Split-Dose Effect of X-Irradiation on the Induction of Cell Death in the Fetal Mouse Brain

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Pregnant mice were exposed to whole-body X-irradiation at a total dose of 0.25 Gy split into two 0.125 Gy exposures at 0.5-, 2- or 6-hour interval on day 13 of pregnancy. Fetuses were obtained from dams at various post-exposure periods and their brains were processed for microscopy. Undifferentiated neural cells in the ventricular zone of telencephalon (ventricular cells) were examined, and incidence of cells involved in pyknosis was evaluated. The curves of incidence of pyknotic cells plotted against time after the exposures to two split-doses at 0.5-hour and 2-hour intervals overlapped that of a single 0.25 Gy exposure; they had a common peak at 8-10 hours after the first exposure. Following two 0.125 Gy exposures at 6-hour interval, two peaks with similar elevations from background levels appeared at 6 and 12 hours after the first exposure. These results indicated that low-dose X-irradiation shows simply additive effects of split doses on cell death, without induction of adaptive response of ventricular cells of the telencephalon at day 13 of pregnancy in mice.

INTRODUCTION

The developing fetal brain is known to be highly vulnerable to ionizing radiation.¹) Among atomic bomb survivors in Hiroshima, the incidence of microcephaly was highest in children prenatally exposed at 8-9 weeks of gestation.²) Severe mental retardation was also induced by the atomic bomb radiation even though the radiation dose was less than 0.5 Gy, when the exposure was at 8-15 weeks of gestation.³) This highest radiosensitive stage in humans (a few weeks from 8 weeks of gestation) in development of microcephaly and mental retardation corresponds to day 13 of pregnancy in mice, and day 15 in rats, i.e., the

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ventricular cells of fetal telencephalon are most susceptible to radiation-induced cell death in this stage of development.\textsuperscript{4,5)}

It is generally accepted that response of organisms to ionizing radiation varies depending on the dose rate and fractionation of dose where the total exposure dose is identical. For instance, fractionated dose-response curves of survival of human fibroblasts in vitro are distinctly different from the single dose exposure curves.\textsuperscript{6)} Adaptive response of human lymphocytes to ionizing radiation is known to be induced by low doses of X-rays in vitro.\textsuperscript{7,8)} For the tissue response, Brizsee \textit{et al.}\textsuperscript{9)} examined effects of fractionation of a total dose of 150-R X-irradiation on the developing rat brain, and reported that tissue damage was more severe in fetuses exposed to a single dose than in those receiving two 75-R exposures. In this experiment, cellular effect of split-dose radiation on the developing brain was examined looking at the induction of acute cell death in the ventricular zone of fetal telencephalon on day 13 of gestation in mice.

\textbf{MATERIALS AND METHODS}

The animals used were from a closed colony of Slc:ICR mice. They were housed in a room at 23±2°C with a 12-hour light-dark cycle (7:00 am-7:00 pm). A solid diet CA-1 and tap water were made available ad libitum. Females aged more than 9 weeks were caged with potent males in pairs overnight, and the following morning females with vaginal plugs were considered to be in day 0 of pregnancy.

The pregnant females were exposed to whole-body X-irradiation at a dose of 0.125 Gy at 1:00 pm–2:00 pm on day 13 of pregnancy, followed by another 0.125 Gy exposure 0.5, 2 or 6 hours after the first exposure. Animals exposed to a single radiation dose of 0.125 or 0.25 Gy, and untreated controls were also prepared. The physical factors of X-rays were 200 kVp, 15 mA, 0.5 mm Cu + 0.5 mm Al added filtration, 83 cm target distance, and 0.243 Gy/min exposure rate. After irradiation, the dams were put to death by cervical dislocation at 1- or 3-hour intervals up to 36 hours. Their fetuses were removed from the uterus and fixed in Bouin’s solution. More than two dams were used for each time interval, and two fetuses were selected at random from each litter for sectioning. Fetal brains were embedded in Paraplast, coronally sectioned at 5 μm thickness, and stained with hematoxylin and eosin. More than 1,500 cells in the ventricular zone of telencephalon were observed in each fetus under a light microscope, and those involved in cellular pyknosis were counted, as described previously in detail.\textsuperscript{5)}

\textbf{RESULTS}

The brain mantle of the day-13 mouse fetus is composed of about 15-cell-thick ventricular zone and 4- to 5-cell-thick intermediate zone. In this stage, cortical plate has not yet been formed at the dorso-medial part of the brain mantle, while at the lateral part, 2- or
SPLIT-DOSE EFFECT ON FETAL BRAIN

Fig. 1. A coronal section of the fetal mouse brain on day 13 of pregnancy, showing the region of examination. Cells in the ventricular zone (arrowhead) in between the two parallel lines about 150 μm apart were counted. LV, lateral ventricle. Scale bar = 0.3 mm, H.E. stain.

Fig. 2. A section of the telencephalon 9 hours after exposure to 0.25 Gy, showing many cells involved in pyknosis in the ventricular zone (VZ). P, pial surface; LV, lateral ventricle. Scale bar = 20 μm, H.E. stain.

3-cell-thick cortical plate is forming (Fig. 1). The pyknotic cells were present in the ventricular zone of untreated brain and increased in incidence after exposure to X-irradiation (Fig. 2).

Fig. 3 shows incidences of pyknotic cells plotted against time after the exposures to single doses of 0.125 and 0.25 Gy and split-doses of 0.25 Gy in total. Following a single dose of 0.25 Gy, pyknotic cells began to increase 3 hours after exposure, reached the peak incidence 9 hours after exposure, and then decreased to the control level (0.2 %) by 36 hours. The curves for exposures to two split-doses at 0.5-hour and 2-hour intervals overlapped that for a single 0.25 Gy exposure (Fig. 3a).

Following a single dose of 0.125 Gy, the incidence of pyknotic cells peaked at 6 hours after exposure and decreased to the control level by 24 hours. When mice were exposed to

Fig. 3. Time course of the incidences of cell death in the ventricular zone of primordial brain mantle following X-irradiation at single doses of 0.125 and 0.25 Gy and three split doses split into two 0.125 Gy at 0.5-, 2- or 6-hour intervals.
another dose of 0.125 Gy 6 hours after the exposure (split-dose group at 6-hour interval), pyknotic cells still decreased for 2 hours, and increased again to reach the second peak incidence with an elevation similar to the first one at 12 hours after the first exposure, i.e., 6 hours after the second exposure, then decreased gradually (Fig. 3b).

DISCUSSION

The present experiment demonstrated that the split-dose effects of X-irradiation on undifferentiated neural cells are rather simple in terms of the induction of acute cell death. About 6% and 13% of ventricular cells in the fetal brain mantle were involved in pyknosis after single doses of 0.125 Gy and 0.25 Gy, respectively. When exposures to 0.25 Gy, split into two 0.125 Gy were adopted at intervals of 0.5 and 2 hours, about 13% of the cells were also involved in pyknosis in both cases. Following two 0.125 Gy exposures at 6-hour intervals, 6% of cells were involved in pyknosis after the first exposure and another 6% was killed by the second exposure. These results indicated that low-dose X-irradiation shows simply additive effects of split doses up to 6-hour-interval on the cell death of ventricular cells.

Cultured human lymphocytes exposed to low doses of 0.005–0.2 Gy X-irradiation were reported to have become less susceptible to subsequent exposure to 1.5 Gy. A maximal reduction in chromatid breaks induced by 1.5 Gy was observed when a low dose of 0.01 Gy was given 5–6 hours before the 1.5 Gy irradiation. In the present experiment the fetal brain was exposed to two doses of 0.125 Gy at 6-hour intervals, but no adaptive response of ventricular cells to the second exposure was observed.

Ultrastructural features of acute cell damage caused by X-irradiation in the ventricular zone of fetal brain were previously described in mice. The dying cells undergo nuclear and cytoplasmic condensation and fragmentation, and then they are phagocytosed by their viable neighbors. These morphological features characterize the cell death as apoptosis, which is an active and controlled rapid process of selective cell deletion on endogeneous lethal mechanisms. Undifferentiated primordial cells are known to commit suicide (apoptosis) to eliminate acquired genetic abnormalities from their population rather than make DNA repair, which is rarely complete. This may lead to the high radiosensitivity of undifferentiated cells of fetal brain.

In the present experiment the peak incidences of apoptosis following split doses of X-irradiation of 0.25 Gy in total at 0.5- and 2-hour intervals were quite similar to that following a single 0.25 Gy in the ventricular cells. Sellins and Cohen demonstrated that the “signal” of apoptosis produced in the cell by ionizing radiation can persist for at least 6 hours if the apoptosis is inhibited. In the fetal brain on day 13 of gestation, a small number of cells begin to manifest nuclear and cytoplasmic condensation 2–3 hours after exposure to X-irradiation, and were followed by many cells afterward (Fig. 3). The signal of apoptosis produced by 0.125 Gy, if not sufficient to trigger endogenous lethal mechanisms, might remain in the cell until the second exposure within 6 hours. The second
exposure might make the signal sufficient for apoptosis in the additive fashion. Thus the lethal effect of low-dose X-irradiation on the undifferentiated cells of fetal brain may be in the simple additive mode.

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