IMPAIRED CEREBRAL CORTICAL GRAY MATTER GROWTH
FOLLOWING TREATMENT WITH DEXAMETHASONE FOR
NEONATAL CHRONIC LUNG DISEASE

Brendan P. Murphy, MB BCh1, Terrie E. Inder, MD2,5, Petra S. Huppi, MD3,5, Simon Warfield4, Gary P. Zientara, PhD4, Ron Kikinis, MD4, Ferenc A. Jolesz, MD4, Joseph J. Volpe, MD5.

1Division of Newborn Medicine, Harvard Medical School and Brigham & Women's Hospital, Boston, MA.

2Department of Paediatrics, University of Otago, Christchurch School of Medicine and Hospital, Christchurch, New Zealand

3Department of Pediatrics, University of Geneva, Geneva, Switzerland

4Department of Radiology, Harvard Medical School and Brigham & Women's Hospital, Boston, MA.

5Department of Neurology, Children's Hospital, Boston, MA

Running Title: Impaired cerebral cortical growth after dexamethasone therapy

Address Correspondence to:
Joseph J. Volpe M.D., Department of Neurology, Children's Hospital, 300 Longwood Avenue,
Fegan 11, Boston, MA 02115
Telephone: (617) 355-6386
Fax: (617) 738-0598
E-mail: volpe_j@a1.tch.harvard.edu
ABSTRACT. Objective. The specific aim of this study was to quantify at term the influence of postnatal systemic dexamethasone treatment for neonatal chronic lung disease on subsequent brain growth and development in premature infants without evidence of severe intraventricular hemorrhage or white matter injury.

Methods. Eighteen premature (23 to 31 weeks) infants, 7 treated with dexamethasone and 11 not treated, were studied at term, i.e., 38 to 41 postconceptional weeks by an advanced quantitative volumetric 3D magnetic resonance imaging (MRI) technique to quantify cerebral tissue volumes. Fourteen healthy term infants also were studied for comparison. A sequence of image processing algorithms was used to segment each of the MRI slices into the following separate tissue classes: cerebral cortical gray matter, basal ganglia/thalami, unmyelinated white matter, myelinated white matter, and cerebrospinal fluid, all classified on the basis of MR signal intensity and anatomical location. A final summing of voxels for each tissue class was performed to compute absolute volumes in ml.

Results. Cerebral cortical gray matter volume in premature infants treated with dexamethasone was reduced 35% when compared to gray matter volume in premature infants not treated with dexamethasone (mean SD, 130.3 ± 54.0 vs. 200.6 ± 35.1 ml, respectively). Subcortical gray matter volumes (basal ganglia and thalami) and myelinated and unmyelinated white matter volumes were not significantly different among the treated and untreated groups. However, premature infants treated with dexamethasone exhibited a reduction (30%) in cerebral tissue volume compared with total cerebral tissue volume in both the premature infants not treated with dexamethasone and the control term infants.
(312.7 \_ 43.7 vs. 448.2 \_ 50.2 and 471.6 \_ 36.4 ml respectively). This latter finding relates primarily to the decrease in cerebral cortical gray matter volume.

**Conclusions.** The data suggest an impairment in brain growth, principally affecting cerebral cortical gray matter, secondary to systemic dexamethasone therapy. Although the premature infants who received dexamethasone were smaller with more severe respiratory disease, these findings are consistent with growing evidence of a potential deleterious effect of dexamethasone on neonatal brain and subsequent neurodevelopmental outcome. This apparent deleterious effect should be taken into consideration by clinicians when weighing the potential risks and benefits of this therapy for low birth weight infants with neonatal chronic lung disease.

**Keywords:** prematurity, dexamethasone, cerebral cortical gray matter, volumetric 3D-MRI
INTRODUCTION

Modern obstetrical and neonatal management has resulted in substantial improvements in the survival rates of very low birthweight infants, particularly those infants who weigh less than 1000 grams and who are at greatest risk of later neurological deficits.\(^1\) Multiple antenatal and postnatal factors appear to contribute to an increased risk of adverse neurological outcome in such infants.\(^2\) However, identification of these factors is incomplete, and the possibility is real that commonly used therapies beneficial for one organ system are deleterious for the central nervous system.

Postnatal systemic dexamethasone therapy has been demonstrated to exert short-term beneficial effects on the neonatal lung, such as improvements in gas exchange, lung mechanics, and time until extubation and/or removal from ventilatory support.\(^3\)\(^-\)\(^4\) However, recent reports of randomized clinical trials of early postnatal steroid therapy for the prevention of chronic lung disease have raised concerns regarding an increase in the rates of adverse neurological outcome among infants who received the steroids as compared to the rates in those not so treated.\(^5\)\(^-\)\(^13\) Moreover, in one series, while the frequency of use of postnatal dexamethasone for management of neonatal chronic lung disease was reported to have risen from 43% in 1990-92 to 84% in 1993-95, rates of cerebral palsy showed an increasing trend from 10% to 16% and the rate of subnormal (<70) cognitive function increased significantly from 20% to 48%.\(^1\) Reduced head growth determined by measurements of occipito-frontal head circumference and poor weight gain also have been reported following postnatal steroid administration.\(^14\) Moreover, experimental studies have demonstrated deleterious effects on central nervous system development due to steroid
administration.\textsuperscript{15-20} Taken together, the data raise the possibility of a deleterious effect of early postnatal dexamethasone administration on development of the neonatal human brain.

The objective of this study was to use advanced quantitative volumetric 3D-magnetic resonance imaging (MRI) techniques\textsuperscript{21, 22} to assess the impact of postnatal steroid therapy on subsequent regional brain development at term in three groups of infants who had no other evidence of neurological insults. The three groups were premature infants without any evidence of intraventricular hemorrhage or white matter injury by conventional brain imaging who (1) had received dexamethasone (n=7) or (2) had not (n=11) and (3) a group of healthy term infants (n=14). We hypothesized that postnatal steroid therapy in the premature infant would result in altered brain development at term.

METHODS

Patients

Eighteen VLBW infants selected in this study (gestational age 23 to 31 weeks) were part of a larger prospective series of very low birthweight infants born at the Brigham and Women’s Hospital during 1997-1999 who were recruited for MRI studies after informed parental consent. The current study infants all had an MRI scan at term, i.e., 38 to 41 weeks' postconceptional age, and in addition no evidence on ANY cranial ultrasound or MRI study of white matter injury (defined as cystic lesions on MRI or ultrasound scan, or T2-hyperintensities in the periventricular white matter on MRI, or marked ventriculomegaly
related to periventricular white matter volume atrophy on MRI) or of intraventricular hemorrhage.

The 7 infants who were treated with dexamethasone were smaller (757 ± 112 vs. 1259 ± 218g, p<0.001) and more immature than the 11 infants who did not receive dexamethasone (25.4 ± 2.0 vs. 28.8 ± 1.5 weeks, p<0.001). The infants who did not receive dexamethasone were discharged at earlier corrected gestational ages, i.e., 36.6 ± 1.3 weeks, compared to 40.2 ± 1.2 weeks in the dexamethasone group (p=0.003), and had slightly lighter discharge weights (no dexamethasone, 2212 ± 192; dexamethasone, 2450 ± 322 grams, p=0.2). The dexamethasone infants trended toward lower weight gains per day, 16.4 ± 2.6 grams per day, compared with 18.8 ± 1.9 grams per day in the no dexamethasone group (p=0.21). On correcting for weight in determination of rate of weight gain (grams per kg per day), this difference was actually reversed due to the smaller infants in the dexamethasone group (dexamethasone, 22.1 ± 4.2 gms/kg/day, no dexamethasone, 17.6 ± 4.3 gms/kg/day, p=0.09). The pattern of growth also was slightly different between the two groups, with initially poorer growth in the infants on dexamethasone followed by much larger weight gains. However, there was no evidence of significantly poor weight gains at any stage in the dexamethasone treated infants. There was no significant difference between these two groups during their hospital course with respect to head growth as assessed by measurement of the occipito-frontal circumference (OFC). The CRIB score, which utilizes birthweight, gestational age, congenital abnormality, base excess, maximum and minimum appropriate FiO2 to calculate a total score (maximum score 23) and predicts mortality and morbidity, was determined on all infants in the first 12 hours of life as a measure of severity of early illness. The CRIB score was higher in the premature infants.
that later received dexamethasone (median 6, range 2-8), compared with the premature infants that did not receive dexamethasone (median 3, range 1-5, \( p<0.05 \)).

The infants exhibited a variation in the starting time, dose and length of their courses at the discretion of the consultant neonatologist. The steroid doses were carefully investigated by analysis and recording against the drug sheet of the infant with cross checking and recording of the current weight of the infant. Five of the seven infants began their course in the first 14 days of life, with four of the seven infants receiving conventional courses of approximately 42 days. One of these four infants had this course extended to 57 days due to a fluctuating respiratory course. No multiple courses were given. Thus, the mean duration of dexamethasone therapy was 28 ± 22 days, (range 6-57 days), with infants having received a median dose of 0.25 mg/kg/day (range 0.19-0.90 mg/kg/day). Infants had been off dexamethasone therapy for 54 ± 18 days (range 26-72 days) at the time of their MRI study at 38 to 41 weeks PCA.

Healthy control term infants (\( n=14 \)) born between 39 and 41 weeks PCA were selected from the neonatal nurseries to undergo MRI, after parental consent. The mean gestational age of the term infants was 40.2 ± 0.6 weeks, and these infants had their MRI scans in the first two days of life. Thus, quantitative volumetric analysis was performed at term PCA in a total of 18 premature and 14 control term infants.

### 3D Volumetric MRI

For the MR studies, all infants were accompanied by a physician specializing in newborn care to the MRI suite after informed parental consent for the study had been
obtained. They were positioned in a special crib in the scanner and monitored with ECG and pulse oximetry (MR Equipment Corp, Bay Shore, NY). Earmuffs were used to minimize noise exposure. No sedation was used for any of the studies. The Institutional Review Board of the Brigham and Women's Hospital approved this longitudinal cohort study of the influence of perinatal factors, including steroid therapy, on neurological outcome in the premature infant.

MRI scanning was performed with a 1.5 T GE Signa System (GE Medical Systems, Milwaukee, WI). Images were acquired and analyzed by an established imaging sequence and post-acquisition processing protocol\textsuperscript{21, 22} to reduce imaging system noise, to reformat the axial images to coronal orientation, to classify tissue according to signal behavior and anatomical localization, and finally to summate voxels for each tissue class to compute absolute volumes in milliliters (ml).

**Statistical Analyses**

Statistical analyses were performed with SyStat and SPSS for Windows (SPSS Inc, Chicago, IL, USA) to compare the volume of cerebral tissues between the three groups by one way analysis of variance (ANOVA) with pair-wise multiple comparison procedures. To isolate the group or groups that differ(s) from the others, a multiple comparison procedures using the Tukey test was performed. Perinatal variables were analyzed by t-test for parametric data and Mann-Whitney rank sum test for parametric and non-parametric data.
Among the premature infants, dexamethasone therapy was the main variable analyzed. To adjust for all potential confounding variables simultaneously, continuous variables were analyzed by multiple linear regression, and dichotomous variables were analyzed by logistic regression. In these analyses, variables analyzed included CRIB score, birthweight, gestational age, sex and weight gain. There was no evidence of white matter injury or intraventricular hemorrhage in any infants, thereby removing the major potential confounding influence of brain injury on cerebral outcome in this study. The other major confounding variables controlled for were gestational age and the CRIB score, as a measure of severity of early illness.\textsuperscript{24} Birthweight was not included in the analysis due to its colinearity with the other factors.

**RESULTS**

Quantitative volumetric MR analysis was performed at 38 to 41 weeks PCA in the 7 premature infants who had been treated with a systemic course of dexamethasone for neonatal chronic lung disease, the 11 premature infants who never received dexamethasone, and the 14 healthy term infants. The findings were as follows.

**Gray Matter Volumes**

Premature infants treated with systemic dexamethasone for neonatal chronic lung disease had a marked reduction at term in cerebral cortical gray matter volume compared with cortical gray matter volumes both in premature infants not treated with dexamethasone and in control term infants (Table 1 and Figure 1; Tukey F = 15.3, \(p < 0.001\); dexamethasone vs. no dexamethasone, difference of means = 70.3, \(p < 0.001\);
dexamethasone vs. term, difference of means = 88.6, \( p < 0.001 \); no dexamethasone vs. term, difference of means 18.2, \( p = 0.4 \). Cerebral cortical gray matter volume was reduced by 35% in premature infants treated with dexamethasone relative to volume in premature infants not treated with dexamethasone. In contrast, there was no significant difference in cerebral cortical gray matter volume in premature infants who had not been treated with dexamethasone relative to healthy control term infants. After adjusting for gestational age and CRIB score, cerebral cortical gray matter volume remained significantly lower in premature infants who had received postnatal dexamethasone therapy (mean difference 65.3, 95%CI 4.3 to 114.58). Subcortical gray matter volumes (basal ganglia and thalami) were not significantly different between the three groups of infants (see Table 1).

**White Matter Volumes: Unmyelinated and Myelinated**

There was a significant reduction in myelinated white matter volumes at term in both premature infants treated with dexamethasone and untreated premature infants, relative to the volumes in the healthy term infants (Tukey \( F = 12.2, p < 0.001 \); dexamethasone vs. no dexamethasone, difference of means = 5.6, \( p = 0.39 \); dexamethasone vs. term, difference of means = 18.0, \( p < 0.001 \); no dexamethasone vs. term, difference of means 12.4, \( p = 0.004 \)). However, there was no significant difference in myelin volumes between premature infants previously treated with dexamethasone and those premature infants that did not receive dexamethasone. The volumes of unmyelinated white matter did not differ significantly between the three groups (see Table 1).

**Cerebrospinal Fluid Volumes**
There was a significant increase in CSF volumes in both premature infants treated with dexamethasone and untreated premature infants, relative to the volumes in the healthy control infants (Tukey F = 8.2, p = 0.002; dexamethasone vs. no dexamethasone, difference of means = 4.3, p = 0.87; dexamethasone vs. term, difference of means = 22.9, p =< 0.025; no dexamethasone vs. term, difference of means 27.2, p = 0.002). However, there was no significant difference in CSF volumes between premature infants previously treated with dexamethasone and those premature infants not treated.

**Total Intracranial and Total Cerebral Tissue Volumes**

Total cerebral tissue volume (a summation of cerebral cortical gray matter, basal ganglia and thalami, myelinated and unmyelinated white matter, and CSF, both intraventricular and extracerebral) was also calculated, as an overall assessment of brain growth. At term, premature infants treated with systemic dexamethasone had a marked reduction in total cerebral tissue volume, relative to volumes in both premature infants not treated with dexamethasone and control term infants (Table 1; Tukey F = 11.1, p < 0.001; dexamethasone vs. no dexamethasone, difference of means = 110.7, p < 0.001; dexamethasone vs. term, difference of means = 106.0, p < 0.001; no dexamethasone vs. term, difference of means 4.7, p = 0.9). Total cerebral tissue volume was reduced 22% in the premature infants treated with dexamethasone relative to the premature infants not treated with dexamethasone. After adjustment for gestational age and CRIB score total cerebral volume remained lower in premature infants who had received postnatal dexamethasone (mean difference 111.2, 95%CI –3.4 to 148, NS). However, with this analysis, although the mean difference in total cerebral tissue volume between these two groups of premature
infants remained unchanged (110.7 vs. 111.2 ml), the increased confidence intervals associated with small numbers of infants in each group resulted in loss of statistical significance.

Total cerebral tissue volume (total intracranial volume excluding CSF volume) was also analyzed. Premature infants who received dexamethasone had significantly lower mean total cerebral tissue volumes compared with premature infants who had not received dexamethasone (Table 1); dexamethasone 312.7 ± 43.7 ml; no dexamethasone 448.2 ± 50.2 ml; term controls 471.6 ± 34.6 ml, ANOVA p<0.001). As with total intracranial volume adjustment for gestational age, CRIB score did not affect the magnitude of the reduction in total cerebral tissue volume between the two groups (dexamethasone vs. no dexamethasone, 131 ml) but increased the confidence intervals such that statistical significance no longer remained.

**DISCUSSION**

This study utilizing advanced MRI techniques documents for the first time in vivo impaired cerebral development at term in premature infants exposed to postnatal dexamethasone therapy for neonatal chronic lung disease. The impairment is demonstrated specifically by a reduction in cerebral cortical gray matter volume. Because cerebral cortical gray matter volume is the single largest cerebral tissue class and increases more dramatically than any other class during the time period of this study, the additional finding of a reduction in total brain volume in the dexamethasone treated patients is not surprising. This study selected premature infants with no evidence of brain injury on MRI and ultrasonography to minimize potential confounding factors. This selection was
important because we have previously demonstrated with these quantitative MRI techniques that the presence of white matter injury is followed by impaired cortical gray matter development at term. However, despite the apparent removal of this potential confounder it is important to note that there are limitations in this study due to the small sample number and the potential sample group bias. The steroid-treated infants in this study were smaller and more premature than the untreated infants, raising the possibility that the differences in volumes at term were related to prematurity, lower birth weight and severity of illness, rather than to dexamethasone therapy. The CRIB score was chosen to control for severity of illness because it is an established index of neonatal risk and has been found to be more useful than the SNAP score. Logistical regression analysis to control for gestational age and CRIB score had no impact on the magnitude of difference in cerebral cortical gray matter volumes between these two groups of premature infants.

Our finding of a reduction in cortical gray matter volume in vivo are consistent with concerning findings of recent randomized clinical trials of early postnatal steroid therapy for the prevention of chronic lung disease. These reports document an increase in the rates of cerebral palsy among children who received the steroids as compared to controls, and one report suggests a disturbance in cognitive function in the steroid–treated infants. A reduction in total cerebral cortical gray matter is also consistent with the recent report of a reduction in head growth in infants receiving postnatal steroids. Our findings are also supported by experimental models that demonstrate deleterious central nervous system developmental effects due to steroid administration.
In conclusion, these findings obtained with advanced MRI techniques provide *in vivo* evidence of altered cerebral development in premature infants who received postnatal dexamethasone therapy. These findings are consistent with both experimental and human clinical studies. A prospective randomized controlled trial incorporating neurodevelopmental outcome with volumetric MRI as primary endpoints is required to fully evaluate the structural and functional impact of postnatal dexamethasone treatment for chronic lung disease. In the interim we conclude that the alteration in brain development demonstrated in this study should be taken into consideration by clinicians when weighing the potential risks and benefits of this therapy for premature infants for treatment of neonatal chronic lung disease.
REFERENCES


8. Tarnow-Mordi W, Mitra A. Postnatal dexamethasone in preterm infants is potentially lifesaving, but follow up studies are urgently needed [editorial]. *BMJ*. 1999; 319:1385-6.


Table 1. Quantitative 3D-MRI Volumes of Cerebral Tissues at Term in Premature Infants at Term Treated with Dexamethasone for Neonatal Chronic Lung Disease, Premature Infants at Term never Treated with Dexamethasone and Healthy Term Infants

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Volume (ml)*</th>
<th></th>
<th></th>
<th>ANOVA Pairwise Multiple Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premature at Term DEX (n=7)</td>
<td>Premature at Term No DEX (n=11)</td>
<td>Healthy TERM (n=14)</td>
<td></td>
</tr>
<tr>
<td>Cerebral cortical gray matter</td>
<td>130.3 ± 54.0</td>
<td>200.6 ± 35.1</td>
<td>218.8 ± 21.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal ganglia/thalamus</td>
<td>23.2 ± 13.2</td>
<td>25.5 ± 11.8</td>
<td>24.7 ± 8.9</td>
<td>0.91</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>55.8 ± 19.6</td>
<td>60.1 ± 21.4</td>
<td>32.9 ± 13.5</td>
<td>0.87</td>
</tr>
<tr>
<td>Myelinated white matter</td>
<td>9.6 ± 3.9</td>
<td>15.2 ± 8.5</td>
<td>27.6 ± 10.1</td>
<td>0.39</td>
</tr>
<tr>
<td>Unmyelinated white matter</td>
<td>178.6 ± 27.3</td>
<td>206.9 ± 41.1</td>
<td>200.3 ± 32.9</td>
<td>0.25</td>
</tr>
<tr>
<td>Total cerebral tissue volume</td>
<td>312 ± 43.7</td>
<td>448 ± 50.2</td>
<td>471 ± 34.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Total intracranial volume</td>
<td>397.5 ± 61.1</td>
<td>508.2 ± 64.1</td>
<td>503.5 ± 39.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.
DEX, treated with dexamethasone; 3D-MRI, three dimensional volumetric magnetic resonance imaging; ANOVA, analysis of variance.
FIGURE LEGEND

Fig. 1. Cerebral cortical gray matter volumes in term infants (n=14), premature infants at term never treated with postnatal dexamethasone for neonatal chronic lung disease (n=11) and premature infants at term previously treated with postnatal dexamethasone for neonatal chronic lung disease (n=7) (expressed as medians with 25th/75th centile box, 10th/90th centile error bars and outliers).