

Histological and ultrastructural studies of liver parenchyma in ALPPS procedure

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BACKGROUND

Associated liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a recently introduced novel technique which can resect all liver tumors despite the small size of future liver remnant (FLR). This procedure induces rapid growth of the FLR comparing any other technique. We previously reported volumetric gain of FLR is very fast, but liver function is not enough. The aim of this study is to clarify differences of regeneration by morphologic features in the non-neoplastic liver on ALPPS procedure, comparing those of hepatectomy following portal vein embolization (PVE).

PATIENTS and METHODS

Eight patients with unresectable multiple liver metastases from colorectal cancer who received the ALPPS procedure were analyzed. For comparison, 14 patients were treated with hepatectomy following PVE were also analyzed. Specimens for pathological examination was obtained at the second operation. We investigate the histologic findings in the non-neoplastic liver of ALPPS in treating metastatic liver disease by comparing those of hepatectomy following PVE.

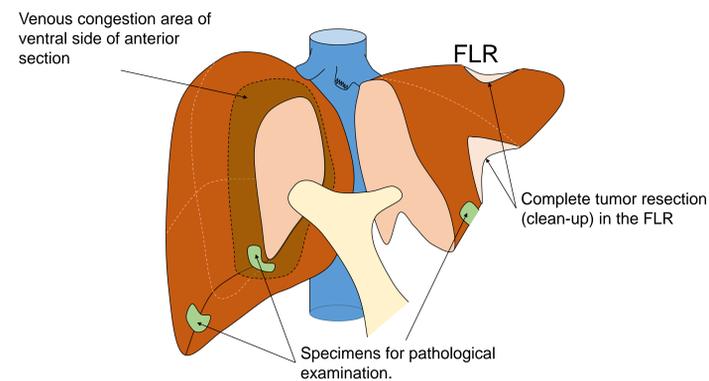


Figure 1. Our ALPPS procedure is extended right hemihepatectomy with middle hepatic vein (MHV). Right portal vein was ligated at first operation. There is no complete devascularized area. Specimens for pathological examination were obtained at the second operation from 3 different areas within FLR, ventral side of anterior section, posterior section (above shown).

Table 1. Demographic and clinical characteristics of patient groups defined by procedure.

Variables	ALPPS (n=8)	PVE (n=14)	P-value
Mean and median age, years (range)	67.5 ± 5.1 (68, 62-78)	67.4 ± 12.6 (72, 35-81)	0.297
Gender			0.662
Male	4 (50%)	9 (64%)	
Female	4 (50%)	5 (36%)	
ICGR15, %	15.3 ± 7.6 (16.4, 2.5-25.9)	14.3 ± 7.0 (14.1, 5.0-25.8)	0.57
Diagnosis			>0.999
CRLM	8 (100%)	12 (86%)	
Gbc	0 (0%)	1 (7%)	
IPNB	0 (0%)	1 (7%)	
Tumor-related factors			0.764
Number	12.8 ± 4.7 (13, 4-19)	13.0 ± 11.5 (12, 1-46)	
Maximum size, mm	22.6 ± 9.1 (21, 1-37)	50.4 ± 46.0 (35.5, 20-200)	0.010
Chemotherapy before Hx.			0.515
Yes	8 (100%)	12 (86%)	
No	0 (0%)	2 (14%)	

ALPPS, associated liver partition and portal vein ligation for staged hepatectomy; PVE, portal vein embolization; ICGR15, indocyanine green retention rate at 15 min; CRLM, colorectal liver metastasis; GB-ca., Gallbladder cancer; IPNB, Intraductal papillary neoplasm with an associated invasive carcinoma; Hx, hepatectomy

RESULTS

Table 2. Changes in eFLRV, ratio between before first operation and before second operation.

Variables	ALPPS (n=8)	PVE (n=14)	P-value
Pre-eFLRV (ml)	303.9 ± 61.1	290.2 ± 72.5	0.188
Second-eFLRV (ml)	457.6 ± 79.5	437.6 ± 95.3	0.441
Ratio	1.5 ± 0.2	1.6 ± 0.4	0.525
Duration (day)	10.9 ± 2.0	51.6 ± 32.8	< 0.001
Volume Gain (ml)	153.6 ± 46.0	147.4 ± 81.6	0.616
Volume Gain/day (ml)	14.4 ± 4.8	3.6 ± 2.2	< 0.001

eFLRV, estimated future liver remnant volume calculated by CT volumetry; Pre-eFLRV, eFLRV before first operation; Second-eFLRV, eFLRV before second operation. Ratio was calculated according to the formula: pre-eFLRV/Second-eFLRV. Duration means days between first operation and second operation. Volume gain/day was calculated according to the formula: volume gain/duration. Values is the mean ± SD.

Table 3. Histological findings at the second operation from Ventral side of anterior section and posterior section.

Variables	ALPPS (n=8)	PVE (n=14)	P-value
Ventral Side of Anterior section			
Hepatocyte Atrophy	8/8 (100%)	3/14 (21.4%)	0.001
Congestion	7/8 (87.5%)	2/14 (14.3%)	0.001
Sinusoidal Dilatation	8/8 (100%)	4/14 (28.6%)	0.002
Fibrosis	7/8 (87.5%)	0/4 (0%)	< 0.001
Degeneration and Necrosis	7/8 (87.5%)	1/14 (25%)	< 0.001
Posterior section			
Hepatocyte Atrophy	2/8 (25.0%)	2/14 (14.3%)	0.602
Congestion	1/8 (12.5%)	3/14 (21.4%)	>0.999
Sinusoidal Dilatation	5/8 (62.5%)	6/14 (42.9%)	0.659
Fibrosis	1/8 (12.5%)	0/14 (0%)	0.364
Degeneration and Necrosis	2/8 (25.0%)	1/14 (7.1%)	0.527

Table 4. Histological findings at the second operation from FLR.

Variables	ALPPS (n=6)	PVE (n=12)	P-Value
FLR			
Brightness of Cytoplasm	3/6 (50.0%)	0/12 (0.0%)	0.025
Narrowing of Sinusoids	3/6 (50.0%)	1/12 (8.3%)	0.025
Hepatocyte Density	306.7±19.2 (cells/HPF)	192.6±49.0 (cells/HPF)	< 0.001
Size of Hepatocyte	20.3±1.0 (µm)	30.9±5.2 (µm)	< 0.001

FLR, future liver remnant. Hepatocyte density in FLR was average cell counts of four high-power fields (x400). Size of hepatocyte was average cell size of selected ten hepatocyte in high-power field (x400).

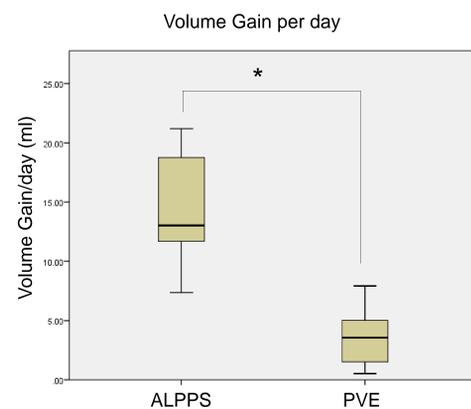


Figure 2. The volume gain per day is shown. *, P < 0.001.

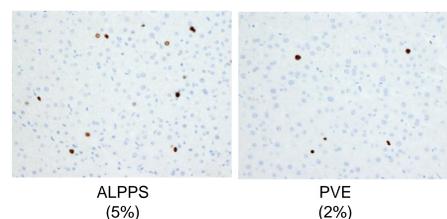


Figure 3. Ki-67 labeling index.

Light microscopy of three different areas

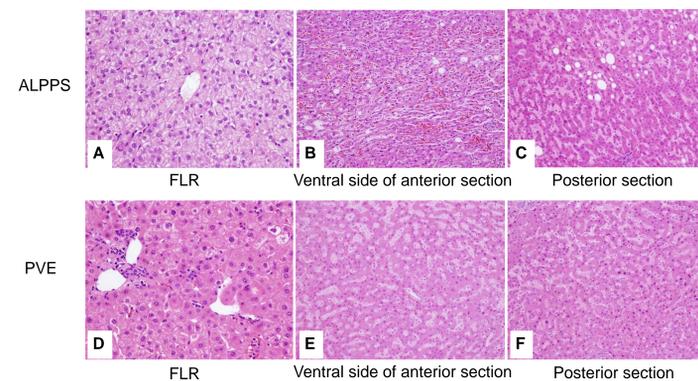


Figure 4. Light microscopy of three different areas. A, B and C are images of ALPPS procedure. D, E and F are images of PVE procedure. Magnification: A and D: x400, B, C, E and F: x200

- In the ALPPS group, specific findings in the area within FLR were brightness of cytoplasm because of glycogen abundant and sinusoid was narrowing due to increase number of hepatocytes (Fig 4A, Table 4). Moreover, individual hepatocytes are small (Table 4). On the other hand, hepatocytes in the PVE group were swollen and size of hepatocytes are larger than that of ALPPS group, and hepatocytes were well-stained in eosin. It means that abundant mitochondria exist in hepatocytes (Fig 4D, Table 4).

- In the ventral side of anterior section, sinusoidal dilatation was seen both group, and in ALPPS group severe congestion was further enhanced to the deportalized ventral anterior section. As a result, specific findings in this area were marked hepatocyte degeneration and fibrosis (Fig 4B, Table 4)

- In the posterior section, sinusoidal dilatation was detected in both group. But there were no appreciable differences in the posterior section between both group.

Electron microscopy of hepatocytes in FRL

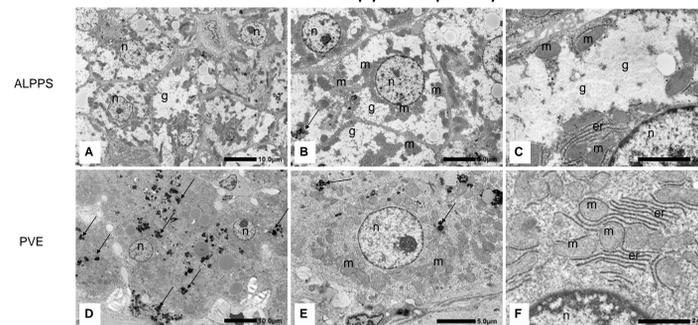


Figure 5. Electron microscopy of FRL in second operation. A, B and C are images of ALPPS procedure. D, E and F are images of PVE procedure. FLR, future liver remnant; n, nuclear; g, glycogen granules; m, mitochondria; er, endoplasmic reticulum. Arrow means lipofuscin depositions. Magnification: A and D: x 2,500, B and E: x 5,000, C and F: x 20,000

- In the ALPPS group, cytoplasmic organelle such as mitochondria, rough endoplasmic reticulum were located in the peri-nuclear sites or cytoplasmic membrane. The vacant cytoplasm is filled with glycogen granules. There are fewer cytoplasmic organelle compared with PVE group. Most of the lipofuscin granules are not recognized (Fig 5A, 5B, 5C). These findings resembles an image observed in recovery state from acute hepatitis.

- In the PVE group, lipofuscin granules in the hepatocyte were more frequently observed and sinusoidal space was larger than in the ALPPS group. A lot of cytoplasmic organelle were recognized in cytoplasm and well developed, it is thought with a mature cell (Fig 5D, 5E, 5F).

Electron microscopy of hepatocytes in VAS

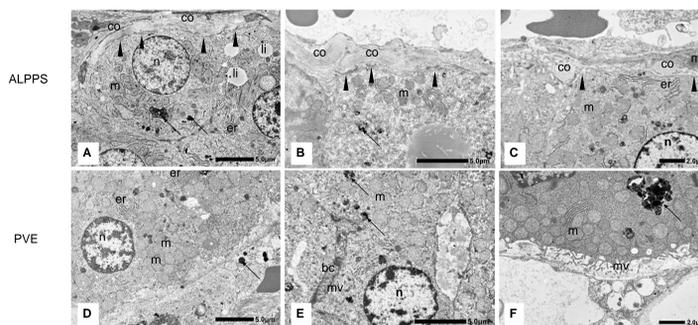


Figure 6. Electron microscopy of VAS in second operation. A, B and C are images of ALPPS procedure. D, E and F are images of PVE procedure. VAS, Ventral side of anterior section; co, collagen bundles; n, nuclear; m, mitochondria; er, endoplasmic reticulum; li, lipid; mv, microvilli; bc, bile canaliculus. Arrow means lipofuscin depositions. Atrophy and loss of hepatocyte microvilli (arrowheads). Magnification: A and D: x 6,000, B and E: x 8,000, C and F: x 10,000

- In the ALPPS group, bundles of collagen fibers were encountered in the space of Disse. Hepatocyte were atrophic change and its cell membrane waves. Hepatocyte microvilli itself were atrophy and shortening, and also the number of microvilli decreased (Fig 6A, 6B, 6C).

- In the PVE group, No hepatocyte microvilli atrophy was not detected in the bile canaliculus (Fig 6E). On the contrary, hepatocyte microvilli in the Disse space were well developed than ALPPS group (Fig 6F).

Electron microscopy of hepatocytes in PS

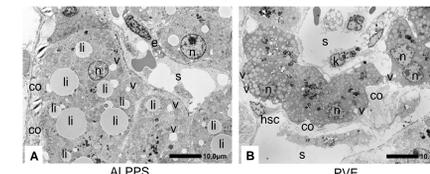


Figure 7. Electron microscopy of PS in second operation. A is an image of ALPPS procedure. B is an image of PVE procedure. PS, posterior section; s, endothelial cell; n, nuclear; co, collagen bundles; s, sinusoid; li, lipid; v, vacuole; k, Kupffer cell; hsc, hepatic stellate cell. Magnification: A and B: x 2,500

- In the ALPPS group, hepatic sinusoids were dilated and vacuoles detected in the hepatocyte. The vacuolation of liver cells is due to the invagination of the liver cell membrane by mechanical force. Bundles of collagen fibers were seen (Fig 7A).

- In the PVE group, dilation of hepatic sinusoids and bundles of collagen fibers were also detected. Hepatocytes were transformed and atrophy, further nuclear membrane changed wavy and transformed. Degree of these findings is stronger than in the ALPPS group.

DISCUSSION

Overall, our results strongly suggested that hepatocytes in FLR with ALPPS procedure could be immature cells morphologically. Therefore, the functional increase in FLR with ALPPS is not reached to a rapid liver volume increase because of immaturity of the hepatocytes.

CONCLUSIONS

ALPPS induces a rapid volume increase of the FLR. However, regenerative hepatocytes of the FLR in ALPPS might be immature, and it is suggested that a function did not catch up with even if estimated FLR volume increase. We believe ALPPS should be performed with caution, especially regarding not only volume increase in FLR but also functional recovery in FLR.