Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Alterations in hepatic lobar function in regenerating rat liver

András Fülöp, MD,[a] *András Budai, MD,*[a] *Zoltán Czigány, MD,*[a] *Gábor Lotz, MD, PhD,*[b] *Katalin Dezső, MD, PhD,*[c] *Sándor Paku, PhD, DSc,*[c,d] *László Harsányi, MD, PhD,*[a] *and Attila Szijártó, MD, PhD*[a,*]

[a] *1st Department of Surgery, Semmelweis University, Budapest, Hungary*
[b] *2nd Department of Pathology, Semmelweis University, Budapest, Hungary*
[c] *1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary*
[d] *Tumor Progression Research Group, Joint Research Organization of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary*

ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form
16 March 2015
Accepted 9 April 2015
Available online 15 April 2015

Keywords:
Bile flow
Indocyanine green
Liver function
Liver regeneration
Portal vein ligation

ABSTRACT

Background: Ligation of a branch of the portal vein redirects portal blood to nonligated lobes resulting in lobar hypertrophy. Although the effect of portal vein ligation on liver volume is well documented, the parallel alterations in liver function are still the subject of controversy. Our aim was to assess the time-dependent reactions of regional hepatic function to portal vein ligation by selective biliary drainage.

Methods: Male Wistar rats ($n = 44$) underwent 80% portal vein ligation. Before the operation as well as 1, 2, 3, 5, and 7 d after circulation, morphology and function (laboratory blood test; hepatic bile flow; plasma disappearance rate of indocyanine green; and biliary indocyanine green excretion) of the liver were examined.

Results: Although portal vein ligation affected liver circulation and morphology to a great extent, serum albumin levels, bilirubin levels, and total hepatic bile flow did not change significantly after the operation. Nevertheless, plasma disappearance rate and biliary indocyanine green excretion indicated a temporary impairment of total liver function with the lowest value on the second day and normalization by the fifth day. Bile production and biliary indocyanine green excretion of ligated lobes decreased rapidly after the operation and remained persistently suppressed, whereas the secretory function of nonligated lobes—after a temporary decline—showed a greater increase than the weight of the lobes.

Conclusions: Portal vein ligation induced temporary impairment of total liver function, followed by rapid recovery mainly by reason of increase in the function of nonligated lobes. Functional increase in nonligated lobes was more pronounced than suggested by the degree of volume gain.

1. Introduction

Curative liver resection provides the best survival rate for patients with liver malignancies [1]. Extended hepatectomy is usually required to achieve negative margins. Excessive removal of the hepatic parenchyma, however, often leads to postoperative liver failure. The most widely used method to overcome this problem is preoperative enlargement of the

* *Corresponding author*. 1st Department of Surgery, Semmelweis University, Üllői út 78, H-1082 Budapest, Hungary. Tel.: +36 20 825 89 25; fax: +36 20 825 21 62.
E-mail address: szijartoattila@gmail.com (A. Szijártó).
0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.04.033

future liver remnant (FLR) volume by portal vein occlusion (PVO) techniques, such as portal vein embolization, two-stage hepatectomy combined with portal vein ligation, and more recently associating liver partition with portal vein ligation for staged hepatectomy. These surgical procedures are applied to redirect portal blood flow away from the liver lobes designated for resection, toward the anticipated FLR resulting in FLR hypertrophy (regeneration) [2].

Currently, computed tomography (CT) volumetry is the standard method for determining whether sufficient regeneration is present after PVO. Nevertheless, increase in the FLR volume (morphologic regeneration) does not necessarily reflect the actual alterations of the FLR function (functional regeneration) [3]. Although the morphologic phenomena have been widely discussed, little is known about the functional alterations occurring after PVO because of the lack of an ideal quantitative test, which would represent multiple aspects of the liver function and would be able to assess FLR function selectively. There are two contradictory theories in the literature. Some studies postulate that liver regeneration is promoted at the expense of liver function, resulting in prolonged and less functional regeneration compared with the rapid increase in FLR volume [4–6]. Other works, based on nuclear imaging techniques (i.e., hepatobiliary scintigraphy [7] or hepatocyte mass scintigraphy [8,9]), indicate that increase in FLR function is more pronounced than implied by the degree of morphologic regeneration. This hypothesis, however, has not yet been confirmed by other widely accepted quantitative liver function tests.

Over the last decades, several quantitative liver function tests have been developed, of which the indocyanine green (ICG) clearance test is the most common. ICG is a fluorescent tricarbocyanine dye exclusively eliminated by the liver without metabolism and enterohepatic recirculation [10]. Although ICG is not metabolized, it follows a path of intracellular transport similar to several exogenous and endogenous molecules; its disappearance from the blood, therefore, provides indirect information about the overall function of the liver. The main limitation of the test is that it does not take into account regional variations in liver quality that may occur after PVO.

Selective biliary drainage, however, enables us to assess biliary ICG excretion selectively in FLR. Literary data have demonstrated that biliary ICG excretion is an excellent indicator of liver function or dysfunction in various pathologic conditions such as liver ischemia–reperfusion, liver transplantation, or severe septic state [11–13]. Furthermore, the capacity of the liver to excrete ICG accurately reflects the intracellular adenosine triphosphate (ATP) level and hence the energy status of hepatocytes, which are among the most decisive factors in terms of functionality and organ viability [14].

The aim of the present study was to selectively assess the time-dependent reactions of regional hepatic function to portal vein ligation by selective biliary drainage and assessment of biliary ICG excretion compared with the conventional parameters of liver regeneration and hepatic circulation.

2. Materials and methods

The experimental design was regulated in accordance with the National Institutes of Health guidelines for animal care and was approved by the Committee on Animal Experimentation of Semmelweis University (license number: PEI/001/313-4/2014). Male Wistar rats weighing 200–250 g were used (Semmelweis University, Central Animal Facility, Budapest, Hungary). Standard rat chow and water were provided *ad libitum*. Before the experiment, the rats were fasted overnight to minimize the effect of food ingestion on the liver blood and bile flow.

2.1. Experimental design and operative procedure

Portal vein ligation was performed as previously described [15]. Briefly, under general anesthesia, induced by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (7.5 mg/kg), the portal branches supplying the median, left lateral, and caudate lobes—approximately 80% of the total liver mass—were ligated. After operation, the animals were returned to their cages. Rats ($n = 36$) were randomly allocated into groups based on the length of the recovery period. Six animal groups were examined as follows: on the preoperative day (day 0, control group) and on postoperative days 1, 2, 3, 5, and 7 ($n = 6$ per time point), respectively. Additional eight animals were used to determine the size of liver lobules before ($n = 4$) and 7 d after ($n = 4$) PVO (Fig. 1).

At the indicated time points, animals were reanesthetized and a 22-gauge polyethylene catheter was placed into the right jugular vein for maintenance of anesthesia (25-mg/kg/h ketamine and 2.5-mg/kg/h xylazine) and for the administration of saline infusion (4 mL/kg/h) as well as ICG. Another 22-gauge polyethylene catheter was inserted into the left carotid artery for hemodynamic measurements. After relaparotomy, surface probes of the laser Doppler flowmeter were placed on the liver's superior right lateral (SRL) and left lateral lobes (LLL). After registration of the liver microcirculatory flow, the bile ducts of portal vein ligated (PVL) and portal vein nonligated (PVNL) lobes were selectively cannulated to assess the bile flow. ICG in a dose of 1.5 mg/mL (ICG-PULSION; PULSION Medical Systems, Munich, Germany) was injected, and then plasma disappearance rate (PDR) and biliary excretion of the dye were determined. After a 150-min period of bile collection, the portal vein was directly punctured with a 24-gauge needle to measure the portal pressure. At the end of the measurements, rats were exsanguinated via the right ventricular puncture and liver samples were excised (Fig. 1).

2.2. Assessment of hemodynamics and liver microcirculation

Blood pressure was measured by an invasive blood pressure monitoring system (Kent Scientific Corporation, Torrington, CT) via the cannulated right carotid artery. Portal pressure was evaluated by direct puncture of the portal vein (Kent Scientific Corporation). Liver microcirculation was assessed before ICG administration using a laser Doppler monitor (DRT4 device with DP1T surface probe; Moor Instruments Ltd, London, United Kingdom). Surface probes were placed on fixed locations of the liver's SRL (PVNL) and LLL (PVL). At least four measurements were performed at different sites of the lobes (5 min for each), and the mean of the measurements was calculated.