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Effect of Intrauterine Growth Restriction on Cell Proliferation in the Fetal Cerebellum of the Guinea Pig

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Abstract : Intrauterine growth restriction (IUGR) is a risk factor for neurological and behavioral deficits in children although the precise underlying biological mechanisms are unclear. It is also unclear whether IUGR affects cell proliferation in the cerebellum, which is vulnerable to prenatal insults during brain development. The aim of this study is to determine whether IUGR affects the alteration of cell proliferation in the fetal cerebellum and whether this change correlate with reduction in the growth of cerebellum.

IUGR was induced via unilateral ligation of the uterine artery in pregnant guinea pigs at 30 days of gestation (dg; term ~67 dg) to produce growth restricted (GR) fetuses. Fetal (control, n=7 and GR, n=7) body and organ weights at 60 dg were measured and Ki67 immunohistochemistry was performed to detect cell proliferation. The width of the external granular layer (EGL) was also measured at 60 dg.

The mean body and organ weights of GR fetuses at 60 dg were significantly reduced. The proportion of proliferating cells to the total cell number in the EGL was not different in GR compared with control animals but the width of the EGL was significantly increased in GR animals.

These results demonstrate that significant reductions in the growth of the cerebellum do not have a well-defined relationship to cell proliferation in IUGR guinea pig fetuses. In addition, despite there was no difference in the proportion of proliferating to post-mitotic cells between control and GR fetuses in the EGL at 60 dg, the width of the EGL was increased in GR fetuses compared to controls. This could be interpreted as a delay in the process of cell production or migration.

Key words : Intrauterine growth restriction, Cell proliferation, Cerebellum, Ki67, Guinea pig

Introduction

Abnormal prenatal brain development resulting from adverse intrauterine conditions is now thought to underlie several neurological disorders that manifest in postnatal life (Nelson and Ellenberg 1986, Blair and Stanley 1988, Naeye et al. 1989, Wadington 1993). Very low birth weight human infants, some of whom are growth-restricted (GR) (Beeby et al. 1996), demonstrate reductions in regional brain volumes (Peterson et al. 2000), increased incidence of sensory deficits (Powls et al. 1997), and long-term difficulties in learning (Lefebvre et al. 1988, Lloyd et al. 1988, Larroque et al. 2001) and aspects of everyday memory and numeracy (Isaacs et al. 2000, 2001). A proportion of such neonates display evidence of chronic malnu-

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trition (Jones and Parer 1983), hypoxemia (Jensen et al. 1996), and altered endocrine balance (Jones et al. 1990); such symptoms are characteristics of chronic placental insufficiency (CPI).

A number of CPI animal models have been established in an attempt to replicate and better understand the mechanisms associated with the development of neuropathologies related to many neurological disorders in the preterm and term infant, including white matter damage and alterations in neuronal survival and growth. In animal studies of intrauterine growth restriction (IUGR), several structural, neurochemical and functional effects are seen in the brain. Both cerebral blood flow (Detmer et al. 1991) and oxygen delivery (Jensen et al. 1996) are reduced in IUGR animals. In a guinea pig model of CPI via uterine artery ligation throughout the second half of gestation (Lafeber et al. 1984), reductions in the volume of the hippocampus at term (Mallard et al. 1999), altered dendritic morphology of hippocampal neurons at term (Dieni and Rees 2003), and fewer CA1 and Purkinje cells in the postnatal hippocampus and cerebellum (Mallard et al. 2000) are reported. Furthermore, in an ovine model of CPI induced by umbilicoplacental embolization in late gestation, a reduction in the number of Purkinje cells and the growth of their processes and significant abnormalities are seen in the development of the cerebral hemispheres and cerebellum at term (Mallard et al. 1998). Thus, although it is clear that CPI can affect brain development, the underlying mechanisms have not been elucidated yet.

Neurogenesis continues in neurogenic zones in the forebrain (SVZ: subventricular zones, SGZ: subgranular zone of the dentate gyrus, olfactory bulb) and cerebellum (EGL: external granular layer) during development (Lichtenwalner and Parent 2006). So far the effects of adverse perinatal factors on neurogenesis have largely addressed the influence of postnatal rather than prenatal insults and results have been controversial. For example, one study has shown that hypoxia-ischemia results in a reduction in neural stem cells and oligodendrocyte precursors in the SVZ (Levison et al. 2001), however another study has shown that acute hypoxia results in apoptosis in the hippocampus followed by neuronal proliferation in the SVZ (Daval and Vert 2004). Similarly, chronic perinatal hypoxia (Fagel et al. 2006) and severe acute hypoxia-ischemia (Ong et al. 2005) can also promote proliferation of the SVZ in the short-term. It is unclear, however, whether these neurons persist, as in one study in which the majority of surviving cells were glia (Ong et al. 2005).

In a recent study using the guinea pig model, although there are significant reductions in the growth of the cellular layers in the cerebellum, no changes in expression of neurotophic factors in the cerebellum following chronic prenatal hypoxia is reported (Dieni and Rees 2005). However, the effects of chronic prenatal insults on cell proliferation are not well known, particularly during fetal life. The relationship between cell proliferation and structural growth after IUGR is also not clear and needs to be clarified. Therefore, the aim of the present study is to ascertain whether a chronic prenatal hypoxemic insult induced in the mid-gestational guinea pig fetus affects the proliferation of the cells in the cerebellum during fetal life and whether this change correlate with reduction in the growth of cerebellum. The guinea pig was chosen as the model since, as in humans, its neurogenesis and a significant proportion of dendritic and axonal proliferation occur *in utero* (Nitsos and Rees 1990, Mallard et al. 2000, Dieni and Rees 2002). The fetal cerebellum was chosen for examination because this region is vulnerable to prenatal insults such as acute and chronic hypoxia (De Haan et al. 1997, Keunen et al. 1999, Mallard et al. 1998, 2000).

Materials and Methods

All procedures were carried out under the approval

of the University of Melbourne Animal Experimentation and Ethics Committee in accordance with international guidelines. The number of experimental animals entered into this study was the minimum required to allow for statistical analysis to be performed for each parameter. All efforts were made to minimize pain and suffering of animals.

1. Surgery

IUGR was induced in time-mated, Dunkin-Hartley guinea pigs by unilateral uterine artery ligation (Lafeber et al. 1984, Nitsos and Rees 1990). Briefly, pregnant guinea pigs (n=10) at 30 days of gestation (dg, term approximately 67 days) were anesthetized with an intramuscular injection of Ketamine (40 mg/kg, Ilium Laboratories, Victoria, Australia) and xylazil (6 mg/kg, Troy Laboratories, Victoria, Australia). A midline incision was made below the umbilicus, the mesometrium associated with one horn of the bicornuate uterus was retrieved, and uterine artery ligation was performed at the cervical end of the arterial arcade. Uterine horns were ligated alternatively in different animals to prevent position bias. The ligature remained in place for the duration the experiment. At 60 dg, guinea pigs were deeply anesthetized with sodium pentobarbitone (i.p., 130 mg/kg, Nembutal, Merial, Australia), and fetuses were removed from the uterine horns by cesarean section. Fetuses were classified as GR if they met previously established criteria (Nitsos and Rees 1990). Fetuses from the unoperated horn served as sham-operated controls. In Fig. 1, prenatal hypoxia period was represented by a shaded bar on the thick black line.

2. Tissue preparation

At 60 dg, fetuses were removed from the uterine horns, and body weights and crown rump lengths were recorded. Each fetus was perfusion-fixed *in situ* via the heart with 0.9% saline solution, followed by 4% paraformaldehyde solution (4% PFA, pH 7.4). Fetal brains were removed, and tissue block including cerebellum was stored in 4% PFA overnight at 4˚C, followed by dehydration through graded ethanol solutions and embedded in paraffin. Serial sagittal sections of the cerebellum were cut 12 μm and mounted on gelatin coated slides. Every 40th section was stained with thionin (0.01%) for analysis.

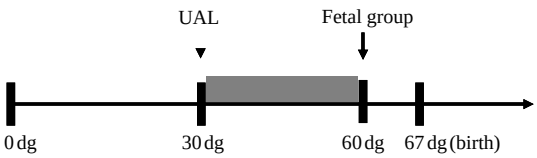

Fig. 1. Timeline of the experiment. Shaded bar indicates period of chronic prenatal hypoxia. An arrowhead indicates uterine artery ligation at 30 days of gestation, and an arrow indicates fetal group at 60 dg. Uterine artery-ligated guinea pigs are allowed to survive for 30 days after surgery. A pregnancy period of guinea pig is approximately 67 days. dg: days of gestation, UAL: uterine artery ligation.

3. Immunohistochemistry

The paraffin was removed from the sections, then the sections were rehydrated, and washed with 0.1 M phosphate buffer (PB, pH 7.4). Immunohistochemical staining for cell proliferation marker Ki67 was performed using a standard protocol (Abraham et al. 2001, 2004). For the demonstration of Ki67 immunoreactivity, slides were put in plastic jars filled with 0.01 M sodium citrate buffer (pH 6.0) and microwave antigen retrieval was performed. After two heating cycles of 7 min each, slides were allowed to cool at room temperature and were repeatedly washed in PB. After washing in PB the sections were pretreated 0.3% H_2O_2 for 20 min. Non-specific binding of immunoreagents was blocked with normal horse serum (10% in PB; Vector, USA) for 30 min, followed by overnight incubation in the primary mouse monoclonal anti-human Ki67 antibody (MIB-1, 1 : 100, Dako, Glostrup, Denmark) in a humidified chamber at 4˚C. Bind-

ing was visualized with biotinylated anti-mouse IgG (Vector, USA) and the avidin-biotin-peroxidase (ABC) detection system (Vectastain ABC Elite Kit, USA), and 3,3′-diamino-benzidine (DAB; Sigma, USA). Following the immunohistochemistry, thionin counterstaining was used, then the sections were dehydrated, cleared with histolene and covered with DPX (BDH chemicals, England).

4. EGL width measurement

For each animal, five Nissl-stained parasagittal sections of cerebellum 480 μm apart were used for measurement of the EGL in lobule I and VIII. The mean width of the EGL was determined using Image Pro Plus Software (Media Cybernetics, Maryland). Sections were viewed under a 40× objective lens on a projecting microscope (Olympus) and the width (μm) of the EGL was then determined along the length of the EGL overlying the molecular layer.

5. Quantitative analysis

Slides were coded prior to quantitative analysis so that the investigator was blind to the animal treatment. Ki67-immunoreactive (IR) cells were counted in the EGL of lobule I and VIII of cerebellum using an image analysis system (Media Cybernetics, Maryland). Three Ki67-IR sections of cerebellum 300 μm apart were used for measurement of the EGL. The proportions of Ki67-IR cells in the EGL of lobule I and VIII were assessed using a counting frame of a defined size placed over the five areas randomly each section. And then results were expressed as percentage (%).

6. Statistical analysis

The mean value of each parameter for control and GR groups was determined by averaging the mean values from each animal. Mean group values of body and organ weights were subjected to a two-sided *t* test. Mean values of proportion of Ki67-IR cells between control and GR groups were compared using the Student's *t*-test. Significance was accepted if $p < 0.05$.

Results

1. Intrauterine growth restriction

A total of 14 fetuses, produced by ten of the pregnant guinea pigs were entered into this study. Of these fetuses, 7 were from the ligated horn and met the criteria for GR, 7 were from the unligated horn and served as controls. Fetuses were classified as GR if as (i) their body weights were at least two standard deviation (SD) below the mean weight of age-matched controls and (ii) the brain/liver weight ratio was at least two SD above the mean of age-matched controls (Table 1). The mean body weight of GR fetuses (n=7) was significantly reduced ($p < 0.0005$) when compared to controls (n=7). GR fetuses also displayed significant reductions in mean crown rump length ($p < 0.0005$) and brain ($p < 0.0005$), cerebellar ($p < 0.0005$), and liver ($p < 0.0005$) weights when compared to controls. The mean brain-body ($p < 0.0005$) and brain-liver ($p < 0.005$) ratios were significantly increased in GR fetuses when compared to controls. These findings reflected the relative sparing of the brain com-

Table 1. Fetal body, brain, cerebellum and liver weights, crown rump length, and brain/body and brain/liver weight ratios at 60 days of gestation

	Control (n=7)	GR (n=7)	P
Body (g)	103.26±4.41	50.23±4.64	***
Brain (g)	2.70±0.07	2.15±0.07	***
Cerebellum (g)	0.30±0.02	0.19±0.005	***
Liver (g)	5.93±0.47	2.31±0.33	***
CRL (Cm)	13.74±0.17	10.33±0.40	***
Brain/Body ratio (%)	2.6±0.001	4.5±0.003	***
Brain/Liver ratio (%)	46.9±0.04	107.1±0.17	**

Values are expressed as Mean±SEM, $^{**}p < 0.005$, $^{***}p < 0.0005$ compared to age- matched controls (Student's *t* test). Brain/Body and Brain/liver ratios expressed as percentages. CRL: crown rump length.

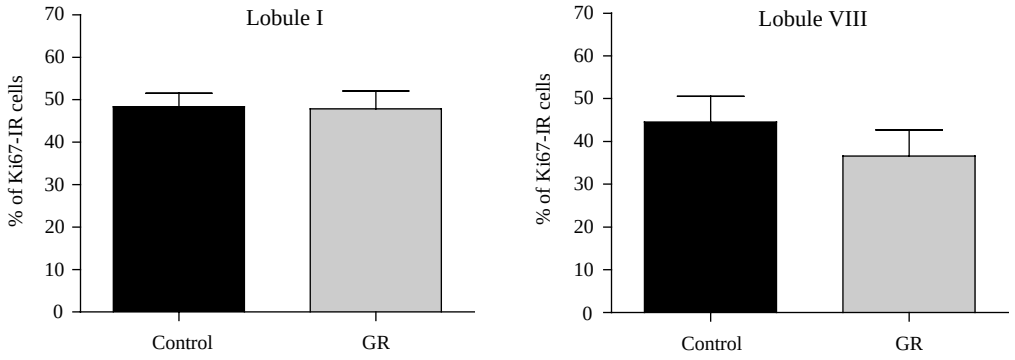

Fig. 2. Proportion of Ki67-IR cells to the total cell number in the EGL of lobule I and lobule VIII from controls and GR fetuses at 60 dg. The proportion of proliferating cells was not different in both lobules of GR fetuses compared to controls at 60 dg ($p > 0.05$). Student's *t*-test.

pared to the whole body and liver, as in a previous report (Dieni and Rees 2005). No evidence of gray and white matter necrosis or infarction was observed in the brains of GR fetuses, in agreement with previous studies of this model (Nitsos and Rees 1990, Rees et al. 1997, Dieni and Rees 2005).

2. The proportion of Ki67-IR cells to the total cell number in the EGL

There was no significant difference in the proportion of Ki67-IR cells to the total cell number in the EGL between control and GR fetuses in lobule I and VIII at 60 dg ($p > 0.05$) (Fig. 2). The proportion of Ki67-IR cells to the total cell number was around 50% in lobule I and below 50% in lobule VIII in both groups. Overall, IUGR did not seem to significantly affect cell proliferation in the cerebellum (Fig. 2).

3. Structural analyses

When compared to controls, the width of the EGL ($p < 0.01$) was significantly increased in the cerebella from GR fetuses compared to controls. This is illustrated in Figs. 3, 4 and 5. There was a reduction in the size of lobules, particularly in lobule I and VIII in the

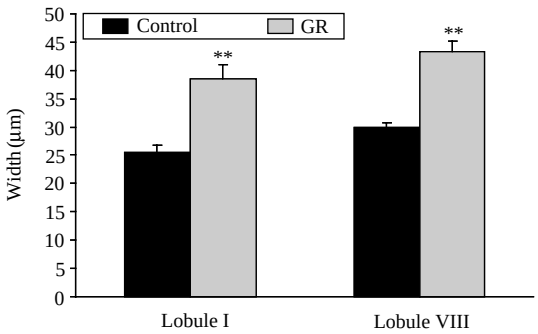

Fig. 3. The width of the EGL in lobule I and lobule VIII from controls and GR fetuses at 60 dg. The width of the EGL was significantly increased in GR fetuses compared to controls. Values are expressed as a Mean ± SEM. $^{**}p < 0.01$ compared to age-matched controls (Student's *t*-test).

cerebella of GR fetuses, when compared to controls (Fig. 4). When compared to controls, the thickness of cellular layers particular in the molecular layer and Purkinje cell layer except the EGL were reduced in both lobules in the cerebella from GR fetuses (Figs. 4, 6). In Fig. 6, when compared to controls, cerebella from GR fetuses were reduced in the widths of the molecular layer and Purkinje cell layer including Purkinje cell.

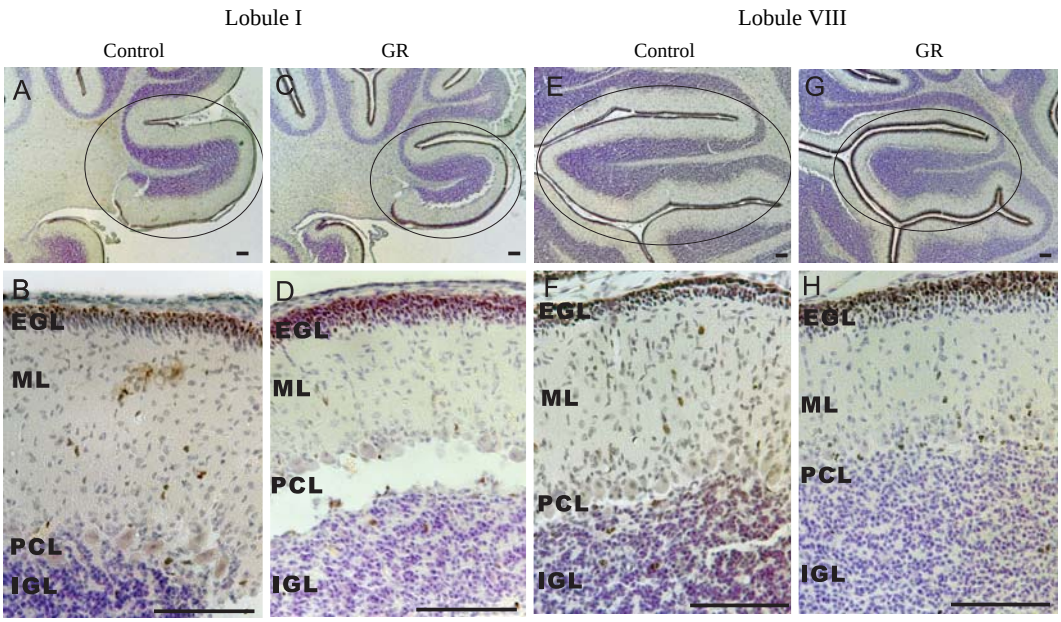

Fig. 4. Ki67 immunoreactivity in the lobule I (A-D) and VIII (E-H) from a control (A, B, E, F) and a GR fetus (C, D, G, H) at 60 dg. When compared to control, the sizes of lobules reduced in GR fetus (circles) and thickness of cellular layers particular in molecular layer, except the EGL also reduced in GR fetus. EGL: external granule layer; IGL: internal granule layer; ML: molecular layer; PCL: Purkinje cell layer. Scale bars=100 μm

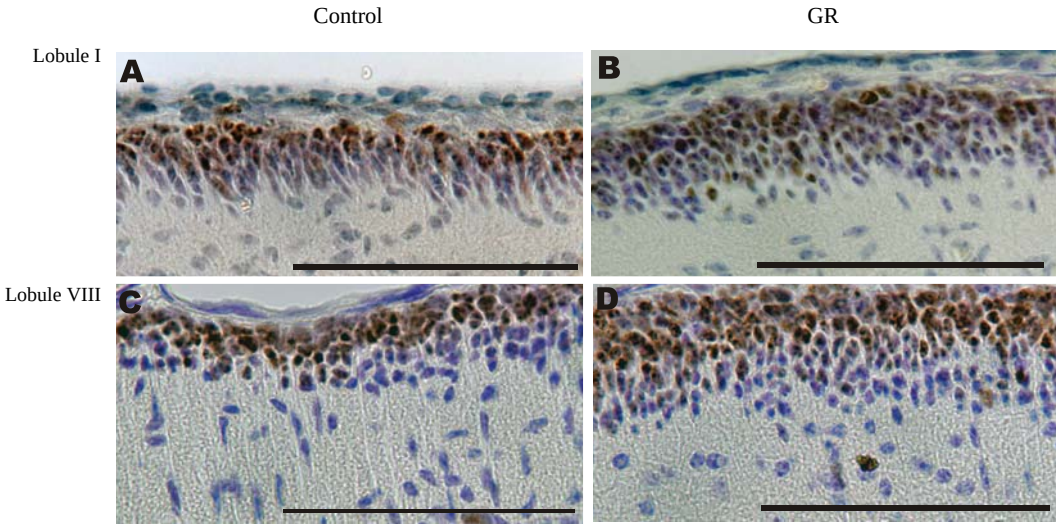

Fig. 5. Representative photomicrographs of the Ki67 immunoreactivity in the EGL of lobule I (A, B) and VIII (C, D) from a control and a GR fetus at 60 dg. The EGL contained large numbers of Ki67-IR cells in both groups but the width of the EGL appeared greater in a GR compared with a control fetus. Scale bars=100 μm

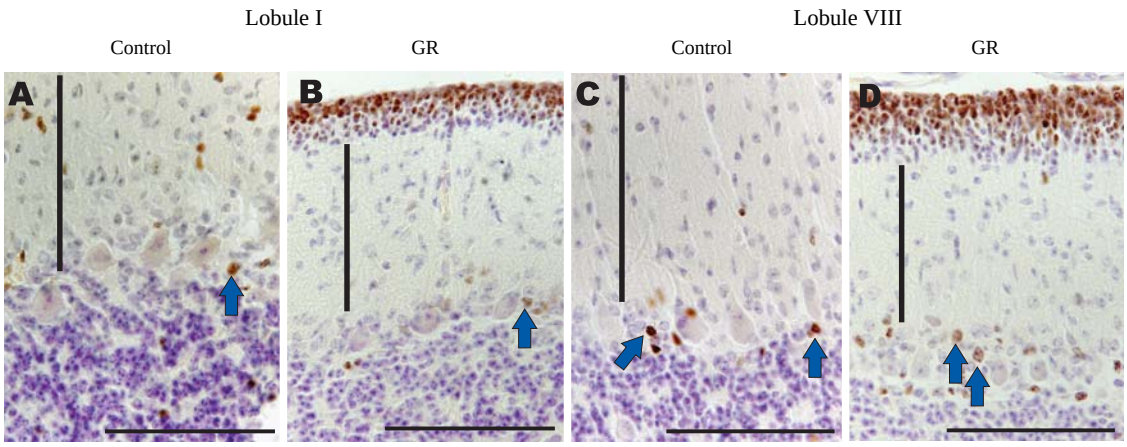

Fig. 6. Ki67 immunoreactivity in the cerebellar layers around Purkinje cell layer of lobule I (A, B) and VIII (C, D) from a control and a GR fetus at 60 dg. The molecular layers in a GR fetus were thinner than in a control fetus (black arrows). Purkinje cell size was smaller and this cell layer was thinner in a GR fetus than in a control. Cells with large cell nuclei among and below the Purkinje cells were Bergmann glia cells in both groups (blue arrows). Scale bars=100 μm

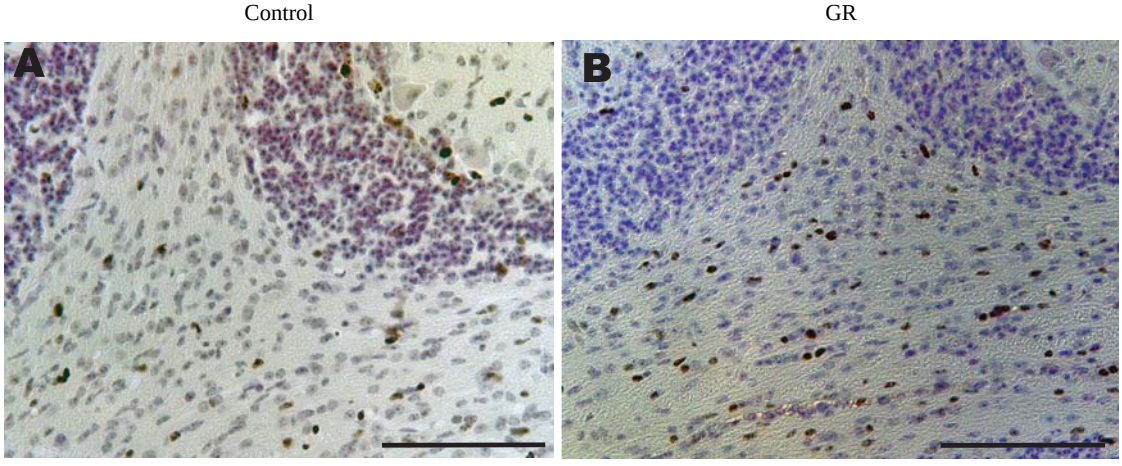

Fig. 7. Representative photomicrographs of the Ki67 immunoreactivity in the white matter of cerebellum from a control (A) and a GR fetus (B) at 60 dg. Strong-stained and a large number of Ki67-IR cells were observed in the white matter in both groups. Scale bars=100 μm

4. Ki67 immunoreactivity

Strong Ki67 immunoreactivity was observed in the EGL, scattered cells in the molecular layer, Purkinje cell layer and the internal granule layer in control animals. A similar distribution of Ki67-IR cells was observed in GR fetuses (Figs. 4-6). The majority of Ki67-IR cells was observed in the outer two thirds of the EGL which contained the progenitor cells in both control and GR fetuses. Whereas in the inner zone containing the postmitotic, premigratory cells, only a few Ki67-IR cells were observed in both control and GR

fetuses (Fig. 5). Bergmann glia cells with large nuclei among and below the Purkinje cells were observed in both control and GR fetuses (Fig. 6). Cells in the white matter were also Ki67-positive. Ki67 immunoreactivity in the white matter from control and GR fetuses showed similar staining pattern (Fig. 7).

Discussion

This study has unequivocally demonstrated that chronic prenatal hypoxia, induced via a reduction in uteroplacental blood flow throughout the second half of gestation and coincided with the period of rapid cerebellar growth in the guinea pig, leads to unmarked alterations in cell proliferation at least in the EGL concomitantly with a reduction in the growth of cellular layers in this structure. These findings show that IUGR does not have a global effect on cell proliferation in the brain. According to our results, the proportion and number of proliferating cells were not altered in the fetal cerebellum although the growth of the cerebellum was significantly reduced.

In the present study, Ki67 immunoreactivity was used for detecting cell proliferation. Rakic and Sidman (1970) used ^{3}H-thymidine autoradiography. It is known that thymidine is incorporated into the cell nuclei only during the S-phase of the cell cycle. Expression of nuclear protein, which is the antigen for Ki67 antibody, is detectable through the S, G2, M phases of cell cycle (Gerdes et al. 1984, Verheijen et al. 1989a, b). Therefore, the Ki67 antibody can bind to more cells at a given time than the supravitally applied radio-labeled thymidine (Abraham et al. 2001). In a previous study, most of labeled cells were located in the outer zone of the EGL but not in the inner zone composed of postmitotic neurons using the Ki67 antibody (Mares et al. 1970). In this study, the majority of Ki67-IR cells was observed in the outer two thirds of the EGL, whereas in the inner zone, only a few Ki67-IR cells were observed in both control and GR fetuses. Distribution and morphology of Ki67-IR cells in the EGL in both groups were similar to those in the normal developing cerebella of other animals and human experiments (Mares et al. 1970, Abraham et al. 2001). The EGL appeared thicker in GR fetuses compared with control fetuses. This suggests that the process of cell formation and/or migration may delay in GR fetuses. In this study, cells with large nuclei among and below the Purkinje cells were observed in both control and GR fetuses. They are thought to be Bergmann glia cells. Proliferation and multiplication of Bergmann glia cell are known to happen throughout cerebellar development, and Bergmann glial processes play a crucial role in the migration of postmitotic granule cells in the growing cerebellar cortex (Rakic 1971, Abraham et al. 2001). The EGL is the sole source of granule cells, but basket, stellate and Golgi cells, as well as glial cells originate from the white matter of the cerebellum (Zhang and Goldman 1996). In the present study, large amounts of Ki67-IR cells were observed in the white matter in both groups. The generation of basket, stellate and Golgi cells precedes the generation of granule cells (Mares et al. 1970, Abraham et al. 2001), accordingly, a proportion of the Ki67-IR cells in the white matter may be proliferating interneuron-precursors, but most Ki67-IR cells may be proliferating glial cells. On the other hand, cell formation is known to be accompanied by cell death (Mares et al. 1970, Abraham et al. 2001). We have yet to determine whether there is an increase in apoptosis following chronic prenatal hypoxia. Therefore, the frequency of pyknotic cell nuclei needs to be examined in the cellular layers in GR fetuses in the future studies.

In agreement with previous studies of chronic adverse intrauterine conditions in the sheep (Rees and Harding 1988, Rees et al. 1988, 1997) and guinea pig (Mallard et al. 2000, Dieni and Rees 2005), we have shown that the developing cerebellum is susceptible to

chronic prenatal hypoxia. Cerebellar weight was reduced in GR fetuses when compared to controls. In addition, cerebella from GR fetuses were reduced in the widths of the molecular layer and Purkinje cell layer including Purkinje cell when compared to controls. Dieni and Rees (2005) also reported that the volume of the molecular and internal granule layers and white matter in GR fetuses were reduced compared to controls. These may be reflected reductions of Purkinje cell dendritic growth, granule cell numbers, and axonal growth or myelination, respectively. Furthermore, the reduction in the size of lobules and Purkinje cells of early developing lobule I and late developing lobule VIII observed in GR fetuses suggest that the normal growth pattern can be either delayed or altered by IUGR.

Fetal hypoxemia is a component of the uterine artery ligation model and hypoxia during brain development in the newborn rat has been shown to have several effects on cerebellar development including retardation of neuronal processes and inhibition of mitosis in the EGL (Yu and Yu 1980). The precise period of neurogenesis is not known in the guinea pig, but it is supposed that the majority of neurons are formed before 40 dg (Dobbing and Sands 1970). In a previous study, the fetal guinea pig brain at 30 dg was very immature with mitotic figures present in the cerebellum, which indicates that neurogenesis is still occuring at this age (Mallard et al. 2000). It is therefore possible to suppose that the uterine artery ligation may affect the numbers of proliferating cells by such direct effect of hypoxia on neurogenesis. Recently, Ong et al. (2005) reported that acute hypoxia-ischemic injury stimulated SVZ proliferation and neurogenesis. Similarly, Fagel et al. (2006) reported that cortical neurogenesis was enhanced by chronic perinatal hypoxia. However, unlike previous studies, there was no increase in cell proliferation after chronic prenatal hypoxia in the fetal cerebellum in this study. Additionally, in contrast to other brain region, the altered cerebellar growth pattern was not associated with alteration in cell proliferation, at least at the time point examined in this study. Although the EGL is important neurogenic zone during cerebellar development (Lichtenwalner and Parent 2006), IUGR might not adversely influence cell proliferation in the EGL during prenatal development. The phenotype of these proliferating cells could not be confirmed in this study. Immunostaining using migrating, neuronal and glial cell markers is necessary to identifying other processes of neurogenesis following chronic prenatal hypoxia and explaining unchanged cell proliferation in the fetal cerebellum of GR animals.

One study has shown that cortical volume and cortical neuron number were recovered 1 week after cessation of the hypoxia (Fagel et al. 2006). This was not a transient phenomenon because the recovery in cortical volume and cortical neuron number are maintained postnatal days. This report suggests that an increase of cell proliferation may be observed in the brain only during the initial week of recovery. Therefore, to understand whether recovery phase following chronic prenatal hypoxia correlates with transient proliferation of cells, further studies with different fetal ages of GR animals are needed.

This study has shown that a chronic reduction in uteroplacental blood flow leads to reduced growth of the fetal cerebellum, but this is not associated with alterations in cell proliferation. While there is no well-defined relationship between alteration in cell proliferation and structural abnormalities in the fetal cerebellum at 60 dg, altered migration and cell production can have long-lasting effects on postnatal structural and functional outcomes.

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자궁속성장지연이 기니픽 태생기 소뇌의 세포 증식에 미치는 영향

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간추림 : 자궁속성장지연은 유아의 신경학적 결함 및 행동장애를 일으키는 원인이 될 수 있지만 이와 관련한 생물학적 원인은 정확히 밝혀져 있지 않다. 또한 뇌발생시 출생전 손상에 영향 받기 쉬운 소뇌의 세포 증식에 미치는 자궁속성장지연의 영향은 잘 알려져 있지 않다. 본 연구는 자궁속성장지연이 태생기 소뇌의 세포 증식에 미치는 영향을 알아보고 세포증식의 변화와 소뇌 성장과의 관계를 규명하고자 시행되었다.

임신 30일 된 기니픽의 한쪽 자궁동맥을 묶어 만성적인 태반 부전으로 인한 성장지연 기니픽 태자를 만들었으며 태생 60일에 희생시켜 태자의 체중과 뇌의 무게를 측정하였다. Ki67 면역조직화학염색을 이용하여 세포 증식의 변화를 관찰하였고 태생 60일의 소뇌 바깥과립층의 폭을 측정하였다.

본 연구 결과, 바깥과립층의 전체 세포 수에 대한 증식세포의 비율의 차이는 정상군과 성장지연군 간에 통계학적 의의는 없었으나 바깥과립층의 폭은 성장지연군에서 유의하게 크게 나타났다.

이상의 연구 결과는 자궁속성장지연이 발생 중인 기니픽 태자의 소뇌 성장을 감소시키지만 이는 태생기 소뇌의 세포 증식과는 연관성이 없음을 나타내 주었다. 또한 태생 60일에 정상군과 성장지연군 간에 바깥과립층 전체 세포 수에 대한 증식세포 수의 비율의 차이는 없었음에도 불구하고 성장지연군의 바깥과립층의 폭은 정상군에 비해 증가되었는데 이는 세포 발생 및 이동 과정의 지연 때문일 것으로 생각된다.

찾아보기 낱말 : 자궁속성장지연, 세포 증식, 소뇌, Ki67, 기니픽

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