

## The Effects of Resveratrol Supplementation on Exercise-Induced Apoptosis of Rat Hepatocytes

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**Abstract:** The present study was designed to determine the effects of resveratrol supplementation on exercise-induced apoptosis of rat hepatocytes. The rats were divided randomly into the sedentary group (Group I) and exhaustive exercise groups (Group I-IV). Each group contains 12 animals. The rats of exhaustive exercise groups received daily oral administration of resveratrol at doses of 0, 200 or 400 mg/kg/day for 30 days. The rats of sedentary group received daily oral administration of vehicle only (10 mL/kg/day) for the same period. Exhaustive exercise was performed on a motor-driven rodent treadmill. Paraffin-embedded liver sections were stained using the Streptavidin Peroxidase (SP) immunohistochemistry technique for BAX and Bcl-2 detection. The results showed that exhausting exercises could make the expression of BAX proteins in the liver and BAX/Bcl-2 ratio dramatically increases, the expression of Bcl-2 proteins obviously decreases. After resveratrol supplementation, the expression of BAX proteins and BAX/Bcl-2 ratio obviously decreased, the expression of Bcl-2 proteins obviously increased. Which indicated that resveratrol supplementation can inhibit exercise-induced apoptosis of rat hepatocytes.

**Key words:** Resveratrol, exercise, apoptosis, rat, hepatocytes, SP

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### INTRODUCTION

Apoptosis or programmed cell death is a normal physiological function essential for the homeostasis of immuno haemopoietic tissues. This process occurs via specific signaling pathways, eventually leading to DNA fragmentation, nuclear condensation, proteolysis and cell fragmentation (Reed, 2001; Albert, 2001). Within the past 10 years, apoptosis has gained the interest of many exercise scientists because in addition to necrotic cell death, evidence indicates that apoptotic cell death also occurs with intense exercise. Intense exercise is often accompanied by an excessive generation of oxygen free radicals. Reactive oxygen intermediates react with cellular components which may cause extensive damage to cellular structures such as DNA. The DNA damage could finally lead to apoptotic or necrotic processes in many organs including skeletal muscles, heart, cells of the immune system, lungs and liver (Phaneuf and Leeuwenburgh, 2001; Song *et al.*, 2006; Kavazis *et al.*, 2008).

Resveratrol (3, 4', 5-tri-hydroxystilbene), a poly phenolic stilbene first isolated from the root of *Veratrum grandiflorum*, occurs naturally in grapes, mulberries and peanuts (Aggarwal *et al.*, 2004; Saiko *et al.*, 2008; Hiroto *et al.*, 2011). In these plants resveratrol is synthesized in response to stress conditions such as an

infection and thus can be considered to be a phytoalexin. Resveratrol has estrogenic activity in mammals and therefore is classified as a phytoestrogen (Niles *et al.*, 2006). Recent studies indicate that resveratrol possesses many biological activities that are protective against atherosclerosis including anti-oxidant, anti-inflammatory, anti-platelet and vasorelaxant activities (Delmas *et al.*, 2005; Xia *et al.*, 2010). In addition, resveratrol is identified as a cancer chemotherapeutic agent and has recently been reported to mimic effects of dietary restriction and extends the lifespans of lower organisms and improves the health and survival of mice on a high-fat diet (Alarcon de la Lastra and Villegas, 2005; Pervaiz and Holme, 2009; Fukui *et al.*, 2010). However, the effects of resveratrol on exercise-induced apoptosis is poorly understood. The purpose of the present study was to investigate the effects of resveratrol supplementation on exercise-induced apoptosis of rat hepatocytes.

### MATERIALS AND METHODS

**Main reagents:** Resveratrol (>98% purity, FW 228.2) were purchased obtained from Cuiyuan Biotechnology Co., Ltd. (Xi'an, China). Rabbit polyclonal antibody against human BAX and Bcl-2 and goat anti-rabbit polyclonal antibody were purchased from Zhongshan Biotechnology Co., Ltd. (Beijing, China). Other chemicals and

biochemicals were of analytical grade and were purchased from Sigma Chem. Co. (St. Louis, MO, USA) and Changsheng Pharmaceutical Co., (Chengdu, China) unless otherwise indicated.

**Animals:** Male Wistar rats weighing 140-180 g were obtained from Sichuan Research Animal Center (Chengdu, China). Rats were housed in a room with alternating 12 h periods of light and dark, ambient temperature of  $24\pm 1^{\circ}\text{C}$  and humidity of  $55\pm 5\%$ . All animals were allowed free access to distilled water and fed on a commercial diet (purchased from Sichuan Research Animal Center). All animals received humane care according to the guideline of Guidebook for the Care and Use of Laboratory Animals. The study protocol was approved by the animal research ethics committee at Sichuan University (Chengdu, China).

**Animals grouping and treatment:** After being adaptively fed for a week, the rats were divided randomly into the sedentary group (Group I) and exhaustive exercise groups (Group I-IV). Each group contains 12 animals. The rats of exhaustive exercise groups received daily oral (gavage) administration of resveratrol at doses of 0, 200 or 400 mg/kg/day for 30 days. Resveratrol was administered in a vehicle of 0.5% (w/v) aqueous methylcellulose containing 0.2% (w/v) Tween 80, a dosing volume of 10 mL/kg/day was used. The rats of sedentary group received daily oral (gavage) administration of vehicle only (10 mL/kg/day) for the same period.

**Exercise program:** After 30 days, the rats of exhaustive exercise groups were exercised vigorously in one session until they were exhausted. The exercise protocol was  $22\text{ m min}^{-1}$  at 10% inclination on a motor-driven rodent treadmill. A rat was considered exhausted when it would not be prompted to run any more. The rats would stop running and when overturned would continue to lie on their backs disregarding gentle prods and electric shock (Chen *et al.*, 2002). Immediately after exhaustive exercise, all the rats were sacrificed by decapitation. The liver were extracted and treated with 4% paraformaldehyde in 0.1 M, pH 7.4 Phosphate Buffered Saline (PBS) for 24 h before the embedding in paraffin. The tissues were cut into serial sections of 4  $\mu\text{m}$  thickness.

**The expression of BAX and Bcl-2 proteins:** Paraffin-embedded liver sections were stained using the Streptavidin Peroxidase (SP) immunohistochemistry technique for BAX and Bcl-2 detection (Bai and Meng, 2005; Wang *et al.*, 2007). Briefly, sections (4  $\mu\text{m}$ ) were

incubated with 3 mL L<sup>-1</sup> hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity, washed in Phosphate Buffered Saline (PBS) and incubated in 100 mL L<sup>-1</sup> normal goat serum for 20 min to reduce nonspecific antibody binding. Sections were then incubated with rabbit polyclonal antibody at a dilution of 1:100 against human BAX and Bcl-2 overnight at 4°C followed by three washes with PBS then incubated with biotinylated goat anti-rabbit polyclonal antibody at a dilution of 1:100 for 30 min followed by 3 washes. Slides were treated with streptavidin peroxidase reagent for 30 min at a dilution of 1:100 and washed 3 times with PBS.

Finally, slides were incubated in PBS containing diaminobenzidine and 10 L L<sup>-1</sup> hydrogen peroxide for 10 min, counterstained with hematine and mounted. The rabbit antibody was replaced by PBS as a blank (Wang *et al.*, 2007). Five high power fields of each section were selected randomly and input to the HM IAS-2000 analysis system for staining intensity (average absorbance) analysis.

**Statistical analysis:** The data are expressed as means $\pm$ SD. Statistical comparisons were compared by one-way Analysis of Variance (ANOVA). The results were considered statistically significant if the p-values were 0.05 or less.

## RESULTS AND DISCUSSION

As shown in Fig. 1 and Table 1, the expression of BAX proteins in the Group II and III were significantly increased compared with that in the Group I ( $p < 0.05$ ). Although, the expression of BAX proteins in the Group IV was also increased, no significant difference was observed ( $p > 0.05$ ). The expression of BAX proteins in the Group III and IV were significantly decreased compared with that in the Group II ( $p < 0.05$ ).

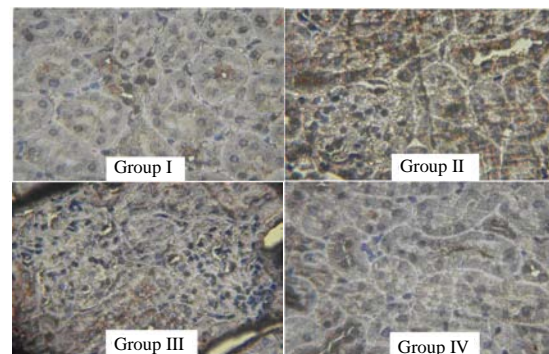


Fig. 1: The expression of BAX proteins in the liver of rats

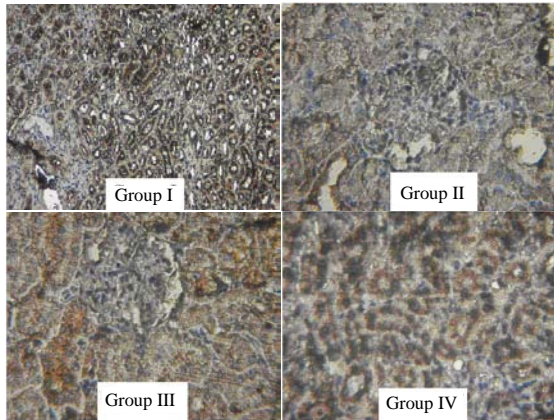


Fig. 2: The expression of Bcl-2 proteins in the liver of rats

Table 1: The comparison of BAX, Bcl-2 and BAX/Bcl-2 ratio among four groups

Groups	BAX	Bcl-2	BAX/Bcl-2
I	14.23±1.17	19.77±2.14	0.72
II	22.49±2.84*	11.64±1.53*	1.93*
III	17.53±1.69**	15.83±1.87**	1.11**
IV	15.15±2.03*	16.42±1.75**	0.92**

Values are mean±SD, \*p<0.05 compared with Group I. \*\*p<0.05 compared with Group II

As shown in Fig. 2 and Table 1, the expression of Bcl-2 proteins in the Group II-IV were significantly decreased compared with that in the Group I (p<0.05). The expression of Bcl-2 proteins in the Group III and IV were significantly increased compared with that in the Group II (p<0.05).

As shown in Table 1, the BAX/Bcl-2 ratio in the Group II-IV were significantly increased compared with that in the Group I (p<0.05). The BAX/Bcl-2 ratio in the Group III and IV were significantly decreased compared with that in the Group II (p<0.05). Apoptosis manifests in two major execution programs downstream of the death signal: the caspase pathway and organelle dysfunction of which mitochondrial dysfunction is the best characterized (Gross *et al.*, 1999; Korsmeyer *et al.*, 2000; Gross, 2001). As the Bcl-2 family members reside upstream of irreversible cellular damage and focus much of their efforts at the level of mitochondria, they play a pivotal role in deciding whether a cell will live or die (Fig. 3). Bcl-2 and BAX are two members of the Bcl-2 family which play different roles in programmed cell death (Huckelhoven *et al.*, 2003; Liu *et al.*, 2005). When BAX was overexpressed in cells, apoptotic death in response to death signals was accelerated, leading to its designation as a death agonist and when Bcl-2 was overexpressed it heterodimerized with BAX and death was repressed (Korsmeyer, 1999; Jang *et al.*, 2002). Therefore, the BAX/Bcl-2 ratio is important in determining susceptibility to apoptosis (Raisova *et al.*, 2001).

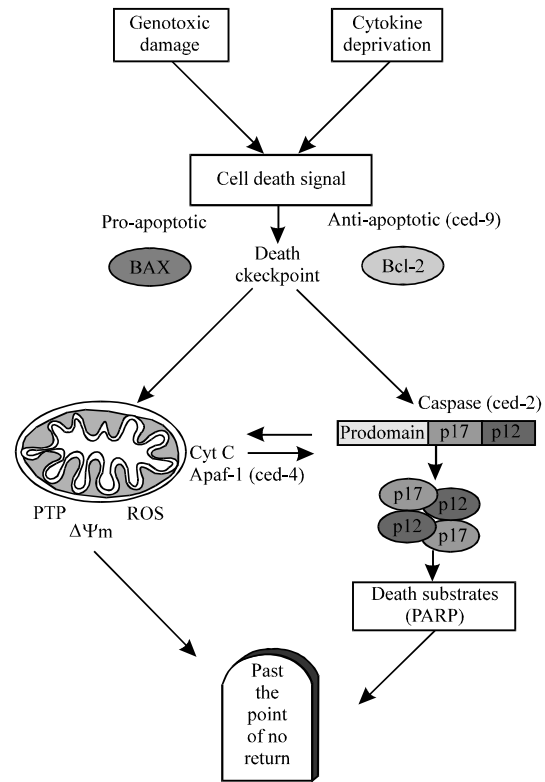


Fig. 3: Schematic Model of mammalian cell death pathway

## CONCLUSION

The data showed that exhausting exercises could make the expression of BAX proteins in the liver and BAX/Bcl-2 ratio dramatically increases, the expression of Bcl-2 proteins obviously decreases. The results indicated that BAX and Bcl-2 co-regulated the exercise-induced apoptosis of rat hepatocytes.

BAX promotes the apoptosis as up-regulated genes while Bcl-2 inhibits apoptosis as down-regulated genes and this may be the gene regulation mechanism of exercise-induced apoptosis. After resveratrol supplementation, the expression of BAX proteins and BAX/Bcl-2 ratio obviously decreased, the expression of Bcl-2 proteins obviously increased. The results indicated that resveratrol supplementation can inhibit exercise-induced apoptosis of rat hepatocytes.

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