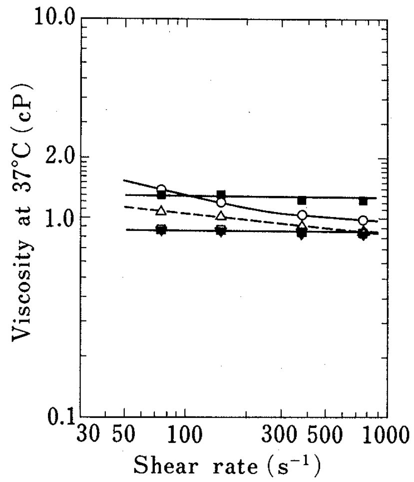

Fig. 2. Viscosity of Rabbit Serum, Hyaluronic Acid Solutions, Chondroitin Sulfate Solution and Saline

The viscosity of various solutions was measured as described in Fig. 1. The viscosity (the ordinate) of rabbit serum (■—■), high-molecular-weight hyaluronic acid solutions from human umbilical cord (100 μg/ml saline, ○—○; 10 μg/ml saline, ▼—▼), chondroitin 4-sulfate solution from whale cartilage (100 μg/ml saline, △---△) and saline (□—□) was plotted against the shear rate (the abscissa) on log-log graph paper.

TABLE I. Viscosity Measurements of Rabbit Pericardial Fluid, Serum, Hyaluronic Acid Solutions, Chondroitin Sulfate Solution, and Saline

	Shear rate (s^{-1})	Viscosity at 37 °C (cP)
Rabbit pericardial fluid		
Sample 1	750	1.04
Sample 2	750	1.03
High-molecular-weight hyaluronic acid from human umbilical cord (molecular weight, $>2 \times 10^6$)		
(1000 μg/ml saline)	750	2.23
(100 μg/ml saline)	750	0.99
(10 μg/ml saline)	750	0.86
Low-molecular-weight hyaluronic acid from hog skin (molecular weight, 4—6×10^4)		
(100 μg/ml saline)	750	0.89
Chondroitin 4-sulfate from whale cartilage		
(100 μg/ml saline)	750	0.86
0.9% (w/v) NaCl (saline)	750	0.86
Rabbit serum	750	1.21

The viscosity of the various solutions was measured with a cone and plate viscometer (biorheolizer) at 37 °C. The sample volume used for measurement was 0.5 ml. For further experimental details, see the text.

viscosity of lower-molecular-weight hyaluronic acid (about 4—6×10^4; from hog skin) solution was less than that of the pericardial fluid (Table I). Also, the viscosity of a lower concentration (10 μg/ml saline) solution of the high-molecular-weight hyaluronic acid was quite similar to that of saline (Newtonian flow) (Fig. 2 and Table I). The viscosity of the chondroitin 4-sulfate solution (100 μg/ml saline) decreased to that (0.86 cP) of saline only at the shear rate of 750 s^{-1} at 37 °C (Fig. 2 and Table I). Thus, these results suggest that high-molecular-weight hyaluronic acid (about 0.01% (w/v)) could contribute to the increase in the viscosity and the rheological characteristics of the pericardial fluid.

Rabbit serum showed Newtonian flow (Fig. 2) and its viscosity was about 1.21 cP at a shear rate of 750 s^{-1} at 37 °C (Table I). Since high-molecular-weight hyaluronic acid is hardly detectable in the rabbit serum,[2)] serum proteins may contribute to the viscosity of rabbit serum.

Other investigators using the same type of viscometer reported that the apparent viscosity of rabbit tear was about 0.946 cP at a shear rate of 752 s^{-1} at 37 °C,[4)] and that of synovial fluid in normal human articular joint was about 73 cP at a shear rate of 384 s^{-1} at 37 °C.[5)] It is known that hyaluronic acid has a high capacity for retaining water and that hyaluronic acid as a lubricant in synovial fluid contributes to the increase in the viscosity of the synovial fluid to a great extent.[6,7)] Since the viscosity of the pericardial fluid is not nearly as high as that of synovial fluid, it is more likely that the high-molecular-weight hyaluronic acid (about 0.01% (w/v)) in the pericardial fluid could provide the heart tissue with resiliency and compliance when the tissue is subjected to stretching or compressive forces.

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