Effects of Continuous Low-Dose Prenatal Irradiation on Neuronal Migration in Mouse Cerebral Cortex

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We investigated the effects of continuous exposure to γ-rays during corticogenesis on the migration of neuronal cells in developing cerebral cortex. Pregnant mice were injected with 0.5 mg of bromodeoxyuridine (BrdU) on day 14 of gestation to label cells in the S phase. The mice were then exposed to 137Cs γ-rays (dose rates of 0.1, 0.3, and 0.94 Gy/day) continuously for 3 days. Brains from 17-day-old embryos and from offspring at 3 and 8 weeks after birth were processed immunohistochemically to track the movements of BrdU-labeled cells. Comparative analyses of the distribution pattern of BrdU-labeled cells in the cerebral cortex revealed that (1) the migration of neurons was delayed during the embryonic period in mice irradiated at 0.94 Gy/day, (2) in 3-week-old mice, there was a significant difference in the distribution pattern of BrdU-labeled cells in the cerebral cortex between the mice irradiated prenatally and control, and (3) in 8-week-old mice, there were no differences in the distribution pattern of BrdU-labeled cells between control and animals irradiated with 0.1 and 0.3 Gy/day. In contrast, in the animals irradiated with 0.94 Gy/day, the significant difference in the distribution pattern of the labeled cells relative to control was maintained. These results suggest that the migration of neuronal cells in mouse cerebral cortex is disturbed by continuous prenatal irradiation at low-dose and some modificational process occurred during the postnatal period.

INTRODUCTION

It is well known that the development of the cerebral cortex in mammals can be severely affected by exposure to ionizing radiation at critical periods of gestation12. Quantitative studies of the effects of low-dose radiation on the migration of neuronal progenitor cells in cerebral mantle in experimental animals have contributed to the pathogenic analysis of mental retardation seen among A-bomb survivors exposed in utero13. Especially, long-term effects on neuronal allocation in adult animals after prenatal irradiation at different neurogenic stages should be evaluated. In our previous paper, we reported that exposure of developing brains of 14-day-old mouse embryos to low doses of X-rays disturbed neuronal migration during early phase of histogenesis in prenatal and early postnatal periods14,15. However, there have been relatively few
studies of long-term effects of low-dose irradiation on prenatal corticogenesis. In the present study, we investigated the effects of chronic exposure to ionizing radiation at different dose-rates at 14 to 17 days of gestation on the migration of neocortical neurons during development, in mice.

MATERIALS AND METHODS

Pregnant C57BL/6J mice crossed with C3H/He were purchased from SLC, Japan 4 days before irradiation and maintained in a temperature- and humidity-controlled animal facility with a light and dark cycle of 12h. The day of vaginal plug formation was counted as embryonic day 0 (E0). Animals were fed on a commercial diet and given water ad libitum. All the animals were maintained in accordance with the guidelines governing the care and use of laboratory mice at the National Institute of Radiological Sciences.

Each pregnant animal received intraperitoneal injection of bromodeoxyuridine (BrdU) to label cells in S phase. BrdU (0.5 mg) was dissolved in 0.5 ml saline and injected at 14 days of gestation. The animals were then irradiated continuously with γ-rays at dose rates of 0.1, 0.3 or 0.94 Gy per day. The irradiation was performed from 11:00 a.m. on E14 to 9:00 a.m. on E17. The three different dose rates were achieved by exposure at different distances (195, 95 and 53 cm) from a 137Cs source of 370 GBq. The dose absorbed by animals was determined with a Victoreen R-meter and Ionex Dose Meter 2500/3 with a conversion factor of 0.95. Irradiation for 22 h/day was controlled automatically. Total dose for 22 h was estimated as daily dose (Gy/day) (Table 1). Six pregnant animals from control and irradiated groups were killed at 10:00 a.m. on day 17 of gestation, i.e., just after the termination of irradiation. The brains from the fetuses were dissected out, and then fixed with 4% paraformaldehyde solution in 0.1 M phosphate buffer at pH 7.4. The other animals were allowed to give birth and rear their litters. Some of the offspring from irradiated animals were perfused transcardially for fixation with 4% paraformaldehyde solution in 0.1 M phosphate buffer at pH 7.4 under deep anesthesia with ether at 3 and 8 weeks of age. Then, the brains were processed for immunohistochemistry.

The brain samples were dehydrated, embedded in paraffin, and sectioned at 4 μm in the frontal plane. The sections through the cerebral cortical area (field 1 of Caviness6), covering the rostral portion of the hippocampus, were selected from each brain for observation. After deparaffinization, the tissue sections were first treated with 1% H2O2 to inactivate the endogenous peroxidase, followed by 0.04% pepsin solution.

Table 1. Number of pregnant mice irradiated continuously from day 14 to 17 and number of embryos or offspring from irradiated and control animals examined for the analysis of neuronal migration

<table>
<thead>
<tr>
<th>Dose rate (Gy/day)</th>
<th>Number of pregnant mice irradiated</th>
<th>Number of embryos or offspring examined</th>
<th>17-day-old embryos</th>
<th>3 weeks after birth</th>
<th>8 weeks after birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>5</td>
<td></td>
<td>12</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
<td></td>
<td>4</td>
<td>11</td>
<td>11</td>
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<tr>
<td>0.3</td>
<td>6</td>
<td></td>
<td>7</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>0.94</td>
<td>4</td>
<td></td>
<td>7</td>
<td>6</td>
<td>8</td>
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and then with 2N hydrochloric acid solution at 40°C for 45 minutes. The samples were then neutralized with 0.1 M sodium borate solution (pH 8.5) for 10 minutes. These sections were incubated for 60 minutes with anti-BrdU monoclonal antibody (Becton Dickinson) diluted 1:100, and rinsed three times for more than 15 minutes. Next, the samples were incubated in biotinated goat anti-mouse IgG-HRP diluted 1:200 for 60 minutes. After rinsing several times, the sections were incubated with a solution containing 3,3'-diaminobenzidine (DAB; 0.5 mg/ml in Tris buffer at pH 7.6) and 0.01% H₂O₂ for 10 minutes. All the sections were thoroughly rinsed with PBS, dehydrated through a series of graded ethanol, and covered with a synthetic medium, Entellan (neu), in a coverslip.

The parietal cerebral cortex, field 1 of Caviness, in the young and adult brains was used for counting the number of labeled cells in the cortical layers as mentioned previously. The sections were observed carefully under a microscope. Cells with heavily immunostained nuclei that obviously differed from the background were considered to be labeled. In the immature cerebral mantle from embryonic mice, the number of BrdU-labeled nuclei was counted in four zones: ventricular zone (VZ), inner-intermediate zone (Ini), outer-intermediate zone (Ino), and cortical plate (CP) of the neocortex using a width of 330 µm. In the young and adult brains, the sections of cerebral walls were divided into five zones: marginal zone I, II/III, IV, V and VI layers. The number of BrdU-labeled nuclei was counted in each layer of the cortex within a width of 650 µm. The position of each labeled cell was plotted on tracing paper using a camera lucida apparatus as described by Roberts et al to survey the distribution of BrdU-labeled cells within the sections. Significance of differences was examined by the t test.

RESULTS

In embryos killed 1 hour after BrdU injection on the 14th day, almost all BrdU-labeled cells were in the ventricular zone with a few in the intermediate zone and the developing cortical plate. The cells arising in the 14th day of gestation migrated outward to form the cortical plate and differentiated into its constituent neurons as described in the previous paper. In the cerebral cortex of adult mouse, most of the labeled neurons were located in layer IV (in the middle third of the cortex), with some in layers II/III or layers V/VI. Effects of continuous irradiation on the initial migration of neuronal cells arising in 14-day-old embryos are shown in Figs. 1 and 2. In the brain mantle of 17-day old embryos, about 70% of the labeled cells were seen in the cortical plate of both control and animals irradiated with 0.1 Gy/day. In the embryos irradiated with 0.3 and 0.94 Gy/day, percent of BrdU labeled cells in the cortical plate on E17 tended to decrease with increasing dose-rate. However, the differences were not significant. In contrast, in the inner intermediate and ventricular zone, significant increases in the percent of BrdU labeled cells were observed after irradiation at 0.94 Gy/day. Moreover, cell density in the cortical plate seems to be lowered and the arrangement of cells in the cortical plate seems to be abnormal in the animals irradiated at 0.94 Gy/day (Fig. 2). Figure 3 shows photomicrographs of the BrdU-labeled cells in the cerebral cortex of 3-week-old mice. In the cerebral cortex of some of the mice irradiated at 0.94 Gy/day, the distribution of BrdU-labeled cells was more scattered than that of control. Figure 4 shows the relative distribution of the BrdU-labeled cells in the cerebral cortex of postnatal mice continuously exposed to γ-rays from day 14 to 17 of gestation.

In 3-week-old mice (Fig. 4A), there was a significant difference in the distribution pattern of the BrdU-labeled cells in the cerebral cortex between control and experimental animals. In animals irradiated with 0.1
Fig. 1. Photomicrographs of BrdU-immunostained sections of the neocortex of 17-day-old mice embryos. A: control, B: mice irradiated with 0.94 Gy/day for 3 days from embryonic day 14 to 17. Most BrdU-labeled cells are located in the cortical plate in the control, whereas they are scattered, occurring not only in the cortical plate (CP) but also in the intermediate (In) and ventricular (VZ) zone, in the irradiated animal.

Fig. 2. The relative distribution of BrdU-labeled cells in the neocortex of 17-day-old embryos after γ-irradiation for 3 days from E14 to E17. VZ, ventricular zone; Ini, inner layer of intermediate zone; Ino, outer layer of intermediate zone. CP, cortical plate. ** and *** Results significantly different from control by t-test at 98 and 99% confidence limits, respectively.
Fig. 3. Photomicrographs of frontal sections through the cerebral cortex of 3-week-old mice. BrdU immunohistochemistry
A: control, B: mice irradiated with 0.94 Gy/day for 3 days from E14 to E17. BrdU-labeled cells are seen mainly in layer IV in the control, whereas they are scattered evenly in the irradiated animal.

Fig. 4. The relative distribution of BrdU-labeled cells in the cerebral cortex of mice after y-irradiation for 3 days from E14 to E17. A: 3-week-old, B: 8-week-old. * and ** Results significantly different from control by t test at 95 and 99% confidence limits, respectively.
and 0.3 Gy/day, the number of labeled cells increased in layers II/III, and decreased in layer IV. In contrast, in the animals irradiated with 0.94 Gy/day, the number of BrdU-labeled cells tended to increase in layers V-VI, and decrease in layer IV. However, the differences were not significant.

At 8 weeks of age (Fig. 4B), there were no significant differences in the distribution pattern of BrdU-labeled cells in the animals irradiated with 0.1 and 0.3 Gy/day. In contrast, in the animals irradiated with 0.94 Gy/day, the pattern of distribution of labeled cells was maintained, i.e., greater numbers in layers V and II/III and fewer in layer IV.

No significant differences in the number of BrdU labeled cells per section in a given unit area of cerebral wall between control and experimental animals were observed, except in 3-week-old mice irradiated with 0.94 Gy/day (Fig. 5).

DISCUSSION

Ferrer et al. (1984) reported abnormal lamination and neuronal micronodules in the cerebral cortex after irradiation with 1.5-2 Gy of X-rays of middle and late fetuses. However, they failed to find any gross malformations in the offspring after birth if fetuses were irradiated with doses less than 1 Gy at the stages of middle and late histogenesis of cerebral neocortex. In the present study, mouse fetuses were exposed to gamma-rays at doses of 0.1, 0.3, and 0.94 Gy/day for 3 days from day 14 to 17 of gestation. Histological anomalies such as neuronal micronodules were not observed in the offspring after birth. Then, the distribution of BrdU-labeled cells in the cerebral neocortex during development was examined and compared with control animals. After irradiation with γ-rays at 0.94 Gy/day for 3 days, BrdU-labeled cells were observed predominantly in the ventricular and inner-intermediate zone compared to control. This suggests a
prolongation of the cell cycle of the ventricular cells and a delay of the migration of postmitotic neurons from the ventricular to the outer-intermediate zone. In young and adult mice after birth, BrdU-labeled cells, i.e., neurons arising in 14-day-old embryos, were distributed in broad layers of the cerebral neocortex: approximately 70% in layer IV, 20% in layers II/III, and 10% in layer V. This observation is consistent with the findings of several studies using ^3H-thymidine autoradiographic methods^6^-^11. 

In 3-week-old mice, there was a significant difference in the distribution pattern of BrdU-labeled cells in the cerebral cortex between the irradiated and control animals. At 8 weeks of age, however, there were no significant differences between the control and animals irradiated at 0.1 and 0.3 Gy/day. In contrast, in the animals irradiated at 0.94 Gy/day, the tendency for abnormal distribution of labeled cells was maintained. These results suggest that the neuronal cells in the cerebral cortex was induced during postnatal maturation. This assumption resulted from the finding that in animals irradiated at 0.1 and 0.3 Gy/day prenatally, no inhibition of the migration of neurons was observed in the cerebral cortex at 17 days of gestation. Similarly, the return to a normal percentage of labeled cells in the cerebral cortex of the animals irradiated with 0.1 and 0.3 Gy/day, at 8 weeks of age is assumed to have resulted from the spontaneous cell death of some aberrant neurons. If the number of dying cells during the period 3–8 weeks postnatal is small, the outcome may be an unappreciable decrease in the number of labeled cells. We presented a similar hypothesis in our previous paper^6^, since we observed that the abnormal distribution of neurons found in the cerebral cortex of the young mice irradiated with X-rays prenatally disappeared in the mature cerebral cortex of adult mice. Inoue et al (1993) also reported that the neuronal migration was impaired and the distribution of the BrdU-labeled neurons was disturbed in the cerebral cortex at 6 weeks of age after prenatal irradiation with 0.24 Gy of ^γ^-rays at 16 days^6^12. Their results showed that the neuronal migration of the cells at embryonic day 16 was inhibited by low-dose irradiation. It is possible that the impaired neuronal migration or abnormal distribution of neurons in murine cerebral cortex are due to the acute low-dose irradiation, though the effect of chronic low-dose irradiation may be reduced as compared to acute irradiation.

In the present study, the number of labeled cells decreased in the cerebral cortex of 3-week-old mice irradiated prenatally with 0.94 Gy. The effects of prenatal irradiation were evident when evaluated in terms of labeled cells per unit area of the cerebral cortex. The result suggests that the labeled cells died during the postnatal maturation after continuous irradiation during the embryonic stages with 0.94 Gy/day since no difference in the number of labeled cells per unit area of the cerebral cortex was observed at embryonic day 17.

At present, there is no direct relationship between the severe mental retardation observed in the A-bomb survivors and impaired migration or abnormal distribution of neurons as seen in the mouse cerebral cortex. It is possible that the disorganized neuronal architecture in the postnatal cerebral cortex results in the dysfunction of the brain. Other factors such as tissue environment or blood supplies may also affect the function of the brain. Indeed, it has been reported that the abnormal development of fetal liver erythropoiesis by fetal X-irradiation in mice impairs oxygen transport^13^19. Further study will be required to evaluate the relationship between severe mental retardation and impaired neuronal architecture or abnormal oxygen transport during brain development.
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REFERENCES