Hiroshima Journal of Medical Sciences
Vol. 32, No. 1, March, 1983
HIJM 32-2

Basic Study of Polyetherurethane Coated Bead-Type Activated Charcoal for Direct Hemoperfusion*)

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(Received November 8, 1982)

Key words: Liver support system, Activated charcoal, Polyetherurethane, Hemoperfusion

ABSTRACT

A new type of activated charcoal for the direct hemoperfusion (DHP) has been developed for use in treatment of fulminant hepatic failure. Bead-type activated charcoal from petroleum pitch (BAC) was coated with polyetherurethane. The coating has permitted the charcoal to minimize the number of released microparticles and yet to show nearly the same adsorption performance as that of the non-coated BAC *in vitro* adsorption test. DHP using a fulminant hepatic failure dog induced by hepatic ischemia has shown improved consciousness and good biocompatibility as well as good clearance of toxic substances. As above, the newly developed bead-type activated charcoal coated with polyetherurethane can be considered effective in treatment of fulminant hepatic failure.

INTRODUCTION

Many cases of application of activated charcoal for use in treatment of fulminant hepatic failure (FHF) have been reported after Gazzard[8)]. It has already been put into clinical practice. Especially, the development of the bead-type charcoal from petroleum pitch (BAC) which is spherical and highly wearproof and the progress in surface coating technique using a biocompatible material have contributed to much safer practice of direct hemoperfusion (DHP). However, since the functional weakness of platelete due to abnormality in the blood coagulation system already existing at the time of FHF has been pointed out[14)], a material of good biocompatibility is being sought.

Noting polyetherurethane to which attention is recently being drawn as a polymer of good biocompatibility, the authors used it to develop a new type of coated BAC for DHP. It was examined by the *in vitro* test and the DHP test using hepatic failure dogs and obtained satisfactory results, as reported hereunder.

MATERIALS AND METHODS

1. Method for Coating Activated Charcoal (Polyetherurethane Coated Bead-type Activated Charcoal, PUAC)

For activated charcoal, BAC-MUL (Kureha Chemical Co., Ltd. Tokyo, Japan) from petroleum pitch, was used. Attached coal dust and adsorbed substances were completely removed by repeatedly washing it with distilled water, HCl and NaOH.

For coating, BAC was dipt in the solution of polyetherurethane dissolved in the solvent of tetrahydrofuran (THF) for 5 min. The concentration of the polyetherurethane was changed to 0.025 W/V%, 0.1 W/V% and 1.0 W/V% to obtain different coat thicknesses. The coated BAC was dried, washed with hot distilled water to remove THF, and kept in dry condition.

2. Charcoal Microparticle Release Test

*) 川西秀樹, 西亀正之, 椙山雅文, 張恒雄, 土谷太郎, 江崎治夫：ポリエーテル型ウレタン被覆ビーズ状活性炭を用いた血液直接灌流の基礎研究

Using 3 types of BAC, their released microparticle counts were compared by both shaking and column reciculation methods. Non-coated BAC, PUAC, and regenerative cellurose coated BAC (Hemocels®, Teijin Co., Tokyo, Japan), 0.25 g each, were put in a 50 ml saline, respectively, and shaked for 2 hr at 37°C and 120 cpm. Each solution was then filtered through a 0.80 μm millipore filter and its microparticle count was measured under an optical microscope for comparison. The 75 g of PUAC filled in a polypropylene column was washed with 2 liters of saline. Recirculating 1 liter of saline at 100 ml/min for 3 hr, its released microparticle count in the total solution was measured.

3. Adsorption Test

Batch Method: A solute was dissolved in a 50 ml phosphate buffer solution (0.15 M, pH 7.4). Adding 0.25 g of BAC into it, the solution was shaked at 37°C and 120 cpm. Samples were taken at each lapse of time of 10, 20, 30, 60 and 120 min and the concentration of the solute of each sample was measured.

Column Recirculation Method: Filling a cartridge of 25 ml internal volume with 10 g of BAC, a test solution was recirculated in it at 50 ml/min for 2 hr. The solute concentration was measured with the lapse of time.

The measured substances and their analysis methods used are, as follows: Creatinine (Jaffe method); bromosulphalein (BSP, Alkali color developing method); inulin (Resorcin method); albumin (BCG method); amino acid (Ninhydrin method); and phenol, pentobarbital-Na, vitamine B_{12}, cytochrome C, brilliant green, and indocyanine green (ICG) (by spectrophotometry).

4. *In Vivo* Perfusion Test

Mongrel dogs (B. W. 15–20 kg) were submitted to an operation for end-to-side port-caval anastomosis. Forty-eight hours after the operation, the dog's common hepatic and gastroduodenal arteries were ligated for 1 hr so that they were made into hepatic failure dogs of acute hepatic ischemia. From the sixth hour after hepatic ischemia, DHP was performed for 4 hr. Further, from the 30th hour after hepatic ischemia, the second DHP was performed, when possible. A polycarbonate column (priming volume: 75 ml) containing 150 g of PUAC (1%) was uses.

The BAC column and all its circuit line were washed with saline, containing heparin (10 IU/ml). Immediately before DHP, 1,000 IU of heparin was generally given. During DHP, 500 IU each hour was injected from the inlet line of the column. The blood flow rate was kept at 4 ml/min/BW. Blood samples were taken from the inlet and outlet lines of the column with the lapse of time to perform various measurements including measurement of blood cell components, blood gas analysis, serum electrolytes and liver function tests, and measurements of NH_3-N, non-essential fatty acid and tryptophan (by modified Denkla and Dewey method[7]).

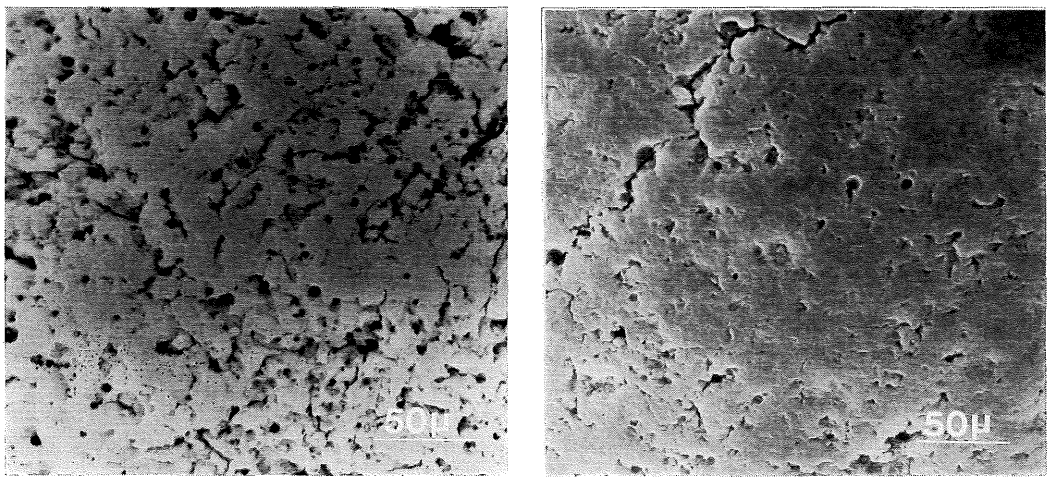

Fig. 1. Presentation of scanning electron microscope finding (×300). Left side; non-coated BAC, Right side; PUAN (1%)

RESULTS

Fig. 1 shows the observed results of the surface condition of PUAC (1%) and non-coated BAC under a scanning electron microscope. The surface has been made smooth by coating.

Table 1. Charcoal microparticles release test

shaking method

		particle (pieces) 1.5-10 μm	over 10 μm
non-coated BAC		69767	3513
PUAC	0.025%	14316	1089
	0.1 %	8749	551
	1.0 %	6272	704

bath ratio 50 ml/0.25 g, 120 cpm, 120 min.

column recirculation method

	particles (pieces) 1.5-10 μm	10-50 μm	over 50 μm
PUAC (75 g)	800	196	0

flow rate 100 ml/min, 3 hrs.

Table 1 shows the results of the charcoal microparticle release test. By the shaking method, the thicker coating decreases the release count. The 1% coated BAC shows a decrease in the release count to approximately 1/10 of that of the non-coated BAC, giving nearly the same value as the Hemocels®. By the column recirculation method, the release count in 3 hr recirculation amounted to approximately 1,000 with the particle size of 1.5-50 μm, which is equivalent to 0.05/ml. This value is much lower than that specified in the standard of water for injection in the U. S. pharmacopoeia (not more than 50/ml with particle size greater than 10 μm). From the above results, it was decided to use the 1.0% coated PUAC.

In Vitro Adsorption Test

Fig. 2 shows the adsorption performance of creatinine, vitamine B_{12} and inulin by the batch method. Less decrease in the adsorption performance of PUAC than that of the non-coated BAC shows little effect to be given by coating. In case of Hemocels®, a decrease in adsorption performance by coating was observed.

In order to compare adsorption performance

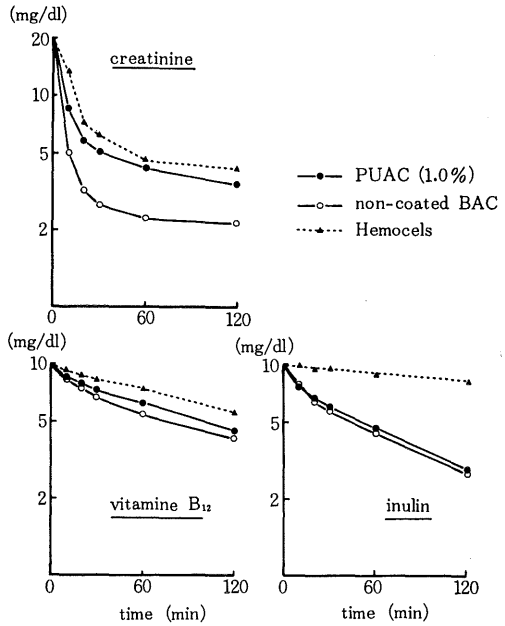

Fig. 2. Adsorption of creatinine, Vitamine B_{12} and inulin Bath ratio 50ml/0.25 g, 120 cpm, 37°C

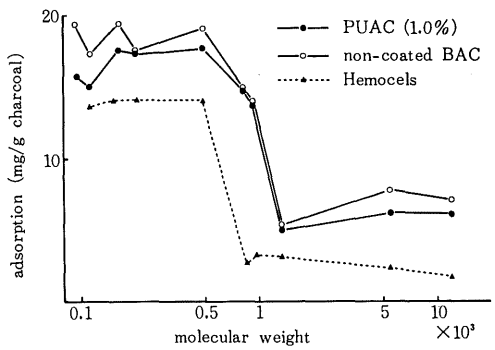

Fig. 3. Comparison of adsorption character in small to middle molecular substances. Bath ratio 50 ml/0.25 g, at 30 min, 37 °C, Solutes; Phenol (94), Creatinine (113)**, Phenylalanine (165), Tryptophan (204), Brilliant Green (483)*, BSP (838)*, ICG (924), Vitamine B_{12} (1355), Inulin (5200), Cytochrome C (12000) Intial conc. 10, 20**, 50* mg/dl

of small to middle molecular substances, PUAC (1%), non-coated BAC and Hemocels® were used. Each adsorption performance from phenol with molecular weight of 94 to cytochrome C of 12,000 was compared by the batch method. As shown in Fig. 3, the adsorption performance of PUAC shows little difference from that of the non-coated BAC for adsorption from small

to middle molecular substances. No dercrease in adsorption performance by coating was observed. In each case, however, a decrease

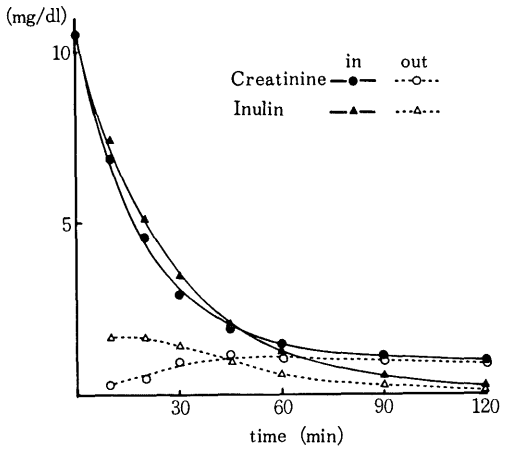

Fig. 4. Adsorption of creatinine, inulin by PUAC. The 1,000 ml solution circulated through 10 g charcoal, flow rate 50 ml/min

in adsorption performance was greater for the molecular weights over 1,000.

Fig. 4 shows the adsorption performance of creatinine (MW 113) and inulin (MW 5200) in the column recirculation test. Both are adsorbed exponentially. Creatinine reaches equilibrium in 90 min and inulin, in 120 min. PUAC shows a good adsorption performance.

For amino acids, the aromatic amino acids such as tryptophan, phenylalanine, tyrosine, were adsorbed above 90%; while the branched chain amino acids such as valine leucine, below 10% (Table 2).

Table 2. Adsorption rate of Amino Acid

	removal rate (%)
Tryptophan	96.3
Phenylalanine	94.5
Tyrosine	93.1
Methionine	29.6
Histigine	24.7
Leucine	8.5
Valine	3.4
Alanine	1.0

initial conc, 10 mg/dl, bath ratio 50 ml/0.25 g, 37°C, 120 cpm, 60 min.

In Vivo Perfusion Test

About 10 hr after hepatic ischemia, the hepatic failure dogs induced by hepatic ischemia fell into a coma. The mean survival time was 57.1±37.0 hr (m±SD, n=17). In the early stage, abrupt increases in GOT, GPT and NH_3-N had been observed. GOT and GPT reached their highest values about 18 hr after and NH_3-N about 30-40 hr after hepatic ischemia. The similar variation was observed in the extension of prothrombin time and the decrease in the amount of fibrinogen.

Four hepatic failure dogs were submitted to 4 hr DHP from the 6th hour after hepatic ischemia. Of the four, two were able to withstand the second DHP. During DHP, little variation in their vital sign and no increase in hemolysis and column internal pressure were observed. The leucocyte count was decreased to 58% of the previous value within 60 min. Thereafter, it was gradually increased reaching

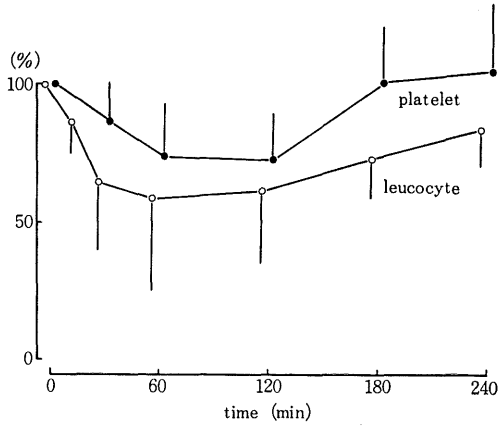

Fig. 5. Changes of platelet and leucocyte during DHP in FHF-dogs. values as mean±SD, n=5

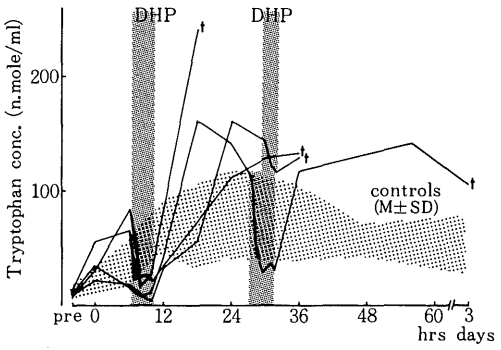

Fig. 6. Changes of free-tryptophan during DHP in FHF-dogs

83% at the end of DHP. Similarly, the platelet count was decreased to 72% within 120 min, but recovered to the previous value at the end of DHP (Fig. 5).

From 60 min after the start of DHP, improvement in the consciousness level was observed. Two hr after the end of DHP, again, it took a turn for the worse with a coma in progress. The serum free-tryptophan value was also abruptly decreased due to perfusion, showing a clearance of about 60 ml/min at all times. After the end of DHP, it was abruptly increased in a variation well correlated with that of the coma level (Fig. 6). The survival time between the minimum 22 hr and the maximum 87 hr showed difference with that of the non-DHP group.

DISCUSSION

In 1964, Yatzidis[15] reported the first application of DHP to the treatment of drug intoxication using coconut shell charcoal. Since then, DHP has been frequently applied, as a removal system, to the treatment of renal failure, drug intoxication, hepatic failure, etc., and its high effect has been clinically recognized.

In the use of activated charcoal, the most difficult problems lie in charcoal microparticle release and blood coagulation and decrease in the blood cell components. To solve such problems, various types of activated charcoal and coating meterial have been studied. Chang et al.[5] have eliminated such disadvantages by coating the charcoal with albumin-collodion. Since then, a number of coating materials have been studied including polyhydroxyethylmethacrylate[2], acrylic hydrogel[8], cellulose acetate[16], cellulose nitrate[4], heparinized hyprophilic polymer[12], etc., which are now in use. For the activated charcoal, the coconut shell charcoal used in the early stage has been replaced with bead-type charcoal with hardness from petroleum pitch that has been developed recently. As above, many types of materials have been studied providing some of them for clincial application at present.

The authors used polyetherurethane which is mainly used, as an anticoagulation material, in connection with the artificial heart and has both strength and anticoagulation property. The stronger the coating material, the thinner the coating can be performed for higher adsorption performance. Especially, it is considered that a thinner coating is required for treating fulminant hepatic failure (FHF) for which the importance of removing middle molecular substances is emphasized. The authors' PUAC has a coated thickness of approximately 0.1 μm. The coated thickness of the coated activated charcoal presently in use is 0.05–3.0 μm. The thinner coat shows superior adsorption of middle molecular substances[6].

Considering the variation in biocompatibility, especially, in platelet count and leucocyte count, the authors' PUAC shows their values in the early stage of DHP decreased to 70% of the previous values, but those improved at the end of DHP. It is nearly the same tendency as given in other DHP's. Weston et al.[14] observing a distinctive decrease in the platelet count of larger (premature) platelet during DHP for FHF, attributed the cause of inducing low blood pressure to the vasoactive substance extricated from such platelet to which activated charcoal had adhered. They used prostacyclin (PGI_2) to prevent activated charcoal from adhering to platelet and obtained a good result[10].

At present, the survival rate from FHF is 15–16%. Even the induction of the artificial liver support system, glucagon-insulin therapy and specially composed amino acid therapy has shown little effect on its improvement. In 1974, the report of the survival rate of 50% by the Williams' Group[8] became a trigger for the hemoperfusion therapy. Afterwards, however, as the number of cases increased, their survival rate has decreased to 37.8%[9] and 8.8%[13]. Also in Japan, the survival rate by the therapy mainly of hemoperfusion is approximately 15%[11]. In most cases, however, the hemoperfusion was performed in the stage of Coma Grade IV when the patient had been in a irreversible condition. If it is performed in the stage of Grade III, the survival rate will be increased three times, as reported[3]. The report emphasizes the necessity of earlier start of the therapy.

As described above, no effective therapy for FHF has yet been established at present. There are many problems to be overcome in future including the development of an effective therapy as well as the determination of hepatic failure in prognosis, the identification of toxic substances, what therapy to be performed in

what stage, etc.

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