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Effects of angelica polysaccharide on hepatocytes apoptosis induced by exhaustive exercise

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The study aims at assessing the effects of angelica polysaccharide (APS) on hepatocytes apoptosis in rats induced by exhaustive exercise. The exhaustive-exercise rat model was established by dividing 32 male wistar rats into four groups randomly. The groups were the normal control group (NC), exhausting exercises control group (EC), low dose APS group (LA) and high dose APS group (HA).The immunohistochemistry staining was used to test the protein expression of Bax and Bcl-2 in liver tissue in rats. Then the Bax/Bcl-2 ratio and hepatocytes apoptosis in each group was analyzed. The result showed that the expression of Bax in rats' liver tissue was decreased, Bcl-2 was increased and the ratio of Bax/Bcl-2 was decreased when exposed to exhaustive exercise after being force-fed with angelica polysaccharide by gastrogavage. Angelica polysaccharide could counteract the hepatocytes apoptosis in exhaustive-exercised rats' liver tissue with better results gotten at high-dose (600 mg/kg·bw).

Key words: Angelica polysaccharide, exhaustive-exercise, apoptosis.

INTRODUCTION

Apoptosis is a basic biological phenomenon that could occur in all cells including the histiocytes. It is a kind of special suicide which occurs in response to stimuli (different stimulation or diseases) . Through this, the body maintains physiological balance by eliminating injured, aged and mutational cells (Hu and Gao, 2005) . As a special stimulation, movement can accelerate the body's metabolism, lead to ischemic and hypoxiemic conditions, cause the reduction of ATP, increase production of free radicals, increase Calcuim ion (Ca^{2+}) concentration, alter mitochondrial structure and function and finally lead to apoptosis (Liu, 2007). Studies have shown that two types of genes can regulate or control apoptosis. These are the stimulative genes and the restraining genes. At present, the Bcl-2 gene family is the most focused gene family involved in the regulation and control of apoptosis. It belongs to a kind of new class of cancer gene family. Bcl-2 and Bax are two important members of this family that Bcl-2 is a major gene in the inhibition of apoptosis (Green and Kroemer, 2004) . Many studies have shown while Bax is a pro-apoptotic gene. They and their family members constitute a complex web of interaction that regulates and control the occurrence of apoptosis (Mounia et al., 2004). At present, the role of apoptosis in medicine and sports is much accounted for.

Angelica is the dry root of the plant angelica, belonging to Umbelliferae. As a well-known traditional Chinese medicine, angelica has many efficacies, such as enriching blood, invigorating the circulation of blood and regulating menstrual function (Meng, 2007). Angelica polysaccharide (APS) is a major element in angelica. In recent years, the studies of the component and pharmacology of angelica polysaccharide have yielded a lot of progress both at home and abroad. These studies have helped in the finding of its apparent effect on the body's immune system and hematopoietic system, as well as the good curative effect of anti-tumor, anti-radiation damage and anti-oxidation (Wang and Shan, 2006; Shang et al., 2001). However, whether angelica polysaccharide can inhibit hepatocytes apoptosis has not been reported. Therefore, this study establishes the exhaustive-exercise rat model, using angelica polysaccharide as a preconditioning drug, and then, observes the apoptosis-related

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genes and the protein expression of Bcl-2 and Bax. It further explores the protective effect and possible mechanism of action of angelica polysaccharide on exhaustive-exercised rats by interfering with hepatocytes apoptosis. This study tries to provide new experimental evidence in the use of angelica polysaccharide as a nutritional supplement in sports.

MATERIALS AND METHODS

Experimental animals and groups

Thirty-two male wistar rats weighing 200-220 g (provided by our Institute of Experimental Animal Center) were used for the study. Conventional sub-cage feeding at room temperature, 24 ± 3°C, humidity (60 ± 1)% was employed. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of China, West Normal University, Nanchong, China, and was carried out according to the ‘‘Principles of Laboratory Animal Care’’ (World Health Organization (WHO) Chronicle, 1985). All the rats were fed normally for a week, and then divided into four groups randomly. The groups made up of eight (8) rats per group included the normal control group (NC), the exhausting exercises control group (EC), the low dose APS group (LA) and the high dose APS group (HA). The rats were subjected to physical exercise except for the NC group. Rats in the LA and HA groups were force-fed angelica polysaccharide solution each day by way of gastrogavage. The dosage of APS was 200 and 600 mg/kg body weight (bw) respectively and 1.0 mL once daily. Rats in the NC and EC groups were force-fed with the same dose of distilled water.

Establishment of exhaustive-exercise rat model

The rats belonging to the exercised groups were made to do adaptive training treadmill exercise for three days at a speed of 10 m/min for 10 min per day on a slope of 0°. In the course of the experiment, the rats were trained under increased exercise load which was established according to the rats' body weight / oxygen consumption regression equation (Shi et al., 2008; Bedford et al., 1979). The rats were made to carry out the exercise until exhausted based on the schedule: First-level load: 0°, 8.2 m/min, 15 min (equivalent to 53% VO_2max); second-level load: 5°, 15 m/min, 15 m (equivalent to 64% VO_2max); third-level load: 10 °, 19.3 m/min (equivalent to 76% VO_2max), until exhausted. The total exercise time was 66 ± 24 min. In order to maintain the exercise intensity, the rats were kept running on the 1/3 foreside of the runway by arousing them with sounds and stimulating their tails with small stick. Electrical means were also used when necessary for stimulation.

Criterion for exhaustion

At the end of the exercise, the rats could not persist in running and were thus stopped on the 1/3 backside of the runway over 3 times. Meanwhile, the stimulation and expulsion were ineffective. The behaviors of the exhaustied rats included difficulty in breath taking, tired look, abdominal supine and unresponsiveness to the further stimulation (Liu et al., 2007).

Detection of the protein expression of Bax and Bcl-2 in liver tissue

The immunohistochemistry staining was used to test the protein expression of Bax and Bcl -2 in the liver tissues of the rats. The process was carried out as follows: the tissues were first cut into 4 m slices, dewaxed and then conventional hydration was carried out. The first antibody used was the rabbit anti-mouse anti-Bax and Bcl-2 polyclonal antibody. The second antibody was labeled by dropping horseradish peroxidase. This was used as a negative control by replacing the first antibody with phosphate buffered saline (PBS). The slices were then coloured with 3,3 -diamino-benzidine (DAB) and then with hematoxylin stain. Finally, the slices were fixed with resin. These were observed through optical microscope and the pictures taken were recorded. Using the high-power microscope, 10 fields of vision in each slice were observed and number of positive cells counted. The percentage of positive cells was used as the expression index (PEI) of protein Bax, Bcl-2 (Zhou et al., 2007). The presence of brown punctate or granular substances in the cells is an indication of positive staining. The same operation was repeated three times and the mean value used for analysis.

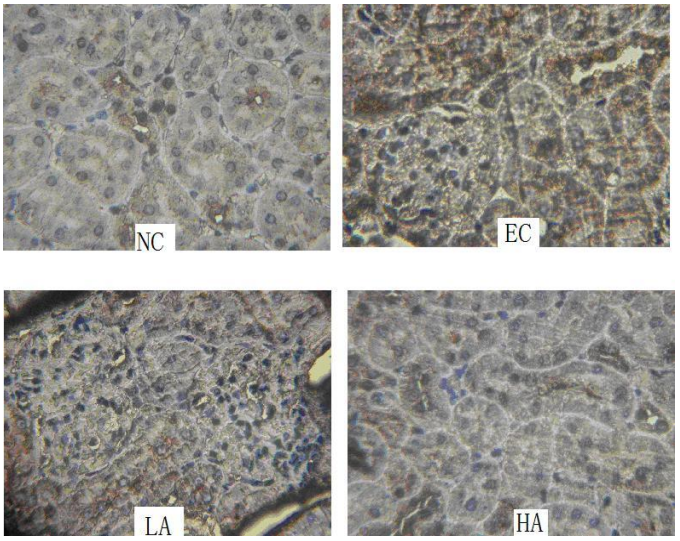

Figure 1. The expression of Bax in liver tissue of rats in each group (the immunohistochemistry staining).

Statistical analysis

Data were expressed as $\overline{x}$ ± s.d and statistical analysis was performed by SPSS15.0 software. The differences between the four groups were analysed using one way ANOVA, $p<0.05$ was considered significant.

RESULTS

Changes in the expression of the apoptosis-related gene Bax in the hepatocyte of each group

The immunohistochemistry staining was used to analyse the expression of Bax in the liver tissues of the rats (Figure.1). The data showed that Bax expression was very low in the NC group, high in the EC group and in between in the LA and HA groups. The results of the Bax gene expression in each group are as shown in Table 1

Table 1. Changes in the expression of Bax and Bcl-2 in liver tissues of rats exposed to exhaustive exercise and the ratio of Bax/Bcl-2 (x±s s, n=8).

Group	Bax	Bcl-2	Bax/Bcl-2
NC Group	14.34±4.72	18.71±3.14	0.77
EC Group	23.58±6.41	12.23±4.15	1.93
LA Group	19.47±3.68	16.14±5.79	1.21
HA Group	16.11±4.83	16.23±4.47	0.99

compared with NC p 0.05 compared with EC p 0.05 compared with LA p 0.05

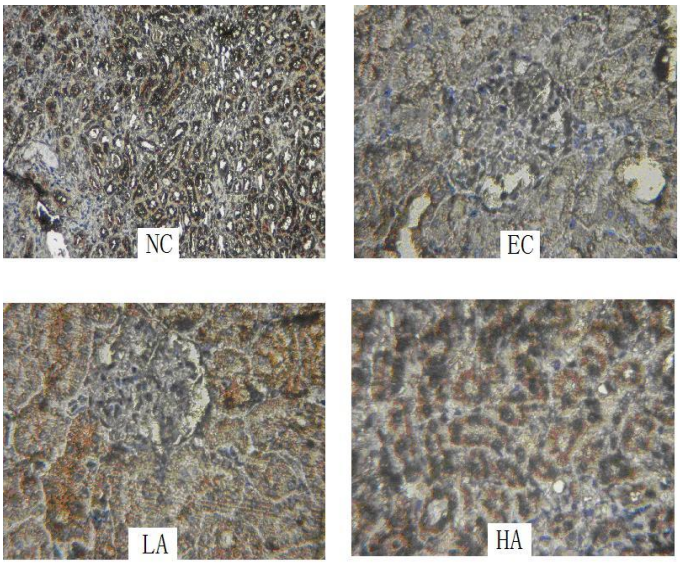

Figure 2. Expression of Bcl-2 in liver tissue of rats in each group (the immunohistochemistry staining)

The result showed that Bax gene expression in the EC and LA groups were statistically significant (p<0.05) when compared to the NC group. Furthermore, the expression of Bax gene in the LA and HA group were statistically significant (p<0.05) when compared to the EC group. It was also shown in the result that force-feeding angelica polysaccharide (APS) by gastrogavage can decrease the expression of Bax in liver tissue in the exhaustive-exercised rats with greater efficiency obtained at high-dose (600 mg/kg.bw) (p<0.05).

Changes in the expression of the apoptosis-related gene Bcl-2 in the hepatocytes of each group

The immunohistochemistry staining was used to analyse the expression of Bcl-2 in liver tissue of rats (Figure.2). The results of the Bcl-2 gene's expressions in each group are as shown in Table 1. The expressions of Bcl-2 were obvious in the NC, LA and HA groups while it was less pronounced in the EC group. In comparison with the NC group, the expression of Bcl-2 in the EC group was statistically significant at p<0.05. In addition, the expressions of Bcl-2 in the LA and HA groups were statistically significant at p<0.05 when compared to the EC group. It was also deduced from the result that force-feeding the exhaustive-exercised rats with different dose of angelica polysaccharide (APS) by gastrogavage can promote the expression of Bcl-2 in liver tissue.

Changes of Bax/Bcl-2 ratio in each group and the pertinence between the ratio and the hepatocytes apoptosis rate

As can be seen from Table 1, comparing with the NC group, the Bax/Bcl-2 ratio in the EC and LA groups was statistically significant at p<0.05. When compared to the EC group, the Bax/Bcl-2 ratio in the NC, LA and HA groups was statistically significant at p<0.05. As the ratio of Bax/Bcl-2 in the rat liver tissues and hepatocytes apoptosis rate were positively correlated (Kim et al., 2005), the results in Table 1 showed that force-feeding angelica polysaccharide (APS) by gastrogavage can inhibit the hepatocytes apoptosis in exhaustive-exercised rats.

DISCUSSION

Since the concept of apoptosis was proposed by Kerr in 1972, reports of apoptosis have increased and it has had great impact on the in- depth study of the life sciences (Wu and Zeng, 2005). Undoubtedly, the amount and intensity of exercises are two important factors affecting apoptosis. In order to carry out apoptosis, some special genes within the cells need to be activated to carry out transcription and synthesize specific proteins. The specific gene products mainly include Bcl-2 protein family, Caspase family, IGF family, the "death genes" p53 with tumor control function and the Fas-Fasl system (Xu et al., 1999; Adams and Cory, 1998). Among the above products, Bcl-2 family members play important roles as proteins in the process of apoptosis and also the most widely studied in sports. Studies have shown that during exercise, there is proper circulation of blood in the body. During the progress of circulation, vasodilation occurs in skeletal muscles, resulting in the increase of the blood flux, while the vasoconstriction occurs in the intra-abdominal organs and skin, which decreases the blood flux. Though the liver helps in proper blood circulation, blood flow into the liver is decreased during exercise. Meanwhile, due to its characteristic high metabolic rate, the liver is one of the organs that are easy affected by ischemia (Liu et al., 2007). No report has been made on the mechanism of apoptosis in liver tissue induced by exhaustive-exercise.

Apoptosis is a protease cascade process. There are

several pro- apoptotic and anti-apoptotic proteins interacting with each other in this process and maladjustment in interaction could lead to apoptosis (Zhou et al., 2007). Studies have shown that apoptosis is regulated by the balance between protein Bcl-2 and Bax. The Bcl-2 and Bax exist as homodimer, though they can exist as heterodimers too. If the Bax exists as homodimer, apoptosis will occur; if the amount of Bcl- 2 is more than Bax when all the Bax are combined, the residual Bcl-2 will play the inhibitory effect, preventing apoptosis. On the contrary, if Bax is more than Bcl-2, Bax will lead to apoptosis (Kim et al., 2005). In other words, the ratio of Bax/Bcl-2 determines the trend of apoptosis (Lee et al., 2005).

The results of this study showed that when compared with the normal control group, the expression of Bax in liver tissue of exhaustive-exercised rats was increased the expression of Bcl-2 was decreased, and the ratio of Bax/Bcl-2 was increased. The results indicated that Bax and Bcl-2 co-regulate apoptosis which was induced by exhaustive-exercise. Bax promotes the apoptosis as up-regulated genes, while Bcl-2 inhibits apoptosis as down-regulated genes and this may be the gene regulatory mechanism of apoptosis in liver tissue induced by exhaustive-exercise. The results also showed that the expression of Bax in liver tissue was decreased, Bcl-2 expression was increased and Bax/Bcl-2 ratio was decreased in exhaustive-exercised rats after being forced-fed with angelica polysaccharide (APS).

In conclusion, angelica polysaccharide (APS) may produce an anti-apoptosis effect on apoptosis in exhaustive-exercised rats by regulating Bax, Bcl-2 and Bax/Bcl-2 ratio. The angelica polysaccharide produces a protective effect on liver tissue with greater effect at high-dose (600 mg/kgbw). The experimental results provided theoretical support for the application of angelica polysaccharide in the field of sports nutrition.

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