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# Perioperative Treatment With Phosphatidic Acid Inhibitor (Lisofylline) Leads to Prolonged Survival of Hearts in the Guinea Pig to Rat Xenotransplant Model

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PHOSPHATIDIC acids (PAs) are a group of molecules that play an important role in intracellular signaling.[1] Of the four species of PA known, one (PA1-α) is rapidly activated during inflammatory responses through lysophosphatidic acid acyl transferase (LPAAT).[2] Lisofylline (LSF) is a potent inhibitor of LPAAT and is known to block the formation of PA1-α, thus attenuating or abrogating a broad array of proinflammatory activities.[3] Given its crucial role in suppressing the inflammatory cascade, we have attempted to study the efficacy of LSF in abating or averting hyperacute xenograft rejection (HAR) in the guinea pig to rat model. The adjuvant affect of steroid therapy was also investigated.

## MATERIALS AND METHODS

*Animals and Surgical Procedures.* Hartley guinea pigs and LEW rats were used as donors and recipients, respectively, of heterotopic cardiac transplants.

*Experimental Design.* Xenograft recipients received perioperatively, a bolus injection of methylprednisolone (MPS) and/or a 5- to 10-minute infusion of Lisofylline (Cell Therapeutics, Seattle, WA) according to the dosage detailed in Table 1. In addition to graft survival, complement activity in the serum of treated and untreated rats was also determined by using sensitized SRBC as targets in hemolytic assays. Tissue biopsies from cardiac xenografts were obtained serially after transplantation for routine histopathological examination. The deposition of rat IgM, IgG, and C3 molecules in the biopsies of xenotransplanted hearts was determined by single-color immunofluorescent staining.

## RESULTS AND DISCUSSION

As depicted in Table 1, untreated rats hyperacutely rejected guinea pig hearts in 12.2 ± 4.3 minutes (group I). Preoperative treatment of the recipient with LSF alone (group II) led to modest prolongation of heart survival (31 ± 9 minutes; group II), which was further enhanced by the addition of a bolus injection of 100 mg MPS (group VI) or by a second infusion of LSF after revascularization (group III). The most significant effect on graft survival was observed, however, when both LSF and MPS were administered pretransplant and postrevascularization (132.2 ± 39 minutes, group VII). It must be noted that MPS given alone, either prior to transplantation (group IV) or else additionally postrevascularization (group V), had a minimal effect on prolonging xenograft survival (groups IV and V). Histopathological examination of biopsies obtained from rejected hearts in untreated recipients displayed characteristic features of HAR with widespread thrombosis, hemorrhage, perivascular edema, and myocardial necrosis. Additionally, immunofluorescent studies revealed marked deposition of rat IgM and C3 on the endothelium of the coronary vessels and the microvasculature. Deposition of rat IgG was undetectable. Biopsies of heart xenografts obtained from animals in group VII 30 minutes after revascularization (while still beating), when subjected to

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**Table 1. Survival of Guinea Pig Cardiac Xenografts in LEW Rat Recipients Treated Perioperatively With Lisofylline (LSF) and Methylprednisolone (MPS)**

| Group | n | Treatment | | Graft survival (min) | |
|---|---|---|---|---|---|
| | | Pre Tx | Postrevascularization | X ± SD | P |
| I | 5 | — | — | 12.2 ± 4.3 | — |
| II | 5 | LSF 125 mg/kg | — | 31.0 ± 9.0 | .01 vs G1 |
| III | 5 | LSF 125 mg/kg | LSF 60 mg/kg | 80.5 ± 87.2 | — |
| IV | 5 | MPS 100 mg/kg | — | 11.6 ± 4.5 | — |
| V | 5 | MPS 100 mg/kg | MPS 50 mg/kg | 23.0 ± 11.3 | — |
| VI | 5 | LSF 125 mg + MPS 100 mg/kg | — | 54.6 ± 34.3 | — |
| VII | 5 | LSF 125 mg + MPS 100 mg/kg | LSF 60 mg + MPS 50 mg/kg | 132.2 ± 39.6 | .01 vs G5 |

immunofluorescent analysis, revealed the deposition of rat IgM and C3; moreover, light microscopic analysis showed retention of normal architecture with minimal changes characteristic of HAR. Histopathological findings at a later time (after rejection), however, were similar to those in animals in group II. In conclusion, the preliminary data obtained from this study suggest that LSF, when used alone or in combination with steroids, prolonged the survival of xenografts transplanted across discordant barriers.

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