Anti-tumor of ILTT Combined with Cisplatin in a Rat Glioma Model

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Abstract: Objective. This study is to evaluate the therapeutic effect of interstitial laser thermotherapy (ILTT) combined with cisplatin chemotherapy on rat glioma model with deeply sited tumors. **Methods.** C_6 glioma cells were injected into the nucleus caudatus by stereotactic technique to induce transplanted gliomas in the rat brains. The rats bearing glioma were treated with ILTT in combination with cisplatin. The survival time of rats was observed up to 40 days after ILTT, and the tumor size of rats at 7th day after ILTT was measured. The data in different groups were statistically analysed. **Results.** The mean survival period of rats was (26.1± 3.6) days in the control group, (27.3±3.9) days in the cisplatin treated alone group, (30.4± 5.3) days in the ILTT alone group and (34.4±4.0) days in the ILTT combined with cisplatin chemotherapy group. At the 7th postoperative day, the maximum diameter of tumors measured in coronal sections of the brains were different between control group and experimental groups: (6.2±0.2) mm of the control group, (6.2±0.1) mm of the cisplatin treated alone groups, (4.8±0.2) mm of ILTT group and (4.9±0.1) mm of the ILTT combined with cisplatin chemotherapy group. **Conclusions.** Based on the above described results, we would conclude that the combination of ILTT and cisplatin chemotherapy might provide a significantly greater antitumor effect in the treatment of glioma. [Life Science Journal. 2005; 2(1): 90-93] (ISSN: 1097-8135).

Keywords: anti-tumor; cisplatin; interstitial laser thermotherapy; rat glioma

1 Introduction

Interstitial laser thermotherapy is a new invasive method for treating cerebral tumors, especially suitable for deep brain tumors[1]. Cerebral glioma is one of the most common tumors in central nerve system. Glioma is difficult to be completely excised by operation for its infiltrative growth, and those located in deep or functional regions are more restricted with operations. The chemotherapy effect is unable to be ensured because most chemotherapy drugs are difficult to penetrate into blood-brain barrier which exists in central nerve system. It is necessary to explore novel treatments because the conventional therapy effect on cerebral glioma is unideal. So we performed the experimental study on treating rat cerebral glioma with ILTT combined with cisplatin. The experimental findings are reported as follows.

2 Materials and Methods

2.1 Cerebral glioma model

C_6 cell line of rat cerebral glioma (presented kindly by Graduate School of Neurosurgery in Shanghai) were cultured for passages with culturing method of monolayer cells in complete culture solution RP-1640 with 10% bovine serum. During phase of logarithmic growth, the cells which were dealt with digestive solution of 0.24% pancretin and then washed with Hanks solution twice were made into suspended solution for inoculation. 64 healthy rats (offered by animal laboratory of Tongji Medical University) weighed 300-350 g were fastened after anesthesia to head arc of Jiangwan type, stereotactic apparatus for rat brain. The indications of rat skull were exposed by operation and the inoculated target was selected: at a point 1.0 mm to the front side of bregmatic midpoint, 3.0-3.5 mm to the right side of sagital sutura, and to a depth of 5 mm under dura. Hole in skull was drilled and $2.0\times10^6/15\mu l$ C_6 cells (livability>90%) were inoculated into each rat with micro-injector directed by stereotactic apparatus. The injection time was over 10 minutes and the injector was slowly withdrawn after being kept for 5 minutes, then the scalp was stitched. The rats fed on food and water freely in cages. The 14-days rats after inoculation were selected as cerebral glioma model in this experiment.

2.2 Treatment method

At 14 days after inoculation of C_6 cells, the rats were randomly divided into 4 groups: 16 rats in each group (10 of 16 were kept for observing survival time, 6 for measuring tumor diameter), Group A acted as control group (without treatment); Group B as cisplatin treatment group; Group C as ILTT group; Group D as ILTT combined with cisplatin group. 1 μg/g cisplatin was given to rats of group B by intravenous injection of rat tail, and afterwards each time equivalent dosage of cisplatin was given every 48 hours up to 3 times. 1μg/g cisplatin was given to rats of group D by intravenous injection of tail 30 minutes before ILTT, and each time equivalent dosage of cisplatin was given every 48 hours up to 3 times. Dexamethason (7 mg/kg) was given by intramuscular injection at 24 hours, 30 minutes before ILTT, and at 24 hours after ILTT, respectively, to prevent cerebral edema. After ILTT, 40,000 U of penicillin was administered by intramuscular injection each day for 3 days in sequence.

2.3 Interstitial laser thermotherapy technique

At 14 days after inoculation of C_6 cells, all rats were fastened to head arc of Jiangwan type, stereotactic apparatus (products of Shanghai) for rat brain after anesthesia with intra-abdominal injection of 2% pentobarbital sodium. Dura was excided along injector pathway after skull was exposed by operation. One side of optic fiber with a core diameter of 0.5 mm was connected with laser and another side (naked optic fiber) implanted into the selected target by stereotactic apparatus. Continuous Nd: YAG laser with wave-length of 1.06 μm and a power of 1.5 W was given for irradiation for 120 seconds, and equivalent quantity of laser was given again after an interval of 120 seconds, then treatment was finished.

2.4 The measurement of largest transverse diameter of tumors

At 21 days after inoculation of tumor, rats were perfused with alcoholic solution containing 0.5% methylene blue through aorta of left ventricle, complete brain was taken out and fixed with 10% formaldehyde, and then coronary section was made along the center of tumor (the center of optic fiber pathway). The largest transverse diameter of each tumor was measured on horizontal direction (including pathological tissues such as necrosis etc.).

2.5 Survival time

After treatment rats were recovered and raised continuously. Rats in each group were observed each day till rats died and the survival times (day) were written down. The survival time of rats without death was counted as 40 days (observation duration was 40 days). Two-sample test of nonparametric tests for multiple samples (Nemenyi method) was applied to analyze the survival time of each group.

3 Results

3.1 Survival time

Rats in all groups had no signs of nerve function deficiency such as hemiplegia et al. and there was no death in late period after treatment. The average survival time is: Group A(26.14 ± 3.58) days, Group B (27.26 ± 3.90) days, Group C (30.40 ± 5.27) days, Group D (34.42 ± 4.05) days. By comparison, there is a significant difference between group D and A ($P<0.01$) and a difference between group D and C($P<0.05$). There is no significant difference among other groups.

3.2 The largest transverse diameter of tumors

Group A (6.2 ± 0.2) mm, Group B (6.2 ± 0.1) mm, Group C (4.8 ± 0.2) mm, Group D(4.9 ± 0.1) mm.

4 Discussion

In recent several decades, the therapeutic effect of malignant glioma is still unsatisfactory. It is difficult to eradicate glioma for its infiltrative growth. Only biopsy can be usually performed and relapse will occur shortly after operation if tumor is situated in deep position or functional region of brain, the average survival time is 9 months[2]. It is necessary to explore novel methods to enhance therapy effect, so we carried out the experimental study on cerebral glioma treated with ILTT combined with cisplatin. The biological behavior of C_6 cells of rat with cerebral glioma are similar to that of human glioma, in recent years C_6 cells are widely applied to establish rat glioma model to expand experimental research. C_6 cell which was inoculated into caudatum could be acted as glioma model in deep position of brain[3].

Nd: YAG laser is a laser that can transmit through a flectional optic fiber which can be implanted into brain tumor tissue with brain tridimentional direction-finder. Its character of striking penetration to tissue ensures to develop more potent thermal effect by contacting irradiation to tissues with less scatter[4], so the laser can be a thermal resource for thermotherapy of brain tumors. It indicated in one research that thermal effect of Nd: YAG laser with ILTT developed an globose coagulated necrotic region surrounded by a band of edema zone[1]. It presented in our research that thermal

effect in edema zone also could induce apoptosis of brain glioma (pending materials for publication). The effect on coagulating tumor with ILTT may kill out an absolute majority of glioma tissue as possible as can, but a small quantity of tumor cells may survive in peripheral zone around effective focus for the infiltrative growth of glioma. If further corresponded treatments such as irradiation or chemotherapy are not applied, the effect of ILTT will be influenced because the residual tumor tissues may grow quickly to develop a relapse of tumor. It shows in this experiment that at 1 week after ILTT the largest transverse diameter of tumors is less than 5 mm in treatment group, and more than 6 mm in control group and cisplatin group; the average survival time of rats loaded with tumor in single ILTT group is longer than that of control group or single cisplatin group, but shorter than that of group of ILTT combined with cisplatin.

Presently the chemotherapy of brain tumors is a hard nut to crack relatively, most chemotherapy drugs are restricted to penetrated into central nerve system for blood-cerebral barrier[5,6], only those drugs with small molecular weight and with lipid-solubility can enter into tumor tissue across blood-cerebral barrier. Although *in vitro* studies proved that many chemotherapy drugs could influence the proliferation of glioma cells, they were not still used for therapy of brain glioma mainly because the drugs can't overpass blood-cerebral barrier[7]. Researches have proved that thermotherapy could open blood-cerebral barrier and promote the transportation of chemotherapy drugs between blood and tissues[8]. The experiment has showed the distribution of Evans Blue in thermal effect focus and its surrounded tissues[9]. Depending on the results we deduced that permeability of blood-cerebral barrier in effective focus and its surrounded tissues enhanced, so cisplatin can enter effective focus and its surrounded glioma tissues and accumulate to a definite concentration on which cisplatin can induce cell apoptosis to kill tumor cells. So we applied cisplatin to kill residual tumor cells right after ILTT. Cisplatin is a kind of non-specific cell-cycle drugs which can be activated in cells with light or heat and its effect is not restricted by cell cycle[10].

At present the reports on therapy of brain glioma with ILTT published increasingly, the animal experiment of EI－Ouahabi[11] presented that the average survival time of rats with brain glioma treated with single ILTT was a little longer than that of control group but there was no difference in statistics. Just as the analysis given by the author, the remained tumor tissue after ILTT and relapse of tumor may be the main cause for animal death. Reports from each clinical research differed[12,13]. Single ILTT was mostly applied for treatments in which temperature in therapeutic zones was controlled by computer system or was monitored by real-time imaging. Our experiment shows that although average survival time can be prolonged with single ILTT, there is no statistic difference by comparison with average survival time of control group. The average survival time of rats loaded with tumor can be more prolonged if cerebral glioma is treated with ILTT combined with cisplatin. There is a significant difference of average survival time between treatment group with ILTT combined with cisplatin and control group or single cisplatin group ($P<0.01$ and $P<0.05$). So the therapeutic effect of treatment group with ILTT combined with cisplatin is markedly superior to that of single ILTT group.

The mechanism of tumor therapy with ILTT may include several aspects as follows: (1) Coagulating tumor tissue: it mainly develops in therapeutic zones with higher temperature nearby optic fiber. Tumor cells in a state of nutrition deficiency acquires energy mainly by anaerobic glycolysis, and lactic acid accumulates in tumor cells; Vessels in tumor tissue which is short of smooth muscle can't dilate in accordance with the increase of temperature; and the vessel structure is not intact and has a low tolerance of heat. All these characters enhance the sensitivity of thermotherapy to tumor cells. (2) Inducing apoptosis of tumor tissue. (3) Activating cytokines, inflammatory media and vascular active substances to influence blood supply for tumor and/or develop a direct killing effect. Even so, the effect of single ILYY is still not ideal. Although average survival time on treatment group with ILTT is longer than that of control group, there is no statistic difference. It may be because that glioma grows with infiltration state but ILTT creates a globose effective focus, so residual tumor cells in some position lead to tumor relapse after the end of treatment. The opening of blood-cerebral barrier enables chemotherapy drugs to enter tumor tissue to kill remained tumor cells.

ILTT is a palliative therapy for glioma. After ILTT the relapse of glioma is inevitable for residual tumor cells. If ILTT is combined with chemotherapy and/or photodynamic therapy, it will helpful to raise the therapeutic effect of malignant cerebral glioma.

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