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-ischaemia (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 48 h, cell death (TUNEL) was evaluated over 7 brain regions. Between 2-3.5h there was a lower heart rate ($p<0.03$) followed by an increased heart rate ($p<0.001$) from 3.5-26h, a lower mean blood pressure 3.5-48h ($p<0.001$), a higher lactate from 3.5-26h ($p<0.05$) and more cardiac arrests ($p<0.05$). Hemodynamic effects occurred despite dexmedetomidine plasma levels within the target sedative range of 1 μg/L. Thalamic and white matter Lactate/N Acetyl aspartate did not differ between groups ($p=0.66$ and 0.21 respectively); whole brain NTP/epp was similar ($p=0.73$) over 48h. TUNEL positive cell death was higher in the hypothermia+dexmedetomidine compared to the hypothermia+fentanyl group (mean count 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 and was neurotoxic following HI in our piglet NE model. These pre-clinical safety and efficacy data suggest dexmedetomidine should not be used with cooling following NE.

Introduction

Intrapartum-related neonatal encephalopathy (NE) leads to a significant 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]. In developed countries therapeutic hypothermia reduces the combined rate of mortality and severe disability in moderate to severe HIE with number needed to treat of 6-7 [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 has 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 opiates 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-ischaemic brain injury [12, 13] and anesthetic brain injury in rodents. 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 [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-ischaemia. We found a significantly reduced dexmedetomidine clearance due to cumulative effects of hypothermia and hypoxia-ischaemia. 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 hypoxia-ischaemia [22].

We hypothesized that hypothermia+dexmedetomidine would lead to better brain protection than hypothermia+fentanyl after global hypoxia ischemia. Our aim was to assess whether an optimized dose of dexmedetomidine started 10 minutes after hypoxia ischemia 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 hypoxia ischemia [23, 24], pattern of injury, neuropathology and cerebral magnetic resonance spectroscopy (MRS) [25]. The efficacy 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 NTP/ exchangeable phosphate pool (epp) [23]; and (ii) Histological assessment of cell death using TUNEL at 48 h 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 48 h varied between normo- and hypothermic groups by 1.0 U, with a standard deviation of 0.65 U (log scale). Assuming similar magnitude of additional effect for hypothermia+dexmedetomidine following hypoxia ischemia versus hypothermia+fentamyl 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 (PaO2) at 8–13 kPa and carbon dioxide (PaCO2) at 4.5–6.5 kPa, allowing for temperature and fraction of inspired oxygen (FiO2) 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 rate of 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 40mm Hg, 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 min.

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 48 h after cerebral hypoxic ischemia.

^{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 hypoxiaischemia

^{31}P MRS data were collected at baseline, during hypoxia ischemia and for one hour after cessation of hypoxia ischemia. Hypoxia ischemia 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 hypoxia ischemia 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, FiO2 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 hypoxia ischemia the carotid arteries were de-occluded and the FiO_2 returned to 21%.

Experimental groups

Following transient hypoxia ischemia and after resuscitation, piglets were randomized 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 48 hours after HI. Both groups were cared for over 48 h after hypoxia ischemia and maintained hypothermic (core temperature 33.5 °C) between 2–26 h. Physiological parameters were compared between groups with Mann–Whitney at each time point.

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 48 h after hypoxia ischemia, piglets were euthanized with pentobarbital, the brain was fixed by cardiac perfusion with cold 4% paraformaldehyde, dissected 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-μmthickness and stained with hematoxylin and eosin to validate the bregma for analysis. For each animal, 2 sections (bregma 00 and −2.0) were stained and 7 different regions in the brain were examined **(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). For each animal and brain region, TUNEL-positive nuclei were counted at two levels and from 7 regions by an investigator blind to the treatment group and the average converted into counts per mm^2.

Statistical Methods

Physiological variables

Mean (SD) values of baseline variables and variables throughout the study at time 0 (end of insult), 2-3.5h after time 0, 3.5-26h after time 0 and 26-48h after time 0 were grouped and compared between groups using the Mann Whitney test.

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 intervals (CIs) 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 hypoxia ischaemia 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% C.I.s and graphically using 95% Least Significant Difference error bars.

Pharmacokinetics

A one compartment linear disposition model was used to fit data to the PK model. Population parameter estimates were obtained using non-linear mixed effects modelling (NONMEM VII, Globomax LLC, Hanover, 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 (fixedeffects). A visual predictive check (VPC) [31], a modelling tool that estimates the concentration prediction intervals and graphically superimposes these intervals on observed concentrations after a standardised dose, was used to evaluate how well the model predicted the distribution of observed dexmedetomidine concentrations. Simulation was performed using 1000 subjects with characteristics taken from 10 studied piglets; these simulations were generated by randomly using values from the estimated parameters and their variability. This is an advanced internal method of evaluation [32, 33] and is considered better than the commonly used plots of observed vs. predicted values [34]. For data such as these where covariates such as weight are different for each piglet, we used a prediction corrected VPC (PC-VPC) [35]. 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 bodyweight, postnatal age, baseline physiological (heart rate and mean arterial blood pressure) measures and the HI severity and duration between groups as shown in **Table 1.** The baseline oxygen was higher in the dexmedetomidine and cooling group but similar for other time epochs.

Heart rate and blood pressure throughout the study

The heart rate in the hypothermia+dexmedetomidine group was lower between 2-3.5h after the start of dexmedetomidine ($p=0.03$). Between 3.5 and 26 h, the heart rate was higher in the dexmedetomidine and cooling group ($p<0.001$). The mean blood pressure remained lower in the hypothermia+dexmedetomidine group from 3.5h to the end of the study ($p<0.001$). The rectal temperature was transiently lower in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group between 2-3.5h ($p=0.03$). Blood lactate was higher in the hypothermia+dexmedetomidine group 3.5-26h after time 0 ($p=0.02$).

The fluid and inotrope requirement comparison between groups is shown in **Table 2**. There were trends to increased geloplasma infusion volume ($p=0.06$), noradrenaline ($p=0.07$) and total adrenaline ($p=0.07$) requirements in the hypothermia+dexmedetomidine group compared to the hypothermia+fentanyl group. There were three fatal cardiac arrests in the hypothermia+ dexmedetomidine group (two at 36h and one at 45h after hypoxia ischemia) and none in the hypothermia plus fentanyl group.

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 3.** There was no difference at 48h between groups for any of the MRS peak area ratios. At 24h, the hypothermia+dexmedetomidine group brain NTP/epp was lower than hypothermia+fentanyl ($p=0.05$), 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 4).** There was evidence of a difference between treatment groups (p=0.036) (**Figure 5B**) with hypothermia+dexmedetomidine with more TUNEL positive cells 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 dexmedetomidine plasma levels (observations) were as predicted and mainly within the clinical sedative range of <1 µg/L.

Discussion

Compared to routine hypothermia+ fentanyl sedation after hypoxia-ischaemia, 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. There was evidence of the haemodynamic effects of dexmedetomidine with a lower heart rate between 2-3.5h followed by an increased heart rate, a lower mean blood pressure and a higher lactate from 3.5-26h. There were more fatal cardiac arrests in the hypothermia+dexmedetomidine group. The hemodynamic effects occurred despite dexmedetomidine plasma levels within target sedative range of 1 μg/L.

In preparation for this efficacy study, we previously studied the pharmacokinetics of dexmedetomidine in the piglet [36]; 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 levels 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 (concentration associated with sedation in human newborns). In the current study, despite achieving plasma levels 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.

Many of the properties of dexmedetomidine, including sedation are transduced via α2-adrenoceptor signaling. 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, 37]. Dexmedetomidine stands out from other sedatives including opioids for its minimal respiratory depression, anti shivering effect and prevention of opioid induced muscle rigidity [38]. The variable hemodynamic changes we saw in our study can be explained by the properties of dexmedetomidine in that there are opposing effects on the cardiovascular system: 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 (≥28-≤44 weeks gestation) suggested that dexmedetomidine is effective for sedating preterm and full-term neonates and is well-tolerated without significant adverse events [39]. 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 [40-42]. In our study the combination of immaturity, hypoxia ischemia and hypothermia are likely to have increased the risk of adverse effects from dexmedetomidine despite plasma levels mainly < 1 µg/L.

There are counteracting and complex interactions between α_2- agonist and α_2-adrenoreceptor subtypes which may be affected by hypothermia. Alpha2-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 [43-45]. 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 [46]; these receptors are sensitive to temperature and become active at temperatures below 37°C [47-49]. All piglets in our study were cooled from 2-26 hours after hypoxia ischemia and the adverse cardiovascular effects (bradycardia and hypotension) may be due to the combination of hypothermia+dexmedetomidine. 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 [47, 48]. Therapeutic hypothermia related bradycardia has been reported in young children following dexmedetomidine infusion [50] [51]. Furthermore, it is known that dexmedetomidine interferes with thermoregulation and promotes hypothermia itself [52, 53]. This may explain our

observation of a slightly lower body temperature between 2-3.5h in the hypothermia+dexmedetomdine group.

It has been shown that anesthesia influences the haemodynamic effects of dexmedetomidine. In anesthetized patients with depressed sympathetic tone, dexmedetomidine increased MBP by peripheral vasoconstriction whereas in awake patients with intact tone, it decreased MBP via centrally mediated effect of α_2 agonists [54]. Rapid dexmedetomidine injection in a group of young children during isoflurane anesthesia for cardiac catheterization transiently increased systolic and diastolic blood pressure [55]. All piglets in our study were anesthetised with isoflurane throughout the experiments. Inhaled isoflurane dose dependently reduces cardiac output and systemic vascular resistance [56]. The negative inotropic and peripheral vasodilatation effects of isoflurane are likely to augment the effects of dexmedetomidine on cardiac output. Isoflurane anesthetised dogs and cats developed greater negative cardiovascular effects with dexmedetomidine as an adjunct compared to isoflurane only anesthesia [57, 58]. Dexmedetomidine reduces cardiac output and the subsequent redistribution of blood flow limits hepatic and other non-vital organ perfusion [59]; this in turn reduces hepatic blood flow and hydroxylation and clearance [60, 61]. In our piglets, it is likely that the interaction between dexmedetomidine (hypotension and bradycardia) and isoflurane anaesthesia (negative inotropic effect and vasodilation) are likely to have increased the depression of the sympathetic activity [62] by the variable binding to peripheral and central α_2 adrenergic receptors. [63].

There are conflicting reports of neuroprotection and neurotoxicity with dexmedetomidine and clonidine. Previous pre-clinical rodent studies have shown that dexmedetomidine protects the brain against focal [64, 65] and global [66-68] hypoxia ischemia by inducing anti-apoptotic effects [69, 70] or via α_2 adrenergic and imidazole 1 receptors [13]. In the developing mouse brain there was dose dependent neuroprotection with dexmedetomidine and clonidine [71]. Other studies however have shown that dexmedetomidine and clonidine induce cerebral hypoperfusion and neurotoxicity in small animal stroke models [72, 73]. 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 [74]. We observed the combination of hypothermia+dexmedetomidine to be neurotoxic with increased TUNEL positive cells (5.1 versus 2.3 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 (CBF) by vasoconstriction in pial arteries and veins [75]. Studies in rats and dogs demonstrated dexmedetomidine dose dependently caused vasoconstriction in pial arteries mediated either by direct action on α_2-adrenoreceptors [76, 77] on the small cerebral arteries distal to circle of Willis [76]. Other studies have shown that systemic dexmedetomidine indirectly induces cerebral vasoconstriction by reducing cerebral oxygen consumption [75, 78, 79].

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 [80]. 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 . [81-83].

Our study suggests that caution is needed when considering the use of dexmedetomidine with hypothermia in the newborn after hypoxia ischemia. 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 such as hypotension and bradycardia; 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.

Acknowledgements

This project was funded by Action Medical Research and supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

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