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Effects of Photodynamic Therapy on the Ultrastructure of Glioma Cells[1]

SHAO-SHAN HU[*], HONG-BIN CHENG[*], YONG-RI ZHENG[*], RU-YOU ZHANG[*], WU YUE[1], AND HAN ZHANG[#,2]

[*]*The Second Affiliated Hospital of Harbin Medical University, Harbin 150086, Heilongjiang, China;*
[#]*Beijing Neurosurgical Institute, Capital Medical University, Beijing 100050, China*

Objective To study the change in ultrastructure of C6 glioma cells after photodynamic therapy (PDT), to compare morphological differences in necrosis and apoptosis before and after PDT treatment, and to evaluate the effect of photodynamic therapy on the blood brain tumor barrier (BTB) of C6 glioma. **Methods** The model was produced by transplanting C6 glioma cells cultured *in vitro* using Peterson method into the caudate nuclei of Wister rats. The experiment group received PDT for two weeks after the operation. The sub-cellular structure, blood-brain-barrier (BBB) and BTB in both groups were observed under electron microscope. **Results** Apoptosis in different phases and necrosis could be observed in some C6 glioma cells. Swelling occurred on the ultrastructure of cellular organs such as mitochondria and endoplasmic reticulum in most of the cells. Damage to the BTB, reduction of the number of cellular organs in endothelial cells of the capillary blood vessels, stretch of the tight junction, and enlargement of the gaps between endothelial cells were also seen in the experiment group. Meanwhile, limited impact on the normal sub-cellular structures and BBB was observed. **Conclusion** PDT could induce apoptosis and necrosis of C6 glioma cells due to the damage to the ultrastructure of mitochondria and endoplasmic reticulum. The weakened function of C6 glioma BTB initiated by PDT makes it possible to perform a combined therapy of PDT and chemotherapy for glioma.

Key words: Photodynamic therapy; Mitochondria; Blood brain barrier; Blood brain tumor barrier; Glioma

INTRODUCTION

Photodynamic therapy (PDT) is a kind of cytotoxic therapy that can induce apoptosis and necrosis. When photosensitizer is allocated on cellular organs and provoked by laser, apoptosis, and necrosis could be induced through some signal pathways, including protein provokation, disphosphorylation, and secondary messengers, such as calcium and cAMP. PDT may also cause change to attachment molecular expression and other surface-receptors. However, the detailed pathways remain uncertain. In this research we studied the change in ultrastructure of C6 glioma cells after PDT, compared the morphological differences in necrosis and apoptosis before and after PDT, and evaluated the effect of PDT on the blood brain tumor barrier (BTB) of C6 glioma.

MATERIALS AND METHODS

Cells

C6 glioma cell line was purchased from the Shanghai Cell Center, Chinese Academy of Sciences.

Animals

Thirty male Wistar rats weighing 280-300 g were purchased from Harbin Medical University.

Apparatus

Following instruments were used in this study: phase contrast microscope (IX-70, Olympus, Japan), CO_2 incubator (Heraeus, China), transmission electron microscope (IEM-1220, Olympus, Japan), and PDT apparatus (LH400, China).

Reagents

Hepes, trypsin, DMSO, HMME, RPMI medium 1640, and fetal bovine serum (FBS) were purchased from Sigma.

Culture of Glioma Cells

C6 glioma cells were cultured with RPMI

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[2]Correspondence should be addressed to Han ZHANG. Tel: 86-10-67028785. E-mail: meishazhang@yahoo.com.cn
Biographical note of the first author: Shao-Shan HU, male, M. D., born in 1970, majoring in microneurosurgery.

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medium-1640 supplemented with 10% FBS, in an incubator containing 5% CO_2 at 37℃, then digested with 0.25% trypsin and washed twice with Hanks to make the suspension.

Establishment of Rat Glioma Model

A rat was fixed on stereotaxic apparatus after anaesthesia, scalp was cut to show the skull markers, hole was drilled 1.0 mm anterior to bregma and 3.0-3.5 mm lateral to sagittal suture[1]. A total of 2.0×10^6 C6 cells suspended in 15 μL Hanks were transplanted into each rat (survival rate >95%) from the hole. The depth was 5 mm below the dura. The injection was continued for more than 10 min, then the needle was drawn out slowly after another 5 min. Scalp was sutured and the rat was fed for further study.

PDT and Microscopy

Twenty rats with giloma successfully inoculated were selected by image examination and served as models. The rats were divided randomly into control (n=10) and experimental (n=10) groups. Each animal was given a photosensitizer (HMME, 5 mg/kg) intraperitoneally and anaesthetized peritoneally with 2% pentobarbital (40 mg/kg). Four hours later, the inoculated tumor was exposed with microneurosurgical method, and received irradiation of LH400 laser photodynamical therapeutic equipment (0.4 W, 120 J/cm^2). The control group received the same treatment except for irradiation. Four hours later, both brain tissue and tumor were fixed with 2.5% glutaraldehyde. Five samples were randomly picked out from each group for observing the change in ultrastructure under microscope.

RESULTS

Experimental Group

The effect of PDT on normal brain was limited. The glioma cells contained intact membranes and well-kept cellular organs. No change in BBB was observed. The glioma cells were consisted of both no-fenestra capillary endothelial cells containing a large number of mitochondria and astrocytes with their feet arranged tightly along the basilemma. Some portions of the endothelial cells containing nuclei protruded into the cavity while some plasm portions showed few parts of the membrane extending into the cavity. The thickness of endothelia was about 500 nm. The tight junction between endothelial cells was seen as tile-shaped. There were many mitochondria in the pericytes. The basilemma of the capillary blood vessels and the neurepithelium were even, showing a medium electron density. The thickness was about 100 nm. Some pinosomes could be observed below the membrane. Some glioma cells attached to the outside parts of capillary blood vessels. The synapses appeared symmetrical with a few of synaptic vesicles.

Compared with the normal brain, the ultrastructure of giloma cells looked obviously ruined. The mitochondria, lysosome, and endoplasmic reticulum became swollen. Apoptosis and necrosis were found in different phases. The damage to BTB was severe, including stenosis of capillary blood vessels with obvious uneven-density membrane and some dissolved endothelial cells. The dysfunction of swollen astrocytes, focal necrosis of the feet, pyknosis of the nuclei, reduction of the number of cell organs, degranulation of some rough endoplasmic reticula, vacuolar degeneration in parts of the mitochondria, and blurred-structure of the tight junction between endothelial cells were observed (Figs.1-6).

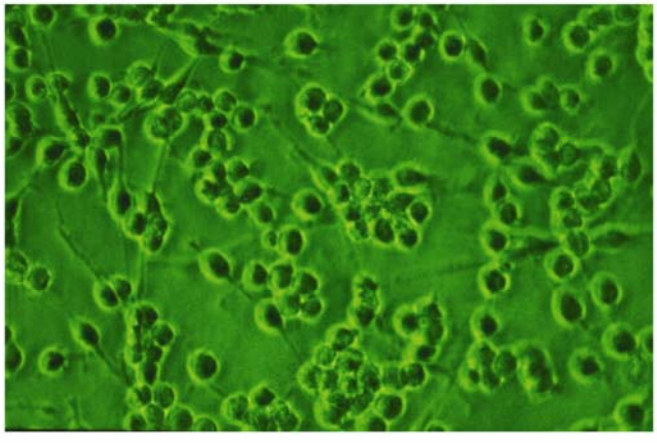

FIG. 1. Phase contrast microscope images of untreated C6 glioma cells (×106).

FIG. 2. Inoculated tumor (MB stained part).

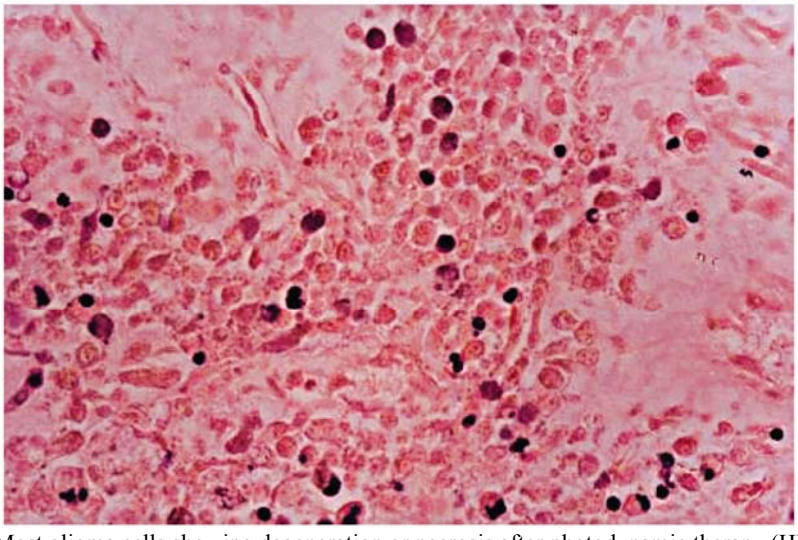

FIG. 3. Most glioma cells showing degeneration or necrosis after photodynamic therapy (HE stain).

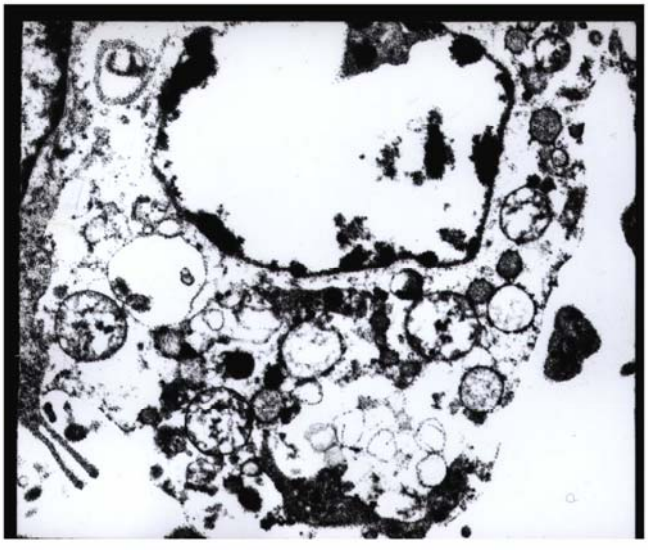

FIG. 4. Necrosis of C6 glioma cells, swollen mitochondrion, and degenerated vacuoles (electron microscope, ×8000).

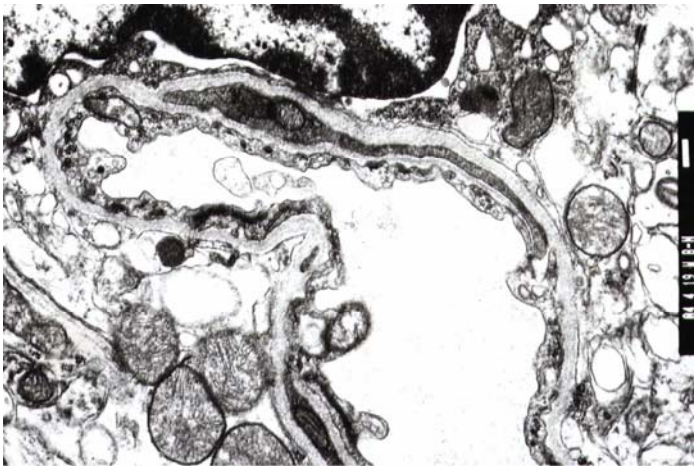

FIG. 5. BBB structure of inoculated tumor with no fenestrae in endothelial cells, uniform basilemma, and well- kept tight connection (electron microscope, ×12000).

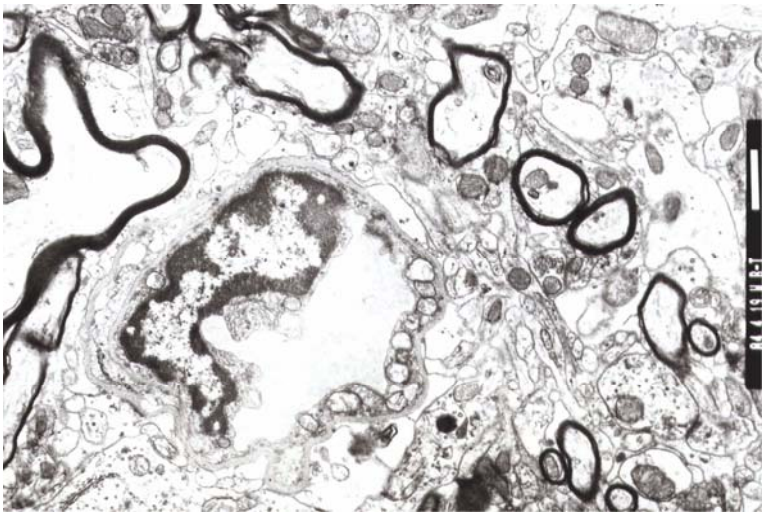

FIG. 6. BTB of glioma cells after PDT with degenerated vacuoles of mitochondrion in the endothelial cells and loose connection (electron microscope, ×6400).

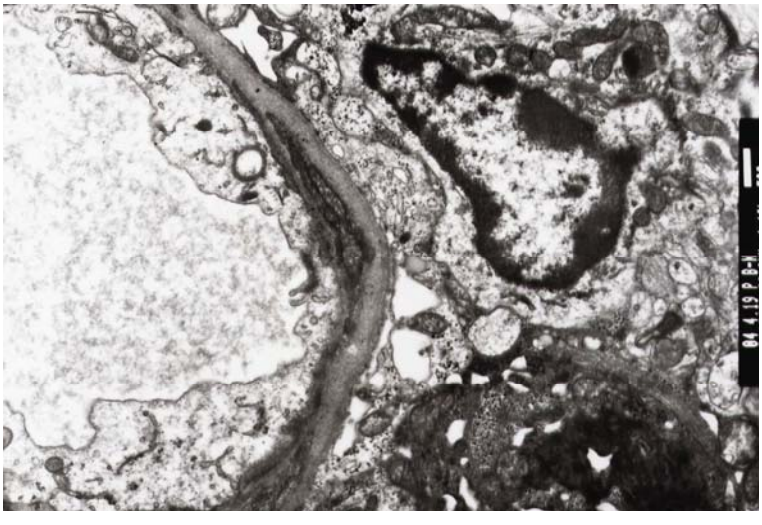

FIG. 7. BTB of glioma cells after PDT with incrassate basilemma, diminished cellular organs of endotheial cells, Swollen, and necrossed endfeet of astrocytes (electron microscope, ×8000).

Control Group

The BBB and BTB of normal brain and the tumor were in good condition, and their structures were well kept.

DISCUSSION

The location of photosensitizer is particularly important, because it can determine the position of original damages. Calcium serving as the secondary messenger is an essential factor for most of the pathways related to the location of photosensitizer. We observed the change in ultrastructure of C6 glioma cells under electron microscope after PDT, suggesting that the original damage might occur at the site of photosensitizer on cellular organs[2-5]. The damage to membrane structures such mitochondria may be due to the morphological change. Since photosensitizers have their own anchor points, the positions could be different when photosensitizers are changed. The specificity of photosensitizers in clinical practice is limited. This kind of photosensitizers causes extensive damages due to their nonspecific locations on membrane, mitochondria, lysosome, and other cellular organs. The photosensitizer used in this study was also nonspecific. The ultrastructural damage and apoptosis in early stage may result from PDT-damaged mitochondrion-cytochrome C releasing caspase 3, thus activating apoptosis pathways[6-8]. The amount of necrotic cells might be due to the secondary damage to the various signal pathways and calcium overload after PDT. Damage to capillary endothelial cells and dysfunction of BTB may lead to the higher permeability of BBB and BTB, so that it is easier for chemotherapeutic drugs to enter tumors. This can be explained as follows: PDT could provoke the photosensitizer in tumors, especially in capillary endothelial cells, and damage the microcirculation, impairing the relative structures, such as basilemma of endothelial cells, tight junction, and end-feet. Another possible reason is that injury of the mitochondria caused by PDT may lead to dysfunction of energy metabolism, during which the cytotoxic effect of PDT may cause the high permeability of BBB[9-11].

The results of this experiment indicate that PDT makes it possible for chemotherapeutic drugs to enter and treat the tumor more easily. We hope that it can significantly improve the therapeutic effect on glioma by opening the BBB selectively. It also can be expected that high blood concentration may be acquired by killing the tumor cells at a relatively low dose of chemotherapeutic drugs. PDT combined with chemotherapy may improve the therapeutic effect and reduce the side effects. However, further studies and clinical tests are still needed.

In conclusion, PDT can induce necrosis and apoptosis of C6 glioma cells, damage to the ultrastructure of C6 glioma cells may be due to the morphological change, break of the integrity of capillary blood vessels leads to disorder of the structure and dysfunction of BTB.

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