Influence on Energy Kinetics and Histology of Different Preservation Solutions Seen During Cold Ischemia in the Liver

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ABSTRACT

Background and Purpose. Cold flush preservation prolongs tissue viability during ischemia. However, there is little understanding of the effects of various preservation fluids on events during this period. A study of cold ischemia in rat livers was undertaken to compare biochemical and histological changes over time, using three preservation solutions: University of Wisconsin (UW), histidine-tryptophan-ketoglutarate (HTK), and Leeds solution (LS) under development at our institution. Leeds solution is a phosphate-based sucrose solution that like UW contains the impermeant lactobionate and the metabolite allopurinol (1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4-one) which acts as a competitive inhibitor of xanthine oxidase, stopping the breakdown of hypoxanthine to xanthine by oxidizing it to alloxanthine, inhibiting both the conversion of hypoxanthine to xanthine and the conversion of xanthine to uric acid.

Materials and Methods. At various time points, samples were analyzed for adenosine triphosphate (ATP) and metabolites by high-performance liquid chromatography as well as for histological changes.

Results. In all livers, ATP, ADP, and AMP degraded over 4 hours. In UW and LS groups, degradation beyond hypoxanthine was halted, and it continued in the HTK group. This blockade led to a significant reduction in the accumulation of xanthine and uric acid. Histological analysis showed protected architecture and maintenance of reticulin scaffolds in the UW and LS groups, whereas tissue breakdown was seen from earlier time points in the HTK group. Additionally, throughout ischemia, signs of pathological injury were more pronounced with UW- than with LS-preserved tissue.

Conclusions. These results implied that cold ischemia in the liver is characterized by dynamic biochemical changes coincident with pathological injury which are initiated from the time of organ perfusion and influenced by the choice of the perfusion fluid. Allopurinol in UW and LS appears to be critical. We hypothesized that it may also affect the degree of subsequent reperfusion injury. The data supported the assertion that LS offered improved preservation over UW, adding to the impetus to shorten ischemic times in clinical transplantation.
UNIVERSITY OF WISCONSIN SOLUTION (UW) has been the solution of choice for preservation of the liver for many years, but in recent years other solutions have been developed which are now used in preference to UW at some transplant institutions. One such solution is histidine-tryptophan-ketoglutarate (HTK). Leeds solution (LS) is under development at our institution.

The present work set out to compare UW and HTK with LS to investigate kinetic and histological changes during preservation. Work has already been performed using LS in kidney,1–2 liver3–7 and heart8 preservation.

METHODS
The experiment was performed under a Home Office Licence according to standard guidelines for animal care. Male Wistar rats (280–340 g) were anesthetized with a single intraperitoneal injection of pentobarbitone (0.6 μg/g). After midline and subcostal laparotomy, the liver was exposed and catheters placed into the portal vein and abdominal aorta. The aorta was sectioned and the liver flushed with ice cold LS, UW, or HTK and stored in 60 mL of the chosen solution at 4°C for up to 48 hours.

At time 0, when the aorta was sectioned, samples of liver were obtained for either histological analysis or determination of energy status. Liver samples taken for histological investigation were fixed in 3% glutaraldehyde or 4% paraformaldehyde. Samples for energy status were stored in liquid nitrogen at −80°C. Samples of liver were taken at time 0 as well as after 5, 10, 20, and 30 minutes and 2, 4, 24, and 48 hours.

Adenosine triphosphate (ATP) and its metabolites were determined using modified reversed-phase high-performance liquid chromatography using the method described by Smolenski et al.9

Paraformaldehyde-fixed tissue embedded in paraffin wax was sectioned at 4 μm and stained with periodic acid–Schiff (PAS) or Gorgon and Sweet stain.10 Glutaraldehyde-fixed tissue was embedded in epoxy resin, 0.2 μm semithin sections cut on a Reichert Ultracut E using glass knives, and placed on glass microscope slides. These semithin sections were stained with azure–methylene blue.11

Histological analysis used PAS stain to examine glycogen content, Semithin azure–methylene blue stain for tissue and cellular structure, and Gordon and Sweet stain for tissue reticulin fibers.

The statistical analysis of the data was performed using either Student t-test or Dunnett’s and Tukey calculations. A significant result corresponded to P < .05.

RESULTS
Figure 1 shows that ATP levels in HTK-perfused livers followed a higher trend than those perfused in either of the other solutions for the first 2 hours, but only reached a significant difference compared with the UW-perfused group after 2 hours (P < .05). By 4 hours this advantage had disappeared.

Over the 48-hour period, AMP levels in LS-perfused tissue appeared to be higher than with the other two solutions (Fig 1), but only reached a significant difference compared with HTK-perfused tissue (P < .05).

ADP levels (not shown) in LS-perfused livers were higher than with the other two treatments throughout the course of the experiment, except at 24 hours, but rarely reached significance.

Little change was seen in hypoxanthine levels among any of the preservation systems until 4 hours (Fig 2). At this point, the amount of hypoxanthine in LS- and UW-perfused tissue started to increase to a higher level than that found at time 0. Over the next 44 hours the levels in these two solutions, but not in HTK, continued to rise.

Little change in xanthine levels was seen in any preservation system until 24 hours (Fig 2). At this point the amount of xanthine in HTK started to increase to a level higher than that found in UW or LS (P < .05), a trend that continued over the next 44 hours.

The level of uric acid in LS- and UW-perfused livers did not change over the whole period from time 0 (Fig 3), but continued to increase in HTK-perfused tissue in a similar fashion to that observed with xanthine.

Five minutes after perfusion, good glycogen content was seen in many cells, but there is less in others, with LS, glycogen was visible in numerous cells with UW, and there were no signs of the presence of glycogen at any time during the 48 hours in any slides of liver preserved in HTK (Fig 4).
24 hours after perfusion, glycogen was still present in some LS-perfused cells and no glycogen was present in UW-perfused cells.

With LS, variability was seen across tissue with semithin azure–methylene blue staining. Morphology was good, but feathery degeneration was starting in places (Fig 5). With UW, variability was seen across tissue, and feathery degeneration was visible and cellular breakdown was starting in places. With HTK, general cellular breakdown and areas of necrosis were seen.

LS and UW produced immediate cell shrinkage, but to a greater extent in UW than LS, whereas liver tissue perfused in HTK solution did not show any change in cell volume. By 60 minutes cellular breakdown was observed in HTK-preserved tissue, but by 24 hours liver tissue in all solutions was beginning to break down.

Five minutes after perfusion, intact strands of reticulin fibers were seen along sinusoids and around veins and bile ducts with LS. Slight shrinkage of cells was visible (Fig 6). Fraying of reticulin fibers was seen, especially around enlarged sinusoids caused by widespread cell shrinkage and around vessels with UW. A large amount of fraying of reticulin fibers was seen, especially around vessels. Two hours after perfusion, fraying of reticulin fibers, especially around portal areas, was seen with LS. Increased fraying and condensing of reticulin fibers was seen with UW,
leading them to appear as smaller thicker strands. Widespread fraying of reticulin fibers was seen with HTK, and some cellular breakdown was occurring.

Reticulin breaks down with irregular degenerative changes, causing loss of connections with the axial reticulin and giving an appearance which has been described as like “fraying rope.” Fraying of reticulin fibres started immediately in HTK and UW solutions, but not in LS solution. This is surprising, because there was little change in cell volume initially in HTK, yet the tissue showed large amounts of reticular fraying after just 5 minutes. Although initially UW showed reticulin fiber damage, this did not develop as quickly as in HTK.

**DISCUSSION**

Leeds solution is a phosphate-based sucrose solution that like UW contains the impermeant lactobionate and the metabolite allopurinol (1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) which acts as a competitive inhibitor of xanthine oxidase, stopping the breakdown of hypoxanthine to xanthine by oxidizing it to alloxanthine, inhibiting both the conversion of hypoxanthine to xanthine and the conversion of xanthine to uric acid (Fig 2 and 3).

Metabolism to xanthine and uric acid which leads to the production of free radicals which are known to damage tissues, occurs during reperfusion, leading to ischemia-reperfusion injury. But with HTK, this happens during the initial ischemia, before reperfusion, suggesting that tissues preserved in HTK show damage occurring earlier than initially believed. This observation upon histological analysis showed more rapid disruption of liver tissue preserved in HTK than in that preserved with either UW or LS. Therefore, the addition of allopurinol to LS and UW appears to prevent this initial damage.

HTK contains α-ketoglutarate, which allows the tricarboxylic acid cycle to continue, thereby using the lactate produced by glycolysis. This in turn means that more glucose is required for glycolysis; therefore, glycogen is broken down in the liver into glucose. Hence the rapid depletion of glycogen in those livers perfused with HTK (Fig 4). Unfortunately this means that upon reperfusion the livers have little energy source in the form of stored glycogen; furthermore, they do not have much uridine (Fig 6), so they are unable to initially produce glycogen.

Two important liver tissue metabolites are preserved better by LS than UW: AMP and uridine (Fig 3 and 6). AMP was higher in LS- than UW-perfused tissue during 48 hours, suggesting that AMP is not broken down as rapidly to adenosine in LS-perfused livers.

Uridine content was greater after 24 and 48 hours among livers preserved in LS rather than UW, which is an important observation, because uridine is required for glycogenesis. Glycogen was preserved in liver tissue after 48 hours of static preservation with LS; however, at 24 hours with UW.
all of the glycogen had disappeared from the liver. Because livers perfused in UW showed lower uridine levels than those perfused in LS, there was less ability for the hepatocytes to synthesize glycogen. Therefore, if a liver has been stored in UW for 24 hours or more, it seems to be equivalent to transplanting a liver from a “starved” person, requiring a longer periods to recover.

Both UW and LS cause tissues to shrink, which was more pronounced among UW-preserved livers, causing the hepatocytes to contract from the veins and the endothelial cells to round up, becoming detached from the vein. This was not seen in LS-perfused liver tissues. Breakdown and disappearance of reticulin fibers showed that the liver was damaged to such an extent that fibrosis may be produced rather than full tissue recovery after reperfusion. “Fraying” and condensing of reticulin fibers was seen after just 5 minutes in UW preservative, which was probably due to the large amount of shrinkage which occurred upon perfusion with this solution. Among LS-preserved livers, this process did not start until 2–4 hours after static preservation, by which time the reticulin in UW-preserved livers was badly damaged. After 24 hours in UW, there was a loss of cellular differentiation, widespread “fraying,” and condensation of reticulin fibers with no glycogen present. By 48 hours, there was widespread tissue and cellular breakdown, with cells becoming “individualized,” because the reticulin was barely

Fig 6. Staining of reticulin.
visible. After 24 hours in LS, there was a small amount of “fraying” and condensation of the reticulin fibers, with cellular differentiation visible and ample glycogen still present. By 48 hours, there was the start of slight cellular breakdown with more “fraying” and condensing of reticulin fibers. Glycogen was still present.

These results suggest that LS is as good as UW for preserving ATP and its metabolites, but histologically it was far superior to UW, which are attributed to less cell shrinkage and subsequent reticulin fracture.

In conclusion, far from being a period of stasis, cold ischemia in the liver is characterized by dynamic biochemical changes coincident with often rapid pathological injury influenced by the choice of the perfusion fluid. The addition of allopurinol to UW and LS appears to be critical. It is reasonable to suggest that this may also affect the degree of subsequent reperfusion injury. Our data supported the notion that LS offers improved preservation over UW and HTK and affirmed the impetus to shorten ischemic times in clinical transplantation, because pathological changes occur within even short periods.

REFERENCES