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

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Article history:
Received 21 May 2012
Received in revised form 14 June 2012
Accepted 15 June 2012
Available online 27 June 2012

Keywords:
Portal vein embolization
Liver regeneration
Bile salts
Triglycerides

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.

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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...
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 O2/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.v.L.) 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 liver 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].
2.4. Biochemical tests

In all blood samples, plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed to examine the degree of liver damage. Plasma bilirubin was determined as indirect measure of hepatic function (hepatic uptake and excretory function). Serum levels of triglycerides were also evaluated to examine liver regeneration. All above-mentioned parameters were determined by routine clinical chemistry. Total serum bile salts were assayed by an enzymatic method as per manufacturer’s instructions (Diazyme Laboratories, Poway, CA).

2.5. Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS 18.0; SPSS, Chicago, Illinois) and GraphPad Prism (GraphPad Software, San Diego, CA). CT volumetry data were compared using a mixed-model analysis based on ranked data. Continuous nonparametric data were compared by the Mann-Whitney U test. The Wilcoxon signed rank test was used for nonparametric continuous data for different time points within groups. Correlation between variables was tested using the Pearson r correlation coefficient. All statistical tests were 2-tailed and differences were considered significant at a P value of <0.05. Data were expressed as means ± SD, unless stated otherwise.

3. Results

3.1. Embolization with PVA particles and coils induces a strong hypertrophy response in rabbits

The PVE procedure was performed successfully in all rabbits. Before embolization, FRL volume, expressed as a percentage...
of total liver volume (%FRL), was 26.3% ± 1.4%, 25.7% ± 3.6%, and 22.4% ± 1.2% for the control, gelatin sponge, and PVAc groups, respectively, with a significant difference between the control group and PVAc (P = 0.009). As expected, %FRL remained constant during the follow-up period in the control group (Fig. 1). After 3 d, %FRL was increased in both embolized groups, with the largest hypertrophy response in the PVAc group. Further increase of %FRL at day 7 post-PVE was most striking in the PVAc group, with a near-doubling of lobular volume (80%) induced by PVE.

Both PVE procedures resulted in a transient elevation of transaminases that peaked after the first day, with normalization of transaminases after 3–7 d (Fig. 2A and B). Bilirubin levels were not significantly altered at the respective time points after PVE (data not shown).

3.2. Bile salts—but not triglycerides—are elevated after PVE

Baseline bile salt levels were similar in the three groups, and remained constant throughout the follow-up period in the control group (Fig. 3A). In contrast, a rapid increase in bile salt levels was apparent already after 3 h in the gelatin sponge group and levels remained elevated for at least 3 d before gradual return to baseline levels. The profile of bile salt levels in the PVAc group differed, peaking at a later time point (3 d) while remaining elevated after 7 d. Unlike bile salts, levels of triglycerides were not significantly changed by either of the PVE procedures (Fig. 3B).

3.3. Correlation of bile salts and triglycerides with FRL volume

Correlation data of plasma bile salt levels and increase in %FRL are shown in Figs. 4 and 5. Analysis of the pooled results of all rabbits showed positive correlations of bile salt levels after PVE, and %FRL or increase in %FRL on days 3 and 7 following PVE. For triglycerides, no significant correlations were observed with (increase of) %FRL (data not shown).

For the separate groups (NaCl, gelatin sponge, and PVAc), no significant correlations between plasma AST or ALT levels on day 1 and (increase in) %FRL were observed. When
combining the groups (n = 15), a significant correlation was found between ALT on day 1 and %FRL on day 7 (r = 0.530, P = 0.042), increase in %FRL on day 3 (r = 0.526, P = 0.044), and increase in %FRL on day 7 (r = 0.603, P = 0.017). For AST on day 1 a positive correlation was seen with the increase in %FRL on day 7 (r = 0.542, P = 0.037).

4. Discussion

Plasma bile salts and triglycerides were examined as predictive factors for the regenerative response following PVE. An established rabbit model of PVE was used [17–19] to study possible correlations between plasma bile salt and triglyceride levels and growth of FRL using two different embolization agents: gelatin sponge and PVAc. This analysis showed that plasma bile salts—but not triglycerides—significantly predicted the hypertrophy response after PVE.

To our knowledge, a predictive correlation between bile salts and FRL growth has not been previously explored in an animal model of PVE, while only a single study has addressed this issue in patients [8]. Several experimental studies showed an increase in serum bile salts or triglycerides during liver regeneration after partial hepatectomy [4–6]. This is partly in line with our observations. Bile salts increased significantly in the rabbit model after PVE, suggesting that bile salt levels are a useful predictor of effective hypertrophy of the non-embolized liver lobe after PVE. However, we did not find significant changes in serum triglyceride levels following PVE in the rabbit PVE model, despite multiple studies reporting a significant increase in serum and hepatic triglycerides during the hypertrophy response [12,13]. The reason for the absence of a significant increase in triglycerides following PVE in our animal study could be 2-fold. First, most studies have been performed in rat models, while we chose a rabbit model, since this model is more compatible with the human situation; and the size of rats brings along surgical and technical difficulties.

Fig. 5 — Significant correlations (r) are shown between bile salts on day 1 (A), day 3 (B), and day 7 (C) and the increase of FRL on day 7. (Color version of figure is available online.)
limitations. Volumetric assessment of liver lobes in rats is more difficult, and introduction of the cannula in the portal vein for embolization is challenging. Second, the above-mentioned studies in literature were performed in combination with partial hepatectomy. Therefore, several pathways were activated, resulting in a hypertrophy response of FRL after liver resection, which could subsequently lead to elevated triglyceride levels. Thus, whereas hepatectomy in rats resulted in increased triglycerides, PVE in rabbits did not affect serum triglyceride levels, suggesting different mechanisms in the regenerative response after resection or PVE [20].

Serum bile salts are increased after PVE, especially upon long-term embolization using PVA and coils (Fig. 3A). Plasma bile salt levels at day 3 predicted FRL volume increase at day 7 with a positive correlation of 0.811. The increase in FRL volume is most pronounced in the first week after PVE measured by CT volumetry in a rabbit model with a maximum hypertrophy response on day 7, although the volume increase of FRL is greatest during the first 3 wk after PVE in humans. Previous clinical studies showed considerable variation in plasma bile salt levels prior to PVE, based on underlying liver disease [8,21–23]. To compensate for these pre-embolization variations, the increase of bile salt levels presumably is a better way to predict FRL growth, as compared to absolute bile salt values.

Our study has a limitation. The initial FRL in the control group (26.3% ± 1.4%) was significantly different compared to the PVAc group (22.4% ± 1.2%) in the rabbit PVE model, although no interventions had been performed. As expected, however, the increase in %FRL was significantly higher in the PVAc group 7 d after PVE, and therefore did not seem to be influenced by baseline values.

In conclusion, a positive correlation was found between the increase in %FRL on day 3 and plasma bile salts in a rabbit model of PVE. In rabbits with a greater hypertrophy response after PVE, the increases in bile salts were also larger. Plasma bile salts, therefore, show predictive value in the assessment of the hypertrophy response after PVE.

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