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A microcalorimetric study on human lung cancer A549 cells whose metabolism is inhibited by medicated liposome

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Abstract

The metabolic thermograms and heat output of human lung cancer A549 cells have been determined in differential inhibition conditions using an LKB 2277 bioactivity monitor. The experimental results indicate that medicated liposome inhibits the metabolism of the cancer cells more strongly than the drug alone. The values of the average heat output of the cancer cells under different conditions have been calculated.

INTRODUCTION

The metabolic thermograms of normal cells reflect their metabolic state, the peak power of the thermograms being directly proportional to the density of the cell's metabolism [1]. Thus, the determination of metabolic thermograms of normal cancer cells and of those inhibited by drugs is very significant in cytological and pharmacological studies. Moreover, it is a very straightforward, simple method. Liposomes are materials whose composition and construction are analogous to those of the cell membrane; therefore, liposome can fuse with the cell membrane, thus changing some of the properties of the membrane, such as its fluidity. Therefore, medicated liposomes can easily enter the cell and remain there, improving the effect of the drug over a longer period of time.

In this work, the metabolic level of human lung cancer A549 cells was determined using an LKB 2277 bioactivity monitor. The metabolic thermograms of the cells were obtained from four kinds of cultural conditions (normal medium, with added drug, medicated liposomes, etc.). From these experiments, we can obtain some significant results.

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INSTRUMENTS AND MATERIALS

Instruments

An LKB 2277 bioactivity monitor was used to determine the metabolic thermograms of the cells; the performance of this instrument and the details of its construction have been described in previous work [2,3].

Materials

Human lung cancer A549 cells, were provided by the Research Institute of Virology of the Hubei Medical Academy.

The medium used was RPMI 1640, containing 10% serum, and penicillin and streptomycin (100 units per ml) at pH 7.2. The wash solution was D-Hands solution.

The liposome was prepared as follows. A mixture was prepared of 1 ml of an ether solution of phosphatidyl choline (concentration 56 mg ml^{-1}), 1 ml of phosphatidyl serine–ether solution (concentration 8 mg ml^{-1}) and 1 ml of cholesterol–ether solution (concentration 16 mg ml^{-1}). These were mixed in a round-bottomed flask (capacity 100 ml) and dried by a vacuum air pump with continual rotation so that the mixture formed an even layer on the bottom. To this was added 3 ml of PBS solution (phosphate buffer solution); the flask was immersed for 4 h and then treated by ultrasound for 20 min. Finally, the solution was centrifuged (4000 per min^{-1}), and the pure liquid liposome sample (concentration 80 mg ml^{-1}) was removed and reserved in a refrigerator (4°C) for use.

The drug employed was MTX (aminopterin). Medicated liposome was prepared by coating the drug with liposome, by mixing MTX with the above liposome solution and applying ultrasound treatment for 20 min (MTX concentration, 0.5 mg ml^{-1}).

All the above materials were provided by the Research Center of Bioengineering of Wuhan University.

EXPERIMENTAL AND RESULTS

In these experiments, the cultural temperature was 37°C, and the amplifier was set at 30 μW.

Determination of the metabolic thermograms of A549 cell samples under the influence of liposome

Experiment 1

An A549 cell sample that had been cultured well on laboratory inoculture was placed into 0.6 ml of RPMI 1640 medium (the cell concentration

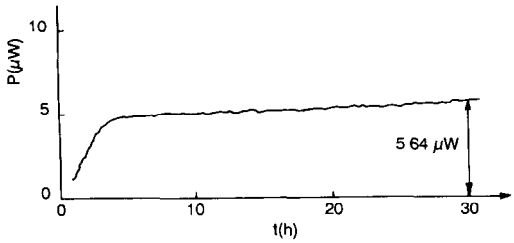

Fig. 1. Thermogram of A549 cells obtained at 37°C, cultured in RPMI 1640.

being about 1.6×10^5 cells/ml) and sealed in a 3 ml ampoule; another ampoule containing 0.6 ml of distilled water was used as a reference. The sample and reference were loaded into the equipment and the metabolic thermogram was continually monitored.

Experiment 2

An A549 cell sample in inoculture was placed into 0.6 ml of RPMI 1640 medium with 8 μl of liposome solution (concentration 80 mg ml^{-1}), sealed in a 3 ml ampoule and loaded into another cell-holder of the equipment to monitor its thermogram at the same time.

The results of these experiments are shown in Figs. 1 and 2.

Determination of the metabolic thermograms of A549 cells under the influence of MTX and medicated liposome

Experiment 3

An A549 cell sample in inoculture was added to 0.5 ml of RPMI 1640 medium with 50 μl of MTX solution (concentration 0.5 mg ml^{-1}) and 50 μl of vacant liposome, i.e. not coating the drug. This was cultured at 37°C for 4 h in an isothermal box. Then the sample was separated by centrifuging, and the clean liquid was removed and washed with medium; this was separated again, and finally, the cells were suspended in 0.6 ml of fresh

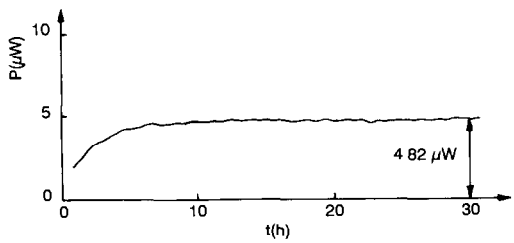

Fig. 2. Thermogram of A549 cells obtained at 37°C, cultured in RPMI 1640+liposome.

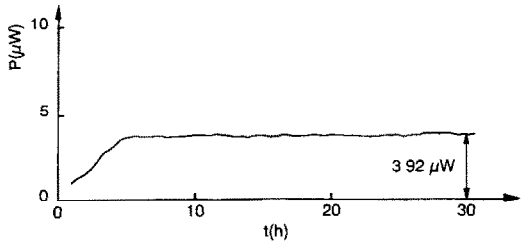

Fig. 3. Thermogram of A549 cells obtained at 37°C, cultured in RPMI 1640+MTX+liposome.

RPMI 1640 medium, sealed in a 3 ml ampoule and its thermogram monitored.

Experiment 4

The cell sample was prepared as in Experiment 3, but this time the cells were cultured in 0.5 ml RPMI 1640 with 50 μl of medicated liposome and 50 μl of PBS solution at 37°C for 4 h. Then the sample was separated, washed, suspended again and monitored as above. The thermograms of these experiments are shown in Figs. 3 and 4.

DISCUSSION

The thermograms of human lung cancer A549 cells were determined in differential inhibition conditions (see Figs. 1–4). From the peak power of the thermograms and the corresponding cell count, we can obtain the average power output for the metabolism of a single cell; the results are shown in Table 1. The results can be summarized as follows:

1. Comparing experiments 1 and 2, it can be seen that the power output was inhibited when the liposome was added to the medium, because the liposome can fuse with the cell membrane, so that the metabolic activity of the cell is changed.

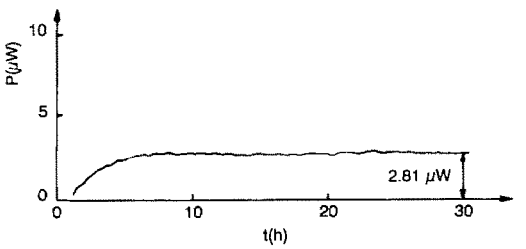

Fig. 4. Thermogram of A549 cells obtained at 37°C, cultured in RPMI 1640+medicated liposome+PBS.

TABLE 1

Average power output data for human lung cancer A549 cells (obtained under different cultural conditions)

Exp. no.	Metabolic power (μW)	Cell number (cells)	Average power (pW/cell)
1	5.64	10.1×10^4	55.8
2	4.82	9.8×10^4	49.2
3	3.92	9.1×10^4	43.1
4	2.81	8.8×10^4	31.9

2. From the results of experiments 3 and 4, it can be seen that the inhibition effect of the medicated liposome is stronger than that of the drug alone, and the inhibition function can be maintained longer. This confirms that the medicated liposome easily enters into the cell and remains there, whereas the drug alone is more easily washed away from the cells. Hence the metabolic power output of experiment 3 is larger than that of experiment 4.

These results have demonstrated that liposome can fuse with the cell membranes, and can coat the drug; therefore, these results are very significant for cytology and pharmacology.

ACKNOWLEDGEMENT

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