Different Effects of *Mahuang* Decoction and *Maxing Shigan* Decoction on Animal Temperature Tropism and Correlation to Differences of Cold and Hot Nature of Chinese Materia Medica

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Abstract: **Objective** To establish an objective method for evaluating the intrinsic characteristics between cold and hot nature of Chinese materia medica (CMM) through the different effects of *Mahuang* decoction (MHD) and *Maxing Shigan* decoction (MSD) on animal temperature tropism. **Methods** The equipment with cold/hot pads was used to investigate the variety of the temperature tropism between two groups of mice treated by MHD and MSD, respectively. Meanwhile, the activities of adenosine triphosphatase (ATPase), superoxide dismutase, succinate dehydrogenase, and malondialdehyde were measured. **Results** After treated by MHD, the macroscopic behavioral index of remaining rate on warm pad (40 ℃) of mice decreased significantly ($P < 0.05$), suggesting the enhancement of cold tropism, meanwhile, the internal indices of ATPase activity and oxygen consuming volume increased significantly ($P < 0.05$), suggesting the enhancement of energy metabolism. On the other hand, the above-mentioned indices in MSD group changed on the inverse way. **Conclusion** The relative drug nature of MHD and MSD revealed in this study is consistent with the theoretical prognostication or definition. It indicates that the internal cold and hot nature of CMM could be reflected in ethological way on the changes of animal temperature tropism which might be internally regulated by body energy metabolism.

Key words: cold/hot nature of Chinese materia medica; *Mahuang* decoction; *Maxing Shigan* decoction; temperature tropism
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Introduction

Mahuang decoction (MHD) and *Maxing Shigan* decoction (MSD), initially recorded in *Shanghanlun* by ZHANG Zhong-jing in Han Dynasty, are two of the most important exterior-releasing (*jiebiao*) prescriptions in traditional chinese medicine (TCM) (Deng, 2003). These two prescriptions are typically semblable in formula composition but of definite difference in drug nature: MHD is composed of *Ephedrae Herba*, *Cinnamomi Ramulus*, *Armeniacae Semen Amarum*, and *Glycyrrhizae Radix* et *Rhizoma* and is considered of hot nature, while MSD cancels *Cinnamomi Ramulus* alternating with *Gypsum Fibrosum* and is considered of cold nature. This raises a question why the drug natures of the two prescriptions are considered completely different due to the change of only one TCM. Is this alternation of drug nature true? How to demonstrate it intuitively, if the alternation is an objective existence?

The former investigations paid more attention to the differences of pharmacological effects (Zhang and Liu, 2007) and the changes of contents in MHD and MSD (He and Luo, 2005; Ma, Wang, and Cao, 2005; Zhou *et al*, 2007). There was a report on investigating the difference between MHD and MSD in view of cold and hot nature which had revealed the objective difference between MHD and MSD at microbial metabolism level (Fan *et al*, 2007), but it was not directly correlated to the drug nature of TCM. Hence, we attempt to investigate the different effects of MHD and MSD on animal temperature tropism and the correlation of the

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nature between the two prescriptions in the animal's macroscopical ethology (Xiao, 2008).

Materials and methods

Experimental drugs

Ephedrae Herba, *Cinnamomi Ramulus*, *Armeniacae Semen Amarum*, *Glycyrrhizae Radix* et *Rhizoma*, and *Gypsum Fibrosum* were bought from Tongrentang Drug Store in Beijing. All were identified by Prof. XIAO Xiao-he.

Medicine preparation: Two prescriptions were soaked for 30 min and then decocted for three times with 10 times of the water volume firstly, 8 times in latter two times, respectively. The decoction was quickly heated to boiling and kept in simmer for 20 min in the first time, and for 15 min in latter two times. The decoction was filtered before cooling and freeze-dried into powder. The samples were preserved at 4 ℃ for latter experiments. Just before experiment, MHD extract and MSD extract were re-dissolved in water respectively with the concentration 2 and 4 g/mL (raw materials to water).

Experimental animals and feedstuff

Male KM mice (18–22) g at cleaning level were purchased from the Laboratory Animal Center of Academy of Military Medical Sciences, with license: SCKX-(army) 2007004.

Basic feed composition: crude protein 19.2%, crude fat 4.1%, crude fiber 2.5%, moisture 9.5%, crude ash 6.6%, total calcium 1.2%, and phosphor 0.9%.

High-protein feed composition: crude protein 24.9%, crude fat 4.2%, crude fiber 2.3%, moisture 8.4%, crude ash 6.6%, total calcium 1.2%, and phosphor 0.9%.

Instruments and reagents

The intelligence monitoring system for detecting animals' behavior was designed by 302 Military Hospital and assembled by Beijing Zhongjiao Instrument Company (Chinese Patent No: ZL2008200004444.2). The principle is that a number of different temperature regions are generated by the automatic temperature control system, which has six channels divided by opaque hard plastic pad (Zhang *et al*, 2009; Zhou *et al*, 2009; Zhao *et al*, 2009; Ren *et al*, 2009). The Mice, which are placed in the channel, are free to stay in any temperature region and perform independently. The video detection system on the control panel is used to track the activities of mice. The residence time, activity frequency, distance, and movement of mice in each temperature region are recorded and converted into quantifiable data. Video recognition software was based on Paulo's algorithms (Aguiar, Mendonça, and Galhardo, 2007), and data analysis soft-ware was written using Visual Basic 6.0 language.

Cary50 Bio UV spectrophotometer (Varian Australia PTY LTD), ATPase test kit (20081113), Coomassie brilliant blue protein test kit (20081113), superoxide dismutase (SOD) test kit (20081113), succinate dehydrogenase (SDH) test kit (20081113), and malondialdehyde (MDA) test kit were brought from the Nanjing Institute of Biological Engineering. Other reagents were of analytical level.

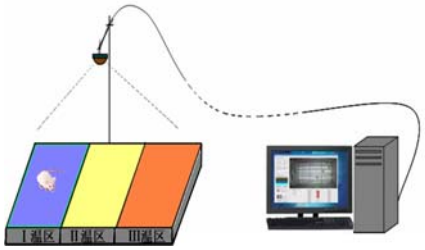

Fig. 1 Intelligent animal temperature tropism behavior monitoring system

Grouping and administration

Before 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 ones with normal body temperature were randomly divided into seven groups: Normal group (NM), weak model group (WM), strong model group (SM), and four medication groups (WM + MHD, WM + MSD, SM + MHD, SM + MSD) treated by MHD and MSD, respectively. MHD and MSD were ig administered once a day for 7 d with the doses of 40.8 and 76.8 g/kg, at which the cold and hot nature difference between MHD and MSD could be exhibited well according to previous experiment. The normal and model groups were administered with physiological saline of the same volume for 7 d.

Establishment of the weak model and strong model

The weak model was established by forcing the mice to swim in water [temperature (20 ± 5) ℃, depth 20 cm] until the mice were exhausted, which were fed with limited feed-intake and given 0.1 g/g of basic feed. The strong model was established by feeding high-protein diet with unlimited access to feed. The weak

model mice were forced to swim one hour before the experiment.

Determination of the temperature tropism of mice on cold/hot pad

The experiment was performed on a bi-zone temperature-controlling pad: 25 ℃ (cool pad) and 40 ℃ (warm pad), respectively, with laboratory temperature of (20 ± 2) ℃. One hour after drug administration, mice were put into the monitoring channel of the cold/hot pad and their temperature tropism behaviors were remotely monitored and the trajectories of each mouse were recorded respectively. Remaining rate (RR) on warm pad = remaining time on warm pad / the total monitoring time × 100%.

Determination of 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 time of breathing for 2.5 mL of air and the air-volume of consumption for 6 min were measured.

Determination of the activities of ATPase, SOD, SDH, and MDA of liver tissue

Mice were killed by cervical dislocation, and then dissected to get livers immediately which were put into physiological saline pre-cooled at 4 ℃ to remove the blood on the tissue surface and wiped up by filter paper. Then the liver tissues were weighted accurately and prepared into tissue homogenate according to the manual in test kit.

Statistical analysis

Data were expressed as $\bar{X} \pm s$, and analyzed using the SPSS statistical software package, version 15.0 (SPSS, Inc., USA). A value $P < 0.05$ was considered statistically significant.

Results

General conditions of animals

The general condition in model groups was similar to pre-experiment findings (Zhang *et al*, 2009; Zhou *et al*, 2009; Zhao *et al*, 2009): The typical features of cold symptom were observed in the WM group, such as fatigue, stagnant weight growth, decreased water intake and swimming time, cold limbs and tail. And the typical features of heat symptom were observed in SM group, such as increased body weight and water intake, hyperactivity. The body weight of model groups intervened by two prescriptions had no significant changes compared with NM group.

Changes about temperature tropism of mice

The temperature tropism of mice from model groups was coincident with preliminary studies, and the remaining time ratio of the WM group on 40 ℃ pad within 7 d was significantly higher ($P < 0.05$) than the NM group, while the remaining time ratio of SM group was significantly lower ($P < 0.05$). The remaining time ratio of the mice on 40 ℃ pad has showed some differences after treated by MHD and MSD, which were summarized as follows:

1. Compared with the WM group, the remaining time ratio on warm pad (40 ℃) of mice treated by MHD was decreased obviously ($P < 0.05$), while the mice treated by MSD was increased;

2. Compared with the SM group, the remaining time ratio on warm pad (40 ℃) of mice were all decreased significantly ($P < 0.05$) after treated by MHD and MSD, but the SM + MHD group decreased more significantly than the SM + MSD group (Table 1).

Table 1 RR on warm pad (40 ℃) and oxygen consumption ($\bar{X} \pm s$, $n = 6$)

Group	Remaining time ratio (40 ℃) pad / %	Time to consume 2.5 mL oxygen / min	Oxygen consumption within 6 min / mL
NM	58.2 ± 6.1	4.4 ± 0.8	3.7 ± 0.9
WM	68.2 ± 3.2[a]	8.0 ± 1.5[a]	1.7 ± 0.7[a]
SM	53.3 ± 11.9[a]	4.1 ± 0.5[a]	4.1 ± 0.4[a]
WM + MHD	54.9 ± 8.6[b]	6.8 ± 1.4[ab]	2.0 ± 0.8[ab]
WM + MSD	68.7 ± 7.4[a]	9.0 ± 1.3[a]	1.7 ± 0.2[a]
SM + MHD	48.1 ± 11.0[ac]	4.4 ± 0.5	3.7 ± 0.4[c]
SM + MSD	48.9 ± 8.2[a]	5.2 ± 0.2[ac]	3.2 ± 0.5[ac]

[a]$P < 0.05$ *vs* NM; [b]$P < 0.05$ *vs* WM; [c]$P < 0.05$ *vs* SM

Changes in oxygen consumption of mice

Compared with the NM group, the time required for mice to consume 2.5 mL oxygen of WM group was found to be increased significantly ($P < 0.05$), while it decreased in SM group ($P < 0.05$). Compared with WM group, it was decreased significantly ($P < 0.05$) after treated by MHD, while increased after treated by MSD; Compared with the SM, both the SM + MHD and SM + MSD group were increased. The difference between them was that the SM + MSD group increased more

significantly ($P < 0.05$) than the SM + MHD (Table 1).

The trend concerning the volume of oxygen consumption within 6 min was opposite to the time required for mice to consume 2.5 mL oxygen, which revealed a similar result about oxygen consumption, but, relatively speaking, the former was more sensitive (Table 1).

Changes of Na^+K^+-ATPase, Mg^{2+}-ATPase, Ca^{2+}-ATPase activities in liver tissue of mice

The activities of Na^+K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase were measured, respectively, and it was found that the change trend of ATPase activities was conformable basically. Compared with the NM group, the ATPase activity of the WM group decreased significantly ($P < 0.05$), while the SM group increased significantly ($P < 0.05$) except the Na^+K^+-ATPase. Compared with the models, the activities of ATPase increased significantly after treatment by MHD and MSD, and it increased more significantly after treatment by MHD (Table 2).

Table 2 Changes of ATPase activities in liver tissue of mice ($\bar{X} \pm s$, $n = 6$)

Group	Na^+K^+-ATPase / ($\mu mol \cdot mg^{-1} \cdot h^{-1}$)	Mg^{2+}-ATPase / ($\mu mol \cdot mg^{-1} \cdot h^{-1}$)	Ca^{2+}-ATPase / ($\mu mol \cdot mg^{-1} \cdot h^{-1}$)
NM	1.67 ± 0.59	0.90 ± 0.66	1.35 ± 0.78
WM	1.28 ± 0.43[a]	0.72 ± 0.14[a]	1.05 ± 0.74[a]
SM	1.40 ± 0.31	0.97 ± 0.25	2.04 ± 1.12[a]
WM + MHD	1.96 ± 1.63[ab]	2.14 ± 0.56[ab]	2.51 ± 0.59[ab]
WM + MSD	1.82 ± 1.21[b]	1.44 ± 1.14[ab]	1.75 ± 1.34[a]
SM + MHD	3.26 ± 1.41[ac]	2.75 ± 1.63[ac]	3.55 ± 1.01[ac]
SM + MSD	2.14 ± 1.40[ac]	1.59 ± 0.81[ac]	2.70 ± 1.07[a]

[a] $P < 0.05$ *vs* NM; [b] $P < 0.05$ *vs* WM; [c] $P < 0.05$ *vs* SM

Changes of SDH activity in liver tissue of mice

Compared with the NM group, the SDH activity of the WM group decreased significantly ($P < 0.05$), while the SM group increased significantly ($P < 0.05$). Compared with the WM group, the SDH activity was increased significantly ($P < 0.05$) after interfered by MHD, but it decreased significantly ($P < 0.05$) after interfered by MSD. The trend of the SM groups was conformable with the WM groups (Table 3).

Changes of SOD activity and the content of MDA in liver tissue of mice

Compared with the NM group, the SOD activity of the WM group decreased significantly ($P < 0.05$), while it increased significantly ($P < 0.05$) in the SM group. Compared with the WM group, the SOD activity increased significantly ($P < 0.05$) after interfered by MHD, but it decreased after treated by MSD. Compared with the SM group, the SOD activity was all decreased significantly ($P < 0.05$) after treated by MHD and MSD, but the activity interfered by MSD apparently declined more. The trend of the content of MDA was nearly opposite to the SOD activity. Compared with the NM group, the content of MDA of the WM group increased significantly ($P < 0.05$), while it decreased in SM group. Compared with the model group, the content of MDA were all increased after treated by MHD and MSD, but it increased more apparently interfered by MSD ($P < 0.05$) (Table 3).

Table 3 SDH and SOD activity, and content of MDA in liver tissue of mice ($\bar{X} \pm s$, $n = 6$)

Group	SDH / ($U \cdot mg^{-1}$)	SOD / ($U \cdot mg^{-1}$)	MDA / ($U \cdot mg^{-1}$)
NM	15.93 ± 8.04	109.37 ± 21.46	3.88 ± 1.32
WM	10.15 ± 5.29[a]	86.83 ± 17.08[a]	8.40 ± 1.79[a]
SM	18.05 ± 4.3[a]	125.04 ± 13.22[a]	2.85 ± 0.87
WM + MHD	16.73 ± 11.43[b]	114.58 ± 36.62[b]	17.99 ± 8.53[ab]
WM + MSD	8.21 ± 4.16[ab]	78.36 ± 17.44[a]	10.03 ± 1.3[a]
SM + MHD	28.03 ± 13.67[ac]	76.02 ± 20.69[ac]	13.14 ± 3.33[ac]
SM + MSD	14.93 ± 5.87	90.71 ± 12.88[ac]	3.47 ± 1.46

[a] $P < 0.05$ *vs* NM; [b] $P < 0.05$ *vs* WM; [c] $P < 0.05$ *vs* SM

Discussion

The differences of cold and hot natures of rhubarb, strobal, ginger, *Gypsum Fibrosum*, red ginseng, American ginseng, and processed products of *Coptidis Rhizoma* were investigated initially by using cold/hot pads at the macroscopical level of animal behavior. The results displayed that the remaining rate of mice on warm pad (40 ℃) was attenuated obviously after interfering by the hot nature medicines such as Strobal, Ginger, and red Ginseng, while the index conversed after treated by cold nature medicines such as rhubarb, *Gypsum Fibrosum*, and American ginseng. The processed materials of *Coptidis Rhizoma* (*Coptis chinensis* Franch.) exhibited a different cold nature in temperature tropism compared with crude *C. chinensis*. The cold nature of bile-processed *C. chinensis* enhanced while the ginger-processed *C. chinensis* changed inversely. This suggested that the correlation between the tropism to high temperature pad and the difference of the cold and hot nature of CMM was indeed existing (Zhang *et al*, 2009; Zhou *et al*, 2009; Zhao *et al*, 2009; Ren *et al*, 2009).

On this basis, the cold and hot nature of MHD and MSD was illustrated objectively in this study based on the cold/hot pads differentiating technology with the index of remaining rate on warm pad (40 ℃). The results showed that the temperature tropism of the models were coincident with the previous studies that the remaining rate of mice of WM on warm pad (40 ℃) increased significantly ($P < 0.05$), while the SM changed adversely. After administration of MHD, it was found that the high-temperature tropism of the WM was reduced significantly ($P < 0.05$). This external behavior of cold tropism might reflect the internal hot nature of MHD in an intuitive and objective way, while the temperature tropism of SM mice was not changed significantly which might be on account of incorrectness between syndrome and prescription. At the same time, MSD increased the high-temperature tropism of the strong model ($P < 0.05$), which reflected the cool characteristic of the formula. These results also coincided with the principle of CMM: Treating Hot with Cold, treating Cold with Hot.

Practically, the state of the body was a variety outcome of physiological and pathological factors by the interaction. The metabolism of organisms was the sum of a series of chemical reactions. According to further analyzed on the energy metabolism as well as oxidation and antioxidant balance which possibly related to the changes of animals temperature tropism, it was found that the activities of ATP, SDH, and SOD in the WM were decreased, while MDA content increased. The result suggested that the performance for the body energy metabolism reduced and the regulation capability decreased. It also coincided with the traditional recognition of deficient cold symptoms in TCM theory. After administration of MHD, the activities of ATP, SDH, and SOD showed a trend of increase, which might be related to the hot nature of *Ephedrae Herba* (Gao, 1992), and MSD revealed adverse effect, which was coincident with the investigations from the perspective of microcalorimetry (Fan *et al*, 2007). The contents of MDA were all significant increased ($P < 0.05$) except the SM administered with MSD probably due to the incorrectness between syndrome and prescription, which also demonstrated that the accordance between drug and symptom was very important to prescription.

In conclusion, the investigation had authenticated the outwardness of the difference between cold and hot natures of MHD and MSD at the level of overall animal behavior when the remaining rate of mice on warm pad (40 ℃) was selected as the macroscopic index. It provided experimental foundation for explaining the differences of similar prescription in cold/hot nature and establishing the evaluation method of cold/hot nature. It also gave some guides for clinical use of drugs.

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