Effects of haemoperfusion through charcoal or XAD-2 resin on an animal model of fulminant liver failure

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SUMMARY In a group of dogs in whom fulminant liver failure had been induced, perfusion of blood through activated charcoal resulted in a significantly longer survival than that of a similar group of dogs whose blood was not so treated. An otherwise progressive rise in blood ammonia concentration was halted in the treatment group. In another group of dogs with fulminant liver failure perfusion of blood through the resin Amberlite XAD-2 was associated with a fall in the serum bilirubin concentration and complete clearance from the blood of ^{14}C -labelled sodium glycocholate. Survival in this group of animals was not significantly prolonged. This was due at least in part to the occurrence of haemorrhage due to thrombocytopenia. Platelets adhere to the resin but do not adhere to the same degree to charcoal coated with a thin layer of polymer.

The pathogenesis of coma in acute liver failure is unknown, although retention of toxic metabolites is likely to be important. These may be water-soluble or lipid-soluble and therefore protein-bound. Recently it has been shown that protein-bound molecules may be removed from plasma by perfusion through columns containing particles of an ion-exchange resin (Willson, Webster, Hofmann, and Summerskill, 1972). Similar perfusion through activated charcoal removes water-soluble substances. Indeed, molecules of intermediate size (molecular weight 300-2000) are removed more efficiently by this method than by haemodialysis (Chang, 1972) and charcoal haemoperfusion has been used to supplement haemodialysis for the treatment of uraemia (Yatzidis, 1964; Dunea and Kolff, 1965; Chang, 1972). Similarly, haemoperfusion over activated charcoal may be used for the removal of glutethimide and barbiturate from patients who have taken an overdose of these drugs (De Myttenaere, Maher, and Schreiner, 1967; Yatzidis, Oreopoulos, Triantaphyllidis, Voudiclaris, Tsaparas, Gavras, and Stavroulaki, 1965), and in our recent studies we also showed that the loss of white blood cells and platelets was minimized by the application of a thin polymer coating to the particles (Gazzard, Langley, Weston, Dunlop, and Williams, 1974).

It is possible that a liver support system could be based on these principles and this paper is concerned with the effects of haemoperfusion through columns of particles of activated charcoal or the resin Amberlite XAD-2 on an animal model of liver failure.

Materials and Methods

PLAN OF STUDY

Liver failure was induced in healthy greyhounds (22-30 kg body weight) by a modification of the method of Rappaport, MacDonald, and Borowy (1953). A side-to-side anastomosis between the superior mesenteric vein and inferior vena cava was performed, and a loose tie with two hitches placed around the hepatic artery. The ends of this tie were brought out through the anterior abdominal wall. The lesser omentum and the left triangular ligament were ligated to exclude any accessory arterial supply to the liver. Twenty-four hours later, when the dogs had recovered, the hepatic artery was occluded by pulling on the ties. Signs of liver failure developed within a few hours.

Sixteen dogs were allocated to a control (six animals) or haemoperfusion (10 animals) group before operation. In five animals of the second group haemoperfusion was carried out from the time of the hepatic artery ligation for 10 hours, using charcoal as

the adsorbent. The other five animals had a haemoperfusion with XAD-2 resin but, because of the severe loss of platelets and white cells, this was only for six hours. Also, the start of the haemoperfusion was delayed for four hours to allow some accumulation of protein-bound substances. All animals received an infusion of 10% dextrose to maintain a positive central venous pressure of 1 cm of water and blood glucose above 100 mg%, measured by Dextrostix. Anticoagulation for the haemoperfusion was obtained with a loading dose of 2000 units of heparin followed by a constant infusion of 2000 units of heparin/hr: the same doses were administered to the control animals.

PERFUSION TECHNIQUE

The dog was connected to the haemoperfusion circuit via an arteriovenous Quintin-Scribner shunt in the neck and blood flow induced with a Watson-Marlow roller pump (MHRE 88). The charcoal columns had been prepared as follows: Sutcliffe-Speakman coconut charcoal (610) with a 10% (by weight) coating of polyhema (polyhydroxyethyl-methacrylate, trade name Hydron) was washed with 10 litres of normal saline and sieved to remove particles smaller than 600 μ in diameter. About 200 g of this charcoal was then packed into a plastic column 3.8 $\times$ 25 cm (Wright Scientific Ltd) which had 600 μ filters at either end.

For the resin haemoperfusions, a 10.2 $\times$ 12.7 cm column with 125 μ filters was used. Amberlite XAD-2, 20-50 mesh (Rohm and Hass Ltd) was soaked in normal saline for 24 hours before use, and then sieved to remove particles less than 125 μ . About 400 g was packed into the column. Both charcoal and resin columns were perfused with heparinized saline (1 unit/ml) immediately before use.

MEASUREMENTS

The blood pressure, central venous pressure, urine output, and conscious state were monitored hourly. Blood samples were obtained immediately after hepatic artery ligation and thereafter at four hourly intervals. The haemoglobin levels, platelet counts, and values of aspartate aminotransferase (used as a measure of the extent of hepatic necrosis) were estimated by standard methods.

Blood levels of ammonia (Kirsten, Gerez, and Kirsten, 1963) and lactate and pyruvate (Hohorst, Kreutz, and Buecher, 1959) were determined as examples of water-soluble substances. In addition, the amino acid chromatogram was obtained immediately after hepatic artery ligation and towards the end of the perfusion with an autoanalyser (Biocal 13C100). Changes in the serum bilirubin were

determined and used as a measure of the removal of protein-bound substances.

The removal of ^{14}C -labelled sodium glycocholate (Radiochemical Centre, Amersham) by the resin was also studied. In two dogs 10 μCi was given intravenously 10 minutes before the perfusion and the radioactivity in subsequent samples from the arterial and venous lines of the column was measured with a liquid scintillation counter (Hewlett Packard, 3220) and an external standard to monitor counting efficiency.

After death all animals had a necropsy to establish (a) the patency of the mesenterico-caval shunt, (b) that the liver had been devascularized satisfactorily, and (c) the extent of any intraperitoneal haemorrhage.

Results

The mean time of survival of the charcoal haemoperfused animals was 17 hours (range 10-22 hours) and that of the controls nine and a half hours (range 6-12 hours) and this was a highly significant difference ($t\ 3.4, = P = 0.001$). The resin-haemoperfused group also survived longer (mean 13 hours, range 8-22 hours) but this was not statistically different from the control group. Following hepatic artery ligation, the times at which precoma and coma developed were variable and there was no difference in these times between the control or either of the haemoperfused groups. The blood pressures of the control and charcoal-haemoperfused animals were stable until three to five hours before death, when a gradual reduction was observed coinciding with oliguria and, eventually, anuria.

In the resin-haemoperfused animals a more precipitous fall of the blood pressure occurred and in four out of five of these animals necropsy revealed extensive intraperitoneal haemorrhage. These animals showed large falls in platelet counts so that at eight hours the count was only 20% of the initial value. In contrast, the charcoal-haemoperfused and control groups showed a much smaller decrease (table I). White blood cell losses during resin perfusion ranged from 75 to 90% of the initial

	Percentage Reduction (± 1 SE) at	
	Four Hours	Eight Hours
Controls	13 $\pm$ 7	29 $\pm$ 10
Charcoal haemoperfusion	13 $\pm$ 5	32 $\pm$ 14
Resin haemoperfusion	5 $\pm$ 10	79 $\pm$ 3.4*

Table I Reduction in platelet count from initial level over the period of study

*Significantly different from other two groups ($P < 0.001$)

Fig 1 Changes in arterial blood ammonia in control and charcoal haemoperfused animals (mean level $\pm$ 1 SD). Controls ----; charcoal haemoperfusion group ———.

Fig 2 Changes in serum bilirubin levels in controls and resin haemoperfused animals (mean level $\pm$ 1 SD). Controls ----; resin haemoperfusion group ———.

arterial level after the first hour but showed a tendency to recover towards the end of the perfusion. Losses were much less severe during the charcoal perfusions.

BIOCHEMICAL CHANGES

As expected, levels of arterial blood ammonia rose progressively in the control animals (fig 1). In the charcoal haemoperfusion group, however, there was no rise and at eight hours the blood ammonia levels were significantly lower than in the controls ($P < 0.05$). When perfusion was stopped arterial ammonia rose steeply.

Similarly, lactate levels rose progressively in the control animals, from 0.5 (SD $\pm$ 0.2) to 3.5 (SD $\pm$ 0.8) m moles/l at eight hours. Levels were lower in the charcoal haemoperfusion group although the differences did not reach statistical significance. Changes in pyruvate levels followed the same pattern. There were no significant differences in the changes of amino acid levels of the charcoal haemoperfused and those of the controls. In the whole series there was a significant rise in glycine, alanine, and phenylalanine (table II).

In both the charcoal-haemoperfused and control

	Mean Values (μ moles/l $\pm$ 1 SE)	
	Initial	Final
Glutamine	1605 (279)	2573 (531)
Glycine	318 (41)	745 (142) ^a
Alanine	380 (53)	959 (168) ^a
Phenylalanine	144 (25)	245 (54) ^a
Histidine	191 (36)	259 (36)
Isoleucine	103 (19)	73 (21)
Leucine	183 (55)	137 (60)

Table II Levels of amino acids immediately after hepatic artery ligation and before death in whole series

^aSignificant difference ($P < 0.005$)

animals there was a progressive rise in serum bilirubin, but in the resin-haemoperfused group there was a rapid fall during perfusion and at eight hours the level was significantly lower than in the controls (fig 2). In the two animals given ¹⁴C-labelled sodium glycocholate sequential blood measurements showed its complete removal during a single passage across the column (fig 3). The ammonia, lactate, and amino acid levels in the resin-haemoperfused animals were similar to those seen in the control animals.

Fig 3 Fall in arterial blood levels of ^{14}C -labelled sodium glycocholate during resin haemoperfusion (mean values of two dogs). Arterial —; venous ----.

Discussion

The animal model of acute liver failure used in the present studies proved reasonably reproducible as was shown by the relatively narrow range of survival times in the control animals. Many of the biochemical features seen in man with fulminant liver failure were present, including a raised arterial blood ammonia and gross amino acid changes. In all animals, the aspartate aminotransferase values rose to more than 1000 iu/l and were thus indicative of severe liver necrosis. However, in this animal model the situation differs in two respects from the syndrome seen in man. First, the course of the liver failure is very rapid and is not potentially reversible. Secondly, the lesion produced, being dependent upon ischaemia of the liver, differs from the pathogenesis of most cases of acute hepatic necrosis in man. Success of this support system could only be assessed, therefore, by prolongation of survival time, whereas in man the objective is complete recovery as a result of the time provided for the liver to regenerate.

There are reports of improvement in the level of consciousness of patients with severe liver failure following both haemodialysis (Kiley, Pender, Welch, and Welch, 1958) and peritoneal dialysis (Jones, Strader, and Berry, 1959; Nienhuis, Mulmed, and Kelley, 1963), and so it is possible that water-soluble toxins are at least partially responsible for the coma seen. Improvement in such patients is often delayed and may be dependent on equilibrium of toxins between cerebrospinal fluid and blood, a process

which is known to take 24 hours or longer for water-soluble substances such as mannitol and insulin (Sisson and Oldendorf, 1971). The production of behavioural and electro-encephalographic changes in experimental animals following the injection of a dialysable extract from the plasma of patients in hepatic coma is further evidence that water-soluble substances may be important (Breenan and Plum, 1971). Although ammonia is adequately dialysed (Kiley *et al*, 1958; Sherlock, 1961), blood levels do not correlate well with coma and its importance is controversial (Schwartz, Phillips, Gabuzda, and Davidson, 1953).

In our charcoal-haemoperfused dogs the blood ammonia fell, but we cannot unreservedly attribute their longer survival to this change.

Protein-bound substances may also play an important part in the aetiology of liver coma. Bile salts, as an example of such, are toxic to brain homogenates (Lascelles, 1971) and it is possible that these as well as other metabolites could inhibit regeneration of the liver. Willson *et al* (1972) demonstrated that protein-bound substances, such as bilirubin and sodium chenodeoxycholate, may be removed effectively from plasma with the resin XAD-2 and it was for this reason that haemoperfusion through this substance was used for our second group of animals. The serum bilirubin fell and extremely rapid removal of a tracer dose of ^{14}C -labelled sodium glycocholate was demonstrated.

The process of molecular adsorption by activated charcoal and polystyrene resins such as XAD-2 is thought to be brought about by hydrogen bonding. The nature of the surface of the molecular matrix is crucial with regard to what substances are attracted to it but so also is the access that the molecules have. Thus, although the surface area available for adsorption in activated charcoal (1500 sq m/g) is high compared with XAD-2 (330 sq m/g), its pores are only a fifth of the diameter of those in XAD-2. It may be for reasons such as these that protein-bound substances are better adsorbed by polystyrene resins. The lack of a major fall in the blood platelet levels of the charcoal-haemoperfused dogs indicates that a satisfactory solution to the problem of platelet and white cell losses may have been found in polyhema. However, we have not yet found a suitable coating for the resin XAD-2 which will still permit the adsorption of protein-bound molecules. The thrombocytopenia and haemorrhage seen when this resin was used for the haemoperfusion of our second group of animals underlines the need to solve this problem before the technique is used to treat patients with liver failure who are already at risk from bleeding.

Although our present studies indicate that charcoal haemoperfusion alone may have a place in the

treatment of patients with fulminant liver failure, it is important that the design of an artificial liver support system should also include a method for the removal of protein-bound toxins.

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