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Digestive and Liver Disease 37 (2005) 689–697
Digestive and Liver Disease
www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Oxidative injury in rat fatty liver exposed to ischemia-reperfusion is modulated by nutritional status

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Received 14 December 2004; accepted 15 April 2005
Available online 1 June 2005

Abstract

Background and aims. Oxidative stress contributes to ischemia-reperfusion injury in fatty livers. This study aimed to determine whether glycogen depletion influences this oxidative injury and whether the administration of glucose can be protective.

Methods. Rats with choline deficiency-induced fatty liver underwent hepatic ischemia-reperfusion. Experimental groups: (1) fed rats; (2) 18 h fasted rats; (3) 18 h fasted rats supplemented with glucose prior to surgery. The thiobarbituric acid-reactive substances, protein carbonyls and total glutathione concentrations were measured in liver tissue and isolated mitochondria as parameters of oxidative stress before and after ischemia and during reperfusion. The mitochondrial F_1-ATPase content and the serum alanine transaminase were also determined.

Results. With respect to fed rats, fasted rats exhibited an increased oxidative injury in both liver tissue and isolated mitochondria throughout the experiment with the only exception of thiobarbituric acid-reactive substances, which were not affected by the nutritional status in liver tissue. Fasted rats showed a significantly lower F_1-ATPase content and higher alanine transaminase levels. Glucose supplementation significantly reduced the fasting-associated exacerbation of oxidative stress and liver injury and the F_1-ATPase exhaustion.

Conclusions. These data indicate that the pre-existing hepatic glycogen content modulates the oxidative damage in rat fatty livers exposed to ischemia-reperfusion injury and that the energetic substrate supplementation may represent a clinically feasible protective strategy.

Keywords: Fatty liver; Ischemia-reperfusion injury; Nutritional status; Oxidative stress

1. Introduction

Ischemia-reperfusion injury contributes greatly to the liver damage occurring during surgical procedures, such as hepatic resection and liver transplantation, and during clinical conditions, such as ischemic hepatitis and multiple organ failure [1–3]. Fatty liver is a very common finding being associated to diseases with high prevalence in the general population of industrialised countries, such as metabolic syndrome, obesity, diabetes, hyperlipidemia, alcohol abuse, endocrine and viral diseases [4]. The presence of fatty infiltration dramatically reduces the tolerance of the liver to ischemia-reperfusion injury [1,2]. This explains both the high incidence of early graft non-function, when donor fatty livers are transplanted and the development of organ failure after partial resection in steatotic livers exposed to periods of vascular clamping which, in contrast, are safely tolerated by control livers [1,2].

The cellular, vascular and metabolic events that occur in a failing fatty liver during ischemia-reperfusion are far from

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1590-8658/$30
doi:10.1016/j.dld.2005.04.015

being characterised. Besides the impairment of the microcirculation, which is considered a major event of reperfusion injury in steatotic livers [5], hepatocyte damage appears remarkably higher in fatty liver than in normal organs [6,7]. Several evidences indicate that an increased sensitivity of fatty hepatocytes to the noxious effects of reactive oxygen species (ROS) plays a pathogenetic role in this event [8,9].

Within hepatocytes, mitochondria are the main intracellular source of ROS [10]. Under physiological conditions, about 1–4% of oxygen reacting with the mitochondrial respiratory chain is incompletely reduced to superoxide anion and, consequently, to hydrogen peroxide [10]. Mitochondrial ROS generation dramatically increases during reperfusion because the electrons released by the respiratory chain can be directly donated to the newly supplied oxygen [10,11]. As a result, mitochondrial structures are exposed to the attack of the ROS generated both outside and within these organelles leading eventually to the dysfunction of important mitochondrial processes including those responsible for the adenosine triphosphate (ATP) synthesis [10]. Indeed, experimental studies and clinical observations indicate that the fatty livers synthesise less ATP than the normal livers during post-ischemic reperfusion [12–14].

The pre-existing nutritional status is a major determinant of hepatocyte injury associated with ischemia-reperfusion [15]. In normal livers, fasting exacerbates warm ischemic injury because depletion of the glycogen stores results in a more rapid ATP fall during ischemia, when the oxidative phosphorylation is inhibited and glycogen must supply glucose for glycolytic ATP generation [15]. Furthermore, starvation can contribute to the ischemia-reperfusion injury by lowering the antioxidant defence systems, and thus predisposing the liver to the ROS-mediated damage [15–17]. Fatty livers appear to be even more susceptible to this adverse effect of fasting, as we have previously shown that starvation produces a significantly greater oxidative imbalance in the whole tissue and in the mitochondrial compartment of steatotic than normal livers [18,19]. However, when an additional insult represented by ischemia-reperfusion occurs, the relation among fatty liver, nutritional status and oxidative damage remains to be determined.

Thus, the aim of this study was to determine in a rat model of choline-deficient fatty liver whether oxidative injury during ischemia-reperfusion is modulated by the pre-existing hepatic concentration of glycogen.

2. Materials and methods

2.1. Animals and induction of fatty liver

Male Sprague–Dawley rats (Charles-River, Calco, LC, Italy), weighing 150–175 g, were allowed to acclimate to the animal quarters and were given free access to a standard chow diet and water for 1 week. Then, rats were fed with a choline-deficient diet (Dyets Inc., Bethlem, PA, USA) for additional 7 days (Table 1). This procedure causes a moderate-massive steatosis, predominantly macrovesicular and involving about two-third of the hepatocytes, without evidence of inflammation and/or fibrosis [20]. As we have previously reported, triglycerides are the main component of accumulated lipids [18].

Table 1
Composition of the choline-deficient diet used to induce fatty liver steatosis

Ingredients	g/kg
Alcohol-extracted peanut meal	90
Soy protein isolate (low in choline)	80
L-Cystine	2
Cellulose fibre	10
Corn starch	100
Dextrin	100
Sucrose	413
Choline bitartrate	–
Vitamin free casein	10
Salt mix #200000[a]	35
Primex (hydrogenated vegetable oil)	100
Vitamin mix #300050	10
Corn oil	50

[a] Contains AIN-76A, vitamin and mineral mixtures (American Institute of Nutrition, 1977).

All procedures involving rats were conducted according to the guidelines for the care and use of laboratory animals approved by our Institutions.

2.2. Experimental design and surgical procedure

The day before the experiment, the rats were divided into three groups as follows: (a) rats with free access to food and water until the time of surgery (*fed group*); (b) rats with access only to water for the 18 h prior to surgery (*fasted group*) and (c) fasted rats with free access to a 40% glucose solution during the 18 h prior to surgery (*glucose supplemented group*). The amount of the solution ingested in 18 h ranged between 30 and 45 ml.

Prior to the experiment, the animal weight was similar in the three groups ranging between 230 and 280 g. Lobar liver ischemia-reperfusion followed by a partial hepatectomy of the non-involved liver was performed in all animals using a modification of the technique described by Kawano et al. [21]. Briefly, under enflurane anaesthesia, the abdomen was opened through a midline incision and the left hepatic artery and portal vein were occluded with a non-traumatic microvascular clip inducing ischemia of the left lateral and median lobes (∼70% of the total liver volume). After 1 h, the microvascular clip was released and the hepatectomy of the non-ischemic right lateral and caudate lobes was performed. This model of partial hepatic ischemia-reperfusion injury avoids splanchnic congestion and, thus, any confounding effects resulting from bowel ischemia and haemodynamic disturbances. Moreover, resection of the non-involved portion forces the animal to survive only on the liver lobes subjected