PRESERVATION OF LIVER WITH EURO-COLLINS SOLUTION: EVALUATION OF ULTRASTRUCTURAL ALTERATIONS

(Received 9 October, 1998)

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ABSTRACT

Objective: The aim of this study was to investigate time-related ultrastructural alterations in the rat liver during Euro-Collins perfusion and storage.

Material-Methods: Rat liver pieces were placed in cold (4°C) Euro-Collins solution and stored for 0, 3, 6 and 12 hour periods. Morphologic investigations were performed using electron microscopy.

Results: Electronmicrographs from the 3 hour and 6 hour perfusions showed a slight degree of hepatocellular degeneration. Extremely swollen and degenerated hepatocytes were observed at the 12th hour of preservation period.

Conclusion: It is concluded that the safe storage period for the rat liver in Euro-Collins solution is less than 12 hours.

Key Words: Liver, tissue preservation, Euro-Collins solution, ultrastructure

INTRODUCTION

It is known that extended ischaemia results in organ infarction. This fact limits the time for availability of donor liver. The increasing use of many organs such as liver, lung, heart, pancreas and small intestine by means of transplantation necessitates the development of successful preservation techniques and solutions (1-10). Euro-Collins (EC) and Beltzer (UW; University of Wisconsin) solutions are the most widely used solutions as the storage media of different organs prior to the transplantation (11-16). Collins et al. in 1969 developed EC solution, which is a common organ preservation solution for kidney preservation (17). EC solution contains a high concentration of K^{++}, a low concentration of Na^{++}, and glucose plus phosphate as agents to suppress cell swelling in the cold. In most transplantation centres, flushing of the organ to be transplanted with EC solution followed by cold storage in the same medium is used as a standard procedure. EC solution is also used as a storage solution for liver in organ transplantation. The degree of ischaemic damage bears importance for the success of implantation. So, as the liver tissue is highly sensitive to ischaemia, the cold storage time was limited to 8-10 hours by using that solution (8).

In the present study, time-related morphological alterations in rat hepatocytes after perfusion and storage in EC solution at 4°C was investigated.

MATERIALS AND METHODS

Animals

Male Wistar Albino mature rats (220-250 gr) (n=5) were used in this study. They were kept in room at a constant temperature of 22°C with 12-h light and dark cycles and fed a standard diet and water ad libitum. Experiments were designed considering accepted ethical standards for animal research.

Liver Perfusion and Storage

They were anesthesized with 0.05 mg/gr body weight i.p. sodium pentobarbital. Following laparotomy, the liver was perfused through the portal vein with about 100 ml Euro-Collins solution (containing in g/L KH_2PO_4:2.04, K_2HPO_4: 7.4, KCL:1.12, $NaHCO_3$:0.84 and glucose:35; osmolarity about 355mOsm/L;pH: 7,2 at 19ºC. The perfusion continued until the liver was completely discoloured. Following the warm perfusion, whole liver was taken out and immersed in cold (4°C) EC solution. Then liver pieces (1cm^3) were taken by needle biopsies at the 3rd, 6th and 12th hours. For control group, liver tissue was fixed immediately after perfusion.

Electron Microscopic Preparation

For the transmission electronmicroscopical examination, liver samples of about 1 mm^3 were fixed in 2.5 % glutaraldehyde in phosphate buffer (0.1 M, pH 7.2) for 2 hours at 4ºC. Postfixation in 1 % phosphate-buffered osmium tetroxide was performed at the same temperature. After dehydration in ascending series of ethyl alcohol, the tissue pieces were blocked in epoxy resin. Thin sections of 600 Aº were cut and were stained with uranyl acetate and lead citrate. For each experimental group (Group 1: control group, Group 2: 3 hours of preservation, Group 3: 6 hours of preservation; Group 4:12 hours of preservation) two grids were randomly examined under a transmission electron microscope (JEOL 1200 EX).

RESULTS

At control group liver parenchyma represented normal ultrastructure of hepatocytes. Nuclei, mitochondria, granular and smooth endoplasmic reticulum were reflecting normal morphology (Fig. 1). Electronmicrographs from 3 hours (Figs. 2 and 3) and 6 hours (Figs. 4 and 5) groups represented slight degree of hepatocellular degeneration. Although some indentations were present, the nuclei were usually in normal ultrastructure. Mitochondria and granular endoplasmic reticulum were reflecting normal morphology, as well. Granular endoplasmic reticulum cisterns showed clusters around the nuclei. Minimal dilatations at smooth endoplasmic reticulum membranes were observed at both groups.

In 12 hours group, nuclear and cytoplasmic degenerations were extremely notified. Nuclei represented inactive stage with unusual chromatin distribution. Granular and smooth endoplasmic reticulum membranes were obviously dilated resulting in vacuolated appearance of the cytoplasm. Ribosomes were decreased in number. Mitochondria were extremely swollen suggesting mitochondrial hypertrophy. Severe mitochondrial degeneration was indicated by total loss in cristae structure. In that group, glycogen depletion was noticed (Figs 6 and 7).

DISCUSSION

Ultrastructural changes in liver cells induced by in vitro ischaemia at 4°C have been compared in liver perfused and stored in EC solution. Modified EC solution has been used as the standard for liver preservation in USA, allowing for safe cold storage up to 9 hours with subsequent excellent liver preservation (18). Glucose, the main ingredient in EC solution, acts as an impermeable in kidneys, but freely crosses the

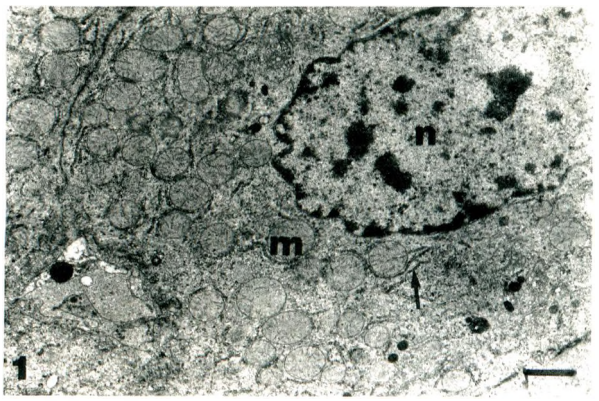

Fig.1.: ***Control Group:*** Liver electronmicrograph represents normal ultrastructure. n:nucleus, →: granular endoplasmic reticulum, m: mitochondrion, bar: 1µm

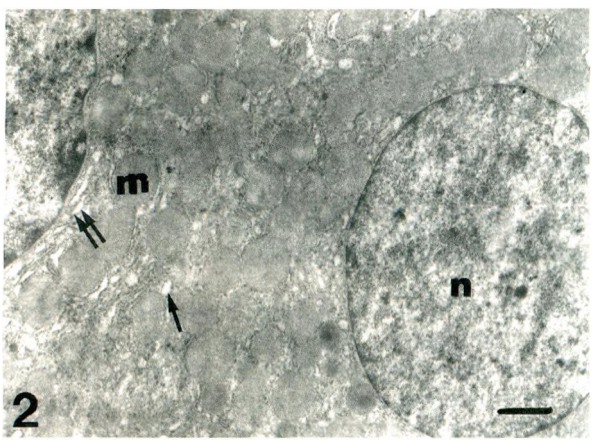

Fig.2.: ***3 hours group:*** Slight degree of dilatations at the smooth endoplasmic reticulum membranes (→). n: nucleus, m: mitochondrion,⇒: granular endoplasmic reticulum membrane, bar: 1µm

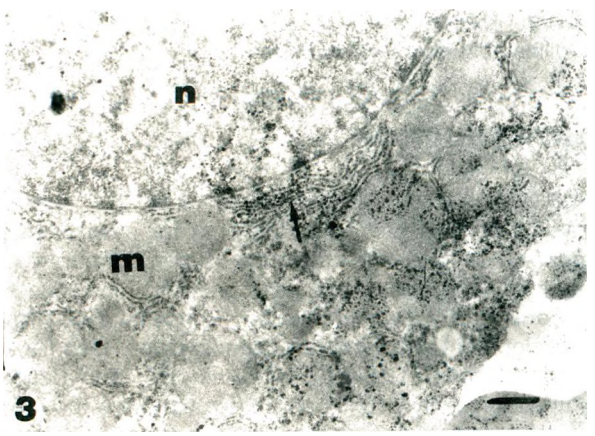

Fig.3.: ***3 hours group:*** Hepatocyte reflects a normal cell ultrastructure. n: nucleus, m: mitochondrion, →: granular endoplasmic reticulum, bar: 500 nm

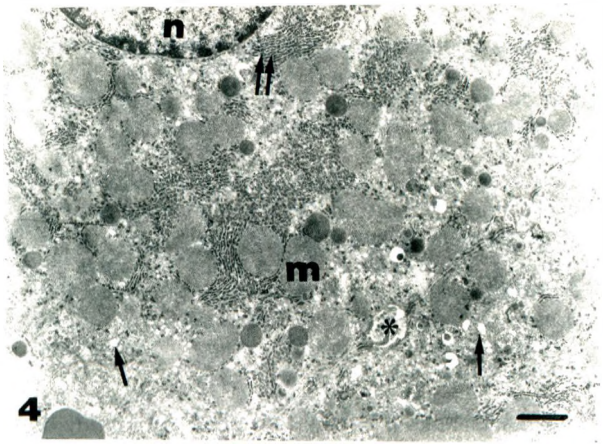

Fig.4.: *6 hours group:* Hepatocyte presents minimal cellular injury with slight dilatations at smooth endoplasmic reticulum membranes (→). n: nucleus, m: mitochondrion, ✱: bile canaliculus, bar: 1μm

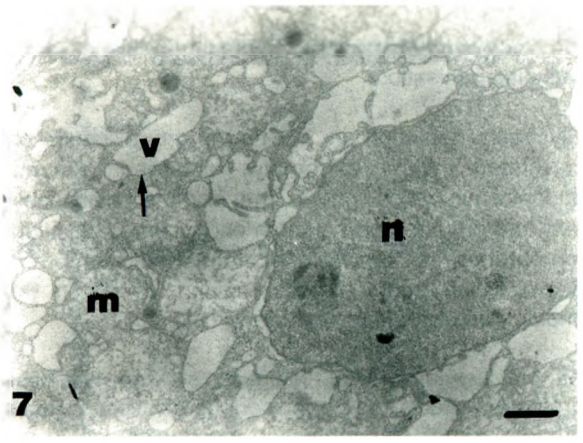

Fig.7.: *12 hours group:* Pyknotic hepatocyte nucleus (n), abnormally enlarged endoplasmic reticulum membranes (→) resulting in vacuoles (v) and mitochondria (m) with damaged cristae, bar: 1μm.

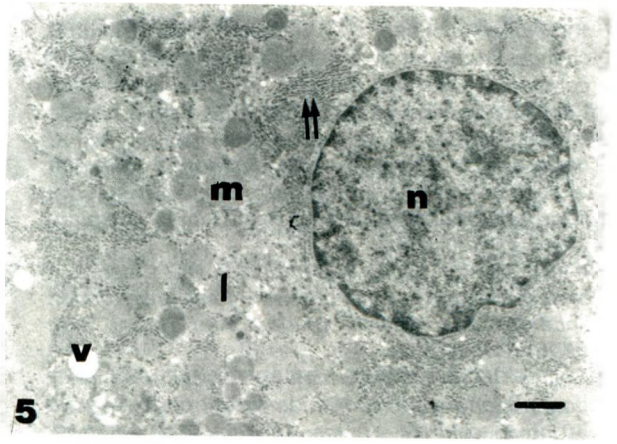

Fig.5.: *6 hours:* Numerous lysosomes (l) and vacuoles (v) in the hepatocyte cytoplasm. n: nucleus, granular endoplasmic reticulum (⇒), bar: 1μm

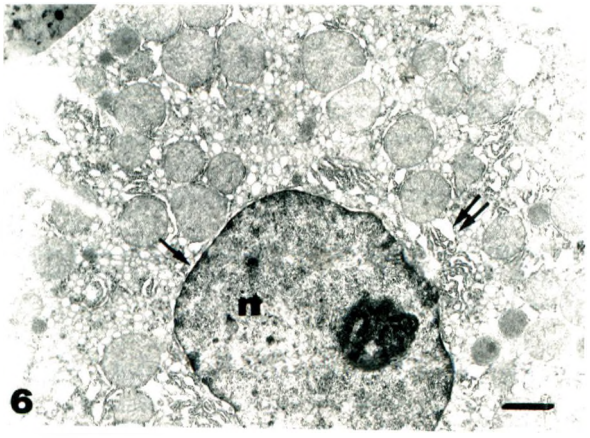

Fig.6.: *12 hours group:* Hepatocyte nucleus (n) with unusual chromatin pattern, dilated perinuclear space (→), prominently enlarged endoplasmic reticulum membranes (⇒) and loss in ribosomal content imply to a severe cellular damage, bar: 1μm

liver cell membrane providing an abundant substrate for glycolysis (18).

Hypothermia retards the effects of ischaemia, but inhibits the Na^{++}/K^{++} dependent ATPase pump, thus causing the cell to lose its membrane potential and the ability to regulate membrane transport which results in cellular swelling. Ischaemia stimulates anaerobic glycolysis (18). The liver was increased in weight during storage in EC solution (19). That case was related to the water influx. Formerly, effectiveness of EC solution was related to its hypertonicity. Later, the importance of impermeable solutes has been appreciated rather than hypertonicity (19). Glucose, which is used as an impermeable in EC solution, is more permeable and more rapidly metabolised in the liver. Thus, a water influx is largely possible for the liver tissue preserved in this study solution. In our study, minimal injury in liver parenchyma was observed at 3 hours and 6 hours, further damage began by the 12th hour. The typical vacuolisation of hepatocyte cytoplasm at 12 hours group could be related to water influx which caused cellular organelle swelling for the liver tissue preserved in EC solution. In livers stored for longer than 8 hours in EC solution lethal injury occurs to sinusoidal endothelial cells. Liver sinusoids were usually observed obstructed in many studies (Michel/Vons). The consequence of the storage of livers stored in EC solution for more than 12 hours was complete destruction of the sinusoidal lining (20). This injury would cause to prohibit graft success (20). Non parenchymal cell injury is related to parenchymal cell blebbing. The blebs might penetrate through sinusoidal fenestrations during storage (20). That case was related to the hepatocyte swelling and vacuolisation. In our study, we focus especially on the hepatocyte dysfunction related to organelle damage.

We could correlate hapatocyte damage with sinusoidal wall disorganisation.

Glycogen depletion in 12 hours group could be correlated with extremely enlarged smooth endoplasmic reticulum. As the intimate relationship between glycogen and smooth endoplasmic reticulum membranes is known the mentioned membrane degeneration could suggest having a role in the impairment of glycogen synthesis (21).

As conclusion, the EC solution storage has less protective effects for longer periods of over 12 hours on liver tissue. This protection mechanism would not be sufficient alone for allograft viability and we think that it needs amelioration.

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