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Analysis of Hepatic Failure Plasma by High Performance Gel-Chromatography*)

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ABSTRACT

Plasma taken from hepatic failure rats induced by D-galactosamine was used to observe the variatiation in its chromatogram obtained by conducting a high performance gel-chromatography (TSK G2000SW used). The progress of hepatic failure caused distinct increase in the 12 abnormal peaks, especially peak b contained middle moelcular weight substances, which showed a good correlation with the condition of progressing hepatic failure. Thus, the measurement of variation in gel-chromatogram, especially, in peak b, is effectives as a parameter of hepatic failure in progress.

INTRODUCTION

Fulminant hepatic failure is a total metabolic disorder that leads to a sudden deterioration in the patient's condition. The purpose of its treatment is how to improve metabolic disorder, how to regenerate liver and how to maintain the patient's life till he reaches a sufficient level of hepatic regeneration. A number of products of metabolic disorder, especially those substances which cause hepatic encephalopathy, have been considered and identified, although all have not yet been clarified. They may be generally classified into water-soluble substances (e. g. ammonia, phenol, biogenic amines, some amino acids and the so-called "middle molecules") and protein-bound substances (e. g. bilirubin and bile acids).

At present, the products of metabolic disorder in the condition of hepatic failure have not been clarified. Therfore, an attempt is being made to determine a parameter for evaluating the degree of hepatic failure by means of high performance liquied chromatography (HPLC)[1, 9]. Special attention is being given to the middle molecular weight substances of 500-5000.

In this study, the authors used the high performance gel-chromatography method to measure variation in the chromatogram of the plasma of fulminant hepatic failure induced by D-galactosamine. Then, the measured variation was examined for the feasibility of its use as parameter to evaluate the degree of the progress of hepatic failure.

MATERIALS AND METHOD

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 1,000 mg/kg BW of D-galactosamine hydrochrolide (Gal) (Shigma Cehmical Co., Ltd., Missouri, USA) intraperitoneally, and used as hepatic failure models. The rats of the normal control group were given the same amount of saline.

Gel-Chromatograhy Method

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A high performance liquid chromatography (HPLC) unit used was Toyo Soda HLC-803D and UV-8 Model II for detection at UV 220 nm. The column used was TSK-GEL G2000SW (Toyo Soda Co., Ltd., Tokyo, Japan) of silica gel, 0.75×60 cm. Elution was achieved with a phosphate buffered saline (0.2 M, pH 6.9) at a flow rate of 0.6 ml/min. at 25°C. Prior to analysis, plasma samples were deproteinized by ultrafiltration through Amicon centriflow CF50A (Amicon Co., Ltd., Massachusetts, U. S. A.). The 10 μl of deproteinized sample was injected into the column. The peaks eluted were named alphabetically according to the elution time. The concentration level of each peak was measured and indicated in PHU/ml (peak height/ml of sample, where 1 PHU is equivalent to 0.01 absorbance unit of UV). All the peaks emerged in 60 minutes.

RESULT

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.

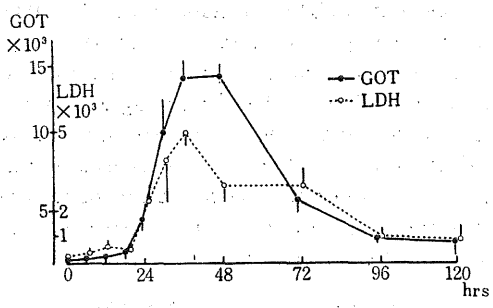

Fig. 1. Changes of S-GOT and LDH in the progress of hepatic failure induced by D-galactosaminein rats

Fig. 1 shows the changes in the liver function test. In the early stage, abrupt increase in GOT and LDH had been observed. GOT and LDH reached their heighest values about 36 hrs after Gal injection.

Variation in Chromatogram of Hepatic Failure Dogs

Fig. 2 shows typical chromatograms of plasma of normal and hepatic failure rat obtained by the UV monitor at 220 nm. The peaks are classified into a-1, showing distinct variation at peaks a, b, c, d and f, in paticular. Of the peaks of the hepatic failure plasma, the peaks a and b are cut off by the 500-dalton cut off membrane (ultra filter UHO5, Toyo Roshi Co., Ltd., Tokyo, Japan) which are considered to contain middle molecular weight substances. On the other hand, the normal plasma is con-

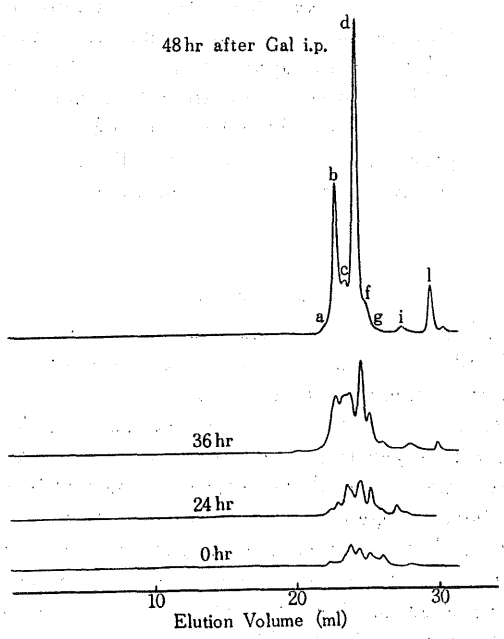

Fig. 2. Changes of the chromatograms taken from ultrafiltrates of rats in the progress of hepatic failure induced by D-galactosamine

Table Changes in the chromatomatographic peak level

peak	Mean peak levels (PHU/ml) at 0 hr	18 hr	24 hr	48 hr	72 hr	96 hr
b	1.02±0.27	1.35±0.15	1.49±0.43	4.46±1.32	2.13±1.89	1.48±0.04
c	1.12±0.14	1.57±0.33	1.61±0.59	2.69±0.98	1.66±1.12	0.92±0.01
d	1.44±0.27	1.78±0.25	1.83±0.41	10.44±5.99	4.83±6.87	1.29±0.08
f	1.14±0.21	1.04±0.81	1.17±0.28	1.36±0.43	1.20±0.70	0.75±0.03

PHU=peak height units. Mean±SD (n=6)

sidered mainly consisting of small molecular weight substances because it has no peaks to be cut off by the UHO5 membrane. In the meantime, the peak b becomes to be hardly detected at over UV 250 nm.

The Table shows the changes of the peaks b, c, d and f measured with time. Each peak increased as hepatic failure progressed. Especilly, the peak b and d showed distinct variations in their increase to 4.5 times and 9.3 times of the normal value after 48 hours of the Gal injection, respectively.

But after 72 hours, each peak decreased and reached the normal value. Thus, the variation of chromatogram, especially the peak b, is significant for use as a parameter for the advance of hepatic failure.

DISCUSSION

Fulminant hepatic failure is a systemic critical sympton that has been developed due to rapid progress of hepatic failure. The importance in its treatment lies in early prediction or the degree of hepatic failure.

Especially, in the case of its treatment by means of an artificial liver support system, to which the extracoporenal circulation system is mostly applied, it is necessary to estimate the amount of abnormal metabolic products emerging from the blood. Since such abnormal metabolic products have not yet been clearly identified at present, particular substances (e.g. ammonia, some amino acids and their unbalance, bilirubin, etc.) are used for its guidance. However, these substances indicates a great difference depending on the individual cases and cannot be said to be capable of fully reflecting the true condition of one's hepatic failure.

Recently, the analysis of hepatic failure plasma has been performed using the high performance liquid chromatography. Hughes et al.[3] have observed an abnormal peak in substances with molecular weights below 10,000 by analyzing the plasma of a hapatic failure patient using the Sephadex G25 chromatography. They assume that these substances would cause activation disturbance of Na·K-ATPase in brain cell microsome leading to brain edema.

Also, Chang et al.[1] and Leber et al.[9] have found an abnormal peak in molecular weights of 500–5000 by analyzing plasmas of galactosamine hepatic failure rats and hepatic failure patients using the Sephadex G15 chromatograpy. Inoue et al.[4] used the polyvinyl alcohol copolymer gel chromatography in an analysis of the plasma of a hepatic failure patient and reported that the variation of the peak could be used in judgement of the effect of plasmapheresis treatment.

All the researchers conducting various studies, as above, point out the importance of the so-called middle molecular (MM) fraction. In particular, the variation in MM is being reviewed in conjunction with the effect of the artificial liver support system.

By comparing cuprophan-hemodialysis (CU-HD) and polyacrylonitrile-hemodialysis (PAN-HD), Opolon et al.[10] and silk et al.[12] have reported the effectiveness of PAN-HD on hepatic failure and indicated the effective removal of MM-fraction by PAN-HD. The usefulness of charcoal and XAD-7 as adsorbent have been discussed by Chang et al.[3] and Hughes et al.[3], respectively, who observed the removal of MM-fraction through hemoperfusion.

In this study, the authors measured the peaks b, c, d and f at 220 nm using the TSK G2000 SW column even in the gel chromatography and indicated their variations as hepatic failure progressed. The authors judged the particular peak b as corresponding to MM.

The authors used hepatic failure dog induced by hepatic ischemia, in the previous study, to analyze their plasma by the same gel-chromatography method and found distinct variations also at the peaks b, c, d, e and f. The peak b, especially, showed good correlation between the degree of progress of hepatic failure and the coma grade[5]. The authors reported that either one of these peaks could be removed by direct hemoperfusion using coated BAC[6] and the newly developed polyetherurethane sheet embedded with powdered charcoal (UPC)[7,8].

This test has not been able to identify what the substances emerging during gel-chromatography are and how they function. Moreover, several substances are considered being overwrapped at these peaks with those of middle molecular weights mainly contained at the peak b, leading to an anticipation of existence of considerable amount of substaces of small mo-

lecular weights at this peak at the same time. Thus, it will be impossible to discuss the substances causing hepatic failure only by the gel-chromatography. However, it is considered useful for predicting the degree of progress and after care because of the good correlation between its peak variation and the degree of hepatic failure.

As to MM, especially, so far considered to be peptide-like substance[9], its molcular weight is only known as lying in the range of 500–5000. However, in both clinical cases and animal tests, it is certain that MM increases as the coma condition progresses. In addition, its increase in the brain passing through the blood brain barrier. Shi and Chang[11] identified MM in the brain by HPLC and observed a reduction in it by ACAC-hemoperfusion. Also, Denis et al.[2] have observed a reduction in the fluorescent MM in the brain by calculating the concentration of such MM from the difference between the tyrosin levels by the fluorimetric method and the resin chromatographic method.

As above, the measurement of MM is significant for use as a parameter for the advance of hepatic failure.

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