The Effect of Hyperthermia on Survival Fraction of DU 145 Human Prostate Carcinoma Cell Line in Monolayer and Spheroid Culture

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Abstract

Background: Hyperthermia (also called thermal therapy or thermotherapy) is a type of cancer treatment in which body tissue is exposed to high temperatures (up to 113°F). Research has shown that high temperatures can damage and kill cancer cells, usually with minimal injury to normal tissues. For many years, biologists have investigated cancer by using monolayer cell culture. It is becoming more and more apparent that two-dimensional cell culture (monolayer) can not completely represent the real structure and characteristics of three-dimensional in vivo solid tumors. These spheroids resemble in vivo tumor models in several aspects. Therefore, studying growth characteristics and behavior of spheroids is beneficial in understanding the behavior of tumors under various experimental conditions.

Methods: In this work we have studied and analyzed the in vitro response of human prostatic carcinoma cell line DU 145 from monolayer and spheroid culture to hyperthermia. For this purpose the DU 145 cells were cultured either as monolayer or spheroids. The thermal response was judged by the survival fraction of colony forming cells in spheroids or monolayer culture following heat treatment.

Results: The result of Survival curves has shown that heating cells at 40°C and 41°C has no significant effect on cell viability and survival fraction at various times of heating but heating the cells at 42°C and 43°C in long period of heat treatment reduce the viability and survival fraction particularly.

Conclusion: Heat shock at 44°C and 45°C has great effect on this cell viability and survival fraction but in any time and temperature, spheroids were more resistant than monolayer's.

Keywords: hyperthermia, spheroid culture, prostate cancer

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Introduction

Prostate cancer is the second most common cause of death from malignant disease in men [1]. For many patients radical surgery is inappropriate [2]. The therapeutic options currently available include hormonal manipulation [3], radiotherapy [4] or chemotherapy [5]. There is a need for new, safe, simple and effective form of treating local progression. Localized hyperthermia is a promising new method for treating certain types of malignant tumors [6]. The body maintains a normal temperature of 37°C. Healthy cells, however, can survive temperatures up to 42°C. According to the National Cancer Institute, hyperthermia cancer treatment kills cancerous cells by elevating their temperatures to the therapeutic temperature range, 42-45°C exponentially over certain time [7]. It can also interact synergistically with ionizing radiation and chemotherapic drugs [8, 9]. Use of hyperthermia in cancer patients is based on two different principles. Mild hyperthermia (up to 42°C) is used to stimulate the immune response for non-specific immunotherapy of cancers. Higher temperatures (around45°C) are used with the hope of inducing regression or outright disappearance of the cancer by direct cell destruction with heat [10].

For many years, biologists have investigated on cancer by using monolayer cell culture. It is clear, that two-dimensional cell culture (monolayer) can not completely represent the real structure and characteristics of three-dimensional in vivo solid tumors [11, 12]. Multicell spheroids (MCSs) are three-dimensional aggregates of cells that mimic micro-tumors and metastases. The multicellular tumor

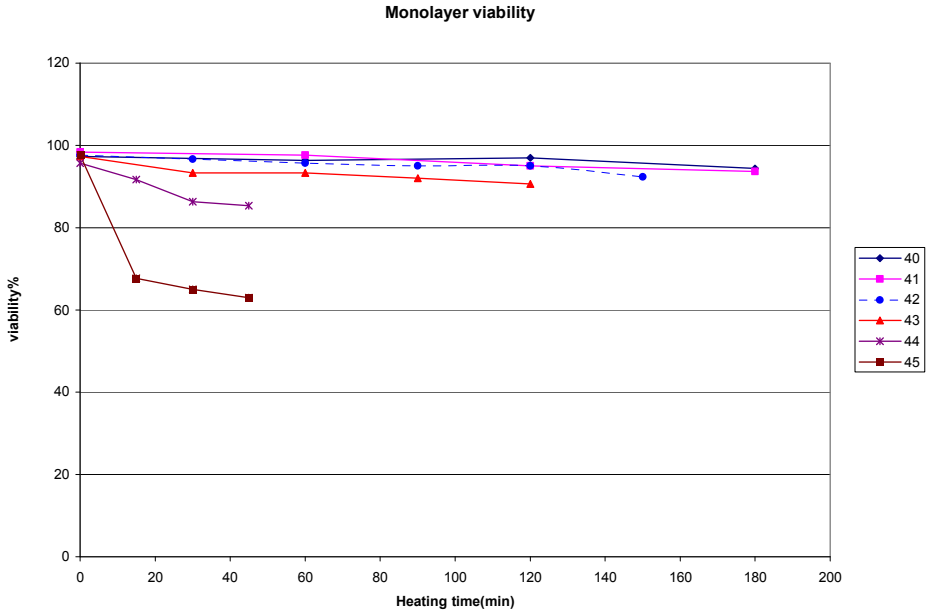

Figure 1 A

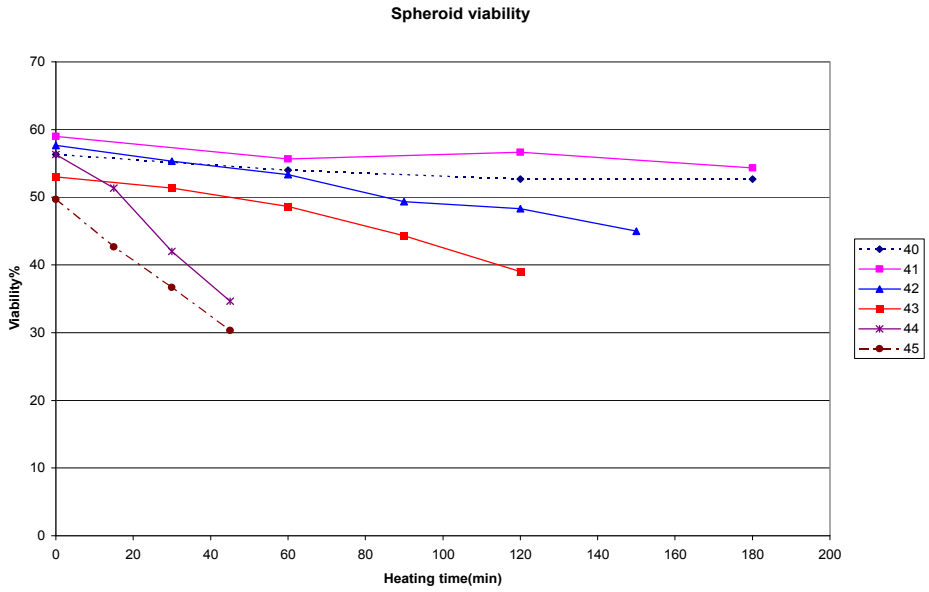

Figure 1 B

Figure1: *The effects of hyperthermia on the viability of DU 145 cells at various times after heat treatment in monolayer (A) and spheroid (B) culture. Immediately after heat treatment, the viability of cells was determined. Mean ± S.E.M of three experiments.*

spheroid represents an in vitro - in vivo transition model which exhibits important in vivo solid tumor correlates [13]. Since, spheroids can develop area of hypoxia and necrosis, conditions that are known to occur in micro regions of in vivo tumors, they have been used to examine numerous aspects of tumor biology [14, 15]. Cells growing as spheroids, have been shown previously to be more resistant than single cells from monolayer cell culture to killing by ionizing-radiation [16], hyperthermia [17, 18] and chemotherapy drugs [19].

DU145 cells are an established cell line which are from metastases of prostatic carcinoma cells in human brain [20] and can self-assemble into large, stable spheroids through a combination of intracellular communication and diffusion [21]. Since many reports

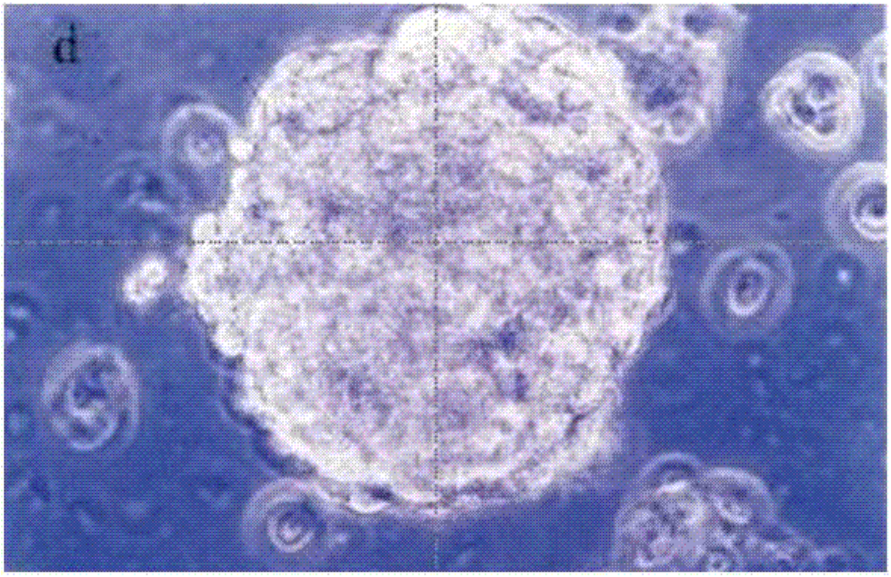

***Figure 2**: Phase contrast micrograph of DU 145 cells as spheroid at the day 11 of the culture.*

have been shown that, human prostatic carcinoma cells (DU 145 and PC-3) displayed a marked sensitivity to mild hyperthermia compared to other human carcinoma cells [20] and it is also the first work that have done on the effect of hyperthermia on spheroid culture of DU 145 prostatic carcinoma cell line, to compare it's result with monolayer cell culture of DU 145 cell line, in this work we have analyzed and studied the thermal dose response curve of DU 145 prostate carcinoma cell line in monolayer and spheroid culture.

A range of heating temperature (40 °C - 45 °C) and heating time has been employed in this study. The overall review shows that this range of temperatures (41–45 °C) in order to aid in their control is applied by many researchers [22, 23, 24, 25, 26, 27].

Materials and Methods

2.1. Monolayer culture:

The DU 145 cells were maintained in RPMI-1640 culture medium (GIBCO) supplemented with 10% heat inactivated fetal calf serum (FCS), 120 mg/l penicillin, 200 mg/l streptomycin. Cells were incubated at 37 °C, 7.5% CO2 and full humidity. Cells were sub cultured every 5 days in order to maintain the cell in constant exponential growth.

2.2. Spheroid culture:

Spheroids were initiated using the Liquid Overlay teqnique [28]. Cells for spheroid formation were obtained by trypsinization from growing monolayer cell culture. Approximately 5 x 105 DU 145 cells were seeded into 100-mm dishes coated with a thin layer of 1% agar (Bucto agar, Difco, Detroit, MI) with 10 ml of RPMI-1640 supplemented with 10% Heat inactivated fetal calf serum(FCS) , 120 mg/l penicillin, 200 mg/l stereptomycin. The plates were incubated at 37°C in humidified 5% CO2 and 95% air atmosphere. After an incubated period of 11 days, spheroids were used for our following experiments.

2.3. Heat treatment of monolayer culture:

Cells from monolayer were collected with 0.25% trypsin and 0.1% EDTA in phosphate-buffered saline (PBS). Counting and viability determination was done by Trypan blue dye exclusion. Cells suspensions in a volume of 8 ml, at a concentration of 2.5 x 105 cells/ml, were placed in a T25 flask. Cells were heated by immersing the culture flasks in a thermo stated water bath (HAKKE f3 with ± 0.1 °C precision). Heat treatment was applied in the range of 40-45 °C for various period of time heating. Cells exposed at 37°C served as control. The time for cell in flasks to equilibrate to the temperature of water bath was six to eight minutes. All experiments were repeated three times.

2.4. Heat treatment of spheroid culture:

To study the effect of hyperthermia on spheroid, cells were cultured at 5 x 105 cells per 100-mm dishes coated with a thin layer of agar. At day 11

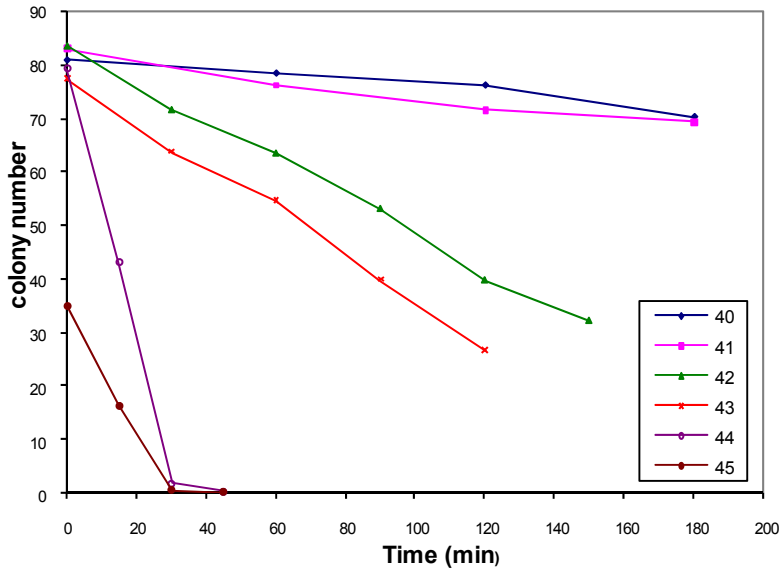

Figure 3 A

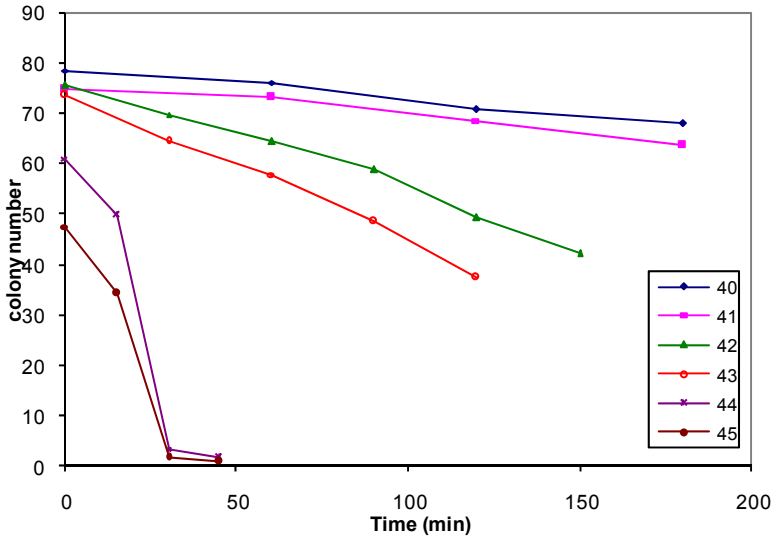

Figure 3 B

Figure 3: *The effect of hyperthermia on the colony formation ability of DU 145 cells from monolayer and day 11 of spheroids as described in section 2. Cells were harvested from these cultures and plated in 60 mm Petri dishes at various concentrations. The colonies formed 9 days after initiation of cultures were counted. Mean ± S.E.M of three experiments.*

spheroids were collected. The culture media was replaced with RPMI-1640 and spheroids were heated as described for monolayer cultures. They were then treated with 300 µl of PBS containing 0.1 mM EDTA/0.25% trypsin (w/v) for 10 min at 37°C. Trypsin was neutralized by addition of 700 µl of the culture medium containing FCS. Spheroids were mechanically disaggregated. Single cells were counted and tested for viability. Cells were then

seeded at a density of 4500 cells per Petri dish for colony formation assay.

2.5. Cell survival determination:

Immediately following heat-treatment, cells were counted and viability was determined by trypan blue dye exclusion.

2.6. Colony formation assay:

Single cells from spheroid and monolayer cultures were diluted .For monolayer 500 cells/Petri and for spheroid 4500 cells/Petri were seeded in triplicate 60-mm Petri dishes (NUNC) in RPMI-1640 supplemented with 10% FCS. Cells were incubated for 9 days. After 9 days visible colonies which have more than 50 cells were counted by an inverted phase microscope (ZEISS, Axiovert 405M) and plating Efficiency (PE) was determined. The PE was defined as:

PE=number of colonies/number of cells cultured* 100

The significance of the differences in mean colony number after exposure at each temperature and 37°C was determined by Student's T test.

2.7. Survival curves:

Survival curves were generated by plotting the log of the ratio of the number of colonies formed at a given heating condition to the number of colonies produced by related control cells,versus the heating time at the given temperature.

2.8. Data analysis:

Each point represents the Mean ± Standard Error of mean of three experiments. The Student's T-test was used for statistical analysis

Results

3.1. Cellular Viability:

Heating cells at 40-43°C did not have any significance effect on the viability of cells in both monolayer and spheroid models.

However, 45 min heat treatment of cells in 44, 45 °C reduces the viability to about 65% in monolayer and 56% in spheroids. The viability curve of monolayer and spheroid cultures is shown in figure1.

3.2. Spheroid formation:

The DU 145 cells could form spheroids by liquid overlay. Figure2 shows the phase contrast micrographs of these spheroids at 11 day of incubation.

3.3. Colony formation ability:

The effects of hyperthermia on the colony forming ability of DU 145 prostatic cell line in two models of monolayer and spheroid culture is shown in figure 3. Cells were heated and assayed for colony formation as described in the method section. As can be seen in Fig3A, a significant reduction ($p<0.05$) in the number of colonies at 42°C after 60 min, at 43 °C after 30 min, at 44°C and 45 °C after 15 min of heating was observed in monolayer. In spheroids we have significant reduction in the number of colonies at 42 °C after 120min, at 43°C after 60min, at 44°C and 45 °C after 15 min of heating [Fig3B]. Hyperthermia also reduced the colony forming ability of both monolayer and spheroid cultures. However, the extent of reduction in the number of colony forming cells from spheroids was much less than monolayer cultures.

3.4. Dose response curves:

Dose response curves were constructed to analyze the data from colony formation assay. The survival curves for DU 145 in monolayer and spheroids cultures are shown in figure 4. Heating at 40°C and 41 °C had no significant effect on the survival of this cell line in both monolayer and spheroid cell cultures. At 42°C and 43°C the survival curves from spheroids apparent to have a wider shoulder as well as more shallow slope compared to monolayer, whereas at 44°C and 45 °C the greater thermoresistance of the spheroids is largely reflected by the wider shoulder of the spheroid surviving curves with a little change in terminal slope.

Disscusion

In this work, we have studied and analyzed the in vitro response of human prostatic carcinoma cell line DU 145 in monolayer and spheroid culture to hyperthermia. Recently hyperthermia has been a focus of all extension as an appropriate arm in cancer treatment for several human tumors [29, 30, 31]. Several clinical reports have indicated that heat may be beneficial for patients with prostate cancer [6, 32, 33, 34].

Many cell lines can form multicellular spheroids [35, 36]. This property is highly dependent on the adhesion molecules such as, the integrin and cadherin families [37]. The multicellular tumor spheroids (MTS) provide a closer in vitro correlate to in vivo malignant tumors than do conventional monolayer cultures [18, 38]. Cells in monolayer cultures, by losing intercellular communication, may have lost protective systems against environmental stresses such as hyperthermia[39,40].Our results presented

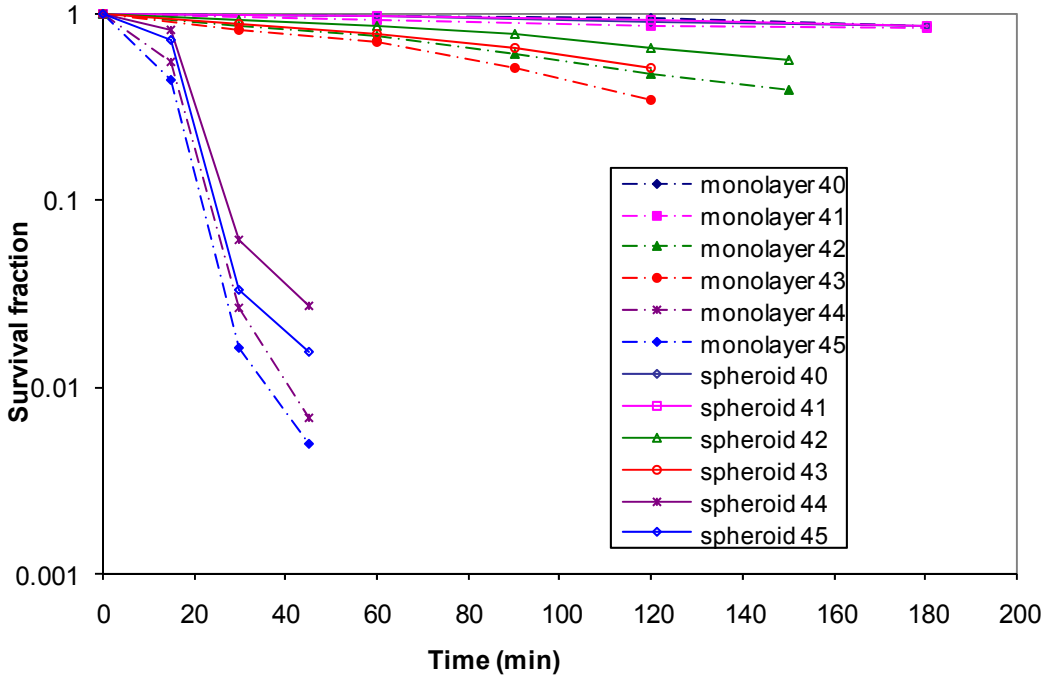

Figure 4: *Colony formation dose-response curves of DU 145 cells from monolayer and spheroids culture. After heating at 40-45°C for defined duration of time, treated and control cells were plated for colony formation assay according to the method section. X-axis shows heating time at each temperature and Y-axis shows survival fraction.*

here[Figs3and4] support this hypothesis. Hyperthermia reduced the clonogenecity of cells from monolayer and spheroid cultures [Fig3]. However, the extent of reduction in clonogenic cells from monolayer cultures was significantly larger than cells from spheroid cultures [Fig4].

Since the cytotoxic effect of hyperthermia on DU 145 human prostatic carcinoma cell line growing as spheroids, has not been investigated, in the present work we have investigated the effect of hyperthermia on DU 145 cell proliferation and surviving in monolayer or as spheroid.

Cells in monolayer and spheroid were both sensitive to heat treatment and viability of the cells decline considerably at 42-45 °C but not any valuable result in 40°C and 41°C for viability decreasing have been seen. The colony numbers of the cells in both cultures were affected from heat shock especially in temperature above 42°C. At 44, 45°C the most reduction in colony number at time 15-45 min was observed.

For comparing cell's resistance to heat shock Dose-response curves (Survival curves) from spheroid and monolayer were judged. The results of the present work indicate that DU 145 cells do acquire increased thermo resistance when growing as spheroids [Fig4]. Cells in spheroids were more resistance to heat treatment than cells from monolayer at all time and temperatures and the shape of the cell survival curves was dependent on the treatment temperatures. Heating cells at 40, 41°C has no significant effect on cell survival, but heating at 42, 43°C for various length of time caused reduction of cell survival. Following more sever treatment with hyperthermia (44, 45°C), the cell survival and viability decline up to 200%, 65% in monolayer respectively. Heating at 44°C decline the cell survival up to 60% while heating at 45°C decrease the cell survival up to 100% in spheroids. Hyperthermia decline the viability of spheroids cells up to 56% at 44 and 45°C.

It has been reported that Chinese hamster V79 cells in spheroids were more heat resistance than cells in monolayer [41]. Such results have gained for different cell lines [42] such as EMT 6 cells which as reported were more heat resistance in spheroids than EMT 6 cells in monolayer cell culture [43].

Our results in agreement with other studies show that cells in spheroid culture are more resistance to heat treatment. The extensive three-dimensional

intercellular contact in multicell spheroids could be hypothesized to influence cell sensitivity to hyperthermia [44]. The result obtained in the present study, although in vitro; provide important information about the effect of heat on human prostatic carcinoma DU 145 cell line in spheroid culture. This information is valuable because it gives us some knowledge about the optimal temperature and duration of heating necessary to achieve a good cell killing response in both monolayer and spheroids.

In summery we found that:

1- Cells in spheroids of DU 145 prostate cancer cell line are more thermo resistant than cells in monolayer.

2- Temperature and exposure time are both critical parameters in achieving maximum cell killing. Thus we must seek a balance between conditions maximizing tumor cell killing and minimizing trauma to adjacent tissue.

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Conflict of interests

The Author has no conflict of interests in this article.

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