LIVER DISEASE

Selective plasma filtration for treatment of fulminant hepatic failure induced by D-galactosamine in a pig model

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Background: Plasma exchange may be useful for treating patients with fulminant hepatic failure but during the procedure growth factors that are important for hepatic regeneration are discarded. Addition of a selective plasma filter to the plasmapheresis circuit could eliminate protein bound toxic substances and retain growth factors for hepatic regeneration. This process is called selective plasma filtration.

Aims: To determine if selective plasma filtration could be a useful treatment modality for fulminant hepatic failure.

Methods: The system was tested in five groups of pigs with fulminant hepatic failure induced by galactosamine: group I, diseased control group (n=5); group II, sham control, (n=6); group III, plasma exchange (n=6); group IV, treatment with AC-1770 selective plasma filter (n=7); and group V, treatment with AC-1730 selective plasma filter which had a smaller pore size than AC-1770 (n=7). Fresh pig plasma was given to replace filtered plasma in pigs of groups III, IV, and V. Treatment was initiated 48 hours after administration of 0.75 g/kg galactosamine. The efficacy of selective plasma filtration was assessed by survival rate and improvement in haematological, biochemical, and immunohistological parameters.

Results: Pigs treated with AC-1770 or AC-1730 selective plasma filters survived longer than the other groups (group I: 55 (10) hours; group II: 68 (7) hours; group III: 91 (10) hours; group IV: 269 (156) hours; group V: 950 (555) hours). One pig in group IV survived for 50 days; one pig in group V survived for 77 days and another pig in group V is still alive (>150 days). After treatment, plasma levels of aspartate aminotransferase, bilirubin, bile acid, ammonia, lactate dehydrogenase, and α-glutathione-S-transferase decreased. Substantial amounts of tumour necrosis factor α (TNF-α) and endotoxin were found in the filtrate. The selective plasma filtration groups retained significantly higher amounts of hepatocyte growth factor than plasma exchange alone. Similar TNF-α clearance was observed in the selective plasma filtration groups and the plasma exchange group. On day 4, significant improvement in liver function, as measured by the indocyanine green clearance test, was observed in groups IV and V but not in the other groups. A higher regeneration index of hepatocytes was also observed in the groups treated with AC-1770 and AC-1730 selective plasma filters.

Conclusion: Selective plasma filtration improved survival time and expedited liver regeneration in pigs with fulminant hepatic failure.

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Fulminant hepatic failure is associated with a mortality rate of 82–97% if liver transplantation is not available.[1 2] Many putative toxins which have accumulated in the body as a result of liver impairment and cytokines released directly from the necrotic liver into the circulation are thought to be responsible for deterioration in the condition of the patient.[3] The necrotic liver may elicit a pan-inflammatory response manifested as fever, vasodilatation, hypotension, adult respiratory distress syndrome, and eventually multiorgan failure leading to death. The presence of these undesirable cytokines and toxic substances may also be responsible for inhibition of hepatic regeneration in fulminant hepatic failure.[4] Liver transplantation remains the best treatment for fulminant hepatic failure because the necrotic liver that elicits the cytokine response is removed and a new liver providing all the essential functions is in place. However, liver graft is not readily available and the "golden" period for liver transplantation is generally short. Management of patients with fulminant hepatic failure therefore aims at stabilising the patient until a suitable donor is available. The use of porcine hepatocytes in an extracoporeal system appears promising[5–7] but none has been adopted clinically. Plasma exchange has been found to be useful in treating patients with fulminant hepatic failure[8] by removal of hepatic toxins and replacement of clotting factors. However, its major disadvantage is loss of substances from plasma that may promote hepatic regeneration.[9] The components in plasma can be separated according to their size using a selective plasma filter. This process is called selective plasma filtration. In this process, protein bound toxins are filtered while useful macromolecules such as hepatocyte growth factor (HGF) are retained. We hypothesised that the introduction of a selective plasma filter in the plasma exchange circuit could be used as a liver detoxifying device. In this paper, we present results of an application of this system to treat pigs with fulminant hepatic failure induced by D-galactosamine.

MATERIALS AND METHODS

Animal model of hepatic failure

We used a porcine model of galactosamine induced fulminant hepatic failure that was previously studied in our laboratory.[10]

Abbreviations: HGF, hepatocyte growth factor; ICG, indocyanine green; TNF-α, tumour necrosis factor α.

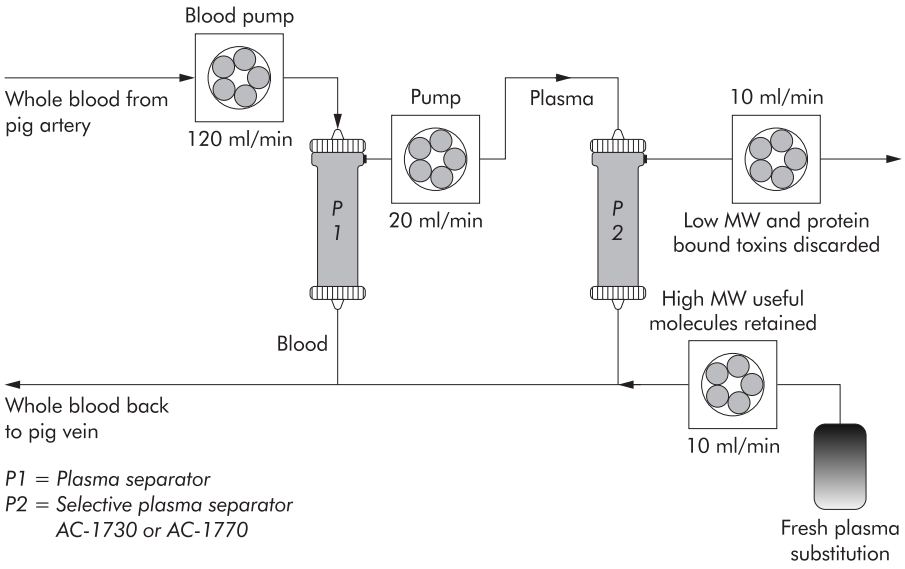

Figure 1 Diagrammatic representation of the arrangement of the plasmapheresis and selective plasma separator in the circuit.

Female inbred Large White pigs, weighing 17–28 kg and 10–15 weeks old, were obtained from the Pig Breeding Centre, the Government of Hong Kong, SAR. Animals were housed in a climate controlled (21°C) room. They were quarantined and allowed to acclimatise in the animal facility for a minimum of one week before being used in this study. All animals were given a standard laboratory diet and water ad labium until the time of D-galactosamine injection. Pigs were initially anaesthetised with ketamine hydrochloride (15 mg/kg) and placed on a mechanical ventilator and anaesthesia machine for continuous administration of isoflurane. The right jugular vein was exposed and cannulated with a 16 G cannula for blood sampling, or drug or fluid administration. The indocyanine green (ICG) clearance test was performed by administering 0.5 mg/kg of ICG intravenously and blood samples were obtained at five minute intervals for 20 minutes to determine ICG clearance. D-Galactosamine (Research Organics Inc, Cleveland, Ohio, USA) 0.75 g/kg was dissolved in 5% dextrose in water and adjusted to pH 6.8 with 1 mol/l NaOH. The solution was then sterilised and used immediately. After administration of galactosamine, pigs were allowed to wake up from general anaesthesia and left in the cage. Only sedation was used for blood sampling in the subsequent days and between treatment sessions.

Five different groups of animals were studied.

Group I: Diseased control group (n=5). Pigs received a bolus infusion of 0.75 g/kg galactosamine. No further treatment was given.

Group II: Sham control group (n=6). Pigs received a bolus infusion of 0.75 g/kg galactosamine. Forty eight hours later, pigs were connected to the plasma separator without a secondary selective plasma filter for the same duration as groups III, IV, and V. Its own blood cells and plasma were then completely returned to the blood circulation without any substitution of fresh pig plasma.

Group III: Plasma exchange group (n=6). Pigs received a bolus injection of 0.75 g/kg galactosamine. Forty eight hours later, they were connected to the extracorporeal system of plasmapheresis. Filtered plasma was replaced by fresh pig plasma.

Group IV: AC-1770 group (n=7). Pigs received the same treatment as group III but a selective plasma filter (Cascadeflo AC-1770; Asahi Medical, Tokyo, Japan) was added to the circuit. Filtered plasma was replaced by fresh pig plasma.

Group V: AC-1730 group (n=7). Pigs received the same treatment as groups III and IV but a selective plasma filter of smaller pore size than AC-1770 (Cascadeflo AC-1730; Asahi Medical) was used.

Extracoporeal circuit of the plasmapheresis and selective plasma filtration

Forty eight hours after administration of galactosamine, all pigs, except the diseased control group, underwent their respective treatments. Pigs were anaesthetised and placed on a mechanical ventilator and anaesthesia was maintained by inhalation of 0.5–1.5% isoflurane and a 1:3 ratio of oxygen and nitrous oxide. Prior to treatment, blood sampling and the ICG clearance test were performed. The plasma filter (PF 2000N; Gambro, Germany) and the selective plasma filters were washed and primed according to the manufacturer's instructions. After cannulation of the external iliac artery and vein, the blood of the pigs was passed through a plasma separation system (PEM 10; Gambro, Lund, Sweden) at a mean flow rate of 120 ml/min. Heparin (250 U/h) was administered as necessary to avoid clotting in the extracorporeal circuit. Plasma separated from blood at a rate of 20 ml/min (fig 1). Plasma then passed through the selective plasma filter in which plasma components were separated according to their size. The filtrate with a flow rate of 10 ml/min was passed into the waste bag and discarded. The amount of filtrate removed was replaced by the same volume of fresh pig plasma. The retained plasma, substituted plasma, and

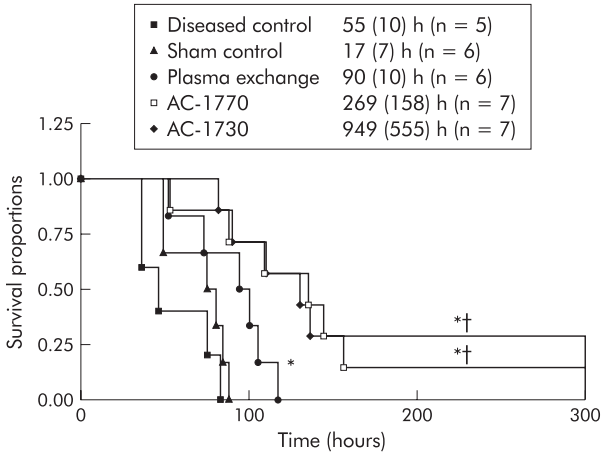

Figure 2 Survival rates of groups I–V. *p<0.05 versus diseased control and sham control groups; †p<0.05 versus plasma exchange group.

Table 1 Laboratory values in group I (diseased control)

Parameter	0 h	24 h	48 h
Sodium (mmol/l)	135 (2)	141 (3)	142 (2)
Potassium (mmol/l)	3.5 (0.1)	4.1 (0.1)	3.7 (0.2)
Calcium (mmol/l)	2.52 (0.08)	2.66 (0.08)	2.34 (0.13)
Protein (g/l)	56 (2)	64 (2)	61 (2)
Total bilirubin (μmol/l)	3 (1)	37 (4)***	73 (4)***
Alkaline phosphatase (U/l)	143 (17)	425 (64)**	1048 (127)***
Aspartate aminotransferase (U/l)	42 (5)	1568 (767)	2753 (1070)*
Lactate dehydrogenase (U/l)	1107 (117)	3814 (1010)*	7475 (1226)***
Ammonia (μg/dl)	92 (30)	133 (55)	565 (251)*
Bile acid (μmol/l)	16 (7)	479 (19)***	358 (23)***
Lactate (mmol/l)	14 (2)	37 (10)	52 (12)*
TNF-α (pg/ml)	70 (2)	76 (3)	103 (13)**
Endotoxin (EU/ml)	1.59 (0.30)	3.98 (1.30)	5.30 (1.69)*
Platelet (10^3/μl)	289 (39)	—	214 (90)
Activated clotting time (s)	129 (8)	163 (11)	205 (5)**
ICG at 15 min (%)	17 (3)	—	57 (5)***

Data are mean (SEM).
ICG, indocyanine green; TNF-α, tumour necrosis factor α.
*p<0.05, **p<0.01, ***p<0.001 versus 0 h (time of galactosamine injection).

original blood cell components were reconstituted and returned to the animal via the venous cannula. The treatment lasted for four hours and approximately 2 litres of plasma were removed from each pigs in groups III, IV, and V. A second treatment session was initiated two days after the first treatment if systolic blood pressure was greater than 80 mm Hg. Pigs were taken care of and monitored closely by an experienced veterinary technician.

Clinical data were recorded hourly. Blood was collected before and after treatment for renal and liver biochemistry determinations. Plasma levels of ammonia, lactate, bile acid, tumour necrosis factor α (TNF-α), endotoxin, and HGF were also measured. Plasma TNF-α was assayed by an in vitro ELISA method (Endogen Inc., Massachusetts, USA). TNF-α clearance was calculated using the following equation:

$$SC = C_f / C_p$$

$$Cl\ (ml/min) = SC \times Q_f$$

where C_f is TNF-α in the filtrate and C_p is TNF-α in plasma before treatment, SC is the sieving coefficient, Q_f is the filtration flow rate, and Cl is TNF-α clearance.

Plasma endotoxin was measured using a limulus amoebocyte lysate pyrochrome kit (Associates of Cape Cod, Massachusetts, USA). Plasma was diluted and heated at 60°C for 30 minutes prior to the assay. As there was cross reactivity between porcine HGF and monoclonal antibody against human HGF,[11] porcine HGF was assayed using a commercially available ELISA kit (R&D Systems, Minneapolis, USA). Percentage HGF retention was used to compare the amount of

Table 2 Comparison of liver enzymes in groups II–V

Parameter	Sham control	Plasma exchange	AC-1770	AC-1730
Alkaline phosphatase (U/l)				
Day 0	96 (21)	154 (29)	141 (17)	154 (30)
Day 1	281 (42)	381 (67)	363 (42)	373 (44)
Pre-T	600 (72)	614 (197)	702 (71)	609 (96)
Post-T	513 (103)	246 (43)	269 (16)***	366 (49)
Filtrate	—	395 (91)	237 (30)	72 (16)
Day 3	725 (127)	856 (199)	664 (97)	671 (202)
Aspartate aminotransferase (U/l)				
Day 0	52 (9)	26 (5)	47 (9)	45 (7)
Day 1	498 (154)	716 (557)	1396 (909)	818 (392)
Pre-T	2875 (976)	2605 (989)	3680 (632)	1387 (421)
Post-T	1812 (458)	673 (169)*	958 (243)***	472 (171)*
Filtrate	—	1259 (425)	1006 (289)	433 (151)
Day 3	3047 (1317)	1159 (225)	1580 (662)*	1108 (675)
Lactate dehydrogenase (U/l)				
Day 0	1176 (225)	953 (109)	1222 (278)	1310 (162)
Day 1	2641 (649)	2323 (836)	2889 (628)	1948 (227)
Pre-T	7090 (1550)	8657 (1944)	8400 (1351)	4704 (755)
Post-T	5298 (1022)	2474 (341)***	2298 (549)***	2460 (249)*
Filtrate	—	3382 (846)	2506 (795)	988 (123)
Day 3	7770 (1128)	8200 (895)	4406 (1069)**	5886 (1427)
α-Glutathione S-transferase (μg/l)				
Day 0	17 (7)	10 (4)	21 (12)	6 (3)
Day 1	21 (9)	201 (166)	35 (10)	49 (24)
Pre-T	130 (44)	282 (147)	235 (73)	126 (34)
Post-T	88 (23)	83 (18)	108 (38)	47 (9)
Filtrate	—	81 (22)	122 (39)	64 (13)
Day 3	129 (46)	106 (49)	89 (6)	125 (29)

Data are mean (SEM).
Pre-T, before treatment; Post-T, after treatment.
*p<0.05, **p<0.01, ***p<0.001 versus pretreatment.

Table 3 Comparison of biochemical parameters in groups II–V

Parameter	Sham control		Plasma exchange		AC-1770		AC-1730	
Total bilirubin (μmol/l)								
Day 0	2	(1)	2	(1)	3	(1)	2	(1)
Day 1	20	(5)	33	(8)	32	(3)	31	(8)
Pre-T	48	(5)	61	(11)	60	(6)	52	(5)
Post-T	43	(3)	29	(7)*	24	(6)***	26	(5)**
Filtrate	—		37	(6)	27	(6)	19	(4)
Day 3	100	(13)***	94	(19)	80	(12)	76	(6)
Bile acid (μmol/l)								
Day 0	5	(1)	6	(1)	5	(1)	6	(2)
Day 1	367	(15)	378	(21)	376	(35)	365	(42)
Pre-T	339	(12)	347	(31)	336	(28)	330	(34)
Post-T	283	(25)	218	(31)**	155	(14)***	181	(37)**
Filtrate	—		251	(26)	166	(10)	147	(21)
Day 3	254	(49)	236	(30)*	219	(33)**	282	(8)
Ammonia (μg/dl)								
Day 0	93	(9)	79	(9)	80	(25)	85	(25)
Day 1	159	(30)	210	(93)	109	(28)	156	(45)
Pre-T	406	(104)	475	(142)	318	(50)	242	(34)
Post-T	408	(89)	570	(381)	215	(61)	120	(18)
Filtrate	—		531	(293)	256	(64)	173	(50)
Day 3	868	(297)*	475	(101)	289	(84)	358	(86)
Lactate (mg/dl)								
Day 0	13	(2)	14	(1)	15	(3)	10	(1)
Day 1	20	(4)	25	(5)	14	(2)	19	(2)
Pre-T	44	(11)	45	(9)	37	(9)	37	(6)
Post-T	38	(17)	52	(21)	47	(16)	22	(4)
Filtrate	—		42	(10)	39	(11)	26	(4)
Day 3	30	(8)	31	(14)	25	(9)	19	(5)*
TNF-α (pg/ml)								
Day 0	63	(4)	71	(16)	57	(5)	47	(9)
Day 1	81	(16)	75	(26)	66	(6)	54	(13)
Pre-T	68	(5)	105	(28)	93	(11)	65	(10)
Post-T	71	(5)	91	(33)	71	(8)	82	(18)
Filtrate	—		89	(21)	68	(5)	67	(10)
Day 3	73	(5)	89	(23)	73	(9)	64	(7)
Endotoxin (EU/ml)								
Day 0	1.85	(0.29)	2.10	(0.14)	2.07	(0.23)	1.49	(0.23)
Day 1	2.18	(0.21)	3.82	(0.49)	2.20	(0.35)	1.70	(0.35)
Pre-T	4.20	(0.98)	4.52	(1.18)	5.18	(1.56)	3.05	(0.47)
Post-T	4.83	(1.32)	2.76	(0.76)	4.10	(0.49)	1.86	(0.22)
Filtrate	—		3.21	(0.59)	2.60	(0.64)	2.40	(0.58)
Day 3	7.73	(2.83)	4.38	(1.46)	4.16	(1.16)	3.33	(1.54)

Data are mean (SEM).
Pre-T, before treatment; Post-T, after treatment; TNF-α, tumour necrosis factor α.
*p<0.05, **p<0.01, ***p<0.001 versus pretreatment.

HGF remaining after treatment between the selective plasma filtration groups and the plasma exchange group, and was calculated using the formula:

$$\%HGF = C_t / C_i \times 100$$

where C_t is the concentration of HGF (pg/ml) after the treatment and C_i is the concentration of HGF (pg/ml) before treatment.

Hepatocyte proliferation activity was compared between groups III, IV, and V after their respective treatments by detection of Ki-67 protein in liver using the immunohistochemical staining method. Liver tissue was taken from a normal pig (acting as a control) and pigs from each group at postmortem examination with comparable survival times. The Ki-67 antihuman monoclonal antibody B56 (PharMingen, Cat No 36215A), shown to cross react with porcine Ki-67, was used. Counting of Ki-67 positive cells was done by two independent observers for at least 1000 cells using an image analyser (MetaMorph Imaging System; Universal Imaging Corporation, Pennsylvania, USA). Hepatocyte proliferative activity was expressed as labelling index, which was defined as the ratio of the number of positive Ki-67 hepatocytes to the total number of hepatocytes counted.

The study was approved by the Committee of Use of Live Animals for Study and Research of the University of Hong Kong.

Statistical analysis

Data are expressed as mean (SEM) and were analysed using the software Instat and Prism 3 (GraphPad Software Inc., California, USA). Sample size was estimated and calculated by Statmate (GraphPad Software Inc.) with the input of SD of 19 hours for the survival time of pigs treated with galactosamine from the pilot experiments, and an α value of 0.05 (the threshold p value). A sample size of seven was chosen for the selective plasma filtration groups after balancing the cost of the experiments and the statistical factors. Survival time was defined as the duration between administration of galactosamine and death of the pig. Survival curves were computed using the Kaplan-Meier method for the different groups and compared using the log rank test. An animal surviving for four days after galactosamine administration was considered a survivor and this definition was used to determine the mortality rate among the groups. Blood biochemical parameters between the treatment groups were analysed using one way ANOVA and the *t* test for multiple comparisons. Fisher's test was used to compare mortality rates. A p value <0.05 was considered significant.

Table 4 Comparison of biochemical and haematological parameters in groups II–V

Parameter	Sham control	Plasma exchange	AC-1770	AC-1730
Sodium (mmol/l)				
Day 0	136 (1)	139 (1)	135 (2)	137 (1)
Day 1	139 (3)	140 (2)	145 (1)	140 (2)
Pre-T	140 (1)	135 (2)	136 (2)	135 (2)
Post-T	135 (5)	138 (1)	137 (2)	136 (2)
Filtrate	—	135 (3)	133 (4)	140 (2)
Day 3	145 (1)	142 (2)	144 (3)	139 (2)
Potassium (mmol/l)				
Day 0	3.7 (0.2)	3.7 (0.2)	3.6 (0.1)	3.4 (0.1)
Day 1	3.7 (0.2)	3.8 (0.3)	3.9 (0.2)	3.9 (0.1)
Pre-T	3.6 (0.3)	3.7 (0.3)	3.5 (0.1)	3.4 (0.1)
Post-T	3.8 (0.2)	3.6 (0.4)	3.7 (0.1)	3.6 (0.1)
Filtrate	—	3.6 (0.4)	3.0 (0.1)	2.8 (0.1)
Day 3	3.7 (0.1)	3.6 (0.2)	3.5 (0.2)	3.2 (0.2)
Calcium (mmol/l)				
Day 0	2.41 (0.05)	2.44 (0.03)	2.46 (0.09)	2.51 (0.04)
Day 1	2.48 (0.10)	2.60 (0.06)	2.67 (0.06)	2.63 (0.08)
Pre-T	2.49 (0.08)	2.49 (0.10)	2.43 (0.08)	2.46 (0.05)
Post-T	2.33 (0.09)	2.44 (0.09)	2.29 (0.12)	2.21 (0.20)
Filtrate	—	2.35 (0.08)	1.92 (0.16)	2.00 (0.13)
Day 3	2.35 (0.12)	2.45 (0.12)	2.34 (0.13)	2.42 (0.06)
Protein (g/l)				
Day 0	63 (5)	54 (2)	56 (2)	59 (2)
Day 1	60 (8)	55 (6)	69 (6)	69 (5)
Pre-T	51 (8)	47 (3)	53 (4)	54 (5)
Post-T	39 (7)	33 (3)*	36 (2)**	40 (3)*
Filtrate	—	32 (3)††	25 (2)†	17 (1)
Day 3	45 (3)	43 (3)	44 (4)	50 (3)
Platelet (10^3/μl)				
Day 0	327 (38)	307 (28)	285 (50)	296 (28)
Pre-T	196 (57)	224 (64)	156 (36)	207 (29)
Post-T	183 (51)	213 (55)	150 (31)	169 (30)
Activated clotting time (s)				
Day 0	136 (6)	136 (21)	128 (10)	119 (11)
Day 1	185 (12)	180 (11)	224 (35)	148 (6)
Pre-T	540 (178)	478 (166)	464 (140)	532 (421)
Post-T	442 (198)	152 (13)*	166 (6)*	152 (8)*

Data are mean (SEM).
Pre-T, before treatment; Post-T, after treatment.
*p<0.05, **p<0.01 versus pretreatment; †p<0.05, ††p<0.01 versus AC-1730.

RESULTS

Laboratory data and survival of the diseased control group

All pigs recovered consciousness from general anaesthesia within one hour after administration of D-galactosamine. Initially, they walked spontaneously around in the cage and drank water. They were still alert 24 hours after drug administration but after 48 hours they went through a state of increasing drowsiness, somnolence, and eventually coma before death.

All pigs in the diseased control group died within 83 hours and mean survival time was 55 hours (fig 2). A progressive increase in levels of liver enzymes, bilirubin, bile acid, and ammonia indicated liver injuries at 24 hours after administration of galactosamine, and these levels remained increased throughout the experiment (table 1). Coagulopathy was manifest as a decrease in platelet count and elevation of activated clotting time (table 1). Liver function impairment was reflected by a gradual increase in ICG retention value at 15 minutes from 17 (3)% on day 0 to 57 (5)% on day 2 (table 1). Plasma TNF-α and endotoxin levels were also elevated significantly compared with day 0 (table 1).

As mean survival time of the diseased control group was 55 hours (fig 2), and the general condition of the pigs and biochemical and haematological parameters deteriorated abruptly after 48 hours of galactosamine infusion, selective plasma filtration was initiated at 48 hours to test the efficacy of the system.

Survival of groups II– V

None of the pigs in the sham control or plasma exchange groups survived for more than five days. Five pigs in the

Table 5 Indocyanine green retention values$_{15min}$

	Disease control	Sham control	Plasma exchange	AC-1770	AC-1730
Day 0	17 (3)	13 (3)	12 (3)	17 (4)	18 (5)
Day 2	57 (5)***	42 (5)***	47 (3)***	58 (3)***	53 (5)***
Day 4	—	—	40 (2)	45 (4)†	37 (3)†
Day 7	—	—	—	25	26 (1)

Data are mean (SEM).
Day 0 was the time of galactosamine injection.
***p<0.001 versus day 0; †p<0.05 versus day 2. There were no statistically significant differences between values in all groups on day 2 (p>0.05).

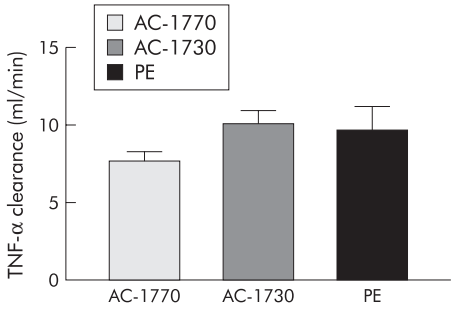

Figure 3 Comparison of tumour necrosis factor α (TNF-α) clearance between the selective plasma filtration groups and the plasma exchange (PE) group.

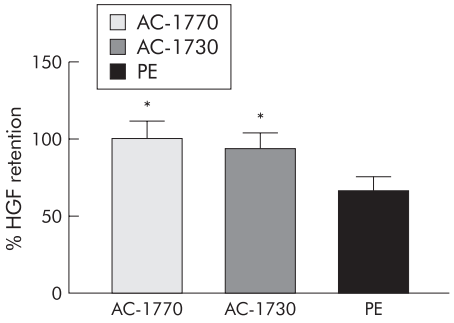

Figure 4 Comparison of the amount of hepatocyte growth factor (HGF) retained after treatment between the selective plasma filtration groups and plasma exchange (PE) group.

AC-1770 group and five in the AC-1730 group survived beyond day 4. Mortality rates in groups AC-1770 and AC-1730 on day 4 were both 29% (five of seven pigs) which were significantly lower than in the sham control group (100%) ($p=0.02$, Fisher's test). The mortality rate in the plasma exchange group was 50% (three of six pigs) which was lower than in the sham control group but was not statistically significant. One pig in the AC-1770 group survived for 50 days; one in the AC-1730 group survived for 77 days and one in the AC-1730 group is still alive at the time of preparation of the manuscript (>150 days). The survival curves of all five groups are shown in fig 2. Three pigs in the AC-1770 group and one pig in the AC-1730 group received the second treatment on day 4. No pig in the plasma exchange group received the second treatment as they were seriously ill even though they survived to day 4.

Laboratory data of groups II –V

All pigs in groups II–V exhibited the same abnormalities in plasma liver enzymes, bilirubin, bile acid, lactate, blood ammonia, ICG retention value, activated clotting time values, and platelet count as the diseased control animals (tables 2–5). After the treatments there was a significant reduction in plasma levels of aspartate aminotransferase, lactate dehydrogenase, bilirubin, and bile acid in groups III–V (tables 2, 3). Plasma levels of alkaline phosphatase, α-glutathione-S-transferase, ammonia, and endotoxin also decreased although these were not statistically significant. Substantial amounts of endotoxin, together with bilirubin, bile acid, ammonia, liver enzymes, and protein were found in the filtrate (tables 2–4). TNF-α clearance rates for the AC-1770, AC-1730, and plasma exchange groups were 8.0 (0.6) ml/min, 10 (0.8) ml/min, and 10 (1.6) ml/min, respectively, indicating that the removal rates of this proinflammatory cytokine were similar in the plasma exchange and selective plasma filtration groups ($p>0.05$) (fig 3).

Percentage HGF retentions for the AC-1770, AC-1730, and plasma exchange groups were 100 (11), 93 (10), and 66 (9), respectively. This indicated that the selective plasma filtration groups could retain significantly larger amounts of HGF after treatment compared with the plasma exchange group ($p<0.05$) (fig 4).

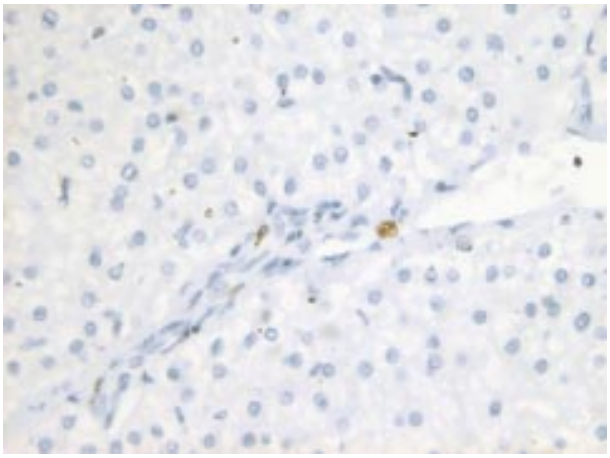

Figure 5 Ki-67 immunohistochemical staining of a normal pig liver.

Table 6 Labeling index (LI) (Ki-67) in the liver of pigs in groups III–V

Group	n	Survival time (h)	LI%
Plasma exchange	4	73–117	20 (1)
AC-1770	4	130–156	32 (3)*
AC-1730	3	82–136	33 (6)*

Data are mean (SEM).
*$p<0.05$ versus plasma exchange group.

On day 3, a rebound increase in plasma levels of bilirubin were observed in all pigs in groups III–V (table 3). However, compared with pretreatment values, the increase was not statistically significant. In contrast, plasma bilirubin levels in the sham control group were significantly higher than pretreatment values. For plasma bile acid levels, day 3 values in the AC-1770 and plasma exchange groups were lower than pretreatment values ($p<0.05$). A significant decrease in plasma aspartate aminotransferase and lactate dehydrogenase on day 3 was observed in the AC-1770 group ($p<0.01$) but not in the other groups. Plasma ammonia levels in the AC-1770 and AC-1730 groups appeared lower than the corresponding values in the sham control and plasma exchange groups. A marked increase in plasma ammonia was found in the sham control group ($p<0.05$).

For groups II–V, there was no major change in plasma sodium, potassium, or calcium levels after treatment (table 4). Plasma protein levels in the AC-1770 and plasma exchange groups were reduced after treatment and concentrations in the filtrate were higher than those in group AC-1730, indicating that the pore size of the AC-1730 membrane was more selective (table 4).

On day 4, a repeat ICG clearance test in the pigs who survived showed that retention values in the AC-1770 and AC-1730 treatment groups were significantly lower than those before treatment (table 5). A similar trend was observed in the plasma exchange group but the reduction was not statistically significant.

Measurement of platelet concentrations after treatment indicated that there was no further reduction in platelet count after treatment (table 4). Activated clotting time values were significantly improved in groups III–V probably because fresh plasma was used in these groups (table 4).

In terms of Ki-67 staining for hepatocyte proliferation activity, positive staining was not seen in normal liver (fig 5)

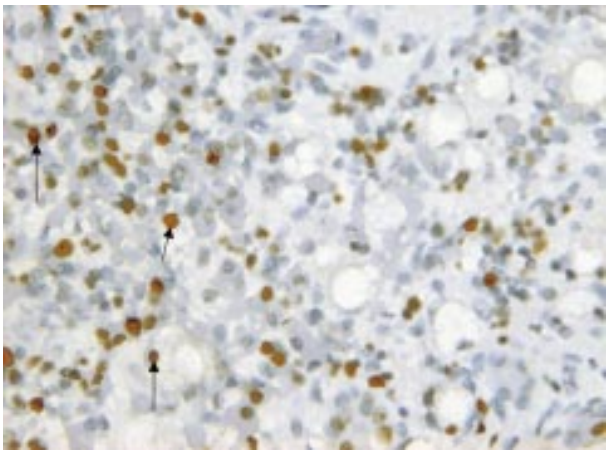

Figure 6 Ki-67 immunohistochemical staining of a pig liver in the AC-1770 group.

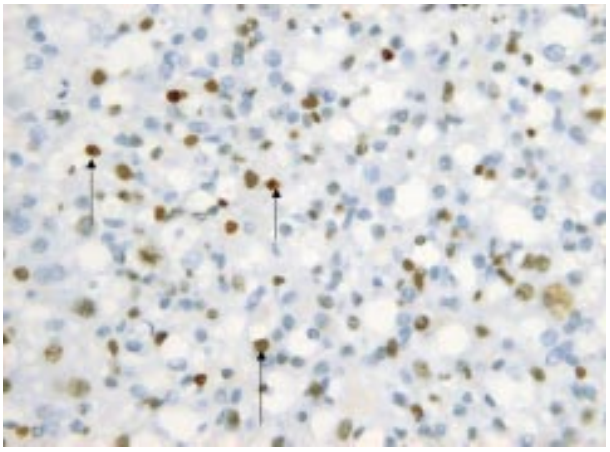

Figure 7 Ki-67 immunohistochemical staining of a pig liver in the AC-1730 group.

but was remarkable in pig liver treated by selective plasma filtration. Compared with pigs treated by plasma exchange only, the labelling index was significantly higher in the groups treated with the AC-1770 and AC-1730 filters (table 6; figs 6–8). Mean labelling index in the plasma exchange group was only 20% which was significantly lower than that for the AC-1770 and AC-1730 groups (32% and 33%, respectively). It appeared that the higher values of HGF retention in the selective plasma filtration groups corresponded to their higher labelling index.

All pigs undergoing treatment procedures remained haemodyamically stable and urine output was normal. At postmortem examination, the livers of the pigs were pale, oedematous, and indurated. Ascites were found and a few scattered mesenteric haemorrhages were observed in some pigs. No abnormality was seen in the kidneys or heart. Histological examination of the liver specimens demonstrated massive hepatic necrosis.

DISCUSSION

Animal models of fulminant hepatic failure with high mortality rates as well as the potential for recovery are required for evaluation of the efficacy of liver supporting therapeutic devices. Animal models of fulminant hepatic failure induced by surgical means, including hepatectomy or complete hepatic ischaemia, are not suitable for this purpose because there is no potential for reversibility or recovery. In contrast, a porcine model of fulminant hepatic failure induced

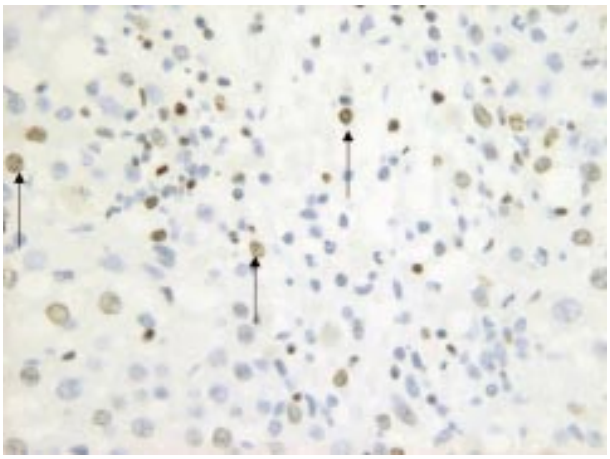

Figure 8 Ki-67 immunohistochemical staining of a pig liver in the plasma exchange group.

by galactosamine was used in our study because this model demonstrated high reproducibility and potential reversibility[10 12] which fulfil the majority of the criteria set by Terblanche and Hickman.[13] Moreover, postmortem examination did not show any damage to organs other than the liver, supporting the fact that the cause of death in experimental animals was attributed to liver failure.

In the present study we demonstrated that selective plasma filtration was efficacious in removing toxic substances which had accumulated in pigs with fulminant hepatic failure (evidenced by a decrease in plasma ammonia, bilirubin, bile acid levels, etc), halting the progression of liver injury (as shown by decreased plasma alanine aminotransferase and lactate dehydrogenase levels), retaining useful materials (evidenced by higher HGF retention), improving liver function (as shown by a better ICG clearance value), promoting liver regeneration, and prolonging survival of pigs after the onset of fulminant hepatic failure. The data also indicated that selective plasma filtration was more effective than plasma exchange alone.

The majority of patients with fulminant hepatic failure will deteriorate and die after the onset of disease but a few may recover spontaneously and resume normal liver function.[14 15] The occasional survivors had rapid regeneration of the liver, probably because the initial cause of the liver injury was not persistent and the accumulated toxic substances that perpetuate liver injury were low in concentration. The exact nature of the toxic substances that produce liver injury and inhibit liver regeneration is not known but may include bilirubin, bile acid, and TNF-α.[16] Impairment of hepatic clearance in liver failure also causes an excessive accumulation of ammonia, mercaptans, indoles, and gamma amino butyric acid that are highly associated with hepatic encephalopathy.[17] Liver regeneration is stimulated by many factors, among which HGF is the most important.[18] High levels of HGF are present in patients with fulminant hepatic failure.[19] Survival of patients with fulminant hepatic failure therefore depends on a fine balance between liver cell necrosis and regeneration, which in turn depends on whether or not toxic substances injurious to liver cells are eliminated.

Haemodialysis[20] and haemofiltration[21] are capable of removing toxic substances from the circulation but efficacy is low because most toxic substances, such as bilirubin, endotoxin, and cytokines are protein bound or large molecules. Plasmapheresis is capable of removing these substances[3 9] and when used in a clinical trial, survival rates of 34% and 41% were recorded.[22 23] However, elimination of the entire volume of the patient's own plasma means exhaustion of important biological factors that could promote liver regeneration. This may explain why the success rate of plasma exchange is not high.

In our present study, a selective plasma filter AC-1770 or AC-1730 was added to the plasmapheresis circuit. AC-1770 and AC-1730 are cellulose diacetate membranes which are characterised by sieving coefficients of approximately 0.85 and 0.7, respectively, for human albumin, approximately 0.7 and 0.3, respectively, for human IgG, and approximately 0.3 and 0.15, respectively, for IgM, according to the manufacturer's information. The pore size of the filters is efficient to filter the protein bound toxins and retain large molecular weight substances. TNF-α has a molecular weight of 17 kDa but could be larger due to binding to proteins or mutimer forms[24 25] in plasma. As a result, its size is larger than 55 kDa but can still be filtered through the AC-1770 or AC-1730 membrane. HGF binding to heparin has a molecular weight of more than 200 kDa[26] and is retained in the circulation. Based on the findings of total protein, TNF-α, endotoxin, and other substances in the filtrate, AC-1770 and AC-1730 probably fulfil the purpose of selective elimination of unwanted substances. Of the two, AC-1770 is perhaps a better choice based on laboratory results.

Plasma levels of TNF-α and endotoxin were not significantly reduced after treatment or the next day. This was probably because constant release of TNF-α and endotoxin into the circulation overwhelmed the function of the system. However, it was found that similar clearance efficiencies of TNF-α were obtained in the AC-1770 and AC-1730 groups compared with that of the plasma exchange group. A longer duration of treatment therefore may be necessary to reduce concentrations to a significant level.

The additional benefit of selective plasma filtration was its ability to retain a relatively large amount of HGF in the pig's circulation. As HGF is a potent mitogen for a variety of types of epithelial cells, including hepatocytes,[27] its higher retention in the body would cause greater liver regeneration in pigs treated by selective plasma filtration than that by plasma exchange alone.

It has been shown that direct contact of peripheral blood mononuclear cell with the filter membrane and adherence of platelets to the membrane leads to complement activation and cytokine production.[28] In our experiments, after treatment with selective plasma filtration, increased levels of TNF-α or decreased platelet concentrations were not found. Indeed, the biocompatibility of the membrane ensured low thrombogenicity and low complement activation.[29] The PF2000N plasma filter, AC-1770, and AC-1730 selective plasma filters are currently used in clinical practice. Moreover, selective plasma filtration did not induce hypocalcaemia as citrate was not used as an anticoagulant. Instead, heparin was used to prevent coagulation within the system. The use of heparin may be advantageous in that HGF is bound to heparin forming a large molecule and therefore is retained in the circulation.

Compared with other liver supporting systems, the drawback of selective plasma filtration is that it does not provide liver function directly to the patient. The bioartificial liver support system currently in clinical trials used porcine or hepatoma cell lines to provide liver function. For porcine support systems, the risk and fear of transmission of endogenous porcine retroviruses to humans have not been totally eliminated.[30] For selective plasma filtration to be applied in clinical practice, the potential risk of transmission of unknown viruses through fresh frozen plasma must be excluded. However, this risk is low if all plasma is obtained from a blood bank.

In conclusion, selective plasma filtration was beneficial in halting liver injury and expediting liver regeneration. As the complication rate related to plasmapheresis is less than 0.3%[31] and addition of a filter membrane already in clinical use to the plasmapheresis circuit may not cause problems, it should be feasible to use selective plasma filtration in clinical trials to determine its efficacy in patients with fulminant hepatic failure.

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