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Thermochemical study on the growth metabolism of human promyelocytic leukemia HL-60 cells inhibited by water-soluble metalloporphyrins[1]

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Abstract

By using an LKB 2277 Thermal Activity Monitor, the thermogenesis curves of human promyelocytic leukemia HL-60 cells growth were determined. It was found that the heat production rate grew almost exponential during the first 10 h. When treated by two series of water-soluble metalloporphyrins, the growth metabolism of HL-60 cells can be suppressed. The extent of inhibitory effect as judged from the multiplication rate constant, *k*, varied with different porphyrins. From these data, it was found that these water-soluble metalloporphyrins can inhibit the growth metabolism of cancer cells even if there is no light.

Keywords: Thermochemistry; Microcalorimetry; Human promyelocytic leukemia HL-60 cells; Water-soluble metalloporphyrins; Inhibition

1. Introduction

Since the first porphyrin complex was separated from nature in the last century, a great amount of research has been performed in this field. The great potential medical and biological applications of porphyrins, the diverse types of porphyrins, the widely developed synthetic methods for porphyrin elaboration, and the extensive and versatile physical properties of porphyrins and metalloporphyrins (fluorescence, intense visible absorption spectra, paramagnetism, etc., depending on the porphyrins) have combined to spark a flurry of diverse types of studies on porphyrins [1,2].

In recent years, porphyrin derivatives have been used in the diagnosis and treatment of malignant diseases. A hemin derivative such as HPD (hematoporphyrin derivative) tends to accumulate specifically in neoplastic tissues and produces irreversible damages via singlet oxygen when the porphyrin is photoactivated by visible light [3,4]. Efforts have been made to prepare new porphyrin derivatives in cancer phototherapy [5,6].

Although there is considerable interest in photodynamic therapy of cancer by porphyrins, very little research has been performed on the anti-tumor activity of porphyrins themselves [7].

The use of microcalorimetry to monitor cell metabolic activity in vitro is well-established. During the past decade, we have applied microcalorimetry to the study on the metabolism of microorganisms [8,9], mitochondria [10,11], cultured tissue cells [12,13] and cultured tissue cells infected by virus [14].

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Thermogenesis curves produced by unperturbed cell population reflect metabolic activity in the experimental conditions. Following drug administration, power dissipation changes may be associated with the underlying events produced by the drug at the level of cells. Parametric evaluation of change in thermogenesis curves provides an estimate of the extent and the kinetics of the drug action.

In this paper, thermogenesis curves produced by human promyelocytic leukemia HL-60 cells under the action of two series of water-soluble metalloporphyrins in different concentrations were determined with an LKB 2277 Thermal Activity Monitor. From these curves, we found the heat production rate of HL-60 cells form exponential lines before and after the additions of porphyrins during the first 10 h of the experiments. A thermokinetic equation as: $\ln P = kt + \ln P_0$ could be established. The values of k before and after HL-60 cells treated by water-soluble metalloporphyrins were calculated. It was found these water-soluble metalloporphyrins can inhibit the growth of cancer cells even if there is no light.

2. Materials and methods

2.1. Materials

Two series of water-soluble metalloporphyrins were synthesized and characterized by the Department of Chemistry, Wuhan University [15]. The structure of porphyrins is shown in Fig. 1.

Human promyelocytic leukemia HL-60 cells were provided by National Research Laboratories of Natural and Biomimetic Drugs, Beijing Medical University.

The cell culture medium consisted of RPMI 1640 medium(GIBCO. USA)+l0% heat-inactivated fetal calf serum±100 IU/ml penicillin+100 IU/ml streptomycin.

2.2. Instrument

An LKB-2277 Thermal Activity Monitor (Thermometric, Järfälla, Sweden) was used. The operation of this instrument and the details of its construction have been described previously [16]. All measurement were made at 37.0°C.

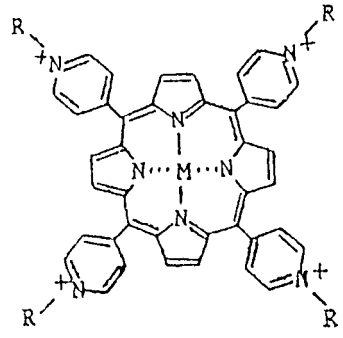

Fig. 1. The structures of water-soluble metalloporphyrins. For M-NPCN series, R=–CH_2CH_2CN, M=Co, Cu, Ni, Zn. For M-NEAE series, R=–$CH_2COOC_2H_5$, M=Cd, Mn, Zn.

2.3. Microcalorimetric measurements

Exponentially growing cells were washed and resuspended in fresh medium to give a final cell concentration of 2×10^5 cells/ml, then 1 ml suspension of cells containing porphyrin at different concentration was removed into 3 ml glass ampoule, and the glass ampoule was put into the microcalorimeter to monitor the thermogenesis curves.

The porphyrin was added from the beginning of the microcalorimetric experiment. The solution of the porphyrin was prepared in RPMI 1640 medium, and was prepared freshly every time.

The LKB 2210 recorder was used in this experiment which allowed continuous recording of the thermogenesis curves.

3. Results and discussion

Fig. 2 shows the thermogenesis curves obtained when a culture of 1 ml of HL-60 cells (at the concentration of 2×10^5 cells/ml) was administrated with Cu-NPCN(for the structure of these porphyrins, see Fig. 1) at concentrations of 0, 1, 2, 5, l0 μmol/l. The others are all similar to this.

From these thermogenesis curves, it is clear that P and t are satisfied for an exponential equation:

$$\ln P = kt + \ln P_0. \quad (1)$$

Using Eq. (1), the multiplication rate constants k of all thermogenesis curves were calculated and the results are shown in Table 1.

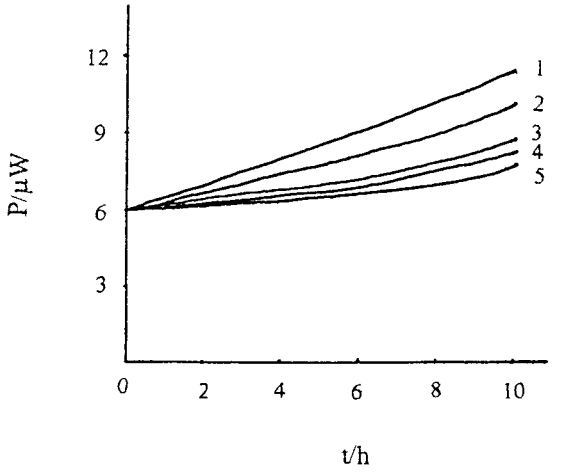

Fig. 2. Thermogenesis curves of 1 ml HL-60 cells at a concentration of 2×10^5 cells/ml treated with Cu-NPCN at concentration of (1) 0, (2) 1, (3) 2, (4) 5 and (5) 10 μmol/l.

These results indicated that these water-soluble metalloporphyrins all have the capacity to inhibit the metabolic growth of HL-60 cells to different extents, and the inhibitory extent varied with different porphyrins.

The depressing effect on the rate constant was concentration-dependent, but the dose–rate constant relationship is not very linear for all the porphyrins.

The results revealed that the action of water-soluble metalloporphyrins on HL-60 cells differs from one another because of their different structures. The cytotoxity is highly dependent on the nature of the central metal atom of these water-soluble metalloporphyrins. For M-NPCN series, the anti-tumor activity is Cu-NPCN>Co-NPCN>Zn-NPCN>Ni-NPCN. For M-NEAE series, the anti-tumor activity is Cd-NEAE> Mn-NEAE>Zn-NEAE. For the porphyrins with the same central metal atom, the anti-tumor activity is Zn-NPCN>Zn-NEAE.

The experiment results demonstrate that microcalorimetry is a good method for cell metabolic studies. Using this method, we can obtain perfect thermogenesis curves closely related to the metabolic process. We believe that isothermal microcalorimetry can be used to monitor the cellular action of many anti-tumor drugs. It can provide important information for cell-drug interaction research.

Table 1
Rate constants k of HL-60 cells in different porphyrins at 37°C

Porphyins	Concentration ($\mu mol\,l^{-1}$)	10^3k (min^{-1})
Control	0	1.16
Ni-NPCN	1	0.95
	2	0.61
	5	0.56
	10	0.47
Zn-NPCN	1	0.91
	2	0.57
	5	0.54
	10	0.44
Co-NPCN	1	0.89
	2	0.56
	5	0.51
	10	0.42
Cu-NPCN	1	0.73
	2	0.32
	5	0.27
	10	0.20
Zn-NEAE	1	0.93
	2	0.62
	5	0.58
	10	0.46
Mn-NEAE	1	0.47
	2	0.22
	5	0.19
	10	0.16
Cd-NEAE	1	0.41
	2	0.21
	5	0.17
	10	0.13

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