

# Impact of Age on Survival after Partial Portal Vein Arterialization for the Treatment of Post-Hepatectomy Liver Failure in a Rat Model

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## Abstract

**Introduction:** Post-operative liver failure (PLF) occurs in approximately 10% of patients undergoing major hepatectomy. Partial portal vein arterialization (PPVA) enhances the regenerative capacity of the resected liver. The aim of this study was to investigate the impact of age on survival after PPVA for the treatment of post-operative liver failure in a rat model.

**Materials and Methods:** 24 rats underwent extended liver resection, that led to PLF. 12 rats were divided in 2 groups treated with PPVA: group 1a-young rats (n=6, age 2 months) and group 2a-old rats (n=6, age 30 months). Two control groups of rats of the same age were not treated with PPVA: group 1b-young and group 2b-old.

**Results:** On postoperative day 7, no significant differences were observed among all groups in terms of ALT levels, prothrombin activity and serum creatinine. As for the liver regeneration markers, the level of mitotic index was greater in the groups treated with PPVA compared to the control groups (without significant differences between young and old groups). The 75% (9/12) of the rats treated with PPVA survived up to 7 days, with no significant differences between young (5/6) and elderly rats 66.7% (4/6).

**Conclusion:** PPVA treatment had the same beneficial effect both in young and old rats.

**Keywords** Hepatectomy; Liver failure; Portal vein arterialization

## Introduction

Extended liver resection may result in postoperative liver failure (PLF) that can be fatal [1]. In some cases liver transplantation is suggested but the organ is not always available. PLF occurs in approximately 10% of patients undergoing major hepatectomy and the main risk factors are the presence of comorbid conditions, pre-existent liver disease and small remnant liver volume [1]. Moreover, it is acknowledged that with increasing age, the liver tissue becomes more sensitive to ischemia-reperfusion injury and its regenerative capacity is reduced [2]. There is experimental [3-6] and clinical [7-13] evidence that the liver hyper-oxygenation through the partial portal vein arterialization (PPVA) enhances the regenerative capacity of the resected liver. This event is probably due to the improvement of the microcirculation flow and the tissue oxygen supply. Indeed, this process would satisfy the increased energy demand occurring during the liver regeneration by favouring the oxidative metabolism of hepatocytes [14,15]. Thus, this study aimed to assess whether age may be a major determinant of overall survival after PPVA procedure for the treatment of post-operative liver failure (PLF) in a rat model.

## Materials and Methods

A total of 24 male Sprague-Dawley rats weighing 200 to 430 g underwent extended liver resection (85%) which was performed by

removing the median, left and caudate lobes. This procedure led to postoperative liver failure (PLF) in all the 12 rats which were divided in 2 study groups treated with PPVA: group 1a-young rats (n=6, age 2 months, weight 200-300 g) and group 2a-old rats (n=6, age 30 months, weight 350-430 g). Two control groups of rats of the same age were not treated with PPVA: group 1b-young and group 2b-old. Under enflurane anesthesia, the abdomen of the study group's rats was surgically opened and a left nephrectomy performed. A 24-G polyethylene tube was subsequently placed to connect the left renal artery to the distal portion of the splenic vein but temporarily closed with a nontraumatic clamp. Afterwards, the spleen was removed and a partial hepatectomy performed. Finally, the shunt was opened by removing the clamp and the peritoneal cavity was closed in two layers. At the end of the operation, 1.0 mL of heparinized physiological saline solution was administered intravenously.

We evaluated the 7-day postoperative survival rate. At the time of death, blood samples (1 mL) collected from the portal vein were immediately assessed for gas content (OSM3 blood gas analyzer, Radiometer, Copenhagen, Denmark) and blood samples (1 mL) obtained from the inferior vena cava were used to measure serum alanine aminotransferase (ALT), prothrombin time (PT) and serum creatinine (SCr). Formalin-fixed sections were stained with hematoxylin-eosin, whereas ethanol-fixed sections were immunohistochemically stained to reveal mitotic index.

Animal care and experimental procedures were approved by the institutional ethical committee and conducted according to the

guidelines for the care and use of laboratory animals approved by our institution.

Statistical differences between groups were analyzed by two-ways analysis of variance. Survivals were evaluated using Kaplan-Meier curves with differences assessed with the log-rank test. Statistical analysis was performed by running the SPSS statistical package on a personal computer. Data are reported as mean values with standard errors. Two-tailed P values of less than 0.05 were considered as significant.

## Results

As expected, in young and old rats PPVA treatment induced an increase in O<sub>2</sub> partial pressure (70 ± 1.8 vs. 70 ± 1.7 mmHg

respectively) and oxygen saturation (89.8 ± 2.4 vs. 88 ± 2.6mmHg, respectively) with a concomitant decrease in CO<sub>2</sub> partial pressure (37.4 ± 2.6 vs. 39.0 ± 2.5 mmHg, respectively). These parameters were registered from the portal blood on postoperative day 7 suggesting that the arterial venous shunt remained patent and functional for such experimental time. Furthermore, no significant differences were observed among all groups in terms of ALT levels, prothrombin activity and serum creatinine up to seven days postoperatively. As for the liver regeneration markers, we detected a greater level of mitotic index in the groups treated with PPVA compared to the control groups (without significant differences between young and old) (Table 1).

Parameter	PPVA treated		PPVA non treated	
	Group 1a-young rats	Group 2a- old rats	Group 1b-young rats*	Group 2b-old rats^
Basal: ALT (U/L)	21+0.4	22+0.7	22+0.3	22+0.5
At sacrifice: ALT (U/L)	45 ± 0.6	48 ± 0.8	50	53
Basal: PT (%)	91+3	89 + 4	90+3	91+2
At sacrifice: PT (%)	90 ± 4	84 ± 4	86	79
Basal SCr (mg/dL)	0.87+0.1	0.88+0.2	0.89+01	0.88+03
At sacrifice: SCr (mg/dL)	0.90 ± 0.1	0.93 ± 0.2	0.90	0.95
N° mitosis/mm2	34.5	29.2	22.4	20.3

Note: \*2 survivors; ^1 survivor.

**Table 1:** Biochemical parameters and mitotic index among young and old rats of both study and control groups.

Weight (gr)	PPVA treated		PPV non treated	
	Group 1a-young rats	Group 2a-old rats	Group 1b-young rats*	Group 2b- old rats^
Rat	200-300	350-430	200-300	350-430
Resected liver	7.8	11.2	8.1	11.5
Remnant liver	2.2	2.8	1.9	2.5
Preoperative liver	10	14	10	14
Liver at sacrifice	6	7.8	4	5.2

Note: \*2 survivors; ^1 survivor

**Table 2:** Liver weights: Differences between control and PPVA treated groups.

After extensive hepatic resection (85% of the liver), the overall survival at 7 days was only 25% (3/12) in rats not PPVA treated without significant differences regarding age (33.7%, 2/6 young vs. 16.7%, 1/6 elderly). In contrast, the 75% (9/12) of the rats treated with PPVA survived up to 7 days (p<0.05), with no significant differences between young 83.3% (5/6) and elderly rats 66.7% (4/6).

Moreover, the liver weights at sacrifice of the two PA treated groups were significantly greater than that of the two respective control groups (group 1a=6 g vs. group 1b=4 g and group 2a=7.8 g vs. group 2b=5.2 g; p<0.05) (Table 2).

## Discussion

Mortality rates after critical major hepatectomy have been reported to be as high as 30% with PHLF representing one of the most dreadful complications [16]. The removal of large portions of liver parenchyma is sometimes necessary to fully excise the neoplastic tissue especially for biliary tract tumors. Aside from cancer surgical treatment, extended hepatic resections are also necessary in case of severe injury to the liver parenchyma (e.g., trauma). In 2011, the International Study Group of Liver Surgery (ISGLS) defined PHLF as “a post-operatively acquired deterioration in the ability of the liver to maintain its

synthetic, excretory, and detoxifying functions, which are characterized by an increased INR and concomitant hyperbilirubinemia on or after postoperative day 5” [17]. However, the severity of its clinical features ranges from mild temporary hepatic insufficiency to fulminant hepatic failure. During the pre-operative assessment in the intent to reduce postoperative morbidity and mortality it is important the evaluation of risk factors like male gender, obesity, diabetes, neoadjuvant treatment with chemotherapy and underlying cirrhosis. The complication severity leading to exitus is related to the quantity of parenchyma removed, but the patient's age plays an important role too. Over the years, the liver parenchyma becomes more sensitive to ischemia-reperfusion injury and its regenerative capacity tends to reduce [14]. The ischemic preconditioning and intermittent clamping by Pringle maneuver are the only intraoperative strategies used during liver resections to reduce the ischemia-reperfusion injury and consequently the risk of postoperative liver failure. Furthermore, the use of PPVA has been widely carried out in clinical practice as a bridge procedure to reduce the risk of acute liver failure (ALF) and to guarantee a better chance for long-term survival. It is well known that the portal blood represents approximately 75% of the total blood flow to the liver, has an oxygen partial pressure <40 mmHg and a low hydrostatic pressure (5-10 mmHg).

Since the PPVA raises the hydrostatic pressure of the portal blood and especially the percentage of oxygen saturation to values similar to those found in the arterial blood, we assume that this strong oxygen increase satisfies the high metabolic demand of the regenerating hepatocytes by favouring the oxidative metabolism. The exact mechanism by which PPVA stimulates liver regeneration is not currently known and further studies should be planned. However, it appears reasonable to hypothesize that the extra oxygen supplied to the liver through the arterovenous shunt [15] mediates the regeneration promotion.

## Conclusion

This study revisited the benefits of PPVA in the promotion of liver regeneration demonstrating that such surgical procedure was protective against PLF induced by hepatectomy in a population of young and old rats. The presence of an additional supply of oxygenated blood in the portal venous system following the PPVA procedure has had positive effects on energy metabolism and has led to a significantly higher survival of arterialized rats. The PPVA treatment had the same beneficial effects on both young and old rats, with no statistically significant differences. Our conclusion suggests that even the elderly liver responds well to the regenerative stimulation induced by the PPVA shunting. However, further large-scale studies and applications are needed to confirm the effectiveness of this procedure.

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