Study on Hot Property of *Aconiti Lateralis Radix Praeparata* and Its Compatibility with *Zingiberis Rhizoma* Based on Animal Temperature Tropism

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Abstract: **Objective** To establish an objective method for evaluating the intrinsic characteristics between hot and cold properties of herbal drugs and to study the hot property of *Aconiti Lateralis Radix Praeparata* (ALRP) and its compatibility with *Zingiberis Rhizoma* (ZR) on animal temperature tropism. **Methods** The equipment with cold/hot pads was used to investigate the variety of temperature tropism among mice treated by ALRP and its compatibility with ZR. Meanwhile, the activities of adenosine triphosphatase (ATPase), total anti-oxidant capability (T-AOC), and total superoxide dismutase (T-SOD) were measured. **Results** Compared with the homologous ALRP group, the compatibility between ALRP and ZR (AZ) had a stronger action against the decreasing of remaining rate on hot pad ($P < 0.05$), suggesting an enhancement of cold tropism. Meanwhile, the internal indexes of Na^+-K^+-ATPase activity, Ca^{2+}-Mg^{2+}-ATPase activity, and oxygen consuming volume increased significantly ($P < 0.05$), suggesting an enhancement of energy metabolism. The changes of T-AOC and T-SOD activity suggested that AZ reinforced the anti-oxidative capability of mice. In addition, it could be inferred from cluster analysis that the activity of AZ was concretely stronger than that of homologous ALRP. **Conclusion** ALRP and ZR are both herbs with hot property, but AZ has an enhanced trend of hot property. This external behavior of cold tropism and the internal activity of energy metabolism and anti-oxidant might reflect the internal hot property in an intuitive and objective way. The changes of ATPase activity of liver tissue might be the mechanism of drug action.

Key words: *Aconiti Lateralis Radix Praeparata*; animal temperature tropism; clustering analysis; compatibility; *Zingiberis Rhizoma*
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Introduction

Drug nature of Chinese materia medica (CMM), the core of theory in traditional Chinese medicines (TCM), is not only the link between CMM and drugs, but also the basis of syndrome differentiation and treatment. The cold (Han) and hot (Re) natures are known as the main drug natures of CMM, which could describe the therapeutic effects and energetic characteristics of herbs and their actions. However, the so-called cold and hot natures of CMM are so abstract that it is rarely to find a scientific way to characterize them (Cheng *et al*, 2010; Xiao, 2008). Herbs, like *Coptidis Rhizoma* (Huanglian) and *Gypsum Fibrosum* (Shigao), which could relieve heat syndromes are characterized as cool or cold, while herbs, such as *Ginseng Radix* (Renshen) and *Aconiti Lateralis Radix Praeparata* (ALRP, Fuzi), which could relieve cold syndromes, are characterized as warm or hot. If the difference between cold and hot properties objectively exist has been the difficult and crucial issue in theoretical research of CMM, and there is no reliable experimental method until now.

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Both ALRP and *Zingiberis Rhizoma* (ZR, Ganjiang) are commonly-used herbs with hot properties in the basic theory of TCM, while there was an outlook of ALRP exerting less hot property without ZR in *Zhengzhi Yaojue* (Principles of Diagnosis and Treatments). Unprocessed ALRP have a strong toxicity, so the ancients attached great importance to the processing methods of ALRP with the aim of efficacy enhancing and toxicity reducing (Yang, Shen, and Zhang, 2000; Zhao, Hou, and Zhao, 2011). Now there are a lot of processed products of ALRP, among which the major categories are Yanfupian (Y), Heifupian (H), Baifupian (B), Danfupian (D), and Paofupian (P). Previous studies on the regulation of the combination between ALRP and ZR concentrated on chemical analysis and pharmacodynamics (Yue *et al*, 2007; Huang *et al*, 2010; Zheng *et al*, 2010). Here, we apply cold-hot pad differentiating assay and intelligent monitoring system for animal thermotropism behaviors, to investigate the enhancing action of the combination between ALRP and ZR and the proper effect of ZR in the combination.

Materials and methods

Materials

Aconiti Lateralis Radix Preparata (Sichuan, China) and *Zingiberis Rhizoma* (Sichuan, China) were purchased from Chengdu Herbal Medicine Market in Sichuan, and the crude materials were identified by Prof. XIAO Xiao-he, a taxonomist in 302 Hospital of PLA. The herbs were grinded and decocted twice with water for 1.5 h each. The resulting extracts were decanted, filtered, and evaporated to dryness under reduced pressure. This dried extract was redissolved and dispersed in warm water and then cooled for ig administration.

Main constituents in ALRP were determined by HPLC with spectrophometric detector. The procedures were as follows: ALRP (0.2 g) was extracted with water (25 mL). The solution was filtrated and then submitted for HPLC analysis. The HPLC was performed on the HPLC Pump (Agilent 1200, USA) using an Agilent Zorbax SB-C_{18} columm (250 mm × 4.6 mm, 5 μm), eluting with A (acetonitrile- tetrahydrofuran 25:15) and B (0.1 mol/L ammonium acetate-water). The flow rate was 1.0 mL/min with detection wavelength at 235 nm. The results were shown in Table 1.

Table 1 Determination of monoester alkaloids in ALRP

Samples	Benzoyl-mesaconine / %	Benzoyl-aconine / %	Benzoyl-hypaconine / %
Y	0.0422	0.0098	0.0269
H	0.1384	0.0207	0.0345
P	0.2327	0.0303	0.0323
ALRP	0.2577	0.0275	0.0274
B	0.1548	0.0185	0.1031
D	0.0565	0.0133	0.0407

Animals

Male KM mice (16—18 g) were purchased from Laboratory Animal Center, Academy of Military Medical Sciences, with license SCKX-(Army)-2007004. Conditions in animal laboratory were controlled at (25 ± 2) ℃, and humidity at 60%—80%, with periodical air changing, and of dark to light period at about 12 h-12 h with artificial lighting. Experiments were performed after gaining formal approval by Institutional Ethical Committee for Research on Animals.

Instruments and reagents

Intelligent Monitoring System for Animal Thermotropism Behaviors was designed by our research team and assembled by Beijng Zhongjiao Instrument Company (Chinese Patent No: ZL20082000 04444.2). The system consists of three major parts, namely, automatic temperature controlling unit, remote monitoring unit, and data processing unit. Before experiment, two stable temperature zones were generated by a temperature-controlling plate (cold/hot pad), then animals were put on it of free motion across the two temperature zones. Parameters for evaluating temperature tropism of animals, such as remaining rate (RR) on hot pad and the distance of movement, could be obtained through a real-time computer processing of artificial intelligence to analyze the video determining the coordinates (X, Y) of each animal in every frame of image, and the trajectories of animals were obtained by joining the coordinates of each animal in all frames $Y_t = f[X_t]$, followed by computer processing of the duration and frequency of each animal in different zones. Video recognition software was based on Paulo's algorithms (Paulo, Luís, and Vasco, 2007), and data analysis software was written using Visual Basic 6.0 languages.

Synergy H_1 Hybrid Microplate Reader (BioTek, USA), SIGMA 3-18K Centrifuger (Germany), ATPase Assay Kit (20110713), Total Superoxide Dismutase (T-SOD) Assay Kit (20110707), Colorimetric Blue

Protein Assay Kit (20110711) and Total Anti-oxidant Capability (T-AOC) Kit (20110715) were purchased from Nanjing Jiancheng Biological Engineering Research Institute. All the reagents were of analytical grade.

Grouping and administration

Before the experiment, the mice were trained to be familiar with the temperature of hot/cold pad and the mice with abnormal rectal temperature were removed from the experiment. The qualified mice with normal body temperature were randomly divided into fourteen groups, namely the control (C), ALRP (A, 10 g/kg, which were equivalent to human dose of 1.1 g/kg), Y (10 g/kg), H (10 g/kg), B (10 g/kg), D (10 g/kg), P (10 g/kg), ZR (Z, 10 g/kg), the compatibility of ALRP and ZR (AZ, 10 g/kg), the compatibility of Y and ZR (YZ, 10 g/kg), the compatibility of H and ZR (HZ, 10 g/kg), the compatibility of B and ZR (BZ, 10 g/kg), the compatibility of D and ZR (DZ, 10 g/kg), and the compatibility of P and ZR (PZ, 10 g/kg) groups. The mice were ig administered with 20 mL/kg. The mice in control group were administered with physiological saline of the same volume for 7 d.

Oxygen consumption

Mice were put in an airtight container with 10 g sodium oxide at the bottom to absorb CO_2 generated by mice and a rubber tube with a graduated pipette was connected at the top. The other tip of graduated pipette was inserted into water vertically. All interfaces were sealed with vaseline. The air consuming volume for 6 min was measured.

Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in liver tissue

Mice were killed by cervical dislocation then immediately dissected to get livers. The liver tissues were put into physiological saline, precooled at 4 ℃ to remove the blood on the tissue surface, and wiped up by filter paper. Then the liver tissues were prepared in 2% tissue homogenate to measure the ATPase activity, counted as 1 μmol inorganic phosphorus produced by ATPase in 1 mg tissue protein within 1 h ($\mu mol \cdot mg^{-1} \cdot h^{-1}$).

Temperature tropism of mice on cold-hot pad

The experiment was performed on a bi-zone temperature-controlling plate: 23 ℃ (cold pad) and 37 ℃ (warm pad), respectively, with laboratory temperature of (25 ± 2) ℃. Ten minutes after drug administration, mice were put into the monitoring channel of the cold-hot plate, their temperature tropism behaviors were remotely monitored, and the trajectories of each mouse were recorded, respectively. RR on hot pad = remaining time on hot pad (s) / the total monitoring time (s)

T-AOC activity

T-AOC activity of 0.1 mL serum of mice was measured according to the introduction of assay kit, counted as the absorbance increased by 0.01 unit of the reaction system at 37 ℃ in 1 mL blood serum per minute.

T-SOD activity

Xanthine oxidase method was taken to measure the SOD activity of 10 μL blood serum. One unit of SOD was counted as the corresponding quantity of SOD when 50% inhibitory ratio of SOD was achieved in 1 mL reaction solution.

Statistical analysis

Data were expressed as $\bar{x} \pm s$. Statistical analysis of RR in the cold pad, ATPase activity, T-AOC activity, and T-SOD activity was performed using ANOVA and *t*-test with SAS 9.0. $P < 0.05$ was considered to be significant.

Cluster analysis

Cluster analysis (CA) is a method of unsupervised learning and a common technique for multivariate analysis, the latter used to sort similar samples into different groups. An unsupervised classification procedure, involving the similarity between the objects to be clustered, was processed using this technique. The similarities or dissimilarities between samples are usually represented in a dendrogram for ease of interpretation (Kong *et al*, 2009). The inter-groups linkage method as the amalgamation rule was used to establish clusters (Kannel *et al*, 2007).

Results

General condition of mice

During the experiment, the skin color, diet, stool and urine, as well as general behavior of mice were monitored and no abnormal phenomenon was found in administration groups. The body weight of mice in administration groups had a trend of an enhancement compared with those in the control group. The water

intake of mice in each group had a trend of an enhancement compared with those in the control group.

Changes in oxygen consumption of mice

The oxygen consumption of mice in 6 min was chosen as the evaluated index. Fig. 1 showed that the oxygen consumption of the administration groups was found to be significantly increased except the P group ($P_P = 0.499$) compared with the control group, while the oxygen consumption of the compatibility groups was found to be significantly increased except the YZ group ($P_{YZ} = 0.166$) compared with ALRP group.

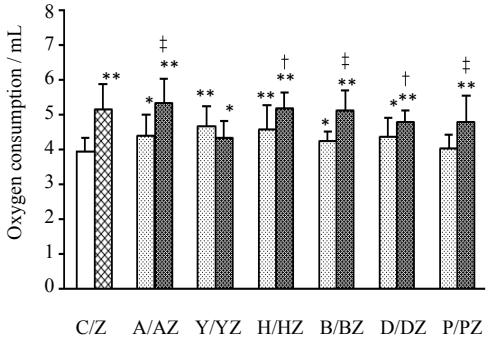

Fig. 1 Oxygen consumption of mice treated with ALRP, ZR, and their compatibility ($\bar{x} \pm s$, $n = 6$)

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* control group; $^{\dagger}P < 0.05$ $^{\ddagger}P < 0.01$ *vs* homologous ALRP group; same as below

Mice were ig administered with 10 g/kg ALRP, ZR, and their compatibility for 7 d. Half an hour after the last administration, mice were put into the device to determine the oxygen consumption.

ATPase activities in liver tissue of mice

Compared with the control group, the Na^+-K^+-ATPase activity of A group was found to be decreased significantly, while it was increased in Y, D, P, YZ, HZ, BZ, DZ, and PZ groups. Compared with the homologous ALRP group, the Na^+-K^+-ATPase activity of AZ, BZ, HZ, and DZ groups was increased significantly (Fig. 2A). And the change of Ca^{2+}-Mg^{2+}-ATPase activity was basically accordant with Na^+-K^+-ATPase activity (Fig. 2B). AZ signifycantly increased the ATPase activities of liver tissue in mice, suggesting an enhancement of energy metabolism.

Mice were ig administered with 10 g/kg ALRP, ZR, and their compatibility for 7 d. After the last administration, mice were killed to get livers and then the liver tissues were homogenized to measure the ATPase activity.

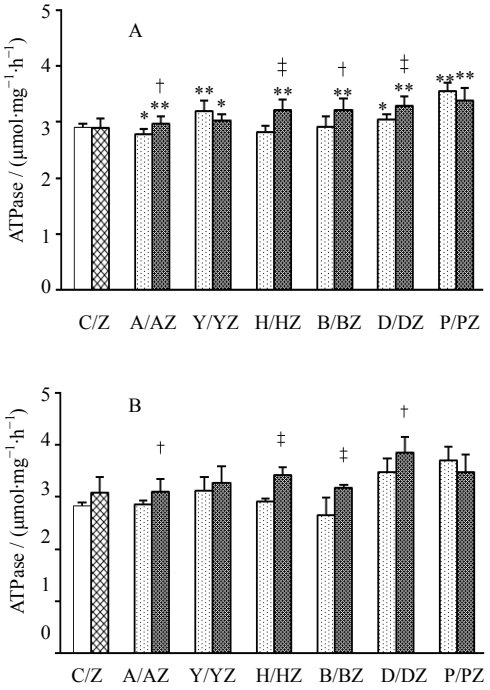

Fig. 2 Na^+-K^+-ATPase activity (A) and Ca^{2+}-Mg^{2+}-ATPase activity (B) of mice treated with ALRP, ZR, and their compatibility ($\bar{x} \pm s$, $n = 6$)

Temperature tropism of mice on cold/hot pad

Compared with the control group, the RR of A, B, D, and P groups on hot pad was not found to be significantly increased ($P > 0.05$), while it was decreased in other groups; Compared with the homologous ALRP group, the compatibility groups had a stronger action against the decreasing of RR on hot pad except the YZ group ($P_{YZ} = 0.219$) (Table 2). To summarize the above results, the compatibility groups enhanced RR on cold pad, suggesting an enhancement of cold tropism and hot production.

Effects of T-AOC and T-SOD activities after drug administration

Compared with the control group, T-AOC activity of Y, H, D, and FZ groups were significantly increased, while it was not significant in A, B, P, and Z groups. Compared with the homologous ALRP group, T-AOC activity of the compatibility group was significantly increased. And the T-SOD activity after drug administration was basically accordant with T-AOC activity (Table 2). The changes of T-AOC and T-SOD activity suggest that the compatibility with ALRP and ZR reinforced the anti-oxidant capability of mice.

Results of CA

In order to assess the tendency of ALRP and the

compatibility, a hierarchical CA of samples was performed. This procedure founded natural groupings of the data set. The results of CA were shown in Fig. 3. It was clear that the samples could be divided into three clusters; Cluster I was formed by samples C, A, B, H and D. Cluster II consisted of the samples Y, Z, AZ, YZ, HZ, PZ, and BZ; Cluster III consisted of samples P and DZ.

Table 2 Comparison on RR on hot pad, T-AOC, and T-SOD activities ($\bar{x} \pm s$, n = 6)

Groups	RR / %	T-AOC / ($U \cdot mL^{-1}$)	T-SOD / ($U \cdot mL^{-1}$)
C	50.2 ± 16.6	7.8 ± 0.35	182.6 ± 8.58
A	52.1 ± 14.6	7.6 ± 0.38	233.9 ± 15.3**
Y	35.6 ± 15.4**	8.7 ± 0.33**	225.8 ± 17.8**
H	41.4 ± 20.0*	8.3 ± 0.25*	304.2 ± 15.4**
B	48.8 ± 14.5	8.4 ± 0.98	228.4 ± 12.3**
D	46.7 ± 12.9	10.9 ± 0.44**	308.0 ± 21.0**
P	49.1 ± 24.4	7.7 ± 0.79	313.9 ± 11.0**
Z	31.9 ± 15.4**	8.0 ± 0.26	254.0 ± 20.2**
AZ	31.3 ± 14.7**‡	8.9 ± 0.33**‡	256.0 ± 7.53**‡
YZ	40.1 ± 17.8**	13.7 ± 0.68**‡	265.4 ± 12.8**‡
HZ	28.0 ± 13.8**‡	12.2 ± 0.79**‡	241.5 ± 10.1**‡
BZ	41.7 ± 17.2*†	13.0 ± 0.31**‡	245.7 ± 7.63**†
DZ	39.1 ± 16.2**†	11.6 ± 0.17**‡	333.3 ± 15.6**†
PZ	30.8 ± 14.1**‡	12.5 ± 0.43**‡	225.4 ± 15.1**‡

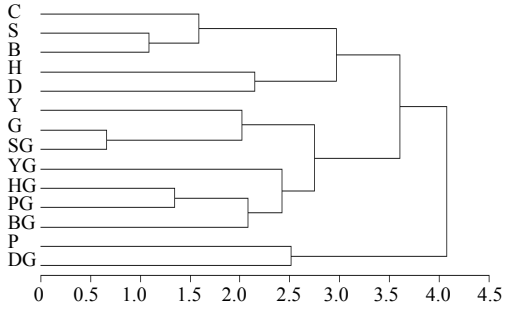

Fig. 3 Cluster analysis

Fig. 3 showed that the A group and the compatibility had a significant difference. And the compatibility had an important effect on this cluster, for example, AZ and A, HZ and H in cluster II had long rescaled distance. Moreover, the different processed ALRP had large influence on this cluster, for example, between A, B and H, D in cluster I had long rescaled distance, while there were long rescaled distance between AZ and YZ, HZ, PZ, BZ. It could also be seen from Fig. 3 that the rescaled distance between clusters I and II was longer than the rescaled distance between cluster I — cluster II and cluster III, which could demonstrate that compared with cluster III, the tendency of cluster I was much similar to cluster II.

Mice were ig administered with ALRP, ZR, and AZ (10 g/kg respectively) for 7 d. Ten minutes after the last administration, the mice were put into Intelligent Monitoring System for Animal Thermotropism Behavior to obtain the result of RR on hot pad and the eyeball was taken for the blood to get the result of T-AOC and T-SOD activities.

Dendrogram of clustering of oxygen consumption, Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities, RR on cold pad, T-AOC and T-SOD activities were performed using SAS 9.0. In addition, it could be inferred from CA that the activity of the compatibility was concretely stronger than that of ALRP.

Discussion

At an appropriate temperature gradient, movable animals were inclined to live in a narrow but the most appropriate temperature range (Liu, Wang, and Sun, 2004; 2005), which was optimum for their metabolism (Zhang *et al*, 2006). This tendency behavior of animals was called thermotropism, which would be influenced by energy state of the body. Under the requested condition of the Intelligent Monitoring System for Animal Thermotropism Behavior (Zhao *et al*, 2010), we could reveal the trend of energy change in animal body by observing the compensatory behavior of automatic selecting, to observe the difference between cold and hot natures of drugs. In this research, the conspicuous decreasing of RR was found that on hot pad after the compatibility groups were ig administered compared with the control group and the homologous ALRP group by cold/hot pad differentiating assay. The result suggested an enhancement of cold tropism to compensate a hot sensation after treated by the compatibility groups, and the method distinguished the minor difference among different processed ALRP. This essay is able to objectively and intuitionally validate the phenomenon of ALRP exerting less hot property without ZR in this experiment. The conclusion from this study was consistent with the theory of TCM.

Besides the behavioral investigation, the oxygen consumption and the ATPase activities of mice were detected to search for possible related indicators

between thermotropism and energy metabolism. In the state of physiology, organic chills and fever reflect the relation of balance between the generation and the utilization of energy. Related studies had reported that energy transfer may course the change of ATPase activity (Matthias, Simon, and Georg, 1997). Cool drugs, just like *Coptidis Rhizoma* (Huang *et al*, 2001) and *Menthae Oleum* (Liu *et al*, 2011), could decrease the ATPase activity, while warm drugs, such as ALRP (Zhang *et al*, 2011) and *Epimedii Herba* (Wang, Fu, and Liu, 2002), would increase the ATPase activity. In this research, the oxygen consumption and ATPase activity were increased after the compatibility groups were ig administered compared with the control group and the homologous ALRP group. It accorded with the increasing effect of exerting hot. The changes of ATPase activity of liver tissue might be the mechanism of drug action.

In the research, we explored not only the increasing of oxygen consumption and ATPase activity because of the hot nature of drug action, but also the significant raise of T-AOC and T-SOD activities. The *D*-galactose exposure also induced an increase in peripheral oxidative stress, including an increase in malondialdehyde (MDA) and decreases in T-AOC, T-SOD, and glutathione peroxidase (GSH-Px) activities (Cui *et al*, 2006). The two indexes increased further after treatment of the compatibility groups. Hence, the results suggested that the hot nature of the compatibility and the homologous processed ALRP could activate the anti-oxidative capability of animals, which might be response to an enhancement of ability to resist external environment.

To sum up, as the researching object to thermo-dynamics and energy metabolism, we investigated the objectivity of ALRP exerting less hot property without ZR based on cold/hot pad differentiating assay. The assay, as the macroscopic object to RR on hot pad, may characterize objectively and quantitatively the object-tivity and authenticity of the phenomenon integrated with the internal indexes of energy metabolism and anti-oxidative capability. In addition, CA of ALRP and the compatibility groups strengthened indirectly the standpoint of ALRP exerting less hot property without ZR and ALRP processed had an effect to this phenomenon.

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