

# Transplantation

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## ARTICLES

### PLATELET-ACTIVATING FACTOR AND HYPERACUTE REJECTION

THE EFFECT OF A PLATELET-ACTIVATING FACTOR ANTAGONIST, SRI 63-441, ON REJECTION OF XENOGRAPTS AND ALLOGRAPTS IN SENSITIZED HOSTS<sup>1</sup>

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The pathogenesis of hyperacute transplantation reactions includes the activation of a cascade of nonspecific inflammatory reactions that precipitates the destruction of the target organ. Platelet-activating factor (PAF) represents an important component of these inflammatory cascades, and we have examined the influence of a specific PAF receptor antagonist (SRI 63-441) on the inhibition of hyperacute rejection in two experimental models, the rejection of rat cardiac allografts by presensitized recipients and guinea pig-to-rat and mouse-to-rat cardiac xenografts. Our results demonstrate that inhibition of PAF function by SRI 63-441 has a variable effect on the survival of cardiac allografts in presensitized rat recipients. In the ACI to sensitized BN cardiac allograft model, the use of SRI 63-441 alone, or in combination with CsA, FK506, or prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), does not prolong graft survival. As we have previously reported, SRI 63-441 does act as a single agent to prolong the survival of ACI to sensitized LEW grafts, and this survival effect is synergistic when combined with CsA. Here we extend these results to demonstrate that this survival is also extended when FK506 is used in the ACI-to-LEW model. Concordant mouse-to-rat cardiac xenografts are also relatively resistant to prolongation of graft survival following treatment with SRI 63-441 alone or in combination with CsA or FK506.

Discordant xenografts appear to be more susceptible to inhibition of the rejection reaction with SRI 63-441. When either donor or recipient animals were treated with SRI 63-441 alone, or in combination with CsA or FK506, there was significant prolongation of guinea pig-to-rat cardiac xenograft survival. These results are consistent with our earlier description of the effectiveness of SRI 63-441 in preventing the rejection of cat-to-rabbit kidney xenografts. We believe that these re-

sults demonstrate that the use of the SRI 63-441 to specifically interfere with the function of PAF has the effect of prolonging graft survival in those systems in which preformed antibody and/or complement activation are important components of the hyperacute reaction. This synthetic drug is representative of a family of compounds whose structure can be modified to balance their therapeutic and toxicity activities, and may prove to be important components of a polytherapeutic approach to the control of graft rejection in sensitized patients or following discordant xenografting.

The hyperacute form of organ graft rejection was originally recognized in patients who had received kidneys from ABO-blood-group incompatible donors (1). The reaction is mediated by the presence of preformed antibody against donor renal graft ABO antigens and is associated with endothelial damage secondary to deposition of antibody and complement (2) and vascular thrombosis (3). Hyperacute rejection (HAR)\* can also be seen in patients who have been stimulated to produce antidonor antibodies to specific HLA antigens (4), either because of the rejection of an HLA incompatible graft, multiple blood transfusions, or, secondarily, because of multiple pregnancies.

Experimental studies of the mechanism of HAR have shown that the primary target for the reaction is the capillary endothelium of the graft (5, 6). Within 1 min after transplantation, widespread blockage of the microvascular system by platelet and neutrophil aggregates and fibrin occurs. The reaction depends upon the activation of complement (7), and results in vasoconstriction and a disseminated intravascular coagulation in the microvasculature. The occlusion of the microvasculature of the graft results in an ischemic necrosis of the transplanted organ that shares many of the features of an experimentally induced generalized Shwartzman reaction (4).

Severe injury to vascular endothelium is associated with the release of a variety of biologically active mediators of coagulation and inflammation, stimulation of platelet aggregation, and modulation of inflammatory cell interaction with the endothe-

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\* Abbreviations: HAR, hyperacute rejection; PAF, platelet-activating factor; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>.

lium. Platelet-activating factor (PAF) is one of the more recently recognized biological mediators, and it represents a class of potent lipid autocoids that have been reported to be important in the pathogenesis of acute inflammation (8), endotoxin shock (9), and anaphylaxis (10). PAF exhibits a broad spectrum of activities, including aggregation and activation of platelets and neutrophils, increased vascular permeability, smooth muscle contraction, and the release of vasoactive amines (8, 11). More recently, PAF has been implicated as a key biological mediator in the pathogenesis of antibody and complement-mediated hyperacute organ rejection (12). When kidney grafts were transplanted to sensitized rabbits, PAF was identified in the blood draining the transplanted kidney. PAF was detected in the blood effluent within 2–10 min after revascularization and was not found in syngeneic controls or unsensitized recipients. Injection of a synthetic, purified PAF into the renal artery induced lesions in the kidney that were identical to those seen in the Shwartzman reaction. Similar results were obtained *in vitro* when perfusion of isolated rabbit hearts with transplantation alloantibodies was associated with a complement-dependent release of PAF (13).

The potential that PAF may play an important role in the early initiation of the lesions of HAR has led us to examine the influence of specific inhibitors of PAF on hyperacute rejection. A variety of specific PAF receptor antagonists have been synthesized and have been reported to block PAF binding to its high-affinity receptor. These compounds are capable of inhibiting and/or reversing PAF-induced hypotension (14), neutropenia (15), bronchospasm (16), endotoxin shock (9), and anaphylaxis (10). Our initial studies indicated that a single bolus of one of these PAF receptor antagonists (SRI 63-441) at a dose of 15 mg/kg was capable of significantly prolonging hyperacute rejection by sensitized LEW rats of ACI strain cardiac allografts (17). This treatment inhibited but did not prevent HAR, and, at somewhat higher doses, there was evidence of a toxic effect on the recipients. The inclusion of either CsA or prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) did not consistently improve the graft survival times. The effect of SRI 63-441 was also tested for its ability to prevent discordant cat-to-rabbit (17) and pig-to-dog (18) xenograft reactions, and our results demonstrated significant prolongation of graft survival, particularly when the SRI 63-441 was used in combination with immunosuppressants or prostaglandins.

The current report evaluates in more detail the effect of SRI 63-441, either alone or in combination with additional pharmacologic agents, on the rejection of rat cardiac allografts in presensitized recipients, and on cardiac survival in a series of concordant and discordant xenografts in rats. The SRI 63-441 compound is a specific PAF receptor antagonist manufactured by the Sandoz Company (East Hanover, NJ). It represents one of a family of agents that can be synthesized to meet various therapeutic requirements, including intravenous or oral administration. Our results indicate that the use of these compounds may be effective in inhibiting humoral effector arms of the inflammatory reaction of hyperacute rejection and that the effective control of this reaction may be possible when these compounds are combined with other therapeutic agents directed at the cellular components of the rejection process.

#### MATERIALS AND METHODS

**Animals.** Commercially available inbred male Lewis (LEW) (RT<sup>1</sup>), Brown-Norway (BN) (RT<sup>1</sup>), and ACI (RT<sup>1</sup>) rats weighing 200–220 g

were purchased from Harlan Sprague Dawley (HSD; Indianapolis, IN). These strain combinations were selected on the basis of strong histoincompatibility differences to provide a reproducible and reliable model of hyperacute rejection. Hartley guinea pigs (200–220 g, HSD), and male inbred B6AF<sub>1</sub> mice (25–30 g; Jackson Laboratories, Bar Harbor, ME) were used for the discordant and concordant heterotopic heart xenografts, respectively. All animals were acclimatized in our animal facility for at least 2 weeks prior to investigation and received standard rat chow (Wayne Lab-Blox F-6, Chicago, IL) and water *ad libitum*.

**Sensitization procedures.** Presensitization of LEW and BN recipients was achieved by the placement of skin allografts from ACI strain rats. The ACI donors were anesthetized with methoxyflurane, and a full-thickness ventral abdominal skin graft removed. A 2.5-cm circular skin graft was transplanted to the dorsum of the LEW or BN recipient and fastened with wound clips (Clay-Adams, Parsippany, NJ). The graft site was wrapped in plaster bandage (Johnson & Johnson, Chicago, IL) for 4 days after the grafting procedure to immobilize the donor graft. The grafts were inspected regularly for rejection, and two (ACI→BN) or three (ACI→LEW) additional grafts were placed on each recipient at 2-week intervals.

**Heterotopic cardiac allografting.** Heterotopic heart transplants were performed 17–20 days following the final skin graft in presensitized recipients. Contemporaneous controls receiving vehicle only were performed for each experimental group. The heart grafting was performed using a modification of the techniques described by Ono and Lindsey (19). The donor animals were anesthetized with sodium pentobarbital (40 mg/kg *i.p.*) and maintained on methoxyflurane via inhalation. The vena cavae and pulmonary veins were ligated with 5-0 silk, and the pulmonary artery and aorta transected 2–3 mm above their origins in the heart. After perfusion of the ventricles and atria with lactated Ringer's solution (containing 200 U/ml of heparin), the donor heart was placed in a saline bath at 4°C. Recipient animals were anesthetized with pentobarbital, a midline incision made, and the great abdominal vessels dissected free from the left renal vein to the bifurcation. The graft was implanted in the abdominal cavity with both anastomoses performed in a running end-to-side fashion with 10-0 Novafil on a TE-70 needle. Operative times ranged from 30 to 45 min with a success rate of approximately 90%. The grafts were evaluated for function by abdominal palpation every 30 min for the initial 15 hr and then twice daily thereafter. Rejection was considered to have occurred when there were no palpable contractions and the condition of the donor heart was confirmed by laparotomy and by histologic evaluation. All experimental animals were weighed daily.

#### Pharmacologic Agents

**PAF antagonist (SRI 63-441).** The PAF inhibitor SRI 63-441 was kindly supplied by Sandoz Research Institute (East Hanover, NJ). This synthetic compound represents one of the most potent receptor antagonists of PAF responses and is a substituted (tetrahydrofuranyl-[methoxyphosphinyloxy]ethyl) quolinium compound (MW 662) (15). The powdered form was reconstituted in 0.68% sodium acetate buffer and 0.9% sodium chloride (pH 5.3) to a final concentration of 10 mg/ml and was placed in a warm water bath (26–28°C) for 5 min. A single bolus injection was administered via the inferior vena cava to the recipient 4 min prior to revascularization of the donor heart. In selected experiments, a single injection of the drug (30 mg/kg) was administered to the donor 4 min prior to harvest of the heart graft. The dosage levels evaluated in the recipients for the different experiments are listed in the individual tables.

**FK506.** A crystalline powdered form of FK506 (MW 882) was supplied by Fujisawa Pharmaceutical Company, Ltd. (Osaka, Japan). A final concentration of 1.28 mg/ml was prepared in 0.9% sodium chloride. FK506 was administered at a dosage of 1.28 mg/kg/day for a duration of 14 days (or until

rejection) by intramuscular injection. Administration of the drug began 3 days prior to transplantation (day -3). In selected experiments, oral FK506 was administered by gavage at a dosage of 3.2 mg/kg/day using the drug preparation and schedule as described above.

**Cyclosporine.** A commercially available formulation of CsA (100 mg/kg) from Sandoz Pharmaceutical Corporation (East Hanover, NJ) was diluted in olive oil to a final concentration of 10 mg/ml and administered orally at a dosage of 15 mg/kg/day beginning 3 days prior to transplantation (day -3) and continued for a maximum of 14 days (or until graft rejection).

**Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>).** Prostaglandin E<sub>2</sub> was supplied by the Upjohn Company of Kalamazoo, Michigan. The PGE<sub>2</sub> was reconstituted in a stock solution of 1 mg in 100 µl of ethanol. This solution was then diluted in 0.9% sodium chloride and administered 30 min prior to revascularization at a dosage of 3 mg/kg/day by continuous intravenous infusion.

**Histology.** Tissue specimens for histologic studies were collected after rejection of the cardiac grafts. At the time of rejection, biventricular segments of the heart and sections of kidney were fixed in 10% buffered formalin solution and embedded into paraffin after fixation. Sections were cut at 3 µ and stained with hematoxylin and eosin.

**Statistics.** Graft survival curves were estimated using the Kaplan-Meier product-limit method. Differences in the median survival times of the various treatment groups were compared using the generalized Wilcoxon test statistics. The *P*-values for pairwise comparisons are given in the tables, and if statistically significant differences (*P*<0.05) exist after adjustment of alpha levels for multiple comparisons the values are indicated in the table footnotes.

## RESULTS

### Hypersensitized Recipients

**SRI 63-441 alone in BN recipients.** Cardiac allograft survival for hypersensitized control and treated animals is summarized in Table 1. Our previous experiments using presensitized LEW rats and ACI donors had demonstrated that significant prolongation of graft survival could be induced by treatment with SRI 63-441 alone, and this set of experiments was attempted to repeat those observations with different strain combinations. As seen in Table 1, the sensitized BN animals consistently rejected their ACI grafts in a hyperacute fashion (MST 68.0±15.1 min; n=39). Treatment of sensitized rats with SRI 63-441 as a single intravenous bolus prior to revascularization did not result in a prolongation of the graft survival. One group (20 mg/kg) actually had shorter survival times than the control group (MST=27.0 min, *P*=0.07).

TABLE 1. Effect of SRI 63-441 alone or in combination with CsA, FK506, or prostaglandin (PGE<sub>2</sub>) on ACI cardiac allograft survival in presensitized BN rats

Treatment	n	Graft survival time (min)	Median ±SE*	<i>P</i> value
Control	39	3, 4, 4, 6, 9, 12, 15, 16, 16, 17, 20, 25, 31, 34, 35, 46, 52, 52, 67, 68, 70, 75, 82, 90, 104, 110, 115, 133, 144, 165, 192, 195, 283, 285, 314, 350, 484, 600, 1080	68.0±15.1	—
SRI 63-441, 15 mg/kg	9	41, 57, 67, 80, 95, 107, 109, 210, 256	95.0±22.4	0.37
SRI 63-441, 20 mg/kg	7	6, 16, 18, 27, 41, 49, 60	27.0±11.8	0.07
SRI 63-441, 30 mg/kg (donor pretreatment)	6	28, 132, 143, 160, 182, 250	143.0±17.2	0.21
CsA alone	7	6, 23, 420, 540, 1440, 1440, 2880	540.0±157.1	0.05
CsA+SRI 63-441, 10 mg/kg	5	99, 159, 262, 273, 307	262.0±112.8	0.08
CsA+SRI 63-441, 15 mg/kg	5	65, 67, 87, 145, 291	87.0±21.9	0.40
FK506 alone	7	120, 169, 212, 230, 240, 247, 468	230.9±23.6	0.02
FK506+SRI 63-441, 15 mg/kg	8	105, 107, 121, 128, 240, 319, 569, 7200	128.0±84.2	0.02
PGE <sub>2</sub> only	7	11, 27, 50, 75, 149, 154, 210	75.0±32.7	0.81
PGE <sub>2</sub> +SRI 63-441, 10 mg/kg	6	35, 48, 60, 166, 368, 420	60.0±72.3	0.30
PGE <sub>2</sub> +SRI 63-441, 15 mg/kg	8	15, 30, 36, 57, 60, 61, 77, 155	57.0±17.0	0.62

\* Asymptotic standard error.

**SRI 63-441 and immunosuppression with CsA or FK506.** Animals treated with either CsA or FK506 alone or in combination with SRI 63-441, also failed to demonstrate prolongation of ACI cardiac grafts transplanted into sensitized BN rats (Table 1). Neither immunosuppressive agent administered alone had the ability to prevent hyperacute rejection, and the combination of either drug with SRI 63-441 does not improve the effectiveness of the treatment. Although there was prolonged survival of the grafts in several groups, the *P*-values were not significant at the adjusted alpha levels of *P*=0.01.

The results we obtained with the treatment of the sensitized BN recipients with CsA were similar to those we observed earlier for the ACI-to-LEW combination (Table 2 and [17]). Although there may be some prolongation of graft survival following treatment with CsA, the

results were not statistically significant in either combination. The ACI-to-LEW donor-recipient combination demonstrated a greater sensitivity to the effect of SRI 63-441, and we tested the effectiveness of FK506 in this combination to establish whether a similar sensitivity could be seen with this drug (Table 3). In this combination a significant prolongation of allograft survival was evident when FK506 was given alone or in combination with SRI 63-441 at dosages of either 10 or 15 mg/kg.

**SRI 63-441 and prostaglandin  $E_2$ .** In order to determine if the effect of SRI 63-441 could be enhanced by using it in combination with other agents that inhibit the inflammatory components of rejection, we included  $PGE_2$  in the treatment regimen.  $PGE_2$  is one of a group of short-lived, local-acting messengers that generally act to protect against

TABLE 2. Effect of SRI 63-441 alone or in combination with FK506 on ACI cardiac allograft survival in presensitized LEW rats

Treatment	n	Graft survival time (days)	Median $\pm$ SE*	<i>P</i> value
Control	74	0.001, 0.002, 0.002, 0.003, 0.003, 0.003, 0.003, 0.004, 0.005, 0.006, 0.006, 0.007, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.03, 0.03, 0.03, 0.03, 0.03, 0.04, 0.04, 0.04, 0.04, 0.04, 0.04, 0.05, 0.05, 0.05, 0.05, 0.06, 0.06, 0.06, 0.06, 0.07, 0.08, 0.08, 0.10, 0.13, 0.13, 0.15, 0.24, 0.24, 0.26, 0.26, 0.27, 0.27, 0.29, 0.31, 0.33, 0.42, 0.42, 0.42, 0.42, 0.60, 0.67, 0.83, 0.88, 1.0, 1.0	0.04 $\pm$ 0.01	—
SRI 63-441, 10 mg/kg <sup>b</sup>	10	0.003, 0.02, 0.04, 0.05, 0.06, 0.14, 1.13, 1.19, 2.19, 4.0	0.06 $\pm$ 0.07	0.08
SRI 63-441, 15 mg/kg <sup>b</sup>	20	0.03, 0.08, 0.09, 0.12, 0.13, 0.15, 0.17, 0.32, 0.42, 0.5, 1.17, 2, 2.7, 3, 3, 3.5, 4, 4, 4, 5	0.12 $\pm$ 1.1	<0.0001 <sup>c</sup>
FK506	11	0.01, 0.03, 0.05, 0.06, 0.1, 0.27, 1.4, 2, 3, 11, 50	0.2 $\pm$ 0.7	0.01 <sup>c</sup>
FK506+SRI 63-441, 10 mg/kg	8	0.85, 4, 4, 8, 13, 20, 29, 39	8.0 $\pm$ 6.4	0.0001 <sup>c</sup>
FK506+SRI 63-441, 15 mg/kg	12	0.06, 0.07, 0.07, 0.10, 5, 8, 9, 15, 19, 27, 31, 50	8.0 $\pm$ 3.5	0.0001 <sup>c</sup>

\* Asymptotic standard error.

<sup>b</sup> Modified data from reference 17.

<sup>c</sup> Significant at the adjusted alpha level (0.02).

TABLE 3. Effect of SRI 63-441 alone or in combination with CsA, FK506, or prostaglandin ( $PGE_2$ ) on guinea pig-to-rat cardiac xenograft survival

Treatment	n	Graft survival time (min)	Median $\pm$ SE*	<i>P</i> value
Control	8	5, 5, 5, 5, 6, 7, 8, 9	5.0 <sup>b</sup>	—
SRI 63-441, 10 mg/kg	6	10, 12, 27, 31, 54, 62	27.0 $\pm$ 11.6	0.0009 <sup>c</sup>
SRI 63-441, 15 mg/kg	7	11, 15, 19, 64, 90, 117, 127	64.0 $\pm$ 58.8	0.0001 <sup>c</sup>
SRI 63-441, 20 mg/kg	5	11, 21, 44, 68, 99	44.0 $\pm$ 25.2	0.002 <sup>c</sup>
SRI 63-441, 30 mg/kg (donor pretreatment)	6	7, 10, 26, 26, 27, 31	26.0 $\pm$ 9.2	0.004 <sup>c</sup>
CsA	6	6, 7, 7, 8, 11, 15	7.0 $\pm$ 0.8	0.07
FK506	8	5, 6, 8, 13, 16, 41, 53, 67	13.0 $\pm$ 5.7	0.02
$PGE_2$	6	6, 6, 10, 11, 12, 19	10.0 $\pm$ 3.1	0.02 <sup>c</sup>
CsA+SRI 63-441, 15 mg/kg	6	13, 15, 19, 55, 68, 75	19.0 $\pm$ 24.5	0.0009 <sup>d</sup>
FK506+SRI 63-441, 15 mg/kg	8	10, 11, 11, 15, 15, 17, 19, 45	15.0 $\pm$ 2.7	0.0001 <sup>d</sup>
$PGE_2$ +SRI 63-441, 15 mg/kg	6	8, 17, 17, 19, 26, 60	17.0 $\pm$ 4.5	0.002 <sup>c</sup>

\* Asymptotic standard error.

<sup>b</sup> Asymptotic standard error cannot be calculated.

<sup>c</sup> Significant at the adjusted alpha level of 0.02.

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and/or limit acute cellular injury. Our previous work with renal transplantation in the pig-to-dog xenograft model (18) suggested that SRI 63-441 and PGE<sub>2</sub> may act synergistically to prolong discordant xenograft reactions. Treatment of sensitized BN recipients of ACI heart grafts with prostaglandin (PGE<sub>2</sub>), either alone or in combination with SRI 63-441, did not, however, result in significantly prolonged cardiac allograft survival (Table 1). In no case did graft survival exceed 7 hr.

#### Xenotransplantation

Both discordant (widely disparate species combinations) and concordant (phylogenetically similar species combinations) animal groups were studied in order to determine whether either of these two xenograft reactions could be influenced by inhibition of PAF function by SRI 63-441.

**Guinea pig-to-rat cardiac xenografts—SRI 63-441 alone.** The median survival time to asystole of the cardiac xenografts transplanted from guinea pig donors to rat (LEW) recipients was 5.0 min (Table 3). Significant prolongation (up to 10-fold) was achieved either when the donor was pretreated with 30 mg/kg SRI 63-441 prior to harvest or when recipients received 10, 15, or 20 mg/kg of this agent prior to revascularization.

**Guinea pig-to-rat cardiac xenografts—SRI 63-441 and additional pharmacologic agents.** Multiple administrations of either CsA or FK506 were not associated with significant prolongation of guinea pig-to-rat cardiac grafts (Table 3). However, when SRI 63-441 was combined with either FK506 or CsA, a 3–6-fold increase, respectively, in graft survival was observed.

As we have described earlier for discordant pig-to-dog xenograft reactions (18), PGE<sub>2</sub> was also effective, particularly when used in combination with SRI 63-441, in modulating HAR associated with the discordant guinea pig-to-rat xenografts (Table 3).

**Histopathology:** When the tissue sections from these grafts were reviewed, the classic histopathologic features of discordant cardiac xenograft rejection were observed. The control grafts exhibited widespread obstruction of small vessels with platelet-fibrin thrombi and, because of the rapid rejection times, few other pathologic changes. In those animals that survived for longer periods of time, there was mild-to-severe intramyocardial hemorrhage and evidence of endothelial damage to small vessels. The endothelial damage consisted of ballooning degeneration of the endothelium, particularly in small arteries. There was no evidence of qualitative differences in the histopathologic changes seen between animals within individual treatment groups, suggesting that the appearance of the endothelial damage and hemorrhage was due to the development lesions with the passage of time. These results are consistent with our previous descriptions of the pathologic features of the hyper-

acute rejection of cardiac allografts in presensitized LEW recipients of ACI hearts treated with SRI 63-441 and/or CsA (17).

**Mouse-to-rat xenografts—SRI 63-441 alone or in combination with pharmacologic agents.** In the concordant species combination of mouse→rat, the control rejection time was consistently 3 days. The administration of SRI 63-441 at 15 mg/kg failed to extend organ survival (Table 4). Similarly, multiple administration of therapeutic dosages of either FK506 or CsA had no effect when given alone. When FK506 was combined with SRI 63-441, 3 out of 5 grafts survived 4 days. This prolongation, however, was not statistically significant (alpha level 0.008). Furthermore, a beneficial response was not evident following the intravenous infusion of 3 mg/kg/day of PGE<sub>2</sub>, either alone or in combination with the PAF antagonist.

#### DISCUSSION

Our experimental results demonstrate that a potent synthetic antagonist (SRI 63-441) to platelet-activating factor is capable of interfering with the hyperacute rejection of foreign allografts and discordant xenografts. We have examined the effectiveness of SRI 63-441 in preventing hyperacute rejection of heterotopic cardiac grafts in two experimental models, heart allografts exchanged between donor and recipient rat strains in which the recipient had been preimmunized with donor skin grafts and rat recipients of discordant (guinea pig) and concordant (mouse) xenografts. The hyperacute rejection of heart allografts by a recipient preimmunized with donor tissues depends upon the presence of circulating antidonor antibodies that bind to the graft immediately following transplantation and precipitate a rejection within a few minutes. The rapid rejection of discordant xenografts is thought to represent a similar antibody-mediated rejection mechanism that depends upon the existence of natural recipient antidonor species antibodies (20), although the formal demonstration of host antidonor antibody is lacking in selected systems (21).

The PAF antagonist SRI 63-441 is capable of prolonging cardiac allograft survival in some, but not all, rat cardiac allograft combinations. We have previously demonstrated that sensitized LEW recipients of ACI heart grafts display a remarkable prolongation (10×) of graft survival when the recipients are treated with SRI 63-441 immediately prior to transplantation (17). In the present study, our results demonstrate that FK506 alone is capable of prolonging graft survival in the sensitized rat model and that the inclusion of SRI 63-441 results in additional improvement in graft survival for this type of hyperacute rejection, perhaps as a result of the simultaneous interference with humoral and cellular inflammatory processes.

TABLE 4. Effect of SRI 63-441 alone or in combination with CsA, FK506, or prostaglandin (PGE<sub>2</sub>) on mouse-to-rat cardiac xenograft survival

Treatment	n	Graft survival time (days)	Median survival*	P value
Control	8	3, 3, 3, 3, 3, 3, 3, 3	3.0	—
SRI 63-441, 15 mg/kg	8	3, 3, 3, 3, 3, 3, 3, 4	3.0	0.32
CsA alone	8	3, 3, 3, 3, 3, 3, 3, 3	3.0	—
FK506	7	3, 3, 3, 3, 3, 3, 3	3.0	—
PGE <sub>2</sub>	5	3, 3, 3, 4, 4	3.0	0.06
CsA+SRI 63-441, 15 mg/kg	6	3, 3, 3, 3, 3, 4	3.0	0.25
FK506+SRI 63-441, 15 mg/kg	5	3, 3, 3, 3, 3	3.0	—
FK506 oral+SRI 63-441, 15 mg/kg	5	3, 3, 4, 4, 4	4.0	0.02
PGE <sub>2</sub> +SRI 63-441, 15 mg/kg	5	3, 3, 3, 3, 4	3.0	0.21

\* Asymptotic standard error cannot be calculated.

Despite the effectiveness of SRI 63-441 in preventing HAR in the ACI/LEW combination, however, the exchange of cardiac allografts between ACI donors and sensitized BN recipients was not associated with significantly prolonged survival following SRI 63-441 treatment. This was true for pretreatment of the recipient or donor with SRI 63-441 alone or in combination with CsA, FK506, or PGE<sub>2</sub>. These results may reflect immunogenetic differences between the LEW and BN strains as recipients (22), or that the intensity of the antidonor antibody response has an important effect on modulating the effectiveness of the treatment.

While the ability of SRI 63-441 to modulate HAR in the presensitized allograft model may be variable, the ability of this compound to interfere with discordant xenograft combinations appears to be more consistent. We have previously demonstrated that pig-to-dog renal xenografts can be prolonged if the recipient is treated with the combination of SRI 63-441 and either one of two prostanoids, PGI<sub>2</sub> or PGE<sub>1</sub> (18). Cat-to-rabbit discordant xenografts can be prolonged by recipient pretreatment of SRI 63-441 alone and do not depend upon the additional action of prostanoids (17). The effectiveness of the prostanoids in enhancing the effect of the PAF antagonist may be the result of their ability to inhibit the acute inflammatory reaction, perhaps via platelet function or their influence on the clotting cascade (23). The current studies demonstrate that SRI 63-441 alone was clearly able to prolong grafts exchanged between the discordant guinea pig-to-rat combination. The addition of CsA or FK506 to the SRI 63-441 treatment was also associated with prolongation of graft survival, although neither of these immunosuppressive agents appeared to enhance the activity of the PAF antagonist. Recipient treatment with PGE<sub>2</sub> alone was associated with significantly prolonged graft survival for the guinea pig-to-rat xenografts, and the addition of SRI 63-441 resulted in improved graft survival times that were comparable to those seen with SRI 63-441 alone and in various combinations with CsA and FK506.

Since the original description of antibody-mediated hyperacute rejection of the kidney, numerous experimental and clinical studies have attempted to define the precise mechanism(s) of this destructive process (24-27). While controversy remains over which antibodies and under what circumstances immediate graft dysfunction is caused, HAR must be regarded as the product of a complex immune/inflammatory reaction. The initial antibody-mediated injury (IgG and IgM) to the vascular endothelium (5) is followed by secondary vasoconstriction (28), the recruitment of polymorphonuclear leukocytes (25), and aggregated platelets (29, 30), with intravascular coagulation (29, 31). The combination of vasoconstriction and the platelet-leukocyte-plugs occlude the small arterioles and capillaries producing ischemic necrosis of the graft. The recruitment of leukocytes and platelets may initially be due to immune adherence and specific aggregation, and then later due to stasis and nonspecific entrapment. This entire process is accompanied by fibrin deposition and rapid occlusion of the vascular system of the organ (4, 32). Platelet-activating factor has been proposed as a key biological candidate in the overall evolution of this type of inflammatory process and a central mediator in the pathogenesis of antibody-mediated hyperacute rejection (12, 13). Hyperacute renal allograft rejection in sensitized rabbits is associated with a local intravascular release of PAF and morphologic and histologic characteristics of HAR in all the recipients (12).

Despite the complexity of the inflammatory and clotting systems involved in the hyperacute rejection of vascularized organs, our results demonstrate that interference with the function of PAF has the effect of prolonging graft survival in those systems in which preformed antibody and/or complement activation are important components of the reaction. The effect of SRI 63-441 alone is not sufficient to provide for a practical method to prevent hyperacute rejection, but these experiments do confirm the central role of PAF in the inflammatory cascade that destroys heart and kidney grafts undergoing HAR in both the highly sensitized recipient and following discordant xenotransplantation. Of particular interest is our observation that donor pretreatment may also influence the outcome of both the allogeneic and xenogeneic hyperacute reactions. These results suggest that SRI 63-441 may be acting to prevent the release of important donor mediators, stabilize the membranes of donor cells, such as endothelium, or perhaps alter the antigenicity of the endothelial cell surfaces. It is very likely that a combination of approaches, affecting both the immunologic and nonimmunologic components of this complex phenomenon, will be necessary to achieve significant control of HAR and that antagonists to the action of platelet-activating factors may play a central role in this form of therapy.

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