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# Comparison of Various Lazaroid Compounds for Protection Against Ischemic Liver Injury

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LAZAROIDS, 21-aminosteroids lacking steroid activity have cytoprotective properties against iron-dependent lipid peroxidation.[1] In addition to their antioxidant properties, lazaroids exert their cytoprotective effect by inhibiting arachidonic acid release, membrane stabilization, suppression of Kuppfer cell activation, and down-regulation of cytokine expression and release.[1–3] While previous reports have shown that lazaroids are effective in reducing ischemia and reperfusion injury to many different organ systems, there have been conflicting reports of the potency of the various lazaroid compounds.[4] In this study, we examined the potency and effectiveness of the three major lazaroid compounds in protecting organs against ischemia and reperfusion injury.

## MATERIALS AND METHODS

Two-hour complete hepatic ischemia was induced in adult female beagle dogs weighing 8 to 12 kg by clamping the vena cava (above and below the liver), the portal vein, and the hepatic artery. Splanchnic and inferior systemic venous beds were decompressed using veno-venous bypass. Total hepatic venous exclusion was maintained for 2 hours. Animals were divided into five groups: Control (n = 10, no treatment), Group F (lazaroid, U74006F, n = 6, 10 mg/kg), Group G (lazaroid U74389G, n = 6, 10 mg/kg), Group A1 (lazaroid U74500A, n = 6, 10 mg/kg), and Group A2 (lazaroid U74500A, n = 6, 5 mg/kg). The lazaroids were given as a continuous infusion over 30 minutes, just before onset of ischemia. Two-week animal survival, aspartate aminotransferase (AST), alanine aminotransferase (ALT), hepatic venous immunoreactive endothelin-1 (irET-1), and plasma malondialdehyde (MDA) were determined serially. Intraoperative hepatic tissue blood flow (HTBF) was measured using a laser-doppler flow meter (ALF21; Advance, Co, Ltd, Tokyo, Japan). Post-ischemic liver tissues (wedge biopsies) were divided and either fixed in buffered formalin, paraffin-embedded and stained with hematoxylin and eosin for histological analysis, or frozen for adenine nucleotide (AN) and purine catabolite (PC) measurement. Results are presented as the mean ± the standard error of the mean. Statistical analysis of the results was performed using the Log Rank test (for survival), and the Mann-Whitney U test (all others). A *P*-value less than .05 was considered significant.

## RESULTS

Except for Group A1, the lazaroid infusion was well tolerated. Infusion of lazaroid A1 (high dose) was associated with arrhythmia and hypotension. Two-week animal survival in the control group (30%) was significantly worse than the treated groups (all but one treated



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animal survival the two-week follow-up). Lazaroid F or A treatment significantly improve liver function. The AST and ALT of control livers 12 hours after reperfusion were 1152 ± 1014 U/L and 13563 ± 1379 U/L, respectively, while the highest AST and ALT of Group F and A livers were less than 5000 U/L. A postreperfusion increase of irET-1 levels in control animals was significantly suppressed in Group A2. Plasma MDA levels were significantly lower in the treated groups than in the control group. Hepatic tissue blood flow after reperfusion was significantly better in the treated groups (>40% of preischemia) than in the controls (26.8 ± 1.7% of preischemia). Compared with control, lazaroid treatment, especially Group A2, allowed less degradation of AN, less accumulation of PC, and prompt restoration of AN after reperfusion. While the histology of post-reperfusion control livers showed marked abnormalities, lazaroid treated livers, particularly Group A2, were well preserved with only minor neutrophil infiltration.

## DISCUSSION

Lazaroid treatment significantly ameliorated ischemia and reperfusion injury to the canine liver. Lazaroid treatment improved animal survival, improved liver function, suppressed endothelin activity, decreased lipid peroxidation, accentuated hepatic tissue blood flow, and decreased adenine nucleotide catabolism.

As suggested in other studies, there appears to be a difference in the effectiveness of the different lazaroid compounds.[4] Lazaroid A. at the lower (5 mg/kg) dose, was more effective in protecting the liver from ischemia and reperfusion injury than Lazaroid F or G. While all three lazaroid compounds are potent anti-oxidants. U74500A is 2 to 10 times more effective in inhibiting iron-dependent lipid peroxidation than U74006F.[5] Conversely, U74006F and U74389G are more effective in scavenging lipid peroxyl radicals, like alpha-tocophenol, than U74500A.[1,6] Thus, the increased potency of Lazaroid A in our model may suggest that early inhibition of lipid peroxidation by iron-chelation is more beneficial than scavenging lipid peroxyl radicals.

The early resumption of energy metabolism, hepatic tissue blood flow, and suppressed endothelin-1 production associated with lazaroid treatment in this study suggests that lazaroids protect not only the parenchymal hepatocytes, but the sinusoidal endothelial cells as well. Indeed, histological architecture in the lazaroid treated groups, especially in Group A2, were well preserved.

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