Function of Trace Metal in Experimental Fulminant Hepatic Failure
—Special Reference to Variation in Zinc Content in Liver—

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ABSTRACT
The authors prepared D-galactosamine (Gal) hepatic failure rats and hepatic failure dogs induced by hepatic ischemia and studied the correlation between the progress of hepatic failure and amount of trace metal in liver measured of the rats and dogs.

After Gal was given, the rats rapidly fell into hepatic failure condition. They were sacrificed with the lapse of time and their zinc (Zn) content in liver was measured, which showed a decrease in relation to hepatic injury reaching a minimum of 67.2±3.8 µg/g dry weight (that of control group: 149.1±23.4, p<0.05) 48 hr later. The hepatic failure dogs induced by hepatic ischemia also fell into hepatic failure after hepatic ischemia and died 17.5–112 hr (average 57.1±38.2) later. Positive correlation between the Zn content in liver immediately after death and the survival time (r=0.653, p<0.01).

As a result, it is suggested that the Zn content in liver can be used as an index to the degree of hepatocyte injury and prognosis of hepatic failure.

INTRODUCTION
In treating fulminant hepatic failure (FHF), it is important to determine the degree of hepatic injury and the course of regeneration, and to predict the prognosis. Zinc (Zn) is known from the long past as an trace element essential to growth and, recently, attention is being drawn to its relation with the synthesis of DNA and RNA11). Especially, Volm15) and Ohtake et al.46) have pointed out that the Zn content in liver of a partial hepatectomized rat is increased in correlation with the process of regeneration of the hepatectomized liver.

For the purpose of determining whether the measurement of trace metal content in liver, especially Zn, could provide a useful parameter to indicate the condition of hepatic failure in progress and prognosis. The authors prepared D-galactosamine (Gal) hepatic failure rats and acute hepatic failure dogs induced by hepatic ischemia and used them to investigate the function of trace metal centering around Zn content in liver.

MATERIALS AND METHODS
1. Preparation of Hepatic Failure Models
Rat: Male rats of Wister (B. W. 280–320 g) subjected to a fast of 24 hr duration before the start of experiment were intraperitoneally injected with 1,000 mg/kg BW of D-galactosamine hydrochloride (Gal) (Sigma Chemical Co., Ltd., Missouri, USA) and used as hepatic failure models. The rats of the normal control group were given the same amount of saline (Control Group).

Dog: Mongrel dogs (B. W. 15–20 kg) were submitted to an operation for end to side port-
caval anastomosis. Forty-eight hr after operation, the dogs’ common hepatic and gastroduodenal arteries were ligated for 1 hr so that they were made into hepatic failure of acute hepatic ischemia. Mongrel dogs of sham-operation (Sham-op Group) were subjected to a 15-min occlusion of portal vein after laparotomy. Forty-eight hr later, a part of the upper abdominal incision was left open for one hr. Thereafter, 2 dogs each were sacrificed every 24 hr within 96 hr.

2. Measurement of Trace Metal in Liver

Immediately after death or sacrifice of the rats and dogs, their livers were excised and used for measuring trace metal in liver. In case of rats, about 1.0 g in wet weight each from each lobe was taken. In case of dogs, 3 cut pieces of 1.0 cm thick of the frontal section were used. The tissue was cut into pieces with a plastic knife, washed with deionized water and dried at 105°C for 24 hr. After measuring its dry weight, 1 ml of conc. HNO₃ per 1.0 g dry weight tissue was added and its acid extraction was performed for 12 hr at 50°C. It was diluted with deionized water and measured for its contents of Zn, Cu, Fe, Mg, Mn, Cd, Na and K with an atomic adsorption spectrophotometer (Shimazu AA-646, Kyoto, Japan). The flameless method (Shimazu GFA-3) was used to measure Cd because of its extremely small content.

The metal content in plasma was measured using freeze-preserved plasma at -20°C that was immediately separated from the blood taken from rats by heart penetration at the time of sacrifice and from dogs by external jugular vein catheter. Zn, Mg, Cd and Mn were measured with an atomic absorption spectrophotometer by directly diluting the plasma and Cu and Fe, by diluting the deproteinized fluid obtained by adding 20% trichloroacetic acid to the plasma. Cd and Mn were measured by the flameless method due to their extremely small content.

RESULTS

1. Hepatic Failure Models

Rat: The mean survival time of the rats with Gal given was 64±15 hr (n=58). The survival rate (120-hr survival) was 7.4%. During observation of the tissue, focal coagulative necrosis occurring from the 12th hr rapidly progressed to a massive necrosis after 48 hr. The 96-hr and 120-hr survived groups maintained fairly good lobular structure and showed a sign of improvement.

Dog: Of the 21 dogs of 1-hr temporary hepatic ischemia, 17 fell into coma and died of hepatic failure within one week. Their mean survival time was 57.1±38.2 hr. The 17 dead dogs were widely divided into two groups—one is the short survived group of 10 that died within 40 hr marking the mean survival time of 28.5±7.4 hr, and the other, the long survived group of the remaining 7 that died between the 40th hr and the 7th day marking the survival time of 98.1±21.7 hr. Four dogs survived for more than 7 days, of which 2 survived for 4 weeks and were sacrificed (Table 1).

Table 1. Survival of fulminant hepatic failure (FHF) dogs

<table>
<thead>
<tr>
<th>Survival time</th>
<th>No. of dogs (%)</th>
<th>Survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>~40 hr</td>
<td>10 (48)</td>
<td>28.5±7.4* hr</td>
</tr>
<tr>
<td>40 hr~7 days</td>
<td>7 (32)</td>
<td>98.1±21.7* hr</td>
</tr>
<tr>
<td>7 days~4 weeks</td>
<td>2 (9.5)</td>
<td>7.5 days, 24.5 days</td>
</tr>
<tr>
<td>Survivors</td>
<td>2 (9.5)</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

(*mean± SD)

Observation of the hepatic failure death group from the histopathological point of view showed various stages from massive necrosis to zonal and focal coagulative necrosis. Even in single cases, there were those showing various necrosis stages existing together.

![Fig. 1. Zn content in liver in hepatic failure rats](image-url)
2. Function of Metal Content in Liver

a. Zn Content

Hepatic Failure Rats induced by Gal: Zn content had begun to be decreased from after 6 hrs when no morphological variation was yet observed. It reached a minimum value of 67.2 ± 3.8 µg/g dry weight (Control Group: 149.1 ± 23.4, p<0.05) 48 hr later. The 96 hr survived group recovered the value nearly the same as for the Control Group (Fig. 1).

Hepatic Failure Dogs induced by hepatic...
ischemia: The short survived group within 40 hr showed a Zn content of 129.0 ± 34.0 µg/g dry weight and the long survived group from 40 hr to 7 days, 217.0 ± 63.7 µg/g dry weight, giving an increase of significant difference (Fig. 2). The hepatic failure death group showed a positive correlation of significance between the Zn content in liver and the survival time (r = 0.653, p < 0.01), in which the former was increased along with the prolongation of the latter (Fig. 3). On the other hand, the Zn content in liver of the Sham-op group was increased to the highest in 48 hr due to the effect of surgical damage and gradually decreased thereafter (Fig. 3).

b) Other Trace Metal Content in Liver
In case of Gal-induced hepatic failure rats, Mg was decreased as hepatic failure progressed, which may indicate the influence of hepatocyte failure. Different from Zn, however, the variation in Mg was first observed when destruction was made obvious in the histopathological find-

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**Fig. 5-a.** Zn, Fe & Mn content in plasma in hepatic failure rats, (—●— hepatic failure group, ...○... control group).

**Fig. 5-b.** Zn, Fe & Mn content in plasma in hepatic failure dogs, (—●— hepatic failure group, ...○... sham-op group).
ing. Cu began to be increased from after 48 hr reaching the highest after 96 hr. No significant variations in Fe, Cd and Mn were observed (Fig. 4).

In case of hepatic failure dogs, a positive correlation of significance was observed between the increases in Mg and K and the length of survival time. Na was in the tendency of showing a correlation between the amounts and the survival time, whereas Cu, Fe, Mn and Cd showed no correlation between their amounts and the survival time (Table 2).

Table 2. Correlation between other metal content in liver and survival in FHF-dogs

<table>
<thead>
<tr>
<th>Metal</th>
<th>Correlation ratio</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Mg</td>
<td>0.599</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>K</td>
<td>0.736</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Na</td>
<td>-0.471</td>
<td>N S</td>
</tr>
<tr>
<td>Cu</td>
<td>0.044</td>
<td>N S</td>
</tr>
<tr>
<td>Fe</td>
<td>0.089</td>
<td>N S</td>
</tr>
<tr>
<td>Mn</td>
<td>0.369</td>
<td>N S</td>
</tr>
<tr>
<td>Cd</td>
<td>0.266</td>
<td>N S</td>
</tr>
</tbody>
</table>

3. Trace Metal in Plasma

Both hepatic failure rats and hepatic failure dogs showed increases in the amounts of Zn, Fe and Mn in plasma as hepatic failure progressed. This may be considered because of their escape from the tissue into blood due to destruction of hepatocyte (Fig. 5a, b). No significant variation in Cu, Cd and Mg in plasma was observed.

DISCUSSION

The roles of Zn in the growth of human body have been discussed so far in various ways and, recently, studies, especially, on DNA synthesis are being under way. In Zn lacking tissue, reduced DNA/RNA ratio\(^{10}\) and thymidine uptake\(^{5}\) have been reported.

RNA-DNA polymerase, deoxythymidine kinase and RNA dependent DNA polymerase, in particular, are Zn-dependent enzyme\(^{11}\) closely related with protein synthesis. Ohtake et al.\(^{3}\) have also reported that a hepatectomized rat showed an increase in Zn content in liver and a decrease in Zn content in plasma at about the 12th hr when the regeneration of its remaining liver grew vigorous, and attributed their cause to the increase in Zn-binding protein suggesting the relation between DNA synthesis and Zn.

Viewing that the Zn content in liver is closely related with the protein synthesis, which leads to cell regeneration, the authors have examined the Zn content in liver using hepatic failure models in anticipation that the Zn content in liver will serve as an index to cell destruction and regeneration at the time of acute hepatocyte injury.

Terblanch et al.\(^{13}\) has specified the minimum conditions for selection of hepatic failure models that they have reversibility and reproducibility and provide the cause of death from hepatic failure. Taking into consideration such conditions, the authors prepared rat models to be injected with Gal into abdominal cavity, as representative of the small animal, and mongrel dog models of 1–hr temporary hepatic ischemia after port-caval anastomosis\(^{1}\), as representative of the relatively large animal.

Hepatic failure by Gal used in this study is caused by inhibition of RNA synthesis and further of protein synthesis. Especially, within 10 hrs after giving Gal, the peak of the reduction in RNA synthesis is said to be observed\(^{10}\). The Zn content in liver is rapidly reduced before hepatocyte volume fraction is reduced after giving Gal, reaching its minimum value after 48 hrs. This well corresponds to the course of Gal hepatic injury, in which the Zn content in liver seems well reflecting the condition of inhibited protein synthesis.

The cause that clearly divided into two groups. The hepatic failure models induced by temporary hepatic ischemia prepared by the authors was considered due to the difference in the degree of hepatocyte necrosis. That is, in the short survived group, the rapidly increased amount of substances escaped from a large part of the hepatocyte that had fallen into a necrosis caused encephalopathy that might lead the models to death. In the long survived group, on the other hand, although the degree of hepatocyte necrosis was a little less and the increase in the amount of escaped substances was not so abrupt that its models were brought to death, the insufficient amount of the remaining hepatocyte absolutely necessary for life maintenance might lead them to death of hepatic failure, thereafter. As described above, it is
considered that the 1-hr temporary hepatic ischemia models are clinically similar to those of fulminant hepatitis.

The Zn content in liver of the hepatic failure rats is in good agreement with the degree of hepatic injury in progress. The hepatic failure death group of the hepatic failure dogs induced by hepatic ischemia also shows a correlation between the Zn content in liver and the survival time. Therefore, the amount of Zn in liver may be considered to be used as a parameter indicating the degree of hepatic injury. Meanwhile, the Sham-op group shows an early increase in Zn content in liver due to the influence of surgical damage. This is probably because of the existence of a hormonal system controlling the transfer of Zn in liver. The hepatic failure death group has shown no such controlling effect probably due to the extremely small amount of cell of liver being the target organ.

How and in what form does Zn exist in hepatocyte, as described above? According to the study by Ohtake et al., using hepatotomized rats, such Zn being increased during DNA synthesis promotion is mostly bound with small molecular weight protein, which is considered to be the same as that of metallothionein. This metallothionein is known as being induced by various stresses. It has also been reported as being induced by CCl4. Such a concept will lead to another that implies the possibility of metallothionein being induced even in hepatic injury of Gal or ischemia. This point is considered to be further studied for clarification.

As to the variation in Zn in other hepatic diseases, Vallee et al. observed a decrease in Zn content in plasma of patients of liver cirrhosis. Wang et al. using alcohol-loaded rat liver have clarified the decrease in Zn content in liver, plasma and muscular and pointed out the decrease in pool and turn over of Zn. Prasad et al. have also clarified clinically and in experiment of rats that the decrease in Zn in body has an effect on the urea synthesis causing an increase in plasma ammonium. From the above, the absence of Zn in liver cirrhosis is assumed to be related with the hepatic coma. Also, Saldeen and Brunk have observed a defense mechanism of Zn against CCl4 hepatic injury. Chavapil et al. are of the opinion that Zn would be taking defensive measures against hepatic injury by disturbing lipid peroxidation of membrane and for stabilization of membrane.

As described above, Zn is an element that has an important effect on liver and, moreover, contained in a considerably greater amount in liver that other trace metal. Therefore, it can be measured easily, only if attention is given to contamination. Using the authors' method, the Zn content in liver can be measured sufficiently with about 20 mg wet weight of tissue specimen and even with a specimen of needle biopsy of liver. Furthermore, even a tissue fixed with formalin can provide sufficiently stable values for easy comparison with histological finding and thus for sufficient possibility of clinical application of this method.

REFERENCE


