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The Role of PAF and Its Antagonism in Transplantation: Organ Ischemia and Preservation

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INTRODUCTION

Human beings have long dreamed of substituting a healthy organ for one no longer capable of functioning, but it is only recently that they have been able to solve some of the technological problems that would permit usccessful organ transplantation. Successful organ transplantation has required the development of new surgical techniques, particularly the improvement of vascular anastomoses. It has also necessitated the development of immunosuppressive methods to prevent allograft rejection. Finally it has stimulated the investigation of new methods of organ preservation to improve organ sharing and procurement over long distances and to maximize optimal donor-recipient matching.

Although the techniques necessary for vascular anastomoses were developed more than 60 years ago by Carrel (1), it was not until 1951 that Lawer and Dubost performed the first clinical renal transplant (2). By 1963 Starzl et al. performed the first human liver transplant (3), and in 1966 Lillehei et al. performed a pancreas transplant (4). In 1967, clinical small bowel transplantation had been attempted as well (5). With the development of extracorporeal

circulation in 1960 by Lower and Shumway, cardiac transplantation became a possibility, and the first cardiac transplant was performed in 1967 by Barnard (6,7).

A better understanding of the process of organ rejection and improved donor-recipient matching have decreased chances of allograft rejection after transplantation. Early methods of immunosuppression included thoracic duct drainage and total body irradiation (8). These were soon replaced by the pharmacological agents azathioprine and antilymphocyte globulin (9,10). The introduction of cyclosporin has greatly improved survival rates for cardiac and liver transplant recipients (11). Although the ideal treatment for rejection has yet to be found, great strides have been made, thus rendering transplantation a viable answer to the patient with a failing organ.

The limited number of organs available for transplantation often necessitates the transportation of organs over great distances. Although all current attempts at organ preservation use some degree of hypothermia, at present there are no consistently successful long-term methods for ensuring organ viability. Maximum acceptable cold storage times in the clinical situation for kidney, heart, and liver are 48–72, 4–6, and 8–10 h, respectively (12–14).

ISCHEMIA AND REPERFUSION INJURY

Techniques currently used for liver preservation have been borrowed and modified from those used for renal preservation. Since these methods involve some degree of hypothermia, the effects of hypothermia as well as those of ischemia must therefore be addressed. Ischemic injury results in morphological changes in cellular membranes and in mitochondria (15). Aerobic metabolism ceases, ATP is depleted, and metabolic products accumulate (16). Eventually all energy-requiring cell functions are terminated. Inactivation of the sodium pump secondary to ATP depletion results in cell swelling due to the passive exchange of ions and water (17,18). Of key importance is determining at what point ischemic injury becomes irreversible. ATP levels in ischemic organs drop precipitously, reaching very low levels long before the tissue is unable to recover (19–23). The loss of total intracellular adenine nucleotides is somewhat more of a prognostic factor (21,22,24,25). Dysfunction of cellular membranes is characteristic of irreversible ischemic injury (26,27). Cellular injury following ischemia may occur during the reperfusion period rather than during the period of ischemia (28). Implicated in this reperfusion injury are calcium ion fluxes and damage mediated by oxygen free radicals (28–32).

Studies by Farber et al. relate the irreversibility of ischemic injury to the inability to restore mitochondrial function and evidence of plasma membrane damage (28). The mitochondrial abnormalities are related to changes in long-chain acyl-CoA metabolism, with inhibition of adenine nucleotide translocation

and potentiation of calcium-dependent increase in permeability in the inner mitochondrial membrane. Disorders in phospholipid metabolism are probably the critical lesions producing irreversible cell injury. The mechanism is thought to be the activation of endogenous phospholipases by increased cytosolic free calcium.

The interruption of the blood supply to the rat liver produces a progressive loss of phospholipids from the ischemic cells. Microsomes from lipid-depleted postmitochondrial supernatants have alterations in structure and function with inhibition of glucose-6-phosphatase and calcium pump activities and 25- to 50-fold increases in passive permeability to calcium (27). EM studies of freeze-fractured microsomes reveal fewer intramembranous particles, bare membrane regions devoid of particles, and areas containing aggregates of intramembranous particles (27).

Microvascular abnormalities have been documented in myocardial reperfusion injury (33). Localized endothelial swelling, "blebs," increased capillary permeability, plugging of the microvasculature with polymorphonuclear leukocytes, and microscopic zones of hemorrhage have all been demonstrated (33).

ORGAN PRESERVATION FOR TRANSPLANTATION

Hypothermia reduces metabolism and, therefore, would appear to be a simple method to minimize ischemic changes. Unfortunately, decreases in temperature do not affect all processes uniformly, and cooling itself may be damaging (34). Nonetheless, all currently used liver preservation protocols, including hypothermic storage, hypothermic pulsatile perfusion, hypothermic hyperbaric oxygenation, intermittent hypothermic perfusion, and freezing, involve hypothermia.

Hypothermic storage remains the technique used in clinical liver transplantation today. In 1960, Starzl et al. experimentally transplanted livers stored at 4°C in heparinized Ringer's lactate for up to 120 min (35). Since that time, modifications in storage solutions have extended the period of hypothermic ischemic storage that allow organ viability. Using an electrolyte-dextran solution, human livers were preserved for 2-3 h and up to 5 h (36-38). Flush perfusion with plasma protein fraction and hypothermic storage in ice has given reliable 4 h preservation of the human liver (39). Lambotte et al. were able to successfully preserve canine livers for 15 h using Collin's C-2 solution with isoproterenol (40). Schalm et al. carried out a controlled study evaluating four different preservation fluids with marked differences in ionic composition, pH, and osmolarity in pigs and dogs and found no significant difference with preservation up to 6 h (41). Benichou et al. found that after 18 h plasma and Collin's solutions permitted survival, whereas lactated Ringer's did not (42). Toledo-Pereyra et al. successfully preserved canine livers for 24 h utilizing hyperosmolar

colloid or crystalloid solutions (43). Monden and Fortner have used modified Sack's solution and prostacyclin to successfully preserve canine livers for 24 and 48 h (44). Recently, Jamieson et al. have reported success with 24–30 h preservation of the canine liver utilizing static hypothermic storage in a unique preservation solution developed at the University of Wisconsin (45).

Extended preservation has been obtained with hypothermic pulsatile perfusion; however, its complexity and expense currently preclude its use in clinical liver transplantation. Using a mixture of Eagle's and Hank's media, Slapak et al. preserved canine livers by perfusion for a period of 6 h (46). Turner and Alican reported greater than 50% survival for more than 4 days for livers perfused for 20 h with homologous serum and low pH (47). Preservation of the canine liver for 24 and 48 h using pulsatile hypothermic perfusion, membrane oxygenation, and cryoprecipitated plasma perfusate has been reported (48,49). Using the model of auxiliary liver allotransplantation, Toledo-Pereyra has shown that a modified silica gel fraction with allopurinol and methylprednisolone improves graft survival and function after 24 h hypothermic pulsatile perfusion (50). In the rat liver, Fuller et al. obtained good preservation of metabolic activity by perfusion with a synthetic solution containing gelatin polypeptides (51).

Furthermore, attempts to prevent or diminish ischemic injury associated with organ procurement and preservation have involved the utilization of several pharmacological agents. Prostacyclin has been used to enhance survival of canine livers preserved by hypothermic storage, and it has been shown to improve the survival of ischemically damaged liver allografts (44,52,53). Chlorpromazine has been found to prevent much of the calcium-mediated injury seen upon reperfusion (28,54). There has been much interest recently in the role of oxygen free radicals in ischemia and reperfusion injury (30–32,55,56). Catalase, superoxide dismutase, and allopurinol have been shown to protect against reperfusion injury mediated by oxygen free radicals. Other antioxidants and calcium channel blockers have also been used in an attempt to ameliorate ischemia and reperfusion injury. Lidoflazine, a calcium channel blocker, has been shown to protect against reperfusion injury during small bowel transplantation (57). α-Tocopherol has been demonstrated to prevent oxygen free radical damage in ischemic rat liver (58). Marubayashi et al. also used coenzyme Q_{10} (ubiquinone) to prevent ischemic hepatocyte damage, presumably by protection of membranes from lipid peroxidation, with preservation of calcium homeostasis and restoration of mitochondrial functions (59).

Platelet-activating factor (PAF) is a key inflammatory mediator elaborated by a variety of cell types, including platelets, neutrophils, and the endothelium, and is intimately involved in the microcirculatory response to both nonimmunologic and immunologic inflammatory stimuli (60–65). Many of the pathophysiological responses and the microcirculatory failure that result from PAF release mimic the progression of responses observed after ischemia and reperfusion injury.

SDZ 63-441 (Sandoz Company) is a potent receptor antagonist of PAF, which can inhibit all known biologic responses to PAF (65). Thus, the availability of such an antagonist could give further insight into the basic mechanisms involved in the ischemic process and could have significant potential as a therapeutic agent for both warm and cold ischemic organ injury.

THE ROLE OF PAF AND ITS ANTAGONISM IN INFLAMMATION AND INJURY TO THE MICROCIRCULATION

PAF is unequaled by any other single autacoid in its potential for being released and for mediating virtually every aspect of acute, basic allergic, and inflammatory reactions (65). In addition to its effects on platelets, platelet-activating factor induces neutrophil aggregation and activation and superoxide anion release and acts directly on vascular endothelium to increase permeability (66). The most prominent and species-constant effect of PAF is increased vascular permeability (67,68). PAF alters the molecular organization of cytoskeletal proteins that control endothelial permeability (69). Human endothelial cells stimulated by PAF retract and lose reciprocal contact. Stress fibers disappear or become less regular, resulting in "bleb" formation (69). Bleb formation is also seen in ischemia and reperfusion injury (70). In addition to the direct effect of PAF on the endothelium, it indirectly elicits endothelial damage by amplifying leukocyte responses (71).

The mediators and cascades involved in the inflammatory response associated with ischemia and reperfusion are quite complex and integrally interrelated and probably represent a nonspecific complicated process resulting in a failure of the microcirculation and organ injury. This interrelationship prompted our laboratory to investigate the role of PAF, which had already been demonstrated to be a potent mediator in inflammation, and PAF antagonism, which we had already demonstrated could prevent a similar pathophysiological phenomenon of antibody-mediated injury to the endothelium and hyperacute rejection in ischemia and reperfusion injury. To further define the role of PAF in the microcirculatory injury seen following ischemia and reperfusion injury, we attempted to modify the injury resulting from both warm and cold ischemia with a potent PAF receptor antagonist, SDZ 63-441 (Sandoz Company), using the isolated perfused rat liver (IPRL) (72). Two sets of experiments were undertaken. The first evaluated the effect of pretreatment with SDZ 63-441, a potent PAF receptor antagonist, on hepatic function following 90 min of warm ischemia. The second set of experiments utilized SDZ 63-441 pretreatment in combination with UW-lactobionate solution (45) to enhance hepatic function following 24 h of static hypothermic preservation. Liver function was evaluated using the isolated perfused rat liver (72).

THE ISOLATED PERFUSED RAT LIVER

The isolated perfused rat liver has proven to be a simple, inexpensive, and useful model for the study of liver function (73-78). The model lends itself to transplantation research as it allows the evaluation and manipulation of the intact organ removed from the donor without requiring immediate transplantation into a recipient. Using the perfusion system, it is possible to maintain near-physiological conditions and yet allow greater freedom in adjusting variables than in the intact animal. The model allows maintenance of the liver in a viable condition for variable periods of time, so that it can be transplanted into a recipient as a final test of the experimental conditions (74).

The perfusion apparatus is a recirculating system that consists of a pulsatile pump, "Hamilton lung" oxygenator, debubbler and reservoir, effluent collecting basin, blood infusion filter, platelet trap, and in-line thermometer (78,79). The temperature in the enclosed system is maintained at 37°C by a circulating water bath. The liver is perfused via the portal vein from a height of 18 cm, which is an appropriate rat portal vein pressure (80). The flow through the liver is determined by the resistance in the intrahepatic vasculature (15-20 ml/min). The blood flow via the portal vein should be approximately 1.25 ml/min per g liver and should not exceed a flow rate of 5 ml/min per g liver or significant barotrauma results (81). Oxygen extraction using the isolated perfused rat liver of the fasted liver has been reported to be greater than 1.0 μmO_2 per min per g liver and in the fed liver is greater than 2.0 μmO_2 per min per g liver and increases fivefold with the addition of taurocholate to the perfusion medium (84). A 95% oxygen-5% carbon dioxide mixture is used to maintain oxygenation (pO_2 = 500 torr).

The perfusate is a dilute blood solution prepared from 2 parts by volume heparinized fresh whole rat blood and 1 part Krebs bicarbonate buffer for a hematocrit of 25, which is adequate for maintenance of tissue oxygenation in this model, and pH 7.5 (85,86).

The isolated perfused rat liver is an excellent screening model for pharmacological agents that may be useful in the prevention of reduction of ischemia and reperfusion injury.

THE PROTECTIVE EFFECT OF A PAF RECEPTOR ANTAGONIST (SDZ 63-441) ON POSTISCHEMIC HEPATIC FUNCTION FOLLOWING 90 MIN OF WARM ISCHEMIA

Our laboratory has evaluated the ability of SDZ 63-441, a potent PAF receptor antagonist (87), to prevent warm ischemic injury to the liver (88).

Materials and Methods

Male Lewis rats purchased from Charles River, weighing 225-300 g, were used as liver donors, and male Lewis rat retired breeders were used as blood donors in these experiments. Animals were acclimatized for 1 week prior to experimentation and housed in a standard animal facility at the University of Pittsburgh. Animals were fed standard rat chow and water ad libitum. Guidelines for the care and use of laboratory animals of the University of Pittsburgh were followed.

Inhalational anesthesia was induced and maintained with methoxyflurane. The abdomen was entered through a transverse incision. All animals received 300 units of heparin IV 5 min prior to cannulation of the bile duct and induction of total hepatic ischemia. Both the hepatic artery and portal vein were ligated. At the time of harvest the liver was flushed via the portal vein with 60 ml cold (4°C) heparinized lactated Ringer's solution from a height of 20 cm immediately after venting the vena cava. The portal vein was cannulated using a blunted 18 gauge needle. After 90 min of total in situ ischemia, the liver was harvested and placed on the perfusion apparatus.

Experimental groups were as follow: group I (control, no ischemia), the livers were immediately harvested (n = 7); group II, cannulation of the bile duct followed by induction of 90 min of total hepatic ischemia, after which the portal vein was cannulated and the livers harvested (n = 9); group III (no ischemia), rats received 20 mg/kg of SDZ 63-441 IV at the time of heparinization (n = 8); group IV, rats received 20 mg/kg of SDZ 63-441 IV at the time of heparinization followed by induction of 90 min of ischemia (n = 9); group V, rats received 10 mg/kg of SDZ 63-441 IV at the time of heparinization followed by induction of 90 min of ischemia. All livers underwent 90 min of warm (37°C), oxygenated ($pO_2 \simeq 500$ torr), dilute sanguinous (Hct = 25; diluent, Krebs bicarbonate buffer, pH 7.4) perfusion (72).

Baseline perfusate levels of SGOT and SGPT were measured using the Technicon RA 500 (Technicon) prior to placing the liver on the perfusion apparatus. Liver function during perfusion was determined by measuring bile production and SGOT and SGPT levels in the perfusate every 30 min. At the completion of the 90 min perfusion period, tissue was immediately freeze clamped in liquid nitrogen and ATP content was determined biochemically (89). Specimens were placed in formalin, embedded in paraffin, and cut and stained with hematoxylin and eosin (HSE) and PAS for light microscopy. An additional three experiments were repeated per group for experimental groups I, II, and IV, and the livers were processed for electron microscopy.

After 90 min of warm sanguinous perfusion the livers were perfused-fixed via the portal vein with 20 ml 2.0% glutaraldehyde in 0.125 M cacodylate buffer at a rate of 5-10 ml/min, minced to 1 × 1 × 1 mm cubes, and placed in 2.0% glutaraldehyde for 2 h (89). The livers were postfixed in buffered 2.0% osmium

tetroxide, dehydrated in ethanol, and embedded in Epon-812. Blocks were cut using a Reichert microtome. Semithin sections were stained with toluidine blue for light microscopy. Ultrathin sections were collected on grids, stained with uranyl acetate and lead citrate, and observed using a Phillips 300 electron microscope (90,91).

All values were corrected to baseline by subtracting the values obtained prior to placing the liver on the perfusion apparatus from the subsequent measurements at 30, 60, and 90 min of perfusion. The statistical evaluation was carried out using an analysis of variance. When indicated, the Student's *t*-test was performed using the variances generated by analysis of variance (ANOVA) method (92). All values reported are expressed as a mean ± the standard error of the mean (SEM). *P* values of less than 0.05 were considered statistically significant.

Results

There was a significant increase in bile production ($p < 0.005$) by those livers harvested from rats that were pretreated with 20 mg/kg IV of SDZ 63-441 (Table 1). Perfusate transaminase levels were significantly lower than in the ischemic livers of animals pretreated with 20 mg/kg SDZ 63-441 ($p < 0.001$, Table 2). There was no significant difference in transaminase release by SDZ 63-441-treated and untreated livers from animals that underwent no ischemic injury. ATP levels were significantly higher in the ischemic livers pretreated with 20 mg/kg of SDZ 63-441 compared with untreated livers ($p < 0.05$, Table 3). Ischemic livers from rats that were pretreated with only 10 mg/kg IV of SDZ 63-441 did not demonstrate improvement when compared to untreated livers in terms of bile production, transaminase release, or ATP content (Tables 1 through 3). There was a significant difference in bile production, transaminase release, and ATP levels in untreated livers when comparing the group that did not undergo an ischemic period (Tables 1 through 3, $p < 0.001$).

All samples for electron microscopy were taken following the 90 min perfusion period. EM was done only for groups I, II, and IV. Electron microscopy of the nonischemic control livers (group I) revealed intact, healthy-appearing hepatocytes as well as intact well-preserved sinusoidal endothelial cells (Fig. 1A). The ischemic livers (group II) exhibited a patchy picture of hepatocellular damage (Fig. 1B). Vacuolated and pale-staining nonviable helpatocytes containing many disrupted mitochondria were found interspersed with viable hepatocytes. The sinusoidal endothelium remained intact when covering viable hepatocytes but was disrupted in areas adjacent to nonviable hepatocytes (Fig. 2). Sections from ischemic livers that were harvested from donors pretreated with SDZ 63-441 (20 mg/kg, group IV) demonstrated relatively normal-appearing hepatocytes with few vacuoles and intact sinusoidal endothelium

Table 1 Effect of SDZ 63-441 Pretreatment on Bile Production

Group	Bile production (μl/min per g liver)		
	30	60	90
I: 90 min perfusion only	1.42 ± 0.15	1.80 ± 0.21	1.80 ± 0.18
II: 90 min perfusion + SDZ 63-441 (20 mg/kg)	1.83 ± 0.30	2.24 ± 0.28	2.29 ± 0.25
III: 90 min ischemia + 90 min perfusion[a]	0.003 ± 0.003	0.09 ± 0.03	0.15 ± 0.04
IV: 90 min ischemia + 90 min perfusion + SDZ 63-441 (20 mg/kg)[a]	0.37 ± 0.07	0.49 ± 0.06	0.61 ± 0.05
V: 90 min ischemia + 90 min perfusion + SDZ 63-441 (10 mg/kg)[a]	0.06 ± 0.06	0.04 ± 0.04	0.02 ± 0.02

[a]Bile production by ischemic liver was essentially nil. Low-dose SDZ 63-441 (10 mg/kg) did not result in an increase in bile production. SDZ 63-441–pretreated (20 mg/kg) livers produced significantly more bile ($p < 0.005$) than untreated ischemic livers.

(Fig. 1C). The patchy distribution of injury combined with the small cross-sectional area observed with electron microscopy introduces the possibility that the sections viewed were not representative of the true morphological picture. In fact, sections of the ischemic nontreated livers could be found that manifested relatively healthy appearing hepatocytes. To obtain a better overview, paraffin-embedded tissue was cut and stained with H&E and PAS for light microscopy.

The nonischemic control livers exhibited normal healthy-appearing hepatocytes (Fig. 3A). PAS-stained sections were glycogen laden (not shown). The ischemic livers contained large patches of pale-staining, nonviable hepatocytes and vacuolization interspersed with normal-appearing hepatocytes (Fig. 3B). PAS staining revealed large patchy areas of glycogen depletion (not shown).

Table 2 Effect of SDZ 63-441 Pretreatment on Transaminase Release

	SGOT (IU/liter)			SGPT (IU/liter)		
Group	30	60	90	30	60	90
I: 90 min perfusion only	8 ± 8	24 ± 11	34 ± 13	9 ± 3	9 ± 6	11 ± 5
II: 90 min perfusion + SDZ 63-441 (20 mg/kg)	20 ± 20	36 ± 31	52 ± 34	14 ± 9	22 ± 16	15 ± 9
III: 90 min ischemia + 90 min perfusion[a]	946 ± 107	1108 ± 143	1237 ± 142	1066 ± 162	1269 ± 132	1349 ± 118
IV: 90 min ischemia + 90 min perfusion + SDZ 63-441 (20 mg/kg)[a]	454 ± 21	599 ± 40	697 ± 48	423 ± 48	529 ± 59	644 ± 58
V: 90 min ischemia + 90 min perfusion + SDZ 63-441 (10 mg/kg)[a]	930 ± 173	1009 ± 116	1018 ± 125	1221 ± 211	1375 ± 133	1289 ± 106

[a] A significant reduction in transaminase liberation was exhibited only by those ischemic livers harvested from donors pretreated with SDZ 63-441 (20 mg/kg) ($p < 0.001$).

Table 3 Effect of SDZ 63-441 Pretreatment on ATP Content

Group	ATP (μmol/g liver)
I: 90 min perfusion only[a]	2.13 ± 0.11
II: 90 min perfusion + SDZ 63-441 (20 mg/kg)	1.74 ± 0.11
III: 90 min ischemia + 90 min perfusion[a,b]	1.24 ± 0.11
IV: 90 min ischemia + 90 min perfusion[b] + SDZ 63-441 (20 mg/kg)	1.54 ± 0.08
V: 90 min ischemia + 90 min perfusion + SDZ 63-441 (10 mg/kg)	1.09 ± 0.12

[a]Ischemic livers manifested a significant reduction in ATP content when compared with nonischemic livers ($p < 0.001$).
[b]The liver tissue ATP content was significantly higher in group IV compared with group III ($p < 0.05$).

Ischemic livers from donors pretreated with SDZ 63-441 (20 mg/kg) also contained some foci of pale-staining, nonviable hepatocytes and vacuolization (Fig. 3C). Although quantitative analysis was not carried out, the foci of injury in the SDZ 63-441-pretreated ischemic livers were generally smaller in size and less abundant than in the untreated ischemic livers. The pretreated ischemic livers were largely glycogen depleted, in fact, to a greater extent than even the untreated ischemic livers (not shown). Nonischemic livers from rats pretreated with 20 mg/kg of SDZ 63-441 had normal-appearing hepatocytes but also demonstrated patchy glycogen depletion (not shown).

Discussion

Ischemia and reperfusion injury appear to be integrally tied to the inflammatory response, resulting in microcirculatory failure followed by necrosis and cell death. The pathways implicated as causative of the injury to the microcirculation that occurs in this process are multiple and complex. These highly interrelated and redundant cascades involved in the mediation of ischemia and reperfusion injury make it highly likely that effective pharmacological modulation of the injury, leading to prevention of both warm and cold ischemic injury and ultimately to prolongation of organ preservation, will depend upon a polypharmaceutical approach.

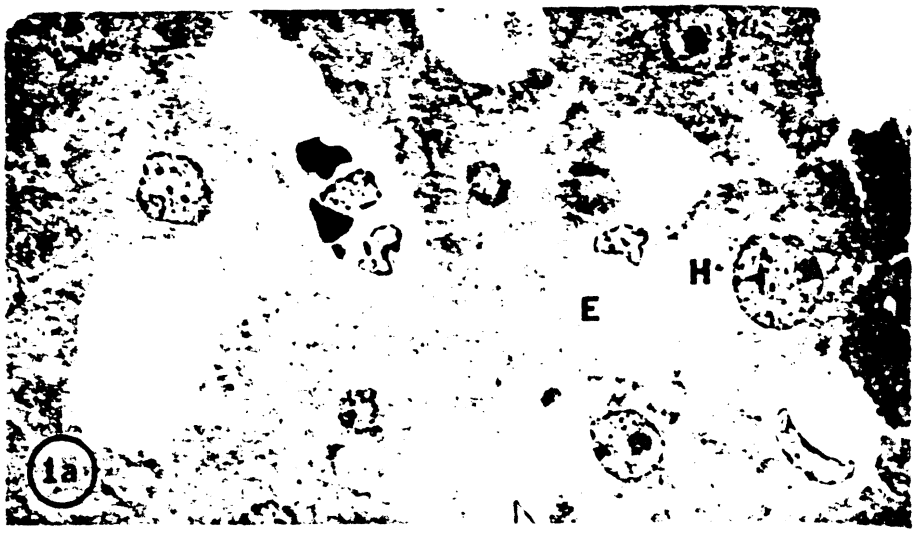

Figure 1 Low-magnification electron micrographs (x 1700) stained with uranyl acetate and lead citrate. (A) Nonischemic control liver. Note intact endothelium (E) and normal-appearing hepatocytes (H). (B) Ischemic liver. Note nonviable hepatocytes (arrows) interspersed with vacuolated but viable hepatocytes (H) and relatively intact endothelium (E). (C) Ischemic liver from an animal pretreated with 20 mg/kg IV of SDZ 63-441. Note normal-appearing hepatocytes (H) in this section along with an intact endothelium (E).

Platelet-activating factor has been proposed as a potential key inflammatory mediator of the microcirculatory failure that results following ischemic organ injury. Platelet-activating factor is released from a number of cells, including endothelial cells, platelets, and neutrophils, and plays a significant role in almost every aspect of the basic inflammatory response (65). In addition to its effects on platelets, PAF induces neutrophil aggregation, activation, and superoxide anion release and acts directly on vascular endothelium to increase permeability. It also has smooth muscle contracting properties (61). SDZ 63-441 is one of the most potent platelet-activating factor receptor antagonists developed to date (87).

A dose-dependent protective effect against warm ischemia and reperfusion injury was clearly demonstrated after donor pretreatment with SDZ 63-441. Bile production, transaminase release, and ATP content all exhibited significant improvement when compared with untreated ischemic controls in those livers from rats pretreated with 20 mg/kg IV of SDZ 63-441. When a lower dose of 10 mg/kg IV of SDA 63-441 was administered, no significant improvement in

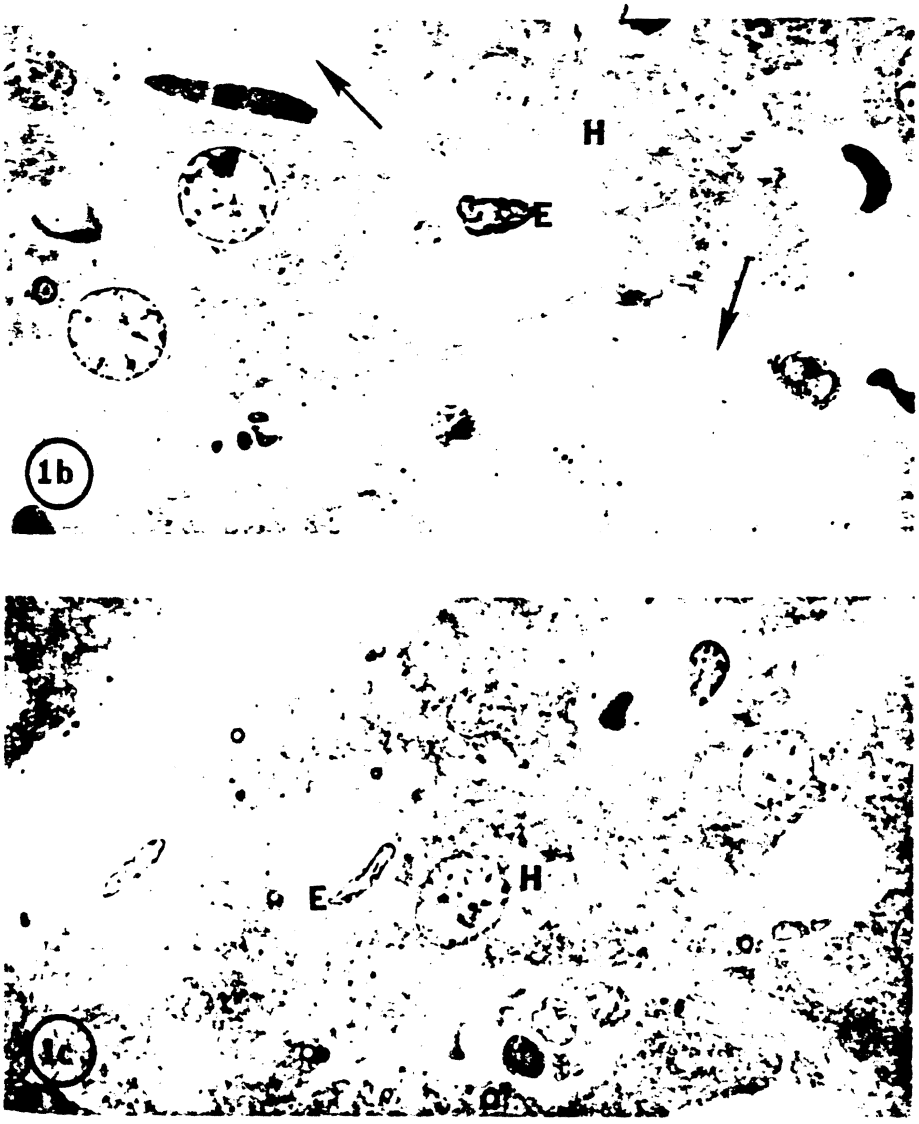

Figure 1 (Continued)

liver function was demonstrated for any parameter measured when compared to ischemic controls. These results substantiate the important role of platelet-activating factor in ischemic organ injury and, moreover, introduces a potential therapeutic approach to organ ischemia and preservation through the inhibition

Figure 2 High-magnification electron micrographs (x 9700) stained with uranyl acetate and lead citrate. (A) Ischemic liver. The endothelium (E) remains intact over the variable hepatocyte (H), which contains vacuoles (V) but is lifted off the nonviable hepatocyte (arrows). (B) Ischemic liver from an animal pretreated with 20 mg/kg IV of SDZ 63-441. Endothelium (E) remains intact over the viable hepatocyte (H).

of PAF by specific receptor antagonism. It is clear from the results reported here that prevention of warm ischemic injury to the liver and improved hepatic function could be achieved by the administration of SDZ 63-441 as a pretreatment to liver donors. Since rat platelets are through to be unresponsive to platelet-activating factor (87), PAF-mediated ischemic liver injury may be neutrophil dependent or, alternatively, may be due to some other PAF-mediated pathway. The improved postischemic hepatic function demonstrated with SDZ 63-441 pretreatment has encouraging implications for liver preservation.

Because the group of animals pretreated with SDZ 63-441 (20 mg/kg) exhibited the most marked improvement in postischemic hepatic function, electron microscopy was performed on these livers to ascertain any histological improvement in organ status. Ischemic pretreated livers (group IV) were compared to ischemic (group II) and nonischemic (group I) controls, which were processed in an identical fashion.

The morphological appearance of the nonischemic control livers was that of normal liver without any evidence of hepatocellular or endothelial injury, indicating that changes in the experimental groups are not artifactual. The general pattern of injury observed in the ischemic livers and the ischemic livers harvested from donors pretreated with SDZ 63-441 (20 mg/kg) was almost identical in nature and consisted of patchy hepatocellular vacuolization and nonviable hepatocytes interspersed with viable cells. However, the degree of injury appeared less severe in the group pretreated with SDZ 63-441 (group IV); the foci of damage were smaller and less abundant than in the untreated ischemic group (group II). The vacuolization observed is characteristic of hypoxic injury (93-95).

The sinusoidal endothelium was relatively spared even in the most severely damaged warm ischemic livers. This is of singular importance since the primary abnormality seen in livers following cold ischemic or preservation injury is endothelial disruption (73). Sinusoidal endothelial injury is apparent in livers injured by cold ischemia while still manifesting normal hepatocellular architecture. Hepatocellular injury manifested by vacuolization does not occur until prolongation of cold ischemic injury well past the point at which endotheial injury becomes apparent (73). This difference in morphology following warm and cold ischemia has only recently been appreciated and lends further credence to the belief that warm and cold ischemia represent two different and distinct types of injury (73). Moreover, it should become possible to use these different and specific pathological changes as predictors of organ function.

The glycogen depletion observed in those livers pretreated with SDZ 63-441 (groups III and IV) was at first puzzling since it would be expected that the more "healthy," better functioning livers would retain their glycogen. However, in addition to its role in the inflammatory response, PAF has a potent agonist effect on the glycogenolytic system in the rat liver (96). It is possible that

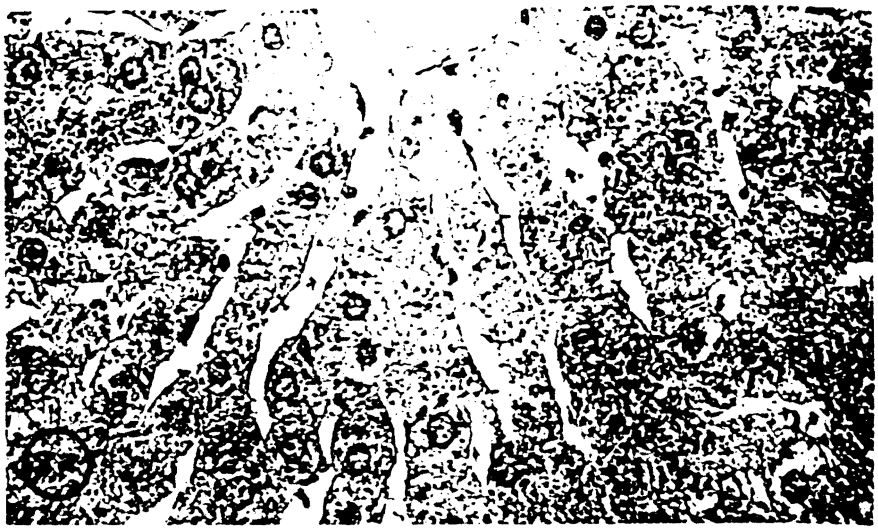

Figure 3 Light micrographs stained with hematoxylin and eosin (×500). (A) Nonischemic control liver with normal-appearing parenchyma. (B) Ischemic liver with a moderate degree of vacuolization: vacuoles (V). (C) Ischemic liver from a rat pretreated with 20 mg/kg IV of SDZ 63-441. Note much lesser degree of vacuolization than in B.

SDZ 63-441, the structurally similar PAF receptor antagonist, may have the same action as PAF itself on the glycogenolytic system activate glycogenolysis.

Briefly, in summary, the platelet-activating factor antagonist SDZ 63-441 significantly reduced hepatic warm ischemic injury in a dose-dependent fashion, thereby substantiating the role of platelet-activating factor in ischemic organ injury and introducing a novel therapeutic approach.

IMPROVED FUNCTION OF LIVERS COLD STORED IN UW-LACTOBIONATE SOLUTION FOR 24 H AFTER PRETREATMENT WITH A PAF RECEPTOR ANTAGONIST (SDZ 63-441)

After demonstrating the protective effect of the PAF receptor antagonist SDZ 63-441 on hepatic function following warm ischemic injury, we evaluated the role of PAF antagonism in livers subjected to a cold ischemic insult, which

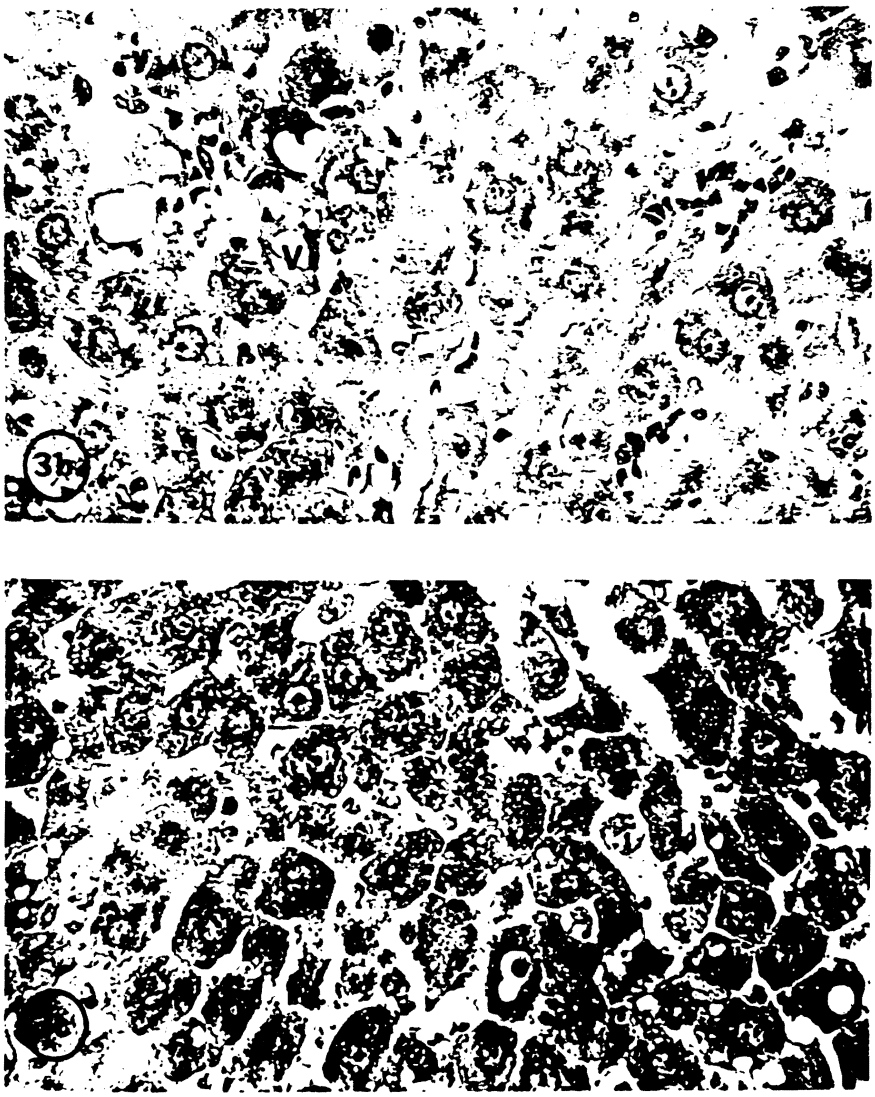

Figure 3 (Continued)

is much more germane to organ preservation (97). This study incorporated the use of UW-lactobionate solution, a new preservation solution initially developed for pancreas preservation but shown to be much more effective for liver preservation than the standard Eurocollin's solution (63,93,98,99).

Materials and Methods

Animals, operative techniques, evaluation of hepatic function, electron microscopy, and statistical analysis are as in an earlier section, with the following exceptions: heparinization occurred 5 min prior to cannulation of the bile duct, and portal vein and the livers did not undergo any period of warm ischemia. The vena cava was vented and the liver immediately flushed via the portal vein with 20 ml cooled (4°C) preservation solution from a height of 20 cm. The liver was excised during the flushing period, weighed, immersed in preservation solution, and stored at 4°C. Prior to placement on the perfusion apparatus, livers were flushed with 8 ml lactated Ringer's solution to removed the preservation solution. Livers were placed on the perfusion apparatus for 90 min as described earlier.

Experimental groups were as follows: group I, controls, levers were flushed with lactated Ringer's solution and immediately placed on the perfusion apparatus (n = 7); group II, livers were flushed and stored with UW solution for 24 h (n = 5); and group III, animals were pretreated with 20 mg/kg IV of SDZ 63-441 5 min prior to harvesting the liver, and the livers were flushed and stored with UW solution for 24 h (n = 6). After storage, all livers were placed on the perfusion apparatus for 90 min.

Livers were weighed immediately after harvesting and after the preservation period to assess tissue edema. SGOT, SGPT, LDH, and glucose levels were measured in the perfusate prior to placing the liver on the perfusion apparatus and every 30 min during perfusion. Bile production was measured after 30, 60, and 90 min. After 90 min of warm sanguinous perfusion the livers were perfused fixed processed for electron microscopy.

Results

There were no significant differences in the weights of livers stored in UW solution for 24 h regardless of pretreatment with SDZ 63-441; that is, neither group gained weight during the preservation period (Table 4). SGOT, SGPT, LDH, and

Table 4 % Change in Liver Weight During Storage

Group	% Change[a]
I: no preservation	–
II: 24 h preservation[b]	-5.7 ± 0.9
III: 24 h preservation + SDZ 63-441[b]	-3.1 ± 1.4

[b]The addition of SDZ 63-441 did not significantly alter weight change during the storage period.

glucose levels in the perfusate are listed in Table 5. The 24 h, UW-stored, SDZ 63-441-pretreated livers (group III) did not release significantly more SGOT than controls (group I) until after 90 min of perfusion ($p < 0.05$), and did not release significantly more SGPT than controls (group I) after 30, 60, and 90 min of perfusion. Although there was a consistent trend of decreased transaminase release in the SDZ 63-441 pretreated, 24 h UW-stored livers (group III) when compared to 24 h UW-stored livers alone (group II), these differences did not reach statistical significance.

Both pretreated (group III) and untreated (group II) stored livers released significantly more LDH into the perfusate than controls (group I, $p < 0.01$), and there was no significant difference between LDH liberation between the two groups that underwent preservation (groups II and III).

Perfusate glucose levels were not significantly different between any of the three groups. Bile production results are listed in Table 6. Bile production by both the pretreated stored livers (group III) and the untreated stored livers (group II) was significantly decreased when compared with nonstored controls (group I). Those livers harvested from animals that were pretreated with SDZ 63-441 and stored produced significantly more bile than those livers that were stored but not pretreated ($p < 0.05$).

Electron microscopy revealed moderate endothelial cell disruption in both groups (groups II and III) of stored livers (Fig. 4A and B). Controls (group I) exhibited a completely intact endothelium (Fig. 4C). The parenchyma of the

Table 5 Effect of SDZ 63-441 Pretreatment on Perfusate SGOT, SGPT, LDH, and Glucose Levels[a]

Group	Perfusion time (min.)	SGOT (IU/liter)	SGPT (IU/liter)	LDH (IU/liter)	Glucose (mg/dl)
No preservation	30	8 ± 8	9 ± 3	63 ± 53	114 ± 11
	60	24 ± 11	9 ± 6	67 ± 70	115 ± 22
	90	34 ± 13	11 ± 5	144 ± 73	112 ± 25
24 h Preservation	30	73 ± 10	43 ± 7	586 ± 105	269 ± 28
	60	128 ± 19	59 ± 11	826 ± 144	221 ± 25
	90	58 ± 15	68 ± 11	902 ± 115	176 ± 23
24 h Preservation + SDZ 63-441	30	52 ± 9	9 ± 18	429 ± 62	209 ± 38
	60	86 ± 16	20 ± 20	665 ± 62	166 ± 29
	90	109 ± 21	20 ± 19	832 ± 53	139 ± 22

[a]There was a consistent trend of decreased transaminase, LDH, and glucose release into the perfusate by group III livers when compared with group II, although this did not reach statistical significance.

Table 6 Effect of SDZ 63-441 Pretreatment on Bile Production

Group	Perfusion time (min.)	Bile production (μl/min per g liver)
I: no preservation	30	1.42 ± 0.15
	60	1.80 ± 0.21
	90	1.80 ± 0.18
II: 24 h preservation[a]	30	0.58 ± 0.08
	60	1.08 ± 0.11
	90	1.14 ± 0.12
III: 24 h preservation + SDZ 63-441[a]	30	0.87 ± 0.16
	60	1.31 ± 0.25
	90	1.51 ± 0.20

[a]Group III livers produced significantly more bile than group II livers ($p < 0.05$).

Figure 4 High-magnification electron micrographs (x 9700) stained with uranyl acetate and lead citrate. (A) Liver preserved at 4°C for 24 h in UW-lactobionate solution. Note focal area of endothelial disruption (arrow) and normal-appearing hepatocyte (H). (B) Liver preserved at 4°C for 24 h in UW-lactobionate solution from an animal pretreated with 20 mg/kg IV of SDZ 63-441. Foci of endothelial disruption (arrows) characteristic of cold ischemic injury are present. Hepatocytes (H) remain normal in appearance. (C) Nonpreserved control liver exhibiting intact endothelium (E), normal-appearing space of Disse (D), and healthy hepatocyte (H).

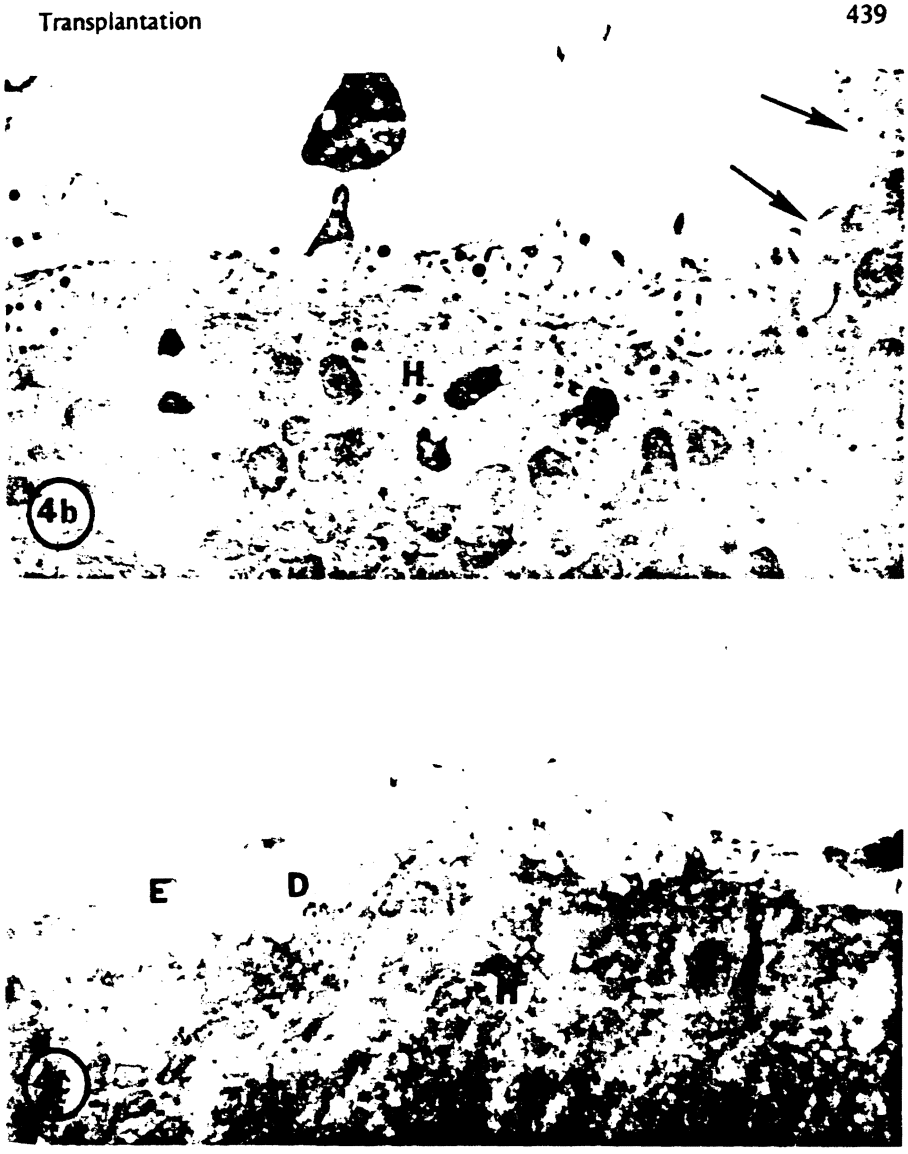

Figure 4 (Continued)

two groups (group II and III) of preserved livers remained healthy in appearance (Fig. 4A and B). There was no distinct difference in the morphology of the pretreated group (group III) when compared to the untreated group (group II). Of particular interest is that the pattern of injury demonstrated following cold

ischemia primarily involves the endothelium, whereas in the same experimental model, warm ischemic injury is primarily parenchymal in nature.

Discussion

Those livers excised from animals pretreated with SDZ 63-441 and stored for 24 h (group III) produced significantly more bile than those that were stored but from untreated animals (group II). Since postrevascularization bile production is considered one of the most important indicators of graft viability and function used in clinical transplantation, this represents a considerable improvement in hepatic synthetic function. Although the other parameters of liver function evaluated in this study did not reach statistical significance, a sustained general trend toward improvement was evident for all variables. This study suggests that pretreatment of donor animals with the PAF antagonist SDZ 63-441 also has a beneficial effect on the reduction of cold ischemic injury of the liver as we previously described for warm ischemic injury.

SUMMARY

Platelet-activating factor is a central mediator of the cell-to-cell interaction involved in response to a multitude of stimuli that incite an inflammatory response. Its role in transplantation and the use of PAF antagonists as a therapeutic approach have implications in drug toxicity, antibody-mediated injury (hyperacute rejection), and ischemia and reperfusion injury. In this chapter we have reviewed data that provide evidence supporting the role of PAF as a mediator of the microcirculatory failure that follows ischemia and reperfusion injury in both warm ischemia and cold ischemia. The use of PAF antagonists could have important clinical applications in organ transplantation.

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