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Study on the thermosensitivity of a tumor cell by microcalorimetry

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Abstract

Power-time curves of the growth of a tongue cancer cell line (CP_2) and a normal human embryonic lung cell line (Fu_2P_1) have been determined by a microcalorimeter at 37°C and 45°C, respectively. The experiments showed that the ratio of the maximal thermal power of both kinds of cell growth, P_{max} (CP_2, 37°C)/P_{max} (Fu_2P_1, 37°C), equalled 1.74 at 37°C, while at 45°C, P_{max} (CP_2, 45°C)/P_{max} (Fu_2P_1), 45°C) = 0.464. The ratio of the metabolic heat for both kinds of cell growing for 63 h at 37°C is, Q (CP_2, 37°C)/Q(Fu_2P_1, 37°C) = 2.295, while at 45°C after both kinds of cells grow for 15 h, Q(CP_2, 45°C)/Q(Fu_2P_1, 45°C) = 0.686. As a comparison, Q(CP_2, 37°C)/Q(CP_2, 45°C) = 1.734, Q(Fu_2P_1, 37°C)/Q(Fu_2P_1, 45°C) = 0.502. The results indicate that the metabolic activity value of the tumor cell is distinctly higher than that of non-tumor cell at 37°C, but lower at 45°C. By means of microcalorimetry, it is demonstrated that temperature has a remarkably selective destructuring effect of heat against tumor cell. The results of 6 other kinds of cancer cells and normal cells at different temperatures are also in agreement with the results obtained. Also, the power-time curves of the two passages of the tongue cancer cell (P_6 and P_7) are determined. The difference between the two passages is shown clearly on the power-time curves. It is concluded that microcalorimetry could be used as a powerful tool to characterize the passages of the cell. © 1997 Elsevier Science B.V.

Keywords: Human embryonic lung cell line; Microcalorimetry; Thermal power; Thermal sensitivity; Tongue cancer cell line

1. Introduction

It is well known that surgery, irradiation and chemotherapy currently play a major role in the treatment of cancer. Recently, hyperthermia in the treatment of cancer has been reported clinically and has obtained satisfactory results [1–3]. Enthusiasm for the use of long duration, mild temperature hyperthermia in conjunction with other treatments such as chemotherapy, irradiation in the treatment of cancer has recently developed [4–6]. Results gained from in vitro experiments have demonstrated that heating cancer cells at 40–45°C can greatly enhance cancer cell killing [7]. Successful application of this form of hyperthermia in clinical cases requires an understanding of the role of exposure conditions such as temperature, length of heating time and sequence of heat, etc. Microcalorimetry is a powerful tool in solving these problems. It allows the study of biology at the molecular level as well as at the cellular level [8,9].

The various metabolic events occurring within the cell are all heat-producing reactions. Thus, by monitoring the heat effect with a sufficiently sensitive calorimeter, the metabolic processes of living cells

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can be studied by a direct method. The calorimetry can directly determine the 'biological activity' of a living system and provide a continuous measurement of the heat production, thereby give much useful information in both qualitative and quantitative ways [10]. Although a lot of work on hyperthermia on cancer cells has been reported, the mechanism by which heat-induced initial events lead to ultimate cell death or how thermotolerance is developed remains to be elucidated [4,11], and a few papers on the heat sensitivity of cancer by means of microcalorimetry have been published in current literature.

During the past decade, we have applied microcalorimetry to the study on the metabolism in microorganisms [12–15] and cultured tissue cells [16–19] at cell and subcell level to obtain useful information about the metabolic processes and medical fields. The purpose of our work at present is to elucidate the mechanism responsible for the antitumor effect of exposure to elevated temperature through systematic research on the cancer cells compared with normal cells at different conditions by means of microcalorimetry combined with other methods.

In this paper, we used a human tumor cell line (tongue cancer cell) and a normal human cell line (human embryonic lung cell) to study their thermal sensitivity at normal and high temperatures by means of microcalorimetry. Quantitative data were obtained which demonstrated that the temperature has highly selective destructing effects of heat against cancer cells. We also found that the minute difference between two passages of the tongue cancer cell is shown clearly on the curves. So, it is concluded that microcalorimetry could be used as a powerful tool to characterize the passages of the cell.

2. Material and method

2.1. Cells and cell culture

The cell lines used in this experiment, human tongue cancer cell line (CP_2) and human embryonic lung cell line (Fu_2P_1), are established and were characterized by the China Center for Type Culture Collection, Wuhan University, Wuhan 430072, P.R. China in 1996. The detection for mycoplasmas in the cell lines was negative [20].

Exponentially growing cultures are obtained by seeding 10^5 cell/ml into a T-25 plastic flask containing 8 ml of the medium at 37°C for 3 days. The medium consisted of the Dulbecco's modified Eagle's Medium (DMEM)(GIBCO. U.S.A.) + 10% heat-inactivated fetal calf serum (Sigma) + 100 IU/ml penicillin + 100 IU/ml streptomycin (pH = 7.2–7.3).

2.2. Preparation of cells

Firstly, the cells were scattered exponentially by 0.25% trypsin 1 : 250, Difco) and counted using a Thoma's hemocytometer. Secondly, 1 ml suspension of an exponentially growing cell was removed into 3 ml glass ampoule at a cell number of 10^4–10^5 cells/ml. Then the glass ampoule was put into the microcalorimeter to monitor the growth of the cell.

2.3. Microcalorimeter

A new type of heat-flow microcalorimeter, the LKB 2277 Bioactivity Monitor, is used in this experiment. It is designed to monitor continuously a wide variety of processes and complex systems over a temperature range 20–80°C. A schematic representation of the calorimetric system is shown in Fig. 1. Each measuring cylinder normally contains a sample and a reference in separate measuring cups (twin system). The

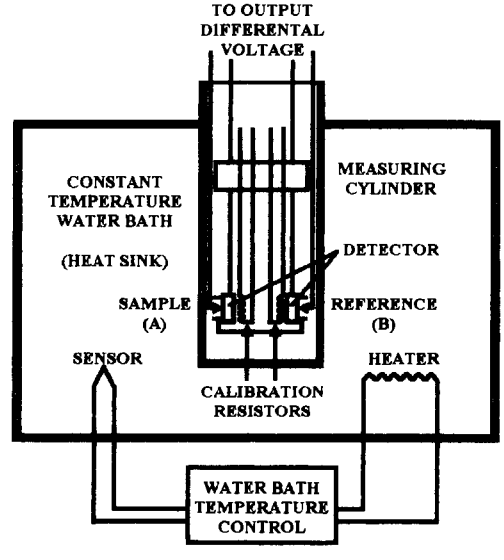

Fig. 1. Simplified operation diagram.

heat output from the sample flows via the thermoelectric detector to the large heat sink (in close contact with the water bath). In response the detector produces a voltage that is proportional to the power output from the sample. In order to minimize the systematic errors and disturbance effects, a differential or twin detector system is used. This system is very sensitive, the detection limit is 0.15 μW and the baseline stability (over a period of 24 h) is 0.2 μW. The performance of this instrument and the details of its construction have been previously described [21].

The LKB 2210 recorder was used in this experiment which allowed continuous recording of the power-time curves for growth.

2.4. Experimental determination

The measurement was carried out at 37°C corresponding to the physiological body temperature and 45°C, hyperthermia respectively; the amplifier of the monitor was set at 100 μW. The paper rate of the recorder was 0.1 mm/min.

For the evaluation of these power-time curves, two parameters were used for the maximal thermal power (P_{max} in μW) and the integral of the curve serving as a measure of the total energy released (Q in J).

3. Results and discussion

If the cell number is N_0 and N_t at time t_0 and t, respectively, and the thermal power of each cell is P, then at time t_0 and t, the thermal power of the cell studied is $P_0 = N_0P$, $P_t = N_tP$, So, P_t which reflects the multiplication of the cell can be used as a parameter to characterize the growth of the cell. Microcalorimetry has great advantage over more conventional bioassay procedures since the experiment directly records dQ/dt (that is P) vs. t, thereby reveals not only thermal data but also kinetic data.

The power-time cures for a normal human embryonic lung cell and tongue cancer cell at 37°C and 45°C are shown in Figs. 2 and 3, respectively.

Fig. 2 shows that the power-time curve of the tongue cancer cell lies above that of the human embryonic lung cell. From Fig. 2, the maximal thermal power of the tongue cancer cell follows

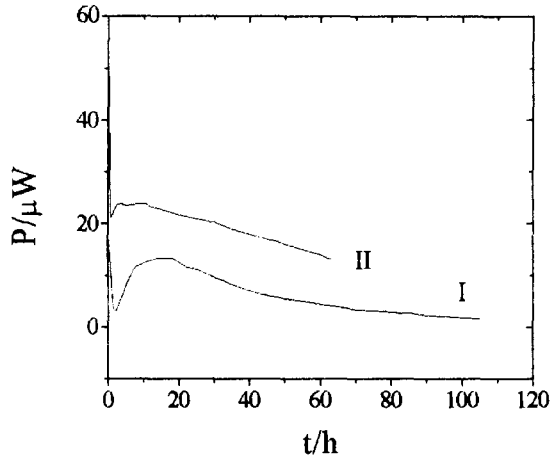

Fig. 2. Power-time curves of Fu_2P_1 and CP_2 with cell number of 2×10^5 cell/ml, 1.7×10^5 cell/ml respectively, in 1 ml DMEM medium containing 10% calf serum at 37°C. (I) Power-time curve of Fu_2P_1, (II) Power-time curve of CP_2.

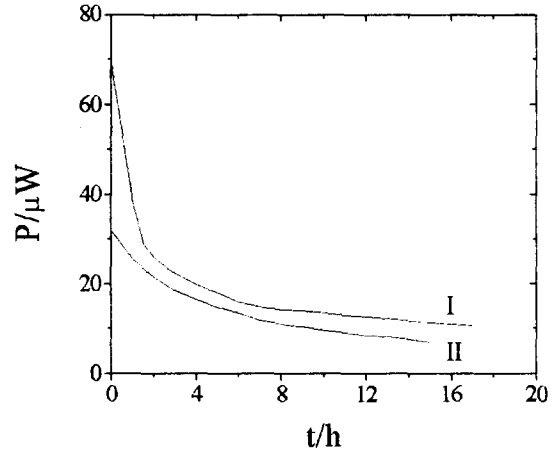

Fig. 3. Power-time curves of Fu_2P_1 and CP_2 with cell number of 2×10^4 cell/ml, 3×10^4 cell/ml respectively, in 1 ml DMEM medium containing 10% calf serum at 45°C. (I) Power-time curve of Fu_2P_1, (II) Power-time curve of CP_2.

as

$$P_{max}(CP_2, 37°C) = 24.0\,\mu W,$$

while $P_{max}(Fu_2P_1, 37°C) = 13.8\,\mu W$.

The ratio of the maximal thermal power of growth of the two kinds of cell is:

$$P_{max}(CP_2, 37°C)/P_{max}(Fu_2P_1, 37°C) = 1.74.$$

Since $P = dQ/dt$, the area under the curve records the total heat released during the experimental period

$$Q(CP_2, 37°C) = 4.429\,J, \quad Q(Fu_2P_1, 37°C) = 1.930\,J.$$

$$Q(CP_2, 37°C)/Q(Fu_2P_1, 37°C) = 2.295$$

The total energy Q released during the measurement period (63 h) is greater for the tongue cancer cell sample by a factor of 2.295 and likewise, the maximal thermal power P_{max} increased by a factor of 1.74.

At this temperature, the cancer cell has been found to be more sensitive to heat than a normal cell. The results of a study on 6 other cancer cell and normal cell lines are also in agreement with the results obtained (and will be discussed in another paper). It is demonstrated again by means of microcalorimetry that the metabolic activity of the tongue cancer cell is stronger than that of the normal human embryonic lung cell.

With the growth of the cell, the amount of oxygen in an ampoule is reduced. As the metabolic activity of the cancer cell at 37°C is higher than that of normal cell, the declining power time-curve of the tongue cancer cell may be due to the shortage of the oxygen.

Fig. 3 shows that both curves are declining. The results demonstrate that at 45°C, both the tongue cancer cell and normal cell did not grow well. But the difference in the curve showed that temperature has different effects on the tongue cancer cell and the normal human embryonic lung cell. Contrary to the case at 37°C, the metabolic activity of the tongue cancer cell is lower than that of the human embryonic lung cell.

Analysis of both kinds of cells growing for 15 h is shown in Table 1.

$$\text{comparing} \quad Q(CP_2, 37°C)/(Q(CP_2, 45°C) = 1.734$$

$$Q(Fu_2P_1, 37°C)/Q(Fu_2P_1, 45°C) = 0.502$$

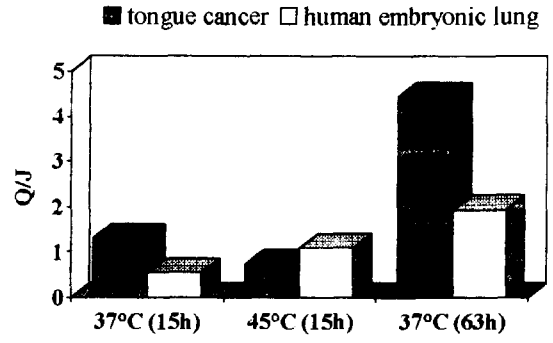

Fig. 4. Comparison of the total released energy (Q) values of CP_2 and Fu_2P_1.

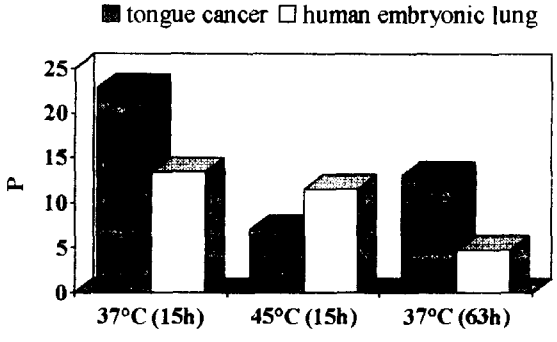

Fig. 5. Comparison of the thermal power (P) values of CP_2 and Fu_2P_1.

The results indicate that at 45°C, the growth of the tongue cancer cell was inhibited more than that of the human embryonic lung cell. The thermal sensitivity of the cancer cell is confirmed again.

Figs. 4 and 5 show for each parameter a direct comparison of the normal human embryonic lung cell (Fu_2P_1) and the tongue cancer cell (CP_2) samples investigated.

At the same experimental conditions as described above, the power-time curves of P_6 and P_7 passages of the tongue cancer cell were also determined at 37°C, as shown in Fig. 6.

Table 1
Comparison of Q (in J) and P (in μW) of human embryonic lung cell and tongue cancer cell growing for 15 h

	Q		$Q(CP_2)/Q(Fu_2P_1)$	P		$P(CP_2)/P(Fu_2P_1)$
	Fu_2P_1	CP_2		Fu_2P_1	CP_2	
37°C	0.5422	1.284	2.368	13.4	22.8	1.70
45°C	1.079	0.7404	0.686	11.5	7.0	0.609

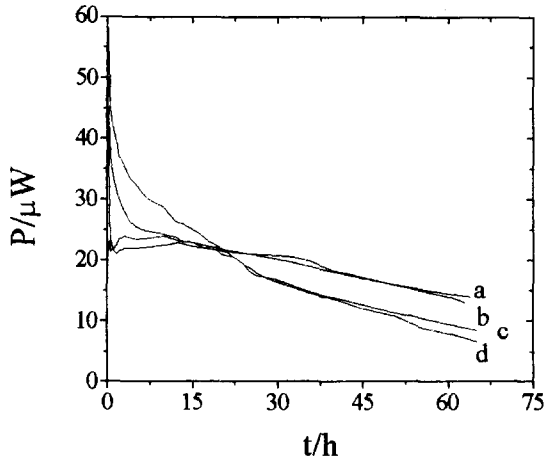

Fig. 6. Power-time curves of CP_2 at 37°C. (a, b) (P_6): Power-time curves with cell number of 8×10^4 cell/mL, 1.7×10^5 cell/mL, respectively, (c, d) (P_7): Power-time curves with cell number of 3×10^4 cell/ml, 2×10^5 cell/ml, respectively.

From Fig. 6, the metabolic heat Q of the P_6 and P_7 tongue cancer cells growing for 63 h was obtained,

$$P_6, \quad Q(P_6, N_0 = 8 \times 10^4/\text{ml}) = 4.442\,\text{J}$$
$$Q(P_6, N_0 = 1.7 \times 10^5/\text{ml}) = 4.429\,\text{J}$$
$$P_7, \quad Q(P_7, N_0 = 3 \times 10^4/\text{ml}) = 3.998\,\text{J}$$
$$Q(P_7, N_0 = 2 \times 10^5/\text{ml}) = 4.027\,\text{J}$$

The results indicated that the metabolic heat of the P_6 CP_2 is almost the same as that of P_7 CP_2, and the difference in the metabolic heat of the same passage, such as a and b or c and d, is also small although at the beginning the cell numbers are different. But the minute difference between the two passages, such as a and c or b and d, of the tongue cancer cell is clearly shown in Fig. 6.

So it is suggested that microcalorimetry could be used as a tool for the characterization of different passages of cells.

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