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Thermo-chemical investigation on the quantity-antibacterial effect relationship of five berberine alkaloids in *Rhizoma Coptidis* on *Escherichia coli* growth

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ABSTRACT

The inhibitory effects of five berberine alkaloids from rhizoma of Rhizoma Coptidis, a traditional Chinese medicinal herb, on Escherichia coli growth were investigated at 37 °C by microcalorimetry. The five alkaloids were: berberine, coptisine, epiberberine, palmatine and jatrorrhizine. The thermogenic power-time curves of Escherichia coli growth with and without berberine alkaloids were by the Thermal Activity Monitor (TAM) Air Isothermal Calorimeter, meanwhile the values of growth rate constants k, growth inhibitory ratio I, maximum heat output Pm and generation time tG were calculated. In accordance with thermo-kinetic model, the relationships of the drugs, such as I - c, k - c, Pm - k were investigated. c was the concentration of the drugs. Half-inbibitory concentration of the drugs, IC50, was obtained by quantitative analysis. From the view of thermo-kinetics and molecular structure, the relationship between quantity and effects of five berberine alkaloids was discussed. Also, the minimum inhibitory concentration (MIC) and the minimal bacteriocidal concentration (MBC) of the five berberine alkaloids on anti-Escherichia coli growth was obtained by tube dilution method. Meanwhile, the action mechanism of antibacterial effect was studied. The efficiency of these five berberine alkaloids on anti-growth of E. coli was as follows: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. This work illustrated that microcalorimetry was a useful tool to investigate the antibacrerial activity of medicinal herbs and provided a general model to study the quantity and antimicrobial effects relationship of medicinal herbs from the view of thermo-chemistry and molecular structure.

KEY WORDS: Rhizoma Coptidis, Berberine alkaloids; Escherichia coli, Microcalorimetry, Quantity and antibacterial effect relationship

INTRODUCTION

Rhizoma Coptidis (Huanglian in Chinese) is a traditional Chinese medicinal (TCM) herb, and is officially listed in the Chinese Pharmacopoeia (1). It is known to show antimicrobial activity against staphylococcus aureus (2), Escherichia coli, Bacillus anthracis and antifungal activity against Candida albicans and Aspergillus niger (3, 4). Its extract has also strong antimicrobial activities and is used for treating dysentery, cholera, leukemia, diabetes and lung cancer (5). The major active components of the herb are berberine alkaloids (BAs), such as berberine, coptisine, epiberberine, palmatine and jatrorrhizine, which are often used as criteria in the quality control of Rhizoma Coptidis products (6, 7). BAs are also active components in large numbers of plant-derived drugs such as antimicrobial from Berberidaceae and Rutaceae family.

In this study, the five BAs were tested against Escherichia coli (E. coli) by microcalorimetry to provide more references for the antimicrobial activity of Rhizoma Coptidis. And some useful informations such as Pm, k, I, IC50 which could not be obtained from other methods was acquired from this study. This information was important to present the antibacterial activity of drugs.

E. coli is one of the most common pathogenic bacterium in clinic. It has been used as an important tool to screen the bioactive part of folium of Isatis indigotica (8) and to evaluate the quality of Rhizoma Coptidis (9).

The microcalorimetric method is one of the important techniques for thermo-chemistry and thermokinetic study. In any living system, the various metabolic

events are all biochemical reactions producing heat. By monitoring the heat effect with a sensitive calorimeter, the microcalorimetric method can directly determine the biological activity of a living system and provide a continuous measurement of heat production, thereby giving much information about the metabolism of organism in both qualitative and quantitative ways. By analysis of thermogenic curves obtained from microcalorimetric measurement, it can reveal many temporal details about the microbial metabolism not observable by other methods (10). The microcalorimetric method has been widely applied in studying metabolism of microorganism and cultured tissue cells (11, 12, 13, 14). As is known that chloramphenicol is a broad-spectrum antibiotic, it can inhibit strongly the growth of Escherichia coli, salmonella and Staphylococcus aureus, etc (15, 16). In this study, chloramphenicol was selected as a standard positive controlling drug to study the antimicrobial activity.

We investigate the relationship between quantity and antibacterial effect of five BAs from Rhizoma Coptidis on E. coli growth based on thermo-kinetic model by quantitative analysis of the thermo-kinetic data. This work provides a powerful method for studying the pharmacodynamic action of Rhizoma Coptidis, which is helpful to discover and search for bioactive components of Traditional Chinese Medicinal compounds.

MATERIALS AND METHODS

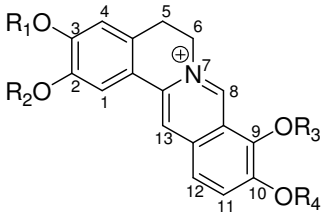
The TAM Air Isothermal Calorimeter, manufactured by Thermometric AB Company of Sweden, was used to measure the heat-output of the metabolism of E. coli. This isothermal micro-calorimeter is an eight-channel twin instrument. The microcalorimeter is thermostated at the range of 5 - 60 °C , with a limitation of detectability of 2 μw. The experiment was performed as the manufacturer's recommend and the report of Wadso (17). Picolog software (Pico Technology Ltd) was used to process the data.

Apparatus

Chemicals

Rhizoma Coptidis (No.084523), which was accredited by professor Xiao-He Xiao, Institute of Chinese Materia Medica, 302 Hospital of PLA (People's Liberation Army), Beijing, 100039, PR China, was the rhizoma of Coptis chinensis Franch, Ranunculaceae, collected from Anguo city, Hebei province, China. was the dried root of Coptis chinensis Franch. Berberine, jatrorrhizine, palmatine, coptisine and epiberberine were supplied by National Institute for the Control of Pharmaceutical and Biological Products. The five BAs were extracted from Rhizoma Coptidis and their structures were given in Fig.1.

E. coli strain (Escherichia coli CMCC B44103) and the standard chloramphenicol were provided by the Chinese Center for Type Culture Collections, National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100051, China. E. coli was grown in a peptone culture medium, which contained 10 g peptone, 6 g beef extract and 5 g NaCl. Medium pH was adjusted to 7.0 - 7.2 with 1 mM NaOH before autoclaving. LB culture medium, which contained 10g peptone, 5 g yeast extract and 5 g NaCl and Medium pH was adjusted to 7.0 - 7.2 with 1 mM NaOH before autoclaving.



	R_1	R_2	R_3	R_4
Berberine	-CH_2-		CH_3	CH_3
Coptisine	-CH_2-		-CH_2-	
Palmatine	CH_3	CH_3	CH_3	CH_3
Jatrorrhizine	H	CH_3	CH_3	CH_3
Epiberberine	CH_3	CH_3	-CH_2-	

Fig.1 Chemical structures of investigated BAs from Rhizoma Coptidis

Experimental procedure

At the beginning of the experiments, E. coli was inoculated into LB culture medium, with 2×106 cells per mL. Cells were suspended in the peptone culture medium, and the fresh prepared BAs solutions by using water with different concentrations were added to the cell suspension.

The microcalorimeter was thermostated at 37 °C, and the measurement made using the ampoule method. All the ampoules containing the bacterial suspension of E. coli and one of the BAs were sealed up and put into 8-channel calorimeter block. After about 30 min (the temperature of ampoules reached 37 °C), the thermogenic power-time curves were recorded until the recorder returned to the baseline. All data were collected continuously by using the dedicated software package.

Then, the MIC and MBC of the five BAs on E. coli growth were determined by tube dilution method. The

berberine alkaloids solution were diluted double decremented continuously and respectively. The different dilutions of BAs were added into LB culture medium respectively, 100 µL microbial suspension of E. coli was inoculated in every tube and was cultivated for 24 h at 37°C to observe the growth of E. coli. The MIC is the average concentration of BAs between the least concentration of the tube in which the E. coli has non-proliferation and the maximum concentration of the tube in which the E. coli proliferates obviously, the MBC was the least concentration of the tube in which the E. coli is killed and has non-proliferation (18).

RESULTS

The thermogenic P-t curves and parameters

The heat-production growth curve of E. coli could be divided into four phases, i.e. lag phase, first exponential phase, second exponential phase and decline phase. The exponential model of E. coli metabolism could be used in the two growth processes:

$Pt = P0 \exp(kt)$ or $\ln Pt = \ln P0 + kt$ (1)

where P0 was the heat output power at time 0, and so was Pt at time t. The thermogenic curve formula of the exponential phase of growth was Eq. (1). According to Equation (1), the growth rate constant (k) was obtained by fitting lnPt and t to a linear equation (Table 1).

Table 1

Table 1 showed k = (0.02899±0.00291) min-1 and all of the correlation coefficients, r, exceeded 0.995, indicating a good reproducibility and relationship.

The thermogenic power-time (P-t) curves of E. coli growth in the presence of 0.10 mg/ml of chloramphenicol and five BAs were showed in Fig 2. It was clear that the addition of drugs delays the maximum peak-time (tm), which also suggests that chloramphenicol and five BAs have inhibitory effect on E. coli. At the same time, the maximum power-output (Pm) decreases correspondingly (as can be seen from the heights of the highest peaks in Fig.2). Compared to the control, the sequence of Pm was chloramphenicol < berberine < coptisine < epiberberine < palmatine < jatrorrhizine, which meaned the strength of anti - E. coli growth was chloramphenicol > berberine > coptisine > epiberberine >palmatine > jatrorrhizine > control.

The thermogenic P-t curves of E. coli in the presence of different BAs were showed in Fig 3. The generation time (tG) of E. coli could be obtained from the formula: tG = (ln2)/k. Table 2 showed k, tG and Pm of E. coli growth in the presence of BAs.

Table 2

Growth inhibitory ratio (I)

I was defined as:

$I\% = [(k0 - kc) / k0] \times 100\%$ (2)

where k0 was the growth rate constant at concentration 0, so was kc at concentration c. Table 2 showed the I of E. coli by different drugs. When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called the half-inhibitory concentration IC50. IC50 can be regarded as the inhibiting concentration of causing a 50% decrease of the E. coli growth.

The power-time curves of E. coli growth in Fig. 3 for the five BAs were similar and the curves could still be divided into four phases. They had same profiles but different peak-heights. Some similarities and differences could be observed from a qualitative point. The curves demonstrated that the lag phase was prolonged and the highest peak degraded with the increasing concentrations of the five BAs. The similar results were showed in Table 2 that the values of k2 and Pm decreased and tG increased with the increasing concentration of five BAs, indicating that the five BAs bona fide inhibited the growth of E. coli. But, with the differences of k2, Pm and tG values, the five BAs had different antibacterial activity.

I - c relationship

I - c relationship could be obtained by fitting I and c to a linear equation. IC50 could be obtained from the linear equation.

For berberine : I % = 6.7512 c + 45.7032, R = 0.9297

IC50 = 0.06 mg/mL (0.05 - 0.35 mg/mL)

For coptisine: I % = 2.6143 c + 45.4013, R = 0.9496

IC50 = 0.08 mg/mL (0.05 - 0.35 mg/mL)

For epiberberine: I % = 7.3429 c + 46.4711, R = 0.9948

IC50 = 0.22 mg/mL (0.05 - 0.35 mg/mL)

For palmatine: I % = 6.8253 c + 30.8902, R = 0.9944

IC50 = 2.80 mg/mL (0.50 - 3.50 mg/mL)

For jatrorrhizine: I % = 2.6799 c + 7.2141, R = 0.9881

IC50 = 13.14 mg/mL (4.5 - 13.5 mg/mL)

Pm - k relationship

Pm - k relationship could be obtained by fitting Pm and k to a linear equation.

For berberine : Pm = 0.0341k + 0.9394, R = 0.9714

(0.05 - 0.35 mg/mL)

For coptisine: Pm = 0.0634 k + 1.0846, R = 0.9922

(0.05 - 0.35 mg/mL)

For epiberberine: Pm = 0.0548 k + 1.0216, R = 0.9761

(0.05 - 0.35 mg/mL)

For palmatine: Pm = 0.0398 k + 1.4112, R = 0.9707

(0.50 - 3.50 mg/mL)

For jatrorrhizine: Pm = 0.0316 k + 1.4011, R = 0.9743

(4.50 - 13.5 mg/mL)

k-c relationship

k-c relationship could be obtained by fitting k and c to a linear equation. k decreased with the drug concentrations increased.

For berberine: k = 0.0033 - 0.0002 c, R = - 0.9287

(0.05-0.35 mg/mL)

For coptisine: k = 0.0034 - 0.0004 c, R = - 0.9508

(0.05-0.35 mg/mL)

For epiberberine: k = 0.0091 - 0.0008 c, R = - 0.9947

(0.05-0.35 mg/mL)

For palmatine: k = 0.0079 - 0.0015 c, R = - 0.9844

(0.50-3.50 mg/mL)

For jatrorrhizine: k = 0.0307 - 0.0021 c, R = - 0.9870

(4.50-13.5 mg/mL)

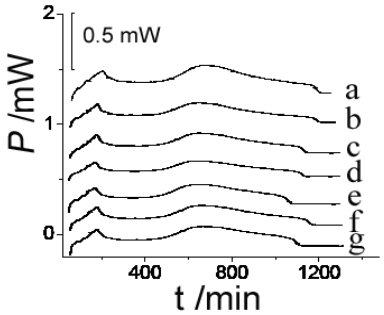
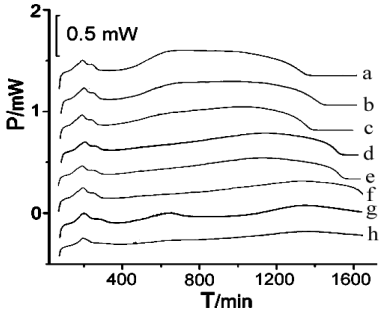
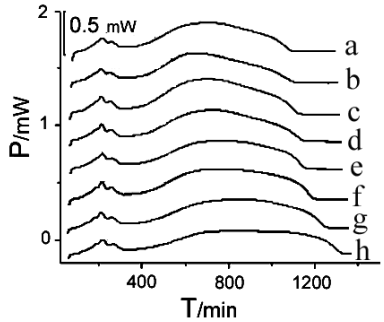


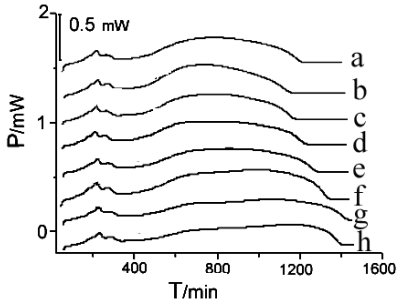
***Fig.2** The power-time (P-t) curves for growth of Escherichia coli at 37 °C without and with drugs.*



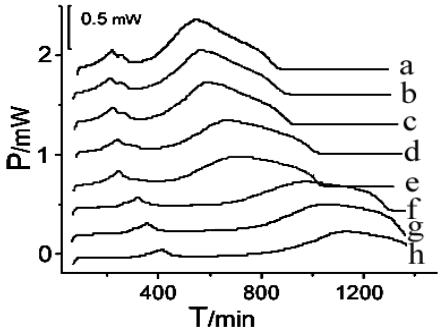
(A) The concentrations of berberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g) , 0.35 mg/ml (h).



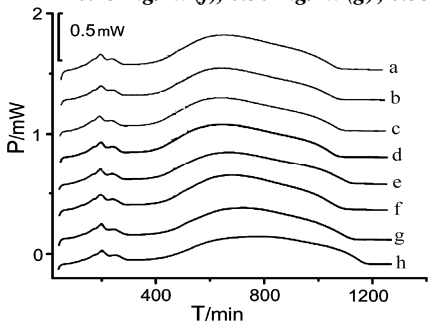
(B) The concentrations of coptisine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g) , 0.35 mg/ml (h).



(C) The concentrations of epiberberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g) , 0.35 mg/ml (h).



(D) The concentrations of palmatine: 0 mg/ml (a), 0.5 mg/ml (b), 1.00 mg/ml (c), 1.50 mg/ml (d), 2.00 mg/ml (e), 2.50 mg/ml (f), 3.00 mg/ml (g) , 3.50 mg/ml (h).



(E) The concentrations of jatrorrhizine: 0 mg/ml (a), 4.50 mg/ml (b), 6.00 mg/ml (c), 7.50 mg/ml (d), 9.00 mg/ml (e), 10.5 mg/ml (f), 12.0 mg/ml (g) , 13.5 mg/ml (h).

***Fig 3.** The power-time curves of E. coli growth in the presence of different concentrations of berberine (A), coptisine(B), epiberberine (C), palmatine (D), jatrorrhizine(E).*

E. coli was cultured in peptone culture medium supplemented with different concentrations of five BAs respectively, and monitored of TAM air at 37 °C. ***(A)*** *The concentrations of berberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g) , 0.35 mg/ml (h).* ***(B)*** *The concentrations of coptisine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g) , 0.35 mg/ml (h).* ***(C)*** *The concentrations of epiberberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g) , 0.35 mg/ml (h).* ***(D)*** *The concentrations of palmatine: 0 mg/ml (a), 0.50 mg/ml (b), 1.00 mg/ml (c), 1.50 mg/ml (d), 2.00 mg/ml (e), 2.50 mg/ml (f), 3.00 mg/ml (g) , 3.50 mg/ml (h).* ***(E)****The concentrations of jatrorrhizine: 0 mg/ml (a), 4.50 mg/ml (b), 6.00 mg/ml (c), 7.50 mg/ml (d), 9.00 mg/ml (e), 10.5 mg/ml (f), 12.0 mg/ml (g) , 13.5 mg/ml (h)*

Table 1 - Growth rate constant (k) of E. coli growth at 37 °C

Experiment	1	2	3	4	5	6	7	8
k /min^{-1}	0.0304	0.0308	0.0284	0.0285	0.0287	0.0279	0.0279	0.0293
R	0.9976	0.9957	0.9984	0.9969	0.9985	0.9978	0.9959	0.9973

^{R}*coefficient correlation*

The growth rate constant (k) of E. coli growth without drugs at 37 °C was obtained by microcalorimetry. The data in the table indicated a good reproducibility and relationship of the results.

Table 2 - The values of k, generation time t_G, inhibitory ratio I and maximum power-output P_m of E. coli growth in the presence of different concentrations of five BAs

BAs	C/mg.mL^{-1}	k /min^{-1}	R^a	t_G /min	I /%	P_m /mW
	0	0.030	0.999	23.1	0	1.744
Berberine	0.05 [b]	0.019	0.992	36.5	36.7	1.161
	0.10 [b]	0.017	0.992	40.8	43.3	1.134
	0.15 [b]	0.015	0.995	46.2	50.0	1.124
	0.20 [b]	0.013	0.994	53.3	56.7	1.102
	0.25 [b]	0.011	0.992	63.0	63.3	1.057
	0.30 [b]	0.009	0.995	77.0	70.0	1.000
	0.35 [b]	0.007	0.996	99.0	76.6	0.954
Coptisine	0.05 [b]	0.022	0.996	31.5	26.7	1.401
	0.01 [b]	0.020	0.996	34.7	33.3	1.396
	0.15 [b]	0.017	0.995	40.8	43.3	1.269
	0.20 [b]	0.013	0.994	53.3	56.7	1.221
	0.25 [b]	0.010	0.995	69.3	66.7	1.168
	0.30 [b]	0.008	0.995	86.6	73.3	1.128
	0.35 [b]	0.006	0.996	115.5	80.0	1.102
Epiberberine	0.05 [b]	0.027	0.995	25.7	10.0	1.521
	0.10 [b]	0.024	0.995	26.6	20.0	1.461
	0.15 [b]	0.022	0.996	29.0	26.7	1.413
	0.20 [b]	0.018	0.995	38.5	40.0	1.335
	0.25 [b]	0.014	0.996	49.5	53.3	1.271
	0.30 [b]	0.011	0.996	63.0	63.3	1.202
	0.35 [b]	0.010	0.995	69.3	66.7	1.123
Palmatine	0.50 [b]	0.028	0.996	24.8	6.70	1.606
	1.00 [b]	0.027	0.996	25.7	10.0	1.583
	1.50 [b]	0.025	0.997	27.7	16.7	1.567
	2.00 [b]	0.022	0.997	29.0	26.7	1.542
	2.50 [b]	0.020	0.996	34.7	33.3	1.515
	3.00 [b]	0.016	0.997	43.3	46.7	1.460
	3.50 [b]	0.010	0.996	69.3	66.7	1.414
Jatrorrhizine	4.50 [b]	0.029	0.995	23.9	3.3	1.662

6.00 [b]	0.028	0.994	24.8	6.7	1.651
7.50 [b]	0.026	0.993	26.7	13.3	1.633
9.00 [b]	0.023	0.994	27.7	23.3	1.591
10.5 [b]	0.020	0.993	30.1	33.3	1.547
12.0 [b]	0.017	0.994	40.8	43.3	1.485
13.5 [b]	0.013	0.994	53.3	56.7	1.443

[a] Correlation coefficient. ; [b] Average of three times experiments.

E. coli was cultured in peptone culture medium supplemented in the presence of different concentrations of five BAs respectively, and monitored by TAM air at 37 °C. ***(A)*** *The concentrations of berberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h).* ***(B)*** *The concentrations of coptisine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h).* ***(C)*** *The concentrations of epiberberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h).* ***(D)*** *The concentrations of palmatine: 0 mg/ml (a), 0.5 mg/ml (b), 1.00 mg/ml (c), 1.50 mg/ml (d), 2.00 mg/ml (e), 2.50 mg/ml (f), 3.00 mg/ml (g), 3.50 mg/ml (h).* ***(E)*** *The concentrations of jatrorrhizine: 0 mg/ml (a), 4.50 mg/ml (b), 6.00 mg/ml (c), 7.50 mg/ml (d), 9.00 mg/ml (e), 10.5 mg/ml (f), 12.0 mg/ml (g), 13.5 mg/ml (h).*

Table 3 *- The values of k and P_m of E. coli growth in the presence of the five BAs at different concentrations*

BAs	k /min^{-1}	R	RSD%	P_m /mW	R	RSD%
Control	0.029			1.733		
	0.031	0.998	0.12	1.730	0.992	0.30
	0.031			1.727		
Berberine	0.009			1.096		
	0.010	0.998	0.10	1.103	0.997	0.47
	0.011			1.094		
Coptisine	0.015			1.212		
	0.017	0.991	0.36	1.218	0.992	0.42
	0.010			1.220		
Epiberberine	0.020			1.341		
	0.021	0.993	0.26	1.335	0.993	0.42
	0.016			1.343		
Palmatine	0.024			1.551		
	0.019	0.995	0.25	1.543	0.991	0.40
	0.022			1.546		
Jatrorrhizine	0.025			1.602		
	0.028	0.998	0.30	1.597	0.995	0.50
	0.022			1.592		

E. coli was cultured in peptone culture medium supplemented in the presence of five BAs respectively, and monitored by TAM air at 37 °C. The concentration was 0.20 mg/ml for berberine, 0.20 mg/ml for coptisine, 0.20 mg/ml for epiberberine 2.00 mg/ml for palmatine and 9.00 mg/ml for jatrohizine.

MIC and MBC

The MIC of the five BAs were 8.6 μM for berberine, 15.79 μM for coptisine, 20.8 μM for epiberberine, 30.32 μM for palmatine and 56.35 μM for jatrorrhizine. And the MBC of the five BAs were 21.4 μM for berberine, 32.8 μM for coptisine, 43.9 μM for epiberberine, 92.3 μM for palmatine and 114.2 μM for jatrorrhizine.

Reliability and stability of microcalarimetry

The "P-t" curves of E. coli in Fig.3 demonstrated that the lag phase prolonged and k and Pm decreased with the increasing concentrations of BAs. Table 2 showed that the values of k and Pm decreased and tG increased with the increasing concentration of OAs, indicating that four BAs bona fide inhibited the growth of E. coli. In order to evaluate the reliability and stability of microcalarimetry, triplicate experiments using these five BAs have been performed under the above-mentioned conditions. Table.3 showed the values of k and Pm of E. coli growth with the five BAs at different concentrations.

Table 3 .

IC50 value -IC50 is used to represent the sensitivity of

bacteria to drugs. The smaller IC50 is, the stronger antibacterial activity the drugs possess. The sequence of the five IC50 was: berberine < coptisine < epiberberine < palmatine < jatrorrhizine. Accordingly, the efficiency of these five BAs on anti-growth of E. coli was as follows: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. Berberine had the strongest activity of anti - E. coli growth. Jatrorrhizine and epiberberine had poor anti - E. coli activity. Jatrorrhizine with the IC50 of 13.14 mg/mL had the poorest activity of anti - E. coli growth.

k value

The value of k can be thought as one of the characteristic constants to illustrate the growth of bacterium. The change of k can be used to estimate the antibacterial strength of drugs when different drugs were added or drug concentration changes at the same conditions. The smaller the absolute value of slope rate of k-c line is, the stronger antibacterial effect the drug has. The order of the absolute values of k-c linear relationship of these five BAs is: berberine < coptisine < epiberberine < palmatine < jatrorrhizine. Accordingly, the sequence of antibacterial activity of the five BAs was: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. Berberine has the strongest inhibitive effects on E. coli growth.

The MIC and MBC for every drug also illustrated that the efficiency of these five BAs on anti-growth of E. coli was: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. The sequence of anti-growth of E. coli was identical to the sequence from the values of IC50 and k, illustrating that microcalorimetry was accurate and useful for investigating the antibacterial activity of five BAs on E. coli growth.

Possible mechanism of action

The thermogenic curves of E. coli growth affected by various BAs from Rhizoma Coptidis indicated that all tested drugs had inhibitory effects on the tested bacteria. The lag phase of bacteria increased with the increasing concentrations of all tested BAs. Berberine, palmatine and coptisine showed stronger inhibitory effects on E. coli than the other two BAs. All BAs belong to berberines of benzyltetrahydroisoquinolines. There are different substituted groups at C2, C3, C9 and C10 of phenyl ring (see Fig.1). The functional groups methylenedioxy at C2 and C3 on phenyl ring improve antimicrobial activity more strongly than methoxyl at C2 and C3 on phenyl ring. However, the effect of bacteriostasis is not significant with methylenedioxy or methoxyl at C9 and C10 on phenyl ring. Combined with the results of test, the analysis of BAs function suggested the possibility that the functional groups methylenedioxy at C2 and C3 would be the principal groups which induce the action of bacteriostasis among the herbs which contain berberines. From the molecular structure of the tested BAs, we could find that the five BAs have different functional groups on benzyltetrahydroisoquinolines ring. So, the number, position and type of functional groups on benzyltetrahydroisoquinolines ring have important influence on the antibacterial activities of the five BAs. The five BAs have different antimicrobial effect on E. coli growth, which is due to the efficiency of a detoxification mechanism with the DNA-helicase activity of bacterial being inhibited. The berberines alkaloids can be connected to the vestibule which is shaped by DNA and ToPopase to form a DNA-drugs-ToPopase ternary complex. The complex affects the duplication of DNA and thus bacterial growth is inhibited (19). The thermo-kinetics informations provided from the thermo-chemical studies illustrated that berberine alkaloids inhibited the microbial growth by prohibiting the DNA synthesis of microbial.

DISCUSSION

Our experiments selected microcalorimetry as a tool to investigate the antibacrerial activity of medicinal herbs (20, 21). Tab.3 showed that the reliability and stability of this method was good. Compared with cup-plate method and nephelometry, microcalorimetry not only supplies a new point of view for the evaluation of bioactivity of drugs but provides more information about the bacterial growth. By using it, the energy changes of four growing periods of E. coli, which represented the regularity of microbial population growth such as the lag phase, logarithmic phase, stationary phase and decline phase could be distinguished from the heat production curve. Values of Pm and k for power-time curve are determined simultaneously which could describe the heat growing production and metabolic process of microbes dynamically and precisely. In this study, we have investigated the antibacterial action of the five BAs in Rhizoma Coptidis on E. coli growth based on biothermo-kinetics, providing more references and insights for studying the mechanism of action of these five natural products, the relationship between drug and bacterium metabolism. In this study, we investigated the quantity-antibacterial effect relationship of five BAs on E. coli growth quantitatively from the view of thermodynamics and molecular structure. Furthermore, the action mechanism of antibacterial effect was studied from the molecular

biology and cellular level. Also, the MIC of the BAs was also investigated. This work also provided a thermo-kinetic model to study the quantity-antibacterial effect relationship of drugs on microbial growth. All these were helpful for searching and discovering more pharmaco-dynamic actions and components of Rhizoma Coptidis.

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