Influence of Hemoperfusion Using Polyetherurethane Sheet Embedded with Powdered Charcoal (UPC) on Middle Molecules of Hepatic Failure Dogs*3

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(Received September 12, 1984)

Key words: Middle molecules, High performance gel-chromatography, Charcoal, UPC, Hemoperfusion, Liver support

ABSTRACT

Plasma taken from hepatic failure dogs induced by hepatic ischemia was used to observe the variation in its chromatogram obtained by conducting a high performance gel-chromatography (TSK G2000SW used). The progress of hepatic failure caused a distinct increase in the middle molecular (MM) fractions (m. w. over 500), which showed a good correlation with the condition of progressing hepatic failure. The reduction of the above number of peaks was observed when the hemoperfusion was performed using the polyetherurethane sheet embedded with powdered charcoal (UPC) that had been newly developed mainly for the purpose of absorbing protein-bound substances. The UPC developed by the authors can successfully remove middle molecular substances in hepatic failure.

INTRODUCTION

Fulminant hepatic failure (FHF) is a total metabolic disorder that leads to a sudden deterioration in the patient’s condition.

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 and protein-bound substances.

So far, various artificial liver support systems have been widely applied to direct hemoperfusion (DHP)2,4,12> and dialysis with high permeability membranes9,10>. These treatments are effective in removing water-soluble substances in the small-to-middle molecular weight region as well as in recovering consciousness. However, they are not yet effective sufficiently in removing protein-bound substances, which is the problem lying in the treatment by removal to be solved, at present. For the purpose of effective removal of protein-bound substances, the authors have developed a new type of adsorbent which is polyetherurethane sheet embedded with powdered charcoal (UPC), which was used in in vitro tests and hemoperfusion tests of jaundiced dogs to prove its satisfactory capability of removing bilirubin, as previously reported93.

At present, since the products of metabolic disorder in the condition of hepatic failure have not been clarified, it is difficult to clearly indicate the efficiency of their removal by means of an artificial liver support system applied to hepatic
failure. In many cases so far, a specific model substance has been used to indicate such efficiency. Therefore, an attempt is being made to determine a parameter for evaluating the degree of hepatic failure and the effect of the liver support system using an abnormal pattern in the condition of hepatic failure detected by means of a high performance liquid chromatography (HPLC)\(^9,10\). Special attention is being given to the middle molecular weight substances (MM) 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 hepatic ischemia. Then, the measured variation was examined for the feasibility of its use as a parameter to evaluate the degree of the progress of hepatic failure and for the effectiveness of UPC developed by the authors on purification of the plasma of hepatic failure.

**MATERIALS AND METHODS**

**Preparation of Hepatic Failure Dogs**

Using mongrel dogs (BW 10-20 kg), acute hepatic ischemia models were prepared in accordance with the Abouna method\(^9\). That is, an end to side porta-caval anastomosis was placed, and 48 hr after operation, each of the common hepatic and gastroduodenal arteries was occluded for 1 hr by a clamping device.

**Hemoperfusion Method**

Hemoperfusion using Polyurethane Sheet embedded with Powdered Charcoal (UPC):

The method for preparing UPC has been detailed in the previous paper\(^9\). In short, specially selected powdered charcoal (particle size 10-40 µm) having a specific pore size distribution was suspended in a polyurethane solution (8 W/V%) and formed in film over a supporting sheet of polypropylene mesh (100 µm thick). Fixing the sheet in water, the solute was extracted in solution (8 W/V%) and formed in film over a supporting sheet of polypropylene mesh (100 µm thick). Fixing the sheet in water, the solute was extracted in solution (8 W/V%) and formed in film over a supporting sheet of polypropylene mesh (100 µm thick). Fixing the sheet in water, the solute was extracted in solution (8 W/V%) and formed in film over a supporting sheet of polypropylene mesh (100 µm thick).

This UPC used in vitro tests is capable of developing a bilirubin adsorption performance about 14 times greater than that of BAC and about 6 times greater than that of Amberlite XAD-7. In hemoperfusion on jaundiced dogs, it shows an amount of adsorbed bilirubin of about 80%. Its adsorption performance of water-soluble substance has been confirmed in vitro test, showing a higher performance than that of BAC with the substances over the molecular weight of 250 but a lower performance with those below 250.

This UPC column (containing 36 g of powdered charcoal) was connected in series with the coated bead-type charcoal (BAC) column\(^6\) (containing 75 g of charcoal) forming a system, which was used in hemoperfusion performed on hepatic failure dogs for 3 hr from the 6th hour of hepatic ischemia. During the hemoperfusion, the blood flow rate was 50 ml/min. Heparin was continuously injected (600 IU/hr.).

**Gel-Chromatography Method**

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 G2000 SW (Toyo Soda Co., Ltd., Tokyo, Japan) of silica gel, 0.75 x 60 cm. Elution was achieved with a phosphate buffered saline (0.2 M, pH 0.9) at a flow rate 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.). Ten 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 unit/ml of sample, where 1 PHU is equivalent to 0.01 absorbance unit of UV).

All the peaks emerged in 60 min. The deproteinized fluid was separated by ultrafiltration through a 500-dalton cut off membrane (ultra filter UHO5, Toyo Roshi Co., Ltd., Tokyo, Japan) and the ultrafiltrate was measured with HPLC in the same manner. The evaluation of analytical capacity of the column, under the authors' experimental conditions was performed by chromatographing the test solutions of the following substances: Bovine Serum Albumin (M.W. 67000), Ovalbumin (M.W. 43000), Chymotrypsinogen (M.W. 25000), Ribonuclease A (M.W. 13700), Bromsulphalein (M.W. 838) and Uric acid (M.W. 116).
RESULTS

Variation in Chromatogram of Hepatic Failure Dogs

Fig. 1 shows typical chromatograms of plasma of a normal and hepatic failure dog (grade IV coma) obtained by the UV monitor at 220 nm, and also, the chromatogram of the filtrate separated through the UHO5 membrane. The peaks are classified into α-1, showing distinct variation in peaks b, c, d, e and f, in particular. Of the peaks of the hepatic failure plasma, the

Fig. 1. Typical patterns of high performance gel-chromatography taken from ultrafiltrates of plasma from a dog in normal and hepatic failure conditions. Ultrafiltrate through GF50A (solid line) and UHO5 (broken line). UV adsorbance at 220 nm.

Fig. 2. Influence of the hemoperfusion using a system formed by connecting UPC with coated BAC on chromatograms of plasma taken from hepatic failure dog.
peaks a and b are cut off by the UH05 membrane, which are considered to contain middle molecular weight substances. On the other hand, the normal plasma is considered mainly consisting of small molecular weight substances because it has no peak to be cut off by the UH05 membrane. In the meantime, the peak b becomes to be hardly detected at over UV 250 nm.

**Variation in Chromatogram by Hemoperfusion**

A system consisting of the UPC column and the coated BAC column connected in series was used in hemoperfusion to observe variation in the chromatogram. Fig. 2 shows the chromatograms at the inlet and outlet of the UPC column and at the outlet of the BAC column at 30, 60, 120, 180-min hemoperfusion. Reduced peaks were observed for both UPC and coated BAC columns after passing each of them. Fig. 3 shows the changes of the peaks b, c, d, e and f of the 4 hemoperfused hepatic failure dogs. The hemoperfusion reduced the peaks d and e to 50% and 64.4% of the initial values, respectively. On the contrary, the peaks b, c and f, although hemoperfused, were gradually increased to 130%, 245% and 206% of initial values, respectively. After the completion of hemoperfusion, all the peaks were increased, showing no difference with those of the non-perfusion groups in 5 hours after the end of hemoperfusion. Fig. 4 shows the changes in clearance by the UPC column only. The peaks d, e and f at 30-min hemoperfusion by the UPC column containing even a small amount of powdered charcoal of 36 g showed the clearances of 30–35 ml/min, but the peaks d and e were reduced to 5 ml/min at the end of hemoperfusion. On the other hand, the peaks b and c showed the clearances of only 12–15 ml/min at 30-min hemoperfusion. However, the UPC hemoperfusion shows nearly the same pattern of variation in clearance as that by the BAC-hemoperfusion. As far as the variation in the chromatogram is concerned, both BAC and UPC shows nearly the same characteristics of

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**Fig. 3.** Changes in the chromatographic peak level of the plasma taken from hemoperfused hepatic failure dogs using the system of UPC and BAC connected. PHU/ml denotes peak height units per ml of sample, DHP is direct hemoperfusion, Value as mean±SD; n=4, non-DHP group (-----, n=6)
Hemoperfusion with UPC

**DISCUSSION**

The use of high performance liquid chromatography in analysis of hepatic failure plasma has been reported by Chang et al.\(^3\), Leber et al.\(^{10}\) emphasizing the importance of the abnormal middle molecular (MM) fraction (MW 500-5000) in particular. By comparing cuprophan-hemodialysis (CU-HD) and polyacrylonitrile-hemodialysis (PAN-HD), Opolon et al.\(^{13}\) and Silk et al.\(^{14}\) 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.\(^{5}\) respectively, who observed the removal of MM-fraction through hemoperfusion.

Many researchers use Sephadex G15 to separate MM-fraction and UV-spectrophotometer to identify it. The authors measured the peaks b, c, d, e and f at 220 nm using the TSK G2000SW column even in the same gel chromatography and indicated their variations as hepatic failure progressed.\(^7,\(^8\)) The authors judged the particular peak b as corresponding to MM. However, it is not always possible to decide that these peaks contain the coma substances. In the hemoperfusion using BAC column for hepatic failure dogs, despite the recovery of consciousness, peaks b and c increased and dissociated with coma grade\(^{11}\). But, the increase of peak b showed a good correlation with the progress of hepatic failure. It cannot be denied that same hepatic failure substance is contained in peak b.

The authors have been making efforts to develop adsorbent that remove protein-bound substances. Noting that the smaller the size of an adsorbent is, the greater the rate of adsorption will be, the authors have developed a new type of adsorbent which is polyetherurethane sheet embedded with powdered charcoal (UPC).\(^9\) Since powdered charcoal having an optimum pore size distribution was used for the purpose of adsorbing protein-bound substances, inferior adsorption of small molecular weight substances to that by BAC was observed, on the contrary. Therefore, UPC and BAC connected in series as a system were used to perform hemoperfusion. In this system, removal of toxic substances, middle molecular and protein-bound substances by UPC and small to middle molecular substances by BAC is sufficiently possible. The variation in the chromatogram obtained by the UPC column showed little clearance for each peak due to the use of a small amount of powdered charcoal as much as 36 g. However, its variation pattern was similar to that in the case of BAC-hemoperfusion.\(^{11}\) Thus, it is considered that even the use of the UPC column alone containing an increased amount of powdered charcoal will be able to provide a removal efficiency sufficiently similar to that by BAC. At present, uniform and rolled UPC, containing 70-80 g of powdered charcoal, produced by a systemized process are being used in a prototype column for experimental and clinical\(^{15}\).

**REFERENCES**

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