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Original Article

Effects of dexmedetomidine on kidney and brain tissue microcirculation and histology in ovine cardiopulmonary bypass: a randomised controlled trial

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Summary

Cardiac surgery requiring cardiopulmonary bypass is associated with postoperative acute kidney injury and neurocognitive disorders, including delirium. Intra-operative inflammation and/or impaired tissue perfusion/oxygenation are thought to be contributors to these outcomes. It has been hypothesised that these problems may be ameliorated by the highly selective α_2-agonist, dexmedetomidine. We tested the effects of dexmedetomidine on renal and cerebral microcirculatory tissue perfusion, oxygenation and histology in a clinically relevant ovine model. Sixteen sheep were studied while conscious, after induction of anaesthesia and during 2 h of cardiopulmonary bypass. Eight sheep were allocated randomly to receive an intravenous infusion of dexmedetomidine (0.4–0.8 $\mu g.kg^{-1}.h^{-1}$) from induction of anaesthesia to the end of cardiopulmonary bypass, and eight to receive an equivalent volume of matched placebo (0.9% sodium chloride). Commencement of cardiopulmonary bypass decreased renal medullary tissue oxygenation in the placebo group (mean (95%CI) 5.96 (4.24–7.23) to 1.56 (0.84–2.09) kPa, p = 0.001), with similar hypoxic levels observed in the dexmedetomidine group (6.33 (5.33–7.07) to 1.51 (0.33–2.39) kPa, p = 0.002). While no differences in kidney function (i.e. reduced creatinine clearance) were evident, a greater incidence of histological renal tubular injury was observed in sheep receiving dexmedetomidine (7/8 sheep) compared with placebo (2/8 sheep), p = 0.041. Graded on a semi-quantitative scale (0–3), median (IQR [range]) severity of histological renal tubular injury was higher in the dexmedetomidine group compared with placebo (1.5 (1–2 [0–3]) vs. 0 (0–0.3 [0–1]) respectively, p = 0.013). There was no difference in cerebral tissue microglial activation (neuroinflammation) between the groups. Dexmedetomidine did not reduce renal medullary hypoxia or cerebral neuroinflammation in sheep undergoing cardiopulmonary bypass.

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Introduction

Cardiopulmonary bypass (CPB) is essential for the safe conduct of many of the two million cardiac surgical procedures which are performed annually around the world [1]. Cardiac surgery (and by extension, CPB) is not without risk. Up to 42% of patients develop an acute kidney injury (AKI), which, if severe, has been associated with a 3–8-fold increase in acute postoperative mortality, prolonged hospital stay and increased long-term mortality which remains elevated until 10 years following the index surgery [2, 3]. Furthermore, up to 50% of patients may have delayed neurocognitive recovery, which has been associated with prolonged hospital stay and long-term neurocognitive decline [4, 5]. While there are multiple possible aetiologies for postoperative neurocognitive disorders, neuroinflammation is hypothesised to play a major role [6].

Intra-operative administration of the α_2-agonist, dexmedetomidine, has been reported to reduce the incidence of both AKI and delirium following cardiac surgery [7]. However, larger prospective studies have cast doubt on these findings [8, 9]. Consequently, the role of dexmedetomidine in preventing these complications following cardiac surgery remains uncertain [2, 10]. It is biologically plausible that dexmedetomidine may reduce the incidence of these events through either anti-inflammatory or centrally acting sympatholytic effects [11]. Rodent studies show that dexmedetomidine may protect against renal and cerebral ischaemic injury [12, 13]. However, to the best of our knowledge, there is no directly observed evidence of improvements in renal and cerebral microcirculatory perfusion or oxygenation and/or reductions in renal histopathological changes or cerebral neuroinflammation from clinically relevant, physiologically and placebo-controlled large animal studies.

In response to this knowledge gap, we performed a randomised controlled trial of intra-operative dexmedetomidine compared with placebo in a clinically relevant ovine model of CPB [14]. We hypothesised that animals that received dexmedetomidine would have better renal medullary and cerebral tissue perfusion and oxygenation and less microglial activation in cerebral cortical tissue relative to animals that received a volume-matched placebo (0.9% sodium chloride).

Methods

We investigated the effects of a clinically relevant dose of dexmedetomidine on tissue perfusion and oxygenation and inflammation within the kidney and brain of sheep during CPB. All experimental studies were approved by the animal ethics committee of the Florey Institute of Neuroscience and Mental Health and are reported here according to the ARRIVE guidelines [15].

Sixteen female non-pregnant Merino ewes (aged 1.5–2.0 y) with a mean (95%CI) body weight 44.0 (42.3–45.7) kg were studied. Our primary outcome was renal medullary tissue oxygen tension, which is a critical mediator of postoperative AKI [16]. Based on our previous investigations, a sample size of eight sheep per group provided a 90% power to detect a 50% reduction in medullary tissue oxygen tension as a continuous variable [16]. Our secondary outcome was neuroinflammation as determined by immunohistochemistry. We have reported previously that four animals per group had 90% power ($\alpha = 0.05$) to detect a 30% increase in microglial activation in cerebral cortices [14].

Before experimentation, animals were housed in metabolic cages with free access to water and 800 g of oaten chaff daily. Animals were allowed to acclimatise for 1 week before preparatory surgery under general anaesthesia. This preliminary procedure involved cannulation of the jugular vein, renal vein and carotid artery, and the insertion of transit-time flow probes and fibreoptic probes in the brain and left kidney as described previously [17]. An indwelling urinary catheter was also inserted. Sheep were given 50 mg intramuscular flunixin meglumine (Norbrook, Tullamarine, Victoria, Australia) for analgesia and 900 mg intramuscular procaine penicillin (Troy Laboratories, Glendinning, NSW, Australia) intra-operatively, repeated at 24- and 48-h postoperatively.

After 5 days of recovery from this preliminary procedure, animals were allocated randomly to receive dexmedetomidine (n = 8) or volume-matched placebo

(n = 8) using block randomisation in groups of 4. Researchers conducting the experiment were not blinded to group allocation. Each experiment was divided into six separate 30-min experimental periods: pre-induction; postinduction; and four periods after commencing CPB (CPB 1–4) (Fig. 1).

Arterial pressure (mmHg), renal blood flow (ml.kg.$^{-1}$ min^{-1}), renal vascular conductance (μl.kg^{-1}.min^{-1}.mmHg^{-1}), cerebral, renal cortical and medullary tissue perfusion (blood perfusion units), tissue oxygen tension (PO_2, kPa) and tissue temperature (°C) were recorded. After induction of anaesthesia, cerebral oxygen saturation (SO_2) was monitored using near-infrared spectroscopy (NIRS; INVOS™, Medtronic, Macquarie Park, NSW, Australia). Arterial blood samples were collected at the midpoint of each experimental period for oximetry, blood gas analysis and measurement of plasma levels of tumour necrosis factor-α, interleukin-6 and interleukin-10 using enzyme-linked immunosorbent assays. Mixed venous and renal venous blood samples were collected at the midpoint of each experimental period for oximetry and blood chemistry (creatinine and sodium).

Following 30 min of unrestrained observation and recording of haemodynamic data in conscious sheep (pre-induction), general anaesthesia was induced with 4 mg.kg^{-1} intravenous propofol (AFT Pharmaceuticals, Burwood, NSW, Australia) and 5 μg.kg^{-1} fentanyl (Hameln Pharmaceuticals, Hameln, Germany), followed by tracheal intubation and mechanical ventilation. Anaesthesia was maintained with inhaled sevoflurane (vaporiser was set to 4% sevoflurane and fresh gas flow set at 500 ml.min^{-1} (250 ml.min^{-1} oxygen and 250 ml.min^{-1} medical air); AbbVie, Mascot, NSW, Australia), intravenous propofol 4 mg.kg^{-1}.h^{-1} and fentanyl 3 μg.kg^{-1}.h^{-1}. All animals received 2 ml.kg^{-1}.h^{-1} intravenous compound sodium lactate (Baxter, Toongabbie, NSW, Australia) as maintenance fluid. After haemodynamic stability was established, animals allocated to the dexmedetomidine group commenced a continuous intravenous infusion at 0.4 μg.kg^{-1}.h^{-1}, with animals allocated to placebo receiving an equivalent volume of 0.9% sodium chloride. After a further 30-min period of observation (postinduction), a right lateral thoracotomy was performed, 300 IU.kg^{-1} of unfractionated heparin (Pfizer, Sydney, NSW, Australia) was administered and CPB was established using methods described previously [14]. A non-pulsatile pump flow of 70 ml.kg^{-1}.h^{-1}, mean arterial pressure (MAP) of 65–75 mmHg and core body temperature of 36°C were maintained on CPB, consistent with current best practice recommendations [18]. Mean arterial pressure was maintained with 0.5 mg intravenous boluses of metaraminol as required (Montrose Pharma, Sydney, NSW, Australia). Once CPB was established, the study drug infusion rate was increased to 0.8 μg.kg.h^{-1} in the dexmedetomidine group, with an equivalent increase in the volume of the vehicle (0.9% sodium chloride) in the placebo group. The ascending aorta was cross-clamped, and antegrade del Nido cardioplegia was administered. After 30 min of stable CPB, a further four experimental periods followed (CPB 1–4), totalling 2 h (Fig. 1).

At the end of the experiment, the animal was euthanised with 20 mg.kg^{-1} sodium pentobarbitone (Virbac, Wetherill Park, Australia) administered into the CPB circuit, and the brain and left kidney were collected for histological assessment. The brain was perfused with 1 l cold saline followed by 1l 4% paraformaldehyde, then removed and one hemisphere was placed in paraformaldehyde for 24 h before being dehydrated in 20% sucrose and frozen for sectioning. The kidneys were cut

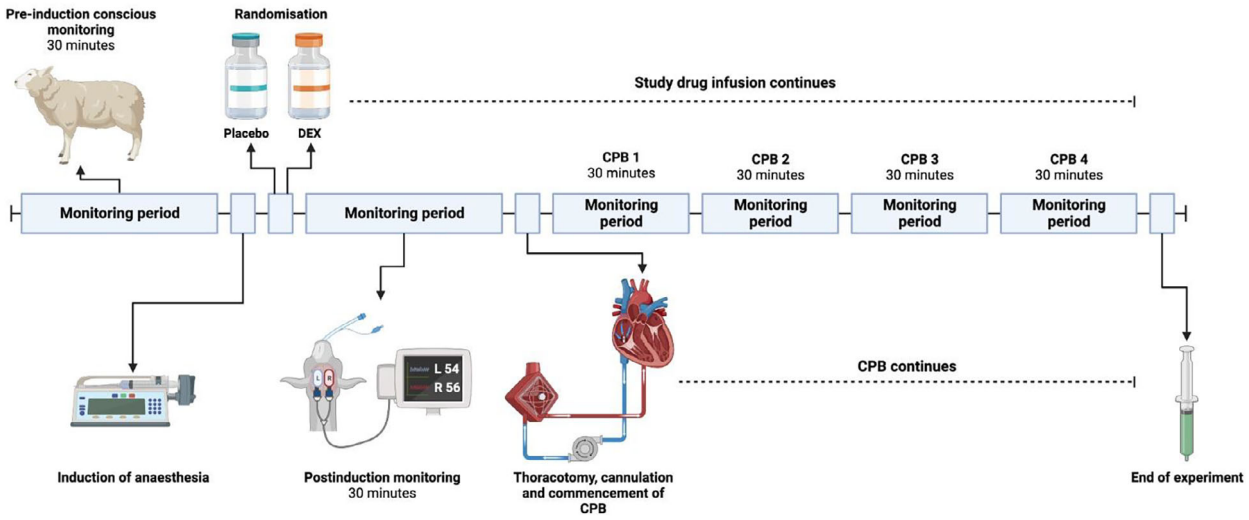

Figure 1 Schematic of study design and experimental timeline (see text for details). Image was created with BioRender.com.

transversely, and with the other cerebral hemisphere, fixed for 14 days in 10% neutral, buffered formalin. Two representative sections of kidney containing papilla, medulla and cortex, and a section of brain extending from cerebrum to corpus callosum, were taken and processed for paraffin sectioning. Additional to the sections of kidney taken from sheep included in this experiment, tissue from two animals that had not undergone any experimental procedure (including anaesthesia) were included as controls for histological analysis. These two animals were euthanised with 20 $mg.kg^{-1}$ intravenous sodium pentobarbitone.

Kidney sections were stained with haematoxylin and eosin, periodic acid Schiff's and Masson's trichrome. Sections were assessed for histological renal tubular injury independently by two histopathologists blinded to treatment allocation according to the presence of tubular dilatation; epithelial thinning; tubular casts; apoptosis; regenerative changes; and tubulitis. The renal interstitium was also assessed for inflammation, oedema and fibrosis. Morphological changes consistent with histological renal tubular injury and inflammation were semi-quantitatively graded as follows: 0 (none, ≤ 5% of total section affected); 1 (mild, 5–25% of total section affected); 2 (moderate, 26–50% of total section affected); and 3 (severe, > 50% of total section affected). Where the semi-quantitative score differed between the two histopathologists, an averaged value was used.

Formalin-fixed brain sections were stained with haematoxylin and eosin and Masson's trichrome. They were assessed for micro-infarcts, thrombi and other overt pathological changes. All histological assessments were performed by two independent histopathologists who were blinded to experimental interventions.

Paraformaldehyde-fixed cerebral tissue sections (40 μm) from each lobe of the brain (frontal, parietal, temporal and occipital) were cut on a cryostat. The sections were washed in 0.1 M phosphate-buffered saline (pH 7.4; 3 × 5 min), and then incubated for 24 h in primary antibody (guinea pig anti-ionised calcium binding adaptor molecule (Iba1), 1:1000; Synaptic Systems GmbH, Gottingen, Germany) at room temperature on a shaker. Sections were washed with 0.1 M phosphate buffered saline and then incubated for 1 h in a secondary antibody (biotinylated donkey anti-guinea, 1:500; Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Sections were then washed in 0.1 M phosphate buffered saline and incubated in the tertiary antibody (streptavidin-488, 1:500; Jackson ImmunoResearch Laboratories) for 1 h. Sections were then mounted on gelatine-subbed slides (24 × 50 mm; Menzel-Glasser, 50 slides, LOMB), and coverslipped with anti-fade histology mountant (Fluoroshield™ with 4′,6-diamidino-2-phenylindole; Sigma-Aldrich, St Louis, MO, USA). Images were taken on the confocal laser-scanning microscope (Leica TCS SP8™; Leica GmbH, Wetzlar, Germany) using a 40× oil immersion objective. Skeletal analysis of microglial morphometry was performed using FIJI (National Institutes of Health, Bethesda MD, USA) and 3DMorph [19].

All data were tested for normality using the Kolmogorov–Smirnov ($n \geq 5$) method. Between-group specific time-point comparisons during the interventional period (postinduction to the end of CPB 4) were made using two-way repeated measures analysis of variance (ANOVA) with factors treatment ($P_{treatment}$) and time (P_{time}) and their interaction ($P_{Treatment} \times P_{Time}$). Between-group comparisons for immunohistochemical and vasopressor data were performed using Student's unpaired t-test and for histopathological data using Fisher's exact test for categorical data or the Mann–Whitney U test for ordinal data. Within-group comparison between experimental periods were performed using Dunnett's test after data were subjected to one-way repeated measures ANOVA with a Greenhouse–Geisser correction. Analyses were performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Two-sided p-values < 0.05 were considered significant.

Results

No between-group differences in whole kidney haemodynamics and function were seen, except for the renal oxygen delivery which appeared to be elevated in the dexmedetomidine group (Fig. 2 and online Supporting Information Table S1). Compared with the postinduction time-point, there were significant decreases in renal vascular conductance at the CPB 1 time-point (dexmedetomidine: mean (95%CI) 47.9 (43.5–52.3) to 32.4 (30.6–34.1) $\mu l.kg^{-1}.min^{-1}.mmHg^{-1}$, $p < 0.001$; placebo: mean (95%CI) 45.5 (40.1–50.9) to 28.8 (25.1–32.6) $\mu l.kg^{-1}.min^{-1}.mmHg^{-1}$, $p = 0.009$). A reduction in renal oxygen delivery was also seen (dexmedetomidine: mean (95%CI) 0.34 (0.30–0.38) to 0.16 (0.12–0.19) $ml.kg^{-1}.min^{-1}$, $p = 0.002$; placebo: mean (95%CI) 0.26 (0.21–0.32) to 0.13 (0.12–0.15) $ml.kg^{-1}.min^{-1}$, $p = 0.018$). However, renal oxygen consumption did not change significantly, so renal fractional oxygen extraction increased between the postinduction and CPB 1 time-points (dexmedetomidine: mean (95%CI) 16.0 (13.5–18.5) to 38.1 (25.7–50.5)%, $p = 0.048$; placebo: mean (95%CI) 18.8 (15.5–21.9) to 54.3 (41.7–66.8)%, $p = 0.047$). There was no difference in our primary outcome between treatment groups. Compared with the postinduction time-point, there were similar reductions in renal medullary tissue PO_2 during CPB (dexmedetomidine: mean (95%CI) 6.33 (5.33–7.07) to 1.51 (0.33–2.39) kPa, $p = 0.002$; placebo: mean (95%CI) 5.96 (4.24–7.23) to 1.56 (0.84–2.09) kPa, $p = 0.001$) (Fig. 3).

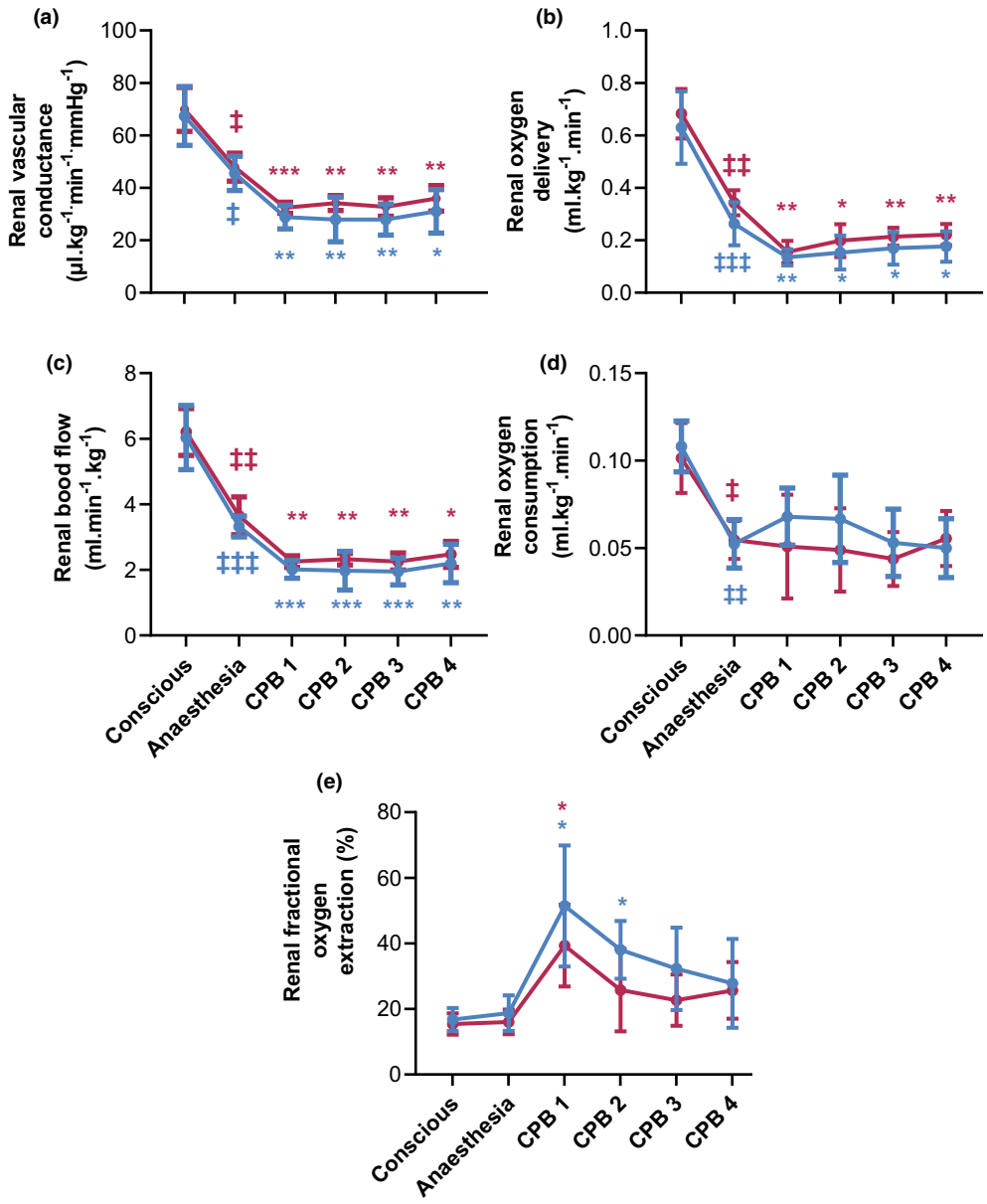

Figure 2 Whole kidney haemodynamics and oxygenation including (a) renal vascular conductance; (b) oxygen delivery; (c) renal blood flow; (d) renal oxygen consumption; and (e) renal fractional oxygen extraction (e). Red, sheep that received dexmedetomidine; blue, sheep that received placebo. Data are presented as mean and 95%CI. N = 8 for renal blood flow and renal vascular conductance for both groups. Due to dysfunction of renal venous cannula n = 7 for renal oxygen consumption for the dexmedetomidine group and n = 6 for the saline group. P_{group} = 0.057 for renal vascular conductance, 0.067 for renal blood flow, 0.038 for renal oxygen delivery, 0.417 for renal oxygen consumption, and 0.059 for renal fractional oxygen extraction. The P_{group} values represent differences between dexmedetomidine and placebo groups from a two-way analysis of variance from post-induction state to the end of cardiopulmonary bypass (CPB) 1–4. $^{‡}p \leq 0.05$, $^{‡‡}p < 0.01$, $^{‡‡‡}p < 0.001$ for comparison between pre-induction and post-induction time-points (Dunnett's test). *p ≤ 0.05, **p < 0.01, ***p < 0.001 (Dunnett's test) for comparison between postinduction and CPB 1–4 time-points.

Commencing CPB induced similar decreases in creatinine clearance between the postinduction and CPB 1 time-points in both groups and similar regional changes in renal cortical perfusion and oxygenation (online Supporting Information Table S1). Compared with the postinduction time-point, neither renal cortical perfusion nor cortical PO_2 was significantly altered (Fig. 3). These findings are again consistent with previous observations in this model [20, 21].

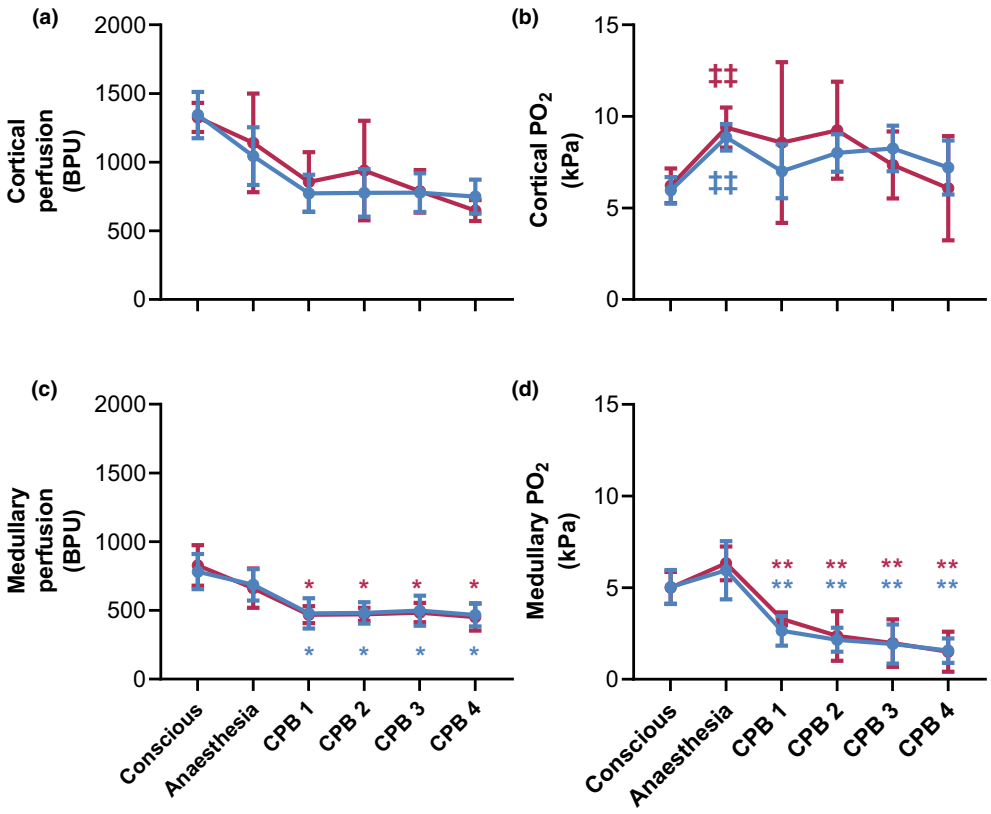

Figure 3 (a) Renal cortical tissue perfusion; (b) renal cortical tissue oxygenation; (c) renal medullary tissue perfusion; and (d) medullary tissue oxygenation. Red, sheep that received dexmedetomidine; blue sheep that received placebo. Data are presented as mean and 95%CI. n = 8 for renal cortical and medullary perfusion and renal cortical PO_2 for both groups. Due to dysfunction of fibreoptic probe, n = 7 for renal medullary PO_2 for both groups. P_{group} = 0.607 for renal cortical perfusion, 0.687 for medullary perfusion, 0.647 for cortical PO_2, and 0.497 for medullary PO_2. p values and statistical analysis are as for Figure 2. ‡‡p < 0.01 for comparison between pre-induction and post-induction time-points (Dunnett's test). *p ≤ 0.05, **p < 0.01 (Dunnett's test) for comparison between postinduction and cardiopulmonary bypass (CPB) 1–4 time-points. BPU, blood perfusion units; PO_2, partial pressure of oxygen; CPB, cardiopulmonary bypass.

Despite no between-group differences in renal macro- and microcirculatory perfusion and oxygenation, the incidence of histological renal tubular injury was significantly greater in sheep that received dexmedetomidine compared with those that received placebo (7/8 vs. 2/8 respectively, p = 0.041) (Table 1). Graded 10 semi-quantitively, median severity of histological renal tubular injury in the dexmedetomidine group was higher (median (IQR [range]) severity score 1.5 (1–2 [0–3]) vs. 0 (0–0.3 [0–1]), p = 0.013) (Table 1 and online Supporting Information Figure S1). No significant differences in inflammation of the renal interstitium were observed between the dexmedetomidine and placebo groups, in both overall incidence (7/8, 87.5% vs. 4/8, 50% sheep, p = 0.28) and median (IQR [range]) severity score (1 (1–1.3 [0–3]) vs. 0.5 (0–1 [0–1]), p = 0.1) (Table 1). No differences in proteinaceous and cellular tubular casts were observed between the dexmedetomidine and placebo groups, in both overall incidence (6/8 vs. 3/8 respectively, p = 0.31) and median (IQR [range]) severity score (1 (0.8–1.3 [0–2]) vs.0 (0–1 [0–1]) respectively, p = 0.12) (Table 1 and online Supporting Information Figure S1). There were no tubular epithelial cell vacuoles, apoptotic and regenerative changes, tubulitis, interstitial fibrosis or oedema observed in either group.

No between-group differences in arterial blood oximetry/chemistry or systemic/renal venous blood oximetry/chemistry were observed (online Supporting Information Tables S2–S4), nor were there differences in total metaraminol requirements between the two groups over the course of the experiment (dexmedetomidine 23.1 (95%CI 18.6–27.5) mg vs. placebo 24.4 (95%CI 6.7–42.0) mg, p = 0.89) that could explain the higher incidence of tubular injury in the dexmedetomidine group.

There were no significant differences in microglial morphology across the frontal, parietal, temporal and occipital cortical regions between the dexmedetomidine

Table 1 Renal pathological changes in dexmedetomidine and placebo treated groups. Reported semiquantitative scores are averaged values from the two independent histological assessments.

Histopathology	Dexmedetomidine n = 8								Saline n = 8								Control n = 2	
Study ID	D1	D2	D3	D4	D5	D6	D7	D8	P1	P2	P3	P4	P5	P6	P7	P8	C1	C2
Tubular injury	+++	+	0	++	+	+	++	++	+	0	+	0	0	0	0	0	0	0
Interstitial inflammation	+++	+	0	+	+	+	++	+	+	0	+	+	0	0	0	+	0	0
Tubular casts	0	+	0	++	+	+	++	+	0	0	+	+	0	0	+	0	0	0

Zero (0), no histological renal tubular injury, inflammation or tubular casts; +, mild histological renal tubular injury, inflammation or tubular casts; ++, moderate histological renal tubular injury, inflammation or tubular casts; +++, severe histological renal tubular injury, inflammation or tubular casts.

and placebo groups (Fig. 4, online Supporting Information Figures S2–S5). Cardiopulmonary bypass produced a significant decrease in cerebral SO_2 on NIRS (dexmedetomidine: mean (95%CI) 63.9 (60.6–67.2) to 44.0 (41.3–47.1)%, $p < 0.001$; placebo: mean (95%CI) 63.5 (57.5–69.6) to 42.8 (38.9–46.8)%, $p = 0.005$) but did not change tissue perfusion or PO_2 (Fig. 5). However, like the kidney, no between-group differences in cerebral cortical tissue perfusion, oxygenation or oxygen saturation were observed in the brain (Fig. 5). Histological analysis of the cerebral lobes showed no cerebral micro-infarcts, microthrombi or other pathological changes in either the dexmedetomidine or placebo groups (online Supporting Information Figure S6).

The plasma concentration of interleukin-6 was significantly increased at the CPB 3 and CPB 4 time-points in the dexmedetomidine and placebo groups compared with the postinduction time-point (Fig. 6). However, there was no significant difference between the experimental groups. Furthermore, there were no significant differences in plasma concentration of tumour necrosis factor-α or interleukin-10, either between the postinduction state and CPB 1–4 in both groups or between the dexmedetomidine and placebo groups at any time-point (Fig. 6).

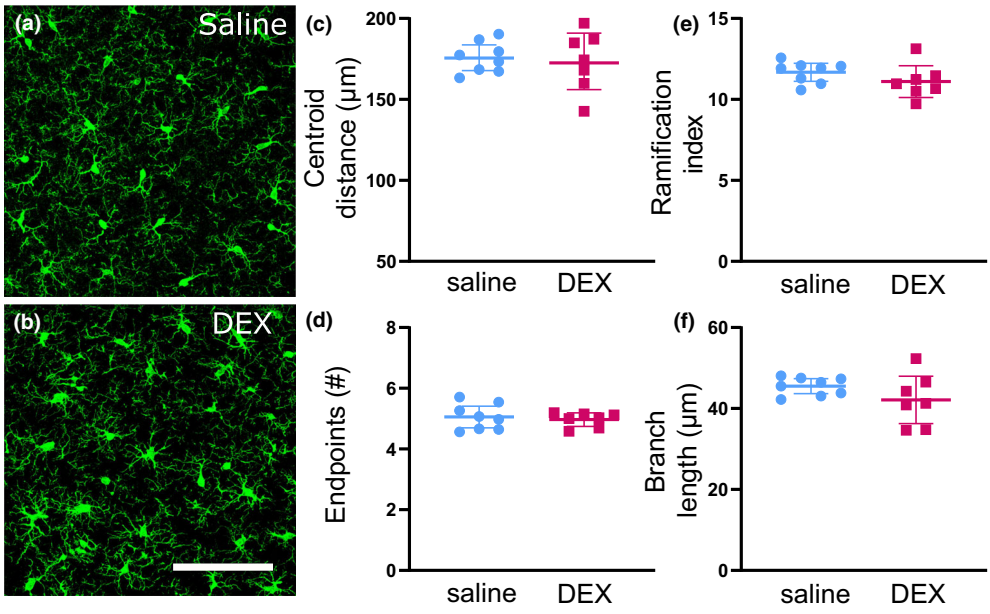

Figure 4 Microglial morphometry in the frontal cortical lobe. Representative photomicrographs of IBA1+ microglia in the frontal cortex of sheep treated with saline (a) and dexmedetomidine (b). (c) frontal lobe microglia centroid distance (µm); (d) number of endpoints (#); (e) ramification index; (f) average branch length (µm). Blue, sheep that received placebo; red, sheep that received dexmedetomidine (DEX). Data are presented as mean and 95%CI. P = 0.758 for centroid distance, 0.219 for ramification index, 0.645 for number of endpoints, and 0.176 for average branch length. P values were derived using a Student's unpaired t-test. Each symbol represents one animal. Scale bar = 100 µm.

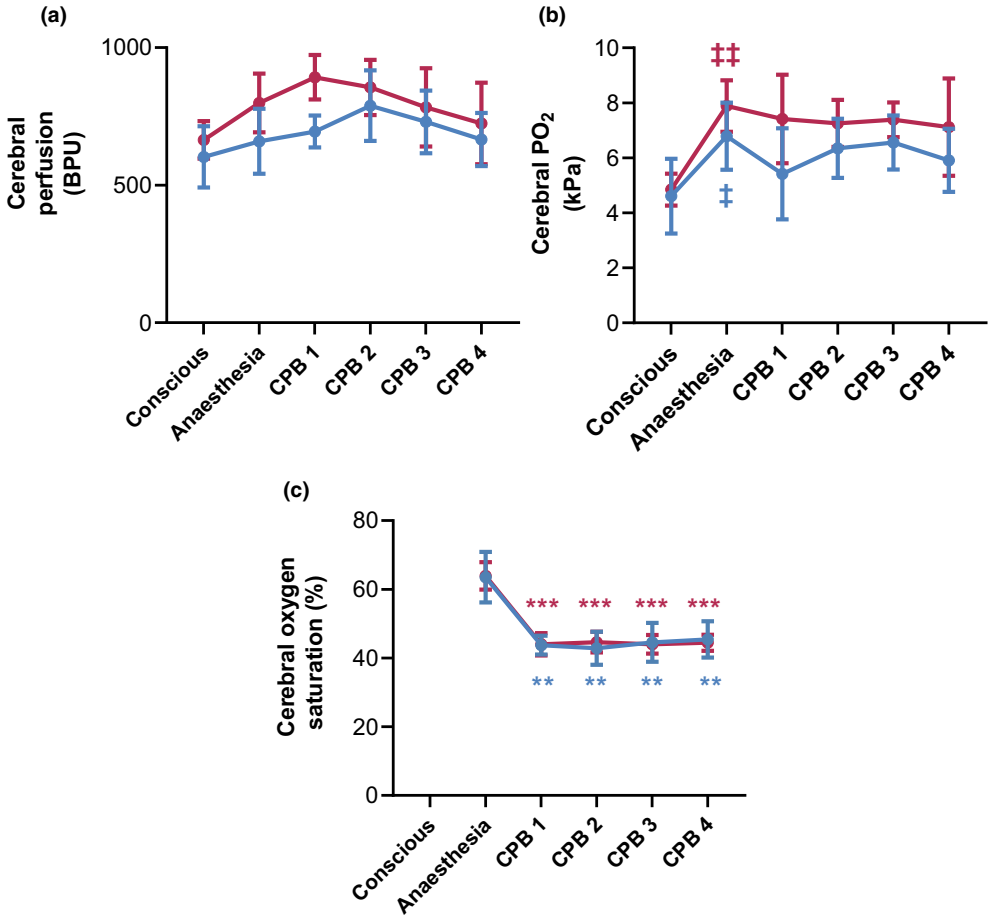

Figure 5 (a) Cerebral cortical tissue perfusion; (b) cerebral cortical tissue oxygenation; and (c) and cerebral oxygen saturation. Red, sheep that received dexmedetomidine; blue, sheep that received placebo. Data are presented as mean and 95%CI. N = 8 for cerebral perfusion and oxygen saturation for both groups and for cerebral PO_2 for sheep that received saline. Due to dysfunction of fibreoptic probe, n = 6 for cerebral PO_2 for sheep that received dexmedetomidine. P_{group} = 0.060 for cerebral perfusion, 0.057 for cerebral PO_2, 0.925 for cerebral oxygen saturation. P values and statistical analysis are as for Figure 2. $^{‡}p < 0.05$, $^{‡‡}p < 0.01$ for comparison between pre-induction and postinduction time-points (Dunnett's test). $^{**}p < 0.01$, $^{***}p < 0.001$ (Dunnett's test) for comparison for comparison between postinduction and cardiopulmonary bypass (CPB) 1–4 time-points. BPU, blood perfusion units; PO_2, partial pressure of oxygen; CPB, cardiopulmonary bypass.

Discussion

We performed a prospective, randomised controlled trial in healthy female sheep to determine if dexmedetomidine, relative to placebo, altered renal and cerebral inflammation and microcirculatory perfusion and oxygenation during CPB. While CPB was associated with a significant decrease in renal vascular conductance, blood flow and oxygen delivery which led to renal medullary hypoxia consistent with previous studies [20, 21], dexmedetomidine did not ameliorate this pathophysiology. Additionally, and unexpectedly, we found that sheep that received dexmedetomidine had greater overall incidence and severity of histological renal tubular injury relative to animals that received placebo. Furthermore, while we have previously shown that our experimental model of CPB induces neuroinflammation [14], no between-group differences in microglial activation were seen in the frontal, parietal, temporal or occipital cortices. While CPB induced cerebral oxygen desaturation (but not cerebral tissue hypoxia), again, no between-group differences were observed. These results suggest that any purported neurological benefit of dexmedetomidine is not mediated by acute reductions in neuroinflammation or intra-operative cerebral malperfusion, hypoxia or desaturation, despite these phenomena being implicated in the pathogenesis of postoperative delirium and cognitive dysfunction [22].

The mechanisms of the reported benefits of dexmedetomidine treatment during the postoperative

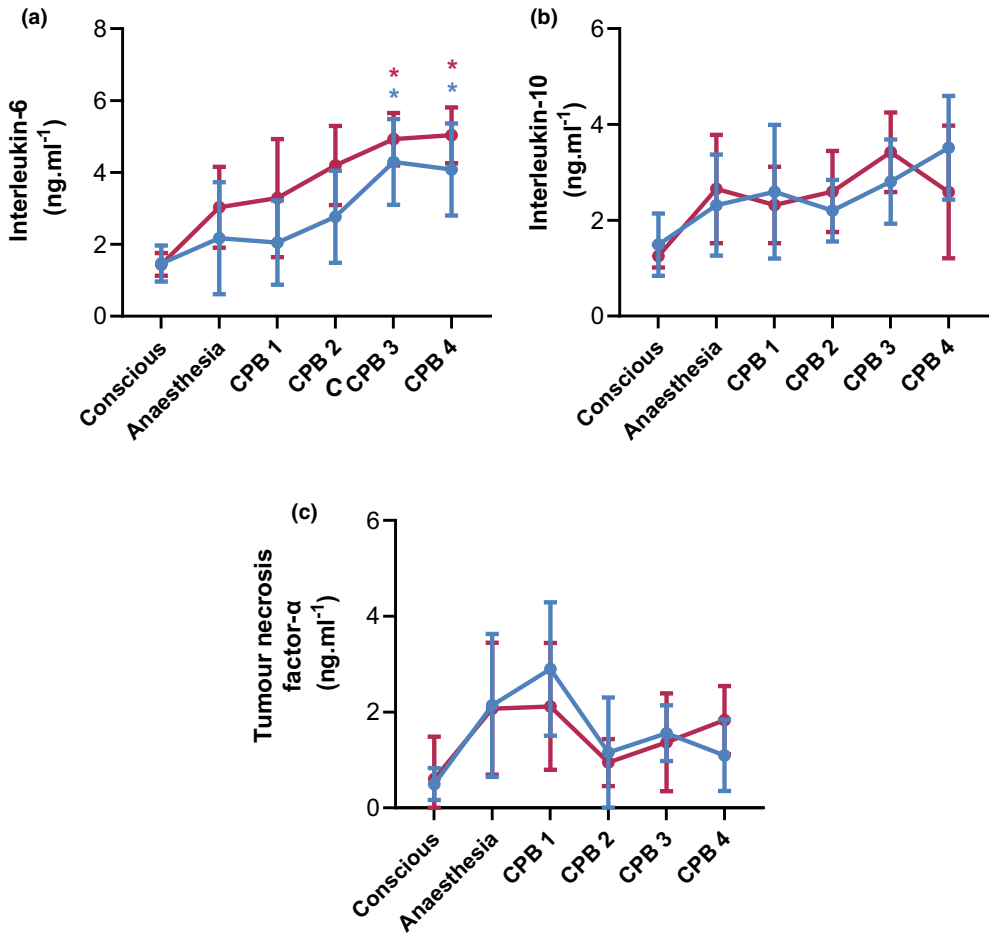

Figure 6 Plasma concentrations of pro- and anti-inflammatory cytokines including (a) interleukin-6; (b) interleukin-10; and (c) tumour necrosis factor-α. Red, sheep that received dexmedetomidine; blue, sheep that received placebo. Data are presented as mean and 95%CI. N = 8 for all the three cytokines for post-induction and cardiopulmonary bypass (CPB) 1–4 time-points for both groups. Plasma levels of the three cytokines in three sheep in each group during the pre-induction period were below measurable concentrations. Thus, n = 5 for all the three cytokines for the pre-induction period for both groups, n = 4 for interleukin-10 for the pre-induction period for saline group. P_{group} = 0.055 for interleukin-6, 0.943 for interleukin-10, and 0.818 for tumour necrosis factor-α. P values and statistical analysis are as for Figure 2. *p ≤ 0.05 (Dunnett's test) for comparison between postinduction and CPB 3 and 4 time-points.

period therefore require further investigation. The cause of the dissociation between cerebral oxygen saturation (measured by NIRS) and cerebral tissue PO_2 (measured by fluorescence lifetime oximetry) in our results is uncertain. It should be noted that NIRS values reflect the weighted average of cerebral oxygen saturation in the cerebral vasculature rather than the tissue per se, and venous haemoglobin saturation accounts for 75–80% of NIRS values [23]. Furthermore, the accuracy of NIRS can be influenced by multiple factors including signals originating from blood circulating in scalp, skull and meninges [24] and haemodilution [25]. In contrast, cerebral tissue PO_2 by fluorescence lifetime oximetry is measured via fibreoptic probes implanted directly [26]. Accordingly, we believe direct measurement of cerebral tissue PO_2 is a more accurate representation of changes in brain tissue oxygen levels than NIRS.

It has been suggested that dexmedetomidine has reno- and neuroprotective effects in patients undergoing cardiac surgery. Cardiopulmonary bypass in sheep induces a marked systemic inflammatory response, which is associated with widespread neuroinflammatory changes across multiple cortical regions [14, 22]. These are largely driven by increased expression of tumour necrosis factor-α, interleukin-1β and interleukin-6 [27]. Upregulation of these cytokines encourages infiltration of neutrophils and platelets in the kidney [28], and microglial activation in the brain after disruption of the blood–brain barrier [29]. There

are multiple purported mechanisms by which dexmedetomidine could reduce kidney and brain injury. First, dexmedetomidine could exert an anti-inflammatory effect, acting centrally on the dorsal motor nucleus of the vagus to enhance efferent activity and the cholinergic anti-inflammatory pathway [30, 31]. Second, dexmedetomidine has been shown to reduce the expression of lipopolysaccharide inflammatory mediators within activated microglia in vitro in a dose-dependent fashion [32]. Third, the centrally acting sympatholytic effects of dexmedetomidine may reduce catecholamine-mediated vasoconstriction in vulnerable organ beds to improve microcirculatory tissue perfusion and oxygen delivery [11]. Finally, dexmedetomidine may exert a direct, neuroprotective effect [12].

These observations spurred a series of studies that were incorporated into systematic reviews. In a meta-analysis incorporating 16 studies and 2148 patients, Liu et al. reported that intra-operative and postoperative dexmedetomidine infusion decreased the incidence of delirium (OR 0.49, 95%CI 0.34–0.72) and AKI (OR 0.46, 95% CI 0.36–0.61) following cardiac surgery [7]. However, these results were not replicated in subsequent large, multicentre randomised controlled trials. In such a trial (stopped early for futility), Turan et al. found a trend for increased incidence of delirium (OR 1.49, 95%CI 0.99–2.43) and AKI (OR 1.40, 95%CI 0.84–2.34) with dexmedetomidine [8]. However, this apparent effect was not statistically significant. Nevertheless, the incorporation of this and other larger randomised controlled trials into a meta-analysis resulted in a loss of the signal for improved renal function (OR 0.85, 95%CI 0.6–1.22), but preservation of the signal for reduced delirium (OR 0.58, 95% CI 0.43–0.71) [33]. In part due to this conflict, recent best practice guidelines state that the overall quality of evidence for dexmedetomidine to reduce AKI is poor, and the evidence that the drug prevents delirium is of variable quality [2, 10].

Our data may partially explain the findings of Wang et al., which showed an increased risk of postoperative AKI in patients undergoing cardiac surgery [9]; but the harm signal in this study was statistically fragile and seemed to be driven by stage 1 AKI, limiting clinical significance. In our study, we found no differences in renal macro- or microcirculatory perfusion or oxygenation that could explain the increased incidence and severity of histological renal tubular injury. Moreover, there were no differences in intra-operative vasopressor requirement or creatinine clearance, suggesting that while histopathological changes were present, AKI was not induced according to conventional criteria [34]. The pattern of histological renal tubular changes observed in ovine CPB was starkly different from that observed in ovine models of septic AKI in which there were no signs of renal tubular injury [35, 36]. Given the lack of a convincing mechanism by which dexmedetomidine could have caused the observed changes (i.e. worse global or regional renal perfusion or oxygenation), we cannot exclude type 1 error. However, while this signal of harm for dexmedetomidine is questionable, our findings also imply that dexmedetomidine is unlikely to protect against renal injury after CPB.

A second important finding was the apparent lack of between-group differences in microglial activation (i.e. neuroinflammation). Despite our previous work showing a neuroinflammatory response in our experimental model [14], we saw no change in microglial morphology in the dexmedetomidine group relative to placebo. This finding does not support the proposition that dexmedetomidine reduces delirium and postoperative cognitive dysfunction after cardiac surgery via a reduction in neuroinflammation. While studies in murine models have been supportive of this concept [37], direct immunohistochemical evidence of microglial activation following cardiac surgery is understandably lacking in humans. To our knowledge, this is the first trial to examine this question in a clinically relevant large animal model of CPB and to examine microglial morphometric activation directly rather than relying on increased plasma levels of neuroinflammatory biomarkers. While our findings cannot show whether dexmedetomidine changes the incidence of delirium or postoperative cognitive dysfunction after cardiac surgery, these data suggest that if such an effect exists, it is not mediated by an acute reduction in neuroinflammation (at least in sheep) intra-operatively. Future studies are required to examine if CPB-associated neuroinflammation persists, is exacerbated, or ameliorated by dexmedetomidine in the postoperative period.

Our study has several strengths. It was performed in a large animal model that closely approximates CPB in humans [14]. The target perfusion pressure, pump flows, extracorporeal circuit and temperature and anticoagulation strategies were consistent with current recommendations [18, 38]. The rate of dexmedetomidine infusion chosen was used in multiple clinical trials [39]. Animals were managed during the experiment by cardiac surgeons, anaesthetists and clinical perfusionists to ensure consistency with human practice. Microglial activation was assessed by a semi-automated computer script (3DMorph) that evaluates the 3D morphology of microglia and analysed by two histopathologists blinded to experimental interventions. This process eliminates biases attributed to manual scoring, morphological assessment and counting used in previous studies [19, 40]. Finally, while the clinicians and scientists who cared for animals during experiments were not blinded

to the group allocation, the presence of histological renal tubular injury and microglial activation were determined by independent assessors who were blinded to the group allocation.

We acknowledge some limitations. The animals used in this experiment were relatively young and healthy, in contrast to the older, multimorbid population commonly encountered in adult cardiac surgery. Additionally, we only used female animals in our experiments as urethral catheterisation of a male sheep is impossible. Furthermore, the availability of uncastrated male sheep is limited, and handling the larger and more aggressive rams carries substantial occupational health and safety concerns. Our model uses a lateral thoracotomy incision to access the mediastinum; this is because the dorsal recumbency required for median sternotomy compromises systemic and renal haemodynamics in sheep [20]. Our approach is analogous to the stimulus of minimally invasive cardiac surgery, but the inflammatory response to this approach is equivalent to that seen following sternotomy in patients having surgery on CPB [41]. Finally, the sheep in our experiment were euthanised immediately after the final experimental period ended, therefore our findings are only relevant to models of care that commence dexmedetomidine intra-operatively before commencing CPB and cannot be extrapolated to patients who commence the drug postoperatively.

In summary, we have evaluated the effects of a commonly used dosing regimen of dexmedetomidine on kidney and brain microcirculatory perfusion and oxygenation, and histopathology in a clinically relevant, large animal model of CPB. We showed that dexmedetomidine does not improve renal or intrarenal perfusion or oxygenation compared with placebo. Thus, the theories that underpinned the possible reno-protective effects of the drug are not supported by our results. Additionally, histological renal tubular injury occurred more frequently in the dexmedetomidine group, and the overall severity of this injury when present was greater. No plausible explanation for these results was found. While we cannot state conclusively that intra-operative dexmedetomidine causes harm, the drug should not be considered reno-protective in this setting until a plausible mechanism has been identified. Additionally, we have shown no difference in microglial activation between the two groups, implying that if dexmedetomidine does act to reduce postoperative neurocognitive dysfunction, it does not do so by mitigating neuroinflammation. Further work elucidating the purported neuroprotective mechanisms of dexmedetomidine in human studies is similarly required.

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Supporting Information

Additional supporting information may be found online via the journal website.

Figure S1. Representative histological images of renal tissue showing acute tubular necrosis, renal interstitial inflammation, and tubular casts.

Figure S2. Analysis of microglial morphology.

Figure S3. Microglial morphometry in the parietal lobe.

Figure S4. Microglial morphometry in the temporal lobe.

Figure S5. Microglial morphometry in the occipital lobe.

Figure S6. Cerebral cortex histopathology.

Table S1. Plasma creatinine and renal oxygenation and function.

Table S2. Systemic variables.

Table S3. Arterial blood oximetry and chemistry.

Table S4. Venous blood oximetry and chemistry.