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Inhibitory study of two cephalosporins on *E. coli* by microcalorimetry

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Abstract The microcalorimetric method has been used to study the effects of cefpiramide and ceftizoxime sodium on the *E. coli* growth. The results revealed that these two cephalosporins may alter the metabolic way of the *E. coli*. Moreover, the lethal doses of cefpiramide and ceftizoxime sodium are 2.000 and 0.2000 μg mL^{-1}, respectively. Combining with the relationships between growth rate constant (k), the maximum power output (P_m), the time corresponding to the maximum power output (t_m) and cephalosporins concentration (C), one can draw the conclusion that the ceftizoxime sodium has a stronger inhibition effects on the growth of *E. coli* than that of cefpiramide and they both have the possibility to induce the drug fever.

Keywords Cefpiramide · Ceftizoxime sodium · Cephalosporins · *E. coli* DH5α · Metabolism · Inhibition · Microcalorimetry

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Introduction

In recent years, the microcalorimetry bioassay has attracted extraordinary attention due to the high sensitivity and a great deal of useful information [1–3]. By monitoring the heat production of the reaction in situ, the metabolic process of the living cells can be studied [4–6]. The attraction of this method also lies in the non-invasive, non-destructive and allows biochemical analysis of the samples after the metabolic power–time curves has been measured [7–9]. Therefore, microcalorimetry has been widely used in the study of bioactivity screening and metabolism [9, 10].

Cefpiramide and ceftizoxime sodium are two kinds of the third-generation cephalosporins with a broad spectrum of antibacterial activity, they both effectively on aerobic and anaerobic gram-positive and gram-negative bacteria [11–14]. Herein, by utilizing the method of microcalorimetry, we estimate the inhibitory effects of cefpiramide and the ceftizoxime sodium on the *E. coli* growth. The metabolic power–time curves of *E. coli* under action of different concentrations of these two cephalosporins were obtained. The relationship between the thermokinetic parameters were discussed so as to offer useful information for clinic cure.

Experimental

Materials

Escherichia coli (*E. coli*) strain DH5α was offered by the Biomass Conversion Technology Group (Dalian Institute of Chemical Physics, CAS, Dalian 116023, P. R. China). The Luria–Bertani (LB) culture medium in a total volume of 1,000 mL contained 10 g tryptone, 5 g yeast extract powder, 10 g NaCl, pH = 7.0–7.2. The LB culture medium was sterilized in high-pressure steam 121°C for 20 min before the experiment. The strain of *E. coli* DH5α was stored at −20°C before the experiment, then it was routinely cultivated on LB culture medium and the procedures have been reported previously [15].

The cefpiramide and the ceftizoxime sodium were kindly afforded by Dalian Institute of Drug Detection, P. R. China.

Instrument

A TAM Air Isothermal Calorimeter (Thermometric AB, Sweden) was used to measure the heat production. It has equipped with eight twin calorimetric channels with one side for the sample and the other for a static reference. The measurements were carried out in sealed 20 mL glass ampoules and the thermostat was maintained at 37°C with an absolute accuracy of 0.02°C. The signal generated was recorded in-situ by a computer. The operation of the instrument and the details of its construction have been described in previously [16, 17].

Methods

The metabolic power–time curves of *E. coli* were determined using ampoule method. After the 20 mL glass ampoules have been sterilized, the LB culture mediums with a volume of 10 mL were placed in it, containing of different concentrations of cephalosporins. Then 200 μL *E. coli* suspension (optical density is about 0.5 at $\lambda =$ 600 nm) were inoculated into each ampoule. After that, the ampoules were sealed with a cap and placed into the microcalorimeter. Meanwhile, the power-time signals were began to record at 1-min intervals by a computer.

Results and discussion

Thermokinetics

The power–time curves of *E. coli* in the logarithmic phase of growth obey the exponential equation [18]:

Table 1 Thermokinetic parameters of *E. coli* growth at different concentrations of cefpiramide

C (μg mL^{-1})	k (10^{-3} min^{-1})	R	P_m (μw)	t_m (min)
0	1.854	0.9997	542.7	2,271
0.5000	0.8893	0.9981	319.4	2,977
0.8000	1.432	0.9972	503.1	3,672
1.000	1.471	0.9994	536.2	4,221
1.500	2.000	0.9972	725.0	4,482
1.800	2.623	0.9993	777.3	5,152
2.000	0	–	–	–

k Growth rate constants, R correlated coefficients for k, P_m maximum power output, t_m the time of P_m

Table 2 Thermokinetic parameters of *E. coli* growth at different concentrations of ceftizoxime sodium

C (μg mL^{-1})	k (10^{-3} min^{-1})	R	P_m (μw)	t_m (min)
0	1.854	0.9997	542.7	2,271
0.01000	1.683	0.9984	269.3	1,678
0.05000	1.781	0.9997	583.1	3,927
0.08000	2.292	0.9986	697.8	4,982
0.1000	3.112	0.9998	935.2	4,903
0.2000	0	–	–	–

$$\ln P_t = \ln P_0 + kt \quad (1)$$

where P_0 and P_t represent the power output at time 0 and t, respectively; k is the constant of cell growth rate. According to Eq. 1, the rate constant k of cell growth could be calculated using the data P_t and t obtained from the power–time curves, and those values are given in Tables 1 and 2.

Power–time curves

The power–time curves of heat evolution are shown for the metabolism of the *E. coli* growth in the existence of cefpiramide and ceftizoxime sodium in Figs. 1 and 2, respectively. As seen form the curves, when these two cephalosporins concentration increased, the shape of the curves almost keep the same except the prolongation of the lag time and the rising of the peaks heights. The results suggest that these two cephalosporins have an inhibitory action on *E. coli* growth and probably alter the metabolic pathway of *E. coli*.

Relationship between k and C

The effects of various concentrations of cefpiramide and ceftizoxime sodium on the heat production rate of *E. coli* cells are demonstrated in Tables 1 and 2. It is conspicuous

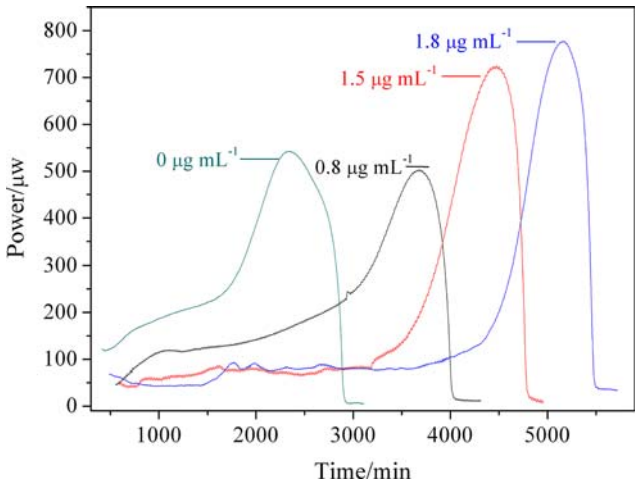

Fig. 1 Power–time curves of *E. coli* at various concentrations of *cefpiramide*

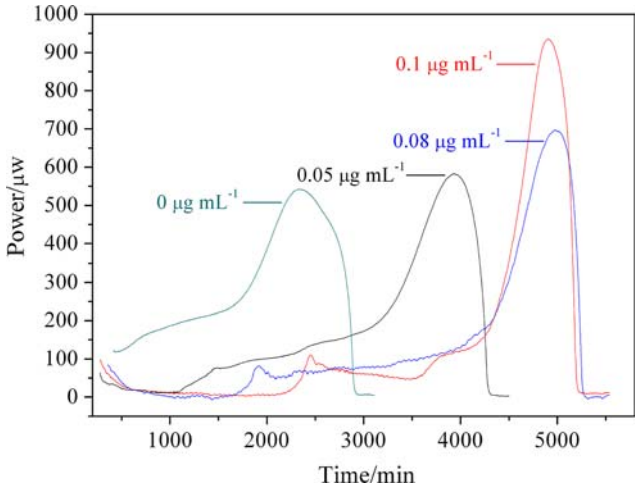

Fig. 2 Power–time curves of *E. coli* at various concentrations of *ceftizoxime sodium*

that the k of the *E. coli* gradually ascends with the increase of the concentration of drugs (C). When the cefpiramide concentration is below 1.500 μg mL^{-1}, the value of k is lower than the control value of 1.854, but once the C reaches 1.500 μg mL^{-1} and higher than this concentration, the k goes beyond the control value, and attains the value of 2.623 when C is 1.800 μg mL^{-1}. Generally speaking, the k is decreasing with the increase of the drug concentration which attribute to the reduction of the cell numbers, the phenomenon in this study revealed that the cefpiramide possible alter the metabolism of the *E. coli*. When the concentration reaches 2.000 μg mL^{-1}, the growth of *E. coli* has been completely inhibited and the value of k is close to 0. Making a linear regression of k versus C, one can obtain the relationship between k and C:

$$k = 1.224C + 0.3107 \quad r = 0.9831.$$

In the case of growing under various concentrations of ceftizoxime sodium, the change tendency of *E. coli* growth rate constant k is resemble to that of cefpiramide. The k is under the control value when C is below 0.08000 μg mL^{-1}; as the C reaches 0.08000 μg mL^{-1}, the k exceeds the control value, even up to 3.112 when C is 0.1000 μg mL^{-1}. From Table 2, it can be seen that the k of *E. coli* is close to the control level in despite of under lower concentration of ceftizoxime sodium. When the concentration reaches 0.2000 μg mL^{-1}, the lag phase is so long that the growth of *E. coli* hardly observes among the experimental period and the k value is near 0. It is concluded that the metabolism of *E. coli* could be changed with a lower concentration of ceftizoxime sodium. The result suggests that the ceftizoxime sodium could potentially have a stronger inhibition on the *E. coli* growth than that of cefpiramide. The correlations between k and C could be formulated according to the following equations:

$$k = 289.2C^2 - 16.44C + 1.828 \quad r^2 = 0.9927.$$

Relationship between P_m and C

As seen from Table 1, the values of P_m rise with the increase of cefpiramide concentration. When the cefpiramide concentration range is below 1.500 μg mL^{-1}, the P_m is under the control value of 542.7 μw and then it is rising with the increasing of C; when the C increases to 1.500 μg mL^{-1}, the P_m is over 542.7 μw and reaches to 725.0 μw. The relationship between P_m and C is linear which can be described as:

$$P_m = 343.6C + 187.4 \quad r = 0.9829.$$

According to the data in Table 2, one could found that the P_m values also elevate with increasing the ceftizoxime sodium concentration. Under the higher concentration, the P_m is above the control value and obtains a largish value of 935.2 μw when the C reaches 0.1000 μg mL^{-1}. The relationship between P_m and C is linearly, it can be exhibit as the follow equation:

$$P_m = -6971C + 203.1 \quad r = 0.9864.$$

Normally, the P_m values decreased with the increase of C, which showed the decrease cell number of the survivors. Nevertheless, in our study, the P_m is ascending gradually with increasing the cephalosporins concentration, the phenomenon implies that the use of cephalosporins may resulted in drug fever and change the metabolism way of

the *E. coli*, which resulting in a higher maximum power output.

Relationship between t_m and C

When the concentration of cefpiramide and ceftizoxime sodium increased, the growth of *E. coli* was inhibited, and the t_m was postponed (Tables 1, 2). When the cefpiramide concentration reaches 1.500 μg mL^{-1}, the retardation of t_m is more visibly and up to 4,482 min. On the other hand, the t_m obtained a value of 4,982 min when the ceftizoxime sodium concentration is 0.08000 μg mL^{-1}. Combining with the values of k and P_m, one can get the conclusion that these two cephalosporins have altered the metabolism of the *E. coli*. The correlations between t_m and C could be formulated according to the following equations:

For *cefpiramide*:

$$t_m = 1573C + 2328 \quad r = 0.9840.$$

For *ceftizoxime sodium*:

$$t_m = -445714C^2 + 85773C + 843.3 \quad r^2 = 0.9959.$$

Conclusions

We have investigated the inhibitory effects of cefpiramide and ceftizoxime sodium on the growth of *E. coli* using microcalorimetric method. Considering the relationship between t_m, P_m and C, one can know that these two cephalosporins probably alter the metabolism of the *E. coli*. In addition, the lethal doses of cefpiramide and ceftizoxime sodium are 2.000 and 0.2000 μg mL^{-1}, respectively. The results show that the ceftizoxime sodium has a stronger inhibition effects than that of cefpiramide on *E. coli* growth, and they both have the possibility to induce the drug fever which should be pay more attention in the clinic treatment. Furthermore, our work also shows that the microcalorimetry provides a useful analytical tool for the characterization of the microbial growth process and the estimation of the drugs efficiency.

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