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Research Report

Neuronal apoptosis and gray matter heterotopia in microcephaly produced by cytosine arabinoside in mice

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ABSTRACT

Primary microcephaly can be accompanied by numerous migration anomalies. This experiment was undertaken to examine the pathogenesis of gray matter heterotopia and microcephaly that is produced after administering cytosine arabinoside (Ara-C) to mice. Pregnant mice were intraperitoneally injected with Ara-C at 30 mg/kg body weight on days 13.5 and 14.5 of gestation, and then their offspring were examined. On embryonic day 15.5, in the ventricular zone of the cingulate cortex, the neuroepithelial cells lacked BrdU immunoreactivity. Nestin-immunoreactive radial glial fibers and calretinin-positive subplate fibers were disrupted. TUNEL reaction was remarkable throughout the cerebral hemisphere. Subcortical heterotopia in the cingulate cortex and subependymal nodular heterotopia in the dorsolateral part of the lateral ventricles became detectable by the first day after birth. Thirty-two days after birth, microcephaly was apparent; subcortical heterotopia was observed to have increased in size while it was still located in the frontal and cingulate cortices. This experiment demonstrated that Ara-C induces neuronal apoptosis throughout the cerebral hemisphere. The immunohistochemical characteristics in the gray matter heterotopia suggest that both the subcortical and the subependymal heterotopias were formed by neurons originally committed to the neocortex. We conclude that the gray matter heterotopia that accompanies the microcephaly was produced by a disturbance of radial, tangential, and interkinetic neuronal migrations due to the toxicity of Ara-C in the immature developing brain.

1. Introduction

Microcephaly is clinically defined as a reduced occipital–frontal head circumference of less than −3 SD (given as a standard deviation score compared to age- and gender-matched controls) (Woods, 2004). It can be divided into primary microcephaly, in which the brain fails to grow to the correct size during pregnancy, and secondary microcephaly, in which the brain is the expected size at birth but subsequently fails to grow normally (Woods, 2004). Primary microcephaly is the result of a variety of genetic and chromosomal defects, as well as environmental insults, such as irradiation, infection, and chemical agents (Menkes et al., 2006). Microcephaly can be also accompanied by numerous migration anomalies, including schizencephaly, lissencephaly, pachygyria, polymicrogyria, and gray matter heterotopia (Menkes et al., 2006).

The nucleoside analog cytosine arabinoside (1-β-D-arabinofuranosylcytosine, Ara-C) is one of the most effective

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chemotherapeutic agents in the treatment of acute myelogenous leukemia and a variety of other hematologic malignancies (Besirli et al., 2003). Ara-C can inhibit DNA synthesis in proliferating cells by being incorporated into elongating DNA strands and causing retardation of DNA elongation, as well as chain termination (Besirli et al., 2003). The administration of Ara-C to mice during pregnancy gives rise to microcephaly with dysgenetic cytoarchitecture and gray matter heterotopia in their offspring (Ono-Yagi et al., 2000). This process leads to hypoplasia of the part of the fetal brain undergoing neuronal proliferation at the time of the injection, thus resulting in microcephaly. Microcephaly is therefore likely due to a reduced production of neurons. However; the exact mechanism of the neuronal migration disorders that accompany microcephaly remains to be elucidated. This experiment was undertaken to examine the pathogenesis of gray matter heterotopia that is accompanied by microcephaly and it is produced by Ara-C in mice.

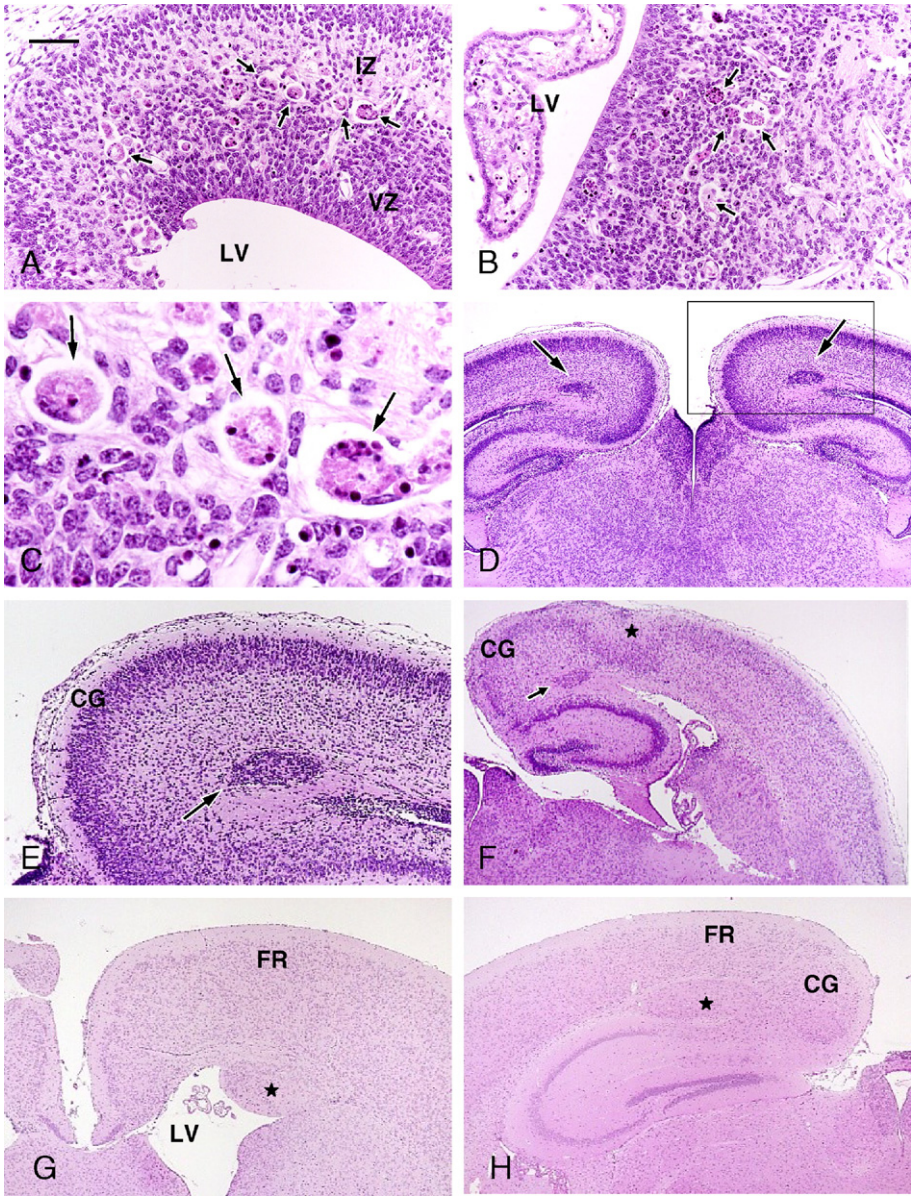

Fig. 1 – The development of the disorganized cerebral cortex in the Ara-C-injected brains. Hematoxylin and eosin (HE) stain. (A–C) E15. Clusters of degenerative neurons in the ventricular and intermediate zones (arrows in panel A), and the ganglionic eminence (arrows in panel B). Note the nuclear condensation and fragmentation (arrows in panel C), thus suggesting apoptosis. (D, E) P0. Bilateral small subcortical heterotopia in the cingulum (arrows in panel D), and its higher magnification (E, showing rectangle in panel D). This heterotopia in the cingulum (arrow in panel E) was located in the caudal part of the cerebral hemisphere. (F) P3. Note the abnormal laminar structure in the frontal cortex (star in panel F) overlying the subcortical heterotopia (arrow in panel F). (G) P13. Subependymal nodular heterotopia (star) in the dorsolateral part of the lateral ventricles. (H) P32. Subcortical heterotopia (star), increased in size, located in the frontal and cingulate cortices. IZ = intermediate zone; VZ = ventricular zone; LV = lateral ventricles; CG = cingulate cortex; FR = frontal cortex. Scale bar: (A, B) 60 μm; (C) 15 μm; (D, F–H) 300 μm; (E) 120 μm.