APOPTOSIS INDUCED BY HYPERTHERMIA IN HUMAN GLIOBLASTOMA CELL LINE AND MURINE GLIOBLASTOMA

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ABSTRACT

Objective: To study the role of apoptosis in tumor cell of malignant glioma death following treatment with hyperthermia and calcium ionophore. Methods: The apoptosis induced by hyperthermia and calcium ionophore, A23187, in human glioblastoma cell line TJ905 and murine glioblastoma G422 was evaluated by characteristic findings in DNA agarose gel electrophresis, ultrastructural examination and flow cytometric analysis. Results: Apoptosis could be induced by moderate hyperthermia, but not by mild hyperthermia, calcium ionophore enhanced significantly the effect of mild hyperthermia on the induction of apoptosis. Conclusion: This result indicates that apoptotic cell death is one of the mechanisms of hyperthermic therapy for malignant glioma and taking measures to increase the cytolic calcium may enhance the effect of hyperthermia.

Key words: Apoptosis, Hyperthermia, Calcium ionophore, Glioblastoma

Apoptosis is a type of cell death that plays an important role in the early development and growth of normal tissue. It is modulated by a variety of stimuli. In multicellular organisms, homeostasis is maintained through a balance between cell proliferation and cell death. Recent evidence suggests that derangement of apoptosis also contributes to a number of human diseases including tumors.[1] More attention has been paid to cell proliferation rather than apoptosis in the study of tumorigenesis. However, it is now suggested that inhibition of apoptosis is also important in the development of malignancies than what we previously believed. Thus, maneuvers that can induce apoptosis will be of considerable benefit in the treatment of malignancies.[2]

It is well known that the efficacy of treatment of malignant gliomas is not favorable even when the currently available combined therapies including surgical resection, radiation and chemotherapy have been used. In a number of reports as well as in our previous studies,[3-5] it has been shown that hyperthermia may be used as an effective adjuvant therapy for malignant gliomas and, in addition, there have been some reports that apoptosis can be triggered by hyperthermia.[6,7] In order to provide insights into the mechanism of hyperthermic cell killing, apoptosis induced by hyperthermia was investigated in the present study. Meanwhile, it was reported that Ca^{2+} dependent endonuclease.[8] Whether Ca^{2+} ionophore A23187 can enhance the effect of hyperthermia on the induction of apoptosis was also examined. This study employed three different diagnostic techniques to identify cell apoptosis i.e., DNA gel electrophoresis, electron microscopic examinatioin and flow cytometric analysis.[9-11]

MATERIALS AND METHODS

Cell Line, Agent and Animal

The human glioblastoma cell line TJ905 was established in the Laboratory of Neuro-oncology, Tianjin Neurological Institute and maintained as a monolayer culture in Waymouth MAB 87/3 medium (Gibco, MD, USA) supplemented with 15% fetal calf serum at 37℃ in 5% CO_2. The cells were subcultured every 2 - 3 days. Kungmin mice bearing the G422 glioblastoma in unilateral cerebral hemisphere induced by methylchloroethane were provided by the

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Heating TJ905 Cells and Mice

TJ905 cells (5×10^6) were plated in each 75 ml flask containing 10 ml of culture medium. After 48 hr incubation they were heated in a water bath at 43℃ and 45℃ for 30 minutes respectively. To assess the effect of the calcium ionophore A23187, a final concentration of 5 μ mol/ml of A23187 was added to the cell culture immediately prior to heating at 43℃ for 30 minutes and controls comprised cultures that had not been heated in the presence of A23187, then the flasks were returned to an incubator at 37℃ in 5% CO_2. The samples of heated cells were taken sequentially at 2, 6, 12 and 24 hours for examination after completion of the heating. Six flasks of cells were examined at different time points for each type of treatment. As to mice bearing G422 gliomas, they were heated in an incubator set at 45℃ and their body temperature actually achieved was approximately 43–44℃ measured by thermometer. The heating was continued for 30 minutes. After completion of the heating, they were returned to room temperature, decapitated at 2, 6, 12, 24 and 48 hour. Ten mice were taken for examination at each time point. The gliomas were removed and preserved at - 70℃.

Extraction of DNA and Electrophoresis

DNA was extracted from the control and heated TJ905 cells as well as G422 tumor tissues sampled at different time points. The cells were rinsed twice with cold 10 mmol/L Tris HC1 (pH8.0) buffer and collected with policeman in 0.5 ml of TBS. The cell pellet was collected by centrifugation at 1500 g for 10 minutes at 4℃ and washed once with 5 - 10 ml of TBS. The cell concentration was adjusted to 5×10^6 cells/ml. To each ml of cells, 10 ml of extraction buffer [containing 10 mM Tris-Hcl (pH8.0), 0.1 mmol/L EDTA (pH8.0), 20 μg/ml Rnase, 0.5% SDS] was added for 1 hr incubation, then proteinase K at the final concentration of 100 mg/L was added. The cells were lysed at 50℃ for three hours. Then the lysates were extracted with an equal volume of saturated phenol and centrifuged at 5000 g for 15 minutes. The supernatant was removed to fresh tube and fully mixed with 0.2 volume of 10 mmol/L ammonium acetate, 2 volumes of ethanol to precipitate DNA. The precipitate was washed with 70% ethanol and air-dried. The DNA was dissolved in 0.5 ml of TE (pH8.0).

The tumor tissue from mice bearing G422 gliomas was weighed and ground in liquid nitrogen, PBS was added and centrifuged at 5000 g for 10 min twice. Then 10 ml of extraction buffer was added to each gram of tissue. The subsequent procedures for preparation of DNA were the same as described above.

DNA (40 μg) was underwent electrophoresis on an 1.5% agarose gel containing ethidium bromide for approximately 2 hours at 100 volts. Lambda DNA/HindIII was used as standard for DNA fragment size.

Study Using Electron Microscope

The TJ905 cells were centrifuged and washed in PBS before being fixed with 6% glutaraldehyde phosphate buffer solution for 2–3 hours. The G422 gliomas were fixed with 6% glutaraldehyde as soon as they were removed from the brain. After a repeated washing with 0.1 mol/L phosphate buffer, they were postfixed in 1% osmium tetroxide for 2 hours, dehydrated with acetone, embedded in Epon-821. Ultrathin sections were stained with sodium acetate and lead citrate, and observed under the H600 transmission electron microscope.

FCM Analysis

The G422 murine gliomas sampled at different time points after heating at 45℃ for 30 minutes were preserved at –70℃. 200–300 mg thawed tumor tissues were homogenized to prepare the cell suspension. After washing with PBS three times, Rnase (0.5 ml, 100 Ku) was added to the cell suspension at 4℃ for 20 min. Then the cells were stained with 1 ml (50 μg/ml) propidium iodide for 10 minutes at room temperature and filtered with 35 mm nylon mesh. PI fluorensce of individual cells was analyzed by FACSTAR flow cytometer (FCM), (Becton Dickinson, CA, USA) equipped with an Argon ion laser. 10,000 events/sample were acquired in list mode by a Hewlett-Packard consort 32 minicomputer.

RESULTS

Electrophoresis of DNA extracted from both TJ905 cells and G422 murine gliomas at 2, 6, 12, 24 hours after heated at 45℃ for 30 min showed that internucleosomal cleavage of DNA into fragments that are multiples of 180 - 200 base pairs had occurred. This DNA cleavage results in a characteristic “ladder” pattern on gel electrophoresis. It appeared during the entire time course of