CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

LEUKOCYTE FILTRATION IMPROVES BRAIN PROTECTION AFTER A PROLONGED PERIOD OF HYPOTHERMIC CIRCULATORY ARREST: A STUDY IN A CHRONIC PORCINE MODEL

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Background: Ischemic cerebral injury follows a well-attested sequence of events, including 3 phases: depolarization, biochemical cascade, and reperfusion injury. Leukocyte infiltration and cytokine-mediated inflammatory reaction are known to play a pivotal role in the reperfusion phase. These events exacerbate the brain injury by impairing the normal microvascular perfusion and through the release of cytotoxic enzymes. The aim of the present study was to determine whether a leukocyte-depleting filter (LeukoGuard LG6, Pall Biomedical, Portsmouth, United Kingdom) could improve the cerebral outcome after hypothermic circulatory arrest.

Methods: Twenty pigs (23-30 kg) were randomly assigned to undergo cardiopulmonary bypass with or without a leukocyte-depleting filter before and after a 75-minute period of hypothermic circulatory arrest at 20°C. Electroencephalographic recovery, S-100 β protein levels, and cytokine levels (interleukin 1 β , interleukin 8, and tumor necrosis factor α) were recorded up to the first postoperative day. Postoperatively, all animals were evaluated daily until death or until electively being put to death on day 7 by using a quantitative behavioral score. A postmortem histologic analysis of the brain was carried out on all animals.

Results: The rate of mortality was 2 of 10 in the leukocyte-depletion group and 5 of 10 in control animals. The risk for early death in control animals was 2.5 (95% confidence interval, 0.63-10.0) times higher than that of the leukocyte-depleted animals. The median behavioral score at day 7 was higher in the leukocyte-depletion group (8.5 vs 3.5; $P = .04$). The median of total histopathologic score was 8.5 in the leukocyte-depletion group and 15.5 in the control group ($P = .005$).

Conclusion: A leukocyte-depleting filter improves brain protection after a prolonged period of hypothermic circulatory arrest.

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Hypothermia is the most frequently used method to protect the brain when antegrade flow is interrupted during operations on the aortic arch.¹ Its efficacy is limited, however, as a result of the safe duration of hypothermic circulatory arrest (HCA) being 40 to 50 minutes at brain temperatures of 10°C to 15°C.² Ischemic cerebral injury follows a well-attested sequence of events including 3 phases: depolarization, biochemical cascade, and reperfusion injury. The importance of the failure of neurotransmitter transport as a common pathway in the pathogenesis of many neurologic disorders, including ischemic cerebral injury, has been widely demonstrated in the past few years.³

Previous studies suggest that the reperfusion phase plays a significant role in the pathogenesis of ischemic brain injury.¹ After ischemic insult of the brain, leukocyte infiltration can be detected. Blood vessels are filled with leukocytes (primarily neutrophils), and edema will develop. Leukocyte adhesion to the wall of blood vessels and infiltration of leukocytes into ischemic brain tissue activates an inflammatory reaction that is driven by cytokines. Impaired microvascular perfusion and release of cytotoxic enzymes are involved in inflammatory reaction, exacerbating neuronal injury.⁴⁻⁶

Leukocyte depletion is demonstrated to mitigate reperfusion injury after myocardial injury.⁷⁻⁹ Leukocyte depletion during and after cardiopulmonary bypass (CPB) was also found to improve pulmonary function¹⁰ and to ameliorate lung injury mediated by free radicals.^{11,12} We have been studying the possible means to improve the safety of HCA with a surviving porcine model.¹³⁻¹⁵ The specific purpose of the current study was to find out whether leukocyte depletion by filtration (Leukoguard LG6; Pall Biomedical, Portsmouth, United Kingdom) could mitigate ischemic brain injury and improve neurologic and neuropathologic outcomes after HCA.

Materials and methods

Twenty female juvenile (8-10 weeks) pigs of a native stock, weighing 23 to 30 kg, were randomly assigned to undergo CPB with or without a leukocyte-depleting filter (L-DF) before and after a 75-minute period of HCA at 20°C.

Preoperative management. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (National Institutes of Health publication No. 85-23, revised 1985). The study was approved by the Research Animal Care and Use Committee of the University of Oulu.

Anesthesia and hemodynamic monitoring. Anesthesia was induced with ketamine hydrochloride (10 mg/kg administered intramuscularly) and midazolam (1 mg/kg administered intramuscularly), and muscular paralysis was maintained with pancuronium bromide (0.1 mg/kg administered intravenously). After endotracheal intubation, the animals were maintained on positive-pressure ventilation with 35% oxygen; anesthesia was maintained with isoflurane (1.1%-1.2%). The arterial catheter was positioned in the left femoral artery. A thermodilution catheter (Criticath, 7F; Ohmeda GmbH & Co, Erlangen, Germany) was placed through the femoral vein to allow blood sampling, pressure monitoring in the pulmonary artery, and recording of cardiac output. The intracranial temperature probe was placed through a drill hole in the epidural space and was isolated with bone wax. The

drill hole was positioned 1 cm to the right from a sagittal joint above a parietal line. Other temperature probes were placed in the esophagus and rectum, and a 10F nelaton catheter was placed in the urinary bladder to monitor urine output.

Electroencephalographic monitoring. Cortical electrical activity was registered from 4 stainless steel screw electrodes (5 mm in diameter) implanted in the skull over the parietal and frontal areas of the cortex by using a digital electroencephalography (EEG) recorder (Nervus, Reykjavik, Iceland) and an amplifier (Magnus EEG 32/8, Reykjavik, Iceland). Sampling frequency was 1024 Hz, and bandwidth was 0.03 to 256 Hz. All EEG recordings are referenced to a frontal screw electrode, which, together with a ground screw electrode, is implanted over the frontal sinuses. The recordings were made by digital EEG, with sensitivity on screen set at 70 μ V/cm. Isoflurane level was adjusted so that EEG showed a steady burst suppression pattern. After this, isoflurane end tidal concentration was kept at this steady level until the end of monitoring. EEG was recorded for 10 minutes to get a baseline recording of steady burst suppression activity before the cooling period. After HCA, EEG recording was restarted and continued until the first postoperative day. The durations of EEG were measured from 5-minute EEG samples at fixed time points, first with half-hour intervals and later with 1-hour intervals. From each 5-minute sample, artifact periods were excluded, and from the rest, the sum of bursts was counted as a percentage of the sum of artifact-free bursts and suppressions. This percentage was used as a measure of EEG activity in the analysis.

CPB. Through a right thoracotomy in the fourth intercostal space, the right thoracic artery was ligated, and the heart and great vessels were exposed. A membrane oxygenator (Midiflow D 705; Dideco, Mirandola, Italy) was primed with 1 L of Ringer's acetate and heparin (5000 IU). After heparinization (300 IU/kg), the ascending aorta was cannulated with a 16F arterial cannula, and the right atrial appendage was cannulated with a single 24F atrial cannula. Nonpulsatile CPB was initiated at a flow rate of 100 mL/kg per minute, and afterward, the flow was adjusted to maintain a perfusion pressure of 50 mm Hg. A 12F intracardial sump cannula was positioned in the left ventricle for decompression of the left heart during CPB. In a randomly assigned group an L-DF (Leukoguard LG6, Pall Biomedical) was used during CPB. A heat exchanger was used for core cooling. The pH was maintained with alpha-stat principles at 7.40 ± 0.05 with an arterial P_{CO_2} of 4.0 to 5.0 kPa, uncorrected for temperature. All measurements were performed at 37°C.

The cooling period of 60 minutes was carried out to attain a rectal temperature of 20°C. Cardiac arrest was induced by injecting potassium chloride (1 mEq/kg) into the aortic cannula, and topical cardiac cooling was then maintained throughout the aortic crossclamp period. The ascending aorta was crossclamped just proximal to the aortic cannula.

Experimental protocol. After cooling to a rectal temperature of 20°C and crossclamping of the aorta, the animals underwent a 75-minute period of HCA with the head packed in ice. After this 75-minute period, antegrade CPB rewarming was initiated.

The left ventricular vent cannula was removed. Weaning from CPB occurred approximately 60 minutes after the start of rewarming with administration of furosemide (40 mg), mannitol (15.0 g), methylprednisolone (80 mg), and lidocaine (40–150 mg). Cardiac support was provided with dopamine. Animals were kept in isoflurane anesthesia until the following morning, extubated, and moved into a recovery room.

During the experiments, hemodynamic and metabolic measurements were recorded at 5 different time points as follows: at baseline; at the end of cooling (at 20°C, immediately before HCA); during rewarming (at 30°C); 2 hours after the start of rewarming; and 4 hours after the start of rewarming.

Postoperative evaluation. Postoperatively, all the animals were evaluated daily by an experienced observer who was blinded to the study group by using a species-specific quantitative behavioral score, as reported earlier.¹⁶ The assessment quantified mental status (0, comatose; 1, stuporous; 2, depressed; and 3, normal), appetite (0, refuses liquids; 1, refuses solids; 2, decreased; and 3, normal), and motor function (0, unable to stand; 1, unable to walk; 2, unsteady gait; and 3, normal). Numerical summing of these functions provides a final score: the maximum (score of 9) reflects apparently normal neurologic function, and lower values indicate substantial neurologic damage. A score of 8 means that the animals were able to stand unassisted and were likely to recover fully. Each surviving animal was electively put to death on day 7 after the operation. The entire brain was immediately harvested and weighed for subsequent histologic analysis.

Histopathologic analysis. During autopsy, the brain was excised immediately, and the hemispheres were separated. One half was immersed in 10% neutral formalin and allowed to fix for 2 weeks en bloc. Thereafter, 3-mm thick coronal samples were sliced from the frontal lobe, thalamus (including the adjacent cortex), and hippocampus (including the adjacent brain stem and temporal cortex), and sagittal samples were sliced from the posterior brain stem (medulla oblongata and pons) and cerebellum. The pieces were fixed in fresh formalin for another week. After the fixation, the samples were processed as follows: rinsing in water for 20 minutes; immersion in 70% ethanol for 2 hours; immersion in 94% ethanol for 4 hours; and immersion in absolute ethanol for 9 hours. Thereafter, the pieces were kept for 1 hour in absolute ethanol-xylene mixture and 4 hours in xylene and embedded in warm paraffin for 6 hours. The samples were sectioned at 6 µm and stained with hematoxylin and eosin. The sections of the brain samples of each animal were screened by a single experienced senior pathologist (J.H.) unaware of the experimental design and of the identity and fate of individual animals. Each section was carefully investigated for the presence or absence of any hypoxic or other damage.

Visual estimation of the injuries in the sampled regions was made as follows: 0, no morphologic damage; 1, edema or eosinophilic dark neurons or dark-shrunk cerebellar Purkinje cells; 2, at least mild hemorrhage; and 3, clearly infarctive foci. Total score is the sum of scores in each specific brain area (cortex, thalamus, hippocampus, posterior brain stem,

and brain stem). To allow semiquantitative comparisons between the animals, we calculated a total histologic score by adding all the regional scores. A score of greater than 4 means that the animal had a distinct brain injury.

Serum S-100β. Serum S-100β levels were determined from mixed venous blood samples by use of a luminescence immunoassay kit (Sangtec-100, LIA-mat; Sangtec Medical AB, Bromma, Sweden). Serum S-100β protein levels were measured at baseline, end of cooling, and 30 minutes, 2 hours, 4 hours, 7 hours, and 20 hours after the start of rewarming.

Cytokines. Cytokine levels (interleukin [IL] 1β, IL-8, and tumor necrosis factor [TNF] α) were recorded on the first postoperative day. Serum specimens were drawn, placed in aliquots, and stored at -70°C until tested. Cytokine concentrations were determined by the enzyme-linked immunosorbent assay method according to the manufacturer's instructions. Enzyme-linked immunosorbent assay kits (Cytoscreen) for swine IL-1β, IL-8, and TNF-α were obtained from Biosource International (Camarillo, Calif). Lower detection limits for the tests were 15 pg/mL for IL-1β, 10 pg/mL for IL-8, and 6 pg/mL for TNF-α.

Other measurements. Systemic arterial and venous blood samples were obtained to determine pH, oxygen tension, carbon dioxide tension, oxygen saturation, oxygen content, hematocrit, hemoglobin, and glucose (Ciba-Corning 288 Blood Gas System; Ciba-Corning Diagnostic Corp, Medfield, Mass). Lactate was analyzed by means of a YSI 1500 analyzer (Yellow Springs Instrument Co, Yellow Springs, Ohio). Leukocyte count was done with a Cell-Dyn analyzer (Abbott, Santa Clara, Calif). Temperatures were recorded at intervals throughout the study. Hemodynamics, temperatures, and respiratory gases were monitored by the Datex AS/3 anesthesia monitor (Datex Inc, Espoo, Finland).

Statistical analysis. Summary statistics for continuous or ordinal variables are expressed as the median with interquartile range (25th and 75th percentiles) or means with SD.

The analysis was performed by analysis of variance for repeated measurements. Comparison between relevant time points and baseline (reference category) was performed by the paired-sample *t* test or the Wilcoxon matched pairs signed rank test. Differences between groups were determined by means of *t* tests or the Mann-Whitney *U* test. Sidák inequality was used to control the multiple comparison problem. Analyses were performed by a standard commercially available statistical program (SPSS version 9.0; SPSS Inc, Chicago, Ill).

Results

Physiologic data

Comparability of experimental groups. The mean ± SD weight of the animals was the same in both of the groups (26 ± 2 kg). The mean ± SD total CPB time was 131 ± 12 minutes in the L-DF group and 138 ± 10 minutes in the control animals. The difference was not statistically significant (*P* = .19). The mean ± SD CPB

Fig 1. Epidural temperatures of 20 pigs undergoing CPB with or without an L-DF before and after a 75-minute period of HCA. Values are shown as medians with interquartile range.

Table I. Hemodynamic data, blood gases, and hematocrit levels in 20 pigs undergoing CPB with or without an L-DF before and after 75-minute period of HCA

Group	N	Baseline (37°C)	End of CPB cooling (20°C)	After start of rewarming		
				30°C	2 h	4 h
MAP (mm Hg)						
L-DF	10	75 (70-82)	51 (50-52)†	57 (54-69)†	62 (60-66)†§	66 (63-70)*
Control	10	78 (75-85)	50 (50-56)†	55 (54-60)†	57 (50-61)†	63 (58-65)*
Cardiac output/CPB flow (L/min)						
L-DF	10	3.3 (3.2-3.7)	2.4 (2.2-2.6)*	2.9 (2.5-3.0)	3.5 (3.1-3.8)	2.7 (2.6-3.1)¶
Control	10	3.5 (3.2-3.8)	2.7 (2.5-2.9)*	2.9 (2.4-3.1)*	3.5 (3.3-4.3)	3.5 (3.3-3.8)
Vascular resistance (dyne × s × cm ⁻⁵)						
L-DF	10	1711 (1353-1956)	1587 (1476-1747)	1634 (1350-1806)	1370 (1191-1500)§	1851 (1569-2088)¶
Control	10	1729 (1586-1773)	1486 (1185-1762)	1582 (1371-1771)	1113 (842-1240)‡	1218 (1173-1354)*
Arterial pH						
L-DF	10	7.5 (7.5-7.5)	7.3 (7.3-7.4)*	7.4 (7.3-7.4)	7.3 (7.3-7.4)†	7.4 (7.4-7.5)
Control	10	7.5 (7.5-7.5)	7.3 (7.3-7.3)†	7.5 (7.4-7.5)	7.4 (7.3-7.4)*	7.5 (7.5-7.5)
Paco ₂ (mm Hg)						
L-DF	10	6.4 (5.9-6.4)	7.4 (6.2-7.9)	5.5 (5.1-6.0)	6.4 (6.3-6.6)	6.9 (6.5-7.0)*
Control	10	5.9 (5.7-6.2)	7.9 (7.2-8.3)†	5.2 (4.9-6.0)	6.1 (6.0-6.2)	6.5 (6.0-6.6)
Hematocrit						
L-DF	10	27.2 (25.5-29.9)	17.0 (15.5-17.6)†¶	17.6 (16.5-18.8)†¶	23.1 (22.0-26.4)†	23.8 (21.8-25.5)†
Control	10	28.2 (26.8-30.9)	18.8 (18.2-19.0)†	21.0 (19.6-22.1)*	25.5 (24.1-26.8)	26.3 (25.9-27.4)

Values are shown as medians with interquartile range.

MAP, Mean arterial pressure; Paco₂, arterial carbon dioxide tension.

* $P < .05$, † $P < .01$, and ‡ $P < .001$ compared with baseline.

§ $P < .05$ and ¶ $P < .01$ between groups.

cooling time group was 61 ± 10 minutes in the L-DF group and 65 ± 6 minutes in the control group. Rewarming times were 70 ± 7 minutes and 74 ± 12 minutes, respectively. Temperatures during the experiment period did not differ between the groups (Fig 1).

Hemodynamic data. All animals were stable before, during, and after CPB. As seen in Table I, both groups had a significant decrease in mean arterial pressure compared with baseline values during rewarming at 30°C at 2 hours after the start of rewarming ($P = .001$)

Table II. Venous lactate levels, oxygen extraction, and oxygen consumption during the experiment in 20 pigs undergoing CPB with or without an L-DF before and after 75-minute period of HCA

Group	N	Baseline (37°C)	End of CPB cooling (20°C)	After start of rewarming		
				30°C	2 h	4 h
Venous lactate (mmol/L)						
L-DF	10	1.0 (0.9-1.2)	1.9 (1.8-2.1)‡	6.0 (5.4-6.7)‡	4.5 (2.7-5.8)‡	2.4 (1.7-2.9)‡
Control	10	1.0 (0.8-1.0)	1.5 (1.2-2.0)‡	5.3 (5.0-5.7)‡	3.9 (3.5-4.8)‡	1.7 (1.5-2.0)‡
O ₂ extraction (mmol/L)						
L-DF	10	2.5 (2.4-2.6)	1.7 (1.6-1.8)	3.2 (3.0-3.5)	2.6 (2.2-2.9)	3.1 (2.6-3.6)
Control	10	2.5 (2.1-2.8)	1.6 (1.3-1.8)	3.4 (2.2-2.7)	2.5 (2.2-2.7)	2.6 (2.3-2.8)
O ₂ consumption (mL/min)						
L-DF	10	91.0 (80.8-93.5)	39.1 (36.4-50.4)†	83.3 (78.2-105.1)	98.7 (80.7-111.4)	95.6 (80.5-101.7)
Control	10	90.4 (70.2-110.5)	45.1 (34.2-52.0)*	87.6 (66.7-98.4)	95.0 (81.7-106.6)	84.5 (81.3-99.5)

Values are shown as medians with interquartile range.

* $P < .05$, † $P < .01$, and ‡ $P < .001$ compared with baseline.

Table III. Leukocyte counts in 20 pigs undergoing CPB with or without an L-DF before and after 75-minute period of HCA

Group	N	Baseline (37°C)	End of CPB cooling (20°C)	After start of rewarming		
				30°C	2 h	4 h
WBC (×10 ⁹ /L)						
L-DF	10	18.4 (14.6-20.9)	3.6 (1.7-4.3)‡	3.6 (2.1- 4.7)‡§	6.8 (4.6-12.1)†	16.0 (13.5-21.0)
Control	10	19.1 (13.8-20.6)	4.0 (2.9-5.2)‡	4.4 (3.8- 6.2)‡	6.0 (4.1-15.5)†	16.5 (12.7-23.9)
Neutrophils (×10 ⁹ /L)						
L-DF	10	9.2 (6.01-12.0)	1.7 (0.8-2.6)*	1.7 (1.0-2.2)*	3.8 (1.0-8.1)	12.3 (10.3-16.0)
Control	10	9.5 (5.8-9.8)	2.1 (1.6-2.8)*	1.6 (1.5-2.9)*	2.9 (1.8-10.0)	12.7 (10.1-20.2)
Lymphocytes (×10 ⁹ /L)						
L-DF	10	8.2 (7.9-9.2)	1.4 (0.9- 1.9)‡	1.8 (1.2-2.3)‡	3.2 (2.9-3.6)‡	3.3 (2.8-3.6)‡
Control	10	9.0 (7.9-10.5)	2.0 (1.7-2.3)‡	2.7 (2.2-3.4)‡	3.4 (2.6-3.7)‡	3.2 (2.6-3.6)‡

Values are shown as medians with interquartile range. WBC, White blood cell counts.

* $P < .05$, † $P < .01$, and ‡ $P < .001$ compared with baseline.

§ $P = .069$ and || $P = .016$ between the groups.

and 4 hours after the start of rewarming ($P < .01$). Mean arterial pressure was higher in the L-DF group 2 hours after the start of rewarming ($P < .01$), and cardiac output was higher in control animals 4 hours after the start of rewarming ($P < .01$, Table I). Vascular resistance decreased significantly in control animals compared with baseline values 2 hours after the start of rewarming ($P < .001$) and 4 hours after the start of rewarming ($P < .01$). Vascular resistance was significantly higher at the same time points in the L-DF group ($P < .05$ and $P < .01$, Table I).

Blood gas and hematocrit measurements are shown in Table I. Both groups had a significant decrease in pH at the end of cooling and 2 hours after the start of rewarming compared with baseline values ($P < .01$), but no significant differences between the groups were seen. Arterial PCO₂ was higher in the control

group at the end of cooling ($P < .001$) compared with baseline. In the L-DF group, arterial PCO₂ was higher 4 hours after the start of rewarming ($P < .01$), but no significant differences between the groups were detected. The hematocrit level was lower at all time points compared with baseline in the L-DF group ($P < .001$) and at the end of cooling and during rewarming at 30°C in control animals ($P < .01$). The hematocrit level was found to be lower in the L-DF group than in control animals at the end of cooling and during rewarming at 30°C ($P < .01$).

Metabolic data. The venous lactate increased significantly during cooling and especially after HCA in both groups ($P < .001$). Oxygen consumption decreased significantly in both groups during cooling ($P < .05$). There were no significant changes in the oxygen extraction during the experiment (Table II).

Fig 2. EEG burst recovery of 20 pigs undergoing CPB with or without an L-DF before and after a 75-minute period of HCA. **Bold lines** indicate medians, and **broken lines** depict animals with early mortality.

Fig 3. Daily scores indicating behavioral recovery of 20 pigs undergoing CPB with or without an L-DF before and after a 75-minute period of HCA. A score of 8 or 9 indicates essentially complete recovery; lower scores indicate substantial impairment, and 0 indicates coma or death. *P* values are given for the difference between groups in score by time.

Leukocyte count. White blood cell count decreased significantly during cooling and after intervention in both groups ($P < .01$) and tended to be lower in the L-DF group during rewarming at 30°C ($P = .07$). Neutrophil count decreased significantly during cooling and rewarming in both groups ($P < .05$).

Lymphocyte count decreased significantly during cooling and after intervention in both groups ($P < .001$) and was lower in the L-DF group during rewarming at 30°C ($P = .02$, Table III).

EEG. No statistically significant differences between groups in the rate of EEG burst recovery was detected.

Table IV. Histopathologic scores after the experiment in 20 pigs undergoing a 75-minute period of HCA either with or without an L-DF

Protocol	Pig No.	Cortex	Thalamus	Hippocampus	Posterior brain stem	Cerebellum	Total score
L-DF group	1	1	0	1	1	3	6
	2	3	3	1	3	3	13
	3	1	0	0	2	2	5
	4	1	0	1	2	1	5
	5	4	3	1	1	1	10
	6	4	1	1	3	3	12
	7	1	1	1	2	0	5
	8	4	1	1	3	1	10
	9	1	0	1	2	3	7
	10	3	2	2	3	3	13
<u>Medians</u>		<u>2</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>2.5</u>	<u>8.5</u>
Control group	1	4	3	2	3	4	16
	2	6	6	4	3	3	22
	3	2	1	1	3	3	10
	4	1	0	0	0	1	2
	5	5	3	3	2	3	16
	6	3	1	4	2	3	13
	7	7	3	2	2	4	18
	8	4	1	2	4	4	15
	9	4	6	2	3	3	18
	10	4	4	2	3	1	14
<u>Medians</u>		<u>4</u>	<u>3</u>	<u>2</u>	<u>3</u>	<u>3</u>	<u>15.5</u>
<i>P</i> values between groups		.004	.026	.001	.025	.015	.005

Scores are defined as follows: 0, no morphologic damage; 1, edema or eosinophilic dark neurons or dark-shrunk cerebellar Purkinje cells; 2, at least mild hemorrhage; and 3, clearly infarctive foci. *Total score* is the sum of scores in each specific brain area (cortex, thalamus, hippocampus, posterior brain stem, and brain stem). To allow semiquantitative comparisons between the animals, we calculated a total histologic score by adding all the regional scores.

EEG did not recover to a recordable level in one control pig. In one pig of the control group, EEG burst disappeared at 12 hours, and in one pig of the L-DF group, burst disappeared at 14 hours (Fig 2).

Morbidity and mortality. All animals were in stable condition during the surgical procedures and survived at least to the first postoperative day. Thirteen (65%) of the 20 animals survived 7 days after the operation and were electively put to death. In the L-DF group 8 (80%) of 10 animals survived for 7 days compared with 5 (50%) of 10 animals in the control group. The risk for early death in control animals was 2.5 (95% confidence interval, 0.63–10) times higher than that of animals in the L-DF group.

Behavioral outcome. The results of behavioral scoring for both groups are shown in Fig 3. A score of 8 or 9 indicates an essentially complete neurologic recovery. Animals that died early were given a score of zero beginning at the time of death. The median behavioral score at day 7 was higher in the L-DF group (8.5 vs 3.5; $P = .04$). The median behavioral score at day 7 among the surviving animals was 9 in the L-DF group and 8 in the control animals ($P = .08$).

Histopathologic results. In terms of histopathology, better outcomes were found in all 5 studied brain regions in the L-DF group than in the control group

(Table IV). The total histopathologic score was calculated by adding the quantitative assessment of histopathologic findings in the investigated regions of the brain for each of the animals. The median of total histopathologic scores was 8.5 in the L-DF group and 15.5 in the control group ($P = .005$). As seen in Fig 4, the total score tended to be higher in animals with early death compared with animals surviving 7 days ($P = .13$). The median of total histopathologic score among surviving animals was 6.5 in the L-DF group and 14 in the control group ($P = .08$).

Cytokines. TNF- α levels increased after intervention in both groups. The medians of TNF- α levels in the L-DF group was 18 pg/mL at 30°C during rewarming and 107 pg/mL after 2 hours after the start of rewarming. At the same time points, medians in the control group were 3 pg/mL and 861 pg/mL. The changes were found to be statistically significant compared with baseline values ($P < .05$), but there were no statistically significant differences between the groups. IL-1 β and IL-8 did not differ during the experiment between the groups (Table V).

S-100 β . S-100 β levels increased after intervention in both groups, being higher compared with baseline values in the L-DF group during rewarming at 30°C ($P = .03$). After this and during rewarming, S-100 β levels

Fig 4. Total histopathologic scores of 20 pigs undergoing CPB with or without an L-DF before and after a 75-minute period of HCA. The total histopathologic score was calculated by adding the quantitative assessment of histopathologic findings in the investigated regions of the brain for each of the animals. The scores in the L-DF group were lower than those in the control group ($P = .005$).

Fig 5. S-100 protein levels of 20 pigs undergoing CPB with or without an L-DF before and after a 75-minute period of HCA. Medians with interquartile range are shown. There were no statistically significant differences between the groups.

Table V. $TNF-\alpha$, $IL-1\beta$, and $IL-8$ levels in 20 pigs undergoing CPB with or without an L-DF before and after 75-minute period of HCA

Group	N	Baseline (37°C)	End of CPB cooling (20°C)	After start of rewarming				
				30°C	2 h	4 h	7 h	24 h
TNF-α (pg/mL)								
L-DF	10	0 (0)	0 (0-5)	18 (0-88)*	107 (4-863)*	0 (0-16)	0 (0-11)	0 (0-9)
Control	10	0 (0)	0 (0-3)	3 (0-9)	851 (9-977)*	5 (0-15)	0 (0-1)	0 (0-1)
IL-1β (pg/mL)								
L-DF	10	0 (0-151)	34 (0-195)	28 (0-61)	31 (0-300)	125 (0-280)	1 (0-359)	54 (0-529)
Control	10	0 (0-132)	0 (0-140)	6 (0-117)	5 (0-99)	4 (0-177)	31 (0-127)	0 (0-2,8)
IL-8 (pg/mL)								
L-DF	10	0 (0)	0 (0)	0 (0)	0 (0-18)	0 (0)	0 (0)	0 (0)
Control	10	0 (0)	0 (0)	0 (0)	7 (0-78)	0 (0-10)	0 (0)	0 (0)

Values are shown as medians with interquartile range.

* $P < .05$ compared with baseline values.

decreased below baseline in the L-DF group, and a statistically significant difference was seen 4 hours after the start of rewarming ($P = .05$). No difference between the groups was detected (Fig 5).

Discussion

The present data indicate that leukocyte filtration during CPB improves brain protection after a prolonged period of HCA. The differences were seen in terms of behavioral and histopathologic data. These findings suggest that leukocyte-depleting filtration mitigates reperfusion injury in the brain.

The most frequently used method to protect the brain during operations on the aortic arch is hypothermia.¹

Hypothermia produces a state of decreased oxygen metabolic activity, thereby reducing tissue damage by increasing the length of permissible period of HCA. Hypothermia specifically prevents the release of neurotransmitters and delays the onset of the fatal biochemical cascade.^{1,2} The knowledge of biochemical cascade has opened up new possibilities to improve cerebral protection against ischemia. We have learned thus far that energy failure itself is not particularly toxic to neurons. What makes energy failure neurotoxic is subsequent activation of glutamate receptor-related mechanisms. If these are blocked by suitable antagonists, the neurons may survive over the period when the supply of oxygen and substrate are compromised. The most studied antagonists are glu-

tamate receptor blockers and Ca^{2+} and Na^{+} channel antagonists. Many of these antagonists have turned out to be neurotoxic in a clinical setting.¹⁷ We have demonstrated very recently that the Na^{+} channel blocker lamotrigine improves brain protection during HCA.¹⁴

The third subsequential phase in ischemic cerebral injury is reperfusion injury occurring after depolarization and biochemical cascade. Leukocyte infiltration and cytokine-mediated inflammatory reaction are known to play pivotal roles in this phase.^{4,5} There are different strategies to mitigate inflammatory injury in the brain; direct cytokine inhibition is one such strategy. Cytokine inhibition by anti-TNF- α monoclonal antibodies can reduce focal ischemic injury. Another possibility is the inhibition of endothelial interactions with leukocytes (ie, blocking the endothelial side of the adhesion molecule). The use of antileukocyte treatments is also a potential strategy.⁶ Heparin has been used to inhibit leukocyte accumulation in cerebral ischemia.¹⁸ All of these strategies are found to be promising in the experimental setting.

It has been previously shown that leukocyte depletion can ameliorate myocardial reperfusion injury.^{7,8} Leukocyte-depleting filtration is also shown to reduce ventricular dysfunction during prolonged postischemic reperfusion⁹ and to attenuate reperfusion injury in patients with left ventricular hypertrophy.¹⁹ When more than 3 blood transfusions are required after a cardiac operation, leukocyte depletion of transfused blood results in a significant reduction of postoperative mortality.²⁰ Leukocyte depletion during and after CPB has been demonstrated to improve pulmonary function¹⁰ and to ameliorate free radical-mediated lung injury.^{11,12} The present study is the first attempt to mitigate HCA-related brain injury by using leukocyte-depleting filtration.

The hardest data in the present study are undoubtedly the histopathology. The total histopathologic score was significantly lower in the L-DF group, and the differences were also seen in all studied regions of the brain. In line with this was the mortality, with the rate of early deaths being 5 of 10 in the control group and 2 of 10 in the L-DF group, supporting the favorable effect of leukocyte filtration after HCA. Because leukocyte filtration is known to mitigate reperfusion in other tissues, such as the heart and lung, the possibility that improved outcome was a consequence of better preservation of these organs after perfusion cannot be excluded. The behavioral outcome was also better in the L-DF group. Control animals showed more severe initial neurologic impairment, and recovery to score 9 was not seen. Looking at the overall consistency of all this outcome data obtained by using this chronic animal model and the fact that brain was exposed for major injury in

this particular protocol, it is likely that the favorable effect of leukocyte-depleting filtration is due to better maintenance of neuronal function. This hypothesis was supported by the finding that animals with early mortality had higher total histopathologic scores compared with animals that survived for 7 days (Fig 4). To study this hypothesis further, we tested the outcomes in the surviving animals in both groups. In this comparison histopathologic scores tended to be lower ($P = .08$) and behavioral scores tended to be higher ($P = .08$) in the L-DF group. These findings give further evidence to the claim that the beneficial effect of white cell filtration is a consequence of improved brain protection.

There were no statistically significant differences in EEG burst recovery. When we studied the Na^{+} channel blocker lamotrigine with this same model, we found that the lamotrigine group had better EEG recovery after HCA.¹⁴ Lamotrigine and other neuroprotective drugs have an effect on biochemical cascade, and leukocyte-depleting filtration exercises an effect on reperfusion injury; this can explain the difference between the studies. In 1 control animal there were no EEG bursts after HCA, and there were 2 animals (1 in each group) whose EEG bursts decreased after earlier recovery. All 3 animals died soon after extubation. This is in line with other data and supports the hypothesis that early mortality was due to neurologic damage (Fig 2). EEG was the only method that could depict the time point when the cerebral cortex probably died.

White blood cell count decreased during and after intervention in both groups but returned to baseline level at 4 hours after the start of rewarming. White blood cell counts tended to be lower in the L-DF group shortly after the start of rewarming. Neutrophil levels were in line with this, but no differences between the groups were detected. Lymphocyte levels decreased during and after intervention in both groups, and lymphocyte counts did not return to baseline during experiment. The lymphocyte levels tended to be lower in the L-DF group during rewarming at 30°C. It has been demonstrated by investigating the expression of antigens on neutrophils that the LG-6 filter selectively removes activated neutrophils but does not have a significant enough effect to reduce the total leukocyte count.²¹ This may explain why only marginal differences between the groups was seen in present study.

TNF- α increased after HCA in both of the study groups, and the levels tended to be lower in the L-DF group than in the control group. There were no differences between groups in IL-1 β and IL-8 levels, and the changes were much smaller compared with baseline values as concerns TNF- α levels. These findings were

surprising because TNF- α and IL-1 β are shown to play an important role in brain immune and inflammatory activities and ischemic brain injury.⁴⁻⁶ On the basis of current data, we have to admit that the cytokines measured here might not be the mediators we should be looking for in this context.

Serum S-100 β protein has been suggested to be a neurobiochemical marker of brain injury after cardiac and aortic arch operations.²² S-100 β release has been associated with length of HCA.^{23,24} A recent study has demonstrated a correlation between early changes in neuropsychologic performance and S-100 β elaboration after HCA.²⁵ In the present study there were no differences between the groups in S-100 β release. The reason for this remains open. In our previous article studying the use of lamotrigine before HCA, there was a statistically significant difference between groups in S-100 β release.¹⁴ One explanation for this could be the fact that leukocyte-depleting filtration has an effect on reperfusion injury and not biochemical cascade.

In conclusion, the present data strongly suggest that leukocyte-depleting filtration before and after prolonged periods of HCA improves brain protection.

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