Activation of Hematoporphyrin in Alternating Magnetic Field: Possible Implications for Cancer Treatment

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A new mechanism of cell damage by alternating magnetic field with hematoporphyrin is described. C6 glioblastoma cell suspensions were exposed to an alternating magnetic field with frequency 180 kHz up to 60 min in the presence of hematoporphyrin in H_2O and in D_2O. The results presented suggest that an alternating magnetic field is able to activate hematoporphyrin, and this method may be a basis for cancer treatment.

Introduction

Photodynamic therapy (PDT) has been shown as a promising means of cancer treatment (Dougherty *et al.*, 1998; Popovic *et al.*, 1996) is based on injection of photosensitizing and in tumor-localizing dyes (e.g. hematoporphyrin (HP) and its derivatives) followed by exposure of the tumor to intensive light source (with wavelength about 650 nm), usually from a laser. This therapy generates highly cytotoxic singlet oxygen ($^1O_2$) which cause irreversible damage of tumor tissue. Unfortunately PDT is efficient only in cases where the entire tumor can be reached by light. Therefore tumors thicker than 4–5 mm are not completely eradicated by PDT.

Recently has been found that similar effects of ($^1O_2$) production exist upon ultrasonic irradiation of hematoporphyrin (Yumita *et al.*, 1989) and this method was applied to the treatment of rat sarcoma 180 (Yumita *et al.*, 1990). The advantage of this approach (termed sonodynamic therapy) is the much longer penetrance length of ultrasound in body tissues as compared to light. Another method proposed to overcome the difficulties of PDT is usage of short electric pulses for HP activation (Ward *et al.*, 1996).

In this report a new method of HP activation is described, which uses an alternating magnetic field.

Material and methods

For this investigation we have modified an experimental setup (Fig. 1) used previously in the study of liposomes (Babincová, 1993; Babincová, 1994; Babincová, 1995; Babincová and Babinec, 1997). The magnetic induction with the amplitude 5.8 mT and frequency 180 kHz was produced inside a water-cooled cooper induction coil with radius r = 17 cm (n =10 turns, with turn to turn distance z = 0.7 cm). The induction B of the magnetic field at the coil center was calculated using the equation (Jordan *et al.*, 1993

$$B = \mu_0 \left(\sum_{i=1}^{n} \frac{2r^2}{[r^2+(iz)^2]^{3/2}} + \frac{1}{r} \right) \frac{I}{2}, \qquad (1)$$

where I is the current through the coil and μ_0 is the absolute magnetic permeability ($4\pi \times 10^{-7}$ Am^2).

C6 glioblastoma cells, cloned originally from rat glioma (Benda *et al.*, 1968) and obtained from the American Type Culture Collection (Rockville, MD, USA), were maintained in a monolayer culture in Dulbecco's modified Eagle's medium containing

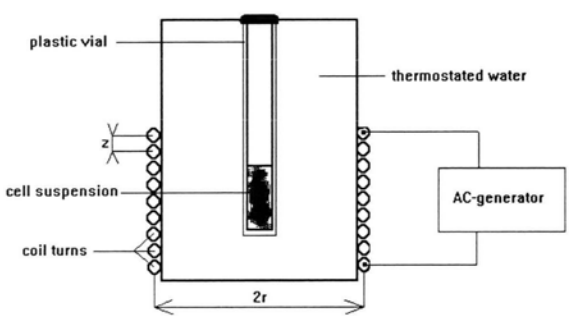

Fig. 1. Scheme of the experimental set-up for the irradiation of cancer cell suspension with an alternating magnetic field.

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10% (v/v) horse serum and 2.5% (v/v) fetal-calf serum in 100 mm Petri dishes. They were maintained at 37 °C in humidified air with 5% CO_2. Before the experiments the growth medium was replaced by a medium containing 0.1 mg/ml of hematoporhyrin dichloride (Sigma, St. Louis, MO, USA). A cell suspension (10^8 cells/ml) in a plastic vial was placed at the centre of the coil (the temperature increase in the cell suspension during irradiation was lower than 1 °C).

Results and Discussion

After irradiation cells were suspended in normal growth medium and the cell survival fraction was estimated by staining the cells with Trypan Blue dye. The integrity of the cells was determined by counting the number of unstained cells under the optical microscope. Dependence of cell survival fraction on the AC magnetic field treatment time is shown in Fig. 2. Without HP there was no effect of the magnetic field on glioblastoma cells. With HP present a 60-min treatment resulted in a damage of 80% of neoplastic cells.

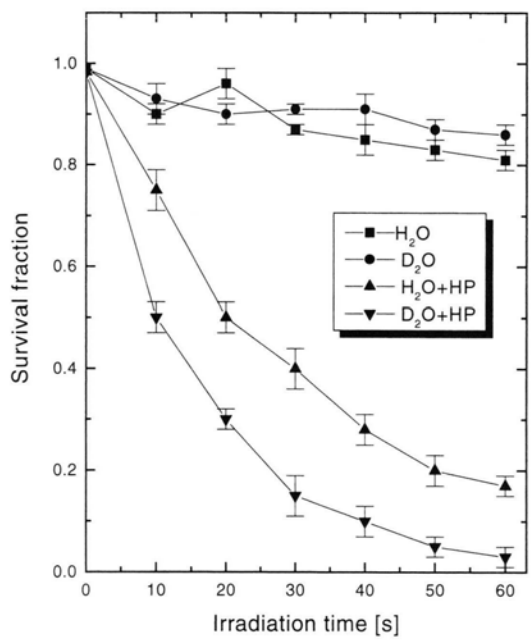

Fig. 2. Dependence of glioblastoma cell survival fraction on AC magnetic field treatment time. Each point represents the mean ± standard deviation from five independent experiments.

The mechanism of HP activation is probably analogous to that of light, sono, or electro-activations, namely the production of strongly toxic singlet oxygen. It seems that exposure of HP to alternating magnetic field influences electron mobility, electron redistribution and subsequently produces singlet oxygen $^1O_2$. We have monitored direct infrared emission of singlet oxygen at 1270 nm during the irradiation of HP solution (work in progress).

Since the life-time of $^1O_2$ in D_2O (60 μs) is substantially longer than in H_2O (4 μs) (Rogers, 1983), we have also investigated the influence of D_2O instead of H_2O. Cell suspension was centrifuged down at 1000 x*g* for 10 min and the pellet was resuspended in D_2O. This procedure was repeated three times. The results in Fig. 2 clearly demonstrate a more pronounced cytotoxic effect of HP, which supports the assumed mechanism of cell damage mediated by singlet oxygen.

In conclusion it is interesting to note that various variants of a free-radical mechanism were proposed to explain the influence of extremely weak electromagnetic fields on living organisms (Kaiser 1996). In the light of these studies it is not surprising that intense electromagnetic fields are also capable to produce free-radicals upon irradiation of some compounds, but now with the beneficial effects a destroying neoplastic cells. There are several parameters that may be optimized for this method, e.g. intensity and frequency of electromagnetic field, exposure time, and also the nature of chemical compounds used for activation. We hope that the proposed method after elaboration would be useful for the cancer treatment.

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