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Bile salts predict liver regeneration in rabbit model of portal vein embolization

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ABSTRACT

Background: Portal vein embolization (PVE) is employed to increase future remnant liver (FRL) volume through induction of hepatocellular regeneration in the nonembolized liver lobe. The regenerative response is commonly determined by CT volumetry after PVE. The aim of the study was to examine plasma bile salts and triglycerides in the prediction of the regenerative response following PVE.

Methods: PVE of the cranial liver lobe was performed in 15 rabbits, divided into three groups: NaCl (control), gelatin sponge (short-term occlusion), and polyvinyl alcohol particles with coils (PVAc, long-term occlusion). In all rabbits CT volumetry and blood sampling were performed prior to PVE and on days 3 and 7. Plasma bile salts and triglycerides were correlated with volume increase of the nonembolized liver lobe.

Results: After 3 and 7 d, respectively, FRL volume was increased in both embolized groups, with the largest hypertrophy response observed in the PVAc group. Plasma bile salt levels were increased after PVE, especially in the PVAc group at day 3 ($P < 0.01$ compared to gelatin sponge). Plasma bile salts at day 3 predicted FRL volume increase at day 7 showing a positive correlation of 0.811 ($P < 0.001$). Levels of triglycerides were not significantly altered in either of the PVE procedures.

Conclusions: Plasma bile salt levels early after PVE strongly correlated with the regenerative response in a rabbit model of PVE, showing more pronounced elevation with larger volume increase of the nonembolized lobe. Therefore, plasma bile salts, but not triglycerides, can be used in the prediction of the regenerative response after PVE.

1. Introduction

Resection of hepatic tumors is being performed with increasing frequency worldwide [1]. Complete resection of hepatic tumors remains the first choice for curative treatment of malignant liver tumors. The remnant liver, however, is sometimes too small to meet the needs of liver function and volume, and for this reason, these patients are considered

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unresectable. Various procedures have been developed to increase the size and function of the future remnant liver (FRL) preoperatively [1,2].

One way to increase the FRL in unresectable patients is portal vein embolization (PVE) of the lobe to be resected. PVE was first described in 1986 in Japan by Kinoshita [3]. Today, PVE is increasingly used in the preoperative management of patients proposed for liver resection in whom FRL volume is deemed insufficient. The usual method to assess liver hypertrophy in the nonembolized lobe following PVE is CT volumetry, performed 3–6 wk after PVE. A drawback of PVE is concomitant enhancement of tumor growth as a result of the release of regenerative factors after PVE. Prediction of effective hypertrophy at an earlier time point is therefore desirable, in order to minimize the waiting time between PVE and subsequent liver resection.

An experimental study by Huang *et al.* [4] showed increased serum bile salt levels during regeneration following partial hepatectomy in mice. In addition, this study showed that an elevation in serum bile salt levels accelerated liver regeneration, whereas a decrease in serum bile salts inhibited liver regrowth after partial liver resection [4]. The latter effect was confirmed in rat studies by Ueda *et al.* [5] and Dong *et al.* [6]. Another study demonstrated increased bile salt levels within the nonligated lobes after portal vein ligation in rats [7]. Subsequently, Hayashi *et al.* [8] showed a significant relation between increased bile salt levels and the degree of hypertrophy in the nonembolized lobe in humans [8]. Apart from these studies, little is known about the relation between bile salts, PVE, and the hypertrophy response of the liver.

In addition to the existence of a relation between liver regeneration and bile salts [9–11], triglycerides also accumulate during liver regeneration. Previous studies have shown that triglycerides accumulate in the rat liver 15–20 h after partial hepatectomy [12,13]. Miyamura *et al.* also revealed an accumulation of triglycerides in regenerating mouse livers 24 h after partial hepatectomy [14]. The precise role of bile salts and triglycerides in liver hypertrophy and regeneration is still unknown. The aim of this study, therefore, was to examine plasma bile salts and triglycerides in the prediction of the regenerative response following PVE in a rabbit model of PVE.

2. Materials and methods

2.1. Animal study

Fifteen female New Zealand White rabbits (Harlan, Gannat, France) with a mean weight of 3.0 ± 0.5 kg were acclimatized for 1 wk under standardized laboratory conditions in a temperature-controlled room. The animals were individually housed, had free access to standard laboratory food and water, and were subjected to a 12-h light/dark cycle per d. Experimental protocols were approved by the Institutional Animal Ethics Committee.

2.2. Experimental design

The rabbit liver consists of four liver lobes, three of which are positioned cranially, with the fourth located caudally [15]. In our rabbit PVE model the cranial liver lobes, which account for approximately 80% of the total liver volume, were embolized. The rabbits were divided into a control group receiving NaCl ($n = 5$) and two groups in which the portal vein to the cranial liver lobes was embolized by either liquefied gelatin sponge (short-term occlusion; $n = 5$) or polyvinyl alcohol particles and coils (PVAc, long-term occlusion; $n = 5$). The rabbit was placed in a supine position after subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic; Reckitt Benckiser Healthcare Limited, Hull, UK) and 0.02 mg/kg enrofloxacin (Baytril; Bayer Healthcare, Berlin, Germany). Rabbits were given enrofloxacin 0.02 mg/kg subcutaneously once a day for 3 d postoperatively. Animals were anesthetized by intramuscular injection of 25.0 mg/kg ketamine (Nimatek; Eurovet, Bladel, The Netherlands) and 0.2 mg/kg dexmedetomidine (Dexdomitor; Orion Corporation, Espoo, Finland). Isoflurane 1%–2% (Forene; Abbott Laboratories, Kent, UK) with O_2/air (1:0.7 L/min) was used to maintain anesthesia. Heart rate and arterial oxygen saturation were measured by pulse oximetry (Hewlett Packard M1165A model 56S; Hewlett Packard, Andover, MA) continuously throughout the procedure.

To identify the individual portal branches, a portography was made. After passing the portal branch to the caudal liver lobe, a microcatheter was positioned into the main portal branch supplying the cranial liver lobes. Control animals received 2.0 mL NaCl via the microcatheter. In the short-term occlusion group, liquefied gelatin sponge (Spongostan; Ferrosan, Soeborg, Denmark) was delivered until flow ceased. Animals in the long-term occlusion group received an initial mixture of contrast (Visipaque; GE Healthcare, Waukesha, WI) and 90–180 μm PVA particles (Cook, Bloomington, IN), followed by injection of 300–500 μm PVA particles until cessation of flow and placement of three platinum coils (6 mm, Tornado Embolization Microcoil; Cook, Bloomington, IN). All embolizations were performed by an interventional radiologist (K.P.vL.) with over 10 y experience. Further details of the embolization technique have been described elsewhere [16].

Portography directly after PVE confirmed total occlusion of the cranial portal blood flow in the embolization groups. The hypertrophy response of the caudal lobe was measured using CT volumetry before embolization and on day 3 and 7 postembolization. Serum bile salt and triglyceride levels were determined at baseline, at 3 h, and at days 1, 3, and 7 after PVE.

2.3. Liver volume

Multiphase contrast-enhanced CT scans were carried out in rabbits using the multislice helical scanner (Philips Medical Systems, Eindhoven, The Netherlands). Total liver, tumor, and FRL were delineated manually, after which the total liver volume (TLV), tumor volume (TV), and FRL volume (FRLV) were calculated with integrated software (Mx-View 3·52; Philips Medical Systems). The percentage of FRL was then calculated by the following formula: %FRL = (FRLV × 100%)/(TLV − TV). A detailed description of CT volumetry in rabbits is described elsewhere [16].