Pentosan polysulfate (fig. 1) is a semisynthetic modified polymer (MW 4000–6000 daltons) derived from beech tree bark. The molecule is a mucopolysaccharide derivative that chemically and structurally resembles other GAGs, including the anticoagulant heparin. In contrast to heparin and other GAGs, PPS is orally bioavailable and has demonstrated few toxic side effects, even when administered in high doses (Marshall et al., 1997; Odlind et al., 1987). PPS has a higher degree of sulfation than heparin, and therefore a greater negative charge density. Similar to other GAGs, its structural and chemical properties allow PPS to bind preferentially to the glyocalyx of circulating blood cells and endothelial cells (Maffrand et al., 1991; Ghosh and Hutadilok, 1995). PPS is 1/15 as effective as heparin in its anticoagulant activity. Its multiple effects on coagulation and fibrinolysis, on platelet functions and on vascular cells have led to the study of PPS in a wide array of clinical disorders such as antagonism of enzymatic activities (leukocyte elastase, heparinase, protein kinases and reverse transcriptase) and inhibition of HIV infectivity (Maffrand et al., 1991). PPS inhibits cancer cell metastasis in the rat and promotes increased lipoprotein lipase and hepatic triglyceride lipase activity (McLeskey et al., 1996; Knabbe et al., 1992; Barrowcliffe, et al., 1986). PPS also may serve as an antiinflammatory agent and has been of benefit in an experimental model of inflammatory arthritis most likely through the inhibition of interleukin-6 production (Smith et al., 1994). Clinically, PPS has been used as an antithrombotic agent and is approved in the United States for the management of patients with interstitial cystitis (Parsons et al., 1993; Parsons and Mulholland, 1987).

ABBREVIATIONS: PPS, pentosan polysulfate; MAC, membrane attack complex; GAG, glycosaminoglycans; NHP, normal human plasma; GVB, gelatin veronal buffer; CK, creatine kinase; LVEDP, left ventricular end-diastolic pressure; LVPD, left ventricular developed pressure; RBC, red blood cell; EGTA, ethyleneglycol-bis(β-aminoethyl ether)-N,N,N’N’-tetraacetic acid; ELISA, enzyme-linked immunosorbent assay.

Received for publication July 21, 1997.

1 This study was funded by the Cardiovascular Research Fund, University of Michigan Medical School, Department of Pharmacology. KSK was supported, in part, by the American Heart Association, Michigan Affiliate (no. 36GB967).

2 Awarded a summer research student fellowship from the American Heart Association, Michigan Affiliate, during the tenure of this study.

The Semisynthetic Polysaccharide Pentosan Polysulfate Prevents Complement-Mediated Myocardial Injury in the Rabbit Perfused Heart

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Accepted for publication February 16, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

Pentosan polysulfate (PPS) is a highly sulfated semisynthetic polysaccharide possessing a higher negative charge density and degree of sulfation than heparin. Like other glycosaminoglycans, the structural and chemical properties of PPS promote binding of the drug to the endothelium. Glycosaminoglycans, including heparin, inhibit complement activation independent of an action on the coagulation system. This ability provides a compelling argument for the implementation of this class of compounds in experimental models of cellular injury mediated by complement. The objective of this study was to examine whether PPS could reduce myocardial injury resulting from activation of the complement system. We used the rabbit isolated heart perfused with 4% normal human plasma as a source of complement. Hemodynamic variables were obtained before addition of PPS (0.03 01 mg/ml) and every 10 min after the addition of human plasma. Compared with vehicle-treated hearts, left ventricular end-diastolic pressure was improved at the conclusion of the 60-min protocol in hearts treated with PPS (58.9 ± 13.6 vs. 15.2 ± 4.8 mm Hg). Further evidence as to the protective effects of PPS was demonstrated by decreased creatine kinase release compared with vehicle (86.5 ± 28.5 U/l vs. 631.0 ± 124.8 U/l). An enzyme-linked immunosorbent assay for the presence of the membrane attack complex in lymph and tissue samples demonstrated decreased membrane attack complex formation in PPS-treated hearts, which suggests inhibition of complement activation. This conclusion was supported further by the ability of PPS to inhibit complement-mediated red blood cell lysis in vitro. The results of this study indicate that PPS can reduce tissue injury and preserve organ function that otherwise would be compromised during activation of the human complement cascade.
The GAGs possess anti-inflammatory properties (Engelberg, 1996). It has been known for a long time that heparin and related GAGs inhibit both the classical and alternative pathways of complement activation (Ecker and Gross, 1929). Thirteen of 22 human complement proteins bind to heparin (Sabu and Pangborn, 1993) as well as native heparin sulfate proteoglycans, thereby inhibiting complement activation independent of any affinity for antithrombin (Weiler et al., 1992). GAGs, having sulfated structural domains, are able to regulate one or more of the serine proteases in the complement cascade (Raepple et al., 1976; Weiler et al., 1992; Sharath et al., 1985; Meri and Pangburn, 1990). Recent studies demonstrating that GAGs inhibit complement activation in vivo, independent of an action on the coagulation system, provide a compelling argument for the implementation of this class of compounds in experimental models of cellular injury that directly involve the complement-mediated tissue injury (Weiler et al., 1992; Black et al., 1995; Gralinski et al., 1996a).

Although PPS possesses many pharmacologic actions including antithrombotic effects and the ability to inhibit numerous enzymes (elastase, heparinase, protein kinases), its effects on activation of the complement system in the myocardium have yet to be ascertained. Complement activation has been demonstrated to occur in myocardial ischemia/reperfusion injury, for example during thrombolysis and clinical procedures, including antithrombotic effects and the ability to inhibit numerous enzymes (elastase, heparinase, protein kinases), its effects on activation of the complement system in the myocardium have yet to be ascertained. Complement activation has been demonstrated to occur in myocardial ischemia/reperfusion injury, for example during thrombolysis and clinical procedures, including heart transplantation and balloon angioplasty (Kilgore et al., 1994a; Fox, 1990; Steg et al., 1993). The objective of the present study was to examine the ability of the highly sulfated carbohydrate polymer, PPS, to inhibit activation of the complement cascade. The rabbit isolated heart perfused in the presence of human plasma was used as a model to determine whether PPS could reduce myocardial injury resulting from activation of the human complement system. The rationale for the use of human plasma for induction of complement-mediated tissue injury derives from the studies demonstrating that the interaction of human plasma or serum with rabbit red blood cells or lung tissue results in the activation of the alternative complement pathway (Platts-Mills and Ishizaka, 1974; Seeger et al., 1989). In the isolated perfused heart, activation of the alternative pathway of human complement involves a direct interaction between the complement components and reactive endothelial cell surfaces of the rabbit heart (Gralinski et al., 1996a; Homeister et al., 1992). This experimental model is well characterized and has been used successfully to assess the potential of pharmacologic agents to inhibit activation of the human complement system in the functioning myocardium (Gralinski et al., 1996b, 1997; Homeister et al., 1993; Park et al., 1997; Kilgore et al., 1994b).

**Methods**

**Guidelines for human and animal research.** The procedures used in this study are in agreement with the guidelines of the Internal Review Board of the University of Michigan and with the regulations of the United States Department of Health and Human Services for the Protection of Human Research Subjects (part 46 of title 45 of the Code of Federal Regulations, as amended); and the University of Michigan Committee on the Use and Care of Animals. Veterinary care was provided by the University of Michigan Unit for Laboratory Animal Medicine. The University of Michigan is accredited by the American Association for Accreditation of Laboratory Animal Care, and the animal care use program conforms to the standards in “The Guide for the Care and Use of Laboratory Animals,” DHEW Publication No. (NIH) 86–23.

**Drugs.** Pentosan polysulfate sodium (PPS) was provided by Baker Norton Pharmaceuticals, Inc. (Miami, FL). PPS was dissolved in sterile 0.9% sodium chloride solution immediately before use and used at a final concentration of 0.03 mg/ml added to the perfusion medium. Control hearts received vehicle (0.9% sodium chloride) only. The pH of both solutions was 7.4.

**Preparation of normal human plasma.** Normal human plasma was collected as described previously (Homeister et al., 1993). After informed consent, human blood was obtained by venipuncture from fasted donors who had not ingested any form of medication in the past 7 days. The blood was placed into tubes containing a minimal amount of heparin (14 U/ml final concentration) and mixed with gentle agitation. The blood was centrifuged at 2000 × g for 10 min, and the plasma was collected. The plasma from a minimum of three donors was pooled. The pooled, NHP was stored at −70°C until used (not more than 72 hr). Immediately before use, the plasma was thawed, centrifuged (40 min at 27,200 × g, 4°C) and filtered through an ethanol-activated C-18 cartridge (Millipore) to remove plasma lipids. Heat-inactivated plasma was used as an addition control. Plasma was thawed and heated to 56°C for 60 min to inactivate complement proteins, centrifuged and processed as mentioned above.

**Assessment of complement inhibition with rabbit erythrocytes.** A red blood cell lysis assay was used to determine the ability of PPS to inhibit the human complement system in vitro and is based on the C5b-9-dependent lysis of antibody-sensitized sheep red blood cells by human plasma. Complement-mediated RBC hemolysis was assessed by a turbidimetric method described previously (Pascual et al., 1990). The hemolysis assay is an accepted method of assessing the complement titer of plasma or serum samples (Whaley, 1985). Rabbit red blood cells were obtained from a donor rabbit with ethylenediaminetetraacetic acid as the anticoagulant, washed three times in 5 ml GVB (Sigma), pH 7.4 (assay buffer), and diluted in GVB to achieve a final RBC concentration of 1 × 10⁸ cells/ml. EGTA was added to the GVB buffer to inhibit the classical pathway. Hanks’ balanced salt solution (20 μl; vehicle) was mixed with 100 μl of 1 × 10⁶ cells/ml and warmed to 37°C for 5 min. The assay was initiated by the addition of 180 μl of 4% human plasma, and the light transmittance was monitored for up to 5 min. The final assay volume was 300 μl. One hundred percent light transmittance was set with RBCs lysed with NHP. PPS was tested at final concentrations of 0.001 mg/ml, 0.003 mg/ml, 0.01 mg/ml, 0.03 mg/ml, 0.1 mg/ml, 0.3 mg/ml, and 1.0 mg/ml. The assay was repeated three times (n = 3).

**Langendorff perfused rabbit heart.** Male, New Zealand White rabbits (2.2–2.4 kg) were sacrificed by cervical dislocation. The...
hearts were removed rapidly and attached to a cannula for perfusion to the aorta. The perfusion medium consisted of a recirculating volume (250 ml) of modified Krebs-Henseleit buffer (pH 7.4, 37°C) delivered at a constant rate of 20 to 25 ml/min that established an initial mean coronary artery pressure perfusion pressure of 60 to 65 mm Hg. The composition of the buffer medium in millimoles per liter was as follows: NaCl, 117; KCl, 4.0; CaCl₂·H₂O, 2.4; MgCl₂·6H₂O, 1.2; NaHCO₃, 25; KH₂PO₄, 1.1; glucose, 5.0; monosodium L-glutamate, 5.0; sodium pyruvate, 2.0, and bovine serum albumin, 0.25% wt/vol.

The Krebs-Henseleit buffer passed through a gas porous “lung” consisting of Silastic Laboratory Grade Tubing (Dow Corning, Midland, MI), 5.49 mm in length, with an inner diameter of 1.47 mm and an outer diameter of 1.96 mm. The membranous “lung” was exposed continuously to a mixture of 95% O₂/5% CO₂ to obtain an oxygen partial pressure within the perfusion medium equal to 500 mm Hg. The hearts were paced throughout the protocol via electrodes attached to the right atrium. Pacing stimuli (3 Hz, 4-msec duration, 4-msec delay) were delivered from a laboratory square wave generator (Grass SD-5, Quincy, MA). The pulmonary artery was cannulated with polyethylene tubing to facilitate collection of the pulmonary artery effluent, representing the coronary venous return to the coronary sinus. The superior and inferior vena cava and the pulmonary veins were ligated to prevent loss of perfusate from the severed vessels. A left ventricular drain, thermistor probe and latex balloon were inserted via the left atrium and positioned in the left ventricle. The fluid-filled latex balloon was connected with rigid tubing to a pressure transducer to permit measurement of left ventricular systolic and end-diastolic pressures. The left ventricular developed pressure was derived by obtaining the difference between the left ventricular systolic and end-diastolic pressures. The intraventricular balloon was expanded with distilled water to achieve an initial baseline LVEDP of 5 mm Hg. Coronary perfusion pressure was measured with a pressure transducer connected to a side-arm of the aortic cannula. All hemodynamic variables were monitored continuously by a multichannel recorder (Grass Polygraph 79D, Quincy, MA). Hearts were maintained at 37°C throughout the experimental period by enclosing the heart in a temperature-regulated double-lumen glass chamber and passing the perfusion medium through a heated reservoir and delivery system.

Experimental protocol. Two treatment groups were used to determine the ability of the sulfated glycosaminoglycan, PPS, to inhibit the effects of complement activation in the human plasma perfused rabbit isolated heart: group 1, control hearts were perfused with vehicle (0.9% NaCl) and 4% NHP (n = 6); group 2, treatment group, consisted of hearts perfused with 4% NHP in the presence of PPS (n = 6). A concentration-response curve was performed with PPS concentrations between 0.1 mg/ml and 0.03 mg/ml. All hearts underwent 10 min of equilibration on the Langendorff apparatus before the addition of vehicle or PPS to the perfusion medium. Ten minutes after addition of vehicle (control group) or PPS, NHP (4% vol/vol) was added to the perfusion medium. Hemodynamic variables, including LVEDP, left ventricular systolic pressure and LVDP, were recorded before the addition of vehicle or PPS (base line, BL), before the addition of 4% NHP (zero, 0) and every 10 min thereafter.

Creatine kinase release. Aliquots (1 ml) of pulmonary artery effluent were collected from hearts treated with vehicle or PPS (0.03 mg/ml) at base line (before the addition of human plasma), and at 10-min intervals throughout the 60-min protocol. Samples were analyzed for total creatine kinase activity (U/l) by UV spectroscopy with a commercially available assay kit (CK-20, Sigma, St Louis, MO).

Measurement of the soluble membrane attack complex (SC5b-9) and Bb. The appearance of the SC5b-9 neoantigen and Bb fragment in the lymphatic fluid collected from the heart was monitored by enzyme-linked immunoassays commercially available from Quidel (San Diego, CA) (Kolb et al., 1988). Appropriate corrections were made for variations in the lymphatic fluid flow rate among the groups. Lymphatic flow rates were determined by monitoring the time to collect 0.5 ml of lymphatic fluid. Because damaged hearts generate lymphatic fluid at a greater rate than normal hearts, SC5b-9 and Bb concentrations were normalized to lymphatic flow (neonantigen concentration multiplied by lymphatic flow rate). Supplied standards and control sera were used to determine the amount of neoantigen present in the effluent samples using derived standard curves as indicated in the manufacturer’s directions. Each sample was assayed in quadruplicate. The ELISAs were repeated three different times.

Determination of tissue-associated SC5b-9. The preparation of the tissue samples and quantitation ELISA for membrane-bound SC5b-9 is described elsewhere (Mathey et al., 1994). Upon completion of the protocol, hearts were perfused with fresh buffer for 5 min to remove vascular-associated SC5b-9. Samples of left ventricle were obtained from both vehicle and PPS-treated hearts. Tissue samples (1 g) were homogenized with a Polytron homogenizer (4 × 10 sec at setting 5; Tekmar Co., Cincinnati, OH), centrifuged (3000 rpm for 10 min) and the pellets washed twice with cold physiologic saline. After centrifugation, pellets were resuspended in 0.75 ml of 130 mM ocetylglucoside (Sigma, St. Louis MO) and allowed to incubate at room temperature for 15 min. At the end of the incubation period, samples were vortexed and centrifuged. The resulting supernatants were assayed by ELISA to quantitate SC5b-9 by enzyme-linked immunoassays commercially available from Quidel (San Diego, CA). Each sample was assayed in quadruplicate. The ELISAs were repeated three different times.

Statistical analysis. The data presented in the text, figures and tables are expressed as mean ± S.E.M. for the indicated number of determinations. Analysis of variance was used to examine the influence of time upon all repeated measurements. If significance was determined, a Fisher’s Protected Least Significant Difference test was used as a post hoc analysis to determine statistical differences between groups at specific time points. All test comparisons performed were two-tailed. One-way analysis of variance (repeated measures) was used to analyze data for the ELISA experiments. A P value < .05 was considered significant. All statistical evaluations were performed with a Macintosh 8500 computer (Apple Computer, Cupertino, CA) with Statview software (Abacus Concepts Inc., Berkeley, CA).

Results

Inhibition of human complement activation: in vitro analysis. The erythrocyte hemolysis assay was used to determine the ability of PPS to inhibit the alternative pathway of complement (fig. 2). PPS prevented RBC hemolysis in a concentration-dependent manner as determined by the percent hemolysis of rabbit erythrocytes. No significant reduction in red blood cell lysis occurred with PPS concentrations of less than 0.03 mg/ml. In contrast, concentrations greater than and including 0.03 mg/ml significantly inhibited lysis compared with control. These data indicate that PPS retains the ability to inhibit the alternative pathway of complement.

PPS concentration-response. Left ventricular developed pressure, an indicator of contractile function, was used for the determination of the concentration-response relationship of PPS (fig. 3). LVDP was determined at the 60-min time point in hearts treated with 0.01 mg/ml, 0.03 mg/ml, 0.10 mg/ml or 0.30 mg/ml PPS by analysis of the difference between peak systolic left ventricular pressure and LVEDP. The LVDP of hearts perfused with 4% NHP and 0.01 mg/ml PPS was not significantly different from that noted in hearts perfused with vehicle. In contrast, the LVDP of hearts treated with 0.03 mg/ml was significantly greater than that noted for control. Similar results were noted for concentra-
tions greater than 0.03 mg/ml. Perfusion of hearts with PPS (0.03 mg/ml) in the absence of 4% NHP did not significantly effect LVDP compared with base line.

**Left ventricular end-diastolic and systolic pressures.** Figure 4 represents tracings derived from isolated hearts perfused with 4% NHP in the absence (top portion) or presence of 0.03 mg/ml PPS (lower portion). Tracings from the vehicle-treated heart demonstrate that, by 30 min after addition of plasma, there is a marked increase in both the left ventricular end-diastolic and systolic pressures that continued for the 60-min duration of the protocol. However, the observed increase in systolic and diastolic pressures noted in control hearts was absent in hearts perfused in the presence of PPS. Addition of 4% heat-inactivated plasma, used as negative control, in place of 4% NHP did not affect end-diastolic or systolic pressures (data not shown).

As shown in figure 5A, a progressive increase in the LVEDP was observed in control hearts as the duration of exposure to 4% NHP increased. By 20 min, the LVEDP of control hearts was increased significantly compared with base line (P < .05), reaching a maximum by 30 min. Hearts perfused with 4% NHP in the presence of PPS (0.03 mg/ml) did not demonstrate the time-dependent increase in LVEDP (fig. 5B). A significant increase in the systolic pressure of vehicle-treated hearts (fig. 5A) perfused with 4% NHP was noted at the 20-min time point and remained significant for the remainder of the protocol. Hearts perfused in the presence of 0.03 mg/ml PPS did not exhibit the increase in systolic pressure (fig. 5B). Therefore, exposure of the perfused hearts to PPS provides a significant degree of protection against the human plasma-induced increase in LVEDP.

**Coronary perfusion pressure.** Coronary perfusion pressure during periods before and after addition of NHP is shown in figure 6. Coronary artery perfusion pressure increased progressively after addition of human plasma to the perfusion medium, which indicates an increase in coronary vascular resistance. Coronary perfusion pressure was significantly lower in PPS-treated hearts than vehicle-treated hearts (P < .05).

**Creatine kinase release.** The release of total myocardial CK into the pulmonary effluent was used as an indicator of cellular damage to the cellular membrane of cardiac cell types and is shown in figure 7. The coronary venous drainage was collected from the pulmonary artery for the determination of CK activity before and after addition of NHP to the perfusion medium. Base-line values for CK were similar for all groups. CK activity increased progressively in the venous effluent after addition of human plasma to the perfusion medium, which indicates alterations in target cell membrane integrity. Hearts perfused with PPS exhibited significant protection as illustrated by attenuation in the release of CK (P < .05).

**Determination of Bb fragment and soluble C5b-9 (SC5b-9).** Aliquots of the lymphatic fluid collected from cardiac lymphatic vessels were analyzed by ELISA for the appearance of Bb fragment and the soluble form of membrane attack complex (SC5b-9). Each sample was assayed in quadruplicate with the ELISA repeated three different times with similar results. The time course for Bb fragment and SC5b-9 formation in the lymphatic drainage of PPS and vehicle-treated hearts is shown in figure 8. To account for variations in the rate of lymphatic fluid flow between groups, the results are expressed as the amount of antigen (nanograms) released into the lymphatic flow per minute. The flow rate of vehicle-treated hearts ranged from 0.72 ml/min at base line to 5.0 ml/min at the conclusion of the 60-min protocol, whereas the rate for PPS-treated hearts ranged from 0.96 ml/min at base line to 1.25 ml/min at the conclusion of the protocol. As seen in figure 8A, lymphatic effluents from hearts perfused with PPS displayed a time-dependent decrease in the immunoreactive Bb fragment, which indicates decreased complement activation. SC5b-9 formation (fig. 8B)
was attenuated in the lymphatic fluid from PPS-treated hearts compared with vehicle-treated hearts (P < .05), which indicates that preservation of functional parameters noted in PPS-treated hearts correlated with the inhibition of MAC.

**Discussion**

The present study examined the ability of a semisynthetic polysaccharide, PPS, to protect the rabbit isolated perfused heart against injury resulting from activation of the complement cascade.
The data put forth in the present study further extend the body of information demonstrating that the GAGs are potent inhibitors of the complement cascade. The inhibitory effects of sulfated GAGs on complement activation have been documented in several experimental models of cellular injury that directly involve the complement cascade (Weiler et al., 1992; Sabu and Pangborn, 1993; Black et al., 1995; Engelberg, 1996; Gralinski et al., 1996b; Edens et al., 1994). The ability of the GAGs to inhibit complement makes this class of compounds potentially useful in the treatment of pathologies where complement activation plays a major role in mediating tissue injury including transplant rejection and ischemia/reperfusion injury. The ability of the GAGs to protect the transplanted organ is best exemplified by DiStefano et al. (1994), who used heparin in conjunction with C1 inhibitor to prevent discordant rejection of cardiac transplants and activation of endothelial cells in a model of xenograft hyperacute rejection. Furthermore, heparin and its non-anticoagulant derivative N-acetylheparin have been shown to protect the reperfused myocardium after a period of regional ischemia (Black et al., 1995; Gralinski et al., 1996b). In the present study, we sought to identify an orally available GAG that retains the ability to inhibit complement activation and therefore may be of benefit in preventing myocardial injury in vivo.

It is well accepted that exogenous heparin has a high binding affinity for both arterial and venous endothelial cells and remains bound to the cell surface for many days (Engelberg, 1988). Light and electron microscopy studies have revealed the in vivo location of anticoagulant heparan sulfate proteoglycans on the luminal surface of endothelial cells with larger quantities deposited in the subendothelial space (Marcum and Rosenberg, 1989). Thus the GAGs are localized strategically at the cell surface (Hiebert and McDuffie, 1989, 1990; Hiebert et al., 1993), where activation of complement is expected to occur when an organ from a discordant species is perfused with human plasma. Heparin concentrations in endothelium versus plasma are approximately 100 times greater after intravenous administration with less than 1% of the administered dose remaining in the plasma, and 45% associated with endothelium within 6 min after administration. The endothelium, therefore, is the main site of heparin distribution. (Hiebert et al., 1993). Both heparin and related non-anticoagulant glycosaminoglycans such as N-acetylheparin inhibit complement activation, which suggests that these compounds are acting independently of any action on the coagulation system. In addition, low molecular weight
heparins possess anticomplement activities. For example, Gralinski et al. (1997) and Park et al. (1997) demonstrated that the highly sulfated GAGs, LU 51198 and reviparin-sodium (LU-47311), protect the ex vivo perfused rabbit heart from complement-mediated injury. The ability of heparin and other GAGs to inhibit complement activation suggests that this class of compounds may be of benefit in modulating the activation state of the complement system.

Initial evidence for the ability of PPS to inhibit complement was derived from the observation that it could inhibit complement-mediated lysis of antibody-sensitized sheep red blood cells in a concentration-dependent manner (Berthoux et al., 1977a, b). In the present study, we have extended these findings by investigating the ability of PPS to inhibit the alternative pathway. The classical pathway was inhibited by the addition of EGTA-Mg+ to the lysis buffer. PPS prevented RBC lysis at concentrations of 0.03 mg/ml and greater, which indicates that the compound has the ability to inhibit the alternative pathway. Further evidence for the ability of PPS to inhibit human complement activation was provided when the rabbit heart was perfused in the presence of human plasma without the development of cardiac injury. Previous studies have demonstrated that perfusion of the rabbit isolated heart with NHP promotes activation of the complement system, which results in an increase in LVEDP and a decrease in LVDP (Homeister et al., 1992, 1993). The alterations in the functional parameters are accompanied by changes in cellular morphology and release of intracellular enzymes. The model, therefore, allows for the direct assessment of complement activation and for the evaluation of pharmacologic compounds designed to inhibit the human complement system (Platts-Mills et al., 1974; Seeger et al., 1989; Homeister et al., 1992).

Rabbit isolated hearts were perfused with 4% human plasma and the functional and biochemical parameters were determined in the presence or absence of 0.03 mg/ml PPS. In the absence of any pharmacologic intervention, hearts perfused with NHP exhibited alterations in LVEDP and LVDP with accompanying loss of cytosolic CK. In contrast, the presence of PPS (0.03 mg/ml) prevented the alterations associated with activation of complement as evidenced by the absence of significant functional changes. Hearts perfused with 4% NHP in the presence of PPS did exhibit a release of CK, although not to the same extent as noted in control hearts. The data suggest that complement activation is occurring, but at a rate insufficient to promote changes in the functional parameters or to impose irreversible damage. The inhibitory effects noted in the isolated heart substantiate those seen in the red blood cell hemolysis assay.

Inhibition of complement activation by PPS resulted in a decreased generation of the soluble form of C5b-9 (SC5b-9) appearing in the lymphatic fluid effluent and decreased myocardial cell membrane-associated immunoreactive C5b-9. Previous studies with C8-depleted plasma demonstrated that the principal mediator of tissue injury and subsequent alterations in cardiac function in this model are caused by the formation of the membrane-associated C5b-9 complex (Homeister et al., 1992, 1993). Immunohistochemical analysis at the ultrastructural and light level of plasma-perfused hearts show deposition of the C5b-9 complex on both endothelial cells and myocytes (Homeister et al., 1993). Formation of the complex, in large numbers, promotes increased membrane permeability to ions, proteins and water, subsequently leading to swelling of the affected cells and cell death. Although the decreased appearance of SC5b-9 in the lymph provides information as to the ability of PPS to inhibit complement activation, it does not address the question as to whether deposition of C5b-9 within cellular membranes is decreased in hearts perfused in the presence of PPS. To determine the degree of membrane-associated C5b-9, tissue samples were homogenized and C5b-9 extracted from myocardial cell membranes. ELISA analysis of C5b-9 deposition revealed that C5b-9 concentrations were decreased in hearts perfused with PPS. Thus, the ability of PPS to inhibit complement activation prevents formation of the MAC and, as a result, protects the target organ from cellular injury.

The mechanism by which the GAGs modulate complement activity has received increased attention. Although the tissue injury, biochemical and functional changes observed in the plasma-perfused isolated heart model, depends on activation of the alternative pathway (Gralinski et al., 1996a), which indicates a possible effect on complement components common to both pathways (Rashmir-Raven et al., 1992; Meri and Pangburn, 1994). The studies by Meri and Pangburn (1994) show that the interaction of the polyanion binding site on factor H with GAGs depends on the number, orientation and polymeric arrangement of sulfate groups and suggest that most, but not all, sulfated GAGs participate in the protection of host tissues from complement damage by promoting inactivation of tissue-bound C3b. It is apparent that this class of compounds acts early in the complement cascade, likely at the level of C3. In the physiological setting, activation of complement is mediated by the binding of the C3 activation product, C3b, to the target cell membrane and the ultimate proteolytic conversion of C3b to C3bBb, which functions as a C3 convertase. Weiler et al. (1978) showed that C3bBb formation is inhibited when heparin masks the factor B binding site on C3b. Heparin may also act to prevent the activation of the alternative pathway C3 convertase by binding to factor D, which in turn mediates C3bBb activation. Highly sulfated GAGs including PPS may function to modulate complement activation by inhibiting the binding of factor B to C3b whereas inhibiting the ability of factor D to convert C3bB to C3bBb. The actions of GAGs on modulating convertase activity does not rule out the possibility that PPS acts at the level of the terminal complement components. Baker et al. (1975) demonstrated that heparin interferes with the formation of MAC by preventing the incorporation of the complex into the target cell membrane. It is apparent, therefore, that PPS may be decreasing myocardial injury by inhibiting complement activation at multiple sites within the cascade.

The results of this investigation present evidence that the semisynthetic polysaccharide PPS can modulate activation of the human complement cascade in the isolated heart. Several GAGs including heparin and its non-anticoagulating derivative N-acetylheparin have been demonstrated to possess anticomplement activities. However, unlike heparin and other GAGs, PPS is orally bioavailable and therefore may be of benefit for the treatment of conditions where long-term inhibition of the complement system is required. The absence of the high concentrations of the MAC in lymphatic drainage and within the myocardium, combined with the corresponding preservation of functional parameters, suggests that
PPS, like other GAGs, has the ability to modulate the degree of complement activation in the functioning myocardium.

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