Ex Vivo Reversal of Heparin-Mediated Cardioprotection by Heparinase After Ischemia and Reperfusion

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ABSTRACT
Glycosaminoglycans, including heparin, have been demonstrated both in vitro and in vivo to protect the ischemic myocardium against reperfusion injury. In the present study, we sought to determine whether the cardioprotective effects of heparin administration could be reversed by the heparin-degrading enzyme heparinase. New Zealand white rabbits were pretreated with heparin (300 U/kg i.v.) or vehicle (saline). Two hours after treatment, hearts were removed, perfused on a Langendorff apparatus, and subjected to 25 min of global ischemia, followed by 45 min of reperfusion. Hemodynamic variables were obtained before ischemia (baseline) and every 10 min throughout the reperfusion period. Compared with vehicle-treated rabbits, the left ventricular end-diastolic and left ventricular developed pressures were improved significantly \((p < .05)\) in the heparin-treated group. Ex vivo administration of heparinase (5 U/ml) immediately before the onset of global ischemia was associated with a reversal of the heparin-mediated cardioprotection. The uptake of a radiolabeled antibody to the intracellular protein myosin and creatine kinase release were used to determine membrane integrity and discriminate between viable and nonviable myocardial tissue. The uptake of radiolabeled antimyosin antibody and release of creatine kinase after reperfusion were increased in heparin-pretreated hearts exposed to heparinase, indicating a loss of membrane integrity and increased myocyte injury. These results demonstrate that neutralization of heparin by heparinase promotes increased myocardial injury after reperfusion of the ischemic myocardium.

Heparin and related glycosaminoglycans (GAGs) have been shown to be of benefit to the ischemic myocardium by preserving contractile function and reducing tissue injury (Friedrichs et al., 1994; Black et al., 1995; Gralinski et al., 1996; Park et al., 1999). It is of interest therefore to determine the effect of heparinase administration in the presence of tissue-bound heparin. Therefore, the present study was designed to determine whether the previously observed cardioprotective effects of heparin administration would be negated by the heparin-degrading enzyme heparinase. It was found that the presence of heparinase during reperfusion was associated with a loss of heparin-mediated protection as demonstrated by changes in cardiac functional parameters and loss of membrane integrity.

Materials and Methods

Guidelines for Animal Research. The procedures used in this study were in accordance with the guidelines of the University of Michigan Committee on the Use and Care of Animals. Veterinary care was provided by the University of Michigan Unit for the 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.

ABBREVIATIONS: GAGs, glycosaminoglycans; CK, creatine kinase; KH, Krebs-Henseleit.

**Treatment Groups.** The present study consisted of three experimental groups in which each group was treated in vivo 2 h before the hearts were removed and subjected to perfusion by the Langendorff method. The treatment regimens were as follows: group I animals received an i.v. dose of heparin sulfate (300 U/kg) 2 h before removal of the heart. The dose of heparin was determined based on a previous study demonstrating the ex vivo cardioprotective effects of the GAG when administered i.v. in a dose sufficient to increase the activated partial thromboplastin time, 2- to 3-fold above the control value (Friedrichs et al., 1994). The hearts were removed 2 h after heparin pretreatment and subsequently treated with placebo immediately before the onset of global ischemia. Animals assigned to group II were treated with heparin as described above, but the isolated hearts from this group were exposed to heparinase (5 U/ml) immediately before induction of global ischemia. Group III animals were treated in vivo with 0.9% sodium chloride solution (drug diluent), 2 h before removal of the hearts. The concentration of heparinase was obtained from preliminary studies examining the effect of heparinase alone on cardiac function. The concentration of heparinase (5.0 U/ml) was obtained from preliminary studies examining the effect of heparinase alone at concentrations of 1, 5, and 10 U/ml using cardiac contractile function as a determinant of drug effect. The range of heparinase concentrations examined in the preliminary studies did not produce an observable change in left ventricular peak systolic pressure or left ventricular end-diastolic pressure during 45 min of exposure to the enzyme in the perfusion medium. Based on the preliminary examination, a concentration of 5.0 U/ml was selected for further study. Heparin (porcine intestinal mucosa) and heparinase III were purchased from Sigma Chemical Co. (St. Louis, MO).

**Langendorff-Perfused Heart.** The Langendorff preparation used in this study has been described in detail previously (Homeister et al., 1992). Briefly, male New Zealand White rabbits (1.8-2.2 kg) were rendered unconscious by cervical dislocation. The hearts were excised quickly and the aorta attached to a cannula for perfusion with a modified Krebs-Henseleit (KH) buffer (pH 7.44, 37°C) at a constant flow (22-28 ml/min). Buffer was composed of 117 mM NaCl, 4.0 mM KCl, 1.2 mM MgCl2, 6.0H2O, 1.1 mM KH2PO4, 25.0 mM NaHCO3, 2.6 mM CaCl2, 2.0H2O, 5.0 mM glucose, 5.0 mM l-glutamate, and 5.0 mM pyruvic acid. The KH buffer passed through a membrane “lung” composed of 18 feet, 0.058 inches i.d., 0.077 inches o.d. Silastic Medical Grade Tubing (Dow Corning, Midland, MI). The membrane lung was gassed continuously with a mixture of 95% O2/5% CO2 to achieve an oxygen partial pressure of 500 mm Hg. An in-line oxygen electrode and digital meter (Instech Laboratories, Plymouth Meeting, PA) continuously monitored the oxygen tension in the KH buffer. The hearts were paced through the right atrium with electrodes attached to a laboratory stimulator (180 impulses/min, 2 ms duration, 4 V; Grass Instruments SD-5, Quincy, MA).

The pulmonary artery was cannulated to facilitate collection of fluid from the coronary venous circulation. The pulmonary veins and the superior and inferior vena cava were ligated. A left ventricular drain, thermistor probe, and a latex balloon entered via the left atrium and were secured with a purse string suture at the atrial appendage. Isovolumetric left ventricular diastolic and systolic pressures were measured with the left ventricular fluid-filled latex balloon. The balloon was filled to achieve a left ventricular end-diastolic pressure of 5 mm Hg. The monitored physiologic parameters included the left ventricular systolic and diastolic pressures and the cardiac electrogram, which were recorded continuously on a multichannel polygraph recorder (Grass polygraph model 79D).

**Experimental Protocol.** Isolated hearts were stabilized under normoxic conditions for 15 to 20 min before the induction of global ischemia. The perfusion medium was recirculated throughout the protocol. Induction of global ischemia was accomplished by stopping the flow of perfusate to the heart. Reperfusion of the heart was achieved by turning on the pump to the original flow rate (20-24 ml/min). All hearts were subjected to 25 min of global ischemia followed by 45 min of reperfusion. Functional parameters were recorded every 10 min during the reperfusion period until termination of the protocol. A constant temperature of 37°C was maintained throughout the periods of ischemia and reperfusion.

**Preparation of Antimyosin Antibody.** Murine monoclonal antimyosin antibody (Mifarmonab F(ab)') was provided by Centocor Inc. (Malvern, PA). Radiodination of the antibody was performed by the chloramine-T method (Sakahara et al., 1987). After incubation with Na125I and chloramine-T, the free 125I was removed by Sephadex G-50 column chromatography. The specific activity of the radiolabeled molecule was between 6 and 12.5 Ci/mg protein.

**Determination of 125I-Antimyosin Uptake.** Uptake of labeled antimyosin was determined by perfusing 1.0 μCi of antibody through the isolated heart for 5 min before terminating the protocol. On administration of the antimyosin antibody, the hearts were washed for an additional 5 min with buffer to remove antibody not bound to myosin. The hearts were dried overnight, weighed, and the uptake of antimyosin determined by a well-type auto-gamma counter (Minaxi Auto-Gama; Packard Instrument Co., Downers Grove, IL). The amount of antimyosin antibody uptake was expressed as a percentage of the perfused dose bound per gram dry weight of the tissue.

**Electron Microscopy.** On completion of the designated protocol, hearts were perfused for 3 min with 2.5% glutaraldehyde and 1% LaCl3 in 0.1 M sodium cacodylate buffer (pH 7.44). The electron-dense LaCl3 served as an indicator of arterial capillary endothelium permeability (Jokelainen et al., 1976). Tissue samples from the left ventricular myocardium were removed and cut into pieces measuring approximately 1 mm on a side. The samples were fixed for an additional 2 h at 4°C in the above-mentioned buffer. After washing with 0.1 M sodium cacodylate buffer, the samples were dehydrated in an ethanolic series and embedded in EM bed-812 (Electron Microscopy Sciences, Ft. Washington, PA). Tissue blocks were sectioned with a Reichert ultramicrotome and placed on formvar-coated copper grids and then stained with 4% uranyl acetate. Sections were observed with a Philips CM-10 electron microscope and representative micrographs from each treatment group obtained.

**Release of Creatine Kinase (CK).** Effluent from the pulmonary artery (coronary venous return) was sampled at baseline and at regular time intervals during the reperfusion period for analysis of creatine kinase release. CK activity was determined using a kit purchased from Sigma Chemical Co. (procedure 47-UV; St. Louis, MO). The assay is based on the modified procedure developed by Szasz et al. (1976). Briefly, the assay measures the increase in absorbance at 340 nm produced by the reduction of NAD (nicotinamide adenine dinucleotide) to NADH (nicotinamide adenine dinucleotide, reduced form). The rate of change is proportional to the CK activity. One unit is defined as the amount of enzyme that produces one micromole of NADH per minute.

**Statistical Analysis.** Results are expressed as mean values ± S.E.M. Comparisons of parameters measured over time were performed using a two-way repeated measures ANOVA to test for group differences over time followed by Dunnett’s post hoc test. If only two groups were compared, the Student’s t test for unpaired comparisons was used. In all cases, a p value of < 0.05 is regarded as significant and is denoted by an asterisk. Statistical analyses were performed on a Macintosh computer using Statview SE + Graphics (Abacus Concepts, Berkeley, CA).

**Results**

**Cardiac Contractile Parameters.** Preliminary studies examining the effects of heparinase alone indicated concentrations of 1, 5, and 10 U/ml (maximum concentration examined) did not alter cardiac function. Based on these results, the subsequent studies with heparinase were carried out using a concentration of 5 U/ml added to the perfusion me-
dium. The decision to use the midpoint concentration was influenced in part by the high cost of the enzyme.

The observed changes in left ventricular systolic and left ventricular end-diastolic pressures for each of the three groups are presented in Fig. 1, A and B, respectively. The contractile parameters of hearts from vehicle-pretreated animals (group 3), subjected to 25 min of global ischemia followed by 45 min of reperfusion, were characterized by increases in the left ventricular end-diastolic and systolic pressures occurring within 10 min after restoration of coronary artery perfusion. The observed responses in the group 3 control hearts are characteristic of what is observed when the heart is subjected to a limited global ischemic insult followed by reperfusion. Left ventricular pressure development ceases within minutes after initiation of global ischemia and the left ventricular end-diastolic pressure increases gradually during the period of global ischemic arrest. On reperfusion there is a return of contractile function along with a progressive increase in the left ventricular end-diastolic pressure and a simultaneous increase in the recorded left ventricular peak systolic pressure. The difference between the peak systolic pressure and the end-diastolic pressure represents the left ventricular developed pressure. Both the end-diastolic and systolic pressures remained increased throughout the 45 min of reperfusion as compared with baseline values. The end-diastolic pressure in hearts from animals pretreated (2 h) with heparin (group 1) was significantly less ($p < .05$), compared with the corresponding time points in hearts from vehicle-treated animals (22.9 ± 4.8 versus 52.9 ± 5.1 mm Hg at 45 min of reperfusion). The peak systolic pressure also was significantly less ($p < .05$) in hearts from animals pretreated with heparin, compared with vehicle-treated animals (48.8 ± 4 versus 89.1 ± 6.7 mm Hg at 45 min of reperfusion). Thus, the combined effects of a reduction in both the left ventricular peak systolic and end-diastolic pressures are consistent with a cardioprotective effect in the heparin pretreated heart compared with the hearts from vehicle-treated animals.

In contrast, the addition of heparinase (5.0 U/ml) to the perfusion medium of hearts from heparin-pretreated animals (group 2) was associated with the reversal of heparin’s cardioprotective effects. The mean left ventricular end-diastolic pressure at the 45 min time point after reperfusion was 66.0 ± 5.1 mm Hg in hearts made ischemic in the presence of heparinase versus 22.9 ± 4.8 mm Hg noted for heparin-pretreated hearts administered drug diluent. A similar change was noted for the left ventricular peak systolic pressure recordings between hearts from groups 1 and 2. The mean peak systolic pressure in heparinase-perfused hearts was 84.5 ± 3.9 mm Hg at 45 min of reperfusion versus 48.8 ± 4 mm Hg in hearts from heparin-pretreated animals. Both the end-diastolic and peak systolic pressures in heparin-pretreated hearts exposed to heparinase were similar to that noted for vehicle-treated animals. As indicated previously, significant functional changes were not observed when hearts were perfused under normoxic conditions for 45 min in the presence of heparinase (1–10 U/ml).

Determination of 125I-Labeled Antimyosin Uptake as a Indicator of Tissue Injury. The 125I-labeled antimyosin antibody provides a convenient approach to quantify the extent of myocardial tissue injury in hearts subjected to global ischemia and reperfusion. Compared with hearts from vehicle-treated animals subjected to global ischemia and reperfusion, the uptake of the radiolabeled antibody was decreased significantly in hearts from animals pretreated with heparin. In contrast, perfusion of the heparin-pretreated hearts with heparinase was accompanied by a marked accumulation of the antibody in myocardial tissue. The results are summarized in Fig. 2 and are consistent with the changes noted in myocardial contractile function, indi-

![Fig. 1. Contractile parameters of isolated hearts exposed to 25 min of ischemia and 45 min of reperfusion. A, systolic pressures from hearts of animals pretreated with vehicle (triangles), heparin (squares), or heparin made ischemic in the presence of heparinase (circles). Values are mean ± S.E.M. *$p < .05$ versus vehicle pretreatment at the same time point. B, end-diastolic pressures from hearts of animals pretreated with vehicle (triangles), heparin (squares), or heparin made ischemic in the presence of heparinase (circles). Values are mean ± S.E.M. *$p < .05$ versus vehicle at same time point.](image-url)
Determination of antimyosin uptake in hearts subjected to global ischemia and reperfusion. Antimyosin uptake was decreased significantly in hearts from heparin-pretreated animals after 25 min of ischemia and 45 min of reperfusion. In contrast, exposure of heparin-pretreated hearts to heparinase resulted in an increase in antimyosin uptake. Control hearts from animals administered 0.9% sodium chloride solution exhibited an intermediate degree of antimyosin antibody binding. Values are mean ± S.E.M. *p < .05 versus vehicle pretreatment; p < .05 versus heparin pretreatment.

Electron Microscopy/Lanthanum Chloride Distribution. Transmission electron microscopy using lanthanum chloride as an indicator of vessel damage provided morphological data for the analysis of cellular ultrastructure. The specimen in Fig. 3A was obtained from an animal in group 3. The perfused heart was exposed to drug vehicle. As observed in the electron photomicrograph, the effect of ischemia/reperfusion in the presence of vehicle resulted in the failure of lanthanum to accumulate on the endothelial surface. Extravasation of lanthanum into the perivascular space is apparent. There is a loss of morphologic structure in both the myocytes and mitochondria. The observed changes are representative of the group and suggest irreversible injury as a result of ischemia/reperfusion.

The morphological appearance of myocardial tissue from a heparin-pretreated animal is presented in Fig. 3B. Densely packed mitochondria containing an intact matrix and normal-appearing cristae are apparent. Amorphous densities do not appear within the mitochondria. The myofibrils are intact with well-aligned Z-bands. The endothelial cells lining blood vessels in hearts from heparin-pretreated animals showed a continuous layer of lanthanum on the luminal surface of the tissue with minimal evidence of extravasation to the perivascular space. In contrast to the illustration in Fig. 3A, the heparin-pretreated heart subjected to 25 min of global ischemia and 45 min of reperfusion retained many normal morphological features, especially the presence of a glycocalyx, suggesting that tissue-bound heparin may provide a cardioprotective effect.

The ultrastructural preservation noted above with heparin pretreatment (Fig. 3B) was absent when hearts from heparin-pretreated animals were exposed to heparinase before being subjected to reperfusion. The observed morphological alterations are consistent with those previously associated with ischemia/reperfusion injury. Endothelial cells exhibited severe swelling with the appearance of intraluminal protrusions in many of the vessels. The luminal surfaces of the vessels were disrupted and contained little lanthanum deposition, whereas heavy deposition of lanthanum occurred within the perivascular space. The mitochondria were swollen with disrupted matrices and contained large, amorphous densities (arrow), suggestive of irreversible injury. Extensive myofibrillar damage is apparent, as seen by blurring of the Z-bands and disputation of the myofibrils. The notable feature in Fig. 3, A and C is the lack of a glycocalyx, as evidenced by the failure of lanthanum to deposit on the endothelial surface.

CK Release. The release of the cytosolic enzyme, CK, into the pulmonary effluent was used as an additional indicator of an alteration in membrane integrity (Fig. 4). Baseline CK values were similar in all groups. In control hearts undergoing 25 min of global ischemia and 45 min of reperfusion, there was an increase in CK activity in the pulmonary artery effluent after the onset of reperfusion. The CK activity increased within 10 min after initiation of reperfusion and continued to increase throughout the remainder of the protocol. In hearts from animals pretreated with heparin, CK activity was significantly less than that noted for control hearts. In contrast, exposure of heparin-pretreated hearts to heparinase resulted in an increase in CK activity similar to that observed in control hearts. CK release from hearts of heparin-pretreated animals was significantly less at the 20- and 30-min time points when compared with heparin-pretreated hearts perfused in the presence of heparinase.

Discussion

The current study derives from an earlier investigation indicating that the isolated heart of Cynomolgus monkeys was protected from the damaging effects of global ischemia and reperfusion if the donor animal had been administered heparin 2 h or more earlier (our unpublished observations). The studies were repeated in the rabbit with a similar outcome and with the additional observation that cardioprotection was achieved by pretreating with nonanticoagulant derivatives of heparin, but not if the GAGs were added directly to the perfusion medium (Friedrichs et al., 1994). The time-dependent nature of the cardioprotective effect, and the fact that protection persisted when the hearts were perfused in the absence of exogenous GAGs, suggested that the compounds were bound to the cardiac tissue (Hiebert and Jaques, 1976a,b). Subsequent investigation using periodate-Schiff reagent staining of heart tissue indicated that i.v.-administered heparin or heparin analogs were bound to the endothelial cell glycocalyx (Friedrichs et al., 1994; Gralinski et al., 1996). These observations were extended using in vivo models of myocardial ischemia/reperfusion injury. Heparin (Black et al., 1995), or its nonanticoagulant derivative N-acetylheparin (Park et al., 1999), protected the myocardium and resulted in a reduction in infarct size due to ischemia/reperfusion. Several other laboratories have reported results in full agreement with our current observations (Hobson et al., 1988; Kouretas et al., 1998, 1999). There is evidence that a 2-h in vivo pretreatment regimen with heparin or N-acetyl-
heparin preserves coronary endothelial function after a brief period of ischemia/reperfusion by a mechanism which is, in part, due to activation of the nitric oxide-cGMP pathway (Kouretas et al., 1999).

Despite the extensive clinical use of heparin, the cytoprotective and anti-inflammatory actions of the GAGs have not been subjected to a critical evaluation. A number of limited clinical studies have been reported in which heparin is observed to possess an anti-inflammatory and/or cytoprotective action (Saliba, 1997; Evans et al., 1997; Downing et al., 1998). The interested reader is referred to an excellent discussion of heparin and its often neglected actions that go beyond its well known anticoagulant effects (Edens et al., 1993). Heparin and related GAGs are among the most effective inhibitors of the complement cascade and the anticoagulant property is distinct from the complement inhibitory activity (Ecker and Pillemer, 1941), thereby providing the opportunity to develop pharmacologic interventions targeted to one or more of the serine proteases of the complement cascade.

Heparin and related GAGs inhibit both the classical and alternative pathways of complement activation (Ecker and Gross, 1929; Weiler et al., 1978). Heart tissue is capable of expressing genes and proteins of the complement system, although it is not yet known which cell types are responsible. Myocardial ischemia/reperfusion promotes a rapid expression of mRNA encoding the complement proteins C3 and C9. These levels exceed those found in normal liver. The observations are consistent with the hypothesis that local production of complement proteins may contribute significantly to the degree of ischemic injury to the myocardium, and that complement expression is augmented by reperfusion (Yasojima et al., 1998). The inhibitory action of heparin on the local tissue complement cascade may provide a partial explanation for the protective effects observed when hearts are pretreated with heparin before being subjected to ischemia/reperfusion.

GAGs bind to the glycocalyx of endothelial cells and myocytes, thereby protecting these cell types against injury (Cranowska and Karwatowska, 1995). The importance of heparin and other GAGs is exemplified further by the recognition that neutralization of heparin or heparin sulfate by protamine or administration of the heparin-degrading enzyme, heparinase, adversely affects the biochemical regulation of endothelial cell-mediated vascular repair (Han et al., 1997). Many GAG-induced actions have been attributed to their ability to associate with the glyocalyx (Ruoslaiti and Yamauchi, 1991), as well as binding to endothelial cell receptors and being internalized (Castellet et al., 1985).

Heparin use may precipitate adverse events (i.e., hemorrhage), or may require that its anticoagulant action be neutralized rapidly. Recently, the endoglycosidase heparinase has been investigated as an alternative to protamine for the neutralization of heparin (Michelsen et al., 1997). We sought to investigate the consequences of heparinase administration on heparin-mediated cardioprotection. We hypothesized that

Fig. 3. Representative electron micrographs of hearts from control animals and heparin-pretreated animals after 25 min of ischemia and 45 min of reperfusion, and hearts made ischemic in the presence of heparinase. A, illustrates the effect of ischemia/reperfusion in the presence of vehicle. There is a loss of morphologic structure in both the myocytes and mitochondria. Little lanthanum accumulation is noted within the vascular lumen on the endothelial surface. The endothelial cells lining blood vessels in hearts from heparin-pretreated animals (B) show a uniform layer of lanthanum (arrows) on the luminal surface (LS), whereas the morphological appearance of the tissue is well preserved. Hearts obtained from heparin-pretreated animals and perfused in the presence of heparinase (C), show deposition of lanthanum (arrowheads) within the perivascular space (PS) and extensive morphological alterations consistent with that previously noted for ischemia/reperfusion injury. Original magnification, 8500×.
the cardioprotective benefits derived from in vivo heparin pretreatment would be abolished and/or reduced in the presence of the heparinase. The results in the isolated heart support the concept that the association of heparin with the cardiac tissue provides a protective action that can be negated by enzymatic cleavage of the GAG from its binding sites, thereby rendering the heart vulnerable to injury during ischemia/reperfusion.

A radiolabeled antibody to the cardiac myosin, served as a specific marker of irreversible injury (Haber et al., 1982). Membrane damage allows entry of the antibody to the cell and binding to myosin. At the microscopic level, antimyosin is bound to myocytes that have undergone extensive damage, as seen by the loss of cytoplasmic features and nuclear structure (Khaw et al., 1979). Decreased antibody binding in the hearts of heparin-pretreated animals provides support for the protective role of heparin. In contrast, when hearts from heparin-pretreated animals were made ischemic in the presence of heparinase, there was an increase in the uptake of the antibody. The amount of antimyosin binding in response to global ischemia and reperfusion was significantly greater in heparin-pretreated hearts exposed to heparinase compared with vehicle-treated hearts subjected to the same ischemic stress, suggesting that the former group incurred a greater degree of injury. On the other hand, hearts from heparin-pretreated animals exhibited a better degree of recovery from the ischemic insult. The reason for an exaggerated response to heparinase in the heparin-pretreated heart is unknown. One may speculate that formation of a heparin–heparinase complex exacerbates the extent of injury in a manner similar to untoward reactions that occur when protamine is administered to neutralize heparin (Shastri et al., 1997). Heparin-like molecules may be essential to the biochemical regulation of vascular repair provided by endothelial cells. The pharmacodynamic actions of heparin neutralization with agents such as protamine or heparinase should be examined with a focus on tissue viability as affected by ischemia and reperfusion (Han et al., 1997). Pretreatment of endothelial cells with heparinase to alter their glycocalyx composition substantially enhances the formation of reactive oxygen species (Gorog et al., 1988). It is not known to what extent this latter mechanism contributed to the heparinase-associated deterioration of cardiac function. The combined cytotoxic effects resulting from activation of the complement cascade along with an augmented formation of reactive oxygen species may provide an explanation for the enhanced antimyosin antibody uptake in the group 3 hearts compared with those in group 1.

Electron microscopy was performed using lanthanum chloride as a marker of vascular injury and increased permeability to correlate the uptake of the antimyosin antibody with myocardial damage (Hoffstein et al., 1975; Haack et al., 1981). Lanthanum chloride binds to a fine filamentous layer composed of an acidic mucopolysaccharide that lines the luminal surface of intact endothelial cells (Jokelainen et al., 1976; Weihe et al., 1977). Disruption of the luminal membrane as a result of ischemia/reperfusion results in the loss of the filamentous layer and a subsequent decrease in lanthanum binding (Haack et al., 1981). Vehicle-pretreated hearts showed an increase in lanthanum-associated electron opacity in the perivascular space. Hearts from animals pretreated with heparin showed the presence of a continuous layer of lanthanum associated with the luminal surface of the vascular endothelium. Hearts from heparin-pretreated animals and subjected to ischemia/reperfusion in the presence of heparinase showed little lanthanum retention on the endothelial cells along with vascular injury and loss of myocyte viability, not noted in hearts pretreated with heparin and not exposed to heparinase. The latter tolerated the stress of global ischemia and reperfusion with a lesser degree of injury assessed by monitoring functional, biochemical, and ultrastructural parameters.

CK is released when myocytes are damaged and membrane integrity is altered (Shell et al., 1971). The increased release of the enzyme in heparin-pretreated hearts exposed to heparinase supports the view that heparinase reverses the protective effects of heparin against ischemia/reperfusion injury. Formation of a heparin–heparinase complex may serve to amplify the injury associated with the stress imposed by ischemia/reperfusion.

The data derived from the present study may offer insight into the cytoprotective actions of heparin. Heparin binds to the endothelium through charge interactions with glycoproteins and polysaccharides that compose the glyocalyx (Marcum and Rosenberg, 1989). These glycoproteins and polysaccharides are involved in determining tissue structure and have a role in mediating cell adhesion, migration, and the activities of membrane-bound chemokines (Hoogenwerf et al., 1997; Marcum and Rosenberg, 1989). Control of vascular permeability and membrane integrity is, in part, dependent on the glyocalyx. Heparinase is known to digest the extracellular matrix, thereby facilitating tissue damage. Perfusion of isolated lungs with heparinase results in an increase in radiolabeled albumin uptake and a subsequent increase in total lung water content (Sunnergren et al., 1987). Based on the antimyosin and CK data, it is clear that preadministration of heparin acts to preserve endothelial cell and myocyte membrane integrity of the perfused heart, whereas treatment with heparinase counteracts the protective effect. Electron microscopy using lanthanum chloride indicates that the glyocalyx of heparin-pretreated hearts exposed to ischemia/reperfusion in the presence of heparinase undergoes extensive disruption.

The results of this investigation provide support for a cardioprotective action of heparin that is dependent on binding of the GAG to the endothelial cell. It is not known whether these findings have a bearing on the potential clinical application of heparinase as a therapeutic intervention for heparin neutralization. Although the study was not designed to address this question, it does call attention to the fact that further investigations are needed that focus on the pharmacodynamic events associated with formation of the heparin–heparinase complex under conditions of altered tissue oxygenation and reperfusion.

References

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