PROSTACYCLIN ELIMINATES THE BIOINCOMPATIBILITY OF CHARCOAL HAEMOPERFUSION

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Summary

During charcoal haemoperfusion (CHP) in dogs, use of prostacyclin (PGI$_2$) in addition to heparin reduced the loss of platelets (25 ± 6 vs 83 ± 2%), the formation of platelet aggregates as judged by screen filtration pressure (65 ± 6 vs 249 ± 25mm Hg) and fibrinogen consumption (20 ± 5 vs 46 ± 6%). Prostacyclin also delayed neutralisation of heparin during CHP. This improvement of biocompatibility may now allow a proper assessment of CHP in liver failure.

Introduction

Widespread assessment of charcoal haemoperfusion has been hampered by problems of bioincompatibility, particularly in patients with fulminant hepatic failure. Polymer coating of the charcoal particles [1–4] seemed a promising method of overcoming these problems but inevitably reduced adsorption efficiency [3,4]. Despite coating with polyHEMA, charcoal haemoperfusion in patients with fulminant hepatic failure caused thrombocytopenia, deterioration in platelet function and the formation of platelet aggregates in the blood returning to the patient [5]. Antiplatelet agents, such as sulphipyrazone and dipyridamole, failed to ameliorate the problem significantly [6]. The recent availability of synthetic prostacyclin (PGI$_2$), [7], the most potent inhibitor of platelet function known, and our experience with its use during haemodialysis [8] prompted an evaluation of PGI$_2$ in charcoal haemoperfusion in healthy dogs.

Materials and Methods

Charcoal Columns and Haemoperfusion Technique

All haemoperfusions were carried out with Becton Dickinson Haemodetoxifier columns containing approximately 100g of uncoated charcoal immobilised on a polyester film. After induction of anaesthesia with intravenous thiopentone
(30mg/kg) fourteen healthy greyhounds had arteriovenous shunts inserted between the carotid artery and the jugular vein. Connections between the columns and the AV shunts were with standard dialysis blood lines and blood flow in the circuit was maintained at 200ml/min using a Watson Marlow roller pump. Haemoperfusions lasted for 60 to 120 minutes, and anaesthesia without assisted ventilation was maintained throughout.

**Heparin and PGI₂ Administration**

Thirteen animals received a bolus of heparin (200IU/kg) by intravenous injection 15 minutes before haemoperfusion. At the same time seven of these animals were given a continuous intravenous infusion of PGI₂ (50ng/kg/min), which was re-sited in the inlet blood line to the column at the start of haemoperfusion and continued at the same rate during the procedure. One animal was haemoperfused with PGI₂ alone.

**Blood Sampling**

Before haemoperfusion and at intervals during the procedure blood samples were withdrawn from the inlet blood line for estimation of platelet counts [9], plasma fibrinogen [10] and calcium thrombin clotting time [11]. At 15, 30, 45, 60, 90 and 120 minutes samples of blood returning from the column were withdrawn from the outlet line for measurement of whole blood screen filtration pressure [12].

Statistical analyses were performed using a one-tailed Student t test.

**Results**

**Haemoperfusion with Heparin or Heparin Plus PGI₂**

During charcoal haemoperfusion with heparin alone the platelet count fell to 17 ± 2% of the initial value and the screen filtration pressure of blood leaving the column rose dramatically at 30 and 45 minutes. Thereafter the screen filtration pressure fell spontaneously to original levels.

On the other hand, in the animals infused with PGI₂ in addition to heparin the platelet count was maintained above 75% throughout haemoperfusion and no elevation of the screen filtration pressure was observed (Figure 1).

To measure the duration of heparin activity in animals without charcoal haemoperfusion 6 greyhounds received heparin (200IU/kg) intravenously while under thiopentone anaesthesia. Heparin activity was monitored at 15 minute intervals by calcium-thrombin clotting time estimation (Ca–TCT). The results (mean ± ISD of Ca–TCT) shown as the shaded area in Figure 2, differed little from the values found in dogs undergoing charcoal haemoperfusion with heparin plus PGI₂. However, during haemoperfusion with heparin alone this response curve was shifted to the left, indicating more rapid removal or inactivation of heparin.
Figure 1. **Upper Panel.** Mean ± SEM of platelet counts (% of initial) during charcoal haemoperfusion with heparin alone (○—○) and heparin plus PGI₂ (●—●). **Lower Panel.** Mean ± SEM of screen filtration pressure of blood leaving the column during charcoal haemoperfusion with heparin alone (closed) and heparin plus PGI₂ (open).

The plasma fibrinogen level during CHP with heparin alone fell to approximately 55% of the initial value. Although there was an early, smaller fall in fibrinogen level during haemoperfusion with heparin plus PGI₂, this was reversed towards the end of the procedure (Figure 2).
Figure 2. Upper Panel. Mean ± SEM of calcium-thrombin clotting time (secs) during charcoal haemoperfusion with heparin alone (○—○) and with heparin plus PGI₂ (●—●). The shaded area represents mean ± SD of Ca-TCT in animals which received heparin but were not haemoperfused (see text). Ca-TCT greater than 10 minutes were not quantified further. Lower Panel. Mean ± SEM of plasma fibrinogen (% of initial) during charcoal haemoperfusion with heparin alone (○—○) and with heparin plus PGI₂ (●—●).

Explanatory note to figures: * p < 0.025, ** p < 0.005, *** p < 0.0005, **** p < 0.00005

Haemoperfusion with PGI₂ Alone

Charcoal haemoperfusion in one dog without heparin but with PGI₂ resulted in a brisk fall in platelet count and an immediate rise in screen filtration pressure
above 300mm Hg, the maximum recording capacity of the transducer. A con-
sumption coagulopathy resulted with the plasma fibrinogen falling from 1.4 to
0.3g/l within 30 minutes and the Ca-TCT exceeded 10 minutes. Despite these
changes the column was not occluded by blood clot.

Discussion

The dramatic preservation of platelet numbers with infusion of prostacyclin
during charcoal haemoperfusion is probably a reflection of the prevention of
platelet aggregate formation. Neutralisation of heparin during extracorporeal
circulation is mediated by release of platelet factor 4 [13,14], and the preserva-
tion of calcium thrombin clotting times with prostacyclin may be another mani-
festation of platelet inhibition. It is possible that prostacyclin also prevented
release of platelet factors which polymerise fibrin monomer, and thus reduced
fibrinogen consumption. Cationic proteins from leukocytes and platelets [15]
are capable of polymerising fibrin monomer, which we have detected by the
serial dilution protamine sulphate test during haemodialysis in dogs and this
'paracoagulation' is not inhibited by heparin [16]. It is interesting to note that
another antiplatelet agent, sulphipyrazone, reduces fibrin deposition on dialy-
sers [17]. The preservation of platelets and fibrinogen, and the minimisation of
heparin which occurs with use of prostacyclin should minimise the risk of bleedin-
g when charcoal haemoperfusion is used to treat patients with fulminant hepatic
failure.

The spontaneous disappearance of platelet aggregates later during the char-
coal haemoperfusions in which heparin alone was used may simply reflect the
depletion of active circulating platelets such as has been described previously
during haemoperfusion [5] and during cardiopulmonary bypass [18]. Alterna-
tively, as the lungs are the main source of circulating PGI₂ [19,20] the arrival
of platelet aggregates in the pulmonary circulation may have stimulated release
of large amounts of prostacyclin into the systemic circulation with subsequent
inhibition of any further platelet aggregation on the charcoal column. Since
prostacyclin is also a vasodilator such a phenomenon might also have explained
the severe hypotension reported during charcoal haemoperfusion in patients
with fulminant hepatic failure that occurred when platelet aggregates were form-
ed [5]. The dispersal of platelet aggregates by infusion of prostacyclin that has
been reported with in vitro haemoperfusion provides some support for this
hypothesis [21].

Unlike haemodialysis [8], use of prostacyclin alone was insufficient to pre-
vent a consumption coagulopathy during charcoal haemoperfusion, and this
experiment underlines the marked thrombogenicity of uncoated charcoal com-
pared with cuprophane membranes used for dialysis.

As with other forms of extracorporeal circulation [22] the use of currently
available antiplatelet agents such as dipyridamole and sulphipyrazone during
charcoal haemoperfusion [6] has produced unremarkable results. It is possible
that these drugs are more effective when administered chronically as we have
shown for sulphipyrazone during haemodialysis [17]. Prostaglandin E₁ and
the calcium chelators, EDTA and citrate, are potent inhibitors of platelet func-
tion and their use has been described in experimental haemoperfusion with effective platelet sparing [22]. However, the cardiovascular and gastrointestinal side effects of PGE₁, and the toxicity of chelators with the necessity for titrated infusions of divalent ions into the blood returning to the patients, make the clinical use of these agents unattractive. On the other hand, at doses of PGI₂ which inhibited platelet activation and fibrinogen consumption during charcoal haemoperfusion, as reported here, we did not observe any adverse effects. The short duration of action and rapid reversibility of the effects of PGI₂ [7] may be a positive advantage, as effective platelet function should be restored shortly after discontinuing haemoperfusion without the need for administering an antidote.

The availability of prostacyclin and its efficacy as demonstrated in this paper may now afford the opportunity for a full assessment of the efficiency of charcoal haemoperfusion particularly in patients with liver failure.

References

Open Discussion

REMUZZI (Bergamo) Your data are very impressive and support the concept that PGI₂ will be useful in extracorporeal circulation. One of the major uses for PGI₂ as a drug, might be during haemodialysis or charcoal haemoperfusion for treatment of uraemia. However platelet behaviour in uraemia is likely to be different from that in healthy subjects; firstly intraplatelet cyclic AMP levels are elevated and secondly a different generation rate of prostacyclin in uraemic animals and humans was reported. My question therefore is, have you done any extracorporeal circulation using PGI₂ in uraemic animals to investigate the response of uraemic platelets to the inhibitory effect of PGI₂?

WESTON We have not done any experiments in uraemic animals yet but have already started to treat patients with prostacyclin during haemodialysis. I take your point that the uraemic platelet is suppressed and therefore there may be no need for a potent antiplatelet agent like prostacyclin. Winchester included Charcoal columns in the dialysis circuits of uraemic patients and found no elevation of screen filtration pressure, and this supports your supposition. In fact I think dialysis with cuprophan membranes for uraemia may be one of the happy accidents in medicine in that the patients have suppressed platelets and we use a biocompatible membranes. However, the heparin requirements during dialysis for uraemia are very variable. The data that I presented today suggest that platelet activation during dialysis may be responsible for the release of antiheparin. It is possible that potent antiplatelet agents such as prostacyclin will find a role in dialysis to reduce heparin requirements.