Light Microscopic and Electron Microscopic Study on Morphologic Features Resulting in the Delay of ICG Elimination in Diabetic and Non-Diabetic Fatty Liver

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Disturbances of intravenously administered indocyanin green (ICG) elimination are related to the effective circulating blood volume and the amount of binding protein for transportation in blood plasma through the liver because of the narrowed sinusoidal space due to the enlarged liver cells with fullness of confluent fat droplets in the cytoplasm. However, morphologic changes of the liver resulting in disturbances of ICG elimination could not be actually clarified until the present. Therefore, morphologic changes of the liver resulting in delayed ICG elimination in fatty liver occurring in diabetes mellitus were investigated in contrast with those in fatty liver in non-diabetic, non-alcoholic diseases of the liver.

An electron microscopic study of the liver with delayed ICG elimination revealed thickening and amorphous growth of the sinusoidal wall with obscure pores followed by membraneous formation, narrowness of Disse’s space, rarefaction of sinusoidal microvilli and proliferation of collagen fibers, in fatty livers derived from both diabetes mellitus and other diseases. The term “intrasinusoidal block” in fatty liver should be utilized on the basis of these electron microscopic features of the liver.

(Key Words: ICG elimination, Fatty liver, Electron microscopy of the liver, Liver biopsy, Intrasinusoidal block)

INTRODUCTION

In most cases of fatty liver, elimination of administered dye, such as bromosulphalein (BSP) or indocyanin green (ICG), is more or less delayed. Disturbances of administered dye elimination are related to the effective circulating blood volume and the amount of binding protein for transportation in blood plasma through the liver because of the narrowed sinusoidal spaces due to the enlarged liver cells with fullness of confluent fat droplets in the cytoplasm. However, morphologic changes of the liver resulting in the disturbance of dye elimination could not be actually clarified until now.

Fatty liver is a manifestation or result of various diseases and metabolic disorders. Therefore, a broad array of clinicochemical methods must be utilized to discover the cause and the severity of the fat deposits. The disturbance of dye elimination can be observed in 70-80% of cases of fatty liver no matter what the cause of the disease (5).

It is well known that most cases of diabetes mellitus are associated with fatty liver. Therefore, morphologic changes referable to the disturbance of administered dye elimination in fatty liver occurring in diabetes mellitus were investigated in contrast with those in fatty liver in non-diabetic diseases. In this study, alcoholic fatty liver was excluded.

MATERIALS AND METHODS

1) The subjects were 85 patients with fatty liver associated with diabetes mellitus, in whom diagnosis of fatty liver was made on the basis of histologic examination of the biopsied liver,
I. Incidence of abnormal ICG-clearance (Table 1)

In 11 or 29% of 38 patients with fatty liver associated with diabetes mellitus who belonged to the group of overweight patients, abnormal ICG clearance could be observed. In five or 12% of 41 patients with fatty liver associated with diabetes mellitus who belonged to the group of patients with normal weight, pathologic ICG clearance could be detected. In one or 17% of six patients with fatty liver associated with diabetes mellitus who belonged to the group of underweight patients, abnormal ICG clearance could be found. In summary, abnormal ICG clearance could be recognized in 17 out of 85 patients with diabetic fatty liver, i.e., in 20% of the 85 cases. In contrast, abnormal ICG clearance could be confirmed in seven out of 33 patients with non-diabetic fatty liver with unknown etiology, i.e., in 21% of the cases. These findings are shown in Table 1.

II. ICG clearance in each group of patients with diabetic and non-diabetic fatty liver

In Figure 1, the disappearance rates of plasma ICG in each group of patients of diabetic and non-diabetic fatty liver are shown as mean values. Concerning the disappearance rate $K_1$, no difference in the values could be found between groups of patients with diabetic fatty liver and the non-diabetic fatty liver group in the range of 0.107 to 0.116. In the overweight and normal weight groups of diabetic patients, the $C_m$-point seemed to be somewhat higher than in the other groups, and these findings are probably caused by parenchymatous changes in the liver. Generally speaking, however, the $C_m$-points in each group of patients with diabetic and non-diabetic fatty liver were lower than those in healthy subjects, which was suggestive of an intrahepatic blood flow disorder.

III. Morphologic findings of the liver

A. Light microscopic findings

In Figure 2-a, the fatty liver in a 54 year-old female patient with diabetes mellitus, in whom ICG elimination test was normal, is shown. In Figure 2-b, histologic examinations of hematoxylin-eosin-stained slides revealed fatty liver in a 55 year-old female patient with diabetes mellitus in whom ICG elimination was pathologic. These two patients belonged to the overweight group. In a comparison of the histologic findings in both patients, the authors could not detect any changes of the liver, by which ICG elimination would be delayed. No distinctive histologic features of the liver due to Bielschowsky's silver impregnation method for normal or abnormal ICG elimination could be found in these diabetic fatty livers (Fig. 3-a, b). These facts were common in each type of patient with diabetic and non-diabetic fatty liver. It can also be said in general that the more the fatty metamorphosis was evident, the more the ICG elimination tended to be delayed. In most cases of diabetic and non-diabetic fatty liver in this study, a close parallel between the severity of fatty metamorphosis of the liver and the delay of ICG elimination was also shown.
but in some cases, the severity of fatty metamorphosis of the liver did not always parallel the severity of disturbed ICG elimination. In any case, no distinctive light microscopic features connected with normal and abnormal ICG elimination could be found on the basis of hepatic materials of the liver.

B. Electron microscopic findings
In general, circulatory changes, parenchymatous alterations and pathologic findings of the biliary tract must be taken into consideration in cases where electron microscopic features can be investigated (Tab. 2).

In Figure 4-a, electron microscopic features in a 57 year-old male patient with normal ICG elimination and in figure 4-b, electron microscopic features in a 55 year-old male patient with abnormal ICG elimination are shown. These two patients belonged to the group of severe fatty liver with diabetes mellitus and were overweight. In the former, electron microscopic features of the liver showed normal arrangement of the sinusoidal space, cellular elements of the sinusoidal wall with apparent fenestration, Disse’s space and the microvilli of the hepatocytes towards Disse’s space. In the latter, however, electron microscopic features of the liver showed thickening and amorphous growth of the sinusoidal wall with obscure pores, so that the sinusoidal wall resembled a membraneous formation. Narrowness of Disse’s space, rarefaction of the sinusoidal microvilli and proliferation of collagen fibers were also recognized.

In short, pathologic findings of the liver could be clarified electron microscopically in diabetic fatty livers with abnormal ICG elimination, while no pathologic features of the liver could be recognized electron microscopically in diabetic fatty livers with normal ICG elimination. These findings were also seen in other patients with diabetic fatty liver of each type in this series.

In Figure 5-a, electron microscopic features of a 49 year-old male patient with normal ICG elimination and in Figure 5-b, electron microscopic features of a 48 year-old male patient with abnormal ICG elimination are shown. These two patients belonged to the group of patients with severe fatty liver without diabetes mellitus. In the former, similar electron microscopic features to those in diabetic fatty liver with normal ICG elimination could be recognized, while in the latter, there were similar electron microscopic features such as thickening and amorphous growth of the sinusoidal wall with obscure pores resulting in a membraneous formation, narrowness of Disse’s space, rarefaction of sinusoidal microvilli and proliferation of collagen fibers. Therefore, it can be said that the morphologic characteristics of delayed ICG elimination in the fatty liver can be summarized by the above-mentioned electron microscopic features, no matter whether the fatty liver was derived from diabetes mellitus or other diseases.

In Figure 6, electron microscopic features in mild fatty liver with abnormal delay of ICG elimination were shown. This 47 year-old male patient belonged to the diabetes mellitus group with normal body weight. Although the fatty metamorphosis was mild, an electron microscopic investigation revealed the above-mentioned morphologic findings of the liver in diabetic fatty liver with delayed ICG elimination. In other words, the characteristic electron microscopic features in fatty liver with the delayed ICG elimination seemed not to depend on the type of diabetes mellitus according to body weight.

In Figure 7, electron microscopic features of a fatty liver with delayed ICG elimination are shown. This 37 year-old male patient with diabetes mellitus was underweight, and microscopic investigation of the biopsied liver revealed quite a large number of vacuolated nuclei in hepatocytes and very few fine fatty droplets in the liver cells. In this case, proliferative fibrosis in Disse’s space was the most outstanding finding, and the above-mentioned electron microscopic characteristics in the sinusoidal wall in fatty livers with delayed ICG elimination could be confirmed to a small extent.

Electron microscopic features of the bile canaliculi and its region must be taken into consideration. In Figure 8-a, electron microscopic features in a 37 year-old male patient with diabetes mellitus, normal body weight and delayed ICG elimination are shown and in Figure 8-b, electron microscopic features in a 39 year-old male patient with diabetes mellitus, normal body weight and normal ICG elimina-
tion are shown. In the both patients, microvilli of the bile canaliculus and Golgi apparatus remained unchanged. Therefore, no pathologic factors in the liver cells in the vicinity of the bile canaliculus which might cause a delay in ICG elimination could be detected electron microscopically.

DISCUSSION

On the basis of the results of the authors' investigation, the following points can be made: 1) as a morphologic basis for abnormal ICG elimination in diabetic and non-diabetic fatty livers, the very core of the problem is to investigate the intercellular spaces of the hepatocytes; 2) no distinctive light microscopic features of the liver which might result in abnormal ICG elimination could be found from biotopic materials of the liver; 3) it is very important to observe the intercellular spaces of the hepatocytes, especially the sinusoidal space very carefully at the time of electron microscopic investigations of diabetic and non-diabetic fatty liver; 4) pathologic findings of the liver could be clarified electron microscopically in the diabetic fatty liver with abnormal ICG elimination, but no pathologic features of the liver could be recognized electron microscopically in the diabetic fatty liver with normal ICG elimination; 5) the electron microscopic characteristics of delayed ICG elimination can be summarized as thickening and amorphous growth of the sinusoidal wall with obscure pores followed by membranous formation, narrowness of Disse's space, rarefaction of sinusoidal microvilli and proliferation of collagen fibers, no matter whether or not the fatty liver was derived from diabetes mellitus; and 6) in patients with diabetic and non-diabetic fatty liver, no pathologic findings in the vicinity of bile canaliculi of the liver cells which might cause a delay in ICG elimination could be detected electron microscopically.

In 36 out of 45 patients with diabetes mellitus (80%), liver biopsy revealed fatty liver and in eight out of the 36 patients with fatty liver (22%), a delay in ICG clearance was confirmed in the authors' earlier study (8). It is well known that about 50% of patients with diabetes mellitus have fatty liver (2, 3, 9, 10), and that diabetes mellitus occurs in 4 to 46% of patients with fatty liver, with an average of 25% (4). No correlation exists between the degree of control or the duration of diabetes mellitus and fatty infiltration, and in about 75% of the cases the fat is either centrilobular or diffuse in distribution (6, 8, 14). It seems that obesity is the major factor in fatty infiltration seen in diabetics, and not the diabetes mellitus itself (1, 8). The majority of obese patients with fatty liver have abnormal glucose tolerance tests, but they are not severely diabetic. In the present study, patients with diabetes mellitus, in whom the diagnosis of diabetes mellitus was made on the basis of family history, 100 g glucose tolerance test (GTT), immunoreactive insulin (IRI) and insulinogenic index, were investigated and patients with non-diabetic fatty liver were also investigated as a control group.

Since Rosenthal and White introduced bromosulphalein (BSP) as the basis for a simple test of liver excretory function in 1925, this diagnostic procedure has been utilized for effectively diagnosing fatty liver. Bromosulphalein should be distributed only within the vascular space and only be removed from the blood by the liver. In cases of fatty liver, elevated BSP retention is the most frequent abnormal liver function test, i.e., it has been effective for diagnosis of fatty liver in more than 50% of patients (Tab.3) (7, 11, 13).

On the other hand, indocyanine green (ICG), which was introduced into clinical medicine by Fox et al. for measuring cardiac output, is rapidly and completely bound to plasma protein, distributed within the vascular compartment after intravenous injection and eliminated from the blood exclusively by the liver. After its excretion into the bile, ICG is not reabsorbed from the intestine, i.e., it does not participate in enterohepatic circulation. ICG is also incorporated into hepatocytes through the sinusoidal liver cell membrane for removal from the blood. Within the hepatocytes, ICG is bound to acceptor proteins Y and Z and not chemically altered during its passage through the liver (12).

In spite of different metabolic pathways, delay of ICG elimination could be found in patients with both diabetic and non-diabetic fatty liver. In 20–22% of the authors' cases, a delay in ICG elimination could be recognized but the occurrence of delayed ICG elimination in 50% of fatty liver cases was also reported (15). In any
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case, functional investigations of ICG metabolism have hitherto been performed, but morphologic changes referable to the delay in ICG elimination have not always been sufficiently investigated.

This electron microscopic study clarified principally the above-mentioned morphologic findings of the sinusoidal wall which could be recognized in patients with fatty liver associated with the delay in ICG elimination no matter whether the fatty liver was derived from diabetes mellitus or other diseases. These electron microscopic changes could also be found no matter whether the severity of fatty metamorphosis of the hepatocytes was moderate or mild. The mechanism of morphogenesis of the sinusoidal wall is now insufficiently clear. It might be supposed, however, that fatty liver or metabolic disorders resulting in fatty liver facilitate the morphogenesis of the sinusoidal wall and that diabetes mellitus is one of the main causative factors in fatty liver. The sinusoidal space and Disse’s space become narrow due to the above-mentioned morphologic findings so that the blood flow there decreases. Therefore, it is reasonable to use the term “intrasinusoidal block” for patients with fatty liver, and the morphologic changes causing the delay in ICG elimination could be clarified electron microscopically in this study.

ACKNOWLEDGEMENT

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REFERENCES

Table 1 Incidence of Abnormal ICG-Clearance in Diabetic & Non-Diabetic Fatty Liver Cases

<table>
<thead>
<tr>
<th>Fatty Liver</th>
<th>Cases</th>
<th>Cases of Abnormal ICG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over</td>
<td>38</td>
<td>11 (28.9%)</td>
</tr>
<tr>
<td>Normal</td>
<td>41</td>
<td>5 (12.2%)</td>
</tr>
<tr>
<td>Under</td>
<td>6</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>17 (20.0%)</td>
</tr>
<tr>
<td>Non-Diabetic</td>
<td>33</td>
<td>7 (21.2%)</td>
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Table 2 Sites of electron microscopic examination

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<thead>
<tr>
<th>Circulatory</th>
<th>Parenchyma</th>
<th>Biliary Tract</th>
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<tbody>
<tr>
<td>Sinusoidal Lumen</td>
<td>Nucleus</td>
<td>Bile canaliculi</td>
</tr>
<tr>
<td>Space of Disse</td>
<td>Mitochondria</td>
<td>Lumen</td>
</tr>
<tr>
<td>Lumen</td>
<td>Microbody</td>
<td>Microvilli</td>
</tr>
<tr>
<td>Sinusoidal lining-cell</td>
<td>Lysosomes</td>
<td></td>
</tr>
<tr>
<td>Fenestration</td>
<td>Rough &amp; smooth</td>
<td></td>
</tr>
<tr>
<td>Microvilli</td>
<td>Endoplasmic reticulum</td>
<td></td>
</tr>
<tr>
<td>Collagen fiber</td>
<td>Ribosomes</td>
<td></td>
</tr>
<tr>
<td>Kupffer Cells</td>
<td>Golgi apparatus</td>
<td>Bile canaliculi</td>
</tr>
<tr>
<td>Ito Cells</td>
<td>Glycogen field</td>
<td>Lumen</td>
</tr>
<tr>
<td></td>
<td>Lipocyte</td>
<td>Microvilli</td>
</tr>
</tbody>
</table>

Table 3 Correct positivity in laboratory findings for diagnosis of liver (13)

<table>
<thead>
<tr>
<th></th>
<th>I (n = 73)</th>
<th>II–III (n = 40)</th>
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<tbody>
<tr>
<td>GPT</td>
<td>37%</td>
<td>47%</td>
</tr>
<tr>
<td>GOT</td>
<td>37%</td>
<td>63%</td>
</tr>
<tr>
<td>GPT + GOT</td>
<td>37%</td>
<td>81%</td>
</tr>
<tr>
<td>BSP</td>
<td>57%</td>
<td>80%</td>
</tr>
<tr>
<td>BSP + GPT + GOT</td>
<td>66%</td>
<td>89%</td>
</tr>
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</table>
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Fig. 1  ICG-clearance in each group of fatty liver cases

- $C_0$: Serum ICG concentration at $T = 0$ (mg/dl)
- $K_1$: ICG disappearance rate from the plasma to the liver
- $K_2$: ICG transfer rate from the liver to the bile
- $R_{15}$: Percent retention at $T = 15$ minutes
- $C_m$: Intersection point of $K_1$ and $K_2$

Fig. 2-a  Diabetic fatty liver in a 54 year-old female patient with normal ICG elimination (H-E stain, 100×)

Fig. 2-b  Diabetic fatty liver in a 55 year-old female patient with abnormal ICG elimination (H-E stain, 100×)
Fig. 3-a  Diabetic fatty liver in the same patient as shown in Fig. 2-a (Bielschowsky's silver impregnation method, 200×)

Fig. 3-b  Diabetic fatty liver in the same patient as shown in Fig. 2-b (Bielschowsky's silver impregnation method, 200×)

Fig. 4-a  Electron microscopic features in a 57 year-old male patient with diabetic fatty liver and normal ICG elimination (2000×)

Fig. 4-b  Electron microscopic features in a 55 year-old male patient with diabetic fatty liver and abnormal ICG elimination (2000×)
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Fig. 5-a  Electron microscopic features in a 49 year-old male patient with normal ICG elimination and non-diabetic fatty liver

Fig. 5-b  Electron microscopic features in a 48 year-old male patient with abnormal ICG elimination and non-diabetic fatty liver (3000 x)

Fig. 6  Electron microscopic features in a 47 year-old male patient with diabetic fatty liver and abnormal ICG elimination (1600 x)
Fig. 7  Electron microscopic features in a 37 year-old male patient with diabetic fatty liver and abnormal ICG elimination (2600 ×)

Fig. 8-a  Electron microscopic features in a 37 year-old male patient with diabetic fatty liver and abnormal ICG elimination (2600 ×)

Fig. 8-b  Electron microscopic features in a 39 year-old male patient with diabetic fatty liver and normal ICG elimination (2600 ×)