Abnormal Development of the Cerebellar Vermis in Children Prenatally Exposed to Alcohol: Size Reduction in Lobules I–V


Abnormalities of the cerebellar vermis have been well documented in animal models of fetal alcohol syndrome. At this point, it is not known if the same brain region is affected in humans prenatally exposed to alcohol. In this study, the area of the cerebellar vermis was measured from brain magnetic resonance images of 9 children and young adults with prenatal alcohol exposure and 24 control subjects in the same age range. Six of the exposed children met standard criteria for fetal alcohol syndrome. The remaining three subjects had significant histories of prenatal exposure to alcohol, but did not have enough of the classic facial features for the diagnosis. For each subject with a suitable midsagittal section, three vermal areas were circumscribed: anterior vermis (vermal lobules I–V), posterior vermis (vermal lobules VI and VII), and the remaining vermal area (including lobules VIII–X). Statistical analyses revealed that the anterior region of the vermis was significantly smaller in subjects with prenatal alcohol exposure, whereas the posterior region and the remaining vermal area did not differ between groups. Previous findings from an animal model of neonatal alcohol exposure have documented Purkinje cell loss in vermal lobules I–V and IX–X, with notable sparing in lobules VI–VII. Thus, the results of both studies indicate similar patterns of abnormal brain development in the anterior vermal region, with apparent sparing in the posterior vermal region. Our findings, for the first time, suggest that regionally specific Purkinje cell death may also occur in humans prenatally exposed to alcohol.

Key Words: Fetal Alcohol Syndrome, Cerebellum, Vermis, Magnetic Resonance Imaging, Prenatal Alcohol Exposure.

The first reported published report of structural brain defects observed in an infant with fetal alcohol syndrome (FAS) occurred two decades ago. The postmortem evaluation of that infant revealed microcephaly, migration anomalies, and agenesis of the corpus callosum. Since that time, few studies have been published documenting the gross morphological abnormalities observed in persons exposed to alcohol prenatally. Carlton reviewed the known autopsy studies of FAS that included a total of 16 subjects. The neuropathology found in these subjects ranged widely. Five of the 16 subjects suffered from severe central nervous system (CNS) disorganization, and 4 of these 5 subjects were noted to have dysgenesis of the cerebellum. Although there is some concern that the children who have died are not representative of children with FAS who survive, in vivo neuroimaging studies in part support the postmortem findings. A recent study using magnetic resonance imaging has confirmed gross neuroanatomical abnormalities in four children prenatally exposed to alcohol. Hypoplasia of the cerebellum was noted in all four children prenatally exposed to alcohol (2 with FAS and 2 with prenatal exposure to alcohol); cerebral hypoplasia and proportional decreases in the basal ganglia were also noted in these subjects.

In the 20 years since FAS was first reported, surprisingly few in vivo and autopsy studies have been published. However, studies using animal models of FAS are numerous and provide invaluable information about alcohol's teratogenic effects on the CNS. A few of the CNS effects observed in rats after perinatal exposure to alcohol are microencephaly, delayed myelination, and alterations in hippocampal mossy fiber branching patterns. Of particular relevance to the current report are studies that examine cerebellar Purkinje cell death in rats prenatally exposed to alcohol. For example, Goodlett et al. demonstrated that the number of Purkinje cells was significantly reduced in earlier maturing regions of the cerebellar vermis (lobules I–V and lobules IX and X) after a single critical day of neonatal alcohol exposure. Notably, the Purkinje cells in the later maturing neocerebellar vermis (lobules VI and VII) were apparently spared. In these animals, the overall weight of the cerebellum at postnatal day 10 was reduced 25–28% relative to control animals. The authors speculate that the regional specificity of the effects of alcohol exposure is related to maturational factors, such as the timing of dendritic outgrowth in the Purkinje cells of the cerebellum. At this point, it is difficult to determine whether similar processes are occurring in humans prenatally exposed to alcohol.

The current study was designed to examine the effects of
prenatal alcohol exposure on the cerebellar vermis. Findings from animal models and autopsy studies discussed herein lead us to hypothesize that we would be able to observe, in vivo, regionally specific vermal abnormalities in subjects prenatally exposed to alcohol relative to controls. We hypothesized that the region most affected in the rat perinatally exposed to alcohol (vermal lobules I–V) would be the most affected in our group of children and young adults prenatally exposed to alcohol. In vivo quantitative analyses such as these provide valuable information about the teratogenic effects of alcohol on the brains of a group of subjects who could otherwise be evaluated only at autopsy.

METHODS

Subjects

The patient group consisted of nine children and young adults between the ages of 8 and 22 years (mean age = 15.1 years; 3 females and 6 males) who had been prenatally exposed to alcohol. They were assessed as part of a larger project studying the effects of prenatal alcohol exposure. All of the children and young adults had histories of behavioral problems, cognitive impairment, and heavy prenatal alcohol exposure. Six of them had the characteristic facial appearance that allowed them to be diagnosed with FAS. The other three subjects did not have the facial appearance to allow a diagnosis of FAS, but instead are referred to as subjects with prenatal exposure to alcohol (PEA). Two of the FAS subjects in this study were also described in a previous report. Whereas specifics about the amount of alcohol consumed by their mothers are not available, all nine were also described in a previous report. The other three subjects did not have the facial appearance that allowed them to be diagnosed with FAS. They were chosen from a larger group of control subjects and matched as closely as possible to the gender and age (mean difference < 1 year) distribution of the patient population. Control subjects 21 years of age and younger were recruited as normal controls for a large, multidisciplinary neurodevelopmental research center. Subjects between 21 and 24 years of age participated as controls in neuropsychiatric studies. All control subjects were screened by medical and psychiatric interviews (of parents or of subjects themselves) for evidence of significant disease, substance abuse, developmental intellectual abnormality, or psychiatric illness. Informed consent was obtained from all subjects and from their parents when appropriate.

It should be noted that the original sample included 12 FAS and 27 control subjects with technically adequate imaging data (e.g., no movement artifact). Three FAS and three control subjects were subsequently excluded because the primary and/or prepyramidal fissures could not be identified because of minor head rotation in the imaging plane.

Imaging Protocol and Analysis

Magnetic resonance images were acquired with a 1.5 T superconducting magnet (Signa; General Electric, Milwaukee, WI). All of the control subjects and 7 of the 9 ALC subjects were imaged without sedation. A multisection, sagittal T1-weighted (repetition time = 600 msec; echo time = 20 msec) sequence was obtained for each subject with images centered at the midsagittal plane. Slice thickness was 5 mm with a 2.5 mm gap between sections.

RESULTS

A significant effect for Region was observed ($F_{2,62} = 47.70; p < 0.01$), not surprisingly, which means only that the brain regions measured differ in size. The main effect for Group approached significance in the analysis ($F_{1,31} = 2.06; p = 0.16$). The result of greatest interest was a Group × Region interaction ($F_{2,62} = 6.30; p < 0.01$), which suggests that regionally specific differences exist between the groups on vermal area measures. Follow up t tests indicated that only one region actually differed between the two groups, as can be seen in Table 1. Vermal lobules I–V were significantly smaller in the ALC subjects. Group differences on measures of vermal lobules VI and VII and the remaining vermal area did not reach significance. In fact, the group means on these measures were nearly identical. Data for each subject are also presented in Fig. 2. As can be seen, only two of the ALC subjects fall within the range of the control group for the area measure of vermal lobules I–V. Complete overlap is observed between the two groups on the area measures of vermal lobules VI and VII and the remaining vermal area.
Fig. 1. Illustration of the vermal measurement technique. The top image displays the defined regions on the midsagittal section from a 15-year-old ALC subject. Shown in light gray is the anterior vermal region (lobules I–V), in the medium shade of gray are vermal lobules VI and VII, and in black is the remaining cerebellar vermis (including lobules VIII–X, and other cerebellar structures). The bottom two images are from a 14-year-old control subject. On the left, vermal regions are defined and color-coded the same as the ALC subject. On the right, the primary and prepyramidal fissure boundaries can be clearly identified.

<table>
<thead>
<tr>
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<th>CON (n = 24)</th>
<th>ALC (n = 9)</th>
<th>t tests (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermal areas I–V</td>
<td>529 (71)</td>
<td>416 (50)</td>
<td>t = 4.38 (p &lt; 0.001)</td>
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<tr>
<td>Anterior vermis</td>
<td>391 (71)</td>
<td>398 (105)</td>
<td>t = 0.243 (p = 0.81)</td>
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<tr>
<td>Vermal areas VI and VII</td>
<td>580 (108)</td>
<td>580 (84)</td>
<td>t = 0.009 (p = 0.99)</td>
</tr>
<tr>
<td>Remaining vermis</td>
<td>1500 (187)</td>
<td>1393 (193)</td>
<td>t = 1.44 (p = 0.16)</td>
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Values are mean areas (±SD). CON, control.

**DISCUSSION**

The results of this study indicate that the area of vermal lobules I–V is disproportionately reduced in size in children prenatally exposed to alcohol compared with matched controls. Vermal lobules VI and VII and the remaining cerebellar vermis, however, seem to be of normal size in the ALC group. Postmortem studies of FAS to date have not examined the cerebellar vermis at the cellular level. Thus, it is difficult to confirm that our in vivo observation of reductions in the size of vermal lobules I–V is in fact caused by regionally specific Purkinje cell death in this population. However, our findings are consistent with findings of regionally specific Purkinje cell depletion in the vermal lobules of rats perinatally exposed to alcohol. The striking similarity in regional patterns of cell death observed in rats and size reductions observed here in humans leads us to conclude that there may be some relationship between Purkinje cell loss and the size of the cerebellar vermis observable in vivo.

In earlier publications, Mattson et al. reported that the total volume of the cerebellum was reduced in children prenatally exposed to alcohol relative to control subjects. Although the total area of the vermis was not significantly reduced in the ALC subjects in the present study, it was somewhat smaller in this group; this comparison may have reached significance with a larger sample. Also, the sensitivity of the measure of total vermal area may have been affected by the unavoidable inclusion of the cerebellar tonsil in the region of the remaining vermal area in some
subjects. Interestingly, there was a group difference ($r = 0.38; p < 0.05$) in the size of the vermis when the anterior and posterior regions were summed, excluding the remaining vermal area. Future studies of vermal morphology in this population should include measures of total cerebellar volume to elucidate this issue further.

Studies of other types of developmental disorders (such as autism, Down syndrome, and Williams syndrome) have shown patterns of cerebellar vermal dysmorphology that are distinct from the pattern observed in children prenatally exposed to alcohol. Using similar methods, Courchesne et al. observed size reductions in vermal lobules VI and VII in patients with autism, with apparent sparing in the anterior vermis. The authors hypothesized that their finding of a size reduction in only the neocerebellum could be related to the timing of Purkinje and granule cell migration to that area. Jernigan and Bellugi found that children with Williams syndrome had neocerebellar vermal regions (lobules VI and VII) that were actually larger than those of control subjects. The two Down syndrome subjects in their study, conversely, seemed to have hypoplasia of the anterior vermis (lobules I–V), with relative sparing of neocerebellar regions, which is similar to our findings in children prenatally exposed to alcohol. Perhaps the differences in cerebellar vermal morphology are related to the distinctive pattern of behavioral and cognitive anomalies that characterize each of these groups of developmentally disabled persons. However, at this point, the functional significance of the regional vermal size reduction is unclear. Further studies correlating neuropsychological and neuroanatomical results are in progress.

REFERENCES