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The Protective Effect of SRI 63-441 on Ischemic Liver Injury Using the Isolated Perfused Rat Liver: Combined Protocol With Superoxide Dismutase

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PREVENTION or reduction of ischemia/reperfusion injury would be of great benefit in organ transplantation. Here we briefly report preliminary studies evaluating a combined protocol using SRI 63-441 (Sandoz Pharmaceuticals, Raritan, NJ), a potent platelet-activating factor receptor antagonist,[1] and superoxide dismutase (SOD), an oxygen free radical scavenger, in an attempt to reduce ischemic injury to the liver.

MATERIALS AND METHODS

Male Lewis rats (250 to 300 g) were used as liver donors, and retired breeders were used as blood donors. Methoxyflurane-anesthetized rats received 300 U of heparin intravenously (IV) five minutes prior to cannulation of the bile duct and induction of total hepatic ischemia. After 90 minutes of ischemia, the portal vein was cannulated and the liver harvested. Group 1, no drug treatment (N = 11); group 2, 12 mg/kg of SOD was added to the flush at harvest and 8 mg/kg of SOD was added to the perfusate at zero, five, and ten minutes of perfusion (N = 4); group 3, rats received 12 mg/kg of SOD IV at the time of heparinization and 12 mg/kg of SOD was added to the flush at harvest (N = 4); group 4, rats received 12 mg/kg of SOD IV at the time of heparinization, 12 mg/kg SOD was added to the flush at harvest, and 8 mg/kg of SOD was added to the perfusate at zero, five, and ten minutes of perfusion (N = 4); group 5, rats received 20 mg/kg of SRI 63-441 at the time of heparinization, 6.5 mg/kg of SOD was added to the flush at harvest, and 6.5 mg/kg of SOD was added to the perfusate at zero minutes of perfusion (N = 6). Upon harvest, the liver was flushed with 60 mL cooled, heparinized (10 U/mL) Ringer's lactate solution (4°C), placed on a perfusion apparatus,[2] and perfused with a warm, oxygenated (pO_2, 500 torr), dilute sanguinous (hct, 25; diluent, Krebs bicarbonate buffer) solution at 37°C, pH 7.4 for 90 minutes. Bile production and perfusate SGOT and SGPT levels were measured every 30 minutes. Results are expressed as mean ± SEM and compared using an independent Student's *t* test.

RESULTS

The three groups using SOD alone (groups 2, 3, and 4) all show an increase in bile production and a decrease in perfusate transaminases when compared to controls (group 1) (Table 1). Only in group 2 was a significant difference seen in all three parameters measured ($P < .05$). There was a significant increase in bile production in group 5 compared to group 1 ($P < .005$); however, there was no significant difference between transaminase levels of the two groups.

DISCUSSION AND CONCLUSIONS

It has recently been suggested that platelet activating factor (PAF) may be a central mediator of the microcirculatory failure that ensues following ischemic organ injury. PAF is released from a number of cells and plays a significant role in almost every aspect of the inflammatory response.[3] SRI 63-441 is the most potent PAF receptor antagonist developed to date. In our previous study, there was a significant increase in bile production and decrease in perfusate transaminases in animals pretreated with 20 mg/kg of SRI 63-441 prior to induction of ischemia compared with controls.[4] It was anticipated that the addition of a second agent, which acted through a

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Table 1. Effect of SRI 63-441 and SOD on Parameters of Hepatic Function

Group*	N	Time (min. perfusion)	Bile (mL)	SGPT (IU)	SGOT (IU)
1	11	30	0.00 ± 0.00	1,074 ± 99	1,006 ± 103
		60	0.03 ± 0.01	1,274 ± 108	1,165 ± 120
		90	0.07 ± 0.02	1,390 ± 99	1,319 ± 119
2	4	30	0.03 ± 0.03	424 ± 67	474 ± 120
		60	0.11 ± 0.05	488 ± 93	562 ± 140
		90	0.19 ± 0.08	673 ± 202	721 ± 184
3	4	30	0.05 ± 0.03	678 ± 216	809 ± 206
		60	0.09 ± 0.03	868 ± 208	990 ± 204
		90	0.13 ± 0.03	1,048 ± 181	1,184 ± 214
4	4	30	0.05 ± 0.02	402 ± 78	604 ± 137
		60	0.09 ± 0.04	434 ± 145	685 ± 137
		90	0.11 ± 0.05	671 ± 130	866 ± 178
5	6	30	0.04 ± 0.01	989 ± 122	952 ± 92
		60	0.12 ± 0.04	1,058 ± 116	1,128 ± 103
		90	0.23 ± 0.06	1,097 ± 134	1,248 ± 106

NOTE. All values expressed are $\bar{X}$ ± SEM. Significance: $P < .05$ group 2 *v* group 1 all parameters; $P < .005$ group 5 *v* group 1 bile production only.

*Groups: 1, control; 2, SOD 12 mg/kg flush and 8 mg/kg × 3 perfusate; 3, SOD 12 mg/kg pretreatment and 12 mg/kg flush; 4, SOD 12 mg/kg pretreatment, 12 mg/kg flush and 8 mg/kg × 3 perfusate; 5, SRI 63-441 20 mg/kg pretreatment + SOD 6.5 mg/kg flush and 6.5 mg/kg perfusate.

different mechanism, might further improve postischemic hepatic function. SOD has been shown to be protective in kidneys subjected to warm ischemia.[5] The decision to add SOD was based on the preliminary results in groups 2, 3, and 4. A lesser dose of SOD was added in the combined study because earlier experiments (not reported) at the higher dosage did not show an increase in bile production and there was some concern of toxicity. The preliminary studies with SOD indicated that, as expected, SOD must be present at the time of reperfusion to exert a beneficial effect. The addition of SOD to SRI 63-441 pretreatment did not result in an improvement over SRI 63-441 pretreatment alone. In fact, at this time, the results are equivocal with an improvement in bile production, but none in perfusate transaminases. The complexity and multiplicity of cascades involved in the mediation of ischemia/reperfusion injury make it highly likely that effective pharmacologic modulation of the injury, leading to prolongation of organ preservation, will involve a polypharmacy approach. The ultimate "cocktail" would contain several agents that act at different critical points along the multiple pathways. It is not unexpected, as in the study reported here, that agents anticipated to behave synergistically could upset the fine balance in the cell's homeostatic mechanisms and invoke a detrimental response.

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