Neurobiology of Disease 32 (2008) 499–509

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Combined effects of prenatal inhibition of vasculogenesis and neurogenesis on rat brain development

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ARTICLE INFO

Article history:
Received 4 June 2008
Revised 21 August 2008
Accepted 9 September 2008
Available online 30 September 2008

Keywords:
Blood–brain barrier
Cerebrovascular function
Angiogenesis
In utero
Epilepsy
Hydrocephalus

ABSTRACT

Malformations of cortical development (MCD) are one of the most common causes of neurological disabilities including autism and epilepsy. To disrupt cortical formation, methylazoxymethanol (MAM) or thalidomide (THAL) has been used to affect neurogenesis or vasculogenesis. Although previous models of MCD have been useful, these models primarily attack a single aspect of cortical development. We hypothesized that simultaneous prenatal exposure to MAM or THAL will lead to the development of a novel and specific type of brain maldevelopment. Rats were prenatally exposed to MAM and THAL. At early postnatal days, brains displayed abnormal ventricular size and hemispheric asymmetry due to altered brain water homeostasis. The postnatal brain was also characterized by gliosis in regions of focal leakage of the blood brain barrier. These morphological abnormalities gradually disappeared at adult stages. Although the adult MAM-THAL rats showed normal cortical morphology, abnormal hippocampal connectivity and mossy fiber sprouting persisted well into adulthood.

Introduction

Dysplastic lesions are a common cause of epilepsy and developmental delay, and the associated clinical spectrum of epilepsy is broad (Schwartzkroin and Walsh, 2000; Sisodiya, 2004; Battaglia and Bassanini, 2006). Other consequences of malformations of cortical development (MCD) are cognitive impairment, spasticity, congenital hemiparesis and visual field defects. The overall progression to MCD occurs during cortical formation and results from disrupted neuronal and glial migration. While these neuroglial mechanisms have been well characterized, vasculogenesis also plays an important role in brain development (Hallene et al., 2006; Bassanini et al., 2007). For example, while the effect of prenatal exposure to MAM is one affecting neurogenesis (Bardosi et al., 1985a; Battaglia et al., 2003a,b; Fonnum and Lock, 2004), MAM exposure produces a similar result as prenatal exposure to thalidomide, which causes MCD by primarily affecting vasculogenesis (Franks et al., 2004; Hallene et al., 2006).

Abnormalities of cortical structure, generated as a product of aberrant patternig of brain development, are often associated with but not limited to seizures and chronic epileptic condition (Barkovich et al., 1992; Sancini et al., 1998). Both motor and cognitive delays are frequently impaired as a consequence of MCD. Teratogen exposure (drugs and/or environmental poisons), maternal trauma, infection and stroke are all factors that might interfere with the normal progression of brain development and give rise to aberrant patterns of cortical structure. Acquired cortical dysplasia appears to result from a progressive process (i.e., that may continue beyond the time of insult), affecting not only the primary region of lesion but also surrounding 'normal' tissue (Marin-Padilla, 2000).

Injection of MAM acetate into pregnant rats at day 14/15 of gestation exposes the fetuses to an agent that disrupts cell proliferation at a time when neocortical and hippocampal neurons and glia are being formed (Bassanini et al., 2007; Battaglia et al., 2003b; Chevassus-Au-Louis et al., 1999). The most salient result of this manipulation is some cortical thinning and the generation of cortical heterotopia—abnormally placed clusters of cortical neurons with abnormal intrinsic properties, altered receptor characteristics, and aberrant connectivity (Chevassus-Au-Louis et al., 1999; Colacitti et al., 1998, 1999). According

Table 1
Summary of the animals and drugs used for the experiments (5 control dams were also used)

Drug used	Dosage	Interval	Number of dams	Number of pups
MAM	15 mg/kg of maternal body weight	12 h	3	39
THAL	30 mg/kg maternal body Weight	12 h	3	32
MAM+THAL (High dose)	MAM: 15 mg/kg of maternal body weight THAL: 30 mg/kg maternal body weight	12 h	3	0
MAM+THAL (Low dose)	MAM: 7.5 mg/kg of maternal body weight THAL: 15 mg/kg maternal body weight	12 h	5	63

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2008.09.007

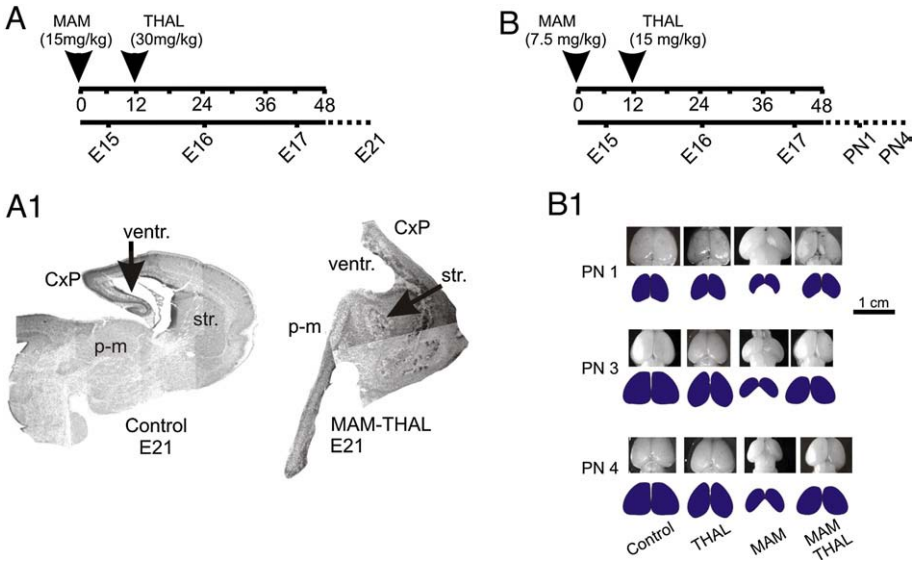

Fig. 1. Schematic representation of the time course of MAM and THAL exposure paradigms and analysis of gross anatomical alterations in control, MAM, THAL and MAM-THAL rat brain. (A) Protocol of the prenatal lethal treatment at day E15 with MAM in sterile saline (15 mg/kg maternal body weight) and THAL in DMSO (30 mg/kg maternal body weight), administered 12 h apart. (A1) Morphology of E21 brain sagittal sections of control and MAM-THAL rats. *CxP*: cortical plate; *Str*: striatum; *Ventr*: ventricle; *p-m*: pons–medulla. Note the gross morphological alterations in treated animals. See text for details. (B) Protocol of treatment with MAM (7.5 mg/kg maternal body weight) and THAL in DMSO (15 mg/kg maternal body weight), 12 h apart. (B1) Gross anatomical alterations of brain in control, MAM, THAL and MAM-THAL rats at postnatal day 1, 3 and 4. Note the significant microcephaly in MAM brain, and the less pronounced delay in growth in MAM-THAL animals.

to a recent study, the affected animals have spontaneous seizures (Harrington et al., 2007). A number of laboratories have shown that these animals have lower seizure thresholds than normal controls in response to a variety of epileptogenic agents (flurothyl, hyperthermia, kindling, etc) (Baraban and Schwartzkroin, 1996).

In addition to neurogenesis, vasculogenesis also plays an important role in MCD and epilepsy or autism. Some reports have emphasized that MAM-treated animals display an array of pathological features including vascular dysgenesis (Bardosi et al., 1985a,b; Bardosi et al., 1987) and prenatal exposure to thalidomide is a recognized cause of autism (Trottier et al., 1999). E15 exposure in rats to the angiogenesis inhibitor thalidomide inhibits vasculogenesis in vitro at concentrations that result in significant morphological alterations in cortical and hippocampal regions of rats prenatally exposed to this vasculotoxin.

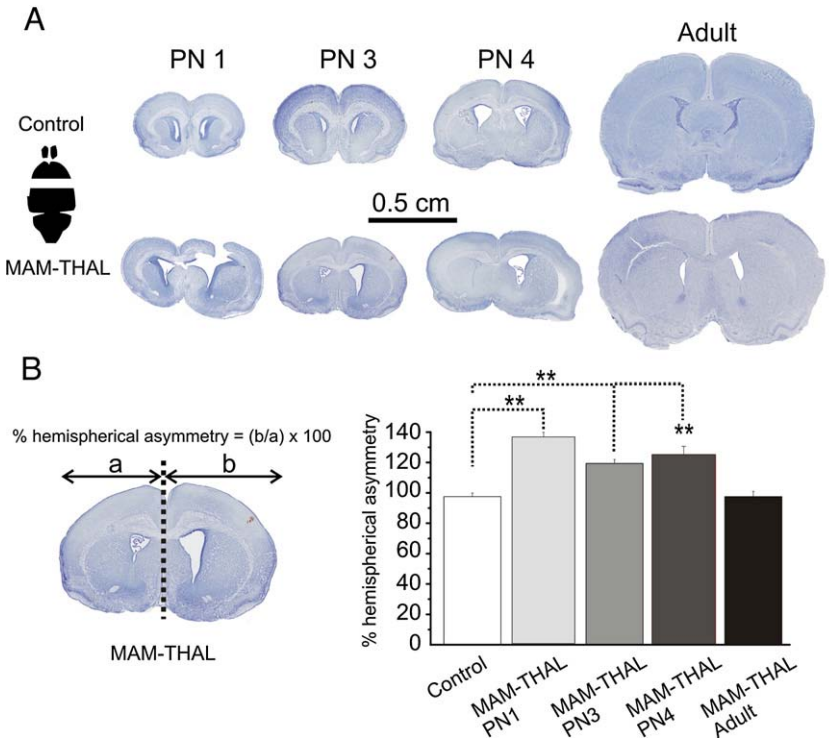

Fig. 2. Hemispheric asymmetry and altered ventricular size in MAM-THAL progeny. (A) Cresyl violet stained brain sections of control and MAM-THAL rats at early postnatal day 1, 3, 4 and adult stage reveal reversible changes occurring postnatally. The schematic of the rat brain shows the approximate level at which the image was taken. The quantification in (B) shows the presence of hemispheric asymmetry at birth and the subsequent return to normal size in adulthood. We analyzed 10 contiguous sections from 3 animals per group. ** indicates $p<0.001$.