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DISCLAIMER

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DNA DAMAGE IN MAMMALIAN CELLS FOLLOWING HEAVY-ION IRRADIATION [1]

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INTRODUCTION

Therapeutic irradiation of intracranial lesions inevitably exposes normal brain tissue to potential injury. Such effects may appear after a long latency period and are often associated with demyelination and vascular damage. The critical cell types implicated in early and late delayed radiation injury in brain are the oligodendroglial cells and the endothelial cells, respectively [4]. Consequently, their cellular response during the long latency period is important in assessing the underlying cellular basis of late CNS damage. There are several reasons to study DNA damage to the brain *in vivo*. First, the DNA molecule is the most sensitive target in the cell; damage to

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DNA during irradiation has been shown to be a sensitive index of response, for doses as low as 100 mGy. In addition, DNA damage is a reliable measure of the direct effects of irradiation because it is least influenced by effects in the surrounding cells and cell-to-cell interactions.

Experimental studies have suggested that the slope of the dose-response of DNA strand breaks *in vivo* represents an index of radiosensitivity [8]. Furthermore, it has been suggested that the process of DNA repair is related to the radiosensitivity for different cell types in the mouse brain [6]. For cultured cells, the DNA repair, or the residual DNA damage after a given time, has been used as a measure of relative biological effectiveness(RBE) [2,9].

In our laboratory we have been investigating DNA damage and repair in the endothelial and oligodendroglial cells of the mouse brain after irradiation using two different types of heavy ions, helium and neon. The method used, the unwinding technique with subsequent staining of the DNA with acridine orange, has been proven to be useful for nondividing cells [1,11] and analysis using a microscope photometric technique. Our primary goal has been to obtain a measure of RBE, in the dose range used in clinical treatment of various brain disorders using heavy charged particle radiosurgery.

METHODS AND MATERIALS

Animals: Female mice of the CB6F1 strain (age 6 wk) were used.

Irradiation procedure: The plateau region of the helium ion beam from the 184-inch Synchrocyclotron (LET = 2 keV/μm) or the plateau region of the neon ion beam at the Bevalac (LET = 30 keV/μm), Lawrence Berkeley Laboratory, were used. The cerebrum was locally irradiated. The dose rate was 3 to 5 Gy per min.

Cell preparation technique: The method has been described in detail [1] and is briefly summarized below. After irradiation, the anesthetized mice were perfused,

and the capillaries and oligodendroglial nuclei from the cerebral cortex were prepared in cell culture medium, Eagles's MEM (Sigma), at 0° C. The cell nuclei and the capillaries were embedded in agarose on microscope slides and after treatment with alkaline solution (pH > 11), they were stained with acridine orange. Then, each single cell nuclei was measured in a microscope photometer (Leitz MPV2) after excitation with light from a Hg lamp. For this input spectrum, green emission (λ = 525 nm) reflected binding of the dye to double stranded DNA and red emission (λ > 610 nm) reflected for binding to single stranded DNA. The ratio of the measured intensities in each wavelength band (red/green) was taken as an index of DNA damage.

In a separate experiment, the prepared glial cell nuclei and the capillaries in cold medium were irradiated *in vitro* with He ions. These cells were treated with alkaline solution immediately after irradiation, and were stained with propidium iodide (concentration 25 μg/ml in PBS), a stain specific for double stranded DNA.

RESULTS

Initial damage/dose response: Initial DNA damage was measured as soon as possible after irradiation, i.e., 5 to 6 minutes. The estimated number of DNA strand breaks after varying doses of He ion and Ne ion irradiation are shown in Fig. 1 and 2, respectively; approximately 20–40 cells from 1 or 2 mice were used for each data point. The analysis of variance (Barlett test) gave a significant difference in response between the oligodendroglial and endothelial cells for Ne ion irradiation. For He ion irradiation however, no significant difference in response was obtained. The ratio of the slopes for the dose response curves for the glial cells is approximately 3. The dose response immediately after irradiation *in vitro* is shown in Fig. 3. Here, a clear difference in response between the two cell types is demonstrated.

Repair of DNA after irradiation: DNA damage after irradiation with 15 Gy He ions or 2 Gy Ne ions is plotted in Fig. 4 and 5, respectively. For irradiation with

15 Gy He ions, both cell types apparently repair fully within 4 hours. After 2 to 4 days, DNA degradation is seen in the glial cell nuclei but not in the capillary cells. After 2 Gy Ne irradiation, the time for repair is longer in the glial cells (approx. 10 h) than the endothelial cells (approx 7 h).

DISCUSSION

The DNA damage induced for proliferating cells *in vitro* (single and double strand breaks, base damage, alkali-labile sites) after high-LET irradiation shows fewer initial number of lesions per cell per Gy as compared to low-LET irradiation but a higher proportion of breaks appear that are repaired slowly or not repaired at all [9,10]. Incomplete repair is considered to be the cause of death of the cell. However, Keng et al [5] have shown that in retinal photoreceptor cells irradiated *in vivo*, the repair of DNA damage is completed even if it takes a long time, up to several weeks. Wheeler et al [12] have demonstrated full repair of DNA damage cerebellar neurons in the rat 8 hours following 12.5 Gy of low-LET (gamma rays) irradiation.

We have shown that glial and endothelial cells in the brain repair the DNA damage after high dose, low-LET (15 Gy He) and low dose, high-LET (2 Gy Ne). However, the temporal pattern of repair is different for the two cell types. Based on histological data, Law [7] also suggested that the two cell types have different repair capabilities. The DNA degradation seen in the glial cells after 15 Gy He ion irradiation starts at the same time as pycnotic oligodendroglial cells are seen after higher doses of X-rays [3]. The difference in dose response immediately after Ne irradiation could be due to a fundamental difference between the cell types, but could also be due to a difference in the fast repair component, which cannot be detected at 6 minutes after irradiation. The lack of difference between cell response after He ion irradiation *in vivo* could also be caused by the protective hypoxia of nembutal anesthesia; the glial cells might be more hypoxic than the endothelial

cells. This conclusion is confirmed by the result for irradiation *in vitro*; here, a difference in response is seen for the two cell types. It can be assumed that the *in vitro* condition gives the same oxygen level to both cell types. It has been shown previously that there is no difference in the yield of DNA damage in endothelial cells if the capillaries are irradiated *in vivo* or *in vitro* [1]. These factors may prove important in clinical treatment of intracranial lesions exhibiting regions of varying hypoxia.

CONCLUSIONS

The conclusions from these preliminary data are: (1) DNA damage in oligodendroglial cells occurs at lower doses of heavy ion irradiation than in endothelial cells, i.e., the glial cells are more sensitive to heavy ion irradiation than the endothelial cells; (2) the DNA repair in oligodendroglial cells after heavy ion doses of 2 to 6 Gy occurs more slowly than in the endothelial cells; (3) based on dose response slopes of DNA damage in oligodendroglial cells at 6 minutes following irradiation of 2 to 6 Gy, an RBE of 3 is obtained for plateau Ne ions as compared to plateau He ions. This suggests that cell-mediated early delayed radiation damage (demyelination) would be due to direct cellular injury and repair in the oligodendroglial cell population, and would be dose-dependent, and would be expected to occur earlier and after doses less than would occur in late delayed brain injury. Furthermore, the dose response RBE values suggest considerable caution in the use of high LET radiation in the treatment of brain lesions.

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FIGURE CAPTIONS

Figure 1: Estimation of DNA strand breaks following helium ion irradiation. Intensity of red fluorescence ($F_{>610}$) divided by intensity of green fluorescence (F_{525}) from acridine orange in single endothelial or oligodendroglial cells immediately (6 min) after irradiation *in vivo*.

Figure 2: Estimation of DNA strand breaks following neon ion irradiation. Intensity of red fluorescence ($F_{>610}$) divided by intensity of green fluorescence (F_{525}) from acridine orange in single endothelial or oligodendroglial cells immediately (6 min) after irradiation *in vivo*.

Figure 3: Intensity of propidium iodide staining (estimate of double-stranded DNA) in single cells irradiated with helium ions *in vitro* at 0°. The values are normalized to control values.

Figure 4: Estimation of DNA strand breaks vs time after irradiation with 15 Gy helium ions *in vivo*.

Figure 5: Estimation of DNA strand breaks vs time after irradiation with 2 Gy neon ions *in vivo*.

DNA DAMAGE, HE ION IRRADIATION (150 MeV/u), MOUSE BRAIN in vivo

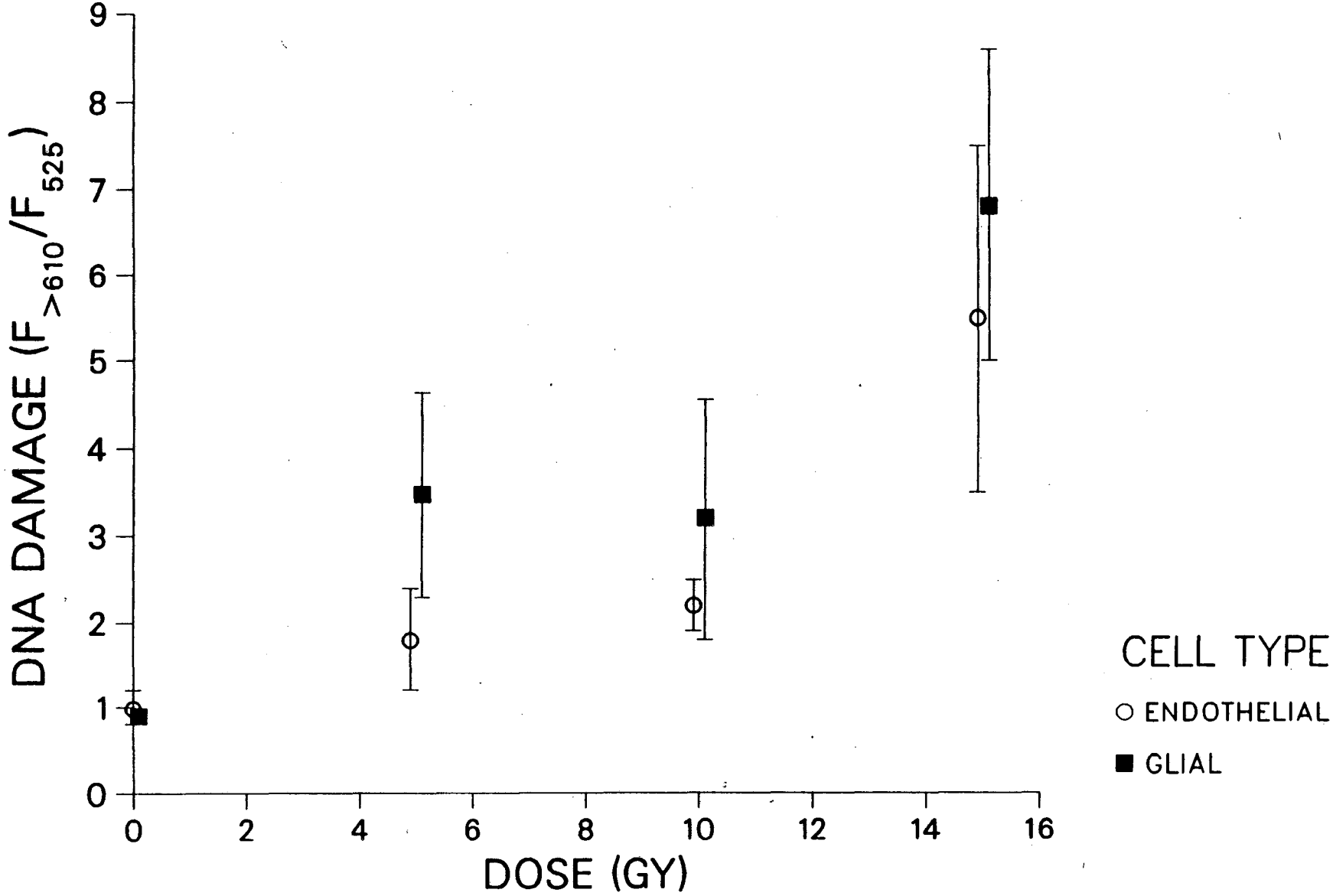

FIGURE 1

DNA DAMAGE, NE ION IRRADIATION (425 MeV/u), MOUSE BRAIN in vivo

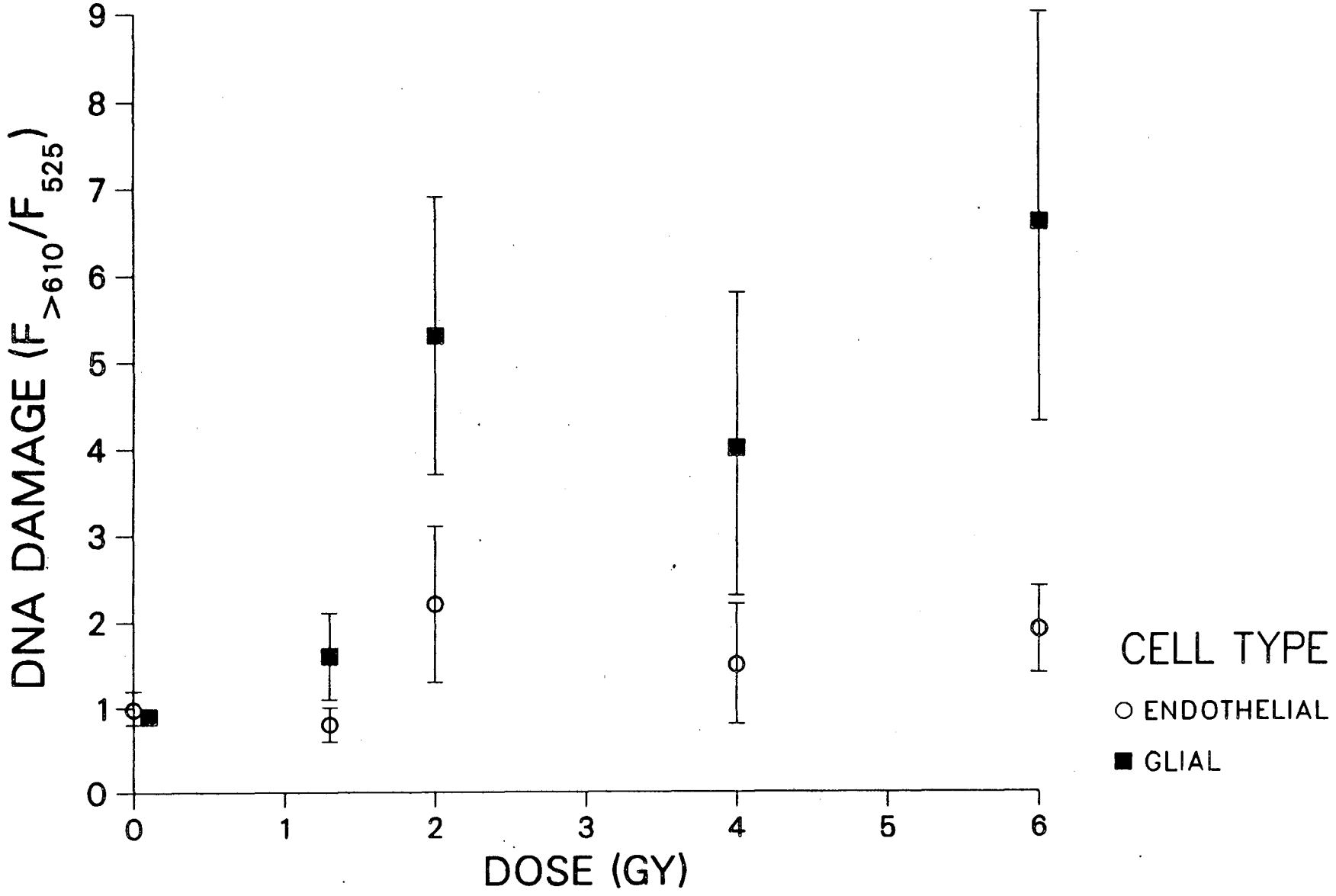

FIGURE 2

DNA DAMAGE, HE ION IRRADIATION (150 MeV/u), MOUSE BRAIN in vitro

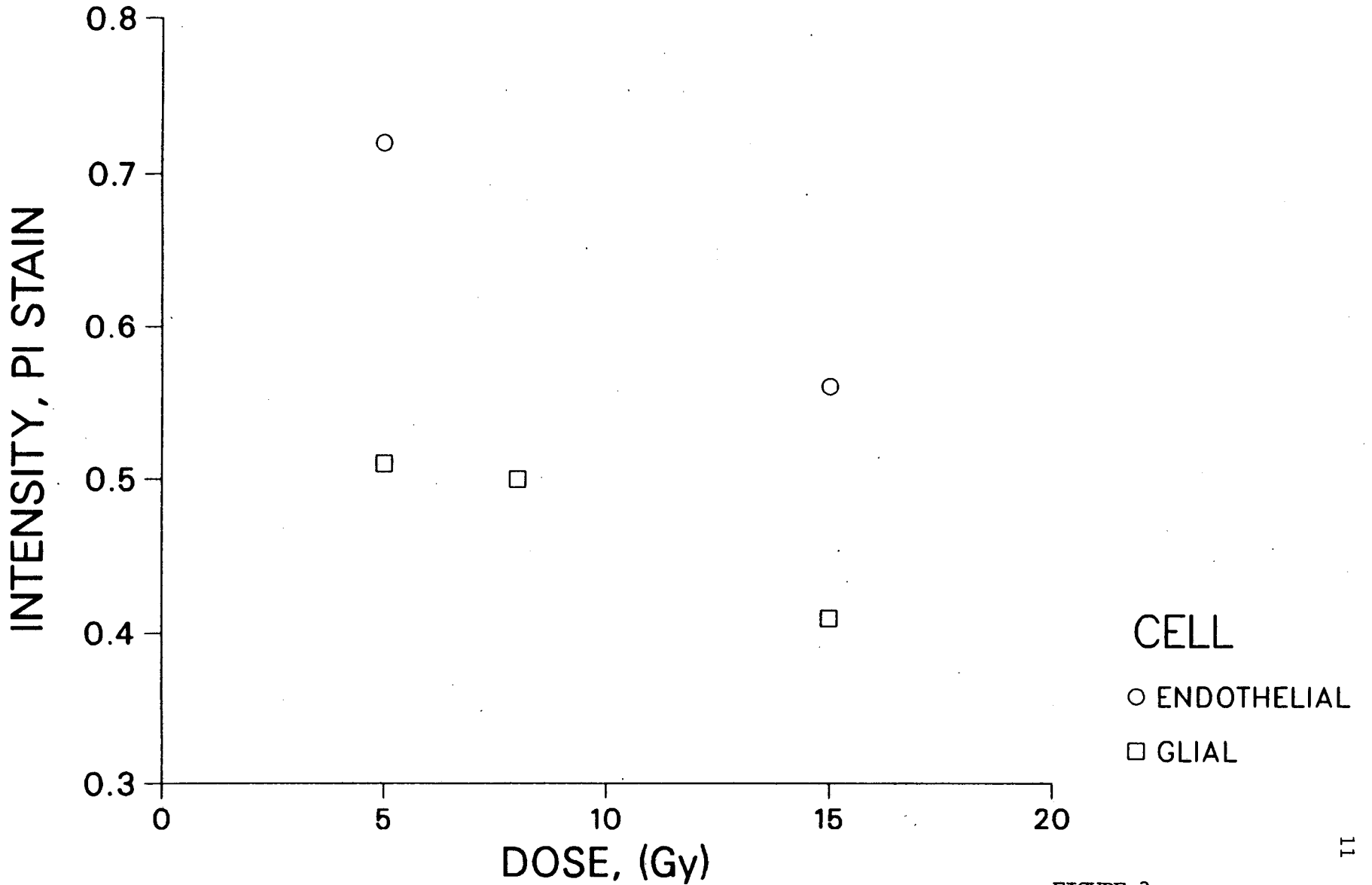

FIGURE 3

DNA DAMAGE REPAIR, HE IONS (150 MeV/u, 15 Gy), MOUSE BRAIN in vivo

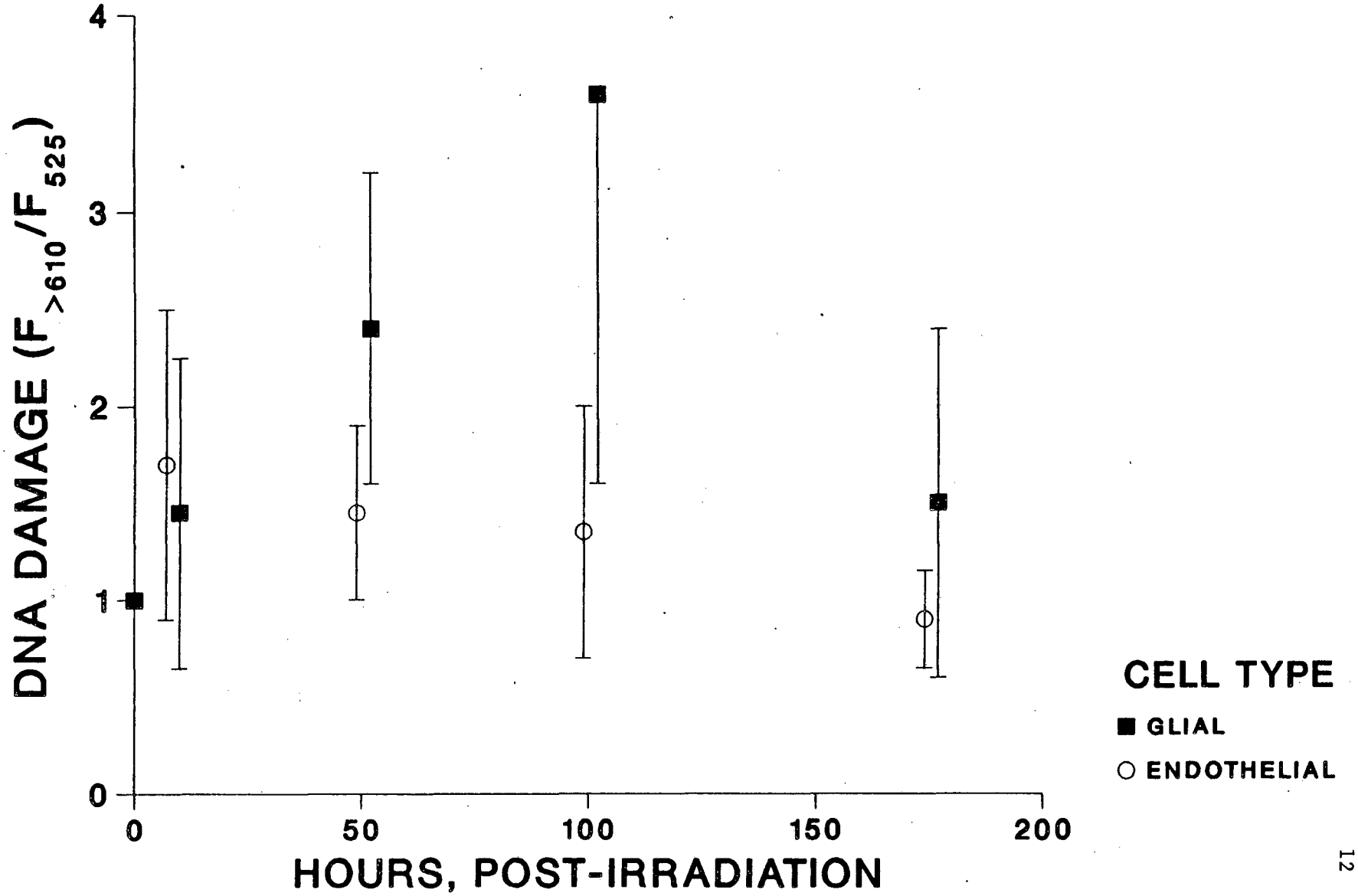

FIGURE 4

DNA DAMAGE REPAIR, NE IONS (425 MeV/u, 2 Gy), MOUSE BRAIN In vivo

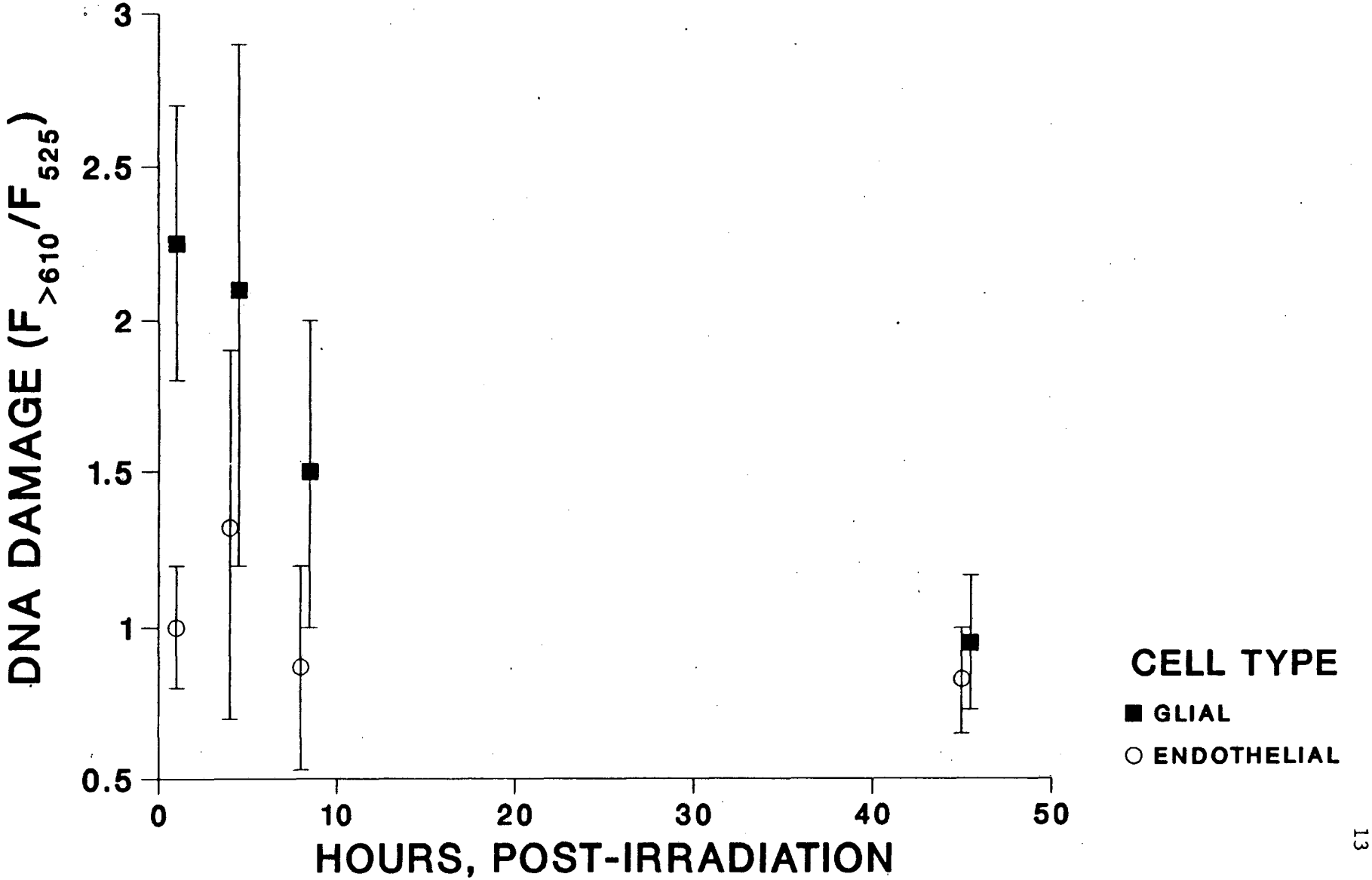

FIGURE 5

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