Nagoya J. Med. Sci. 48. 11 ~ 20, 1986

THE INFLUENCE OF PORTAL VEIN OCCLUSION ON LIVER MITOCHONDRIA IN RATS AFTER RELEASING BILIARY OBSTRUCTION

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ABSTRACT

Biliary obstruction often follows advanced cancers of the biliary system, such as pancreas cancer, which requires a resection of the involved portion of the portal vein. To improve the patient's state of obstructive jaundice, bile drainage should be performed before the operation. Temporary occlusion of the portal vein is inevitable for a resection and reconstruction of the portal vein, and its detrimental influence on the liver remains to be resolved. In this study, the effect of portal vein occlusion on rat liver mitochondria was investigated after releasing biliary obstruction. The common bile duct of the male Donryu rat was ligated and, after a certain duration, was recanalized to allow for external bile drainage. Then the portal blood flow was blocked for either 15 or 30-min. At the end of the experiment, the following parameters were examined: the respiratory functions and ultrastructures of liver mitochondria, and the serum levels of hepatic enzymes. A significant reduction of the respiratory functions was observed in the 30-min occlusion group regardless of the length of the biliary obstruction period; also the ultrastructures of the liver mitochondria showed severe changes associated with increased s-GPT and m-GOT. These results indicate that 15-min occlusion of the portal vein after releasing biliary obstruction has a negligible effect on the liver functions, but 30-min occlusion induces dysfunction of liver cells. Therefore, additional countermeasures to clear such liver dysfunction should be employed in biliary tract surgery, provided that a prolonged portal vein occlusion is inevitable.

Key Words: occlusion of the portal vein, liver mitochondria, respiratory function, obstructive jaundice, bile drainage

INTRODUCTION

Temporary occlusion of the portal vein in a resection of the involved portion of the portal vein must be considered to result in liver ischemia, as the portal vein is one of the afferent blood supplies to the liver. Various investigations (1–5) of and countermeasures (6) for the detrimental influence on a living body due to portal vein occlusion have been conducted. However, few studies have dealt with the influence of portal vein occlusion, on the liver. Especially the influence after release of the biliary obstruction.

Clinically, high incidence of obstructive jaundice is observed in cases of advanced cancer such as pancreas cancer; therefore, surgery is generally performed after bile drainage. The author investigated the important points to note during and after an operation by studying the influence of portal vein occlusion on the liver after biliary decompression using an experimental model (7) thought to be the most similar to the patients.

In the present study, obstructive jaundice was induced in Donryu rats and, after a certain period, biliary decompression was conducted. Temporary occlusion of the portal vein was then performed in the model after sufficient biliary decompression. The detrimental influence on the

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Received for Publication May 28, 1985

liver after portal vein occlusion was examined by the respiratory function and the ultrastructures of liver mitochondria, the main energy-producing organella in liver cells, and by biochemical data of serum.

MATERIALS AND METHODS

1. *Experimental animals and their maintenance*

Male Donryu rats aged 3–4 months and weighing 300–400 g were maintained about one month in an air-conditioned room preoperatively. Pelleted food (Oriental Kobo, Ltd., MF) was given with water *ad libitum.*

2. *Biliary obstruction, biliary decompression, and occlusion of the portal vein*

Under anesthesia with Nembutal (40 mg/kg body weight) injected into the abdominal cavity, a median laparotomy was conducted and a vinyl tube with an outer diameter of 1 mm and an inner diameter of 0.5 mm (Hakko Denki Seisakusyo, Ltd.) was inserted into the extrahepatic bile duct according to the cut-down technique in order to excrete bile. Complete obstructive jaundice was induced by ligating the end of the tube drawn out of the abdominal cavity. After either 3 days or 14 days of biliary obstruction, the ligation of the vinyl tube was released to allow for external bile drainage (7). After relaparotomy under anesthesia, induced as above, and after dissection of adhesions around the portal vein, occlusion of the portal vein was done by occluding the superior mesentric artery and the portal vein by small clips (Maruho Hatsujo Kogyo, Ltd.)

3. *Experimental method*

First we made two models with biliary decompression as follows: 1) After 3 days of biliary obstruction, biliary decompression was conducted and conditions of mitochondria were observed on the 1st, 3rd and 5th day (J-3-R group); 2) After 14 days of biliary obstruction, biliary decompression was done and mitochondrial conditions were observed on the 1st, 3rd, 5th and 10th day (J-14-R group).

As the second step in the occlusion of the portal vein, 2 cases from both groups as follows: J-3-R-5 group, 3 days of biliary obstruction and 5 days after biliary decompression; and from J-14-R-10 group, one with 14 days of biliary obstruction and one 10 days after biliary decompression and also it was performed in the control group (C group). i) Occlusion of the portal vein for 15-min or 30-min was conducted in J-3-R-5 group (J-3-R-5-O-15, J-3-R-5-O-30). ii) Occlusion of the portal vein for 15-min or 30-min was conducted in J-14-R-10 group (J-14-R-10-O-15, J-14-R-10-O-30). iii) Occlusion of the portal vein for 15-min or 30-min was conducted in C group (C-O-15, C-O-30). Each group contained from 6 to 9 rats.

The rat was decapitated and the fresh liver was collected after blood gathering. Liver mitochondria were prepared by the method of Aoyama *et al.* (8). Mitochondrial function was measured polarographically using an oxygen electrode (Beckman oxygen sensor 39550) according to the method of Chance and Hagihara (9). Oxygen consumption of mitochondria was measured from State 1 to State 5 according to the definition of Chance and Williams (10). From the tangent and its intraseption point of the obtained curve, oxygen consumption and its rate were calculated. Respiratory control index (RCI) was determined by the ratio of the rate of oxygen consumption in the presence of ADP and that in the absence of ADP.

$$\text{RCI} = \frac{\text{Rate of oxygen consumption in State 3}}{\text{Rate of oxygen consumption in State 4}}$$

ADP/O ratio was determined by the ratio of moles of ADP phosphorylated to atoms of oxygen consumed.

$$\text{ADP/O} = \frac{\text{Amount of ADP (1 } \mu\text{mol)}}{\text{Amount of oxygen consumed in State 3}}$$

State 3 respiration was determined as the rate of oxygen consumption in the presence of ADP. ATP formation was determined as the product of ADP/O ratio and State 3 respiration. Mitochondrial protein was determined by the biuret method.

Biochemical examinations were performed in serum which was taken after decapitation. Total bilirubin was measured by alkaliazobilirubin method; s-GOT and s-GPT, by UV method; and mitochondrial GOT (m-GOT), by column UV method. These are general biochemical methods of the respective measurements.

A part of the liver was used for electron microscopic observation. Fresh livers isolated from rats were fixed immediately in 2% glutaraldehyde followed by 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Epon 812. Ultrathin sections were double-stained with uranyl-acetate and lead-citrate and observed by an electron microscope (Hitachi H-500). The various fields were selected at random to compare the morphology of mitochondria with the biochemical data.

The data were expressed as the mean ± standard deviation and statistical analyses were made by Student's t-test. P values less than 0.05 were determined to be significant.

RESULTS

1. *Recovery of mitochondrial functions after biliary decompression*

Recovery of mitochondrial functions after biliary decompression in J-3-R group were observed in the time course. RCI showed a significant P value of less than 0.05 on the 3rd day compared with that in C group, although it recovered to 4.49 ± 0.27, which was equal to the normal level, on the 5th day.

In J-14-R group, RCI was significantly lower ($P<0.01$) than that in C group until the 5th day, but it recovered to the normal level on the 10th day (Fig. 1).

Serum biochemical data recovered earlier than mitochondrial functions as described by Miyata (11). They recovered on the 3rd day in J-3-R group and on the 5th day in J-14-R group. To clarify the delay in the recovery of mitochondrial functions, J-3-R-5 group and J-14-R-10 group were considered suitable for testing portal vein occlusion after release of biliary obstruction. Experimental occlusion of the portal vein was also performed in C group (Sham operation group) which was without jaundice.

2. *Changes in mitochondrial functions by occlusion of the portal vein*

In C group, RCI did not decrease after 15-min occlusion but it decreased to 3.99 ± 0.25 ($P<0.01$) after 30-min occlusion. ADP/O ratio showed no reduction after 30-min occlusion. State 3 respiration and ATP formation showed decreases ($P<0.01$) after 30-min occlusion (Fig. 2).

In J-3-R-5 group, RCI did not decrease after 15-min occlusion but decreased to 3.64 ± 0.32 ($P<0.001$) after 30-min occlusion. ADP/O ratio showed a decrease after both 15-min and 30-min occlusion (Fig. 3).

In J-14-R-10 group, RCI did not decrease after 15-min occlusion but decreased to 3.40 ± 0.32 ($P<0.001$) after 30-min occlusion. ADP/O ratio, State 3 respiration, and ATP formation did not decrease significantly, but they showed a decreasing tendency (Fig. 4).

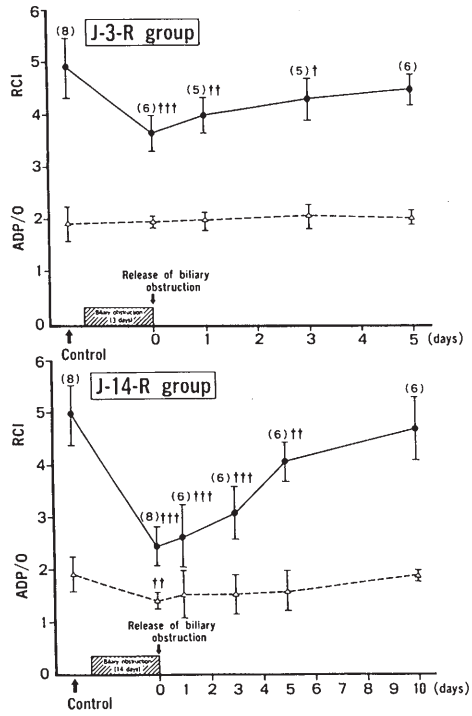


Fig. 1

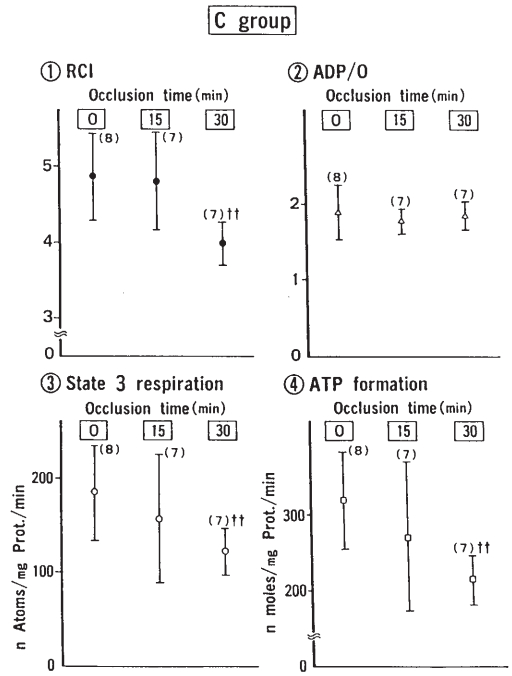


Fig. 2

Fig. 1 Mitochondrial function after release of biliary obstruction.
●—● : RCI; Δ---Δ : ADP/O. Data were expressed as the mean ± standard deviation. † : $p<0.05$, †† : $p<0.01$, ††† : $p<0.001$, compared with the control group. Numbers inparentheses indicate numbers of animals.

Fig. 2 C group. Mitochondrial function after occlusion of the portal vein. Data were expressed as the mean ± standard deviation, †† : $p<0.01$, compared with the non-occlusion group. Numbers in parentheses indicate numbers animals.

As shown in these results, the decrease in RCI in each group, regardless of the duration of biliary obstruction, means that mitochondrial dysfunction appeared after 30 min occlusion of the portal vein. RCI did not decrease after 15-min occlusion.

3. *Changes in serum biochemical data by occlusion of the portal vein*

In C group, s-GOT value increased significantly ($P<0.05$), but not greatly, after both 15-min and 30-min occlusions of the portal vein. s-GPT and m-GOT values increased significantly ($P<0.001$) after 30-min occlusion (Table 1).

In J-3-R-5 group, s-GOT value did not increase after either 15-min or 30-min occlusion, but s-GPT value increased ($P<0.001$) after 30-min occlusion. m-GOT value increased ($P<0.05$) after both 15-min and 30-min occlusions of the portal vein (Table 2).

In J-14-R-10 group, s-GOT value increased slightly ($P<0.05$) after 30-min occlusion. s-GPT and m-GOT values increased significantly ($P<0.001$) after both 15-min and 30-min occlusion (Table 3).

These changes in the biochemical data showed that s-GOT value increased only slightly, except in J-3-R-5 group, despite long duration of occlusion, and s-GPT value increased signifi-

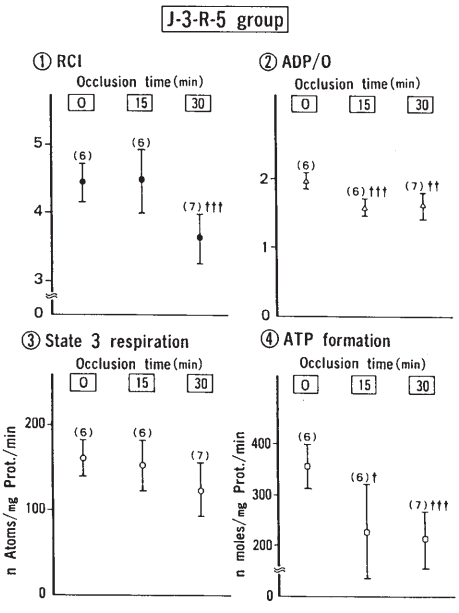


Fig. 3

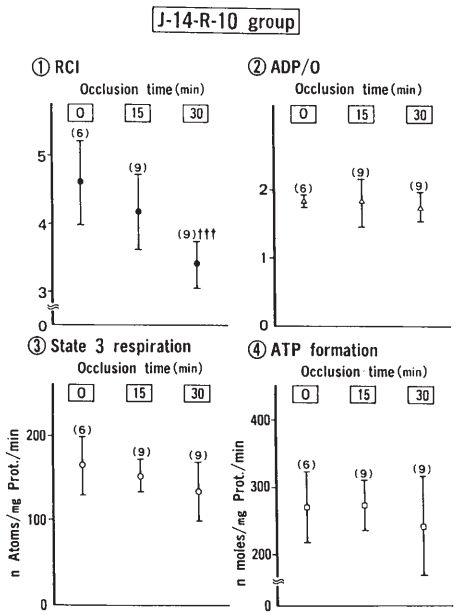


Fig. 4

Fig. 3 J-3-R-5 group. Mitochondrial function after occlusion of the portal vein. Data were expressed as the mean ± standard deviation, † : $p<0.05$, †† : $p<0.01$, ††† : $p<0.001$, compared with the non-occlusion group. Numbers in parentheses indicate numbers of animals.

Fig. 4 J-14-R-10 group. Mitochindrial function after occlusion of the portal vein. Data were expressed as the mean ± standard deviation, ††† : $p<0.001$, compared with the non-occlusion group. Numbers in parentheses indicate the numbers of animals.

Table 1. Biochemical data after occlusion of the portal vein (C group)

Parameters	Occlusion time (min)		
	0 (8)	15 (7)	30 (7)
T-Bil. (mg/dl)	0.4 ± 0.1	0.3 ± 0.2	0.2 ± 0.1††
s-GPT (Unit)	103 ± 22	147 ± 35†	153 ± 43†
s-GPT (Unit)	18 ± 7	29 ± 9	32 ± 8†††
m-GOT (mU/ml)	11.0 ± 4.6	29.5 ± 23.4	46.1 ± 19.5†††

Data were expressed as the mean ± standard deviation.
†$P<0.05$, ††$P<0.01$, †††$P<0.001$, compared with the non-occlusion group.
Numbers in parentheses indicate numbers of animals.

cantly in every group after 30-min occlusion. The ratio of m-GOT and s-GOT showed higher increases with longer duration of occlusion.

4. *Changes in the ultrastructure of liver mitochondria by occlusion of the portal vein*

In C group, swelling of mitochondria and, accordingly, removal of cristae to the periphery were observed after 30-min occlusion. However, these changes were not obvious after 15-min occlusion.

Table 2. Biochemical data after occlusion of the portal vein (J-3-R-5 group)

Parameters	Occlusion time (min)		
	0 (6)	15 (6)	30 (7)
T-Bil. (mg/dl)	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1
s-GOT (Unit)	108 ± 5	150 ± 50	136 ± 31
s-GPT (Unit)	18 ± 6	26 ± 6	36 ± 7†††
m-GOT (mU/ml)	13.1 ± 0.3	27.5 ± 11.5†	30.1 ± 14.7†

Data were expressed as the mean ± standard deviation.
†P<0.05, †††P<0.001, compared with the non-occlusion group.
Numbers in parentheses indicate numbers of animals.

Table 3. Biochemical data after occlusion of the portal vein (J-14-R-10 group)

Parameters	Occlusion time (min)		
	0 (6)	15 (9)	30 (9)
T-Bil. (mg/dl)	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
s-GOT (Unit)	145 ± 26	195 ± 59	231 ± 93†
s-GPT (Unit)	19 ± 9	48 ± 12†††	62 ± 22†††
m-GOT (mU/ml)	13.5 ± 2.4	42.2 ± 14.9†††	55.8 ± 25.7†††

Data were expressed as the mean ± standard deviation.
†P<0.05, †††P<0.001, compared with the non-occlusion group.
Numbers in parentheses indicate numbers of animals.

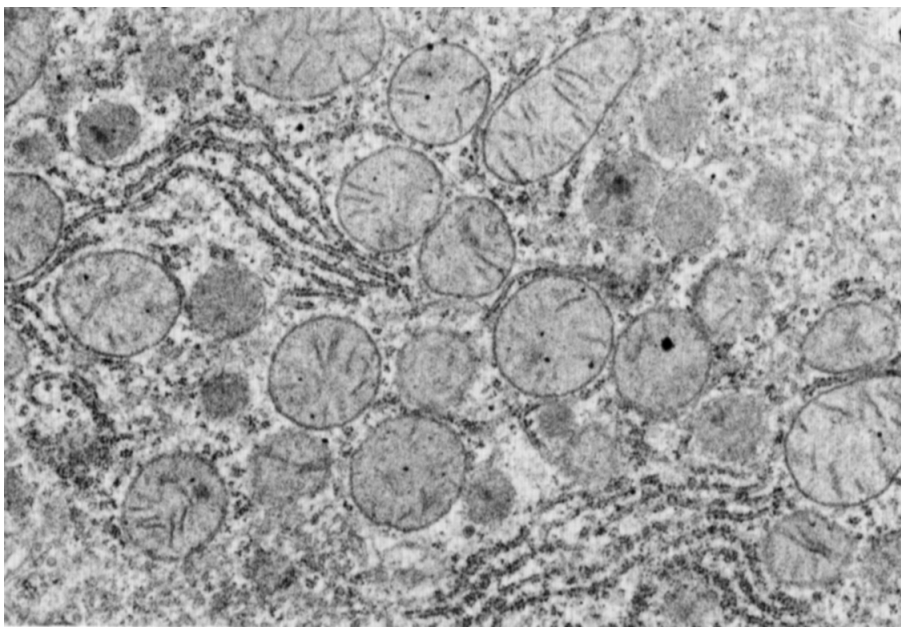
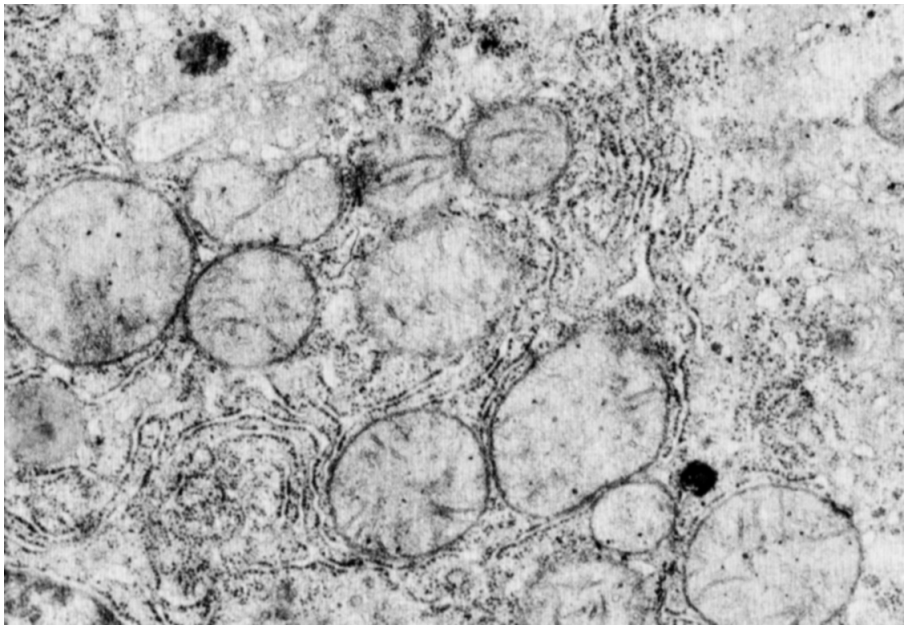


Fig. 5 (A)



(B)

Fig. 5 (A) Electron photomicrograph of liver cells of the non-occlusion group in C group; Mitochondria are almost normal. Magnification × 64,500 (original)
(B) Electron photomicrograph of liver cells of the 30-min-occlusion of the portal vein in J-3-R-5 group. Swelling of mitochondria is evident. Magnification × 52,100 (original)

In J-3-R-5 group, morphological changes similar to those in C group were observed after 15-min occlusion. After 30-min occlusion, remarkable swelling of mitochondria and disappearance of cristae from the matrix center were observed (Fig. 5B).

In J-14-R-10 group, after 15-min occlusion, swelling of mitochondria was observed with inner membrane approaching outer membrane. After 30-min occlusion, these changes became more remarkable, but there was no destruction of mitochondrial membrane as in other groups.

DISCUSSION

Recently, in order to increase perfect resectability in advanced cancers, such as pancreas carcinoma, gallbladder carcinoma, or bile duct carcinoma, a combined resection of the portal vein with the neoplasm has been commonly performed (21, 22). The portal vein has an important role in supplying blood to the liver as well as to the hepatic artery. Especially, the afferent blood supply through the portal vein makes up 65–85% of the total hepatic blood flow (12), and the oxidative content of the portal venous blood is close to that of the arterial blood. Therefore, occlusion of the portal vein induces liver ischemia resulting in dysfunction of liver cells. The cases in which carcinoma involves the portal vein are often accompanied by obstructive jaundice, and operations are usually performed after bile drainage.

The author performed the experimental occlusion of the portal vein to see how much damage on liver mitochondria, the energy-producing organella, would be produced by liver ischemia after biliary decompression. An animal experiment of portal vein occlusion was first intended by

Oré *et al.* (5). Several analyses on unexpected deaths of the experimental animals have been done (1–5) and most of them concluded that such deaths resulted from reduction of circulative blood flow due to splanchnic pooling of the portal venous blood (2–4), and some remedies for this phenomenon have been recently recommended (6). The present study is the first trial to investigate the influence on the liver by occlusion of the portal vein after releasing obstructive jaundice.

It is clinically significant to clarify the relationship between the occlusion period of the portal vein and liver cell damage. The recovery process of mitochondrial functions obtained in this study using the rat liver after releasing biliary obstruction was the same as that in the study by Miyata (11). Therefore, J-3-R-5 group and J-14-R-10 group were chosen as bile drainage models and were considered suitable to estimate the timing of the clinical operation. Energy is obtained from mitochondrial respiratory function. RCI shows intactness of respiratory control of mitochondria, and a reduction in RCI is probably caused by uncoupling of oxidative phosphorylation as the first factor (11). RCI decreased after 30-min occlusion of the portal vein in all three groups: C group, J-3-R-5 group and J-14-R-10 group. But ADP/O ratio decreased only in J-3-R-5 group. State 3 respiration showed no decrease even after 30-min occlusion, except in C group, indicating there was no damage in the electron transport system (11).

In the biochemical data, s-GOT value showed no remarkable increase after either 15-min or 30-min occlusion of the portal vein, although s-GPT increased in every group after 30-min occlusion, probably as a result of dysfunction of liver cells due to ischemia. m-GOT value increased after only 15-min occlusion, except in C group, and further increased in all three groups after 30-min occlusion. When m-GOT is released into the blood, severe liver cell dysfunction and an acceleration of mitochondrial membrane permeability (14) inevitably occurs because m-GOT is one of the enzymes from liver cells located on the crista of mitochondrial membrane (13). The increase in m-GOT value after 30-min occlusion could be related to the impaired mitochondrial respiratory function reported by Shirakawa (15) and Sato (16).

Mitochondria are known to swell with liver ischemia or hypovolemic shock (17). Hift and Srawitz (18) considered this phenomenon a condition of potential hyperactivity. The changes in liver cells caused by portal vein occlusion are probably similar to the condition of complete liver ischemia or hypovolemic activity.

The measurements of mitochondrial respiratory functions and biochemical data only gave us information about the whole liver; therefore, morphological observations of the liver mitochondria were done from view fields selected at random.

After 15-min occlusion of the portal vein, swelling of mitochondria with removal of cristae to the periphery and an approach of the inner membrane to outer membrane with bright matrix were observed only in J-14-R-10 group. After 30-min occlusion, swelling was observed in all three groups and was very remarkable in all except C group. However, destruction of the outer membrane of mitochondria did not occur even after 30-min occlusion. The results showed that dysfunction appeared after 30-min occlusion of the portal vein in mitochondrial respiratory functions, biochemical data, and the ultrastructure, i.e., these three parameters are related to one another.

The author set the occlusion period of the portal vein as 15-min and 30-min because the occlusion period of the portal vein in patients is usually less than 30-min for the resection and reconstruction of the portal vein (19, 20, 21). The average time is less than 20-min in our patients.

Therefore, the great significance of the present data is that 30-min occlusion of the portal vein induces severe damage to mitochondrial respiratory functions associated with the ultrastructural and enzymological changes of liver mitochondria. If sufficient bile drainage were

performed until mitochondrial functions recover, there would be no significant changes in liver functions by 15-min occlusion of the portal vein. But severe liver dysfunction appears after 30-min occlusion. These facts obtained by the present study should be taken into consideration when managing a patient with obstructive jaundice who requires a simultaneous resection of the involved portal vein.

ACKNOWLEDGEMENTS

I greatly appreciate Prof. Shigehiko Shionoya and Prof. Takayuki Ozawa for their guidance and instruction. I also would like to thank Prof. Junpei Asai, Dr. Yuji Nimura, Dr. Kanji Miyata and Dr. Shunpei Yokoi for their suggestions throughout the experiment and all the doctors in the Division of Oncology for their assistance.

A summary of this paper was presented at the 22nd (July 22, 1983) and 24th (July 19, 1984) Annual Congress of The Japanese Society of Gastroenterological Surgery.

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