# LIVER PRESERVATION WITH LIDOFLAZINE AND THE UNIVERSITY OF WISCONSIN SOLUTION: A DOSE-FINDING STUDY[1]

Over the last decade, two major advances have made liver transplantation a widely applied and successful treatment of endstage liver disease. First, improvements in immunosuppression made possible by cyclosporine (*1*) and the recently introduced, even more powerful, immunosuppressant FK 506 (*2*) have reduced graft loss due to rejection. Second, a breakthrough in preservation of the liver for transplantation was recently achieved by the University of Wisconsin cold storage solution (UW)* (*3*). Thus, it is now possible to safely preserve a human liver for up to 24 hr (*4*). This has simplified the logistics in liver transplantation and also contributed to an improved quality of the organs that are transplanted. In spite of this, there is still a need for methods to further improve both the duration and the quality of preservation.

Numerous studies have demonstrated that cell and organ injury from ischemia can be reduced by avoiding the occurrence of elevated intracellular $Ca^{2+}$ levels, e.g., by the use of $Ca^{2+}$ antagonists (*5–8*). Previously, a protective effect was found when lidoflazine, a $Ca^{2+}$ antagonist, was added to UW solution for preservation of the rat kidney (*9*). In the present study, we have investigated the effect of lidoflazine on liver preservation with UW solution, using the isolated perfused rat liver model.

The experiments were performed on male Lewis rats, weighing 240–340 g (Harlan, Indianapolis, IN). The animals had free access to standard pellet diet and tap water, and were not fasted before surgery. Anesthesia was with inhalational methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ).

Heparin, 300 IU, was given i.v. 10 min before harvesting. Ligatures were placed around the inferior vena cava, cranially to the renal vein and artery, and around the portal vein. For bile collection, polyethylene tubing (PE-10, I.D. 0.28 mm, O.D. 0.61 mm; Clay Adams, Parsippany, NJ) was inserted into the bile duct. Immediately after cannulation of the portal vein with a 16-gauge catheter (Critikon, Inc., Tampa, FL), this catheter was connected to an extension set and a 60-ml syringe (Abbott Laboratories, North Chicago, IL) filled with ice-cold preservation solution and immediately flushed in situ. The vena cava was transected distally to the ligature. Using this procedure, the warm ischemia time was always shorter than 20 sec. The liver was flushed with 30 ml at a hydrostatic pressure of 15–20 cm $H_2O$. During flushing, a 14-gauge catheter (Deseret Medical Inc., Sandy, UT) was inserted into the infrahepatic vena cava; the suprahepatic vena cava was transsected and the hepatectomy was completed. Each liver was weighed and stored in 100 ml of preservation solution for 72 hr, kept surrounded by ice.

The perfusions were performed in a thermostatically controlled Plexiglass cabinet (65×32×60 cm; Fischer Scientific, Pittsburgh, PA). Each liver was continuously perfused through the portal vein with 200 ml of recirculating buffer at 38°C for 90 min, using a Masterflex pump controller (Cole-Parmer Instrument, Chicago, IL) calibrated to maintain a flow rate of 3.5 ml/min/g. The perfusate was oxygenated while passing through oxygen-permeable tubing (T57111115-6, I.D. 1.5 mm; American Scientist, Warrendale, PA) inside a plastic container with a 95% $O_2$ and 5% $CO_2$ mixture at a flow rate of 6 ml/min, giving an oxygen tension of 480–550 mmHg on the arterial side of the system. Air bubbles were avoided by connecting a disposable Nylon filter (blood set 64; Abbott Laboratories) in line between the oxygenator and the inflow. The portal vein pressure was continuously monitored with an in-line manometer (Abbott Laboratories).

Krebs-Hanseleit bicarbonate solution containing 2% albumin (Sigma, St. Louis, MO) and 5 mM glucose (Abbott Laboratories) was used as the perfusate. The initial pH was adjusted to 7.38–7.42, but was not further adjusted during the perfusion, since the pH had earlier been found to decrease by not more than 0.10–0.15 (unpublished data). The preserved livers were weighed, then flushed with 3 ml of lactated Ringer's solution (Baxter Healthcare Corp., Deerfield, IL) at room temperature through the portal vein and immediately connected to the perfusion apparatus. The bile was collected in preweighed test tubes placed outside the cabinet to avoid dilution of the bile by condensed water, and the amount of bile produced was established by weighing the bile samples, assuming the bile density to be equal to water. Perfusate samples were taken every 30 min for measurement of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH), and purine nucleotide phosphorylase (PNP). Perfusate samples were also taken for immediate measurements of pH, $pO_2$, and $pCO_2$ from the inflow as well as the outflow using a pH-blood gas meter (ABL 2-acid-base laboratory; Radiometer, Copenhagen, Denmark). After the perfusion, the liver was again weighed and the bile volume was measured. The release of the hepatocellular enzymes was determined with colorimetric methods (Sigma), using a Technicon RA-500 analyzer. Levels of PNP in the perfusate were measured, using the method of Hoffee et al. (*10*).

Six different doses of lidoflazine (4-[4,4-bis(4-fluorophenyl)butyl]-*N*-(2,6-dimethylphenyl)-1-piperazineacetamide; Kabi Pharmacia, La Jolla, CA) were dissolved in UW solution (DuPont, Wilmington, DE) on the day of the harvest. The doses tested were 0.078 mg/L ($1.6\times10^{-7}$ M), 0.312 mg/L ($6.3\times10^{-7}$ M), 1.25 mg/L ($2.5\times10^{-6}$ M), 5 mg/L ($1.0\times10^{-5}$ M), 20 mg/L ($4.1\times10^{-5}$ M), and 80 mg/L ($1.6\times10^{-4}$ M). The same solution was used for both flush-out and cold storage. In a control group, plain UW solution was used. Six experiments were performed in each group.

Results were calculated as mean ± SD. Statistical comparisons were performed using the Wilcoxon rank sum test. A *P*-value less than 0.05 was considered statistically significant.

As seen in Figure 1, in which the amount of ASAT released into the perfusate is presented in control livers with 0, 24, 48, and 72 hr of cold ischemia time, a clear-cut difference between the 48-hr and 72-hr preserved livers was seen. Since a certain amount of damage is required in a model, in which quantification of improved preservation is a major goal, 72 hr was chosen for the experiments. The other enzymes tested (ALAT, LDH, and PNP) behaved in a similar fashion in these control experiments, and the differences reached the same levels of significance (data not shown).

The levels of enzymes in the 72-hr preserved livers, released

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* Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; LDH, lactate dehydrogenase; PNP, purine nucleotide phosphorylase; UW, University of Wisconsin solution.

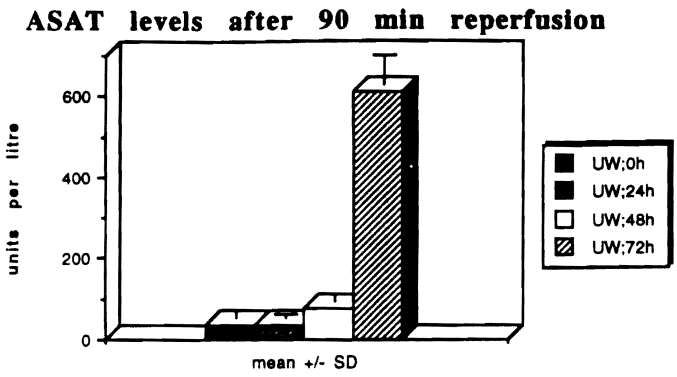


FIGURE 1. ASAT levels after 90 min of reperfusion, after 0, 24, 48, and 72 hr of cold storage in plain UW solution. The differences between the 72-hr group and the other groups were all significant ($P<0.001$).

into the perfusate during reperfusion, are given in Figure 2. For simplicity, only the 90-min values are presented. The 30-min and 60-min values were proportionally lower than the corresponding 90-min values, and the statistical comparisons in the 30- and 60-min values reached the same levels of significance as these. The four lowest doses of lidoflazine (0.078–5 mg/L) were all effective in preventing enzyme release, as compared with when plain UW was used. The ASAT, LDH, and PNP 90-min values from these groups were all significantly lower than the values from the control group. When comparing the ALAT values, the 0.312- and 5-mg/L groups had significantly lower values. However, the two highest doses (20 and 80 mg/L, groups 5 and 6) were not significantly different in UW for any enzyme tested.

In contrast to our experiences from the isolated perfused rabbit liver (*11–15*), measurement of the bile production was technically difficult, since biliary sludge would sometimes obstruct the thin catheter in the groups of preserved livers, or kinking of the catheter would occur, which was not easily detected because of the low volumes produced. Therefore, a number of experiments in each group had to be excluded, and no valid conclusions could be drawn from this parameter only. Instead, the results of the enzyme analyses were regarded as more reliable indicators of liver cell injury after preservation in this rat model. However, as seen in previous studies (*11, 15*), bile production in control livers gradually decreased with in-

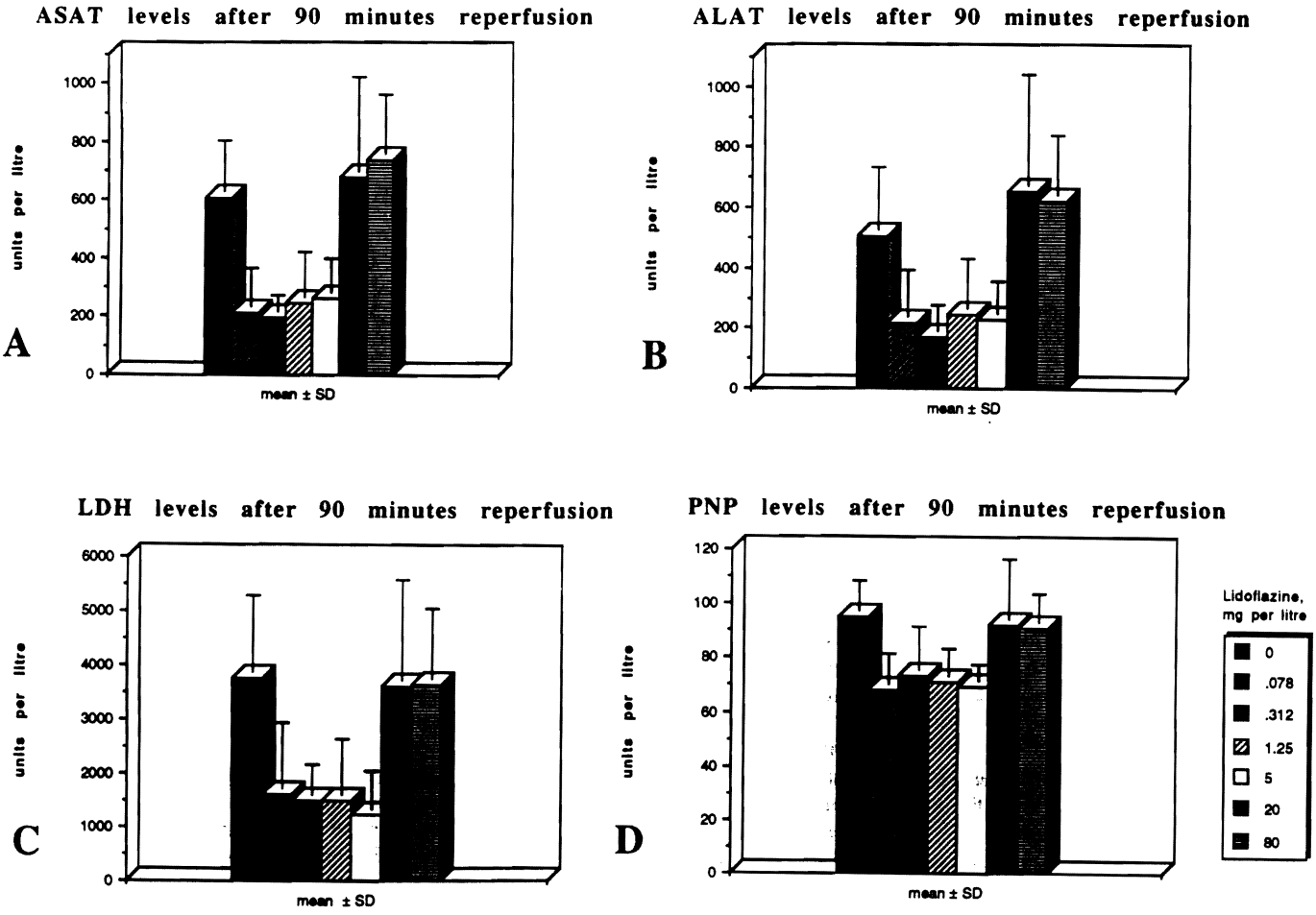


FIGURE 2. Enzyme levels in the perfusate 90 min after reperfusion. A. ASAT: The levels of statistical significance for the different doses of lidoflazine (mg/L) compared with controls were: $P<0.01$ for 0.078, 0.312, and 5; $P<0.05$ for 1.25; and NS for 20 and 80. B. ALAT: The levels of statistical significance for the different doses of lidoflazine (mg/L) compared with controls were: $P<0.01$ for 0.312; $P<0.05$ for 5 and 1.25; and NS for 0.078, 1.25, 20, and80. C. LDH: The levels of statistical significance for the different doses of lidoflazine (mg/L) compared with controls were: $P<0.01$ for 0.078, 0.312, and 5; $P<0.05$ for 1.25; and NS for 20 and 80. D. PNP: The levels of statistical significance for the different doses of lidoflazine (mg/L) compared with controls were: $P<0.01$ for 0.078 and 5; $P<0.05$ for 0.312 and 1.25; and NS for 20 and 80.

creasing ischemia time (1.1±3.4 [n=6], 0.59±0.22 [n=5], 0.27±0.15 [n=3], and 0.19±0.06 [n=4] ml/90 min in the 0-, 24- 48-, and 72-hr preserved livers, respectively. Bile production after 72 hr appeared to improve with addition of at least moderate to higher doses of lidoflazine (0.17±0.06, [n=4], 0.21±0.04 [n=3], 0.25±0.09 [n=4], 0.25±0.06 [n=3], 0.25±0.08 [n=5], and 0.25±0.05 [n=4] ml/90 min for the 0.078-, 0.312-, 1.25-, 5-, 20-, and 80 mg/L groups, respectively, but due to the low number of evaluable experiments, no statistically significant differences could be calculated. The bile in 72-hr preserved livers was pale as compared with fresh or 24-hr preserved livers. The portal vein pressure was 11.0±2.2 cm $H_2O$ for all groups without any significant differences. One experiment in the 1.25-mg/L group was excluded because of air embolism. There was no difference in liver weights after flush-out between the groups. During preservation, all livers decreased in weight, which is a well-known phenomenon with UW (*13*). The addition of lidoflazine did not affect the liver weight during cold storage.

As indicated by the enzyme data, lidoflazine significantly improved the capacity of UW to preserve rat livers. Since the concentrations of the hepatocellular enzymes (ASAT, ALAT, and LDH) and the endothelial enzyme (PNP) were lower for the four lowest doses of lidoflazine tested, compared with UW alone, the present data suggest that lidoflazine improves the preservation of hepatocytes as well as liver endothelial cells. Preservation of the endothelial cells is of special importance, since injury to these cells can cause an aggravation of hepatocellular injury during reperfusion. Furthermore, the endothelium is the first target of allogenic blood after transplantation, and injury here may cause an increase in the immunogenicity of the liver (*16*).

It was found that lidoflazine increased the flush-out rate during harvesting, for all doses that also reduced the release of enzymes, except for the lowest dose (6.2±1.0, 6.8±0.4, 8.1±0.5 [$P<0.01$], 7.9±0.8 [($P<0.05$], 9.1±1.2 [$P<0.01$], 6.8±0.4, and 6.8±1.3 ml/min for the 0, 0.078, 0.312, 1.25, 5, 20, and 80-mg lidoflazine/L groups, respectively; levels of significance compared with the control group are within brackets). This effect is probably mediated by vasodilation. Theoretically, vasodilation may be beneficial by contributing to a more rapid cooling of the organ and by improving the distribution of the preservation solution within the parenchyma. However, in a small animal, such as the rat, both mechanisms are probably of little importance, since the duration of the harvesting procedure is short, and the small organ can quickly achieve a low temperature, once surrounded by ice. Also, in cold storage of rat organs with a relatively large surface area/volume ratio, simple diffusion may be sufficient to supply a satisfactory distribution of the preservation solution. Thus, the present data suggest that the capacity of lidoflazine to improve liver preservation in this model is not caused mainly by dilatation of the vascular bed. However, for the preservation of larger organs such as the human liver, vasodilation may be an additional beneficial effect in a dose-related manner.

The two highest doses of lidoflazine used here had no ameliorating effect on the release of liver enzymes, nor did they increase the flush-out rate. Nevertheless, lidoflazine appears to have a wide therapeutic window, as it was effective in a concentration $10^{-7}$ M–$10^{-5}$ M. It cannot be ruled out that an even lower dose than $10^{-7}$ could be effective, although this was never tested.

The results from this investigation are in agreement with those of a previous study, in which lidoflazine was found to improve the preservation of rat kidneys (*9*).

We conclude that lidoflazine improves the quality of liver preservation with UW solution in this in vitro model. However, further evaluation of this drug in a transplant model is required before the clinical usefulness can be determined.

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## FALSELY ELEVATED FK-506 LEVELS CAUSED BY SAMPLING THROUGH CENTRAL VENOUS CATHETERS

FK-506 (Tacrolimus, ProGraf) is a potent immunosuppressive agent that is currently being investigated at several centers in solid organ transplantation. Initial reports of efficacy have generated great interest among clinicians in the transplant community (*1–4*). Although immunosuppressive regimens with FK-506 appear to be effective, the drug has been associated with numerous adverse effects, most notably nephrotoxicity and neurotoxicity (*4–8*). The challenge remains to titrate the FK-506 dosage to its optimal immunosuppressive effect with minimal toxicity. This will require pharmacodynamic studies to define the therapeutic range for FK-506. For this to be accomplished, it is imperative that accurate drug concentration monitoring be carried out in conjunction with clinical evaluations. A potential complication with this process has recently been discovered.

The University of Nebraska Medical Center (UNMC) was involved in a multicenter randomized trial comparing the efficacy of CsA versus FK-506 in orthotopic liver transplantation. After transplantation, patients randomized to receive FK-506 were begun on a continuous infusion of FK-506 solution (0.02 mg/ml) through a central venous catheter. The initial FK-506 infusion was administered through an Edwards Swan-Ganz catheter (7.5 French 110 cm device manufactured by Baxter HealthCare, model #93A-8314) until patients were hemodynamically stable, at which time the Swan-Ganz catheter was replaced by a triple-lumen central venous catheter (7 French 30 cm polyurethane device manufactured by Arrow product #AK14703). The infusion was continued for 72 hr or until the patient was able to take oral medication.

A subset of patients in the primary FK-506 trial gave informed consent to participate in a concurrent pharmacokinetic protocol that consisted of multiple blood sampling over a 10-day period. In this protocol, while FK-506 was being infused through a Swan-Ganz catheter, serial blood samples were obtained through indwelling peripheral arterial catheters. After the Swan-Ganz catheter was removed, blood samples were obtained as above or through an unused lumen of the triple-lumen catheter. The sample technique was consistent throughout the study; all blood samples were preceded by withdrawing and discarding 5 ml of blood (to purge the lumen of flush solution) and were followed by a 5- to 10-ml flush of 0.9% sterile sodium chloride solution.

During the pharmacokinetic trial, it was noted that spuriously high whole blood and plasma concentrations, as measured by ELISA, were reported during the 12-hr period between discontinuation of i.v. FK-506 and the administration of the first oral dose. In multiple subjects, a 10- to 50-fold increase in the anticipated concentration was observed intermittently (*9*). An examination of procedures led us to suspect that the samples in question may have been drawn from the same catheter lumen previously used to infuse FK-506 solution. We postulated that FK-506 or some component of the i.v. solution interacted with the triple lumen catheter, which led to sorption of FK-506 in that lumen.

To confirm our suspicions, simultaneous blood samples were obtained from the catheter lumen used to infuse FK-506, and from either a peripheral catheter or a lumen of the central venous catheter not used for FK-506 infusions in 2 subsequent patients enrolled in the pharmacokinetic trial. Samples were obtained at various times after termination of i.v. FK-506. Dramatic differences between the sampling sites were observed in whole blood and plasma concentrations in both patients (Table 1). The greatest degree of disparity between sampling sites occurred within the first hour after the end of i.v. FK-506 infusion. Although the concentration ratio of the catheter lumen used to infuse FK-506 to the alternate lumen showed an obvious diminishing trend in both patients, an impressive difference (260%) persisted between sampling sites in patient 2, even though several days had elapsed.

In a recent report, Taormina et al. (*10*) investigated the loss of FK-506 diluted in 5% dextrose or 0.9% sodium chloride in various containers and administration sets. Their results indicate that over a 24-hr period, less than 10% of the initial FK-506 concentration is lost when (1) FK-506 is diluted in 5% dextrose and stored in glass bottles or polyolefin bags, or (2) FK-506 is diluted in 0.9% sodium chloride and stored in plastic syringes (Becton Dickinson & Co. brand) or polyolefin bags. However, greater than 10% of the initial FK-506 concentration was lost with both solutions after only 6 hr in polyvinyl chloride bags. In the same study, a 2-hr infusion of FK-506 in 5%

TABLE I. Whole blood and plasma FK-506 concentrations (ng/ml) as drawn from a catheter lumen used to administer FK-506 (site B) and an uncontaminated alternate site (site A)

| | Time (hr)[a] | Site A (uncontaminated) | | Site B (FK-506) | |
|---|---|---|---|---|---|
| | | Plasma (ng/ml) | Whole blood (ng/ml) | Plasma (ng/ml) | Whole blood (ng/ml) |
| Patient 1 | 0.5 | 0.98[b] | 30.0[b] | 94.84 | >240.0 |
| | 1.0 | 1.38[b] | 34.3[b] | 213.1 | >240.0 |
| | 1.5 | 0.82 | 40.3 | 34.5 | 231.0 |
| Patient 2 | 1.0 | 3.09 | 29.7 | 231.0 | >240.0 |
| | 1.5 | 2.66 | 32.5 | 183.0 | >240.0 |
| | 2.0 | 2.58 | 28.4 | 35.6 | 198.0 |
| | 12.0 | 1.29 | 15.5 | 11.9 | 151.0 |
| | 114.0 | 4.86 | 32.5 | 11.5 | 102.0 |
| | 164.0 | 3.52 | 26.8 | 9.14 | 93.3 |

[a] Time in elapsed hours from end of FK-506 infusion to sample collection.

[b] Indicates samples drawn through an arterial line; all other samples were obtained through a triple lumen catheter.