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Calorimetric investigation of the effect of hydroxyanthraquinones in *Rheum officinale* Baill on *Staphylococcus aureus* growth

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Abstract

The inhibitory effects of five hydroxyanthraquinones (HAQs) from root and rhizoma of *Rheum officinale* Baill, a traditional Chinese medicinal (TCM) herb, on *Staphylococcus aureus* growth were investigated by calorimetry. The power–time curves of *S.aureus* with and without HAQ were acquired and the extent and duration of inhibitory effects on the metabolism evaluated by growth rate constants (k_1, k_2), half inhibitory ratio (IC_{50}), maximum heat output (P_{max}) and peak time (t_p). The value of k_1 and k_2 of *S. aureus* in the presence of the five HAQs decreased with the increasing concentrations of HAQs. Moreover, P_{max} was reduced and the value of t_p increased with increasing concentrations of the five drugs. The inhibitory activity varied for different drugs. IC_{50} of the five HAQs was 4 μg ml^{-1} for emodin, 3.5 μg ml^{-1} for rhein, 10 μg ml^{-1} for aloe-emodin, 1000 μg ml^{-1} for chrysophanol, 1600 μg ml^{-1} for physcion. The sequence of antimicrobial activity of the five HAQs: rhein > emodin > aloe-emodin > chrysophanol > physicion.

Keywords: Hydroxyanthraquinones; Calorimetry; *Staphylococcus aureus*; Inhibitory effects

1. Introduction

Rheum officinale Baill (Dahuang in Chinese) is a traditional Chinese medicinal (TCM) herb, and is officially listed in the Chinese Pharmacopoeia [1]. *R. officinale* Baill extract has strong antibacterial activities and is used for treating dysentery, cholera, leukemia, diabetes and lung cancer [2,3]. The major active components of the herb are hydroxyanthraquinones (HAQs), which are often used as criteria in the quality control of Dahuang products. HAQs are also active components in a large number of plant-derived drugs such as laxatives from *Rheum*, *Cassia*, *Aloe* and *Polygonum* species. Most of the HAQs are present as pharmacologically inactive glycosides in plant extracts but are thought to be activated by glycoside cleavage in vivo by microorganism in the intestinal flora [4]. In this study, five HAQs in *R. officinale* Baill were tested against *Staphylococcus aureus*.

Calorimetry has been previously used to investigate the interaction between drugs and microbial cells [5,6]. This technique has been used to investigate the inhibitory effects of selenomorpholine compounds on *Escherichia coli* [7] and *S. aureus* [8] and in the study of toxic action of heavy metals on the filamentous fungus *Rhizopus nigricans* [9].

This paper briefly describes the application of the calorimetric method to obtain power–time curves produced by *S. aureus* suspension cells under the action of five HAQs in different concentrations.

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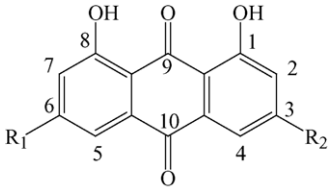

	R_1	R_2
emodin	OH	CH_3
rhein	H	COOH
aloe-emodin	H	CH_2OH
chrysophanol	H	CH_3
physcion	OCH_3	CH_3

Fig. 1. Chemical structures of investigated HAQs in *R. officinale* Baill.

2. Experimental

2.1. Instrument

An LKB 2277 Bioactivity Monitor (Thermometric AB, Sweden) was used to determine the metabolism of *S. aureus* cells.

2.2. Materials

S. aureus (CCTCC AB910393), was provided by the Chinese Center for Type Culture Collections, Wuhan University, Wuhan 430072, PR China. *S. aureus* was grown in a peptone culture medium, which contained 10 g peptone, 5 g beef extract and 5 g NaCl l^{-1}. Medium pH was adjusted to 7.0–7.2 with 1 mol l^{-1} NaOH and 1 mol l^{-1} HCl before autoclaving. Emodin, rhein, aloe-emodin, chrysophanol and physcion were supplied by National Institute for the Control of Pharmaceutical and Biological Products. The five HAQs were extracted from *R. officinale* Baill and their structures are given in Fig. 1.

2.3. Preparation of samples

At the beginning of the experiments, *S. aureus* was inoculated into the peptone medium, with 2×10^6 cells ml^{-1}. Cells were suspended in the peptone culture medium, and the fresh prepared HAQs solutions with different concentrations were added to the cell suspension.

2.4. Experimental procedure

The calorimeter was thermostated at 37 °C, and the measurement made with the stopped-flow method. In all of the experiments, the flow cell was completely cleaned and sterilized by pumping sterilized distilled water, 0.1 mol l^{-1} solution of HCl, 75% alcohol solution, 0.1 mol l^{-1} NaOH and sterilized distilled water in sequence by an LKB-2132 microperpex peristaltic pump, each for 15 min at a flow rate of 50 ml h^{-1} [9,10].

Once the system was cleaned and sterilized and a stable baseline obtained, the cell suspension, containing *S. aureus* and one of the HAQs, was pumped into the flow-cell at a flow rate of 50 ml h^{-1}. When the measuring cell (0.6 ml) was full, the pump was turned off and the thermogenic curve recorded until the recorder returned to the baseline. Since the bacterial metabolic process was monitored in isothermal and isochoric conditions, the nutrient and oxygen consumed by cells was surely limited.

3. Results

3.1. Growth rate constants (k_1, k_2) of S. aureus

Fig. 2 shows the thermogenic curves of *S. aureus* at 37 °C with and without drugs. The heat-production growth curve of *S. aureus* could be divided into four phases, i.e. lag phase, first exponential phase, second exponential phase and decline phase. The exponential model of metabolism of *S. aureus* could be used in the two growth processes [11,12].

$$P_0 = P_0 \exp(kt) \text{ or } \ln P_t = \ln P_0 + kt \quad (1)$$

The thermogenic curve formula of the exponential phase of growth was Eq. (1). The growth rate constants (k_1, k_2) were obtained by fitting $\ln P_t$ and t to a linear equation. The second exponential phase could be regarded as a part of stationary phase because the growth rate constant of phase II (k_2) was much less than that of phase I (k_1).

The power–time curves of *S. aureus* in the different concentrations of drugs showed that the lag phase was prolonged and the growth rate constants (k_1, k_2) and maximum power-output (P_{max}) decreased with the increasing concentrations of HAQs, indicating that HAQs inhibit the growth of *S. aureus*. Emodin, rhein and aloe-emodin suppressed the growth of *S. aureus* more than chrysophanol and physcion. Emodin and rhein completely inhibit growth of *S. aureus* at 10 and 6 μg ml^{-1}, respectively. The different inhibitory action of five drugs could be clearly demonstrated by the plots shown in Fig. 3.

3.2. Inhibitory ratio I and the half inhibitory concentration IC_{50}

Inhibitory ratio I was defined as

$$I = [(k_0 - k_c)/k_0] \times 100\% \quad (2)$$

where k_0 was growth rate constant of the control, k_c was rate constant in the exponential phase of bacterial growth inhibited by inhibitor concentration c. IC_{50} is the inhibitor concentration causing a 50% decrease of the growth rate constant. IC_{50} was 4 μg ml^{-1} for emodin, 3.5 μg ml^{-1} for rhein, 10 μg ml^{-1} for aloe-emodin and

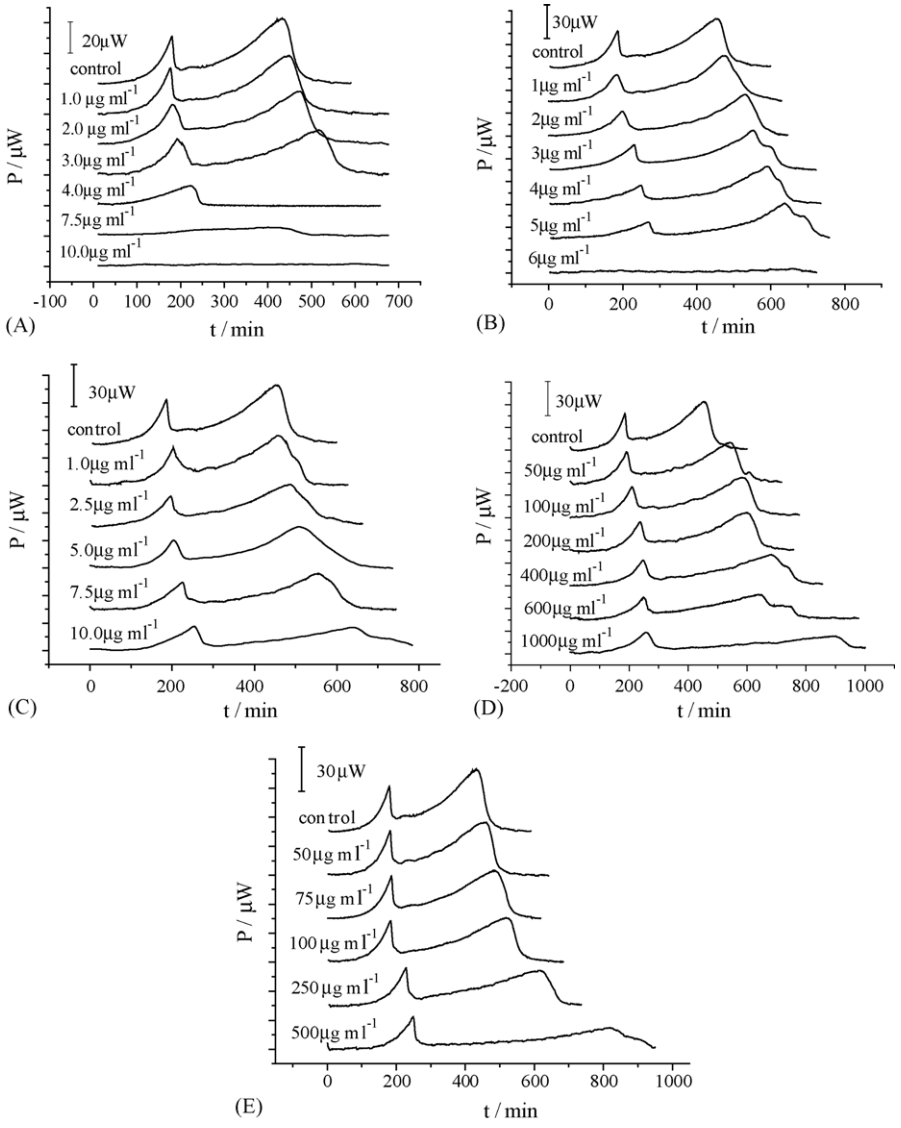

Fig. 2. The power–time curves of *S. aureus* growth in the presence of HAQs at different concentrations: (A) emodin; (B) rhein; (C) aloe-emodin; (D) chrysophanol; (E) physcion.

1000 μg ml^{-1} for chrysophanol and 1600 μg ml^{-1} for physicon. Thus, the sequence of anti-microbial activity of these five HAQs was rhein > emodin > aloe-emodin > chrysophanol > physicion.

According to the previous studies, the ranges of IC_{50} for the five compounds were close to the ranges of minimum inhibitory concentrations (MICs) assessed by a standard method. MICs were 12.5–160 μg ml^{-1} for emodin,

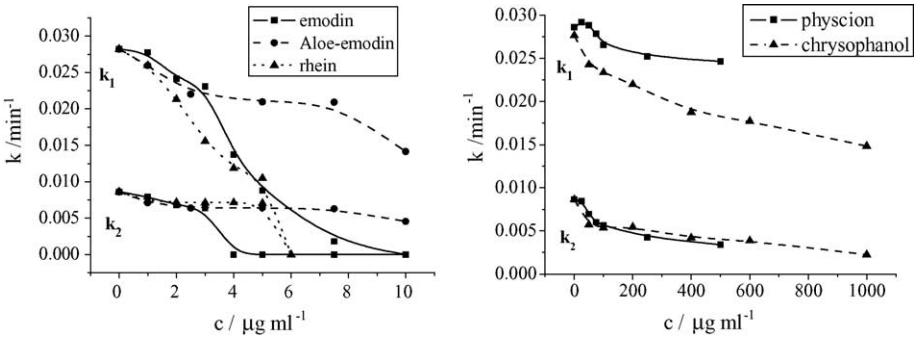

Fig. 3. Plot of k_1, k_2 for the growth *S. aureus*vs. concentration (*c*) for HAQs.

16–32 μg ml^{-1} for rhein, 6.25–80 μg ml^{-1} for aloe-emodin, approximately 500 μg ml^{-1} for chrysophanol and more than 200 μg ml^{-1} for physcion [13–16]. Basically the range and orders of antibacterial activity were the same except for physicion.

3.3. The maximum heat-output (P_{max}) and peak time of thermogenic curve (t_p)

S. aureus produced more energy in the second exponential growth phase than in other phase, so P_{max} was chosen as the maximum output in that phase and t_p as the peak time in thermogenic curve. The *P–t* curves of *S. aureus* and the HAQs in different concentrations showed the quantity of P_{max} decreased and t_p was prolonged with increasing concentrations of the drugs.

4. Discussion

The thermogenic curves of *S. aureus* growth affected by various HAQs from *R. officinale* Baill 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 HAQs. Emodin and rhein showed stronger inhibitory effects on *S. aureus* than the other three HAQs. All of HAQs have two ketone groups at C9 and C10, two hydroxyl groups at C1 and C8. There are different substituted groups at C3 and C6 of phenyl ring (see Fig. 1). The functional groups hydroxyl, hydroxylmethyl and carboxyl on phenyl ring in HAQs improve the anti-microbial activity.

Acknowledgements

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