ELSEVIER

Comparison of Two Preservation Solutions in the Protection of the pH Regulation Mechanism of Perfused Rat Livers After 24 Hours of Cold Storage

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LIVER has a pH stabilizing mechanism that can be impaired by ischaemic damage such as occurs during organ preservation for transplantation. We compared the effectiveness of University of Wisconsin solution (UW) and phosphate-buffered sucrose for liver (PBSL) on pH regulation and pH stabilization following flush and storage at 0 to 4°C for 24 hours, and reperfusion at 37°C.

During both warm and cold ischemia hypoxia leads to the accumulation of metabolically generated intracellular acid. In hepatocytes, the pH regulatory system has been partly characterized. Roles for the Na^+ and H^+ exchanger and the Na^+ and HCO_3^- cotransporter have been suggested.[1] Upon reperfusion, hepatocytes have a requirement to eliminate acid to restore their intracellular pH to a normal physiologic level. It has been shown that intracellular pH buffering power of the hepatocytes remains intact even after 48 hours of storage in the preservation solutions.[2] This correction of pH is of vital importance to the liver, as impairment will affect cell membrane potential; energy supply; and the intracellular concentration of ions, including Ca^{++}, K^+, and Na^+. Hepatocytes have two major mechanisms for eliminating H^+ from the cell. These are the electroneutral Na^+/H^+ exchanger and electrogenic Na^+/HCO_3^- transporter. Both of these transporters increase their activity with an increase of intracellular H^+ ion concentration. As the hepatocytes eliminate intracellular acid, the extracellular fluid tends to become more acidic. During reperfusion hepatocytes normally correct the pH to the physiologic level. It is important that the cells of a liver graft retain effective function of this mechanism, particularly in the early stages of reperfusion. This may depend on the effectiveness of the preservation fluid. We compared the effects of UW on control of pH with those of a (combined) series of phosphate-buffered sucrose-based preservation solutions developed for use on the liver (PBSL).

MATERIALS AND METHODS

The experiment was carried out under a Home Office licence according to standard guidelines for animal care. Male Wistar rats weighing 280 to 300 g obtained from Biomedical Services, University of Leeds, were anaesthetized by a single intraperitoneal injection of pentobarbital. (0.6 μg/g body weight) without prior fasting. The abdomen was opened by a longitudinal midline and transverse subcostal incision. The common bile duct was visualized and cannulated with a fine cannula (0.28-mm diameter) secured with a cotton thread. The bile duct was then divided at the distal end.

Heparin (1000 U) was injected intravenously. The abdominal aorta was isolated and cannulated with a 2-mm diameter polyethylene catheter held in position by a bulldog clamp. The thoracic cavity was opened, and the supra-diaphragmatic aorta clamped. Thirty milliliters of ice-cold experimental preservation solution was passed through the aorta to perfuse the liver via the hepatic artery and allowed to escape through hepatic veins and cut IVC into the chest cavity.

The portal vein was visualized; a 2-mm polyethylene cannula was inserted and secured with a cotton tie. Another 20 mL of ice-cold preservation solution was allowed to run through the portal vein. The cannula was then capped making sure no air bubbles entered.

Two preservation solutions were used; UW and PBSL.

The liver was mobilized, removed, and stored in 60 mL of experimental solution at 0 to 4°C for 24 hours.

Following storage, the liver was attached to an isolated perfused liver apparatus and perfused at 37°C with MOPS buffered saline containing 5% bovine serum albumin to maintain the oncotic pressure and 40% washed bovine red cells to carry oxygen to the liver.

Isolated Perfused Rat Liver (IPRL)

The perfusate was drawn at a constant flow rate of 15 mL per min^{-1} from the reservoir situated on a magnetic stirrer by the roller peristatic pump. It was equilibrated with 95% oxygen and 5% carbon dioxide in the oxygenator, passed through a debubbler, and perfused through the liver. It was allowed to escape from the cut vena cava from which it was returned to the reservoir. Perfusion pressure was monitored continuously at the portal vein catheter. A sampling port positioned immediately prior to the portal vein cannula facilitated collection of portal vein samples. Samples were also obtained from the emerging fluid. Perfusion continued for a period of 2 hours. Taurocholic acid (4.84%) was added throughout (Fig 1).

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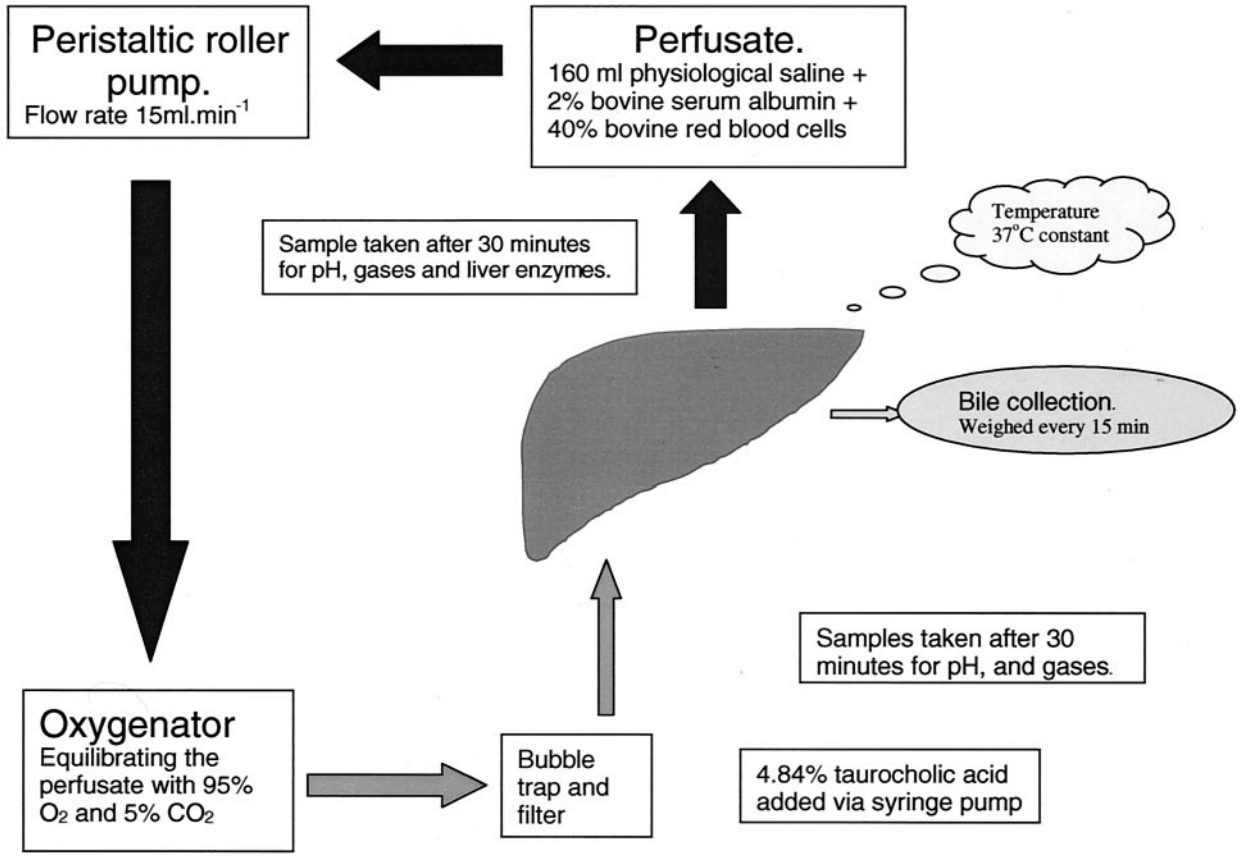

Fig 1. Isolated perfused rat liver model.

Samples were collected from the portal vein and hepatic vein after every 30 minutes and measurements made of pH, partial pressure of oxygen, and carbon dioxide.

RESULTS

The pH of the perfusate decreased in the UW group as compared to the PBSL group, where it was restored toward the physiologic pH (Table 1).

DISCUSSION

In hepatocytes the pH regulatory systems have been partly characterized. Roles for the Na^+/H^+ exchanger and the Na^+/HCO_3^- cotransporter have been suggested.[1] In HCO_3^- free medium, the Na^+/H^+ exchange system responds to the fall in intracellular pH by extruding protons in exchange for extracellular Na^+. The energy required for this extrusion is provided by the large inwardly directed Na^+ gradient. The Cl^-/HCO_3^- exchanger is also present on the canalicular membrane. Under physiologic conditions the Cl^-/HCO_3^- exchanger would transport Cl^- into and HCO_3^- out of the cells, resulting in a net increase in extracellular pH. Evidence for a Cl^- independent, Na^+ dependent, HCO_3^- electrogenic cotransport mechanism, located on the basolateral membrane of the hepatocytes has also been found. These systems act to buffer the pH against extracellular variations. It has been shown that the intracellular buffering power of the hepatocytes is not impaired, even after 48 hours of cold storage in the preservation solutions.[2]

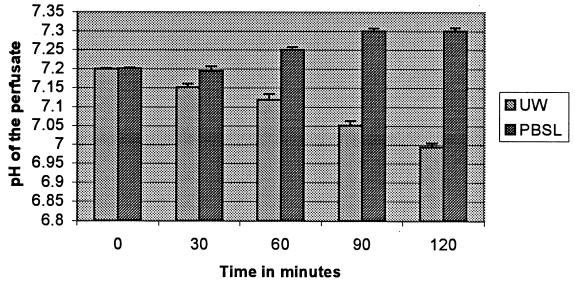

Fig 2. Adjustment of pH in two groups.

The ideal pH for liver preservation is not known. Some studies suggest that the preservation solution should be at the physiologic pH (7.4).[3] A few studies suggest that pH should be adjusted at the physiologic intracellular pH (7.2 to 7.4), whereas a few studies suggest more alkaline[4] or acidic pH.[5–9] The pH of UW solution is 7.4, while that of PBSL is 7.0. After preservation, the pH of the preservation

Table 1. pH in the Two Groups

Time	0	30-	60	90	120
UW ($n = 12$)	7.2 ± 0.002	7.152 ± 0.01*	7.119 ± 0.015**	7.052 ± 0.012***	6.995 ± 0.11***
PBSL ($n = 52$)	7.2 ± 0.002	7.195 ± 0.013*	7.251 ± 0.007**	7.301 ± 0.008***	7.302 ± 0.007***

All values expressed as means ± SEM.
*$P < .05$.
**$P < .001$.
***$P < 0.0001$.
One-way analysis of variance was used.

solution (in which the livers were stored) dropped in both cases, presumably due to lactic acid production in the livers due to anaerobic metabolism.

We set the pH of the perfusate (MOPS buffered Ringers solution + 2% serum albumin + 40% washed RBCs) at 7.2 because this corresponds to the physiological pH in the portal vein of the rat. During reperfusion pH was observed but was not adjusted. The livers in the UW group acidified the perfusate, while livers stored in all of the modifications of PBSL corrected the pH toward the physiological pH of the body (Table 1, Fig 2). The exact reason for the different responses between PBSL and UW is not known at present and is currently under investigation.

CONCLUSION

PBSL flush fluids were associated with alkalinisation of the perfusion fluid after reperfusion while UW fluid was associated with acidification.

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