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Zinc Content in Liver of Experimental Fulminant Hepatic Failure Rat

—Significance as Index to Hepatic Injury and Regeneration—*)

Hideki KAWANISHI[1)], Masayuki NISHIKI[1)], Taro TSUCHIYA[2)] and Haruo EZAKI[1)]

1) *The Second Department of Surgery, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan*
2) *Akanekai Tsuchiya Hospital, Hiroshima 733, Japan*

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ABSTRACT

A rat model of fulminant hepatic failure induced by D-galactosamine (Gal) was given glucagon and insulin. The amount of Zn contained in its liver was measured and investigated for use as an index to the progress of hepatic failure and to the hepatic regeneration.

The results showed that the Zn content in the rat's liver had been rapidly reduced to the lowest value 48 hours after Gal was given before the morphological change occurred. Also, a significant improvement was observed in the Zn content in liver by giving glucagon and insulin. As a results, it is suggested that the Zn content can be used as an index to the degree of hepatocyte injury and regeneration.

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 RNA[14)]. Especially, Volm[16)] and Ohtake[11)] et al. 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 quatitatively clarifying the condition of hepatocyte in FHF, the authors have examined the change of trace metal using D-Galactosamine (Gal) hepatic failure rats[6)] and hepatic failure dogs induced by hepatic ischemia[7)], and reached the conclusion that the Zn content in liver is well correlated with the degree of hepatocyte injury. However, no obvious evidence has been obtained as to whether or not such correlation can be used as an index to regeneration.

In this study, using Gal hepatic failure rats, the authors made an attempt to verify whether or not the measurement of Zn content in liver can be used as an index to hepatic regeneration by giving them glucagon and insulin.

MATERIALS AND METHODS

1. Preparation of Hepatic Failure Model

Male rats of Wister (B. W. 280-320 g) subjected to a fast of 24-hour duration before the start of experiment were injected with 1000 mg/kg BW of D-galactosamine hydrochrolide (Gal) (Sigma Chemical Co., Ltd., Missouri, USA) intraperitoneally, and used as hepatic failure models (Gal-Group). The rats of the normal control group were given the same amount of saline (Normal-Group).

*) 川西秀樹，西亀正之，土谷太郎，江崎治夫：実験的急性肝不全ラットにおける肝亜鉛量の変動——肝障害及び再生の指標としての意義——

2. Injection of Glucagon and Insulin

Glucagon and regular insulin mixed with 5 ml of 5% glucose solution respectively, were injected intraperitoneally at the 24th hour after Gal was given. The rats were divided into the following groups by the amount of glucagon and insulin so given: glucagon-insulin (G I) 1 mg 1 u/kg BW, 1 mg 2 u/kg BW, 1 mg 4 u/kg BW, 1 mg 8 u/kg BW, and glucagon 1 mg/kg BW and insulin 4 u/kg BW. Further, another group was given 5 ml of 5% glucose solution (Glucose-Group).

3. Measurement of Zn content in Liver

Immediately after sacrificing the rats, the liver was excised and partially cut for use as histological specimen. Its remaining part was out into pieces and washed with deionized water. About 1.0 g in wet weight was dried for 24 hours at 105°C. After measuring its dry weight, 1 ml of conc. HNO_3 was added to it. Its acid extraction was performed for 12 hours at 50°C. Then, it was diluted with deionized water and measured with an atomic-absorption spectrophotometer (Shimazu AA 646, Kyoto, Japan).

4. Histopathological Finding

The tissue was fixed with 10% buffer formalin and stained with hematoxylin-eozin. Then, its hepatocyte volume fraction (HVF) was measured in accordance with the Scotto's method[15].

RESULTS

Gal induced Hepatic Failure Rats

The mean survival time of the rats with Gal given was 64±15 hours. The survival rate (120-hour survival) was 7.4%. During observation of the tissue, focal coagulative necrosis occurring from the 12th hour rapidly progressed to a massive necrosis after 48 hours.

Fig. 1 shows the variations in Zn content in liver and HVF. A more rapid reduction in Zn content than in HVF had begun to be observed even from about the 6th hour before any morphological change was observed. It reached the minimum value of 67.2±10.0 μg/g dry weight (Normal-Group 149.1±46.7, $p<0.05$) by the 48th hour. The values for the 96-hour survival group were nearly the same as for the Normal-Group.

From the above results, it has been proved that the reduction in Zn content in liver observed earlier than any morphological chang in liver can be used as a useful index to determining the degree of hepatic failure in progress.

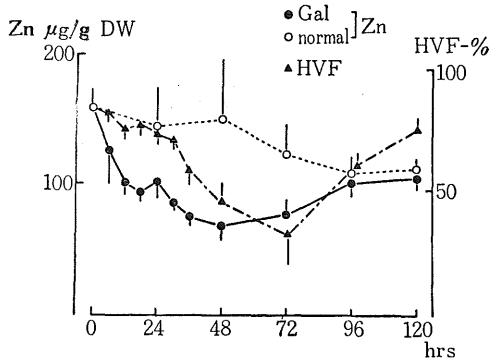

Fig. 1. Zn content in liver and hepatocyte volume fraction in hepatic failure rats induced by galactosamine. (mean±SD)

Influence of Glucagon and Insulin

Fig. 2 shows the Zn content in liver of the 48- and 72-hour groups after glucagon and insulin were given. There was no significant difference between the Glucose and Gal-Groups. Both the 48- and 72-hour groups with glucagon and insulin given showed high values. Significantly high 48-hour values were shown by the groups of G, I 1 mg 2 u/kg, 1 mg 4 u/kg, 1 mg 8 u/kg, and glucagon 1 mg/kg and insulin 4 u/kg, in comparison with those by the Glucose-Groups. However, no difference was indicated depending on the amount and ratio of glucagon and insulin given.

Fig. 3 showes the HVF of the 48- and 72-hour groups. In the 48-hour value, the G, I 1 mg 8 u/kg group showed a significant difference only from the Glucose-Group but none at all from the Gal-Group. Thus, there was no results obtained showing any HVF improvement by giving glucagon and insulin. In the 72-hour value, the G, I-Group showed a tendency of higher values.

DISCUSSION

The roles of Zn acting 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 RNA/DNA ratio[13] and thymidine uptake[4] have been reported. Ohtake et al[11]. have also reported that a hepatectomized rat showed an increase in the Zn content in liver and an decrease in the Zn

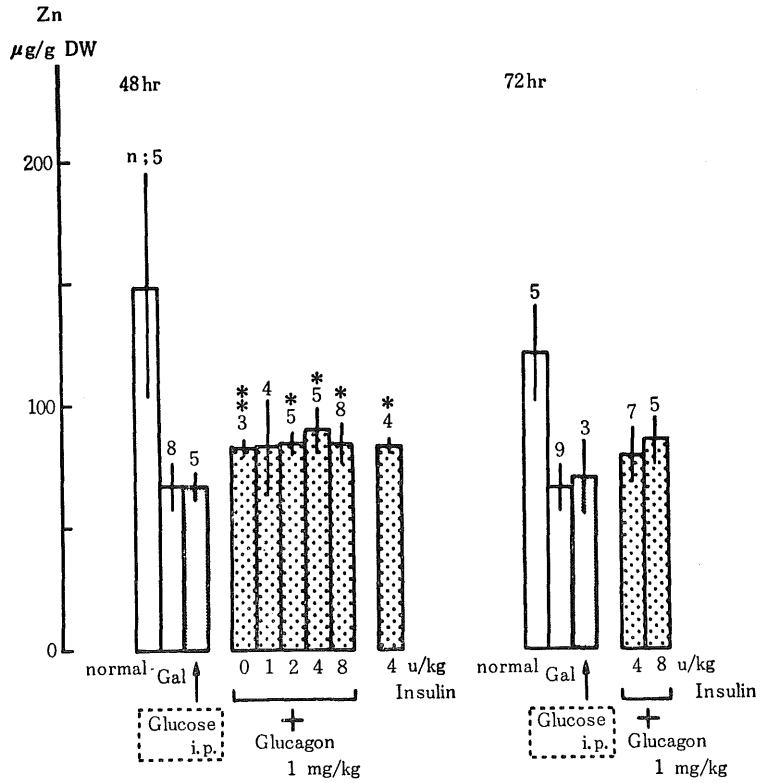

Fig. 2. Zn content in liver in hepatic failure rats induced by galactosamine (mean±SD), *p<0.05, **p<0.01, compared with glucose group.

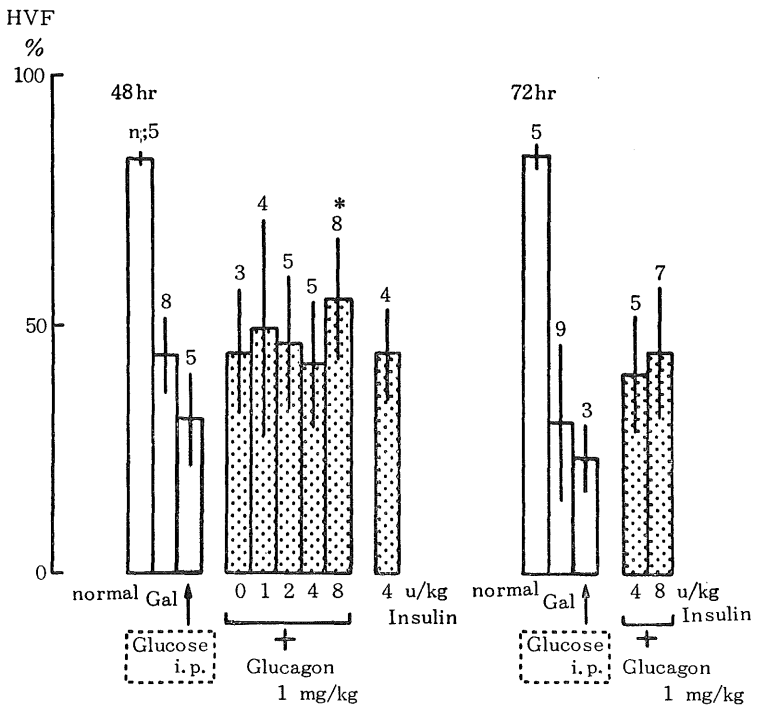

Fig. 3. Hepatocyte volume fraction in fulminant hepatic failure rats induced by galactosamine (mean±SD), *p<0.05, compared with glucose group.

content in plasma at about the 12th hour 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.

In the previous study[7] it has been clarified that, since there is a correaltion among the Zn content in liver, the survival time and HVF in hepatic failure dogs induced by acute hepatic ischemia, the Zn content in liver can be an index to indicating the degree of hepatocyte injury and its prognosis.

Hepatic failure by Gal used in this study is caused by inhibition of RNA synthesis and further of protein synthesis. Especially, within 10 hours after giving Gal, the peak of the reduction in RNA synthesis is said to be observed[2]. The Zn content in liver is rapidly reduced before HVF is reduced after giving Gal, reaching its minimum value after 48 hours. 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.

Once injured hepatocyte always has its own regeneration function to act on and a possible factor expediting such function, that is, the hepatotropic factor is considered to exist, as represented by such as glucagon, insulin, etc. Since the synergistic effect in promoting hepatic regeneration of glucagon and insulin was reported by Bucher et al.[1], many experiments have been conducted to prove its promotion of DNA synthesis and cell multiplication. As an index to hepatic regeneration in such experiments, DNA-synthesis[1], hepatic cyclic-AMP and -GMP levels and hepatic α-fetoprotein level[8], hepato cellural energy charge[17], etc., were used. To investigate whether or not the Zn content in liver can be the index to hepatic regeneration, the authors measured the Zn content in liver of rats which had fallen into the hepatocyte destruction condition at the 24th hour after Gal was given, by giving them glucagon and insulin. As a result, the Zn content in liver in the glucagon-insulin given group was significantly increased showing its usefulness as an index to hepatic regeneration. However, no difference was observed depending on the amount or ratio of glucagon and insulin given. Even only glucagon or only insulin given group showed an increase to the same extent. The amount and ratio of glucagon and insulin given have so far been studied in various ways. Bucher et al.[1] have reported that the amounts of glucagon and insulin showing the highest DNA synthesis promotion are 2 mg 2 u/kg/day and 4 mg 4 u/kg/day, respectively. Farivar et al.[3] have also reported that 0.3 mg 20 u/kg/day prolongs the survival time of the mouse given with Murine A59 hepatitis virus. However, from the fact that the authors' data were not able to suggest anything on the amount and ratio to be given, it is considered necessary to study hepatic regeneration including other types of its index.

How and in what form does Zn exist in hepatocyte, as described above? According to the study by Ohtake et al.[11,12] using hepatectomized 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 methallothionein. This methallothionein is known as being induced by various stresses[10]. It has also been reported as being induced by CCl_4[9]. Such a concept will lead to another that implies the possibility of methallothionein being induced even in Gal hepatic injury. This point is considered to be further studied for clarification.

The authors' study so far conducted has made clear that the measurement of Zn content in liver can be used as an index to the condition of hepatocyte to be observed. Furthermore, it is more stable than other biochemical examination regardless of any method used, only if attention is given to contamination. Even a tissue fixed with formalin can provide sufficiently stable values for easy comparison with histological finding. In practical application to a clinical case, the greatest problem lies in whether or no hepatic biopsy can be carried out at the fulminant stage of hepatic failure. Kagawa et al.[5] have performed biopsy under laparoscopy and Scott et al.[15] needle biopsy at the fulminant stage. Thus, it is con-

sidered possible to obtain a biopsy specimen even if at the fulminant stage of hepatic failure provided sufficient care be exercised.

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