Use of Opioids in Asphyxiated Term Neonates: Effects on Neuroimaging and Clinical Outcome

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ABSTRACT

Perinatal asphyxia is a common cause of neurologic morbidity in neonates who are born at term. Asphyxiated neonates are frequently treated with analgesic medications, including opioids, for pain and discomfort associated with their care. On the basis of previous laboratory studies suggesting that opioids may have neuroprotective effects, we conducted a retrospective review of medical records of 52 neonates who were admitted to our neonatal intensive care unit between 1995 and 2002 and had undergone magnetic resonance imaging (MRI) of the brain. Our review revealed that 33% of neonates received morphine or fentanyl. The neonates who received opioids also had experienced hypoxic/ischemic insults of greater magnitude as suggested by higher plasma lactate levels and lower 5-min Apgar scores. It is interesting that the MRI studies of neonates who were treated with opioids during the first week of life demonstrated significantly less brain injury in all regions studied. More important, follow-up studies of a subgroup of opioid-treated

neonates whose MRI scans were obtained in the second postnatal week had better long-term neurologic outcomes. Our results suggest that the use of opioids in the first week of life after perinatal asphyxia have no significant long-term detrimental effects and may increase the brain's resistance to hypoxic-ischemic insults. (*Pediatr Res* 57: 873–878, 2005)

Abbreviations

BG, basal ganglia
MRI, magnetic resonance imaging
NICU, neonatal intensive care unit
NMDA, N-methyl-D-aspartate
PCPCS, Pediatric Cerebral Performance Category Scale
TE, echo time
TR, repetition time
W, watershed
WBC, white blood cell

Immediately after birth, asphyxiated neonates who are admitted to the neonatal intensive care unit (NICU) experience repetitive invasive and noninvasive procedures, such as endotracheal intubation, arterial line placement, i.v. catheterization, intramuscular injections, and endotracheal suctioning. Although necessary steps in the care of these neonates, such procedures result in pain and prolonged stress during a critical time associated with rapid brain growth and development (1). In addition to causing abnormalities in respiration, heart rate, and blood pressure (2), neonatal pain may alter neuronal and synaptic organization (3) and potentiate the effects of hypoxia (4). Animal studies show that exposure to untreated repetitive pain and stress results in prolonged firing of C-fibers, causing

glutamate-induced N-methyl-D-aspartate (NMDA) receptor activation (5). This may augment hypoxia-induced glutamate release, an important mechanism that contributes to neuronal injury or death.

Opioid analgesics such as morphine and fentanyl are administered to critically ill neonates in an effort to reduce pain and stress (6). The antinociceptive effects of these medications are mediated through a combined presynaptic and postsynaptic hyperpolarization, leading to reductions in the release of and sensitivity to endogenous mediators such as glutamate (7,8). These data suggest that opioids might confer neuroprotection by decreasing excitotoxicity through reductions in glutamate release, leading to greater overall reductions in neurologic damage. Cell culture studies indicate that both endogenous and exogenous opioids can protect cortical neurons from hypoxia-induced cell death (9,10) and induce ischemic tolerance in cerebellar Purkinje cells that are subjected to ischemia-reperfusion conditions (11). Opioid receptor agonists were shown to increase tissue preservation and survival time of

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organs before their use in transplantation surgery (12) and increase survival time during severe hypoxia in intact animals (13,14). In ventilated preterm neonates, clinical trials of low-dose morphine or fentanyl infusion have demonstrated conflicting results. Although several pilot studies (15,16) as well as a large randomized trial supported the neuroprotective role of opioids (17), a recently published study in ventilated preterm neonates of 24–32 wk gestation demonstrated that preemptive low-dose morphine infusion did not reduce the incidence of neonatal death, intraventricular hemorrhage, or periventricular leukomalacia (18). Although these results are important, none of these studies included term infants and all excluded infants with severe perinatal asphyxia. In addition, these studies did not examine long-term outcome but focused on acute neurologic status at 36 wk after conception (16) or at 28–35 postnatal days (17,18).

To evaluate the effects of opioid analgesia on neuroimaging and clinical outcomes, we conducted a retrospective review of medical records of neonates who were admitted to our NICU and had undergone magnetic resonance imaging (MRI) of the brain for perinatal asphyxia. We tested the hypothesis that opioid analgesics that are administered during the first week of life can alter the severity of acute and long-term neurologic injury in full-term neonates who are exposed to perinatal asphyxia. The first postnatal week was chosen on the basis of animal studies on brain energy metabolism that characterized the term human brain's first postnatal week to be particularly vulnerable to the effects of hypoxic energy failure (19). This period of heightened vulnerability corresponded with marked declines in phosphocreatine and nucleoside triphosphates in response to hypoxemia (19) as well as developmentally programmed increases in the expression of specific excitatory amino acid receptors (20). In addition, neonates are subjected to the highest number of painful procedures during the first week of life, with painful procedures occurring 6–14 times per day (21,22). Our aim was to compare clinical, neuroimaging, and long-term outcomes in opioid-treated *versus* nontreated neonates to determine whether opioids play a role in decreasing acute and long-term neurologic injury.

METHODS

Patient data. Medical, radiologic, and research records of newborns with brain injury were reviewed. Inclusion criteria were MRI scan in the first month of life and at least one of the following criteria for perinatal asphyxia: 1) 5-min Apgar score of ≤ 5 , 2) umbilical cord arterial pH ≤ 7.10 , 3) umbilical cord base excess ≥ -10 , and 4) clinical postasphyxial syndrome (encephalopathy, seizure, intraventricular hemorrhage). Exclusion criteria were 1) proven congenital infection or metabolic disease or 2) CNS or other major malformations. This study was reviewed and approved by the Loma Linda University Institutional Review Board.

Review of the medical records was performed and infant physical characteristics such as estimated gestational age, birth weight, and sex were recorded. To define the infant's condition immediately after birth, we also recorded the neonate's initial chemical (blood gas, electrolytes) and hematologic data as well as the results of the first neurologic examination. We also examined maternal and labor and delivery histories to determine the presence or absence of risk factors that are known to affect neurologic outcome. These factors include maternal and fetal conditions such as inflammation or infection (23), alcohol or illicit drug abuse (24), and intrapartum events that result in prolonged and catastrophic asphyxia (*e.g.* cord compression) (25).

Use of opioid analgesics. Individual patient medication administration records, nursing forms, physician's order sheets, and the institution's pharmacy

records were reviewed for documentation of administration of opioid analgesics. The MRI scans of neonates who received morphine, fentanyl, or meperidine in the first week of life were compared with those who did not receive any of these opioids. Treated neonates were found to have received one of the following: 1) morphine 0.1 mg/kg i.v. every 2–6 h as needed or fentanyl 2–5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ continuous i.v. ($n = 3$), 2) fentanyl 2–5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ continuous i.v. ($n = 8$), 3) morphine 0.1 mg/kg i.v. every 2–6 h as needed ($n = 2$), 4) morphine 40 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ continuous i.v. ($n = 1$), or 5) morphine 0.1 mg/kg i.v. every 2–6 h as needed or morphine 30–40 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ($n = 3$). The first and last group of neonates (1 and 5) received either continuous morphine or fentanyl infusion in the first 1–2 d of life, followed by intermittent (as needed) opioid boluses in the next 5–6 d of life or *vice versa*. None of the infants received simultaneous intermittent boluses and continuous i.v. infusions of opioids.

MRI scans. To quantify the extent and the severity of brain damage in opioid-treated and nontreated infants, we used an MRI scoring system that assigns a numerical score to the severity of injury in the basal ganglia and watershed zones, regions that are found on MRI to be particularly vulnerable to hypoxic-ischemic injury in the newborn (26). MRI scans of the identified neonates were scored by an experienced neuroradiologist (N.W.) who was blinded to clinical information and opioid utilization. Scoring was based on a previously validated scoring system for asphyxiated neonates developed by Barkovich *et al.* (26) and described in Table 1. Briefly, each MR scan was scored twice by the same radiologist, with scoring separated in time by >1 mo. The first and second sets of scores were compared, and intraobserver variability was calculated. The κ statistic was 0.85, showing high consistency of scoring and low intraobserver variability. This MRI scoring system was previously shown to discriminate between good and poor neuromotor and cognitive outcomes at 3 and 12 mo in asphyxiated neonates (26).

MRI was performed using a standard circularly polarized head coil in a conventional 1.5T scanner (Vision; Siemens Medical Solutions, Erlangen, Germany). To assess severity of injury in the basal ganglia and watershed-cortical regions, we used two sequences: 1) a sagittal T1-weighted spin echo sequence [repetition time (TR)/echo delay time (TE) = 550/22 ms, 4 acquisitions, 5 mm thick], and 2) an axial triple spin echo sequence (TR/TE1/TE2/TE3 = 3000/22/60/120 ms, 1 acquisition, 5 mm thick). The proton density weighted image (first echo of the triple spin echo sequence; TR/TE = 3000/22 ms) and the T2-weighted image (third echo of triple spin echo sequence; TR/TE = 3000/120) along with the T1 weighted images were evaluated and a basal ganglia (BG) score, a watershed (W) score, and a combined basal ganglia/watershed (BG/W) score were assigned for each type of image.

We first compared the MRI scores of all opioid-treated ($n = 17$) and nontreated neonates ($n = 35$) who met study criteria (total $n = 52$) to demonstrate the early effects of opioid analgesia on brain injury. Subsequently,

Table 1. MRI scoring system

Score	MRI finding
Basal ganglia	
0	Normal or isolated focal infarct
1	Abnormal signal in thalamus
2	Abnormal signal in thalamus and lentiform nucleus
3	Abnormal signal in thalamus, lentiform nucleus, and perirolandic cortex
4	More extensive involvement
Watershed	
0	Normal
1	Single focal infarction
2	Abnormal signal in anterior or posterior watershed white matter
3	Abnormal signal in anterior or posterior watershed cortex and white matter
4	Abnormal signal in both anterior and posterior watershed zones
5	More extensive cortical involvement
Basal ganglia/watershed	
0	Normal
1	Abnormal signal in basal ganglia and thalamus
2	Abnormal signal in cortex
3	Abnormal signal in cortex and basal nuclei (basal ganglia or thalami)
4	Abnormal signal in entire cortex and basal nuclei

Scoring system derived from Ref. 6.

we compared MRI, Pediatric Cerebral Performance Category Scale (PCPCS), and neuromotor scores (see "Long-term neurologic outcome scores" below) in a subgroup of infants whose MRI scans were performed after day 7 of life ($n = 24$; 12 in the opioid group and 12 in nonopioid group) on the basis of previous studies that demonstrated that the appearance of the asphyxiated infant's brain evolves during the first 17 d of life (26). This analysis allowed us to assess the long-term effects of opioid treatment on infants with better defined neuroimaging evidence of brain injury.

Long-term neurologic outcome scores. Neurologic outcomes were measured at discharge and subsequent visits using the PCPCS, a six-point outcome scoring system modified from the Glasgow Outcome Scale score (27). The PCPCS was obtained by an experienced pediatric neurologist (S.A.) who was blinded to MRI or opioid information. This tool quantifies the overall functional and cognitive impairment of neonates and children and has been validated in 1469 pediatric patients after acute CNS injuries (28). It was also shown to correlate with other measures of psychological outcome (29). The score includes the following outcomes: 1 for normal, can perform all age-appropriate activities; 2 for mild disability, conscious, alert, and able to interact in most age-appropriate activities and may have a mild neurologic deficit; 3 for moderate disability, conscious, sufficient cerebral function for age-appropriate activities of daily life, but has significant cognitive impairment; 4 for severe disability, conscious, dependent on others for daily support because of impaired brain function; 5 for persistent vegetative state; and 6 for death. Long-term neurologic outcome was also determined using a validated neuromotor scoring system (30) by a second pediatric neurologist who was blinded to opioid utilization and clinical course. The score includes the following outcomes: 0 = normal, 1 = abnormal tone or reflexes, 2 = abnormal tone and reflexes, 3 = decreased power in addition to tone or reflex abnormality (functional deficit of power), 4 = cranial nerve involvement with motor abnormality, 5 = spastic quadriplegia, and 6 = death.

Statistics. Continuous data (demographic, chemical, and hematologic data) were analyzed using an independent sample t test and reported as the mean and SEM. Ordinal data (MRI, PCPCS, and neuromotor scores) were analyzed using a nonparametric Mann-Whitney test. The κ statistic was used to assess intrarater variability agreement for MRI assessments. All statistical analyses were performed using SPSS Statistics for Windows Version 10 (Chicago, IL). Differences were considered significant at $p \leq 0.05$.

RESULTS

Subjects. From 1995 to 2002, we reviewed the records of 60 neonates who were admitted to the NICU and also underwent MRI. Eight neonates were excluded: two for CNS anomalies such as rolandic polymicroglia and microcephaly, one for congenital herpes infection, one for appearance of prematurity on examination, one for a poor-quality MRI scan, one for large parenchymal hemorrhage, one for vein of Galen aneurysm, and one for absence of documented hypoxic-ischemic events or sequelae (normal arterial blood gases, normal EEG, etc.). Table 2 summarizes the demographic characteristics of neonates who met the study criteria. The majority of the neonates in both groups were born at outside hospitals and were transferred to Loma Linda NICU at 1.5 ± 0.5 d after birth.

Table 3 summarizes the neonates' documented physiologic and neurologic status during labor and delivery and immediately after birth, as well as perinatal risk factors. Neonates in the opioid-treated group had more documented episodes or markers of asphyxial insult such as meconium staining, fetal bradycardia or abnormal heart rate, or multisystem involvement. The majority of neonates in both groups had one or more abnormal neurologic signs consistent with perinatal hypoxic/ischemic injury.

Opioid analgesics and MRI score. Table 4 describes the demographic and clinical characteristics of all neonates who met study criteria and did ($n = 17$, 33%) or did not ($n = 35$, 67%) receive opioid analgesia in the first week of life. The rationale for the clinician's choice to treat or not to treat with

Table 2. Patient demographics

	Received opioid analgesia ($n = 17$)	No opioid analgesia ($n = 35$)	p value*
Mean birth weight (SEM)	3563 $\pm$ 180 g	3320 $\pm$ 109 g	NS
Estimated gestational age	39 $\pm$ 0.4 wk	39 $\pm$ 0.2 wk	NS
Sex	Male, 11 (65%) Female, 6 (35%)	Male, 21 (60%) Female, 14 (40%)	NS
Inborn/outborn status	Outborn, 11 (65%) Inborn, 6 (35%)	Outborn, 25 (71%) Inborn, 10 (29%)	NS
Mean age at time of MRI (SEM)	6.4 $\pm$ 0.75 d	10 $\pm$ 1.3 d	0.014
Mean age at follow-up examination (SEM)	15 $\pm$ 6 mo	21 $\pm$ 4 mo	NS
Discharge status	Alive, 16 (94%) Dead, 1 (6%)	Alive, 33 (94%) Dead, 2 (6%)	NS

* Independent samples t test.

Table 3. Neurologic examination, fetal status, and risk factors

	Received opioid analgesia ($n = 17$)	No opioid analgesia ($n = 35$)
Fetal/neonatal status		
Meconium staining	11 (64%)	12 (34%)
Fetal bradycardia/abnormal fetal heart rate	10 (59%)	17 (49%)
Multisystem involvement	17 (100%)	30 (86%)
Risk factors*		
Uterine rupture	1	3
Abruptio placenta	2	2
Maternal fever	1	0
Prolapsed cord	1	1
Nuchal cord	2	4
Placenta previa	1	0
Substance abuse	4	5
Type 1 diabetes	2	2
Oligohydramnios	1	0
Macrosomia/difficult delivery/shoulder dystocia	4	0
Group B strep infection	0	1
Herpes simplex virus infection	0	1
Premature rupture of membranes	1	4
Abnormal neurologic findings		
Abnormal tone/reflexes	15 (88%)	28 (80%)
Abnormal state of consciousness†	7 (41%)	6 (17%)
Respiratory distress/failure	15 (88%)	25 (71%)
Abnormal EEG	14 (82%)	32 (91%)

* Some infants had more than one risk factor.

† Unable to assess or not documented in majority of infants.

opioid analgesia was not well documented in the medical record and was most likely related to the treating clinician's attitudes about neonatal pain and pain therapy (31). It is interesting that the prescription of opioid analgesia seemed to be related to the severity of the infant's illness. Neonates who received opioids had significantly lower 5-min Apgar score ($p < 0.03$), lower pH ($p < 0.046$), higher P_{CO_2} ($p < 0.048$), higher Hb ($p < 0.036$), and higher serum lactate levels ($p <$

Table 4. Clinical characteristics and MRI score of infants who received/did not receive opioid analgesia in the first week of life

	Received analgesia [n = 17 (31%)]	No analgesia [n = 35 (69%)]	p value*
Demographics			
Birth weight	3563 ± 180	3320 ± 109	NS
EGA	39 ± 0.4	39 ± 0.2	NS
Apgar, 1 min	2.8 ± 0.5	3.3 ± 0.5	NS
Apgar, 5 min	4.3 ± 0.5	5.8 ± 0.4	0.033
Laboratory data			
Blood gas			
pH	6.99 ± 0.05	7.15 ± 0.04	0.046
Pco ₂	67 ± 9	47 ± 5	0.048
BE	-14 ± 3	-11 ± 1	NS
HCO ₃	16.5 ± 2	16.7 ± 1	NS
Chemistry			
Sodium (meq/L)	131 ± 2	131 ± 4	NS
Ionized calcium (meq/L)	1.1 ± 0.07	1.1 ± 0.03	NS
Ammonia (μmol/L)	68 ± 10	56 ± 6	NS
Lactate (mmol/L)	9.5 ± 5	2.3 ± 0.4	0.001
Hematology			
WBC (bil/L)	22 ± 4	18 ± 1	NS
nRBC (per 100 WBC)	34 ± 20	15 ± 3	NS
Hb	17 ± 0.7	15 ± 0.5	0.036
Hct	52 ± 2	47 ± 11	NS
Lymph%	29 ± 4	29 ± 2	NS
Platelets (bil/L)	224 ± 22	245 ± 1	NS
MRI score			
Basal ganglia T1	0.29 ± 0.14	1.83 ± 0.30	0.004
Basal ganglia PD	0.24 ± 0.14	1.69 ± 0.30	0.006
Basal ganglia T2	0.29 ± 0.14	1.83 ± 0.30	0.004
Watershed T1	0.53 ± 0.23	2.14 ± 0.36	0.009
Watershed PD	0.35 ± 0.21	1.94 ± 0.38	0.013
Watershed T2	0.53 ± 0.23	2.14 ± 0.36	0.009
Basal ganglia	0.47 ± 0.21	1.80 ± 0.28	0.008
Watershed T1			
Basal ganglia	0.41 ± 0.21	1.69 ± 0.29	0.011
Watershed PD			
Basal ganglia	0.44 ± 0.22	1.74 ± 0.28	0.008
Watershed T2			

EGA, estimated gestational age; WBC, white blood cell; nRBC, nucleated red blood cell; Hct, hematocrit.

* Independent samples *t* test for demographic and laboratory data; Mann-Whitney for MRI score.

0.001). These results are consistent with previously published findings from clinical trials using open-label morphine (18). More important, we found that neonates who received opioids in the first week of life had significantly lower MRI scores (*i.e.* less evidence of injury) in all brain regions compared with neonates who did not receive opioids (Table 4).

Opioid analgesics and long-term outcome. On the basis of information that the appearance of the injured neonatal brain changes significantly during the first 17 d of life and that correlation with long-term outcome was better in patients whose MRI examinations were performed after the first week of life (26), we evaluated the MRI of a subgroup of opioid-treated (*n* = 12) and nontreated infants (*n* = 12) whose brains were scanned at or after postnatal day 7 and compared their PCPCS and neuromotor scores. Although opioid-treated in-

fants had significantly lower birth weight and higher lactate levels, all other demographic and clinical laboratory data characteristics were similar between the two groups (Table 5). When MRI scores were compared, opioid-treated infants had significantly lower scores than nontreated infants (Table 6). More important, opioid-treated infants whose MRIs were performed at postnatal day 7 and above (*n* = 6) have better long-term neurologic scores (PCPCS and neuromotor scores) than nontreated infants (*n* = 9; *p* < 0.03). In this subgroup, opioid-treated neonates had their MRI at age 12 ± 1 d, whereas nontreated infants had their MRI at 11 ± 1 d (NS); follow-up evaluations were performed at 13 ± 7 mo for opioid-treated infants and 19 ± 10 mo for nontreated infants (NS). Analysis of patients who were not available for long-term neurologic evaluation revealed six opioid-treated and three non-opioid-treated infants. Those who were lost to long-term neurologic evaluation shared similar clinical and laboratory characteristics with those who were available for follow-up, although treated neonates tend to have better MRI scores than nontreated neonates (BG scores: 0 ± 0 *versus* 0.33 ± 0.33; *p* = 0.157).

DISCUSSION

Opioid receptors are widely distributed in the CNS with functions that include pain modulation, cardiorespiratory control, and neuronal activity (32). Opioids are inhibitory neurotransmitters and are known to attenuate neuronal excitability by stimulating potassium ATP channels, leading to hyperpolarization (42). Cell culture studies indicate that endogenous opioids are released during hypoxia (35) and that both endogenous and exogenous opioids can protect cortical neurons from

Table 5. Clinical characteristics of neonates who underwent MRI scan at ≥7 days of life

	Received analgesia (n = 12)	No analgesia (n = 12)	p value*
Demographics			
Birth weight	3747 ± 213	3065 ± 207	0.03
EGA	39 ± 0.4	39 ± 0.2	NS
MRI exam age	12 ± 1.5	11 ± 1.2	NS
Apgar, 1 min	3.2 ± 0.7	4.1 ± 0.9	NS
Apgar, 5 min	4.6 ± 0.7	5.7 ± 0.9	NS
Laboratory data			
Blood gas			
pH	7.07 ± 0.06	7.18 ± 0.08	NS
Pco ₂	61 ± 8	43 ± 4	NS
BE	-10 ± 3	-8 ± 3	NS
HCO ₃	18 ± 2	19 ± 1	NS
Chemistry			
Sodium	129 ± 3	127 ± 11	NS
Ionized calcium	1.1 ± 0.11	1.1 ± 0.05	NS
Ammonia	77 ± 3	63 ± 9	NS
Lactate (mmol/L)	9.5 ± 5	2.6 ± 0.6	0.02
Hematology			
WBC	24 ± 5	15 ± 2	NS
nRBC	41 ± 28	24 ± 7	NS
Hb	16 ± 0.7	15 ± 0.8	NS
Hct	49 ± 2	45 ± 2	NS
Lymph%	29 ± 6	28 ± 4	NS
Platelets	200 ± 24	258 ± 18	NS

* Independent samples *t* test.

Table 6. MRI, PCPCS, and neuromotor scores of neonates who underwent MRI scan at ≥ 7 days of life

	Received analgesia (n = 12)	No analgesia (n = 12)	p value*
MRI scores			
Basal ganglia T1	0.25 $\pm$ 0.18	1.92 $\pm$ 0.54	0.018
Basal ganglia PD	0.17 $\pm$ 0.17	1.92 $\pm$ 0.54	0.008
Basal ganglia T2	0.25 $\pm$ 0.18	1.92 $\pm$ 0.54	0.018
Watershed T1	0.25 $\pm$ 0.18	2.33 $\pm$ 0.63	0.007
Watershed PD	0.00 $\pm$ 0.00	2.17 $\pm$ 0.66	0.002
Watershed T2	0.25 $\pm$ 0.18	2.33 $\pm$ 0.63	0.007
Basal ganglia/watershed T1	0.17 $\pm$ 0.11	2.08 $\pm$ 0.51	0.005
Basal ganglia/watershed PD	0.08 $\pm$ 0.02	2.08 $\pm$ 0.51	0.002
Basal ganglia/watershed T2	0.17 $\pm$ 0.11	1.91 $\pm$ 0.53	0.009
Long-term follow-up			
PCPCS score	1.17 $\pm$ 0.17	3.44 $\pm$ 0.67	0.033
Neuromotor score	0.67 $\pm$ 0.67	3.22 $\pm$ 0.74	0.038

* Mann-Whitney test.

hypoxia-induced cell death (9,10). Because hypoxia and untreated pain lead to the release of excitatory neurotransmitters such as glutamate (5,36,37), efforts to relieve pain may also attenuate neuronal excitotoxicity and cell injury or death.

Our results suggest that the use of opioids in the first week of life after perinatal asphyxia may have beneficial effects and may increase hypoxic-ischemic tolerance. Our study supports earlier pilot studies (15,16) as well as the large randomized study by Simons *et al.* (17) that showed that preemptive morphine infusion decreased neurologic injury by significantly decreasing the incidence of periventricular and intraventricular hemorrhage in preterm neonates as diagnosed by cranial ultrasound. Our findings included neuroimaging and long-term follow-up data that showed more favorable MRI and long-term neurologic outcomes scores in neonates whose MRI examinations were performed after postnatal day 7. Timing of MRI examination may be relevant because of previous observations that tissue changes in the injured brain are more advanced and more easily detected during the second week compared with the first week, resulting in better correlations between long-term outcome and acute structural abnormalities as seen with MRI (26,38).

Despite the potential benefit of opioids on asphyxiated term neonates as indicated in this study, caution must be exercised in the use of this class of medications. Available literature suggests that the routine use of opioid analgesics can be complicated by problems such as tolerance, withdrawal, and ventilator dependence (39). Very few studies have examined the long-term effects of exposure to opioids in the neonatal period. In addition, previous reports indicate that endogenous opioids can suppress DNA synthesis *in vivo* in immature cerebellar and glial cells (40), whereas exogenous opioids can exacerbate neurotoxicity in animal models of cerebral ischemia (41). More important, a recent large, randomized trial by Anand *et al.* (18) in preterm neonates suggested that although morphine infusion decreased clinical signs of pain, it did not improve neurologic outcome at 28 postnatal days. Inconsistencies in the literature regarding the effects of opioids highlight the possibility that multiple factors are involved and that opioids can be beneficial in certain circumstances but not in

others. The challenge lies in determining the nature of these circumstances and the mechanisms involved. Caution therefore must be used in the generalization of findings. Furthermore, although neonates in our opioid-treated group had significantly higher lactate levels and lower pH, higher P_{CO_2} , and higher base deficits, a selection bias still may have occurred whereby encephalopathic neonates who received opioids were those who were more responsive or had less brain injury at the time of birth compared with those who did not receive opioids. Caution is additionally warranted regarding interpretation of these findings because of important study limitations, such as a small sample size, a retrospective study design, a reduced number of infants available for long-term follow-up, and possible selection bias.

Possible mechanism of neuronal opioid effect. The precise role of morphine and other opioids when used to treat neonatal pain is complex and requires further study. Morphine mimics the cardioprotective effect of ischemic preconditioning, acting through a δ -opioid receptor-mediated mechanism (42). A similar mechanism operating in the brain might explain the findings in the current study. Both adenosine and opioids inhibit Ca^{2+} -dependent neurotransmitter release, and both seem to trigger preconditioning. In some animal studies, evidence suggests that there may be cross-talk between adenosine and opioid-receptor-mediated neuroprotection (43–45). In the CNS, adenosine and opioid receptors are physically coupled (46). Morphine is known to elevate adenosine levels *via* inhibition of adenosine kinase, resulting in locally increased adenosine concentration (46). Although morphine might function in part by increasing the levels of neuroprotective adenosine, the metabolic breakdown of adenosine by xanthine oxidase ultimately to urate generates hydrogen peroxide. Alternatively, if intracellular Ca^{2+} levels remain low as a result of opioid-mediated hyperpolarization, then calcium-dependent xanthine oxidase remains inactive and adenosine catabolism is mediated by xanthine dehydrogenase without the generation of hydrogen peroxide and other reactive oxygen species (47). In addition, low intracellular calcium levels prevent the activation of calcium-dependent destructive proteases, endonucleases, and lipases (48,49), further decreasing neurologic damage.

CONCLUSIONS

Our results suggest that the use of opioids in the first week of life after perinatal asphyxia have no significant long-term detrimental effects and may increase the brain's resistance to hypoxic-ischemic insults. However, because of potential complications and neurotoxic effects, additional studies are mandatory. Future prospective randomized trials should allow us to examine these opioid pathways to determine whether there is truly an immediate neuroprotective effect on hypoxic-ischemic brain injury and whether these agents can play a role in improving long-term outcome.

REFERENCES

1. Anand KJ 2000 Effects of perinatal pain and stress. *Prog Brain Res* 122:117–129
2. Heurteaux C, Lauritzen I, Widmann C, Lazdunski M 1995 Essential role of adenosine, adenosine A1 receptors, and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. *Proc Natl Acad Sci USA* 92:4666–4670

3. Anand KJ, Scalzo FM 2000 Can adverse neonatal experiences alter brain development and subsequent behavior? *Biol Neonate* 77:69–82
4. Pokela ML 1994 Pain relief can reduce hypoxemia in distressed neonates during routine treatment procedures. *Pediatrics* 93:379–383
5. Bennett GJ 2000 Update on the neurophysiology of pain transmission and modulation: focus on the NMDA-receptor. *J Pain Symptom Manage* 19:S2–S6
6. Olkkola KT, Hamunen K, Maunukela EL 1995 Clinical pharmacokinetics and pharmacodynamics of opioid analgesics in infants and children. *Clin Pharmacokinet* 28:385–404
7. Lee J, Kim MS, Park C, Jung EB, Choi DH, Kim TY, Moon SK, Park R 2004 Morphine prevents glutamate-induced death of primary rat neonatal astrocytes through modulation of intracellular redox. *Immunopharmacol Immunotoxicol* 26:17–28
8. Yamakura T, Sakimura K, Shimoji K 1999 Direct inhibition of the N-methyl-D-aspartate receptor channel by high concentrations of opioids. *Anesthesiology* 91:1053–1063
9. Zhang J, Gibney GT, Zhao P, Xia Y 2002 Neuroprotective role of delta-opioid receptors in cortical neurons. *Am J Physiol* 282:C1225–C1234
10. Zhang J, Haddad GG, Xia Y 2000 Delta-, but not mu- and kappa-, opioid receptor activation protects neocortical neurons from glutamate-induced excitotoxic injury. *Brain Res* 885:143–153
11. Lim YJ, Zheng S, Zuo Z 2004 Morphine preconditions Purkinje cells against cell death under in vitro simulated ischemia-reperfusion conditions. *Anesthesiology* 100:562–568
12. Chien S, Oeltgen PR, Diana JN, Salley RK, Su TP 1994 Extension of tissue survival time in multiorgan block preparation with a delta opioid DADLE (D-Ala2, D-Leu5]-enkephalin). *J Thorac Cardiovasc Surg* 107:964–967
13. Mayfield KP, D'Alecy LG 1992 Role of endogenous opioid peptides in the acute adaptation to hypoxia. *Brain Res* 582:226–231
14. Mayfield KP, D'Alecy LG 1994 Delta-1 opioid agonist acutely increases hypoxic tolerance. *J Pharmacol Exp Ther* 268:683–688
15. Barker DP, Rutter N 1996 Stress, severity of illness, and outcome in ventilated preterm infants. *Arch Dis Child Fetal Neonatal Ed* 75:F187–F190
16. Anand KJ, Barton BA, McIntosh N, Lagercrantz H, Pelausa E, Young TE, Vasa R 1999 Analgesia and sedation in preterm neonates who require ventilatory support: results from the NOPAIN trial. *Neonatal Outcome and Prolonged Analgesia in Neonates. Arch Pediatr Adolesc Med* 153:331–338
17. Simons SH, van Dijk M, van Ling RA, Roofthoof D, Tibboel, Dui ven voorden HJ, Bunkers C, Smink E, Anand KJ, van den Ander JND 2003 Routine morphine infusion in preterm newborns who received ventilatory support: a randomized controlled trial. *JAMA* 290:2419–2427
18. Anand KJ, Hall RW, Desai N, Shephard B, Bergqvist LL, Young TE, Boyle EM, Carbajal R, Bhutani VK, Moore MB, Kronsberg SS, Barton BA; NEOPAIN Trial Investigators Group 2004 Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. *Lancet* 363:1673–1682
19. Jensen F, Tsuji M, Offutt M, Firkusny I, Holtzman D 1993 Profound, reversible energy loss in the hypoxic immature rat brain. *Brain Res Dev Brain Res* 73:99–105
20. Hattori H, Wasterlain CG 1990 Excitatory amino acids in the developing brain: ontogeny, plasticity, and excitotoxicity. *Pediatr Neurol* 6:219–228
21. Simons SH, van Dijk M, Anand KS, Roofthoof D, van Ling RA, Tibboel D 2003 Do we still hurt newborn babies? A prospective study of procedural pain and analgesia in neonates. *Arch Pediatr Adolesc Med* 157:1058–1064
22. Stevens B, McGrath P, Gibbins S, Beyene J, Breau L, Camfield C, Finley A, Franck L, Howlett A, McKeever P, O'Brien K, Ohlsson A, Yamada J 2003 Procedural pain in newborns at risk for neurologic impairment. *Pain* 105:27–35
23. Nelson KB 2003 Can we prevent cerebral palsy? *N Engl J Med* 349:1765–1769
24. American Academy of Pediatrics Committee on Substance Abuse and Committee on Children with Disabilities 2000 Fetal alcohol syndrome and alcohol-related neurodevelopmental disorders. *Pediatrics* 106:358–361
25. Pasternak JF, Gorey MT 1998 The syndrome of acute near-total intrauterine asphyxia in the term infant. *Pediatr Neurol* 18:391–398
26. Barkovich AJ, Hajnal BL, Vigneron D, Sola A, Partridge JC, Allen F, Ferriero DM 1998 Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR Am J Neuroradiol* 19:143–149
27. Ashwal S, Holshouser BA, Tomas LG, Shu S, Perkin RM, Nystrom GA, Hinshaw DB Jr 1997 1H-magnetic resonance spectroscopy-determined cerebral lactate and poor neurological outcomes in children with central nervous system disease. *Ann Neurol* 41:470–481
28. Fiser DH 1992 Assessing the outcome of pediatric intensive care. *J Pediatr* 121:68–74
29. Fiser DH, Long N, Roberson PK, Hefley G, Zolten K, Brodie-Fowler M 2000 Relationship of pediatric overall performance category and pediatric cerebral performance category scores at pediatric intensive care unit discharge with outcome measures collected at hospital discharge and 1- and 6-month follow-up assessments. *Crit Care Med* 28:2616–2620
30. Hajnal BL, Sahebkar-Moghaddam F, Barnwell AJ, Barkovich AJ, Ferriero DM 1999 Early prediction of neurologic outcome after perinatal depression. *Pediatr Neurol* 21:788–793
31. Green CR, Wheeler JR, LaPorte F 2003 Clinical decision making in pain management: contributions of physician and patient characteristics to variations in practice. *J Pain* 4:29–39
32. Pasternak GW 1993 Pharmacological mechanisms of opioid analgesics. *Clin Neuropharmacol* 16:1–18
33. Deleted in proof.
34. Deleted in proof.
35. Armstead WM 1995 The contribution of delta 1- and delta 2-opioid receptors to hypoxia-induced pial artery dilation in the newborn pig. *J Cereb Blood Flow Metab* 15:539–546
36. du Plessis AJ, Volpe JJ 2002 Perinatal brain injury in the preterm and term newborn. *Curr Opin Neurol* 15:151–157
37. Hamrick SE, Ferriero DM 2003 The injury response in the term newborn brain: can we neuroprotect? *Curr Opin Neurol* 16:147–154
38. Rutherford M 2002 MRI of the Neonatal Brain. WB Saunders, London, pp 99–128
39. Dominguez KD, Lomako DM, Katz RW, Kelly HW 2003 Opioid withdrawal in critically ill neonates. *Ann Pharmacother* 37:473–477
40. Stiene-Martin A, Knapp PE, Martin K, Gurwell JA, Ryan S, Thornton SR, Smith FL, Hauser KF 2001 Opioid system diversity in developing neurons, astroglia, and oligodendroglia in the subventricular zone and striatum: impact on gliogenesis in vivo. *Glia* 36:78–88
41. Kofke WA, Garman RH, Garman R, Rose ME 1999 Opioid neurotoxicity: fentanyl-induced exacerbation of cerebral ischemia in rats. *Brain Res* 818:326–334
42. Schultz JE, Hsu AK, Gross GJ 1996 Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. *Circ Res* 78:1100–1104
43. Reshef A, Sperling O, Zoref-Shani E 2000 The adenosine-induced mechanism for the acquisition of ischemic tolerance in primary rat neuronal cultures. *Pharmacol Ther* 87:151–159
44. Li H, Henry JL 2000 Adenosine action on interneurons and synaptic transmission onto interneurons in rat hippocampus in vitro. *Eur J Pharmacol* 407:237–244
45. Peart JN, Gross GJ 2003 Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors. *Am J Physiol* 285:H81–H89
46. Halimi G, Devaux C, Clot-Faybesse O, Sampol J, Legof L, Rochat H, Guieu R 2000 Modulation of adenosine concentration by opioid receptor agonists in rat striatum. *Eur J Pharmacol* 398:217–224
47. Saugstad OD 1996 Role of xanthine oxidase and its inhibitor in hypoxia: reoxygenation injury. *Pediatrics* 98:103–107
48. Siesjo BK 1989 Calcium and cell death. *Magnesium* 8:223–237
49. Siesjo BK, Bengtsson F 1989 Calcium fluxes, calcium antagonists, and spreading depression: a unifying hypothesis. *J Cereb Blood Flow Metab* 9:127–140