INTRODUCTION

It has been demonstrated that certain common anesthetic agents, such as ketamine and isoflurane, cause widespread apoptosis and neurodegeneration in neonatal rat (Hayashi et al., 2002; Ikonomidou et al., 1999; Jevtovic-Todorovic et al., 2003; Ma et al., 2007; Sanders et al., 2009, 2010; Scallet et al., 2004) and monkey (Creeley et al., 2013; Brambrink et al., 2012, 2010; Slikker et al., 2007; Zou et al., 2009) brains. While ketamine and isoflurane have different pharmacologic actions in exerting their respective anesthetic effects (Brambrink et al., 2010; Haberny et al., 2002; Ma et al., 2007; Mellon et al., 2007), they both induce similar types of neuroapoptotic lesions and in similar locations in developing brains (Brambrink et al., 2010; Slikker et al., 2007; Zou et al., 2009). Dexmedetomidine (Precedex®, Hospira, Inc., Lake Forest, IL, USA) is an anesthetic/sedative that activates a2 adrenoceptors, a different mechanism of action than those of ketamine and isoflurane. It has recently been shown that dexmedetomidine alone did not induce neuroapoptosis in neonatal rat brains (Sanders et al., 2009, 2010) when compared to the control group. In addition, when co-administered with isoflurane, dexmedetomidine attenuated the severity of neuroapoptosis induced by isoflurane (Sanders et al., 2009, 2010).

Original Article

Neurotoxic effects of dexmedetomidine in fetal cynomolgus monkey brains

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ABSTRACT — The neuroprotective effects of dexmedetomidine have been reported by many investigators; however its underlying mechanism to reduce neuronal injury during a prolonged anesthesia remains unclear. In this study, we investigated the neurotoxic effects of dexmedetomidine in fetal monkey brains. In the present study, we compare the neurotoxic effects of dexmedetomidine and ketamine, a general anesthetic with a different mechanism of action, in fetal cynomolgus monkeys. Twenty pregnant monkeys at approximate gestation day 120 were divided into 4 groups: non-treatment controls (Group 1); ketamine at 20 mg/kg intramuscularly followed by a 12-hr infusion at 20-50 mg/kg/hr (Group 2); dexmedetomidine at 3 μg/kg intravenously (i.v.) over 10 min followed by a 12-hr infusion at the human equivalent dose (HED) of 3 μg/kg/hr (Group 3); and dexmedetomidine at 30 μg/kg i.v. over 10 min followed by a 12-hr infusion at 30 μg/kg/hr, 10 times HED (Group 4). Blood samples from both dams and fetuses were measured for concentration of dexmedetomidine. Each fetus was perfusion-fixed, serial sections were cut through the frontal cortex, and stained to detect for apoptosis (activated caspase 3 and TUNEL) and neurodegeneration (silver stain). In utero treatment with ketamine resulted in marked apoptosis and degeneration primarily in layers I and II of the frontal cortex. In contrast, fetal brains from animals treated with dexmedetomidine showed none to minimal neuroapoptotic or neurodegenerative lesions at both low- and high-dose treatments. Plasma levels confirmed systemic exposure of dexmedetomidine in both dams and fetuses. In conclusion, these results demonstrate that dexmedetomidine at both low-dose (HED) and high-dose (10 times HED) does not induce apoptosis in the frontal cortex (layers I, II, and III) of developing brain of cynomolgus monkeys.

Key words: Dexmedetomidine, Ketamine, Neuroapoptosis, Fetal brains, Cynomolgus monkeys

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While this demonstration of the lack of neuroapoptosis following dexmedetomidine exposure to neonatal rat brain is encouraging, similar findings in another species would further strengthen the support of the safety of dexmedetomidine on developing brains. Therefore, the objective of the current study was to determine whether administration of dexmedetomidine to pregnant cynomolgus monkeys would result in neuroapoptosis and cellular degeneration in fetal monkey brains compared to control brains utilizing the ketamine-induced neuroapoptosis monkey model described by Slikker et al. (2007). The study also included association of the histopathology findings with plasma levels of dexmedetomidine in both dams and fetuses.

**MATERIALS AND METHODS**

**Animal selection and study groups**

A sufficient number of female cynomolgus monkeys (*Macaca fascicularis*) from the Macine Pte Ltd. breeding facility (PT Mac Fauna, Bintan, Indonesia) were screened to obtain twenty pregnant animals at the approximate gestation day of 120 (± 7) days for this study. Pregnancy and day of pregnancy were established using ultrasound and expert veterinary opinion. The 20 pregnant monkeys were randomly assigned to the four study groups as indicated in Table 1.

All animal treatments and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Macine Pte Ltd. prior to start of the experimental phase.

**Test compounds and formulations**

Dexmedetomidine hydrochloride was supplied by Hospira, Inc. (Lake Forest, IL, USA) and ketamine hydrochloride was purchased from Troy Laboratories Pty. Ltd. (Glendenning NSW, Australia). Both drug products are clear, colorless solutions in sterile vials. Sterile physiological saline was used to dilute the test substances to the correct dose concentrations. Dexmedetomidine (100 μg/ml in 2 ml vials) and ketamine (100 mg/ml) were supplied ready-to-use. Dilutions were performed as per instructions in the package inserts.

**Study design and dosing procedures**

The study design and dosing regimen (Table 1) were similar to those described by Slikker et al. (2007) with minor modifications. Group 1 represented caged controls in which the animals were not administered vehicle or either of the two anesthetics. Group 2 animals were administered ketamine at 20 mg/kg intramuscularly (i.m.) followed by a 12-hr infusion at 20-50 mg/kg/hr. Animals in Group 3 received the low dose of dexmedetomidine (3 μg/kg intravenously [i.v.] over 10 min followed by a maintenance dose of 3 μg/kg/hr for 12 hr); this low dose represents the human equivalent dose (HED). Group 4 animals received the high dose of dexmedetomidine (30 μg/kg i.v. over 10 min followed by a maintenance dose of 30 μg/kg/hr for 12 hr [10 times HED]). Intravenous infusions of dexmedetomidine and ketamine were performed using an appropriately sized infusion catheter; extension set, and calibrated infusion system. The animals were dosed via the tail or saphenous vein. Throughout the anesthesia procedure, a water-circulated heating pad was used to keep the animals warm and to maintain body temperature. In addition, the dam’s heart rate and body temperature were monitored at regular intervals throughout the 12-hr infusion. The mean values for heart rate and body temperature immediately prior to the start of infusion (time 0 [T0]), 6-hr into the infusion period (T6), and at the end of the 11 to 12-hr infusion period (T11/12) are reported. Control animals were retained in their home cage for 12 hr with water, but without food on the day of procedures.

<table>
<thead>
<tr>
<th>Group (N = 5)</th>
<th>Treatment</th>
<th>Dosing Regimen</th>
<th>Withdrawal Period</th>
<th>C-section Time Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>At random based on study schedule</td>
</tr>
<tr>
<td>2</td>
<td>Ketamine</td>
<td>Loading dose of 20 mg/kg i.m. + 20-50 mg/kg/hr for 12 hr</td>
<td>6-hr post end-of-infusion</td>
<td>End of withdrawal period</td>
</tr>
<tr>
<td>3</td>
<td>Dex (Low Dose)</td>
<td>Loading dose of 3 μg/kg i.v. over 10 min followed by a maintenance dose of 3 μg/kg/hr for 12 hr</td>
<td>6-hr post end-of-infusion</td>
<td>End of withdrawal period</td>
</tr>
<tr>
<td>4</td>
<td>Dex (High Dose)</td>
<td>Loading dose of 30 μg/kg i.v. over 10 min followed by a 30 μg/kg/hr for 12 hr</td>
<td>6-hr post end-of-infusion</td>
<td>End of withdrawal period</td>
</tr>
</tbody>
</table>

Note: Dex = dexmedetomidine
Surgical preparation for cesarean section (C-section)

Six hours after the end of infusion, the dams were deeply anesthetized with ketamine (20 mg/kg i.m.) and placed on dorsal recumbency. A final surgical scrub and site preparation was performed using iodine and a sterile drape applied around the surgical site. The fetus was carefully removed from the uterus by C-section after which its attachment to the placenta through the umbilical cord was carefully clamped and cut. The entire duration of the C-section was normally within 1 hr to minimize the exposure of ketamine to the monkeys (especially the control and dexmedetomidine-treated animals).

Following completion of the C-section, the fetus was checked for viability. Its body weight and gender were also recorded. The fetal forelegs were attached to a dissection board and the pinch response (foot-pad squeeze) was done to determine the depth of anesthesia. With the heart still beating, perfusion of phosphate buffered saline (PBS) followed by 10% neutral buffered formalin (NBF) was performed via a small incision in the lateral wall of the left ventricle of the heart using a gravity perfusion kit. On commencement of the perfusion, a small incision was made in the right atrium to allow the fluid to flow through. The brain was immediately removed upon completion of perfusion and placed in a suitable container of 10% NBF.

Following the C-section, the muscle and skin layers of the dam were sutured using an absorbable suture material. Closure of the skin layer was performed using a subcuticular pattern to eliminate the need for suture removal. Carprofen (Rimadyl®, Pfizer Ltd., Sandwich, England) was given to the females during induction of anesthesia and twice daily for 48 hr post-operatively. In addition, amoxicillin (Betamox®, Norbrook Laboratories Ltd., Northamptonshire, England) was given pre-operatively and for seven days post-operatively. All dams were returned to the colony.

Histologic and immunohistochemical preparation

Perfusion-fixed fetal brains were prepared for histopathologic and immunohistochemical analysis by Comparative Biosciences (Sunnyvale, CA, USA). Brains were examined to characterize the effects of dexmedetomidine in comparison to untreated and ketamine-treated animals. In particular, detailed evaluation, characterization and comparison of the incidence, severity and distribution of induced apoptosis between the treatment groups was compared to control brains.

The orientation, sectioning and sampling of the frontal cortex was based upon the methodology set forth in Brambrink et al. (2010) and Slikker et al. (2007). Each brain was carefully oriented and gross-trimmed into each block to assure correlative symmetry between animals of the frontal cortex. Five to six consecutive blocks were prepared for each brain spanning the entire frontal cortex. (In addition, the remainder of the brain, including the midbrain, cerebellum, medulla and brain stem were also extensively examined.) Approximately 100 unstained sections were microtomed for each block for a total of about 600-800 sections per animal. Preliminary assessments were conducted to assure that the section levels selected and examined correlated well between animals. Serial paraffin sections were cut through the frontal cortex with an emphasis in demonstrating layers I, II, and III. Preliminary assessments were also conducted to confirm that the ketamine-induced lesions were confined to Layers 1 and 2 of the frontal cortex and that the lesions were distributed consistently in this region in all animals.

Approximately 25 coronal sections from the relevant regions of each brain were stained with HE stain to define general histology and morphology. For each of these 25 HE sections for each brain, there were additional adjacent three sections that were stained with activated caspase 3 (AC3), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), and silver stain. Thus, approximately 25 sections spanning the frontal cortex from each brain were stained with each of HE, AC3, TUNEL, and a silver stain (25 HE and 75 immunosections total for each brain were examined).

HE-, silver-, AC3-, and TUNEL-stained sections were qualitatively and semi-quantitatively examined by direct light microscopy. For each stain, the incidence and severity of the lesions (presence of apoptosis and cell injury) were scored semi-quantitatively using the accepted industry scoring system: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate and 4 = severe. The slides were examined by a ACVP-board-certified veterinary pathologist and then peer-reviewed by a second pathologist in a masked manner.

Histomorphometry

Representative AC3, TUNEL, and silver-stained sections from each animal were assessed histomorphometrically to determine the presence and amount of neuroapoptosis. The areas of interest, histologic findings and the histomorphometric findings were correlated by two independent pathologists. The area of apoptotic cells per field was determined by histomorphometry (Olympus MicroFire™ Imaging System, Olympus America, Inc., Melville, NY, USA) using a validated color phase-analysis methodology and an experienced trained pathologist.
masked to the treatment groups. Using imaging analysis, the color corresponding to the region of interest (ROI) was selected. The software calculated the total positively stained area (pixels), which was normalized to the total pixel area of the tissue image. The resulting total area of apoptotic cells was calculated as a percentage of the total area of neuropil from six representative different fields in the lesion area from two blocks from the frontal cortex for each stain. For each stain, three fields per slide were selected, photographed (400X) and histomorphometrically measured, based upon the localization, uniformity and consistency of the neuro-degenerative lesions from typical representative regions of Layers 1 and 2 of the frontal cortex. This led to a total of 18 areas of measurement for each animal. The percentage of total area of apoptotic cells in treated animals was compared statistically to the percentage of total area of apoptotic cells in untreated animals and compared statistically.

Statistical analysis was performed using InStat Version 3.01 (GraphPad Software, San Diego, CA, USA). Histomorphometry data for silver, caspase, and TUNEL staining were analyzed using one-way Analysis of Variance (ANOVA, a parametric test) followed by the Tukey-Kramer test. Data were plotted graphically using Prism Version 3.03 (GraphPad Software).

Pharmacokinetic sampling and plasma processing

Blood samples (approximately 1.0 ml) were collected from the dams using an appropriate peripheral blood vessel of the pregnant females dosed with dexmedetomidine (Groups 3 and 4) using sterile, disposable syringes and needles, and transferred into K2EDTA-containing blood collection tubes. Samples were collected prior to dosing, at 1 hr during infusion, and at the end of the 12-hr infusion period.

Following the C-section and prior to perfusion fixation, blood samples (approximately 1.0 ml) were collected from the fetuses of the pregnant females infused with dexmedetomidine via intracardiac puncture.

All samples (dams and fetuses) were placed on wet ice and processed within 30 min of collection. The samples were centrifuged at 4°C at 4,000 rpm for 10 min. Plasma was transferred into polypropylene screw-cap vials and stored frozen at approximately -80°C in the clinical pathology laboratory at PT Mac Fauna until shipped on dry ice to Maccine Pte Ltd. (Singapore Science Park II, Singapore), and subsequently to CPR Pharma Services (Thebarton SA, Australia) for analysis of dexmedetomidine levels.

Determination of plasma dexmedetomidine concentration

Dexmedetomidine in monkey plasma samples was quantitated using a fully validated high performance liquid chromatographic procedure with mass spectrometric detection. The method extracts dexmedetomidine and the internal standard (detomidine) from monkey plasma by liquid/liquid extraction using tert-butyl ether as the organic extractant. The extract is injected onto an Alltima phenyl column using isocratic elution at 0.4 ml/min. The mobile phase consisted of acetonitrile / 10 mM ammonium acetate pH 5.0 (75/25) and the eluate monitored by an API4000 MS/MS detector in positive MRM mode with ESI source. The single charged Q1/Q3 transition is 201.1 / 95.0 atomic mass units (amu) for dexmedetomidine and 187.3 / 81.1 amu for the internal standard. The method range is from 5.00 to 2,000 pg/ml for the analyte and has a run time of approximately three min per sample. The method validation for dexmedetomidine is an adaptation of the method described by Ji et al. (2004).

RESULTS

Heart rate and body temperature of the dams

The mean heart rate and body temperature of the dams from the dexmedetomidine- and ketamine-treated groups at the three infusion time points (T0, T6, and T11/12) are shown in Table 2. The heart rate results showed that the monkeys’ mean heart rates following anesthesia were lower than the pre-anesthesia values but the mean heart rates were stable throughout the 12-hr infusion period for all three groups. The mean heart rates of the two dexmedetomidine groups were slightly lower than those in the ketamine group, but the mean heart rates for all three groups observed throughout the infusion period were similar to values reported by others for cynomolgus monkeys (Zola-Morgan and Micheletti, 1986).

The mean body temperatures of the dams for all three groups were stable throughout the 12-hr infusion period (Table 2). The mean body temperature values observed in the current study were consistent with those reported when cynomolgus monkeys were anesthetized using barbiturates (Zola-Morgan and Micheletti, 1986).

Plasma concentration of dexmedetomidine

Systemic exposure of dexmedetomidine was confirmed in the plasma of dams at both postdose time points (One hour after start of infusion and at the end of the 12-hr infusion) and in a dose-dependent manner (Fig. 1). At the one hour postdose time point, the mean plasma concentration of dexmedetomidine was 1,730 pg/ml (1.7 ng/ml)
for the low-dose group and 20,580 pg/ml (20.6 ng/ml) for the high-dose group. The mean plasma level at the end of the 12-hr infusion was 1,900 pg/ml (1.9 ng/ml) and 21,260 pg/ml (21.3 ng/ml) for the low- and high-dose groups, respectively. Plasma concentration of dexmedetomidine in the fetal blood circulation was measurable even when the fetal blood samples were collected following C-section, which occurred 6 hr after the termination of infusion. The mean plasma concentrations in the fetal circulation were 50.43 pg/ml (0.05 ng/ml) and 2,200.2 pg/ml (2.2 ng/ml) in the low-dose and high-dose groups, respectively.

**Histopathologic assessment**

The frontal cortex of untreated brains was within normal limits while there was extensive apoptosis and neurodegeneration of Layers I and II in all animals in the ketamine-treated group. The histological changes were uniformly and morphologically consistent with-
were similar to the scores of untreated control brains and were lower than those seen with the ketamine treatment (Table 3).

**Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining**

TUNEL-label is a common method for detecting DNA fragmentation that results from apoptotic signaling cascades. Fetal brain sections exposed to ketamine or both doses of dexmedetomidine stained by TUNEL-label are shown in Figs. 4 (20X magnification) and 5 (400X magnification). The results showed that the extent of TUNEL-label cells in dexmedetomidine-treated fetal brains (Figs. 4C, D and 5C, D) were similar to those found in untreated control fetal brains (Fig. 4A, 5A). In comparison, the number of TUNEL-label cells following ketamine treatment were dramatically increased (Figs. 4B, 5B, and Table 3) in the frontal cortex of the fetal monkey brains.

**Silver staining**

Silver staining detects early as well as chronic neural degenerative cells. Fetal brain sections stained with silver stain in the current study are shown in Fig. 6. Similar to the AC3 and TUNEL staining results, the extent of silver impregnated cells in both doses of dexmedetomidine-treated fetal brains (Figs. 6B, C, F, and G) were similar to those found in untreated control fetal brains (Figs. 6D, and H). Ketamine treatment resulted in significantly more silver-impregnated cells in the frontal cortex of the fetal monkey brains (Figs. 6A, and E) as well as higher severity scores as compared to dexmedetomidine-treated or untreated control brains (Table 3).

**Histomorphometric Assessment**

The histomorphometric results are expressed as a percentage of the total area of neuropil are summarized in Table 3b and presented in detail in the scatter diagrams. Treatment with dexmedetomidine was associated with a significant reduction in apoptosis and neurodegeneration at both dose levels as compared to the ketamine-treated group (Table 3a) as demonstrated histomorphometrically with AC3, TUNEL and silver stained tissue. In addition, the severity scores of apoptosis expressed as a percentage of total neuropil area are presented in Table 3b. There was a statistically significant increase in neuronal apoptosis in ketamine-treated brains in comparison to control and dexmedetomidine-treated brains (p < 0.001).

### Table 3a. Summary of microscopic severity scores of neuroapoptosis and neurodegeneration

<table>
<thead>
<tr>
<th>Group (N=5)</th>
<th>Treatment</th>
<th>HE</th>
<th>TUNEL</th>
<th>Activated Caspase 3</th>
<th>Silver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.5</td>
<td>0.8 ± 0.6</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>Ketamine</td>
<td>1.8 ± 1.2b</td>
<td>1.9 ± 0.9b</td>
<td>3.5 ± 0.7b</td>
<td>2.8 ± 1.2b</td>
</tr>
<tr>
<td>3</td>
<td>Dex (Low Dose)</td>
<td>0.1 ± 0.3</td>
<td>0.7 ± 1.0</td>
<td>1.4 ± 0.6</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>Dex (High Dose)</td>
<td>0.1 ± 0.3</td>
<td>0.2 ± 0.4</td>
<td>1.7 ± 1.0</td>
<td>0.4 ± 0.8</td>
</tr>
</tbody>
</table>

*Note: Values are mean ± S.D., Dex = Dexmedetomidine; Staining Severity Scores: 0 = normal; 1 = minimal scattered apoptosis and cell debris; 2 = mild; 3 = moderate; 4 = severe and extensive. b Statistically significant difference between ketamine-treated and other groups.*

### Table 3b. Summary of phase analysis for apoptosis

<table>
<thead>
<tr>
<th>Group (N=5)</th>
<th>Treatment</th>
<th>% Apoptosis Severity Scores a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TUNEL</td>
<td>Activated Caspase 3</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0.32 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>Ketamine</td>
<td>2.73 ± 0.51b</td>
</tr>
<tr>
<td>3</td>
<td>Dex (Low Dose)</td>
<td>0.32 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>Dex (High Dose)</td>
<td>0.52 ± 0.18</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation. Data are shown as the ratio of the area of apoptosis to the total neuropil area, in %. b There was a statistically significant increase in neuronal apoptosis in ketamine-treated brains in comparison to control and dexmedetomidine-treated brains (p < 0.001).*
Fig. 2. Activated caspase 3 (AC3) immunostained photomicrographs of the frontal cortex of fetal monkey brains of untreated control (A) and monkeys treated with ketamine (B), low-dose dexmedetomidine (C), and high-dose dexmedetomidine (D). Arrows in Fig. 2B indicate AC3-positive cells. 10X magnification.

Fig. 3. Activated caspase 3 (AC3) immunostained photomicrographs of the frontal cortex of fetal monkey brains of untreated control (A) and monkeys treated with ketamine (B), low-dose dexmedetomidine (C), and high-dose dexmedetomidine. The AC3-positive cells shown in Fig. 2B can be clearly seen in Fig. 3B. 20X magnification.
Fig. 4. Immunohistochemical TUNEL-label photomicrographs of the frontal cortex of fetal monkey brains of untreated control (A) and monkeys treated with ketamine (B), low-dose dexmedetomidine (C), and high-dose dexmedetomidine (D). TUNEL-labeled cells as a result of ketamine treatment are clearly seen as brown cells in Fig. 4B. 20X magnification.

Fig. 5. Immunohistochemical TUNEL-label photomicrographs of the frontal cortex of fetal monkey brains of untreated control (A) and monkeys treated with ketamine (B), low-dose dexmedetomidine (C), and high-dose dexmedetomidine (D). TUNEL-label cells are indicated by arrows in all four panels. Fetal monkey brains exposed to ketamine resulted in significantly greater number of TUNEL-label cells compared to untreated controls as well as both doses of dexmedetomidine. 400X magnification.
and dexmedetomidine-treated brains (p < 0.001). There were no statistically significant differences between control and dexmedetomidine-treated brains.

In summary, histopathology evaluation for neuroapoptosis and cellular degeneration of dexmedetomidine-treated fetal monkey brains at both dose levels did not appear significantly different from untreated control fetal monkey brains.

**DISCUSSION**

Common general anesthetics such as ketamine and isoflurane have been reported to induce neuroapoptosis and cellular degeneration in actively developing brains in rodents (Hayashi et al., 2002; Ikonomidou et al., 1999; Jevtovic-Todorovic et al., 2003; Ma et al., 2007; Sanders et al., 2009, 2010; Scallet et al., 2004) and nonhuman primates (Creeley et al., 2013; Brambrink et al., 2012, 2010; Slikker et al., 2007; Zou et al., 2009). Dexmedetomidine is a sedative and anesthetic with a different mechanism of action than ketamine and isoflurane. Specifically, ketamine induces anesthesia by blocking the glutamate N-methyl-D-aspartate (NMDA) receptors (Franks and Lieb, 1994) while isoflurane exerts its effect by potentiating γ-amino butyric-acid type A (GABA A) receptors (Franks and Lieb, 1994), and inhibits receptor activity in the NMDA glutamate receptor subtypes (Shelton and Nicholson, 2010; Brosnan, 2011). Dexmedetomidine is a selective α2 adrenoceptor agonist at low (pharmacologic) doses and α2 adrenoceptors are believed to play an important role in cellular signaling in the central nervous system early in life (Song et al., 2004; Winzer-Serhan and Leslie, 1999).

Recent reports have demonstrated that dexmedetomidine does not induce neuroapoptosis in neonatal rat brains (Sanders et al., 2009, 2010). The aim of the current study was to extend the rodent finding in nonhuman primates with the addition of plasma level measurement of dexmedetomidine using an established ketamine-induced neuroapoptosis monkey model described by Slikker et al.
al. (2007). Based on Slikker’s monkey model, the monkeys in the current study were given a loading dose of ketamine at 20 mg/kg i.m., followed by a 12-hr infusion at 20-50 mg/kg/hr. This ketamine dose in monkeys is approximately 10 times the HED (Ketalar [package insert], 2012). The high-dose of dexmedetomidine used in the current study is also approximately 10 times HED. Therefore, comparison of the neurotoxic effects of ketamine and dexmedetomidine at those respective 10 times HED is considered valid.

Systemic exposure to dexmedetomidine for both the dams and fetuses was confirmed. At both the low- and high-dose levels, dexmedetomidine was detected at one hour following the initiation of infusion as well as at the end of the 12-hr infusion time point for the dams. Importantly, dexmedetomidine was also detected in the plasma of fetuses at both dose levels. This was especially interesting and important because the fetal blood samples were collected following the C-section, 6 hr after the termination of the dexmedetomidine infusion. The mean fetal plasma concentrations of dexmedetomidine were 50.43 pg/ml (0.050 ng/ml) for the high-dose group and 2200.2 pg/ml (2.2 ng/ml) for the low-dose group. This is a significant point because it suggests that the dexmedetomidine level in the fetal circulation was slightly higher than the mean plasma concentration of dexmedetomidine in humans at steady state which is approximately 1 ng/ml (Ebert et al., 2000). This result further confirms the dose level selection for dexmedetomidine was sufficiently high enough to determine its potential neurotoxicic effects. Also important to note is the fact that placental transfer of dexmedetomidine has been demonstrated in an in vitro human placenta study (Ala-Kokko et al., 1997). Furthermore, in vivo placental transfer of dexmedetomidine was observed when radiolabeled dexmedetomidine was administered subcutaneous in rats (Precedex [package insert], 2013). Therefore, detection of dexmedetomidine in the fetal circulation in the present study was consistent with and extends those previous findings. Plasma concentration of ketamine from both the dam and fetal circulation was not measured as the dose of ketamine used in the current study was identical to that used in the Slikker monkey study (Slikker et al., 2007). In addition, the primary objective of the current study was to determine the potential neurotoxic effects of dexmedetomidine; while the neurotoxicity of ketamine has been well documented in a monkey at that dose level.

Following confirmation of systemic exposure of dexmedetomidine in the fetal circulation, we proceeded to determine whether this exposure resulted in neuroapoptosis and cellular degeneration in the fetal monkey brains. The histopathology results from this study show no significant neuroapoptotic lesions present in the untreated control group. However, in utero treatment with ketamine was associated with marked neuroapoptosis and cellular damage, primarily in layers I and II of the frontal cortex in fetal monkey brains. This result reproduced and confirmed the findings on the neurotoxic effects of ketamine in developing monkey brains reported by Slikker et al. (2007). The current monkey neurotoxicity study showed that for the dexmedetomidine-treated groups, there was none to minimal neuroapoptosis noted as a result of the in utero treatment at both the low- and high-dose levels. Not only were the neuroapoptotic lesions as a result of dexmedetomidine treatment clearly much less severe than in the ketamine-treated group, the histopathology findings from brains of dexmedetomidine-treated animals at both dose levels did not appear significantly different from untreated control fetal monkey brains. Although the plasma level of dexmedetomidine in the fetal circulation during the infusion period was not, and could not be determined, the mean concentration of dexmedetomidine in the fetal circulation at the time of C-section (6 hr after termination of infusion) was measured to be 2.2 ng/ml in the high-dose group (10X HED) which is above the mean steady-state clinical plasma concentration of 1 ng/ml and yet, the fetal monkey brains did not show enhanced apoptosis compared to controls.

It has recently been shown that exposure of ketamine to neonatal monkey brains resulted in long-lasting cognitive deficits (Paule et al., 2011). More importantly, in a recent clinical study, Ing et al. (2012) reported that children that had been exposed to anesthesia before the age of 3 years had a higher relative risk of language and cognitive deficits at age 10 years when compared to unexposed children (Ing et al., 2012). Dexmedetomidine has now been demonstrated not to induce neuroapoptosis in rodents (Sanders et al., 2009, 2010) and nonhuman primates. Furthermore, when co-administered with isoflurane to neonatal rodents, dexmedetomidine was able to attenuate the extent of neuroapoptosis induced by isoflurane (Sanders et al., 2010) and that the reduction of the neuroapoptotic lesions in those neonatal rats also resulted in the attenuation of isoflurane-induced neurocognitive impairment in neonatal rats (Sanders et al., 2009). Furthermore, binding of endogenous norepinephrine to α2 adrenoceptors activate cellular survival mechanisms such as the Ras-Raf-pERK pathway (Philipp et al., 2002; Wang et al., 2006). Activation of the Raf-pERK pathway has been associated with neuroprotection against apoptosis induced by NMDA antagonists in developing rat brains (Hansen et al., 2004). Dexmedetomidine has also
been shown to increase expression of anti-apoptotic proteins such as mdm2 and bcl-2 in a model of adult ischemic cerebral injury (Engelhard et al., 2003). It would be important to determine whether dexmedetomidine could attenuate ketamine- or isoflurane-induced neuroapoptosis and neurocognitive deficits in a future monkey study.

There are a few limitations associated with the current monkey neurotoxicity study. First, although heart rate, body temperature, oxygen saturation, and clinical signs were monitored during the treatment and recovery periods, other physiological parameters were not monitored or measured. Secondly, even though the ketamine-induced neurotoxicity monkey model is well characterized in the Slikker publication (Slikker et al., 2007), the plasma ketamine level was not measured in the current study. Finally, although the histomorphometric analysis method used in the current study is considered standard/routine in the histopathology field and that the morphometric analysis was also peer-reviewed by a second pathologist in a blinded manner, this method is still considered semi-quantitative.

In conclusion, the results from the current monkey neurotoxicity study showed that exposure of dexmedetomidine to fetal monkey brains at both the low-dose (3 μg/kg:1X HED) and the high-dose (30 μg/kg:10X HED) levels did not induce or enhance neuroapoptosis and cellular degeneration in the frontal cortex (layers I, II, and III).

REFERENCES


