Dexmedetomidine Combined with Therapeutic Hypothermia is associated with Cardiovascular Instability and Neurotoxicity in a Piglet Model of Perinatal Asphyxia

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Dexmedetomidine: adverse effects with cooling

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Abstract

The selective α2-adrenoreceptor agonist, dexmedetomidine has shown neuroprotective, analgesic, anti-inflammatory and sympatholytic properties that may be beneficial in neonatal encephalopathy (NE). As therapeutic hypothermia is only partially effective, adjunct therapies are needed to optimize outcomes. The aim was to assess whether hypothermia+dexmedetomidine augments neuroprotection compared to routine treatment (hypothermia+fentanyl sedation) in a piglet model of NE using magnetic resonance spectroscopy (MRS) biomarkers, which predict outcome in babies with NE, and immunohistochemistry. After hypoxia-ischemia (HI), 20 Large White male piglets were randomized to: (i) hypothermia+fentanyl with cooling to 33.5°C from 2-26h; or (ii) hypothermia+dexmedetomidine (loading dose 2μg/kg at 10 minutes followed by 0.028μg/kg/h for 48h). Whole-brain phosphorus-31 and regional proton MRS were acquired at baseline, 24 and 48 h after HI. At 48h, cell death (TUNEL) was evaluated over 7 brain regions. Dexmedetomidine plasma levels were mainly within the target sedative range of 1 μg/L. In the hypothermia+dexmedetomidine group there were 6 cardiac arrests (3 were fatal) versus 2 (none fatal) in the hypothermia+fentanyl group. The hypothermia+dexmedetomidine group required more saline (p=0.005) to maintain blood pressure. Thalamic and white matter Lactate/N Acetyl aspartate did not differ between groups (p=0.66 and 0.21 respectively); whole brain nucleotide tri-phosphate (NTP)/epp was similar (p=0.73) over 48h. Cell death (TUNEL positive cells/mm^2) was higher in the hypothermia+dexmedetomidine compared to the hypothermia+fentanyl group (mean counts 5.1 versus 2.3, difference 2.8 (95% C.I. 0.6 to 4.9), p=0.036). Hypothermia+dexmedetomidine was associated with adverse cardiovascular events even within the recommended clinical sedative plasma level; these may have been exacerbated by an interaction with either isoflurane or low body temperature. Hypothermia+dexmedetomidine was neurotoxic following HI in our piglet NE model, suggesting caution is vital if dexmedetomidine is combined with cooling following NE.

Introduction

Intrapartum-related neonatal encephalopathy (NE) leads to a considerable global public health burden resulting in 50 million disability life adjusted years in babies who survive; in 2010 this related to 2.4% of the Global Burden of Disease and 6.1 million years of life with disability [1,2]. Therapeutic hypothermia reduced the combined rate of mortality and severe disability in moderate to severe NE with number needed to treat to avoid death and/or an adverse neurodevelopmental outcome of 6-7 in developed countries [3]. However despite cooling, around 50% of infants have adverse outcomes [4]. Effective and safe adjunct therapies are needed to augment hypothermic neuroprotection.

Dexmedetomidine is a highly selective α2-adrenoceptor agonist that confers sedative, anti-inflammatory, analgesic, sympatholytic and organ-protective properties [5]. Pre-clinical [6] and clinical studies [7] show the importance of sedation during therapeutic hypothermia and recent data have shown the critical importance of the sensitizing effect of perinatal inflammation and infection in both high and low-income socio-economic groups [8-10]. There are concerns with the routine long-term use of opioids in neonates [11]. A sedative that enhances macrophage phagocytosis and bacterial clearance, minimizing inflammation-induced brain injury with no neurotoxicity would be particularly helpful in these patients.

Dexmedetomidine has extensive experimental support for its neuroprotective effects by both α2 and non-α2 adrenoceptor mediated mechanisms. Dexmedetomidine has shown neuroprotection in neonatal models of hypoxic-ischemic brain injury [12] and anesthetic brain injury in rodents [13]. Dexmedetomidine has a dose dependent anti-inflammatory effect on plasma cytokine release following sepsis [14,15] and neuroprotective properties in sepsis-induced brain injury [16].

Dexmedetomidine has been used as a sedative in critically ill children and neonates [17]. A dose dependent hemodynamic response has been seen with hypotension, hypertension and bradycardia particularly with increasing plasma concentrations [18-20]. Dexmedetomidine effects may be opposing and depend on central and peripheral actions. Close monitoring of circulatory dynamics with dexmedetomidine has been recommended especially with therapeutic hypothermia where there is potential for altered pharmacokinetics (PK) and pharmacodynamics (PD) [21]. In our previous study, we investigated dexmedetomidine PK during hypothermia and

rewarming to determine the safe and effective dose for use in a study of neuroprotection after hypoxia-ischemia. We found reduced dexmedetomidine clearance due to cumulative effects of hypothermia and hypoxia-ischemia (HI). Indeed, dexmedetomidine clearance was reduced almost ten fold compared to adult values; PK analysis estimated a loading dose of 2 μg/kg dexmedetomidine followed by 0.028 μg/kg/h would achieve the sedative target plasma concentration of 0.5–0.6 μg/l with hypothermia after HI [22].

We hypothesized that hypothermia+dexmedetomidine would lead to enhanced brain protection than hypothermia+fentanyl after global HI. Our aim was to assess whether an optimized dose of dexmedetomidine started 10 minutes after HI augments routine hypothermic neuroprotection in a piglet perinatal asphyxia model. This model also has strong similarities to newborn infants with NE in terms of the timing of the evolution of injury after HI [23,24], pattern of injury, neuropathology and cerebral magnetic resonance spectroscopy (MRS) [25]. The effectiveness of dexmedetomidine protection was assessed using: (i) Cerebral MRS biomarkers, proton (^{1}H) MRS lactate/ N acetyl aspartate (NAA) [25] and phosphorus-31 (^{31}P) MRS for phosphocreatine/inorganic phosphate (PCr/Pi) and nucleotide triphosphate (NTP)/ exchangeable phosphate pool (epp) [23]; and (ii) Histological assessment of cell death using TUNEL at 48h after hypoxia ischemia.

Material and Methods

Sample size calculation

Our primary outcomes were cerebral lactate/NAA and NTP/epp. Previous work with our model suggested that the change in lactate/NAA during 48h varied between normo- and hypothermic groups by 1.0 U, with a standard deviation of 0.65 U (log scale). Assuming a similar magnitude of additional effects for hypothermia+dexmedetomidine following HI versus hypothermia+fentanyl and similar variability at 48 h and with 5% significance and 80% power, at least eight subjects were required in each group based on a two-sample t-test sample size calculation.

Animal experiments and surgical preparation

All animal experiments were approved by the ethics committee of UCL and performed under UK Home Office Guidelines [Animals (Scientific procedures) Act, 1986]. The study complies with the ARRIVE guidelines. Twenty newborn male piglets with a birth weight of 1.6-2.1kg <48 h of age were anaesthetized and surgically prepared as described previously [24]. The study timeline is shown in **Figure 1.**

Following clinical assessment, piglets were sedated with intramuscular midazolam (0.2 mg/kg). Whilst monitoring arterial oxygen saturation (SpO_2), anesthesia was induced by inhalation of isoflurane (4% v/v) through a facemask to facilitate tracheostomy and intubation. Throughout the surgery, isoflurane was maintained at 2.8–3% guided by peripheral oxygen saturation monitoring (Nonin Medical, Plymouth, MN, USA) and the animal's response to stimulation. Following tracheostomy, a suitable size of endotracheal tube (Smiths Medical, Ashford, Kent, UK) was fixed and the piglet was mechanically ventilated (SLE 2000 infant Ventilator, Surrey, UK). Ventilator settings were adjusted to maintain partial pressure of oxygen (PaO_2) at 8–13 kPa and carbon dioxide ($PaCO_2$) at 4.5–6.5 kPa, allowing for temperature and fraction of inspired oxygen (FiO_2) correction of the arterial blood sample. After the airway was secured, both common carotid arteries were surgically isolated at the level of the fourth cervical vertebra and a vascular occluder (OC2A, In Vivo Metric, Healdsburg, CA, USA) was placed on each side. After completion of surgery, inspired isoflurane concentration was maintained at 2% v/v.

An umbilical venous catheter was inserted for infusion of maintenance fluids (10% dextrose, 60 ml/kg/day before the insult and 40 ml/ kg/day after resuscitation), fentanyl (5 µg/kg/h in the cooling group, and antibiotics (benzyl penicillin 50mg/kg,

every 12 h and gentamicin 4mg/kg, once a day). An umbilical arterial catheter was inserted for invasive physiologic monitoring (SA instruments) for heart rate and arterial blood pressure, and blood sampling for arterial gases and electrolytes (Abbot Laboratories, UK). Hepsal (0.5 IU/ml of heparin in 0.9% saline solution) was infused at 0.3 ml/h to prevent umbilical arterial catheter blockage.

Piglets were cared for under intensive care conditions throughout the experiment. To maintain the MABP above 40mmHg, bolus infusions of 0.9% saline (Baxter; 10 ml/kg), dopamine (5–20 μg/kg/min), dobutamine (5–20 μg/kg/min) and adrenaline (0.1–1.5 μg/kg/min) were used as required by a NICU trained clinician. High serum lactate was treated by optimizing oxygenation and 0.9% saline bolus infusions. Hyperkalemia (K>7.0 mmol/l) was treated with 4 μg/kg salbutamol (10 μg/ml) over 10 minutes.

MR methods

The head was immobilized in a stereotactic frame for MRS acquisition. Piglets were positioned within the bore of 9.4 Tesla Agilent MR scanners. ^{1}H and ^{31}P MRS data were acquired at baseline and at 24 and 48h after cerebral HI.

^{31}P MRS

A 7 cm x 5 cm elliptical transmit-receive MRS surface coil tuned to the ^{31}P resonant frequency was positioned on top of the head. ^{31}P MRS was acquired with 1-minute resolution using a non-localized single-pulse acquisition. MRS data were analyzed using the Advanced Method for Accurate, Robust and Efficient Spectral fitting of MRS data with use of prior knowledge (AMARES)[26] as implemented in the jMRUI software. Prior knowledge of NTP multiplet structure was used. NTP is predominately ATP and the latter contributes approximately 70% of the NTP signal [27]. Thus NTP changes during this experiment predominately reflected ATP changes. Pi was fitted using 4 separate components and PCr with a single component. The following peak-area ratios were calculated: Pi/epp, PCr/epp, and NTP/epp where epp = exchangeable phosphate pool= Pi + PCr + 2γ-NTP + β-NTP.

^{1}HMRS

^{1}H MRS data were collected from voxels located in the dorsal right subcortical white matter at the centrum semiovale level (white matter voxel, 8 Å~ 8 Å~ 15mm) and in the deep gray matter centered on both lateral thalami (deep gray matter voxel, 15 Å~ 15 Å~ 10mm) using a combination of a 65 Å~55mmelliptical receive surface coil, a

150mm diameter transmit volume coil and a LASER acquisition (TR = 5000 ms, TE = 288 ms, 128 averages). Spectra were analyzed using AMARES as implemented in the jMRUI software and the lactate/NAA peak area ratio was calculated.

Cerebral hypoxia ischemia

^{31}P MRS data were collected at baseline, during HI and for one hour after cessation of HI. HI was induced inside the MR scanner by remotely inflating the vascular occluders around both common-carotid arteries, and simultaneously reducing FiO2 to 6% (vol/vol). During HI the β-NTP peak height was continuously monitored using in-house Matlab (Mathworks) software. At the point at which β-NTP had fallen to 50% of its baseline value, FiO_2 was increased to 9%. When β-NTP fell to 40% baseline height the inspired oxygen fraction was titrated to keep the β-NTP peak height between 30% and 40% of its original height for a period of 12.5 min. At the end of HI the carotid arteries were de-occluded and the FiO_2 returned to 21%.

Experimental groups

Following transient HI and after resuscitation, piglets were randomized by computer generated randomization to: (i) hypothermia+fentanyl throughout the study n=10 or (ii) hypothermia+dexmedetomidine n=10. A loading dose of 2 μg/kg dexmedetomidine was started at 10 minutes after resuscitation and infused over 20 minutes followed by a maintenance dose at 0.028 μg/kg/h for the next 48 h. Fentanyl was stopped upon starting maintenance dexmedetomidine. Blood for dexmedetomidine PK assay was sampled at 10, 20, 40 and 60 minutes and thereafter at 2, 4, 6, 12, 24, 36 and 48h after HI. Both groups were cared for over 48h after HI and maintained hypothermic (core temperature 33.5°C) between 2–26h.

Amplitude integrated EEG (aEEG)

After surgical preparation, multichannel six-lead EEG monitoring (Nicolet Care Fusion, Wisconsin, USA) was recorded at baseline and throughout the periods between MRS data acquisitions. Filtered amplitude integrated EEG (aEEG) recordings were classified according to the voltage criteria [28]. Grade 1 was severely abnormal voltage with lower and upper margin of the bandwidth <5μV and <10 μV respectively, grade 2 lower margin and upper margin $\leq$5 μV and >10 μV respectively and grade 3 lower and upper margin >5 μV and >10 μV respectively.

Brain histology

At 48h after HI, piglets were euthanized with pentobarbital, the brain was fixed by

cardiac perfusion with cold 4% paraformaldehyde, disected out and post-fixed at 4 °C in 2% paraformaldehyde for 7 days. Coronal slices (5 mm thick) of the right hemisphere, starting from anterior to the optic chiasma, were embedded in paraffin, sectioned to 8-µm thickness and stained with hematoxylin and eosin to validate the bregma for analysis. For each animal and brain region, TUNEL-positive nuclei were counted at two levels, from 7 regions (3 fields per region) by an investigator blind to the treatment group and the average counts per field of view converted into counts per mm^2 **(Figure 2)**.

To assess cell death, brain sections were stained for nuclear DNA fragmentation using histochemistry with transferase mediated biotinylated d-UTP nick end-labeling (TUNEL) as previously described [29]. Briefly, TUNEL sections were pretreated in 3% hydrogen peroxide, subjected to a protease K pre-digestion (Promega, Southampton, UK) and incubated with TUNEL solution (Roche, Burgess Hill, UK). TUNEL was visualized using avidin-biotinylated horseradish complex (ABC, Vector Laboratories, Peterborough, UK) and diaminobenzidine/ H2O2 (DAB, Sigma, Poole, UK) enhanced with CoSO4 and NiCl2. TUNEL sections were dehydrated and cover-slipped with DPX (VWR, Leighton Buzzard, UK).

Statistical Methods

Physiological variables

The mean level of physiological measurements for the two treatment groups were compared by firstly taking the average of the results taken at 15 minute intervals in the following periods; baseline, T=0, 2-3.5h, 3.75-26h and 26.25-48h for each subject. Analysis of variance was then performed on the least significant (LS) means fitting terms Treatment, Period and a Treatment x Period interaction. The means for each period and differences in means for the two treatments were presented with confidence intervals in **Table 2**.

MRS

All analyses were performed using the SAS JMP® v11.0.0 software. A statistical model was fitted to the ratios NTP/epp, PCr/Pi and Lac/NAA. An analysis of variance (ANOVA) model was fitted and the differences in the means on the log scale for the two treatment groups (hypothermia+dexmedetomidine versus hypothermia+fentanyl) were estimated from the model at each of the three time points with 95% confidence interval (CI) for the differences. The differences in treatment group means are shown graphically using 95% Least Significant Difference error bars.

aEEG

Following the baseline scoring, scores were assigned at 3, 6, 12, 24 and 36h after HI and group averages compared.

TUNEL

An analysis of variance model was fitted to the mean counts to give an estimate of the expected counts per mm^2. The overall difference between the means for the two treatment groups, and treatment differences across regions are presented with 95% CI and graphically using 95% Least Significant Difference error bars.

Pharmacokinetics

A one compartment linear disposition model was used to fit earlier data to the PK model [22]. Population parameter estimates were obtained using non-linear mixed effects modelling (NONMEM VII, Icon Development Solutions, Elliot City, MD, USA) [30]. This model accounts for population parameter variability (between subjects) and residual variability (random effects) as well as parameter differences predicted by covariates (fixed effects). A visual predictive check (VPC) [31], a modeling tool that estimates the concentration prediction intervals and graphically superimposes these intervals on observed concentrations after a standardized dose, was used to ascertain if current observed concentrations were predicted by parameter estimates from that earlier study [22,32]. Concentration prediction intervals from the earlier study were graphically superimposed on those intervals determined from observed concentrations in the current study. Simulation is performed with parameter estimates from the earlier study using 1000 subjects with characteristics taken from new patients. For data such as these where covariates such as dose, weight and height are different for each patient, a prediction corrected visual predictive check [33] is used; observations and simulations are multiplied by the population baseline value divided by the individual-estimated baseline.

Results

There were 10 animals in each group.

Baseline Physiological Data and Insult severity

There were no intergroup differences in postnatal age, bodyweight, duration of hypoxia-ischemia and insult severity measured by acute energy depletion (AED) on

^{31}P MRS **(Table 1).** The baseline blood gas CO_2 was lower (p=0.033, but still within the normal range) and blood lactate higher (p=0.002) in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group **(Table 2).** Baseline mean arterial blood pressure (MAP), heart rate, oxygen saturation, arterial blood gas oxygen, base excess, glucose, blood pH and core body temperature were similar between groups **(Table 2).** The baseline oxygen was higher in the dexmedetomidine and cooling group but similar for other time epochs.

Just after resuscitation, the hypothermia+fentanyl group had a lower oxygen saturation compared to the hypothermia+dexmedetomidine group (p=0.044); in the time periods 2-3.5h and 26-48h, the blood gas oxygen partial pressure was higher in the hypothermia+fentanyl group compared to the hypothermia+dexmedetomidine group (p=0.032 and 0.029 respectively). In the period 2-3.5h after HI, the temperature was lower in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group (p=0.027). There was weak evidence for a lower MAP in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group between 26-48h (p=0.071).

The fluid and inotrope requirement comparison between groups is shown in **Table 3 and 4)**. Comparing hypothermia+dexmedetomidine to hypothermia+fentanyl groups, 5/10 versus 1/10 subjects required noradrenaline, 4/10 versus 2/10 subjects required adrenaline for MBP support, 7/10 versus 3/10 subjects required adrenaline for resuscitation, 6/10 versus 2/10 subjects had a cardiac arrest, 3/10 versus 0/10 subjects had a fatal cardiac arrest. More saline was required for blood pressure support in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group (p=0.005).

MRS analysis

The least squares means plots and 95% Least Significant Difference (LSD) bars for NTP/epp, PCr/Pi and Lac/NAA (on log 10 scale) in thalamus and white matter are shown in **Figure 3.** The differences in the means and CI at 48h are shown in **Table 5.** There was no difference at 48h between groups for any of the MRS peak area ratios. There was weak evidence that the hypothermia+dexmedetomidine group brain NTP/epp was lower than hypothermia+fentanyl (p=0.05) at 24h, but at 48h this difference was not observed.

EEG

There was no difference between the group mean aEEG scores at any time point for the hypothermia+fentanyl compared to the hypothermia+dexmedetomidine groups (**Figure 4**).

Hypothermia+ dexmedetomidine increased TUNEL positive cell counts

Representative photomicrographs of TUNEL staining in the putamen and periventricular are shown in **Figure 5A.** A paired t-test comparing the overall TUNEL count means (across all fields and subjects) of the two treatment groups for each of the 7 regions and overall was performed **(Table 6).** There was evidence of a difference between treatment groups (p=0.036) (**Figure 5B**) with hypothermia+dexmedetomidine with more TUNEL positive cells/mm^2 than hypothermia+fentanyl. The overall mean count for the hypothermia+dexmedetomidine group was 5.1 compared with a mean count of 2.3 for the hypothermia+fentanyl; a mean difference of 2.8 (95% CI 0.6-4.9). The regional brain counts for the two groups are shown in **Figure 5C**.

Dexmedetomidine PK

The mean plasma concentrations of dexmedetomidine following a loading dose of 2 µg/kg over 20 minutes followed by an infusion of 0.028 µg/kg/h were below 1 µg/L, despite the model predicting slightly higher concentrations (**Figure 6**). **Figure 6A** shows prediction corrected observed concentrations. **Figure 6B** shows prediction corrected percentiles (10%, 50%, 90%) for observations (red lines with symbols) and predictions (lines) with 95% confidence intervals for prediction percentiles (grey shaded areas). The median predictions and observations graphically lie almost on top of each other. The dexmedetomidine plasma concentrations (observations) were as predicted and mainly within the clinical sedative range of <1 µg/L.

Discussion

Compared to routine hypothermia+ fentanyl sedation after HI, we did not observe any improvement in cerebral protection based on magnetic resonance biomarkers lactate/NAA and NTP/epp with hypothermia+dexmedetomidine. There was increased cell death across the combined 7 brain regions with hypothermia+ dexmedetomidine compared to hypothermia+fentanyl. All piglets were anesthetized with isoflurane, which may have exacerbated the cardiovascular effects. The hypothermia+dexmedetomidine group required more saline for MBP support than the hypothermia+fentanyl group and there were three fatal cardiac arrests in the hypothermia+dexmedetomidine versus none in the hypothermia+fentanyl group. These effects occurred despite dexmedetomidine plasma levels within target sedative range of 1 μg/L.

In preparation for this study, we had previously studied the PK of dexmedetomidine in the piglet [22]; compared with adult values, clearance was reduced almost tenfold in the newborn piglet following hypoxic ischemic brain injury and subsequent therapeutic hypothermia. This reduced clearance was related to cumulative effects of both hypothermia and exposure to hypoxia. We observed that high plasma concentrations of dexmedetomidine were associated with major cardiovascular complications and were able to estimate from a one compartment model, that a bolus of 2 μg/kg dexmedetomidine over 20 min followed by an infusion of 0.028 μg/kg/h dexmedetomidine during therapeutic hypothermia would likely achieve a plasma concentration of 0.5–0.6 μg/L (this is the concentration associated with safe sedation in human newborns [34]). In the current study, despite achieving plasma concentrations mainly < 1 μg/L with the calculated infusion rate, we saw adverse cardiovascular responses; there was no relation between the plasma concentration and adverse cardiovascular responses.

The baseline physiological data showed a lower CO_2 partial pressure and higher lactate concentration in the blood of the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group, however all other variables were similar at baseline and these differences were not apparent at later time points in the study. The severity of the hypoxic ischemic insult was similar between groups. Mean partial pressure oxygen (PaO_2) was lower in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group at 2-3.5h and 26-48h; this may reflect the cardiovascular and hemodynamic instability observed in the

hypothermia+dexmedetomidine group.

Many of the properties of dexmedetomidine, including sedation are transduced via α2-adrenoceptor signaling. The α2 receptors are located in blood vessels, sympathetic terminals and the brain, where their activation causes vasoconstriction, anxiolysis, sedation and analgesia. Dexmedetomidine is 1600 times more specific to α_2 than α_1 receptors, permitting its use for sedation without cardiovascular side effects of α_1 receptor activation [19]. Dexmedetomidine stands out from other sedatives including opioids for its minimal respiratory depression, anti shivering effect and prevention of opioid induced muscle rigidity [35].. The hemodynamic changes and requirement for MBP support we saw in our study can be explained by the properties of dexmedetomidine: in the central nervous system dexmedetomidine produces sympatholysis and a reduction in blood pressure, in the peripheral nervous system it causes vasoconstriction leading to an increase in blood pressure.

A recent phase II/III multicenter, safety and pharmacokinetic study of dexmedetomidine in term and preterm infants ($\geq$28-$\leq$44 weeks gestation) (predominantly those intubated following surgery) suggested that dexmedetomidine is effective for sedating preterm and full-term neonates and is well-tolerated without major adverse events [36]. There were three adverse events observed (hypo and hypertension and agitation). The PK profile of dexmedetomidine was different in neonates compared with older children with a longer half-life and larger area under the curve drug concentration over time; this confirms our previous findings in the piglet indicating that lower doses are required to achieve the same levels of sedation and avoid adverse effects with newborns. In other clinical studies, bradycardia, hypotension and hypertension have been observed in children to varying degrees depending on the plasma concentration of dexmedetomidine [34,37,38]. In our study the combination of immaturity, HI, hypothermia and the concomitant use of isoflurane are likely to have increased the risk of adverse effects from dexmedetomidine despite plasma levels mainly less than 1 µg/L. However, further work is needed before our results are extrapolated to humans as some recent studies suggest beneficial effects with dexmedetomidine: in 102 patients receiving dexmedetomidine during the first postoperative day after cardiac surgery compared to an age- and procedure-matched cohort not receiving dexmedetomidine, the use of a dexmedetomidine infusion was associated with a decreased incidence of acute kidney injury but no change in clinical outcomes [39]. In a retrospective cohort study of infants undergoing surgical intervention for congenital heart defects requiring

cardiopulmonary bypass, the use of dexmedetomidine in the operating room at the time of sternal closure was associated with reduced need for mechanical ventilation in the immediate postoperative period. No increased adverse outcomes were reported in these infants, despite exposure to deep hypothermic circulatory arrest and intraoperative fentanyl [40].

There are counteracting and complex interactions between α_2- agonist and α_2-adrenoreceptor subtypes which may be affected by hypothermia. The α_2-adrenergic receptors present in the brainstem decrease blood pressure; activation of presynaptic α_{2A}- adrenoreceptors decreases sympathetic outflow and norepinephrine release causing hypotension and bradycardia [41-43]. Postsynaptic α_{2B}-adrenoreceptors on vascular smooth muscle mediate vasoconstriction and are responsible for hypertension. Presynaptic α_{2C}- adrenoreceptors are also inhibitory autoreceptors with a prominent role in peripheral autonomic system [44]; these receptors are sensitive to temperature and become active at temperatures below 37°C [45]. All piglets in our study were cooled from 2-26h after HI and the adverse cardiovascular effects (cardiac arrest) may be due to the combination of hypothermia+dexmedetomidine. We did not assess the effect of dexmedetomidine without hypothermia, as cooling is routine therapy for moderate to severe NE. Cooling has the potential to change the expression of α_{2C}- receptors; silent intracellular α_{2C}- receptors become active and translocate to the cell surface causing vasoconstriction following exposure to temperatures below 37°C [45]. Therapeutic hypothermia-related bradycardia has been reported in young children following dexmedetomidine infusion [35,46]. Furthermore, it is known that dexmedetomidine interferes with thermoregulation and promotes hypothermia itself [47,48]. This may explain our observation of a significantly lower body temperature between 2-3.5h in the hypothermia+dexmedetomdine group, although this was not below the target hypothermia therapeutic range. We have previously shown that the depth of hypothermia is critical for its efficacy in this model [49].

It has been shown that anesthesia influences the hemodynamic effects of dexmedetomidine. In anesthetized patients with depressed sympathetic tone, dexmedetomidine increased MAP by peripheral vasoconstriction whereas in awake patients with intact tone, it decreased MAP via centrally mediated effect of α_2 agonists [50]. Rapid dexmedetomidine injection in a group of young children during isoflurane anesthesia for cardiac catheterization transiently increased systolic and diastolic blood pressure [51]. All piglets in our study were anesthetized with

isoflurane throughout the experiments. Inhaled isoflurane dose has been shown to reduce cardiac output and systemic vascular resistance [52]. The negative inotropic and peripheral vasodilatation effects of isoflurane are likely to augment the effects of dexmedetomidine on cardiac output and may have added to the adverse cardiovascular responses in our piglets receiving dexmedetomidine. Isoflurane anesthetized dogs and cats developed greater negative cardiovascular effects with dexmedetomidine as an adjunct compared to isoflurane only anesthesia [53,54] Dexmedetomidine reduces cardiac output and the subsequent redistribution of blood flow limits hepatic and other non-vital organ perfusion [55]; this in turn reduces hepatic blood flow and hydroxylation and clearance [56,57]. In our piglets, it is likely that the interaction between dexmedetomidine and isoflurane anesthesia (negative inotropic effect and vasodilation) are likely to have increased the depression of the sympathetic activity [58] by the variable binding to peripheral and central α_2 adrenergic receptors [59].

There are conflicting reports of neuroprotection and neurotoxicity with dexmedetomidine and clonidine (an α_2 adrenoceptor agonist approximately eight-times less selective towards the α_2 adrenoceptor than dexmedetomidine). The effect of acute (25 μg/kg) or prolonged (25 μg/kg three times daily, for 2d or 4d) exposure of dexmedetomidine with therapeutic hypothermia on uninjured neonatal Lewis rats was studied. Prolonged dexmedetomidine treatment in neonatal rats was not associated with abnormal brain histology (no increased gliosis, macrophage activation, or apoptosis in either hypothermic or control rats). Although no adverse effects were seen, these were not injured animals, nor did they have fentanyl and isoflurane exposure [60]. Other rodent studies have shown that dexmedetomidine protects the brain against focal [61,62] and global [63,64] HI by inducing anti-apoptotic effects [65,66]} or via α_2 adrenergic and imidazole 1 receptors [12]. In the developing mouse brain there was dose dependent neuroprotection with dexmedetomidine and clonidine [67]. Further studies however have shown that dexmedetomidine and clonidine induce cerebral hypoperfusion and neurotoxicity in small animal stroke models [68]. Dean et al showed a complex dose dependent response to clonidine with low dose but not high dose being neuroprotective after hypoxic brain injury in preterm fetal sheep [69]. We observed the combination of hypothermia+dexmedetomidine to be neurotoxic with increased TUNEL positive cells (5.1 versus 2.3/mm^2 mean counts across the brain) compared to hypothermia+fentanyl. One mechanism leading to this in our model could be a reduction in cerebral blood flow by vasoconstriction in pial arteries and veins [70].

Studies in rats and dogs demonstrated dexmedetomidine dose dependently caused vasoconstriction in pial arteries mediated either by direct action on α_2-adrenoreceptors [71] on the small cerebral arteries distal to circle of Willis [71]. Other studies have shown that systemic dexmedetomidine indirectly induces cerebral vasoconstriction by reducing cerebral oxygen consumption [70].

The reasons for these conflicting reports may be related to differences in species, models, maturity of the brain, dose and timing of the drugs and the integrity of the autonomic system. Different expressions of α- adrenoreceptor subtypes in cerebral arteries in human, monkeys and dogs can lead to different responses to noradrenaline, α_2- agonists and antagonists...An age dependent change in expression and binding capacity of α_2- receptors in postnatal life occurs in human and rat brain and this may affect the sensitivity of the newborn to dexmedetomidine [72] .

Our study suggests that caution is needed when considering the use of dexmedetomidine with hypothermia in the newborn after HI. We saw no improvement in cerebral energetics on MRS with hypothermia+dexmedetomidine versus hypothermia+fentanyl. There was evidence of neurotoxocity with an increased number of TUNEL positive cells across the brain with hypothermia+dexmedetomidine compared to hypothermia+fentanyl. We saw some expected hemodynamic effects of dexmedetomidine; adverse effects and cardiac arrests may relate to interactions between reduced clearance in an immature animal, concomitant anesthesia exacerbating the cardiovascular effects of dexmedetomidine and the effects of hypothermia on the temperature sensitive α_{2C}- receptors. Reduced cerebral perfusion induced by dexmedetomidine is likely to have contributed to the neurotoxicity.

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Table 1. Baseline group data

Population variables	**Hypothermia + Fentanyl Mean (SD)**	**Hypothermia + Dexmedetomidine Mean (SD)**	**P value**
Postnatal age (h)	32.8 (10.4)	33.0 (10.0)	0.51
Body weight (g)	1978 (109)	1900 (190)	0.247
Duration of HI (min)	23.6 (3.04)	25.4 (5.56)	0.4
Insult severity ($x10^{-2}$) measured from acute energy depletion (AED)*	9.63 (3.2)	10.6 (3.3)	0.53

*The insult severity AED was estimated by calculating the time integral of the change in NTP/epp during HI and the first 60 min of resuscitation

Table 2. Means and Differences in Physiological Variables pooled according to Time Period after HI

	Period	LS Mean	SEM	LS Mean	SEM	Difference LS Mean	SED	Lower CL	Upper CL	p-Value
		Control		Dexmedatomidine		Control *cf* Dex				
MAP	Baseline	51.9	2.74	50.3	2.74	**1.66**	3.88	-6.04	9.37	0.669
	T=0	47.6	2.74	42.8	2.74	**4.80**	3.88	-2.91	12.51	0.219
	2 - 3.5h	43.0	2.74	43.9	2.74	**-0.91**	3.88	-8.62	6.79	0.814
	3.5 - 26h	46.4	2.74	43.0	2.74	**3.39**	3.88	-4.32	11.10	0.384
	26 - 48h	53.4	2.74	46.1	2.89	**7.28**	3.99	-0.64	15.20	0.071
HR	Baseline	176.6	7.43	171.1	7.43	**5.52**	10.51	-15.35	26.40	0.600
	T=0	185.3	7.43	172.1	7.43	**13.20**	10.51	-7.67	34.07	0.212
	2 - 3.5h	158.1	7.43	148.0	7.43	**10.09**	10.51	-10.79	30.96	0.340
	3.5 - 26h	152.4	7.43	162.6	7.43	**-10.14**	10.51	-31.01	10.74	0.337
	26 - 48h	175.9	7.43	179.8	7.83	**-3.84**	10.79	-25.29	17.60	0.723
O_2 Sat	Baseline	98.0	1.67	98.3	1.67	**-0.25**	2.36	-4.94	4.44	0.916
%	T=0	83.7	1.67	88.7	1.76	**-4.97**	2.42	-9.78	-0.15	**0.044**
	2 - 3.5h	99.0	1.67	98.8	1.67	**0.20**	2.36	-4.49	4.89	0.933
	35 - 26h	98.4	1.67	98.4	1.67	**-0.01**	2.36	-4.70	4.68	0.998
	26 - 48h	98.7	1.67	98.1	1.87	**0.59**	2.50	-4.39	5.56	0.815
PaCO2	Baseline	6.5	0.51	5.0	0.47	**1.52**	0.70	0.12	2.91	**0.033**
	T=0	5.4	0.40	5.2	0.44	**0.23**	0.60	-0.96	1.41	0.707
	2 - 3.5h	4.2	0.44	3.9	0.40	**0.29**	0.60	-0.89	1.48	0.624

	3.5 - 26h	5.5	0.40	4.9	0.40	**0.58**	0.56	-0.54	1.70	0.304
	26 - 48h	6.5	0.40	6.1	0.42	**0.39**	0.58	-0.75	1.54	0.496
PaO2	Baseline	13.3	2.96	11.1	2.74	**2.17**	4.04	-5.87	10.21	0.592
	T=0	8.9	2.30	8.6	2.57	**0.36**	3.44	-6.50	7.21	0.918
	2 - 3.5h	21.0	2.57	13.5	2.30	**7.51**	3.44	0.65	14.36	**0.032**
	3.5 - 26h	15.9	2.30	13.6	2.30	**2.27**	3.25	-4.19	8.74	0.486
	26 - 48h	25.2	2.30	17.8	2.42	**7.41**	3.34	0.77	14.05	**0.029**
BE	Baseline	2.5	1.73	0.8	1.60	**1.71**	2.36	-2.98	6.41	0.469
	T=0	-5.1	1.34	-4.5	1.50	**-0.60**	2.01	-4.60	3.40	0.766
	2 - 3.5h	5.5	1.50	3.9	1.34	**1.60**	2.01	-2.40	5.60	0.428
	3.5 - 26h	1.4	1.34	-1.0	1.34	**2.38**	1.89	-1.39	6.16	0.212
	26 - 48h	1.3	1.34	-1.3	1.41	**2.61**	1.95	-1.27	6.48	0.185
Lac	Baseline	2.8	0.82	6.3	0.76	**-3.50**	1.12	-5.72	-1.28	**0.002**
	T=0	8.8	0.63	8.8	0.71	**0.06**	0.95	-1.84	1.95	0.954
	2 - 3.5h	4.6	0.71	4.9	0.63	**-0.35**	0.95	-2.25	1.54	0.713
	3.5 - 26h	3.4	0.63	4.7	0.63	**-1.24**	0.90	-3.03	0.55	0.171
	26 - 48h	2.6	0.63	3.2	0.67	**-0.66**	0.92	-2.49	1.18	0.478
Glu	Baseline	6.9	2.22	8.1	2.02	**-1.23**	3.00	-7.21	4.75	0.682
	T=0	10.4	1.65	9.8	1.75	**0.64**	2.41	-4.16	5.43	0.792
	2 - 3.5h	9.3	1.65	9.6	1.57	**-0.28**	2.28	-4.81	4.26	0.903
	3.5 - 26h	12.7	1.57	16.3	1.57	**-3.62**	2.22	-8.04	0.79	0.107
	26 - 48h	12.2	1.57	14.5	1.65	**-2.30**	2.28	-6.84	2.24	0.316
pH	Baseline	7.4	0.04	7.4	0.04	**-0.07**	0.06	-0.19	0.05	0.232
	T=0	7.3	0.03	7.3	0.04	**-0.02**	0.05	-0.12	0.08	0.679

	2 - 3.5h	7.6	0.04	7.6	0.03	**-0.01**	0.05	-0.11	0.09	0.836
	3.5 - 26h	7.4	0.03	7.4	0.03	**0.00**	0.05	-0.10	0.09	0.930
	26 - 48h	7.3	0.03	7.3	0.04	**0.01**	0.05	-0.09	0.11	0.849
TºC	Baseline	38.3	0.22	38.2	0.22	**0.10**	0.31	-0.52	0.71	0.753
	T=0	37.8	0.22	37.9	0.22	**-0.13**	0.31	-0.74	0.48	0.654
	2 - 3.5h	36.0	0.22	35.4	0.22	**0.69**	0.31	0.08	1.31	**0.027**
	3.5 - 26h	33.6	0.22	33.5	0.22	**0.13**	0.31	-0.48	0.75	0.667
	26 - 48h	37.3	0.22	37.2	0.22	**0.09**	0.32	-0.54	0.72	0.787

MAP: mean arterial blood pressure mm Hg; HR: heart rate bpm; O2 saturation: oxygen saturation, PaO2: partial pressure of oxygen kPa; PaCO2: partial pressure of CO_2 kPa; BE: base excess mmol/L; Lac: lactate; Glu: glucose mmol/L; pH: blood pH; T ºC: core temperature.

Significant p values are in bold.

Table 3. Saline, inotrope (dopamine, dobutamine, noradrenaline and adrenaline) requirements and number of subjects requiring adrenaline for resuscitation, and number of cardiac arrests (fatal and non-fatal)

Treatment/ pig	Saline ml/kg/h	Dopamine ug/ml/min	Dobutamine ug/ml/min	*Nor-adrenaline ng/ ml/ min	*Adrenaline ng/ml/ min	*Adrenaline for resus	*Cardiac arrests	*Fatal Cardiac arrests
HT+fentanyl 294	0.947	16.1	10.55	84.56	131	1	1	0
HT+fentanyl 301	0.64	38.5	0	0	0	1	0	0
HT+fentanyl 308	0.689	20.1	2.32	0	0	0	0	0
HT+fentanyl 312	0.625	28.1	2.43	0	0	1	1	0
HT+fentanyl 314	1.041	12.27	0	0	0	0	0	0
HT+fentanyl 316	0.416	2.13	0	0	0	0	0	0
HT+fentanyl 317	0.74	2.39	3.02	0	0	0	0	0
HT+fentanyl 327	0.208	6.77	0	0	0	0	0	0
HT+fentanyl 330	0.208	23.04	0	0	0	0	0	0
HT+fentanyl 337	0.631	21.4	7.36	0	386.3	0	0	0
HT+dexmedetomidine 299	0.869	0	0	0	0	0	0	0
HT+dexmedetomidine 302	1.03	17.39	0	0	0	2	1	0
HT+dexmedetomidine 303	0.52	20.83	3.99	17.2	0	1	0	0
HT+dexmedetomidine 304	1.15	17.7	4.82	79.3	0	0	0	0
HT+dexmedetomidine 322	1.29	22.9	0	100	0	0	0	0
HT+dexmedetomidine 323	1.7	5.9	0	15.15	159.5	1	1	0
HT+dexmedetomidine 325	0.94	16.7	4.87	0	241	1	1	1

HT+dexmedetomidine 328	0.947	18.07	0	0	213	0	0	0
HT+dexmedetomidine 313	2.22	23.6	7.36	172	181	1	1	1
HT+dexmedetomidine 331	1.09	14.93	0	0	0	1	2	1
				***1/10 vs 5/10**	***2/10 vs 4/10**	***3/10 vs 7/10**	***2/10 vs 6/10**	***0/10 vs 3/10**
				p = 0.07	**p = 0.31**	**p = 0.089**	**p = 0.085**	**p = 0.105**

HT: hypothermia.

* Comparison of proportions of subjects with the complication or receiving the therapy (not appropriate to average over so many zeros)

Table 4. Least square mean and confidence limits for the total dose throughout the study of saline, dopamine and dobutamine treatment in the hypothermia+dexmedetomidine versus hypothermia+fentanyl groups

	Control		**Dex**				**Confidence limits**		
	LS mean	SEM	LS mean	SEM	Control - Dex	SED	lower	Upper	p value
Saline ml/ kg/ h	0.61	0.12	1.18	0.12	-0.56	0.17	-0.93	-0.20	**0.005**
Dopamine µg/ml/min	17.1	3.1	15.8	3.1	1.3	4.4	-7.9	10.4	0.773
Dobutamine µg/ml/min	2.6	1.0	2.1	1.0	0.5	1.5	-2.6	3.5	0.755

Table 5. Summary of differences between hypothermia+fentanyl and hypothermia+dexmedetomidine MRS data at 48h.

	Difference in Means (log 10) hypothermia+fentanyl versus hypothermia+ dexmedetomidine	95% C.I. for Difference (log scale)	Ratio of Means (original scale)	95% C.I. for Ratio (original scale)	P value
NTP/epp	-0.024	-0.16 to 0.12	0.95	0.69 to 1.3	0.73
PCr/Pi	0.067	-0.19 to 0.32	1.17	0.65 to 2.1	0.60
Thalamus Lac/NAA	0.101	-0.37 to 0.57	1.26	0.43 to 3.7	0.66
White Matter Lac/NAA	0.345	-0.2 to 0.89	2.21	0.63 to 7.71	0.21

The hypothermia+dexmedetomidine group showed no evidence of a difference compared to hypothermia +fentanyl. epp = exchangeable phosphate pool; PCr= phosphocreatine; Pi= inorganic phosphate; Lac= lactate; NAA= N acetyl aspartate

Table 6. Summary of Differences between hypothermia+fentanyl and hypothermia+dexmedetomidine TUNEL counts for seven brain regions and overall.

Area	Difference in Mean TUNEL Count (hypothermia+fentanyl versus hypothermia+ dexmedetomidine)	Standard Error of the difference	Lower 95% C.I. for Difference	Upper 95% C.I. for Difference	p value
Sensorimotor cortex	3.33	3.46	-3.52	10.19	0.337
Cingulate cortex	-1.04	3.46	-7.90	5.82	0.764
Thalamus	1.05	3.46	-5.81	7.91	0.762
Caudate	4.23	3.46	-2.63	11.08	0.225
Putamen	6.20	3.46	-0.66	13.06	0.076
Internal Capsule	2.69	3.60	-4.43	9.81	0.456
Periventricular white matter	3.11	3.46	-3.75	9.96	0.371
OVERALL	2.79	1.32	0.19	5.40	**0.036**

Figure Legends

Figure 1

Study time-line. Following baseline data acquisition, piglets underwent cerebral hypoxia-ischaemia. At the end of hypoxia-ischemia (time 0), piglets were randomized to (i) hypothermia (33.5oC for 24h from 2-26h)+fentanyl or (ii) hypothermia+dexmedetomidine. For those randomized to hypothermia+fentanyl, the fentanyl infusion was continued. For those randomized to hypothermia+dexmedetomidine, fentanyl was stopped and a loading dose of 2μg/kg dexmedetomidine was started at 10 mins after resuscitation and infused over 20 mins followed by a maintenance dose at 0.028 μg/kg/h for the next 48 h. The time points for the dexmedetomidine PK blood concentrations are shown. Blood gases were done every 4-6h and more frequently if unstable.

Figure 2

Representative piglet brain photomicrographs at the 2 brain levels indicating brain regions assessed for TUNEL immunohistochemistry. (1) cingulate cortex, (2) sensorimotor cortex, (3) periventricular white matter. (4) internal capsule, (5) caudate nucleus, (6),putamen (7) thalamus. Three fields per region were assessed on 2 brain levels. Red fields indicate those brain regions included in the MRS voxel; black fields indicate those brain regions not included in the MRS voxel.

Figure 3

Magnetic resonance spectroscopy peak area ratios of the brain at baseline, 24 and 48h after HI. Least square mean plots with 95% least significant difference (LSD) bars for the NTP/epp and PCr/Pi in whole forebrain and Lac/NAA in thalamus and white matter; non overlapping bars show evidence of a significant difference. There was no difference at 48h between groups for any of the MRS peak area ratios. At 24h, hypothermia+dexmedetomidine NTP/epp was lower than hypothermia+fentanyl ($p=0.05$), but at 48h this difference was not observed. HI: hypoxia ischaemia; NTP: nucleotide triphosphate; epp: exchangeable phosphate pool.

Figure 4

A. Amplitude-integrated electroencephalogram (aEEG). There was no difference between the hypothermia+dexmedetomidine compared to the hypothermia+fentanyl group mean aEEG scores at any timepoint after hypoxia ischemia. B. The classification criteria used to define the aEEG group.

Figure 5

TUNEL histology. **A.** Representative sections are shown at x20 magnification in the hypothermia+fentanyl (left column) and hypothermia+dexmedetomidine (right column) in the putamen and white matter. **B.** There was an overall increase in the TUNEL-positive cells per mm^2 (pooled across region and R0/R1 levels) in the hypothermia+dexmedetomidine compared to the hypothermia+fentanyl groups. C. On regional assessment, there was no significant difference between TUNEL positive cells across the 7 individual brain regions.

Figure 6A. A visual predictive check was plotted for the dexmedetomidine pharmacokinetic (PK) model. All plots show median and 90% intervals (solid and dashed lines). The prediction corrected observed concentrations are plotted. With a bolus of 2 µg/kg dexmedetomidine over 20 minutes followed by an infusion of 0.028 µg/kg/h the concentration in most but not all subjects was below1µg/L.

Figure 6B. Prediction corrected percentiles (10%, 50%, 90%) are shown for observations (lines with symbols) and predictions (lines) with 95% confidence intervals for prediction percentiles (gray shaded areas).

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