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Study of the quantitative quantity–activity relationship of four ginsenosides on splenic lymphocytes growth by microcalorimetry

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Abstract In this study, the activities of four ginsenosides Rc, Re, Rd, and Rf on splenic lymphocytes growth were studied by microcalorimetry. Some qualitative and quantitative information, such as the metabolic power–time curves, growth rate constant k, maximum heat-output power of the exponential phase P_{max} and the corresponding appearance peak time t_{max}, total heat output Q_t, and promotion rate R_p of splenic lymphocytes growth affected by the four ginsenosides were calculated. In accordance with thermo-kinetic model, the corresponding quantitative relationships of k, P_{max}, t_{max}, Q_t, R_p, and c were established, . Also, the median effective concentration (EC_{50}) was obtained by quantitative analysis. Based on both the quantitative quantity–activity relationships (QQAR) and EC_{50}, the sequence of promotion activity was Rc > Re > Rd > Rf. The analysis of structure–activity relationships showed that the number, type, and position of sugar moieties on the gonane steroid nucleus had important influences on the promotion activity of Rc, Re, Rd, and Rf on splenic lymphocytes growth. Microcalorimetry can be used as a useful tool for determining the activity and studying the quantity–activity relationship of drugs on cell.

Keywords Microcalorimetry · Ginsenosides · Splenic lymphocytes · QQAR

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Introduction

Ginseng, the root of *Panax* ginseng C.A. Meyer, is a traditional folk medicine that is reported to have many beneficial effects and has had varied uses in traditional Asian medicine for thousands of years. There are several species of the herb, but all species share the same constituents including ginsenosides, polysaccharides, peptides, and fatty acids [1, 2]. Among these constituents, ginsenosides are generally considered to be the main active features of the plant [3, 4]. Over 30 different ginsenosides have been isolated and identified from the root of *Panax* ginseng, which share a similar basic structure, consisting of gonane steroid nucleus having 17 carbon atoms with sugar moieties attached. They are classified into 20(*S*)-protopanaxadiol type (ppd-type) and 20(*S*)-protopanaxatriol type (ppt-type) according to the position of sugar moieties at carbon-3 and -6 of the rings [5].

Studies have shown the wide pharmacological activities of ginsenosides. For example, ginsenosides exert various effects on diverse living cells and tissues, which include that they can increase the intracellular Ca^{2+} concentration in macrophages, NIH3T3, and endothelial cells [6]. Experimental evidence suggests that ginsenosides can reduce pain produced by chemicals or noxious heat. In studies employing writhing and tail-pressure tests in mice, they were found to be antinoci-captive [7]. Another study has reported that ginsenosides inhibit the development of tolerance to the analgesic and hyperthermic effects of chronic morphine treatment in rodents [8]. With wide pharmacological properties, ginsenosides can be used therapeutically. In mice, a glucosyl ginsenoside compound inhibited metastasis of lung carcinoma [9]. Some ginsenosides have been reported to possess bioactivity in cell culture experiments and can alter cancer cell proliferation, induce

apoptosis, and perturb normal cell cycle events [10]. However, from then on, the effects of ginsenosides on the growth of mice splenic lymphocytes have not been reported.

Splenic lymphocytes are the important cells of immunologic system and play a significant role in host acute or chronic diseases. As the first step for T cell activation, splenic lymphocytes should be proliferated or decreased influenced by different factors [11, 12]. Within the cell growth, various metabolic events occur with heat-producing. Using a sensitive calorimeter, the heat and cell growth progress can be recorded. Microcalorimetry provides a useful analytical tool for this determination, which has been used extensively to investigate the state of interaction between drug and cell with much useful information in both qualitative and quantitative ways [13–20]. By analyzing the information, the activity of drugs on cell growth can be studied.

In this study, we used microcalorimetry to investigate the activities of two 20(*S*)-protopanaxadiol ginsenosides (Rc and Rd) and two 20(*S*)-protopanaxatriol species (Re and Rf) on the growth of mice splenic lymphocytes. By analyzing the power–time curves and some quantitative thermokinetic parameters of the cell growth under the action of the four ginsenosides at different concentrations, the quantitative quantity–activity relationship (QQAR) was established, and the effects of ginsenosides on the growth process of mice splenic lymphocytes were elucidated by QQAR.

Materials and methods

Instrument

The 3114/3236 TAM air isothermal microcalorimeter (Thermometric AB, Sweden) was used to determine the metabolic power–time curves of splenic lymphocytes. This microcalorimeter was an 8-channel heat conduction calorimeter for heat flow measurements in the milliwatt range under isothermal conditions and thermostated at the range of 5–60 °C, with a limit of detectability of 2 μW. All calorimetric channels were of twin type, consisting of a sample and a reference vessel. Each vessel was connected to the surrounding heat sink by a Peltier module, and when heat was produced or consumed because of any process, the temperature of the sample vessel was to be changed. The software supplied to TAM air was used to monitor the baseline drift which was less than 20 μW over 24 h.

Materials

Four ginsenosides Rc, Rd, Re, and Rf (Fig. 1) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing), the purities of which were more than 98% by HPLC analysis. RPMI-1640 culture medium and fetal serum were purchased from Gibico Company, USA.

Animals

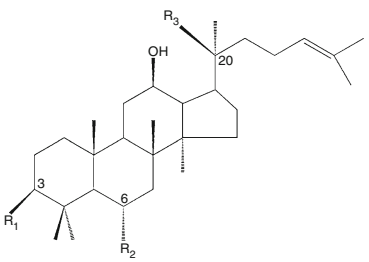
Male balb/c mice, Specific pathogen Free (SPF) grade, weighing from 20 to 22 g were provided by Animal Center of National Institute for the Control of Pharmaceutical and Biological Products (Certificate No: SCXK11-00-0010). All the animals were kept under the same laboratory conditions of temperature from 20 to 22 °C and were given access to standard laboratory chow and tap water. The procedures involving animals and their care conform to the Guiding Principles for the Care and Use of Laboratory Animals of China.

Obtainment of mice splenic lymphocytes

BALB/c mice, approximately 8 weeks old, were sacrificed by cervical dislocation. Spleens were aseptically removed and were placed in cold Hanks solution and teased apart with a pair of forceps and a needle. A single cell suspension from the teased tissue was obtained by passing it through a 200-mesh by the buffer solution containing 1 mmol L^{-1} Tris–HCl and 1% NH_4Cl (pH 7.2). Cells were washed twice with RPMI-1640 medium and subsequently suspended in complete RPMI-1640 culture medium. Cell number and viability were determined by Trypan blue dye exclusion.

Microcalorimetric measurement

At the beginning of the experiments, mice splenic lymphocytes (5×10^6 mL^{-1}) were prepared and transferred



Ginsenosides	R_1	R_2	R_3
Rc	–O–Glc–Glc	–H	–O–Glc–Ara(f)
Rd	–O–Glc–Glc	–H	–O–Glc
Re	–OH	–O–Glc–Rha	–O–Glc
Rf	–OH	–O–Glc–Glc	–OH

Fig. 1 Chemical structure of the four ginsenosides. *Glu* glucose; *Ara(f)* arabinose in furanose form; *Rha* rhamnose

into each ampoule at the same volume. The fresh prepared Rg_1 and Rb_1 solutions (medium as solvent) of different concentrations were added into the cell suspension. The microcalorimeter was thermostated at 37 °C, and the ampoule method was adopted in the study. Ampoules, filled with ginsenosides and cell suspension, were sealed with wax and put into the 8-channel calorimeter block. All the procedures were completely sterilized. After about 30 min (the temperature of ampoules reached 37 °C), the thermogenic curves of splenic lymphocytes were recorded until they returned to the baseline. All the data were collected continuously by using the dedicated software package.

Results

Thermogenic curves of mice splenic lymphocytes growth at 37 °C

The growth thermogenic curves: the power–time (*P–t*) curve (a) and the corresponding ln *P–t* curve (b) of splenic lymphocytes growth in RPMI-1640 culture medium at 37 °C without any substance were shown in Fig. 2. The power–time curve shows the total metabolism profile of splenic lymphocytes and the ln *P–t* curve indicates the changing character of the metabolic heat-power, which could be divided into three stages: stage I is a balance phase of the instrument, stage II is the quick growth phase, and stage III is a decline phase.

Correspondingly, the *P–t* curves of splenic lymphocytes growth at 37 °C affected by different concentrations of the four ginsenosides (Rc, Rd, Re, and Rf) were recorded and are shown in Fig. 3. As the concentration increased, the heights of the highest peaks were all raised in a regular fashion, showing that the growth was accelerated. However, the shapes of growth metabolism curves were basically the same in the presence or absence of organic ginsenosides, i.e., the three phases still existed.

Thermokinetic parameters

The power–time curves of splenic lymphocytes growth show that the quick growth phase obeys the exponential equation [21]:

$$n_t = n_0 \exp(kt) \quad (1)$$

where t is the incubation time, n_t is the cell number at time t, n_0 is the initial cell number, and k is the constant of cell

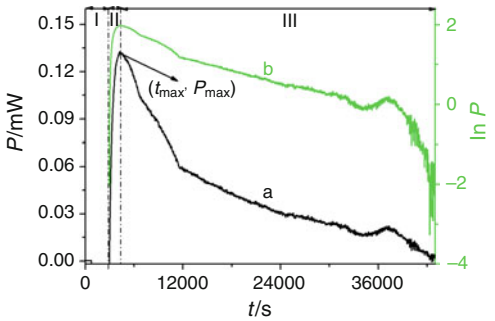


Fig. 2 The metabolic curves: power–time curve (**a**) and the corresponding ln *P–t* curve (**b**) of splenic lymphocytes growth at 37 °C without any substance. It is the metabolic profile of splenic lymphocytes culturing in RPMI-1640 culture medium supplemented without any substance monitored by the microcalorimeter at 37 °C and could be divided into three stages: stage I is a balance phase of the instrument, stage II is the quick growth phase, and stage III is a decline phase

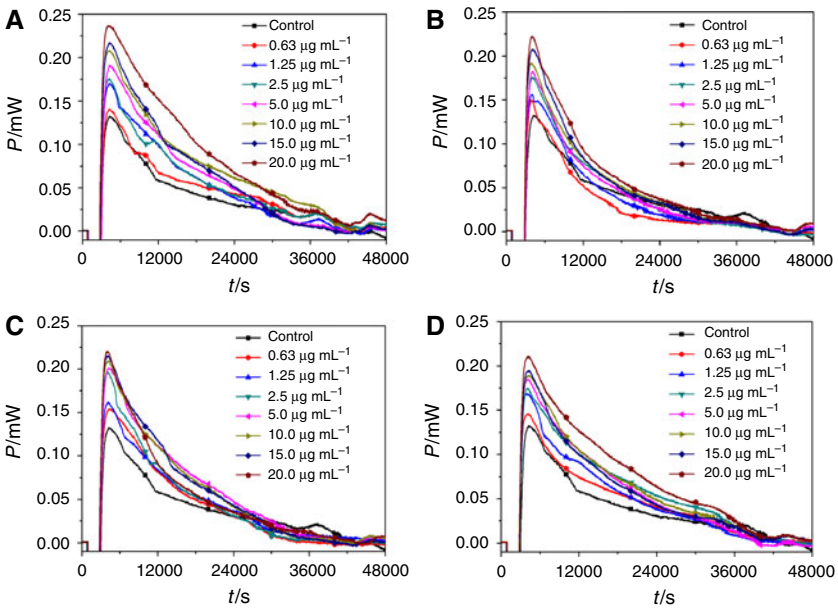


Fig. 3 Power–time curves of splenic lymphocytes growth at 37 °C affected by different concentrations of Rc (**a**), Rd (**b**), Re (**c**), and Rf (**d**). Their concentrations were listed in this figure

growth rate. If the heat output power of each cell is denoted as P_w, then

$$n_t P_w = n_0 P_w \exp(kt) \quad (2)$$

when $n_0 P_w$ is indicated at P_0 and $n_t P_w$ at P_t, and P_t and P_0 are defined as the heat output power at time 0 and t, respectively. Then, Eq. (2) can be rewritten as:

$$P_t = P_0 \exp(kt) \quad (3)$$

or

$$\ln P_t = \ln P_0 + kt \quad (4)$$

Using the Eq. (4), the rate constant of cell growth k was calculated by analyzing the experimental data of P_t and t obtained from the cell growth curves to a linear equation. The values of k with the corresponding RSD of 1.77% are shown in Table 1, which indicated a good reproducibility of the experiments.

Then, based on the above equation 4, the corresponding k values along with their correlation coefficients of splenic lymphocytes growth affected by the four ginsenosides at different concentrations were all calculated and are shown in Table 2. Also, the maximum heat-output power P_{max} and the appearance time t_{max} of the highest peak were obtained. By integrating the peak areas under the curves, the total heat outputs Q_t in the whole growth progress of splenic lymphocytes affected by the ginsenosides were then obtained (Table 2).

Table 2 showed that the values of k, P_{max}, and Q_t increased with the increase of the concentrations of the four ginsenosides, while t_{max} keep fluctuating with the concentration ranges. From the values in Table 2 along with the profiles in Fig. 3, it could be concluded that the four ginsenosides in the concentration ranges of 0.625–20.0 μg mL^{-1} all could promote the growth of splenic lymphocytes and the promotion activities would be enhanced with increasing the concentrations of the four compounds. Therefore, based on the values of k, the promotion ratio (R_p, %) for Rc, Rd, Re, and Rf on splenic lymphocytes growth could be calculated and defined as

$$R_p(\%) = (k_c - k_0)/k_0 \times 100\% \quad (5)$$

where k_0 was the growth rate constant of splenic lymphocytes without ginsenoside (the control), k_c was the growth rate constant in the exponential phase of splenic lymphocytes promoted at promoter concentration c.

For further evaluating the activities and investigating the quantity–activity relationship of the four ginsenosides on splenic lymphocytes growth, the relationships of quantitative thermokinetic parameters in Table 2 and concentration c were established and are shown in Fig. 4.

Relationship between k and c

The values of k in Table 2 illustrated that the promotion activities of Rc, Rd, Re, and Rf on splenic lymphocytes were all clearly dose dependent. The rate constants increased gradually with increasing concentration of the four ginsenosides. The relationships between k and c of the four ginsenosides in Fig. 4a could be expressed as the following equation by using linear regression method:

$$\text{For Rc: } k = (1.21991\text{E}{-4})\,c + 0.00399,\quad R = 0.9901\ (0.625{-}20.0\ \mu g\,mL^{-1})$$

$$\text{For Rd: } k = (1.16302\text{E}{-4})\,c + 0.00396,\quad R = 0.9839\ (0.625{-}20.0\ \mu g\,mL^{-1})$$

$$\text{For Re: } k = (9.07996\text{E}{-5})\,c + 0.00419,\quad R = 0.9858\ (0.625{-}20.0\ \mu g\,mL^{-1})$$

$$\text{For Rf: } k = (1.03282\text{E}{-4})\,c + 0.00386,\quad R = 0.9918\ (0.625{-}20.0\ \mu g\,mL^{-1})$$

The good linearity with $R > 0.9830$ of the $k - c$ relationship for the four ginsenosides showed that the k values were almost linearly increased with the increase of the concentrations of Rc, Rd, Re, and Rf.

Relationship between P_{max} and c

Figure 3 showed that the heights of the highest peaks of splenic lymphocytes affected by the four ginsenosides were all ascended within the concentration range of 0.625–20.0 μg mL^{-1}, which could be reflected from the

Table 1 Growth rate constant k of splenic lymphocytes cultured in 1640 culture medium and monitored by the microcalorimeter at 37 °C

No	1	2	3	4	5	6	Mean ± RSD/%[a]
k/s^{-1}	0.00337	0.00346	0.00352	0.00349	0.00352	0.00351	0.00350 ± 1.77
R[b]	0.9860	0.9886	0.9811	0.9826	0.9855	0.9819	0.9843 ± 0.29

[a] Relative standard deviation

[b] Correlation coefficient

Table 2 Thermokinetic parameters for the growth of splenic lymphocytes growth in the presence of ginsenosides Rc, Rd, Re and Rf

Ginsenosides	c/μg mL^{-1}	k/s^{-1}	R^a	P_{max}/mW	t_{max}/S	Q_t/J	R_p/%
Control	0	0.00349	0.9826	0.1327	4240.0	1.747	0
Rc	0.625	0.00388	0.9861	0.1413	4200.0	2.020	10.32
	1.25	0.00408	0.9856	0.1705	4160.0	2.227	16.91
	2.5	0.00436	0.9864	0.1760	4160.0	0.373	24.93
	5.0	0.00474	0.9868	0.19136	4200.0	2.534	35.82
	10.0	0.00533	0.9838	0.2085	4160.0	3.120	52.72
	15.0	0.00588	0.9875	0.2172	4240.0	3.718	80.23
	20.0	0.00629	0.9884	0.2370	4040.0	3.718	80.23
Rd	0.625	0.00386	0.9898	0.1501	3900.0	1.838	10.03
	1.25	0.00399	0.9853	0.1567	3980.0	1.964	14.33
	2.5	0.00427	0.9873	0.1763	4100.0	2.173	25.21
	5.0	0.00475	0.9882	0.1826	4080.0	2.324	40.11
	10.0	0.00529	0.9889	0.1923	3960.0	2.492	51.58
	15.0	0.00577	0.9872	0.2082	4120.0	2.621	65.33
	20.0	0.00611	0.9866	0.2226	3960.0	2.899	72.21
Re	0.625	0.00406	0.9857	0.1544	4200.0	1.853	13.47
	1.25	0.00427	0.9886	0.1623	4060.0	2.034	22.35
	2.5	0.00452	0.9866	0.1968	4000.0	2.082	38.11
	5.0	0.0048	0.9867	0.2015	4260.0	2.631	44.41
	10.0	0.00516	0.9883	0.2095	4020.0	2.665	50.72
	15.0	0.00548	0.9849	0.2156	4060.0	2.793	57.02
	20.0	0.00599	0.9865	0.2206	3960.0	2.937	71.63
Rf	0.625	0.00378	0.9841	0.1460	4080.0	2.035	2.58
	1.25	0.00399	0.9848	0.1689	3980.0	2.181	8.60
	2.5	0.00412	0.9822	0.1748	4080.0	2.364	18.05
	5.0	0.00452	0.9887	0.1851	4000.0	2.522	31.23
	10.0	0.00499	0.9863	0.1895	4220.0	2.745	42.98
	15.0	0.00532	0.9851	0.1948	4260.0	3.076	52.44
	20.0	0.00591	0.9867	0.2110	4080.0	3.276	69.34

[a] Correlation coefficient

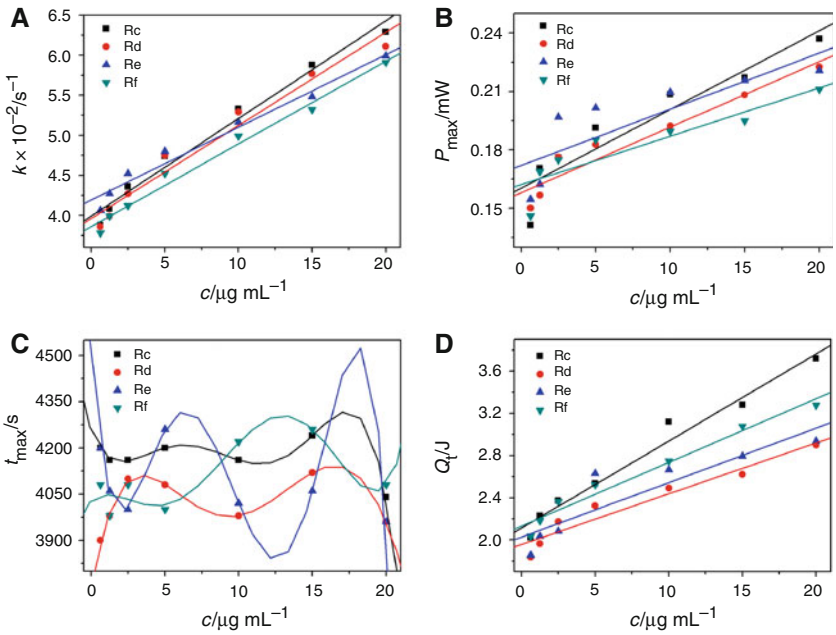


Fig. 4 Relationships between some thermokinetic parameters and concentration c of Rc, Rd, Re and Rf. **a** $k - c$; **b** $P_{max} - c$; **c** $t_{max} - c$ and **d** $Q_t - c$

values of P_{max} in Table 2 and the relationship in Fig. 4b. The relationships between P_{max} and c were as follows:

$$\begin{aligned}
\text{For Rc: } P_{max} &= 0.00404c + 0.16027,\\
R &= 0.9496\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rd: } P_{max} &= 0.00337c + 0.15797,\\
R &= 0.9632\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Re: } P_{max} &= 0.00289c + 0.17195,\\
R &= 0.8319,\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rf: } P_{max} &= 0.00249c + 0.16211,\\
R &= 0.8967\ (0.625 - 20.0\,\mu g\,mL^{-1})
\end{aligned}$$

The above equations showed that relationships of $P_{max} - c$ for Rc and Rd were satisfied with $R > 0.9490$, while for Re and Rf were not so good with $R < 0.9000$.

Relationship between t_{max} and c

From the data in Table 2 and the plot in Fig. 4c, we can see that for the four ginsenosides, the values of t_{max} had the irregular change of "increase–decrease" and the relationships between t_m and c for the two drugs presented double S shape but not linear. The $t_{max} - c$ equations could be described as below:

$$\begin{aligned}
\text{For Rc: } t_{max} &= (-0.00954)\,c^5 + 0.44317\,c^4 - 7.18702\,c^3\\
&\quad + 48.42598\,c^2 - 123.00209\,c + 4256.81316,\\
R^2 &= 0.9976\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rd: } t_{max} &= 0.00287\,c^5 - 0.22479\,c^4 + 5.84326\,c^3\\
&\quad - 62.26879\,c^2 + 256.66759\,c + 3756.91962,\\
R^2 &= 0.9945\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Re: } t_{max} &= (-0.04047)\,c^5 + 1.97753\,c^4 - 33.66373\,c^3\\
&\quad + 234.8119\,c^2 - 602.24429\,c + 4497.34521,\\
R^2 &= 0.9977\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rf: } t_{max} &= 0.00716\,c^5 - 0.33763\,c^4 + 5.15094\,c^3\\
&\quad - 27.28333\,c^2 + 42.53585\,c + 4028.93394,\\
R^2 &= 0.9729\ (0.625 - 20.0\,\mu g\,mL^{-1})
\end{aligned}$$

Relationship between Q_t and c

Usually, the total heat output for growth phase of splenic lymphocytes is increased with the increase of concentration of the four ginsenosides. By analyzing the Table 2 and Fig. 4d, one could see that the total heat output Q_t of growth phase all increased with increasing concentrations of Rc, Rd, Re, and Rf. The relationships between Q_t and c were all nearly linear which were as follows:

$$\begin{aligned}
\text{For Rc: } Q_t &= 0.08246\,c + 2.11232,\\
R &= 0.9862\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rd: } Q_t &= 0.04815\,c + 1.95613,\\
R &= 0.9854\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Re: } Q_t &= 0.05161\,c + 2.02698,\\
R &= 0.9837\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rf: } Q_t &= 0.06033\,c + 2.13121,\\
R &= 0.9845\ (0.625 - 20.0\,\mu g\,mL^{-1})
\end{aligned}$$

Relationship between R_p and c

Based on the above analysis, it could be found that during the concentration range of 0.625–20.0 μg mL^{-1}, with increasing concentration of the four ginsenosides, the growth rate constant k, the maximum heat-out power P_{max} and the total heat output Q_t increased, suggesting that Rc, Rd, Re, and Rf all had strong promotional activities on the growth of splenic lymphocytes. Further, the promotional extent can be quantitatively described by the promotion ratio R_p, which is listed in Table 2, and the relationship between R_p and c is shown in Fig. 5. Then, the $R_p - c$ equations for the four ginsenosides were obtained as follows:

$$\begin{aligned}
\text{For Rc: } R_p &= 3.51376\,c + 14.04736,\\
R &= 0.9891\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rd: } R_p &= 3.16266\,c + 15.25919,\\
R &= 0.9876\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Re: } R_p &= 2.49011\,c + 23.18537,\\
R &= 0.9756\ (0.625 - 20.0\,\mu g\,mL^{-1})\\
\text{For Rf: } R_p &= 3.17855\,c + 7.48149,\\
R &= 0.9788\ (0.625 - 20.0\,\mu g\,mL^{-1})
\end{aligned}$$

With $R > 0.9750$, the quantitative quantity–activity relationships (QQASs) of R_p and c were satisfied for the four

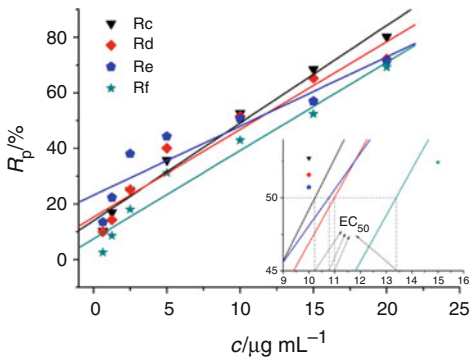


Fig. 5 Relationship between R_p and c of each ginsenoside. From these relationships, the values of EC_{50} of 10.26 μg mL^{-1} for Rc, 11.03 μg mL^{-1} for Rd, 10.81 μg mL^{-1} for Re, and 13.47 μg mL^{-1} for Rf were obtained

ginsenosides, showing that the promotion activities on the growth of splenic lymphocytes were enhanced with the increase of concentrations of Rc, Rd, Re, and Rf. When R_p was 50%, the corresponding concentration of the compound, which produced 50% of the maximum response for this compound, was called the median effective concentration (EC_{50}, required to induce a 50% activity). Based on the above $R_p - c$ equations, the values of EC_{50} of 10.26 μg mL^{-1} for Rc, 11.03 μg mL^{-1} for Rd, 10.81 μg mL^{-1} for Re, and 13.47 μg mL^{-1} for Rf were calculated, showing that the order sequence of promotional effect of the four ginsenosides on splenic lymphocytes was: Rc > Re > Rd > Rf.

Study of structure–activity relationship

The above analysis has shown that all the four ginsenosides had strong promotional activities on splenic lymphocytes, and this activity represented good quantitative quantity–activity relationships: the promotional activities were enhanced with the increase of the concentration of Rc, Rd, Re, and Rf. Rc was found to have the strongest promotional activity to the growth of splenic lymphocytes, whereas Rf had the poorest activity. These results indicated that specific differences in ginsenoside chemical structure would influence the growth of splenic lymphocytes.

Ginsenosides are characterized according to the number and position of sugar moieties on the sterol chemical structure (Fig. 1). Differences of the substitute groups of sugars on C-3, C-6, and C-20 of ginsenosides will influence the hydrophilic character of the compounds required to interact with cell membrane function [22]. Rc and Rd both belong to the 20(*S*)-protopanaxadiol classification, while Rc with four sugars (three glucoses and one arabinose) on gonane steroid nucleus have much stronger promotional activity on splenic lymphocytes than Rd with three sugars (glucose). Re and Rf both belong to the 20(*S*)-protopanaxatriol classification, while Re with three sugars (two glucoses and one rhamnose) on gonane steroid nucleus presented much stronger promotional activity than Rd with two sugars (glucose). These results illustrated that the number of sugar moieties on gonane steroid nucleus had important influences on the activity of ginsenoside. On the other hand, Rd and Re, belonging to different classifications, had same number of sugars on the sterol chemical structure, but Re had much stronger promotion activity on splenic lymphocytes than Rd. The differences between them were that the hydroxyl on C-3 (R1, Fig. 1) for Re was substituted by two glucosides for Rd and the H at C-6 (R2) for Rd was substituted by one glucose-rhamnoside. These results illustrated that the type and position of sugars on gonane steroid nucleus could also influence the activity of ginsenoside. Further studies would be focused on the mechanism of action of these different promotion activities of ginsenosides on splenic lymphocytes.

Discussion

Our study shows that microcalorimetry is a powerful tool for monitoring the kinetics of the cell behavior and estimating the bioactivity of drugs, further studying the quantitative quantity–activity relationship of these drugs. As a non-destructive and noninvasive technique with the high sensitivity of the monitor, the whole metabolism of cell and the influence of drugs on this metabolism can be examined automatically and continuously. Some thermokinetic and thermodynamic information that cannot be obtained by conventional bacteriological techniques is obtained, and all of this information is very significant for the studies of pharmacology [23–25]. Moreover, the microcalorimetric method requires only an observable difference between the power production in the treated and controlled incubations. Unlike many other procedures, transparent solution is not required. Therefore, microcalorimetric method may reveal greater and newer details about the metabolism than the existing methods do.

In summary, microcalorimetry was used to measure the activity of four ginsenosides on splenic lymphocytes. The power–time curves showed that the height of the highest peak of splenic lymphocytes growth was raised with increasing concentrations of the four ginsenosides, at the same time, the total heat output was also increased. From the power curves of splenic lymphocytes growth affected by the four ginsenosides, some quantitative thermokinetic parameters were calculated; then, the quantity–activity relationships of these parameters and concentration c could also be obtained. Based on both the quantity–activity relationships and median effective concentration (EC_{50}), the order sequence of promotional activity was Rc > Re > Rd > Rf. From the analysis of structure–activity relationships, it could be concluded that the number, type, and position of sugar moieties on the gonane steroid nucleus had important influences on the promotion activity of Rc, Re, Rd, and Rf on splenic lymphocytes growth. The mechanism of action of the four ginsenosides on splenic lymphocytes would be the subject for the future study.

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