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Change in mitochondrial membrane potential is the key mechanism in early warm hepatic ischemia-reperfusion injury

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Abstract

The mitochondrion has been proposed to be both a target and a perpetuator of hepatic ischemia-reperfusion (IR) injury because of its reactive oxygen species (ROS) formation. Our hypothesis is that subcellular derangement in mitochondrial function is one of the earliest steps leading to the early IR-mediated loss of hepatocellular integrity. Under chloralhydrate anesthesia (36 mg/kg BW), Sprague–Dawley rats (n = 7) were subjected to 40 min of warm hepatic lobular ischemia followed by 60 min reperfusion. Rats (n = 7) without hepatic IR were used as controls. The fluorochromes rhodamine 123 and bisbenzimide were administered intravenously for observation of changes in mitochondrial membrane potential and hepatocellular viability, respectively. Intravital fluorescence microscopy (IVFM) was performed prior to ischemia and at 15, 45, and 60 min after reperfusion in the experimental group and at corresponding time points in the control group. A parallel relationship between mitochondrial membrane potential and cell viability as reflected in a concomitant reduction in nuclear and cytoplasmic fluorescence intensity during IR was demonstrated (r^2 = 0.76, $P < 0.05$). The diminution in fluorescence intensities also correlated significantly with the elevation in plasma transaminase activities ($r^2 > 0.90$, $P < 0.05$). Our data suggested that alteration in mitochondrial membrane potential is a critical subcellular event leading to hepatocellular damage in the early phase of hepatic IR injury.

Keywords: Liver; Rhodamine 123; Bisbenzimide; Fluorescence intensity; Hepatocyte viability

Introduction

Ischemia-reperfusion (IR) injury has been identified as the major early complication of orthotopic liver transplantation (OLT) (Clavien et al., 1992). One of the major consequences of hepatic IR injury is hepatocyte damage, the precise mechanism of which remains unclear. There is a growing body of evidence suggesting that mitochondria are a major target of IR injury (Schild et al., 1997). These findings are of particular importance as mitochondria have been shown to play a pivotal role in the regulation of cell death under both physiological and pathophysiological conditions (Kroemer et al., 1998; Lemasters et al., 1987; Rosser and Gores, 1995). For a metabolically active organ such as the liver which is rich in mitochondria, an investigation into the changes in mitochondrial function is of particular significance in the understanding of the subcellular mechanisms underlying hepatic IR injury.

Under physiological conditions, the mitochondrial proton pump drives protons out of the matrix of the organelle and thereby generates a proton gradient across the almost impermeable inner mitochondrial membrane. This electrochemical gradient forms a mitochondrial membrane potential ($\Delta\psi$) of up to −160 mV which is vital for the maintenance of normal mitochondrial functions including ATP synthesis from inorganic phosphate and ADP, importation of mitochondrial proteins, calcium homeostasis, reintroduction of protons through the subunit F_0 of complex V, and a variety of other metabolic transport mechanisms (Jassem et al., 2002; Kroemer et al., 1998).

The rapid sequestration of lipophilic cations in the mitochondria driven by the mitochondrial $\Delta\psi$ has led to the development of a number of mitochondrion-selective probes which enable researchers to follow optically mito-

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chondrial activity, localization, and abundance of mitochondria, as well as to monitor the effects of some pharmacological agents on mitochondrial function (Elimadi et al., 1998; Settaf et al., 1999). Rhodamine 123 (Rh123) is a cell-permeant, cationic, and mitochondrion-selective fluorescent dye that is rapidly sequestered by mitochondria (Johnson et al., 1980). This fluorochrome is subsequently washed out of the cells once mitochondrial $\Delta\psi$ is lost (Goldstein and Korczack, 1981; Johnson et al., 1980, 1981). Thus, quantification of the fluorescence intensity of Rh123 has been validated as a measure of mitochondrial membrane potential. Studies performed using this mitochondrion-selective probe have been extensive over the years, with the majority of investigations performed using cell cultures (DeMartinis et al., 1987; Oh et al., 1991; Schneckenburger et al., 1992). Only in the recent past have Suematsu and co-workers successfully established an approach for the ex vivo assessment of hepatocellular mitochondrial function with Rh123 in an isolated perfused rat liver preparation (Kurose et al., 1993; Shiomi et al., 1998; Suematsu et al., 1992). They demonstrated that hepatocellular mitochondrial $\Delta\psi$ was reduced by low flow-induced hypoxia (Suematsu et al., 1992).

In vivo comparison of hepatocellular mitochondrial $\Delta\psi$ in intact rat liver with Rh123 is of particular interest because mitochondrial membrane permeability transition and disruption of the electrochemical gradient of the inner membrane are critical steps preceding the development of apoptotic cell death (Crompton, 1999; Kroemer et al., 1998). Using the technique of intravital fluorescence microscopy (IVFM), the changes in mitochondrial membrane potential in vivo as reflected in the fluctuations of Rh123-derived fluorescence intensity can be visualized and compared.

The fluorescent dye bisbenzimide (Hoechst 33342) rapidly permeates cells and binds to the minor groove of DNA in nuclei. This property of bisbenzimide has been exploited as a probe for cell viability especially in the field of cell biology where it is used to determine DNA content and density of cells. Elstein and co-workers demonstrated that only those viable and early apoptotic cells can be stained by bisbenzimide (Elstein and Zucker, 1994). Vollmar and co-workers have successfully applied this fluorochrome in an in vivo study of hepatic structure during CCl_4-induced liver injury (Vollmar et al., 1998). The fluorochrome has been proven useful in the comparison of the number of viable hepatocytes and also the visualization of hepatic cords and sinusoids after orthotopic liver transplantation in the rat (Sun et al., 2003).

Although it was believed that cellular injury was negligible during the early phase of hepatic reperfusion when neutrophils have not yet been involved (Kohli et al., 1999a,b), a later study demonstrated that up to 10% of necrotic hepatocellular damage occurs as early as 1 h after reperfusion (Gujral et al., 2001). The objective of the present study was an attempt to demonstrate in vivo the hypothesis that warm hepatic IR injury causes transition in mitochondrial membrane potential during the early phase of reperfusion which leads to an impairment in hepatocellular integrity and cell death.

Materials and methods

Animals

Male Sprague–Dawley rats with a body weight of 260–350 g were obtained from the Department of Laboratory Animal Sciences of Otago University Medical School, Dunedin, New Zealand, and were housed in groups of 5 in temperature- and humidity-controlled animal quarters under a 12-h light/dark cycle. The animals were maintained on a standard rat diet with free access to tap water before surgery. This study was approved by the Committee on Animal Ethics in the Care and Use of Laboratory Animals of the Otago University Medical School.

Experimental protocol

Age-matched rats were randomly divided into two groups: (i) hepatic IR group ($n = 7$) that was subjected to 40 min of warm hepatic lobular ischemia followed by 60 min reperfusion; (ii) control group ($n = 7$) which was not subjected to IR treatment of the liver. All animals were administered a bolus of heparin (20 U/kg, intravenously) following cannula placement in blood vessels. In the hepatic IR group, IVFM examination was started 15 min after intravenous bolus injection of Rh123 and bisbenzimide but prior to the commencement of hepatic ischemia (pre-IR). Subsequently, IVFM was performed after 40 min of warm ischemia, and at 15, 30, 45, and 60 min after reperfusion. In the control group, IVFM was performed at 15, 55, 70, 85, 100, and 115 min after intravenous injection of the fluorochromes. These time points corresponded to those chosen for the hepatic IR group after laparotomy. Blood samples were taken for the analysis of plasma enzyme activities prior to IR and at 15, 30, 45, and 60 min after reperfusion.

Surgical procedures

All surgical procedures were performed under anesthesia with chloralhydrate (36 mg/kg body weight intraperitoneal). The animals were placed in the supine position and the body temperature was maintained at 37°C using a thermoregulated heating pad. Cannulae were inserted into (i) the right carotid artery for collecting blood samples and (ii) the right jugular vein for the administration of fluorochromes. A laparotomy was performed by an upper midline incision plus a left side subcostal extension. The ligaments over the left side of the liver were dissected and the left lobe liver was mobilized.