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Protective effects of the lazaroid U74500A and lidoflazine on liver preservation with UW solution

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Abstract. The effect of adding a 21-aminosteroid, U74500A, and a Ca^{2+} antagonist, lidoflazine, alone and together to UW solution was assessed in a rat liver preservation model. Following preservation, the livers were reperfused using a closed circuit, and the release of hepatocellular enzymes (ASAT, ALAT, and LDH) into the perfusate was determined with increasing time. Both drugs reduced the amount of enzymes lost from the liver. The combination of the two drugs was better than either drug alone. These data suggest that both agents may be of value in organ preservation for clinical liver transplantation.

Key words: Liver preservation, rat, UW solution – Rat, liver preservation, UW solution – U74500A, rat liver preservation – Lidoflazine, rat liver preservation

Introduction

Lazaroids are a new group of 21-aminosteroid compounds that have recently attracted interest because of their membrane-stabilizing properties [5, 10, 12]. In particular, they have been shown to reduce iron-dependent lipid peroxidation, which is an important mechanism for oxygen free, radical induced hepatic injury occurring as a consequence of ischemia and reperfusion. These data suggest that these agents may be of value in organ preservation. In the present study, the effect of adding the lazaroid U74500A to University of Wisconsin (UW) solution on the hepatic injury experienced as a result of organ preservation was evaluated using the isolated, perfused liver. The magnitude of the improvement achieved with U74500A was compared to that achieved with a Ca^{2+} antagonist, lidoflazine, which is known to reduce the hepatic injury incurred as a consequence of hepatic preservation using the same model [13]. In addition, the effect of adding both of these drugs to the UW solution was determined.

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Materials and methods

Male Lewis rates (Harlan, Indianapolis, Ind.) weighing 240–340 g were used for the experiments. U74500A (Upjohn, Kalamazoo, Mich.), 21.6 mg/l, or lidoflazine (Kabi Pharmacia, La Jolla, Calif.), 5 mg/l, or a combination of both were dissolved in UW solution (DuPont Critical Care, Wilmington, Del.). No insulin, methylprednisolone, or antibiotics were added. The hepatectomy and rat-isolated perfusion technique used were identical to those previously described [13]. Thirty milliliters of the preservation medium was used to flush the portal vein, and the livers were stored in 100 ml of the same solution. Control livers were flushed and stored with UW solution without additives. The livers were stored at 0 °C for 72 h before reperfusion was started using a closed circuit, with Krebs-Henseleit bicarbonate solution containing 2% albumin and 5 mM glucose as the perfusate. At 30 and 60 min after reperfusion, samples of the perfusate were taken for analysis of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and lactate dehydrogenase (LDH). The amount of hepatocellular enzymes present in the perfusate was determined using a Technicon RA-500 analyzer.

Results were calculated as means ± standard deviations (SD). Statistical comparisons were performed using the Wilcoxon rank-sum test. A p value less then 0.05 was considered statistically significant.

Results

All livers lost weight during cold storage with no detectable difference between groups [12.8% ± 2.0% (lidoflazine + lazaroid) vs 10.7% ± 3.7% (lidoflazine) vs 11.3% ± 4.9% (lazaroid)]. The amount of ASAT, ALAT, and LDH released into the perfusate during the perfusion period is shown graphically in Figs. 1–3. The levels of all three enzymes increased with increasing time of the reperfusion. The addition of U74500A to UW solution was associated with a significant reduction in the release of ASAT and LDH at 30 and 60 min. The reduction in ALAT release was not significant. The addition of both agents to the UW solution resulted in a significantly reduced initial release of ASAT, ALAT, and LDH into the perfusate medium compared to what was seen with either drug alone. After 60 min of reperfusion, however, the levels of all three enzymes in the perfusate had increased to the level achieved with the addition of either agent alone.

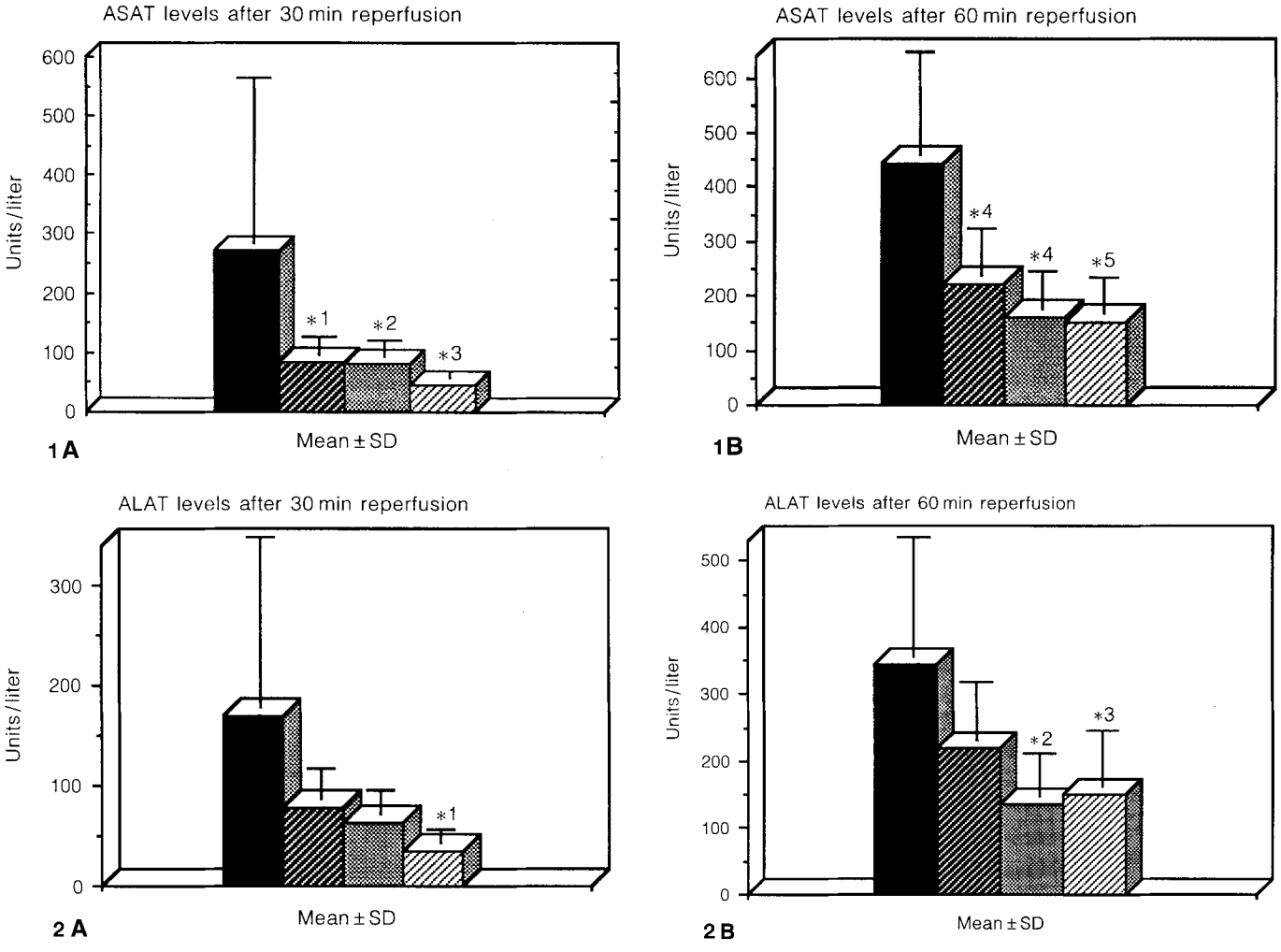

Fig. 1 A, B. The amount of ASAT released into the perfusate was significantly reduced after **A** 30 and **B** 60 min, when U74500A, lidoflazine, or both drugs were added to the preservation medium. At 30 min the drug combination was more effective than either drug alone ($P = 0.016$ for both comparisons), whereas there was no significant difference at 60 min. ■ UW solution, ▨ lazaroid, ▩ lidoflazine, ▨ lazaroid + lidoflazine. *1 $P = 0.01$; *2 $P = 0.011$; *3 $P = 0.001$; *4 $P = 0.025$; *5 $P = 0.007$

Fig. 2 A, B. The amount of ALAT released into the perfusate was not significantly altered **A** after 30 min, when U74500A or lidoflazine was added to the preservation medium. **B** After 60 min a reduction was seen in the lidoflazine group, but not with U74500A. The drug combination was found to reduce the release of enzyme at 30 and 60 min. At 30 min the combination was more effective than lidoflazine alone ($P = 0.031$). ■ UW solution, ▨ lazaroid, ▩ lidoflazine, ▨ lazaroid + lidoflazine. *1 $P = 0.004$; *2 $P = 0.01$; *3 $P = 0.016$

Discussion

A major principle in organ preservation is the use of hypothermia. Hypothermia reduces the rate of cellular metabolism and thereby the number of various metabolic events that occur during ischemia that lead to cell injury and death. Hypothermia is not without side effects, however, that include cell swelling. This effect can be counteracted with the use of a flush solution that contain cell impermeants, such as those present in the UW solution [3, 19]. Organ preseration can be improved further with the use of pharmacological agents that interfere with key processes in the pathogenesis of cell injury occurring as a result of ischemia and reperfusion. Examples of such agents are membrane stabilizers including chlorpromazine [17, 18], glucocorticoids [17], oxygen-free radical scavengers [15], vasodilators [11], and calcium antagonists [2, 13].

Glucocorticoids have been used extensively in experimental studies to reduce injury experienced with trauma, especially neurotrauma [8], ischemia [16] and, in some studies, the injury associated with organ preservation [7, 17]. The putative mechanism behind the protective effects of glucocorticoids in these situations is believed to be their membrane stabilization effects that limit the development and progression of iron-dependent lipid peroxidation [15]. Recently, 21-aminosteroids or lazaroids, a novel group of steroids that lack glucocorticoid or mineralocorticoid effects, have been shown to be potent inhibitors of iron-induced lipid peroxidation [1]. Moreover, these agents have been shown to be scavangers of lipid peroxyl and and phenoxy radicals [16]. In both clinical and experimental studies, these agents have been shown to reduce the severity of brain and spinal cord ischemia [9, 20].

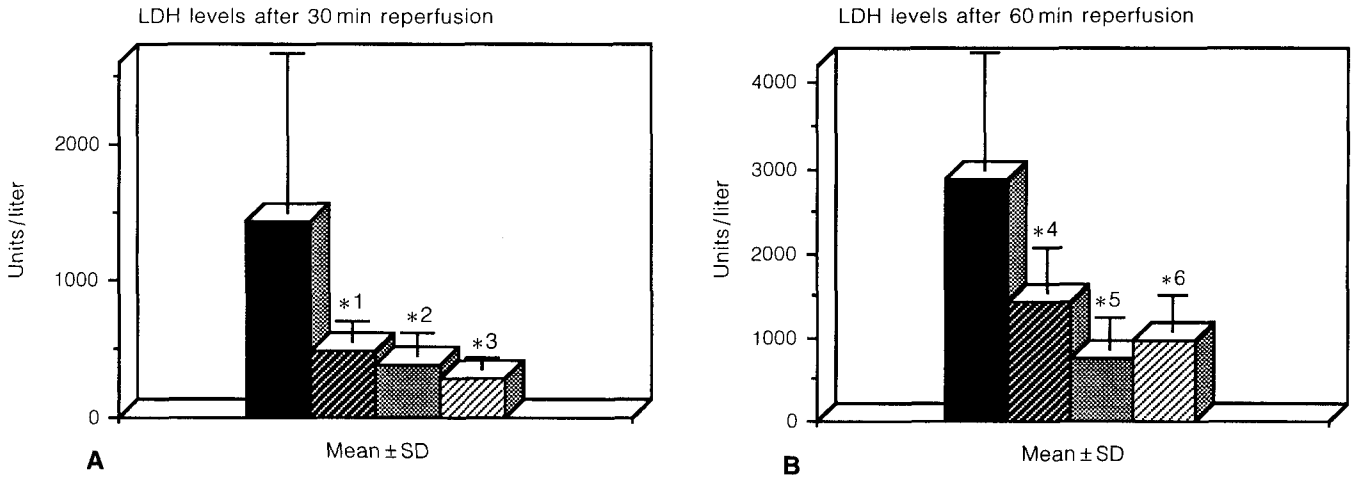

Fig. 3 A, B. The amount of LDH released into the perfusate was significantly reduced after **A** 30 and **B** 60 min, when U74500A, lidoflazine, or both drugs were added to the preservation medium. At 30 min the drug combination was more effective than U74500A alone ($P = 0.016$), whereas there was no significant difference in this comparison at 60 min. At 60 min, lidoflazine was associated with a lesser release of LDH than U74500A alone ($P = 0.037$). ■ UW solution, ▨ lazaroid, ▩ lidoflazine, ▨ lazaroid + lidoflazine. *1 $P = 0.01$; *2 $P = 0.009$; *3 $P = 0.002$; *4 $P = 0.016$; *5 $P = 0.004$; *6 $P = 0.003$

In the present study, the effect of adding the 21-aminosteroid U74500A to UW solution on the hepatic injury occurring as a result of cold ischemia was assessed using the isolated, perfused rat liver. The isolated, perfused liver has been shown to be a valuable tool for screening various preservation techniques, and it has recently been used extensively by us, as well as by other groups of investigators [2, 13, 14, 17–19]. In some of these studies a significant correlation between performance in the isolated, perfused liver and in vivo assessment of liver preservation has been found [12–14]. The dose of U74500A employed (30 μmol/l or 21.6 mg/l) was within the dose range found to be effective in reducing ischemic injury to the central nervous system [9]. The magnitude of the cytoprotection achieved with U74500A was compared to that achieved with a calcium channel blocker, lidoflazine. This latter agent has previously been shown to be beneficial in this model at a dose of 5 mg/l [13], as well as in an orthotopic liver transplant model in the rat [12]. U74500A reduced the amount of hepatocellular enzymes released into the perfusate upon reperfusion after 72 h of cold storage. The improvement observed was similar to that obtained by adding lidoflazine to the UW solution.

The effect of combining a lazaroid and a calcium channel blocker was also studied. After 30 min of reperfusion, the enzyme release into the perfusate with both agents added to the UW solution was reduced, as compared to that observed when either drug was used alone ($P = 0.016$). This indicates that the two agents may have an additive effect at reducing the cell injury that occurs during ischemia, cold storage, and early reperfusion. Since using doses greater than 5 mg of lidoflazine does not result in a greater reduction in enzyme loss with this model [13], the finding of an additional effect with U74500A and lidoflazine together suggests that the two agents work by different mechanisms to prevent cell injury. From prior studies with the lazaroids it appears as if the major action of such agents is to inhibit lipid peroxidation [4]. Another mechanism that may contribute to the cell membrane injury experienced during ischemia is the activation of phospholipases that occurs as a result of increased cytosolic Ca^{2+} levels [6]. Thus, calcium entry blockers, like lidoflazine, have been used and have been shown to reduce the cell injury associated with organ cold storage and reperfusion by limiting the entry of calcium into the cytosol.

In conclusion, in the present study the 21-aminosteroid U74500A was found to reduce the liver injury experienced during cold storage and reperfusion of rat liver in vitro. The magnitude of improvement was similar to that found with a Ca^{2+} antagonist, lidoflazine. Importantly, the effect of both agents, when used in combination, was greater than that achieved with either agent alone, at least during the early (30-min) reperfusion period.

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