J Therm Anal Calorim (2011) 104:357–363
DOI 10.1007/s10973-010-1003-6

Microcalorimetric study of the opposing effects of ginsenosides Rg_1 and Rb_1 on the growth of mice splenic lymphocytes

Yan-Ling Zhao · Jia-Bo Wang · Ping Zhang · Li-Mei Shan · Rui-Sheng Li · Xiao-He Xiao

Received: 14 July 2010 / Accepted: 3 August 2010 / Published online: 29 August 2010

Abstract Splenic lymphocytes play an important role in host acute or chronic diseases. The abnormality of these cells in the spleens of humans might lead to some riskful diseases for human. Hence, in this study, the effects of two ginsenosides Rg_1 and Rb_1 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}, total heat output Q_t of splenic lymphocytes were obtained to present the effects of Rg_1 and Rb_1 on these cells. The values of k, P_{max}, and Q_t from the thermogenic growth curves of splenic lymphocytes were found to increase in the presence of Rg_1, while the change was adverse for Rb_1, illustrating that Rg_1 had promotion effect and Rb_1 had inhibitory effect on splenic lymphocytes growth and these promotion or inhibitory effects were enhanced with increasing the concentration of the two compounds, respectively. The microcalorimetric results were confirmed by MTT assay for determining the MTT optical density (OD) value and [^{3}H] Thymidine incorporation assay ([^{3}H]-TdR) for determining the count per minute (cpm) value: Rg_1 could increase the MTT OD value and the cpm value of [^{3}H]-TdR incorporation into splenic lymphocytes, and these values were increased with increasing the concentration of this compound, while Rb_1 had the adverse results. The structure–activity relationships showed that the glucopyranoside and hydroxyl groups at the dammarane-type mother nucleus skeleton might play a crucial role for the opposing effects of the two ginsenosides on splenic lymphocytes. Compared with the other two assay methods, the microcalorimetric method provided more useful and reliable information for quickly and objectively evaluating the effects of drugs or compounds on the living cells, which would be a highly promising analytical tool for the characterization of the biological process and the estimation of the drugs' efficiency.

Keywords Microcalorimetry · Ginsenosides Rg_1 and Rb_1 · Splenic lymphocytes · Opposing effects · MTT · [^{3}H]-TdR

Y.-L. Zhao · J.-B. Wang · P. Zhang · L.-M. Shan · X.-H. Xiao (✉)
Institute of Traditional Chinese Material Medica, People's Liberation Army, 100, The 4th Ring Road, Beijing 100039, People's Republic of China
e-mail: zhao2855@263.net

R.-S. Li
Animal Laboratory Center, 302 Hospital of People's Liberation Army 100, The 4th Ring Road, Beijing 100039, People's Republic of China

Introduction

Gingseng, frequently used as a crude substance, is taken orally in Asian countries as a traditional medicine for thousands of years. Named by the botanist Carl Meyer, the genus Panax derives its name from the Greek pan (all) and akos (healing). As a commonly used nutraceutical, Ginseng is a key component in traditional Chinese medicine and is also one of the most extensively used products in the West. The most important bioactive components contained in Ginseng are ginsenosides (triterpenic dammaranic saponins). The two major groups of ginsenosides are Rg and Rb, which have 20(S)-protopanaxadiol and 20(S)-protopanaxatriol, respectively, as the sapogenines, which contain an aglycone with a dammarane skeleton and have been reported to regulate a variety of physiological processes. The pharmacological effects were totally different according to different kinds of ginsenosides [1–3].

Interestingly, the ginsenosides of groups Rb and Rg have some opposing pharmacological properties [4]. For example, Rg_1 played dominant role in angiogenesis while Rb_1 showed inhibited effect in the earliest step of angiogenesis [5]. However, the effects of ginsenosides Rg_1 and Rb_1 on the growth of mice splenic lymphocytes have not, so far, been reported (Fig. 1).

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 [6, 7]. Within the cell growth, the various metabolic events are all heat-producing reactions. Microcalorimetry provides a useful analytical tool for the characterization of cell growth progress, which has been used extensively to investigate the state of interaction between drug and cell with much useful information to be furnished [8–14]. Thus, by monitoring the heat effect with a sufficiently sensitive microcalorimeter, the metabolic processes of living cells can be studied by a direct method. Microcalorimetry can directly determine the biological activity of a living system and provide a continuous measurement of the heat production, thereby giving much useful information in both qualitative and quantitative ways [15, 16]. Each type of cell has a unique heat power versus time trace, as reported by the microcalorimeter, under a defined set of growth conditions. Any substance that can modify the metabolic growth progresses involved in cell will change the characterization of curves, not only thermodynamic but also kinetic information can be obtained. By analyzing the information, the activity and potency of drugs on cell growth can be compared.

The use of microcalorimetry to monitor cell metabolic activity in vitro has been well established. During the past decade, microcalorimetry has been applied to study the effect of active components in Chinese medicine on microorganisms [17], mitochondria [18], cultured tissue cells [19], and cultured tissue cells infected by virus [15].

In this article, the curves produced by mice splenic lymphocytes under the action of two ginsenosides Rg_1 and Rb_1 at different concentrations were determined by microcalorimetry. From the power-time curves of these cells, growth rate constant k, maximum heat-output power of the exponential phase P_{max}, total heat output Q_t were obtained to present the effects of Rg_1 and Rb_1 on these cells. Meanwhile, MTT assay for determining the MTT optical density (OD) value and [^{3}H] Thymidine incorporation assay ([^{3}H]-TdR) for determining the count per minute (cpm) value were employed to confirm these effects of ginsenosides Rg_1 and Rb_1 on the growth of mice splenic lymphocytes. The experiments showed that the ginsenosides had opposing effects on mice splenic lymphocytes and these effects from the three assay methods were consistent.

Materials and methods

Instruments

The 3114/3236 TAM air bioactivity monitor (Thermometric AB, Sweden), an 8-channel heat conduction calorimeter for heat flow measurements in the milliwatt range under isothermal conditions, was held together in a single removable block. This block was placed in an air thermostat, which kept the temperature within 0.02 °C. 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 due to any process, the temperature of the sample vessel was to be changed. The temperature of the surrounding was constant, and thus a temperature gradient across the Peltier module was developed. This would generate a measurable voltage, and the voltage was proportional to the heat flow across the Peltier module and to the rate of the processes taking place in the sample vessel. Such voltage signal was recorded continuously and in real-time through an 8-channel data logger. The software supplied to bioactivity monitor which was used to monitor the baseline drift was less than 20 μW over 24 h.

A microplate Reader (Synergy2 Multi-Mode Microplate Reader, BioTek, USA) was used to determine the optical density (OD) value and a liquid scintillation counter (MicroBetu Trilux 1450 type, Perkin Elmer, USA) was used to detect the cpm value of mice splenic lymphocytes cultured with RPMI-1640 medium solution including Rg_1 and Rb_1, respectively, at different concentration.

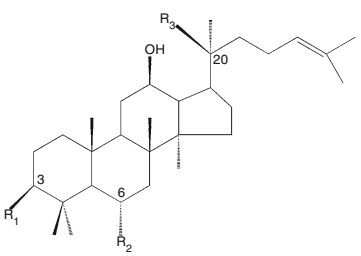

Fig. 1 Chemical structure of the two ginsenosides

Ginsenosides	R1	R2	R3
Rg_1	–OH	–O–Glc	–O–Glc
Rb_1	–O–Glc–Glc	–H	–O–Glc–Glc

Animals

Balb/c mice, Specific pathogen Free (SPF) grade, male, 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 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.

Materials

Ginsenosides Rg_1 and Rb_1 were purchased from National Institute for the Control of Pharmaceutical and Biological Products, the purities of which were more than 98% by HPLC analysis. RPMI-1640 culture medium and fetal serum were purchased from Gibico Company, USA. 96-well plates and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma Company, USA.

Methods

Preparation of mice splenic lymphocytes

BALB/c mice, approximately 8 weeks old, were sacrificed by cervical dislocation, and spleens were removed aseptically. Spleens 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 assay

At the beginning of the experiments, mice splenic lymphocytes (5×10^6 mL^{-1}) were prepared and transferred 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 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 data were collected continuously by using the dedicated software package.

MTT assay

The splenic lymphocytes were set up in 96-well plates (5×10^6 mL^{-1}) and incubated in the freshly prepared Rg_1 and Rb_1 solutions (medium as solvent) of different concentrations, respectively. After the cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h with triplicates, 10 μL MTT was added to every well and continued to culture for 6 h. Another 100 μL hydrochloric acid isopropanol solution was added to the wells after the supernatant of cell culture medium was discarded. The MTT optical density (OD) value of splenic lymphocytes suspension coexisted with one of the two ginsenosides was determined by the Microplate Reader.

[^{3}H]-TdR assay

The splenic lymphocytes were set up in 96-well plates (5×10^6 mL^{-1}) and incubated in the freshly prepared ginsenoside Rg_1 and Rb_1 solutions (medium as solvent) in different concentrations, respectively. After the cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h with triplicates, 10 μCi mL^{-1} [^{3}H], 20 μL was added in 96-well plates splenic lymphocytes. 16 h later, the cpm value of [^{3}H]-TdR of splenic lymphocytes suspension coexisted with one of the two ginsenosides was detected by the liquid scintillation counter.

Results

Power–time curves of splenic lymphocytes growth

The thermogenic power–time curve of splenic lymphocytes growth without any substance monitored by the microcalorimeter at 37 °C was shown in Fig. 2. It was the metabolic profile of splenic lymphocytes without ginsenosides monitored by the microcalorimeter at 37 °C and could be divided into three stages: stage I, II, and III. Stage I is a balance phase of the instrument, stage II is the quick exponential growth phase, and stage III is a decline phase of splenic lymphocytes. The exponential metabolism model of splenic lymphocytes could be used in the growth processes:

$$\mathrm{Ln}\ P_t = kt + \mathrm{Ln}\ P_0 \quad (1)$$

where P_0 represents the heat-output power at the beginning and P_t represents that at time t. According to Eq. 1, growth rate constant k of splenic lymphocytes can be obtained by

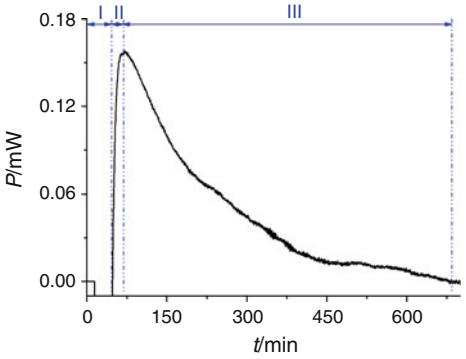

Fig. 2 The power–time curve of splenic lymphocytes growth at 37 °C without any substance

fitting Ln P_t and t to a linear equation. The values of k with the corresponding RSD of 1.30% were shown in Table 1, which indicated a good reproducibility of the experiments.

The corresponding thermogenic power–time (P–t) curves of splenic lymphocytes growth with the two ginsenosides were monitored by microcalorimetry and shown in Fig. 3. As could be seen from the profiles of these curves, the growth of splenic lymphocytes was influenced by the two ginsenosides.

Quantitative thermokinetic parameters of splenic lymphocytes growth

As shown in Fig. 3, there was a characteristic peak of the thermogenic curves. Comparison to control (without ginsenosides), the peak heights of splenic lymphocytes growth raised with increasing the concentration of Rg_1, while they were lowered with increasing the concentration of Rb_1, which could also be presented from the change of the values of the maximum heat-output power P_{max} listed in Table 2, illustrating that Rg_1 promoted the growth of splenic lymphocytes while Rb_1 inhibited the growth of splenic lymphocytes.

Table 2 showed that the k values of splenic lymphocytes growth in the exponential phase were increased with increasing the concentration of Rg_1 while were decreased with increasing the concentration of Rb_1. The relationships between k and c were:

For Rg_1: $k = 0.00312c + 0.19592, \quad R = 0.9593$

For Rb_1: $k = -0.0032c + 0.16416, \quad R = -0.9557$

The high R values of greater than 0.9550 also showed the promotion effect of Rg_1 and inhibitory effect of Rb_1.

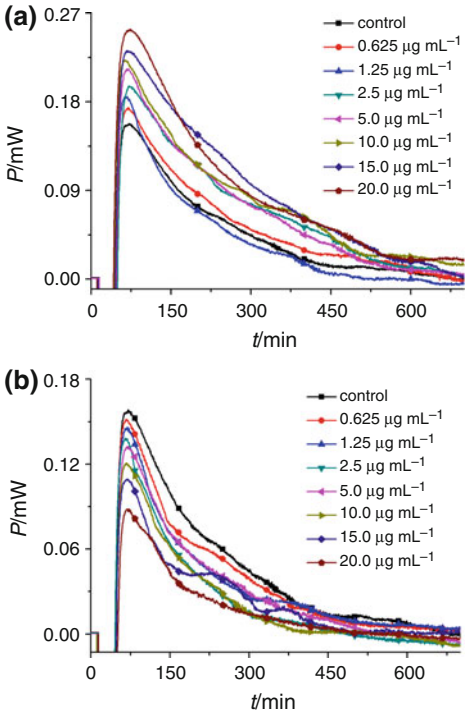

Fig. 3 The power–time curves of splenic lymphocytes growth at 37 °C affected by different concentrations of **a** Rg_1 and **b** Rb_1

Based on the different effects of Rg_1 and Rb_1 on splenic lymphocytes growth, the promotion ratio (R_p, %) for Rg_1 and the inhibitory ratio (R_i, %) for Rb_1 could be calculated based on k. They could be defined as:

$$R_p(\%) = (k_c - k_0)/k_0 \times 100\% \text{ and}$$
$$R_i(\%) = (k_0 - k_c)/k_0 \times 100\%$$

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 or inhibited at promoter or inhibitor concentration c. Both of R_p and R_i values were increased with increasing the concentration of Rg_1 and Rb_1, further illustrating the strong promotion effect of $Rg_1$and inhibitory effect of Rb_1. Then, the relationships between the total heat output Q_t in the whole course of splenic lymphocytes growth were obtained and shown in Fig. 4. The values of Q_t were increased for Rg_1 and decreased for Rb_1 with increasing the concentration of the two ginsenosides. These all illustrated that the Rg_1 had strong promotion effect and Rb_1 had strong inhibitory effect on splenic lymphocytes growth.

Table 1 Growth rate constant k of splenic lymphocytes cultured in 1,640 culture medium and monitored by the microcalorimeter at 37 °C

Experiment No.	1	2	3	4	5	6	RSD[a] %
k/min^{-1}	0.17945	0.18359	0.18204	0.18379	0.18391	0.18666	1.30

Table 2 Thermokinetic parameters for splenic lymphocytes growth affected by Rg_1 and Rb_1 of different concentrations

Compound	c/μg mL^{-1}	k/min^{-1}	r^a	P_{max}/mW	t_{max}/min	Q_t/J	R_p/%	R_i/%
Control	0	0.18359	0.8620	0.158	70.7	1.76	0	0
Rg_1	0.625	0.19336	0.8768	0.174	69.0	1.88	5.32	–
	1.25	0.20287	0.8813	0.185	64.0	1.97	10.50	–
	25	0.21080	0.9134	0.196	70.0	2.72	14.82	–
	5	0.21937	0.9186	0.214	67.0	2.73	19.49	–
	10	0.23112	0.8914	0.222	61.7	3.11	25.89	–
	15	0.24164	0.8905	0.232	67.3	3.46	31.62	–
	20	0.25418	0.9112	0.254	73.7	3.57	38.45	–
Rb_1	0.625	0.16598	0.8952	0.152	66.7	1.54	–	9.59
	1.25	0.15820	0.8956	1.146	69.3	1.39	–	13.83
	2.5	0.14678	0.9386	0.138	66.3	1.21	–	20.05
	5	0.13441	0.8921	0.132	69.3	0.96	–	26.79
	10	0.12517	0.8827	0.121	68.0	0.89	–	31.82
	15	0.11911	0.8801	0.110	69.3	0.81	–	35.12
	20	0.10610	0.9339	0.088	69.3	0.70	–	42.21

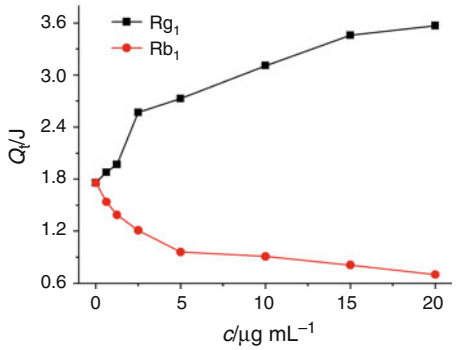

Fig. 4 Relationship between the total heat output Q_t and c

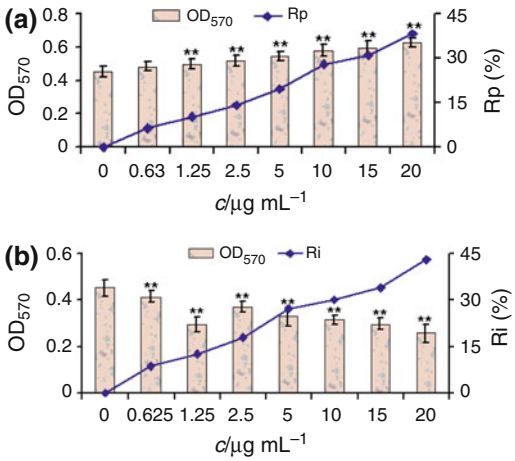

Fig. 5 Effect of **a** Rg_1 and **b** Rb_1 on MTT OD values of splenic lymphocytes. Compared with normal cells, ** $P < 0.01$

Confirmation results of MTT assay

As confirmation of the microcalorimetric results, the MTT assay was used to evaluate the effects of Rg_1 and Rb_1 on MTT OD values of splenic lymphocytes. Figure 5a showed that low concentration (0.625 μg mL^{-1}) of Rg_1 could increase the MTT OD value of splenic lymphocytes and these values were increased with increasing the concentration of this ginsenoside, while Fig. 5b showed that low concentration (0.625 μg mL^{-1}) of Rb_1 could decrease the MTT OD value of splenic lymphocytes and these values were decreased with the increase of the concentration of Rb_1. The corresponding R_p (%) for Rg_1 and R_i (%) for Rb_1 was defined as:

$$R_p(\%) = (OD_c - OD_0)/OD_0 \times 100\% \text{ and}$$
$$R_i(\%) = (OD_0 - OD_c)/OD_0 \times 100\%.$$

The values of R_p and R_i in Fig. 5 were both almost linearly increased with the increase of concentration of the two ginsenoside, illustrating the promotion effect of Rg_1 and inhibitory effect of Rb_1 on splenic lymphocytes.

Confirmation results of [^{3}H]-TdR assay

As another confirmation of the microcalorimetric results, the [^{3}H]-TdR assay was used to study the effect of Rg_1 and Rb_1 on [^{3}H]-TdR incorporation into splenic lymphocytes. Similarly to the above results, Fig. 6a showed that low concentration (0.625 μg mL^{-1}) of Rg_1 could increase the cpm value of [^{3}H]-TdR incorporation into splenic lymphocytes and these values were increased with increasing the concentration of this ginsenoside, while Fig. 6b showed that low concentration (0.625 μg mL^{-1}) of Rb_1 could decrease the cpm values of [^{3}H]-TdR incorporation into

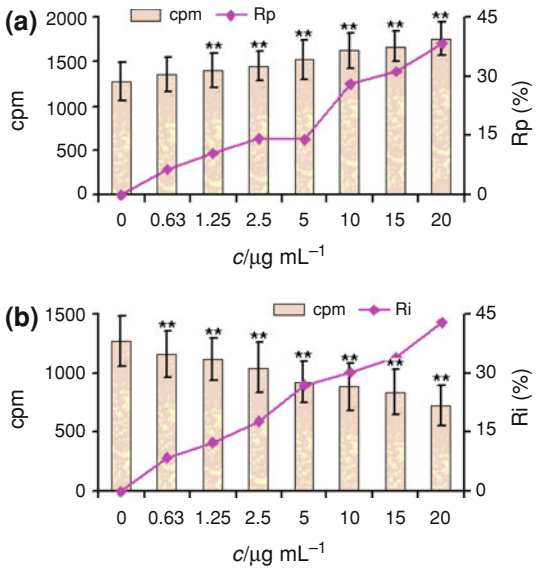

Fig. 6 Effect of **a** Rg_1 and **b** Rb_1 on [^{3}H]-TdR incorporation into splenic lymphocytes. Compared with normal cells, ** $P < 0.01$

splenic lymphocytes and these values were decreased with the increase of the concentration of Rb_1. The corresponding R_p (%) for Rg_1 and R_i (%) for Rb_1 were defined as:

$$R_p(\%) = (\text{cpm}_c - \text{cpm}_0)/\text{cpm}_0 \times 100\% \text{ and}$$
$$R_i(\%) = (\text{cpm}_0 - \text{cpm}_c)/\text{cpm}_0 \times 100\%$$

The values of R_p and R_i in Fig. 6 were both almost linearly increased with the increase of concentration of the two ginsenosides, also showing the promotion effect of Rg_1 and inhibitory effect of Rb_1 on splenic lymphocytes.

Structure–activity relationship

The biological activity of a particular substance depends on a complex sum of individual properties including compound structure, affinity for the target site, and survival in the medium of application. The ways in which Rg_1 and Rb_1 react with splenic lymphocytes vary due to the difference in their structures. Analysis of the two compounds may provide some explanation for the structure–activity relationships. Rg_1 and Rb_1 both belong to dammarane-type tetracyclic triterpenoid saponins, but with different sapogenins and substituent groups on the mother nucleus skeleton. Rg_1 has the 20(S)-protopanaxtriol type structure and Rb_1 has the 20(S)-protopanaxdiol type structure. The substitute groups of glucopyranoside and hydroxyl on C-3, C-6, and C-20 result in their different polarity. Rg_1 with less numbers of glucopyranoside and hydroxyl has poorer polarity and water-solubility than Rb_1, which results in the poorer affinity to lymphocytes of Rg_1 than that of Rb_1. It has been reported that Rg_1 could promote the expression of IL-2R and diminish the production and release of Sil-2R of cell membrane, also could improve the level of cyclic nucleotides of mice splenic lymphocytes, which might be the reason for the promotion effect of Rg_1 on splenic lymphocytes proliferation [20]. While Rb_1 with high affinity for the cells could more easily penetrate the cell membrane of splenic lymphocytes, which changes cell membrane permeability and inhibits Na^+ channel activity [21], further disrupts transport of nutrients and waste across the membrane. Therefore, the proliferation of cells is unable to occur and the growth of these cells is inhibited. The extensional mechanism of action of Rg_1 and Rb_1 on splenic lymphocytes needs further study. All these illustrated that Rb_1 with more glucopyranoside and hydroxyl groups has stronger inhibitory effect than Rg_1. It could be concluded that the glucopyranoside and hydroxyl groups at the dammarane-type mother nucleus skeleton of ginsenosides were important to the activities of the compounds on splenic lymphocytes.

Discussion

Bioassay study is important and necessary in the activity evaluation of drugs and other compounds and is useful for the development of bioactivity test systems [22–25]. New methods and approaches are in need for this assay and this test systems. In this article, we proposed a new microcalorimetric method to detect the bioactivity of ginsenosides Rg_1 and Rb_1 on mice splenic lymphocytes. Then, the commonly used MTT and [^{3}H]-TdR assay methods were used to confirm the results of microcalorimetric assay. Compared with the two conventional methods, microcalorimetry not only offers a new way for evaluating the bioactivity of drugs, but also provides more information on living cells growth. As an essential feature of microcalorimetry, it is based on the universal heat exchange involved in all biochemical reactions. Especially, it can supply thermogram as a profile detected to describe the bioactivity of drug. By using it, the energy changes of the growing periods of mice splenic lymphocytes, which represent the regularity of cells growth, can be distinguished by the heat production profile because the values of P_{max}, k, and Q_t can be determined simultaneously from the thermogenic profile of cells. Therefore, the metabolic process of cells can be described dynamically and precisely.

By analyzing the qualitative and quantitative information from the microcalorimetric assay, such as the power–time curves, P_{max}, k, and Q_t, it could be concluded that Rg_1 could promote the growth of mice splenic lymphocytes while Rb_1 inhibited their growth. These promotion and inhibitory effects were enhanced with increasing the concentration of the two ginsenosides. These consistent results were also obtained from the MTT and [^{3}H]-TdR assay: the

MTT OD values of splenic lymphocytes with Rg_1 and the corresponding R_p were increased, while they were decreased and the corresponding R_i were increased; the cpm values of [^{3}H]-TdR incorporation into splenic lymphocytes could be increased by Rg_1 while they were decreased by Rb_1. All these above-mentioned results showed that the two ginsenosides from Ginseng may modulate mice splenic lymphocytes proliferation in a different manner: Rg_1 could promote the proliferation of splenic lymphocytes and Rb_1 could block the proliferation of them. The opposing effects of Rg_1 and Rb_1 on mice splenic lymphocytes might be related with their molecular structures. The future work should be focused on study of the effects of more ginsenosides on splenic lymphocytes and other living systems to elucidate the concrete and detailed mechanism of action.

This study indicated the potential applicability and development prospect of microcalorimetry for determining the influence of drugs and other compounds on living cells. This method approximates more closely to the in vivo state and may reveal more and newer details about the metabolism than the existing methods, such as MTT assay and [^{3}H]-TdR assay, do. Through microcalorimetry, some thermodynamic and kinetic information of biological specimens can be obtained, and all of these are very important to understand biological progresses.

Acknowledgements This study was supported by Foundation of National Basic Research Program of China (No. 2007CB512607) and National Nature Science Foundation (No. 30772740).

References

1. Nah SY, Kim DH, Rhim H. Ginsenosides: are any of them candidates for drugs acting on the central nervous system? CNS Drug Rev. 2007;13:381–404.
2. Nah SY, Bhatia KS, Lyles J, Ellinwood EH, Lee TH. Effects of ginseng saponin on acute cocaine-induced alterations in evoked dopamine release and uptake in rat brain nucleus accumbens. Brain Res. 2009;1248:184–90.
3. Chen CF, Chiou WF, Zhang JT. Comparison of the pharmacological effects of *Panax ginseng* and *Panax quinquefolium*. Acta Pharmacol Sinica. 2008;29:1103–8.
4. Shibata S, Tanaka O, Shoji J, Saito H. Chemistry and pharmacology of *Panax*. In: Wagner H, Hikino H, Farnsworth NR, editors. Economic and medicinal plant research, vol. 1. London, Orlando, SanDiego, New York, Toronto, Montreal, Sydney, Tokyo: Academic Press; 1985. p. 217–87.
5. Sengupta S, Toh SA, Sellers LA, Skepper JN, Koolwijk P, Leung HW, Yeung HW, Wong RN, Sasisekharan R, Fan TP. Modulating angiogenesis: the yin and the yang in ginseng. Circulation. 2004;110:1219–25.
6. Panayi GS, Lanchbury JS, Kingsley GH. The importance of the T-cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arth Rheumatol. 1992;35:729–35.
7. Murphy KM. T lymphocyte differentiation in the periphery. Curr Opin Immunol. 1998;10:226–32.
8. Kong WJ, Wang JB, Jin C, Zhao YL, Dai CM, Xiao XH, Li ZL. Effect of emodin on *Candida albicans* growth investigated by microcalorimetry combined with chemometric analysis. Appl Microbiol Biotechnol. 2009;83:1183–90.
9. Kong WJ, Jin C, Xiao XH, Zhao YL, Li ZL, Zhang P, Liu W, Li XF. Comparative study of effects of two bile acid derivatives on *Staphylococcus aureus* by multiple analytical methods. J Hazard Mater. 2010;179:742–7.
10. Wadsö I. Characterization of microbial activity in soil by use of isothermal microcalorimetry. J Therm Anal Calorim. 2009;95:843–50.
11. Afzal AB, Akhtar MJ, Svensson LG. Thermal studies of DBSA-doped polyaniline/PVC blends by isothermal microcalorimetry. J Therm Anal Calorim. 2010;100:1017–25.
12. Zhao H, Bennici S, Shen J, Auroux A. Calorimetric study of the acidic character of V_2O_5–TiO_2/SO_4^{2-} catalysts used in methanol oxidation to dimethoxymethane. J Therm Anal Calorim. 2010;99:843–7.
13. Zhao YL, Yan D, Wang JB, Zhang P, Xiao XH. Anti-fungal effect of berberine on *Candida albicans* by microcalorimetry with correspondence analysis. J Therm Anal Calorim. doi:10.1007/s10973-009-0565-7.
14. Kong WJ, Li ZL, Xiao XH, Zhao YL, Zhang P. Activity of berberine on *Shigella dysenteriae* investigated by microcalorimetry and multivariate analysis. J Therm Anal Calorim. doi:10.1007/s10973-010-0778-9.
15. Yang LN, Sun LX, Xu F, Zhang J, Zhao JN, Zhao ZB, Song CG, Wu RH, Ozao R. Inhibitory study of two cephalosporins on *E. coli* by microcalorimetry. J Therm Anal Calorim. 2010;100:589–92.
16. Li X, Zhang T, Min XM, Liu P. Toxicity of aromatic compounds to *Tetrahymena* estimated by microcalorimetry and QSAR. Aquat Toxicol. 2010;98:322–7.
17. Kong WJ, Zhao YL, Xiao XH, Li ZL, Jin C, Li HB. Investigation of the anti-fungal activity of coptisine on *Candida albicans* growth by microcalorimetry combined with principal component analysis. J Appl Microbiol. 2009;107:1072–80.
18. Zhou PJ, Zhou HT, Yi P, Liu Y, Wu ZB, Qu SS, Zhu YG. Microcalorimetric studies on the thermogenesis of energy release of mitochondria isolated from rice. Microchem J. 2001;69:103–9.
19. Tan AM, Xu B, Huang SQ, Qu SS. Thermochemical study on the growth metabolism of human promyelocytic leukemia HL-60 cells inhibited by water-soluble metalloporphyrins. Thermochim Acta. 1999;333:99–102.
20. Liu M, Zhang JT. Studies on the mechanisms of immunoregulatory effects of ginsenoside Rg_1 in aged rats. Acta Pharm Sin. 1996;31:95–100.
21. Liu D, Li B, Liu Y, Attele AS, Kyle JW, Yuan CS. Voltage-dependent inhibition of brain Na(+) channels by American ginseng. Eur J Pharmacol. 2001;413:47–54.
22. Zhang GJ. Bioassay on traditional Chinese medicine. Beijing: China Chemical Industry Press; 2006.
23. Xiao XH, Jin C, Zhao ZZ, Xiao PG, Wang YY. Probe into innovation and development of pattern of quality control and evaluation for Chinese medicine. China J Chin Mater Med. 2007;32:1377–81.
24. Xiao XH, Yan D, Wang JB, Jin C. Some opinions on bioassay for the quality control of Chinese medicines. World Sci Tech/Mod Tradit Chin Med Mater Medica. 2009;11:504–8.
25. Braissant O, Wirz D, Göpfert B, Daniels AU. Use of isothermal microcalorimetry to monitor microbial activities. FEMS Microbiol Lett. 2010;33:1–8.