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THE CHANGE IN IMMUNE FUNCTION OF RED BLOOD CELLS IN RATS AFTER AN 8-WEEK HEAVY EXERCISE TRAINING

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ABSTRACT

THE CHANGE IN IMMUNE FUNCTION OF RED BLOOD CELLS IN RATS AFTER AN 8-WEEK HEAVY EXERCISE TRAINING. **Paul Soong, Qi-Chen Hu, and Ren-Xing Huang. JEPonline. 2003;6(3):1-8.** This study investigated the effect of prolonged high intensity exercise training on immune function of red blood cells and white blood cells. In addition, the effect of nutritional supplementation with the herb Hong Jing Tian (tibetan rhodiola) on immune function of red blood cells and white blood cells was tested. 27 sprague-dawley male rats were randomly assigned to 3 groups: a control group (C), a trained group (T), and a trained group with herbal supplementation. (TH). Groups T and TH completed treadmill running training at a high intensity for 8 weeks. During the first week, rats were exercised at 15 m/min for 10 min/day to familiarize them with the protocol. From the second week, running speed was initially set at 20 m/min for 10 min. Run speed and duration were gradually increased until training bouts of 30 m/min and 90 min run time were reached. This run speed and duration were maintained through the eight-week training period. Blood samples were collected in the morning after training and at least 24 hours after the training. The rosette formation rates of red blood cells CR1 (%), a measure of the immune function of red-blood-cells, were 13.71 ± 3.55 in C, 4.22 ± 1.39 in T and 9.91 ± 2.39 in TH. There were significant differences between the rates in groups C and T ($p < 0.01$), between the rates in groups C and TH ($p < 0.05$), and between the rates in groups T and TH ($p < 0.01$). The migration indexes of white blood cells were 0.76 ± 0.07 in C, 1.09 ± 0.12 in T and 0.74 ± 0.05 in TH, and the changes in white blood cell migration indexes significantly correlated with the changes in rosette formation rates of red blood cell CR1 ($r = -0.68$ $p < 0.01$). The results indicate that immune adherent function of red blood cells is inhibited remarkably by prolonged heavy exercise training. There is a high correlation between the changes in immune functions of red blood cells and white blood cells. Hong Jing Tian, however, can minimize the adverse effect of prolonged heavy exercise training on immune function of red blood cells.

Key Words: Heavy exercise, Red blood cells, Immune function, Hong Jing Tian, Herbs

INTRODUCTION

It is clear that physical exercise and sports training will influence the immune system of the body. Over the past decades, sports scientists have paid more and more attention to the immune function of athletes and how exercise training can alter immune function. Some studies have indicated that moderate to high levels of physical activity lead to immune suppression and an increased risk of some diseases, such as upper respiratory tract infection (10, 16, 23). Similarly, some studies have demonstrated that heavy training or competition may increase the risk of viral and bacterial infections (15, 17, 18).

On the other hand, decreased rates of infection resulting from the chronic benefits of exercise have also been described (14, 20, 28). Some animal researches indicated that chronic exercise could retard, delay, or prevent the incidence, progression, or spread of experimental tumors (6, 25). Animal research also suggests that exercise induces a protective effect to infections (4, 5).

Siegel, Liu and Gleicher (22) demonstrated that red blood cells not only have respiratory function but also immune function. One of the important immune functions of red blood cells is immune adherence. Red blood cells bind to antigen-antibody-complement complexes through complement receptor type 1 (CR1) on their surface. Some studies have shown that different chronic and acute exercise programs induce an increase, or a decrease, or no changes in CR1 activity of red blood cells (9, 10, 25). The immune functions of red blood cells and white blood cells interact with each other in the body, which complete body defense functions against pathogens and immune surveillance against malignant cells (7, 22). It has also been reported that there is a relationship between changes in the immune functions of red blood cells and white blood cells in physical exercise (9).

After making a comprehensive survey of the body of literature available concerning the immune function and physical exercise, most of the research discusses the relationship of white blood cell immune function and physical exercise. However, there is a paucity of data relating to the qualities or functions of the red blood cells in auto immunity. The red blood cell immune system, as a novel concept, may play an important role in explaining the changes in immune function pertaining to physical exercise.

This research observed the change of the activity of red blood cells CR1 in rats after an 8-week treadmill run training program. Also, this research investigated the effect of a Chinese herb, Hong Jing Tian on the change of the activity of red blood cells CR1 in rats after run training. Consequently, the purpose of this research was to investigate; 1) the effect of heavy exercise training on immune function of red blood cells, and 2) factors that may affect the immune function of red blood cells.

METHODS

Animals

27 Sprague-Dawley (SD) male rats (120 days of age) were used for this study. Animals were obtained from the animal laboratory of Shanghai Medical University, P.R. China. The animals were fed a standard rodent laboratory food and allowed free access to food and water. The temperature of the animal room was maintained at 18-20 °C with 50-70 % relative humidity, on a 12/12 hour light-dark cycle.

The animals were randomly assigned to one of three groups. Seven rats were in the control (non-trained) group (C, weight 175.43±23.08g). Nine rats were in the trained group (T, weight 190.07±17.98g) and 11 rats were in the trained and herbal supplementation with Hong Jing Tian group (TH, weight 188.60±21.04g).

Preparation of Hong Jing Tian Co.

The Hong Jing Tian was made in Shanghai University of Traditional Chinese Medicine, P.R. China and mainly consisted of Chinese herbs, including alpine Hong Jing Tian, Huang Qi, Bai Shu, Mai Dong and Wu Wei Zi. The concentration of the herbs was 1.78 g drug/mL. The essential function of Hong Jing Tian is to invigorate the spleen and to replenish *Qi*, a term in Chinese traditional medicine. The concept of *Qi* is absolutely at the heart of Chinese medicine, yet no one English word or phrase can adequately capture its meaning.

Procedures

The rats in C were housed in cages without any treatment. Rats in T and TH completed eight weeks (6 days/week) of treadmill running. During the first week, rats were exercised at 15 m/min for 10 min/day to familiarize them with the surroundings. From the second week, running speed was initially set at 20 m/min for 10 min. Run speed and duration were gradually increased until training bouts of 30 m/min and 90 minutes run time were reached. This run speed and duration were maintained through the eight-week training period. Care was taken to ensure that both T TH groups were matched for running speed and duration throughout the entire training period (1).

During the training period, rats from TH were orally administered 2/day with Hong Jing Tian (1.3mL/100g body wt). The T group was given the same volume of water without the herbs.

Data Collection

Animals were anesthetized with 2% sodium pentobarbital (40mg/kg body wt) 24~28 hours after the final training session. Blood samples were collected from the abdominal aorta in test tubes containing heparin and were stored at 4 °C.

Immune adherent function of red blood cells was measured by the red blood cell CR1 rosette test and red blood cell-immune complex rosette test to assess the activity of red blood cell CR1. Immune function of white blood cells was measured by the inhibition test of white-cell migration.

Method of Red Blood Cell CR1 Measurement

Five hundred μ L of blood were drawn into a clean heparin coated test tube. The cells were washed twice with 0.9% sodium chloride solution. The cells were suspended by gently drawing them in and out of a Pasteur pipette and were centrifuged at 2000 rev/min for 10 min/time and the supernatant was discarded. Red blood cells were adjusted to a concentration of 1.25×10^7 /mL in 0.9% sodium chloride solution. This was hereby known as the red blood cell suspension. Yeast and yeast coated with complement were washed once with 0.9% sodium chloride solution and were adjust to a concentration of 1×10^8 /mL in 0.9% sodium chloride solution.

Seventy-five μ L of each of the red blood cell suspension and yeast coated with complement suspension were added into a 5 mL test-tube and mixed gently. The cells were then incubated in a water bath at 37° C for 30 min. After the incubation, 150 μ L of 0.9% sodium chloride solution was added and mixed with cells gently. Fifty μ L of 0.25% glutaraldehyde were added and mixed with the cells gently to fix the cells for five minutes. After the fixation, 150 μ L of the cells was spread on a microscope slide. The smear was allowed to air dry or a hair dryer was used to hasten the action. A drop of formaldehyde was placed on the smear for fixation. After the smear dried, the cells were stained with Wright's Stain Solution. Two hundred red blood cells were counted using a microscope. Two or more yeast binding to a red blood cell was counted as one rosette. The number of rosette formation of red blood cell CR1 (red blood cell CR1 rosette formation rate) was calculated.

Seventy-five μ L of the yeast suspension replaced the yeast coated with complement suspension. The number of rosette formation of red blood cell-immune complex (red blood cell-immune complex formation rate) were calculated by the same way as described above for measurement of red blood cells cr1 rosette formation rate.

Statistical Analyses

Data of immune function of red blood cells and white blood cells were presented as means \pm standard deviations. Alterations in the data and the relationship between immune functions of red and white blood cells were analyzed with two-way ANOVA and correlation. *P* values of ≥ 0.05 were considered statistically significant.

RESULTS

The rosette formation rate of red blood cell CR1 (RBC-CR1R) and the rosette formation rate of red blood cell-immune complex (RBC-ICR) in different groups are presented in Table 1. The rosette formation rate of red blood cell CR1 in T was significantly different from that in C and significantly lower than that in TH. After the 8-week running training, there was a trend for increased rosette formation rates of red blood cell-immune complex in T and TH compared to C, but no statistical significance was shown.

Table 2 shows that the migration indexes of white blood cells in three groups. Statistical analysis showed the migration index of white blood cells in T was remarkably higher than that in C and TH ($p < 0.05$). There was no significant difference between the migration indexes of white blood cells in C and TH.

There was a negative correlation between the rosette formation rates of red blood cell CR1 and the migration indexes of white blood cells ($r = -0.68$, $p < 0.01$). There was no significant relationship between the changes in the rosette formation rates of red blood cell-immune complex and the migration indexes of white blood cells during the training period.

Table 1 Values of red blood cell CR1 activity in the three groups.

Groups	N	RBC-CR1R (%)	RBC-ICR (%)
C	7	13.71 \pm 3.49	4.29 \pm 1.11
T	9	4.22 \pm 1.31 ^{***}	5.53 \pm 1.58
T+H	11	9.91 \pm 2.27 [*]	4.91 \pm 2.07

Significantly different from value of NT group: * $p .05$,
 ** $p .01$; Significantly different from value of TM group:
 ## $p .01$.

Table 2. Migration indexes of white blood cells in three groups.

Groups	N	WBC-MI
C	7	0.76 \pm 0.07
T	9	1.09 \pm 0.12 ^{* #}
T+H	11	0.74 \pm 0.05

Significantly different from value of NT group ($p .05$)
 # Significantly different from value of TM group ($p .05$)

DISCUSSION

Red blood cells have a simple structure, with no nucleus or additional cytoplasmic organelles. As such, the red blood cell is suited to the simple function of transporting respiratory gases. Interestingly, the immune adherent function of red blood cells was not discovered until the 1950s. Furthermore, it was shown that the red blood cell immune adherence phenomenon led to an enhancement of phagocytosis. It has been demonstrated that red blood cell immune adherence was initiated by C3 receptors on the surfaces of red blood cells, which now is called complement receptor type I. Siegel, Liu and Gleicher (22) put forward a new concept, "the red-cell immune system", in the early 1980s and first pointed out that red blood cells are an important part of the body's immune system, and that the immune function of red blood cells, just like that of white blood cells, cannot be neglected.

It has been reported there are many immune materials in red blood cells. Besides CR1, red blood cells have other immune factors such as lymphocyte function-associated antigen-3 (LFA-3, CD58), decay accelerating factor (DAF, CD55), membrane inhibitor of reactive lysis (MIRL, CD59), homologous restriction factor (HRF), phagocytosis inhibitory factor (PIF) and natural killer enhancing factor (NKEF). These factors, with CR1, regulate complement activation and regulate the immune function of leukocytes. This study principally investigates the capacity of red blood cell immune adherence, which is an important and main immune function of red blood cells.

The Effect Of 8-Week Treadmill Running On Red Blood Cell Immune Function

Human studies showed that there is no significant difference between resting values of red blood cell immune functions in trained and untrained people (9, 12, 25). However, acute exercises at 45% VO_2max and 85 % VO_2max for 10 min, respectively, remarkably decreased the activity of red blood cell CR1 in trained and untrained people. Furthermore, untrained subjects showed greater change in activity of erythrocyte CR1 post-exercise at 85 % VO_2max in comparison to athletes (9). After the step exercises at 700 kgm/min for

three durations; 15, 30 or 45 min, the activities of red blood cell CR1 in healthy young males markedly decreased immediately and 3 hours post-exercise, and recovered 15 hours post-exercise. However, no distinct change in the rosette formation rate of red blood cell CR1 occurred immediately after a set of Wu style Tai Chi (around 20-min) and the rate increased 2-hour post-exercise, that is, the immune adherent function of red blood cells was enhanced (12). Thomsen and his coworkers reported that 60 min of bicycle exercise at 75 %VO₂max did not lead to a change in level of red blood cell CR1 in highly trained athletes. The aforementioned studies indicate that there are different effects of various exercise and training on immune function of red blood cells. Different research results may be obtained due to different research subjects, detect methods, exercise styles, exercise duration and intensities. However, most scientists consider that low or moderate exercise training can improve the immunity of the body, whereas, prolonged and intense exercise may induce immune suppression and an increased risk of some diseases.

In this research, rosette formation rate of red blood cells in T and TH groups was still much lower than that in C group at least 24 hours after 8-week running training. The results further show that heavy exercise training may lead to a significant decrease in red blood cell immune function of the body.

The Relationship Between Immune Function Of Red Blood Cells And White Blood Cells

The mechanisms of changes in the immune function of red blood cells of the body due to physical exercise are still unclear. Our former studies showed that the decline in activity of red blood cell CR1 during exercise was associated with increases in plasma lactate concentration and immune complex level, etc. (9,10). The results of this research indicate that the change in the immune function of red blood cells due to exercise training relates to the change in immune function of white blood cells.

The function of immune system is to protect the body from damage caused by invading microorganisms. This defensive function is performed by nonspecific immunity or specific immunity. The former is achieved by external mechanisms or internal mechanisms, and the latter is attributable to T and B cells that can respond selectively and specifically to a seemingly infinite number of different non-self materials. Besides CR1, red blood cells possess other immune factors such as lymphocyte function-associated antigen-3 (LFA-3, CD58), decay accelerating factor (DAF, CD55), membrane inhibitor of reactive lysis (MIRL, CD59), homologous restriction factor (HRF), phagocytosis inhibitory factor (PIF) and natural killer enhancing factor (NKEF), etc. These factors, with CR1, play roles on regulation of complement activation, regulation of immune functions of monocytes and phagocytes, regulation of immune functions of T and B lymphocytes and enhancement of activities of natural killer cells and lymphokine activated killer cells. Red blood cells improve the activity of leukocyte CR1, CR2 and CR3 through immune adherence by CD58, CD59 and CR1. Moreover, red blood cells activate white blood cells, enhance interleukin 2 (IL-2) receptor expression, and increase IFN- γ secretion. The interaction of LFA-3 (CD58) on the surface of red blood cells with CD2 on T lymphocytes activates lymphocytes and enhances cytokine secretion. Red blood cells are an essential component of the immune system of the body and they, just like white blood cells, may be considered to be an important part of the immune system (5,22). On the other hand, cytokines secreted by white blood cells, such as IL-2, IFN- γ and thymosin, lead to a rise in activity of erythrocyte CR1 and enhancement in the immune adherence of red blood cell in vitro (7,8).

Some clinical studies have observed that there is a significant correlation between changes in immune functions of red blood cells and white blood cells. The activity of erythrocyte CR1 positively correlated to the ratio of T lymphocyte subgroups, CD4/CD8. Compared to healthy people, the immune function of red blood cells and the number of IL-2R positive cells were significant lower in patients with gastric cancer and increased after the gastric cancer was resected. A positive correlation between the ability of red blood cells binding to tumor cells and the number of IL-2R positive cells was found. Furthermore, it was also reported that there was a correlation between red blood cell immune function and activity of NK cells in these studies (8,12,26).

Few reports described the relationship between immune functions of red blood cells and white blood cells in physical exercise. This study showed that there was a negative correlation between the rosette formation rate of red blood cell CR1 and the migration index of white blood cells. The results suggest that an 8-week heavy

training on treadmill induced decreases in the activity of CR1 on red blood cells. Meanwhile, it also led to remarkable suppression in the activation of lymphocytes, which indicates that the decrease in the immune adherence of red blood cells due to physical exercise and training is associated with the immune function of white blood cells. There are mutual relation and effect between immune functions of red blood cells and white blood cells. However, there are few studies in this field and further investigations are needed.

The Effect Of Hong Jing Tian On The Immune Function Of Red Blood Cells

The number of red blood cells in circulation system is predominant. They integrate multifunction, such as nourishing and moistening the body, into one entity. There is a close relationship between red blood cells and "the theory of *Qi* and blood" in traditional Chinese medicine. As such, a brief description and explanation of *Qi* is necessary. That which animates life is called *Qi*. The concept of *Qi* is fundamental to Chinese medical thinking. Life is defined by *Qi* even though it is impossible to grasp, measure, quantify, see or isolate. Immaterial yet essential, the material world is formed by *Qi*. An invisible force as only by its effects, *Qi* is recognized indirectly by what it fosters, generates, and protects. Matter is *Qi* shape. Mountain forming, forests growing, rivers streaming, and creatures proliferating are all manifestations of *Qi*. In the human being, all functions and mind are manifestations of *Qi*: sensing, cogitation, feeling, digestion, exercising and sports performance. *Qi* begets movement and heat. Chinese medicine emphasizes that life cannot separated from the way it manifests. When the heart beats and the breath is warm, it is understood that life exists within the body. When the heart stops beating and the body becomes cold, the life force, or *Qi*, is no longer present. Life force and *Qi* are one. Like fresh air, health *Qi* moves freely; like stale air, stagnant *Qi* is heavy, oppressive, constrictive, and congestive (2,10).

"The theory of spleen and stomach" is also an important part in traditional Chinese medicine. Traditional Chinese medicine believes that spleen and stomach are main organs of the digestive system. Water and food are not only the chief source of the nutrients which man needs to keep his life activities, but also the material basis for producing *Qi* and blood, and what is more, it is the spleen that transports, distributes and transforms nutrients. Thus, "the spleen provides the material basis for the acquired constitution" and "the spleen is the source of producing the *Qi* and blood." Reinforcing the functions of the spleen and stomach can enhance the defensive function of the body (19, 27).

Some studies have demonstrated that the immune function of red blood cells was associated with the changes in *Qi* and blood, and the spleen function. Under physiological condition, rise or fall in human *Qi* and blood is consistent with change in the immune function of red blood cells in different time of a day and different stage of the life. Under pathological condition, the immune function of red blood cells in patients with deficiency in *Qi* and blood is lower, and recovers after the patients take the medicine that has the functions of replenishing *Qi* and enriching blood. Similarly, clinical study and animal research have demonstrated that the activity of red blood cell CR1 decreased in the persons with deficiency in the function of the spleen and the immune function of red blood cells recovered and increased after the method of invigorating the spleen used.

Hong Jing Tian, as above-mentioned, can invigorate the spleen and replenish *Qi*. Some studies have shown that it can regulate the functions of the center nerve system, material metabolism, and anaerobic ability and enhance sports capacity. Scientists found that Hong Jing Tian prolonged livability of rats under decompression hypoxia situation, reduced the accumulation in blood lactate during physical exercise, were favorable for dispelling fatigue and distinctly improved physical work capacity 170 (PWC₁₇₀) of people at high altitude. Scientists also demonstrated that there were the effective components, including amylose organic acid, glycoside, in Chinese medicines, which could enhance, improve and regulate the immune function of red blood cells (13,26).

In this study, the immune function of red blood cells in rats significantly decreased after the 8-week heavy running training on the treadmill. While the rosette formation rate in rats that were administered Hong Jing Tian during the training period was much higher than that of rats without the Chinese medicine, which means that the activity of red blood cell CR1 in rats with Hong Jing Tian was remarkably improved. The results indicate that Hong Jing Tian can attenuate the adverse effect of prolonged heavy exercise training on the immune function of red blood cells.

CONCLUSION

This study showed that the activity of CR1 on the surface of red blood cells in rats significantly decreased after 8-week heavy exercise training on a treadmill and there was close correlation between the change in rosette formation rate of red blood cell CR1 and the migration indexes of white blood cells. The results indicate that heavy exercise training significantly inhibited red blood cell immune function of the body and the change in the immune adherence of red blood cells were closely associated to the change in the function of lymphocytes. There were mutual relation and effect between immune functions of red blood cells and white blood cells. However, Hong Jing Tian could attenuate the adverse effect of prolonged heavy exercise training on the immune function of red blood cells.

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