Laserthermia Kills Acidotic Cancer Cells More Effectively than Conventional Hyperthermia

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Abstract. The aim of this study was to investigate how acidosis affects the sensitivity of F9 embryonal carcinoma cells to conventionally induced hyperthermia and Nd-YAG laser-induced laserthermia. Nigericin, a polyether ionophore, was used to reduce the intracellular pH to 6.8, while the control cells were maintained at a physiological pH of 7.4. Acidosis increased the heat sensitivity significantly. After the water bath only 75% of the acidotic F9S1 cells were viable, whereas all of the control cells at pH 7.4 survived. When the laser was used, the difference was even more pronounced. Only 9% of the acidotic cells were viable after a 2-min treatment and 0.2% after a 4-min treatment. At physiological pH a 2-min treatment left 41% of the cells viable and a 4-min treatment 2%. This study showed that laserthermia is significantly more effective in killing cells with low intracellular pH than it is in killing cells with normal intracellular pH.

INTRODUCTION

The tumour-cell-killing effect of hyperthermia has been known for centuries. Different methods of general hyperthermia, however, typically cause undesired effects on normal tissue. This problem can be solved by using Nd-YAG laser-induced hyperthermia, laserthermia. This method gives a well-demarcated local tissue destruction, sparing normal tissue. In addition to the hyperthermia-mediated killing of cells, the laser apparently has a direct tumoricidal effect (1, 2).

The cores of many tumours are characterized by a pH which is 0.3–0.5 units lower than the normal tissue pH 7.4 (3–10). This phenomenon may be caused by the accumulation of lactic acid resulting from relative hypoxia within the tumour (7, 11). The pH conditions have clinical significance: acidosis is known to increase the thermosensitivity and to decrease the radiosensitivity of cells.

Many of the experiments with acidotic cells have been done by making the cell environment acidotic just before hyperthermia treatment by changing the pH of the culture medium. Thermal sensitivity is, however, related to changes in intracellular rather than extracellular pH (5, 12, 13). The intracellular pH plays an important role in the regulation of many cellular activities including growth (14). Experiments on the effect of hyperthermia on acidotic cells should therefore be carried out with cells at a low intracellular pH. Eukaryotic cells control their intracellular pH by using ion-transport systems situated in the plasma membrane. Cellular acidosis can be produced by using polyether ionophore nigericin which couples potassium and hydrogen gradients across the plasma membrane thus making the intracellular pH equal to the extracellular pH (4, 12, 13, 15).

The aim of the present study was to compare the effect of laserthermia and conventional hyperthermia on acidotic embryonic carcinoma cells. If laserthermia could be shown to be more effective, it would provide a new modality for treating radioresistant acidotic tumour cells and could be assessed as an adjuvant to radiation therapy.
MATERIALS AND METHODS

Cell cultures

The F9S1 embryonal carcinoma cells were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum (FCS) as described previously (16, 17). For the experiments the cells were plated on 48-well tissue culture plates (Costar Cambridge, Massachusetts). On the following day the cytosol of the cells was acidified by incubating the cells in 1 μM nigericin (Sigma, St Louis, Missouri) in isotonic KCl-buffer at 37 °C for 15 min. The pH of the nigericin solution was adjusted to 6.8 (14). In the reference group the physiological pH of 7.4 was maintained by replacing the media with 1 ml of prewarmed HEPES-buffered PB1/bovine serum albumin (18) immediately before the treatments. Thereafter, the wells were treated either with laser or water bath. Some of the wells in both groups were left intact to serve as untreated controls. The control wells were kept at room temperature. The controls for the water bath group included plates kept at 37 °C.

Preliminary experiments were carried out to see how long the cells remained viable in the nigericin solution. The first 15 min were needed for the pH equilibrium to be established (13, 14). The cells started to significantly lose their viability after 90 min. Therefore we did not keep the cells in the nigericin solution for more than 75 min in any of the experiments. Ethanol, the solvent of the nigericin stock solution, applied at the same final concentration as in the actual experiments, did not affect the cultures.

Experimental design

The cells with intracellular pH of 7.4 and 6.8 were treated by exposing them to different temperatures. In the water bath the temperatures were 37, 44, 49, 54 and 59 °C and in laser treatments, 42-44 °C. A standard laboratory water bath was used to apply hyperthermia. Laserthermia was induced by the contact Nd-YAG laser (CL 60, Surgigal Laser Technology, Malvern, Pennsylvania) used in a continuous wave mode with 6 W power setting. A frosted-end probe (MRP 5, SLT) was used. The temperature was continuously controlled with a digital thermometer (Exacon) placed at a distance of 4 mm from the laser tip placed in the middle of the well (1); the diameter of the well was 10 mm. The experimental set-up is shown in Fig. 1. The exposure time in the water bath was 4 min and for the laser, 2 and 4 min. As shown before (1), a laser treatment (42-44 °C) of only 2 min at the physiological pH results in a significant cell loss whereas a water bath at the same temperature (44 °C) causes no cell death even in 15 min.

Each experiment included at least six wells for every variable and was repeated at least three times.

Estimation of viable cells

The relative quantities of cells at 24 h after treatment were estimated as described previously (1) by labelling the cells with 1 μCi ml⁻¹ of ³H-thymidine (New England Nuclear, Boston, Massachusetts). The detached cells were removed by rinsing with PBS. The number of adherent viable cells was then defined by counting the radioactivity in the wells. All figures were corrected by subtracting the mean background measured in the wells without cells.

Statistics

The Kruskal–Wallis one-way analysis of variance was used for the statistical evaluation of the data. A p-value of <0.05 was considered significant.