DOI:10.1111/j.1477-2574.2010.00160.x HPB

ORIGINAL ARTICLE

Intermittent ischaemia maintains function after ischaemia reperfusion in steatotic livers

Mathilde Steenks[1], Mark C.P.M van Baal[2], Vincent B. Nieuwenhuijs[3], Menno T. de Bruijn[2], Marc Schiesser[2], Mike H. Teo[4], Tom Callahan[1], Rob T.A. Padbury[2] & Greg J. Barritt[1]

[1]Department of Medical Biochemistry, Flinders Medical Centre and School of Medicine, Flinders University, [2]The HPB and Liver Transplant Unit, Flinders Medical Centre and School of Medicine, Flinders University, Adelaide, SA, Australia, [3]Department of Surgery, University Medical Centre, Groningen, The Netherlands, and [4]Discipline of Anatomical Sciences, Faculty of Health Sciences, University of Adelaide, SA, Australia

Abstract

Background: Ischaemic preconditioning (IPC) and intermittent ischaemia (INT) reduce liver injury after ischaemia reperfusion (IR). Steatotic livers are at a higher risk of IR injury, but the protection offered by IPC and INT is not well understood. The aim of the present study was to determine the effectiveness of IPC and INT in maintaining liver function in steatotic livers.

Material and methods: A model of segmental hepatic ischaemia (45 min) and reperfusion (60 min) was employed using lean and obese Zucker rats. Bile flow recovery was measured to assess dynamic liver function, hepatocyte fat content quantified and blood electrolytes, metabolites and bile calcium measured to assess liver and whole body physiology. Liver marker enzymes and light and electron microscopy were employed to assess hepatocyte injury.

Results: IPC was not effective in promoting bile flow recovery after IR in either lean or steatotic livers, whereas INT promoted good bile flow recovery in steatotic as well as lean livers. However, the bile flow recovery in steatotic livers was less than that in lean livers. In steatotic livers, ischaemia led to a rapid and substantial decrease in fat content. Steatotic livers were more susceptible to IR injury than lean livers, as indicated by increased blood ALT concentrations and major histological injury.

Conclusion: INT is more effective than IPC in restoring liver function in the acute phase of IR in steatotic livers. In obese patients, INT may be useful in promoting better liver function after IR after liver resection.

Keywords

liver, ischaemia reperfusion injury, intermittent ischaemia, ischaemic preconditioning, bile flow, lipid, obese, rat

Received 29 September 2009; accepted 19 January 2010

Correspondence

Professor Greg J. Barritt, Department of Medical Biochemistry, School of Medicine, Flinders University, GPO Box 2100, Adelaide SA 5001, Australia. Tel: +61 8 8204 4260/8204 4419; Fax: +61 8 8374 0139; E-mail: greg.barritt@flinders.edu.au

Introduction

Ischaemia and reperfusion injury of the liver occurs during reperfusion after liver resection with hepatic inflow occlusion through clamping of the portal triad (Pringle manoeuvre), and after liver transplantation.[1–3] Patients with moderate to severe steatosis are at a higher risk of liver failure and death after major liver resection,[4–6] and after liver transplantation.[7–11] Steatotic livers are more susceptible to ischaemia reperfusion damage than normal livers.[8,11–16] The present organ shortage in liver transplantation could be reduced markedly if livers with a higher degree of steatosis could be confidently used as donor grafts. A larger proportion of obese patients could undergo successful liver resection if there were available improved methods to reduce ischaemia reperfusion damage in steatotic livers.

Ischaemic preconditioning (IPC) and intermittent ischaemia (INT) have been employed to reduce liver injury and enhance survival after ischaemia reperfusion (reviewed in[2,17,18]). While there is some evidence from studies with animal models that IPC and INT can reduce ischaemia reperfusion damage in steatotic

livers,[19–23] the effectiveness of these procedures has not yet been fully evaluated. There is a need for a better understanding of the effects of IPC and INT on steatotic livers in order to develop improved strategies for the protection of steatotic livers from ischaemia reperfusion damage.

Warm ischaemic reperfusion injury is described in terms of two phases: the acute (early, 0–4 h after commencement of reperfusion) phase and the late (4–48 h after commencement of reperfusion) phase.[2,18,24] Bile flow recovery after ischaemia reperfusion is a sensitive indicator of early changes in liver function in the acute phase of ischaemia reperfusion injury, and a valuable parameter for assessing the effects of IPC and INT on dynamic liver function. While bile flow is an established marker of liver function in *ex vivo* perfusion models,[25–27] there is limited information available for *in vivo* models. We and others have shown that bile flow responds very rapidly to ischaemia and reperfusion and that, in rat models of ischaemia reperfusion injury, IPC and INT enhance bile flow recovery.[26,28–32] The aim of the present study was to test the effectiveness of IPC and INT in maintaining liver function in the acute phase of ischaemia reperfusion injury, assessed by measuring bile flow recovery, in steatotic livers of genetically-obese Zucker rats. As bile flow is an established indicator of dynamic liver function (reviewed in[18]), we have used this parameter to assess the effects of IPC and INT on the liver in the acute phase of ischaemia reperfusion injury.

Materials and methods

Animals and experimental design

Zucker rats[33] were obtained from Harlan Sprague Dawley Inc., Indianapolis, Indiana, USA, and were housed in the Flinders Medical Centre Animal House. Male obese (*fa/fa*) (11–45 weeks, 460–910 g) and lean heterozygous (FA/*fa*) rats (11–30 weeks, weighing 430–600 g) were housed at 22°C, 60% humidity, with a 12-h light/dark cycle and free access to food and water. Animals received humane care, and the experimental protocols were conducted according to the criteria outlined in the 'Australian Code of Practice for the Care and Use of Animals for Scientific Purposes' (National Health and Medical Research Council of Australia). Zucker (*fa/fa*) rats express a missense mutation in the leptin receptor[34] leading to an almost complete loss of leptin receptor function and hence, loss of response to leptin. This leads to increased food intake, altered lipid and carbohydrate metabolism, including development of obesity, a fatty liver and decreased energy expenditure.[35] In obese Zucker rats, steatosis of the liver is not associated with inflammation, which is a shortcoming in many other models of obesity, such as those which employ ethanol ingestion or diets deficient in choline.[23]

Lean and obese rats were randomly allocated into groups (four rats per group). Control group: common bile duct cannulation and bile flow measurement for 125 min. Continuous clamping (CC) group: common bile duct cannulation and bile flow measurement for 20 min with normal inflow to the liver followed by clamping for 45 min of the portal vein and hepatic artery to the medial and left lateral lobes, followed by 60 min of reperfusion. Ischaemic preconditioning (IPC 10/10) group: common bile duct cannulation and bile flow measurement for 20 min with normal inflow to the liver followed by 10 min of clamping, 10 min of reperfusion, 45 min of clamping, followed by 60 min of reperfusion. Intermittent ischaemia (INT) group: common bile duct cannulation and bile flow measurement for 20 min with normal inflow to the liver followed by three episodes of 15-min clamping with an interval of 15 min of reperfusion between episodes, then 60 min of reperfusion.

Surgical procedure

The experiments employed an established model of segmental (60%–70%) hepatic ischaemia and reperfusion[36] in which the bilateral median and left lateral lobes were made ischaemic, whereas the right lateral and caudate lobes remained non-ischaemic. Anaesthesia using isoflurane, midline laparotomy, cannulation of the bile duct and collection of blood and liver samples were conducted as described previously.[31] Periodic clamping of the hepatic pedicle was performed as follows: microvascular clamps (straight, 15 mm length, 6 × 1 mm jaw dimensions, 100 g clamping pressure; Fine Science Tools Inc., Foster City, CA, USA) were placed on the portal vein and on hepatic artery branches above those leading to the right lateral and caudate lobes.

Measurement of bile flow

Bile flow was measured as described previously.[31] For each liver, the bile flow recovery was estimated by dividing the value at the end of the reperfusion period by the average of the baseline bile flow values, and was expressed as a percentage of the base line bile flow. For the continuous clamping, IPC and INT groups, total bile secretion during the 60-min reperfusion period was also determined by measuring the area under the curve. Initial rates of increase in bile flow after commencement of the 60-min reperfusion were determined by linear regression (PRISM) using the first five time points.

Concentrations of liver marker enzymes, electrolytes and metabolites in blood and bile

Blood was collected in the presence of an anticoagulant. The concentrations of alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), potassium, creatinine, urea, glucose, calcium, albumin and total protein in plasma, and concentrations of calcium in bile were measured spectrophotometrically using an Hitachi 917 auto analyser (Hitachi Australia Pty Ltd, North Ryde, Australia) and standard Roche-Hitachi methodology.

Assessment of liver histology

Liver tissue fixed in paraformaldehyde was embedded in paraffin, sectioned (3 μm thick), stained with hematoxylin and eosin, and analysed histologically in a blinded fashion. The severity of hepatic cell injury was evaluated by an established point-counting