Efficacy of Petroleum Charcoal Haemoperfusion and Acetate-free Dialysate in 10 Patients with Hepatic Coma

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Summary

Bead-type activated charcoal derived from petroleum pitch releases far fewer charcoal particles than coconut charcoal. Two types of bead charcoal, T601 and BAC-LQ, recently developed by us have excellent adsorption properties for molecules of greater molecular weight than 1,000 daltons. T601 has a larger area of pores of 10Å° – 80Å° than the BAC-LQ, resulting in stronger adsorption of molecules of intermediate size (300–5,000 daltons). The two types of charcoal are coated with 0.5 μ collodion which has been shown not to affect adsorption capacity. Our coating method is one in which the coating does not cover the entire surface of the charcoal. Pores greater than several microns remain uncoated. The BAC-LQ coated with 0.5 μ collodion adsorbs 40% of bromsulphalein (BSP), at equilibrium, in the form of protein-bound BSP. In the present study, prostaglandin E$_1$ adsorbed on charcoal was employed to decrease platelet loss. This chemical inhibits platelet aggregation. Our artificial liver-assist consists of a combination of haemoperfusion over 0.5 μ collodion-coated bead charcoal with haemodialysis using an acetate-free dialysate recirculation system. A total of 32 haemoperfusions were performed in 10 patients: 3 with hepatic cirrhosis (1 with portacaval shunt), 6 with fulminant hepatitis, and one with halothane-induced liver failure. Nine of the ten patients showed varying degrees of improvement in neurological or EEG status.

Introduction

Haemodialysis using cellophane or cuprophane membranes is said to be ineffective in the treatment of hepatic encephalopathy. Therefore the removal, not
only of water soluble substances, but also larger molecules has been considered. Accordingly, activated charcoal has been used successfully for haemoperfusion of patients with hepatic failure. However, coconut charcoal, which is frequently used at present, is not always satisfactory in terms of particle release, and adsorption capacity for larger molecular substances. Our new artificial liver-assist features collodion-coated (Chang et al, 1972) bead-type activated charcoal, derived from petroleum pitch, used as the adsorbent.

METHODS AND MATERIALS

Bead charcoal (diameter 0.6 mm) is a very hard material with a very smooth surface. Compared with other types of activated charcoal, it releases much smaller amounts of heavy metals. In order to test its long-term toxicity, the bead charcoal was implanted in guinea-pigs subcutaneously and intra-abdominally for 12 months. During this period no toxic reaction was observed in the surrounding tissues. The number of carbon grains (1.2 – 5.0 μ) isolated from the charcoal, counted by a Coulter Counter, was far less than from coconut charcoal (Amano et al, 1976). Bead charcoal was coated with 0.5 μ colloidion, for use in direct haemoperfusion, by spraying a 0.1% pyroxylin ethanol solution using 3.75 g of pyroxylin per 1 g of charcoal. This coating is unique in that the entire surface of the charcoal is not covered and relatively large pores (<10 μ) remain open (Figure 1).

The adsorption of the following substances by bead charcoal (BAC-LQ and extra-porous T601) and coconut charcoal (HC-30) was examined under coated and uncoated conditions: urea (MW 60 daltons), creatinine (113), uric acid (168), Yellow 1 (350), Red 102 (640), vitamin B12 (1354), inulin (5,000), and cytochrome-C (13,000).

To solutions of the above substances, 10 mg/100 ml in saline buffered at pH 7.4, was added 0.6 g of charcoals BAC-LQ, T601 or HC-30 and the suspensions were shaken at 120 rpm for 2 hours at 36°C. The concentrations were measured colorimetrically by the UV method. The adsorption of ammonia, amino acids, phenol, indole, amino nucleoside, pyruvic acid, α-ketoglutaric acid and short-chain fatty acids, which are said to be elevated in the serum of patients with hepatic encephalopathy, were examined in saline buffer by the UV, Naphthoquinone, and Dinitrophenyl-hydrazine methods. In order to investigate the absorption of larger molecular substances, bromsulphalein (BSP) as a protein-bound substance was studied. One ml of BSP (0.5 w/v%) was added to 10 ml of human plasma and shaken thoroughly. Into this solution, 0.44 g of uncoated and 0.5 μ colloidion coated BAC-LQ was added. Each mixture was shaken at 120 rpm for 2 hours (36°C). The concentrations were measured by the visual adsorption method.
Figure 1. 0.5 μm collodion coated bead charcoal (T-601) × 200
RESULTS

Wide Spectrum Adsorption Capacity of Bead Charcoal

The BAC-LQ proved to have approximately twice the adsorption for Red 102 (602 MW) and vitamin B_{12} (1354) than did HC-30. Compared with HC-30, BAC-LQ is also more avid in the adsorption of substances with molecular weights up to 5,000. The extra-porous T601 showed 1.5-fold adsorption of any substances compared with HC-30. The adsorption capacity of the charcoal is hardly affected by the 0.5 μm collodion membrane, and even for larger molecules there was no change in adsorption (Figure 2).

Figure 2. The ability of petroleum charcoals and coconut charcoal to adsorb different molecular weight substances

Adsorption of Substances which are Elevated in Patients with Hepatic Failure

The substances adsorbed well by bead charcoal were: amino acids (methionine, phenylalanine, tyrosine, tryptophan, etc), phenol, indole, nucleoside, and short-chain fatty acids. On the other hand pyruvic acid or α-ketoglutaric acid were hardly adsorbed (Figure 3).

In a phosphate-saline buffer, 60% of unbound BSP was adsorbed by 0.5 μm collodion-coated charcoal, BAC-LQ, and in human plasma 40% of BSP adsorbed in the form of protein-bound BSP. The adsorption pattern was not different from uncoated charcoal (Figure 4).

Clinical Results

Our artificial liver-assist is shown in Figure 5. We have found that when acetate
<table>
<thead>
<tr>
<th>Substance</th>
<th>Adsorption</th>
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<tr>
<td>ammonia</td>
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<td>pyruvic acid</td>
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<td>α-ketoglutaric acid</td>
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<td>serotonin</td>
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<td>γ-aminobutyric acid</td>
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Figure 3. The ability of petroleum activated charcoal to adsorb substances which are increased (?) in hepatic coma patients' plasma.

Figure 4. The ability of petroleum charcoal to adsorb protein bound BSP in human plasma (by measuring visible absorption spectra).
is contained in dialysate, abnormalities of blood lactate and pyruvate levels occur and the control of pH becomes difficult. We tried the addition of bicarbonate to dialysate, but calcium carbonate formed with the bicarbonate inflow from dialysate to blood. Therefore we now infuse NaHCO₃ and CaCl₂ intravenously to control electrolytes and pH. A total of 32 haemoperfusions were performed over one year in 10 patients with hepatic coma (Table I): 3 with hepatic cirrhosis (one

### Table I. List of Hepatic Coma Patients Treated by Artificial Liver Assist

| Case | Age | Sex | Cause of coma | Portal vein | Bilirubin (mg/dl) | GGT (Kunitz unit) | GOT (IU/l) | Aspartate Transaminase (IU/l) | Serine Aminopeptidase (IU/l) | Alkaline Phosphatase (IU/l) | Pepsinogen (mg/l) | Creatinine (mg/dl) | Frequency of death | Case of death |
|------|-----|-----|---------------|-------------|------------------|------------------|----------|-------------------------------|-------------------------------|-------------------------|------------------|-----------------|------------------|---------------|----------------|
| 1    | 40  | M   | Cirrhosis     | 22.8        | 1.6              | 56               | 216      | 7.410                         | 140.3.5                      |                         |                  |                 | Boecky - G.I. bleeding | 2             | survive       |
| 2    | 63  | M   | Cirrhosis     | 22.8        | 1.6              | 56               | 216      | 7.410                         | 140.3.5                      |                         |                  |                 | Boecky - G.I. bleeding | 2             | survive       |
| 3    | 55  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 4    | 55  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 5    | 54  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 6    | 55  | F   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 7    | 55  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 8    | 55  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 9    | 55  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
| 10   | 55  | M   | Fulminating   | 15.6        | 2.0              | 44               | 206      | 7.360                         | 128.3.9                      |                         |                  |                 |                 | 32            | survive       |
with portacaval shunt), 6 with fulminant viral hepatitis and one with halothane-induced liver failure. Of the ten patients, nine showed improvement in the EEG, and four survived. There was a 10-30% fall in platelet count, but no patients died of thrombocytopenia.

**DISCUSSION**

Willson et al (1972) paid attention to large molecular substances and reported the effectiveness of resins for the adsorption of protein-bound substances. In another report, Weston et al (1974) performed charcoal haemoperfusion in dogs with acute hepatic failure and could decrease serum ammonia and prolong their survival. Although we investigated the adsorption capacity of bead charcoal for substances of differing molecular weights, the exact chemical and/or physical mechanisms involved in the differentiation process require further study. However, we believe that bead charcoal of wide adsorption spectrum such as T601 will be quite useful where the removal of substances of intermediate molecular weight becomes important. At the same time, it is desirable that advances be made in polymer-coating technique so that platelet and white-cell adsorption may be decreased, and adsorption capacity maintained. In this consideration, a study of the use of prostaglandin E₁, which is able to inhibit platelet aggregation, adsorbed on bead charcoal coated with a collodion membrane is being pursued.

**References**

Chang, TMS, Gonda, A, Dirks, JH, and Coffey, JF (1972), *Transactions of the American Society for Artificial Internal Organs*, 18, 465  
Willson, RA, Webster, KH, Hofmann, AF and Summerskill, WHJ (1972)  
*Gastroenterology*, 62, 1191

**Open Discussion**

DENTI (Saluggia) I have one short comment and one question for Dr. Malchesky, Since 1975 we have been studying adsorbent-filled fibres, using the Enka Glanzstoff fibres. We have tried to quantitate the microparticle release from cartridges thus constructed, using a Coulter counting technique. I am able to confirm the absence of any significant particle release if the unit is well-constructed, without any damaged fibres. No more particles are released than are normally generated by roller-pump action on the tubing.

My question revolves around the difficulties of sterilising this kind of device because ethylene oxide is adsorbed by the carbon, the membrane is temperature
sensitive, and formaldehyde is strongly adsorbed and only slowly released. What kind of sterilisation technique do you use?

MALCHESKY As I have mentioned, we carried out steam sterilisation of fibres when we were carrying out our adsorption studies and the adsorption remains within the range of adsorption of unsterilised fibres. So we have found sterilisation to be a reasonably good procedure at the present time.

KLINKMANN (Rostock) I have a question to the whole Japanese group but mainly to Dr Odaka. First of all, did you see any pyrogenic reactions during the treatment in your patients? The second question is, your colleagues have shown quite clearly that there is a steep decrease and a high adsorption of amino acids. I wonder if you can give us any information on what happened to the amino acid level in your patients?

ODAKA We checked on both pyrogen and bacteria before supply of the column. We tested after 7 days for pyrogen and 10 days for bacteria. If there are no problems, the column is applied to the patient. So we did not see pyrogenic reactions during treatment except 2 cases which were carried out in the beginning of this series.

In answer to the second question, the activated charcoal adsorbs many kinds of amino acid, so we use collodion membrane over charcoal. Each semipermeable membrane has a different cut-off point. The characteristic of the semipermeable membrane is a very important factor for preventing amino acid adsorption.

KLINKMANN Did you actually measure amino acids in your patients?

ODAKA Yes, I measured them in hepatic coma patients. Some kinds of amino acids are adsorbed, but not all kinds.

CAMBI (Parma) I wonder if the removal of the solutes not adsorbed by charcoal, like urea, is affected by the presence of charcoal on the membrane. Did you measure actually the removal of urea?

MALCHESKY Yes, as I showed on the slide there was no urea adsorption in any of the charcoal devices that we studied.

CAMBI Yes, but is the urea clearance through the cuprophane with this system not reduced, actually?

MALCHESKI There was no urea removal by these systems.

CAMBI Do you have any figure for removal of the amino acids per hour?

MALCHESKY We have not studied amino acids yet with this system, but right now we have an on-going programme studying hepatic-assist devices, and we will be looking at that at some later date.

CHAIRMAN (PARSONS London) I have asked the other contributors about this and no one is measuring amino acid loss per hour, which is a very important and worrying feature of this treatment.
KENNEDY (Glasgow) I feel that haemoperfusion will undoubtedly have a therapeutic role in some forms of drug intoxication and it may have a role in hepatic failure, although I think that remains to be proven. As regards chronic renal failure I believe it will have a role only if it can be shown that it can remove substances which are inadequately removed or not removed at all by conventional haemodialysis and I do not think it is enough to demonstrate that a combination of haemoperfusion and haemodialysis removes rather more urea and rather more creatinine and rather more uric acid. After all, we can do that simply by dialysing our patients for longer or by using a bigger surface area. I think the onus is to prove that it is doing something qualitatively different in the correction of uraemia. I wonder if any of the speakers have a comment?

MALCHESKY In the system we are studying we have noted that you can remove substances that are least removed in dialysis better with the charcoal fibre system; for example, uric acid removal is better than creatinine; likewise salicylate is better than creatinine. So, where with dialysis small molecules and water soluble molecules, like creatinine, are removed at a much higher rate, I think you have here a means of regulating the removal of different types of molecules with techniques in addition to dialysis, which is why we are thinking of combination-type devices. It may be important to modify the present fibre or devices. What we have might not be adequate. We might have to open the pore size and we might have to modify the filling materials and use other sorbents.

CHAIRMAN I must say, if I may add a comment on hepatic coma, the patient waking up in three hours is very difficult to achieve with straight-forward haemodialysis. So something is coming out on these columns which may be influencing the degree of coma, which is very interesting. But I have asked the speakers and they do not have any measurements on these molecules that are coma-inducing or bleeding-inducing.

ANDREUCCI (Naples) I could not read these slides with all the data on the patients in hepatic coma. I am talking about the last paper. I was just wondering about the improvement in renal function of these patients. These patients had hepatic coma, perhaps they had the hepato-renal syndrome. Did their renal function improve with this haemoperfusion or not?

CHAIRMAN That's the question addressed to Dr Amano. Could you tell us whether dialysis improved renal function or whether the renal function stayed poor throughout?

AMANO The patients with hepatic encephalopathy had good renal function, but I have as yet no clinical data about the change of intermediate molecules before and after haemoperfusion.

CHAIRMAN Thank you very much. I think that is an important question. The substances that are sending you into coma may not be the same that are shutting down your renal circulation in this syndrome.

CASTRO (Munich) I would like to make a comment. We are testing now a new type of dialysate which combines the two possibilities, dialysis and perfusion. There are hollow fibres with two layers. One layer in the inside has 10 micron
cuprophane and on the outside a second layer which includes a charcoal. We can make in the same device the two mechanisms, dialysis and perfusion. There is the possibility of using the new dialyser with only a small quantity of dialysate.

CHAIRMAN Have you a question? That was very interesting.

CASTRO It is a comment about the problem; as Professor Kennedy said, between dialysers and perfusion. I don’t have a question.

HABERAL (Ankara) A question for Dr Odaka. He showed us on a slide the increase in RBC, WBC and platelet level. How do you explain this increase in level?

ODAKA In each dialysis about 2 litres of water was removed so the concentration is altered.

CHAIRMAN Haemoconcentration.

HABERAL How many times do you dialyse a patient in hepatic coma for him to get well?

AMANO Daily, for three or four hours.

CHAIRMAN For how many days?

AMANO One.

CHAIRMAN He dialysed three hours on one day and that was that!

HABERAL That is enough?

CHAIRMAN That seems enough!

HERWIG (Chur - Switzerland) I have a question. Did you notice a drop in blood pressure in the patients? It seems that hormonal pressor substances may be adsorbed too, such as thyroid hormones.

CHAIRMAN Dr Ota would like to comment on these hormones and other important substances that may be being removed during charcoal perfusion.

OTA I did not measure pressor hormones. But I think they may be removed because the incidence of hypotension was more frequent during haemoperfusion than with conventional haemodialysis.

KOPP (Munich) Do you have any data on adsorption of heparin, or about coagulation factors or increased activation of coagulation, with resultant coagulation within the column, or increased bleeding from the patient?

CHAIRMAN Are there any troubles with coagulation or evidence for heparin adsorption?
ODAKA We used 6000 units of heparin in each dialysis.

CHAIRMAN And that’s enough?

ODAKA Coagulation factors are within the normal range. We use one infusion of 6000 units of heparin per perfusion.

CHAIRMAN This does not seem to be a lot.